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Molecular Therapy
Review

Update on Clinical *Ex Vivo* Hematopoietic Stem Cell Gene Therapy for Inherited Monogenic Diseases

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Gene transfer into autologous hematopoietic stem progenitor cells (HSPCs) has the potential to cure monogenic inherited disorders caused by an altered development and/or function of the blood system, such as immune deficiencies and red blood cell and platelet disorders. Gene-corrected HSPCs and their progeny can also be exploited as cell vehicles to deliver molecules into the circulation and tissues, including the central nervous system. In this review, we focus on the progress of clinical development of medicinal products based on HSPCs engineered and modified by integrating viral vectors for the treatment of monogenic blood disorders and metabolic diseases. Two products have reached the stage of market approval in the EU, and more are foreseen to be approved in the near future. Despite these achievements, several challenges remain for HSPC gene therapy (HSPC-GT) precluding a wider application of this type of gene therapy to a wider set of diseases while gene-editing approaches are entering the clinical arena.

For many years hematopoietic stem cell transplantation (HSCT) has represented the only treatment strategy able to achieve permanent functional reconstitution for some inherited genetic disorders. However, despite the development of registries to obtain the best matched donors and progress in efforts to achieve stable engraftment of hematopoietic stem progenitor cells (HSPCs) through a targeted conditioning regimen and adequate prophylaxis to reduce graft-versus-host disease (GvHD) complications, morbidity and mortality rates remain noteworthy.[1,2] In this scenario, autologous HSCT using gene therapy (GT) with integrating vectors has become an advanced treatment option for some inborn error diseases, both by providing a long-lasting functional copy of the defective gene in the HSPCs and by reducing the allogeneic treatment-related toxicities.

The first clinical application of HSPC-GT was applied to the most severe primary immunodeficiencies (PIDs), which have immunological defects that are intrinsic to the hematopoietic system, making them the ideal target for allogeneic HSCT and, consequently, for HSPC-GT (Figure 1). After successful studies in preclinical murine models, the first GT trials were conducted using gamma retroviral vectors (RVs) in X-linked severe combined immunodeficiency (SCID-X1) and adenosine deaminase deficiency (ADA-SCID).[3,4] The promising early results were subsequently disrupted by the occurrence of genotoxic events in X-linked (X)-SCID patients.[5,6] The scientific community promptly reacted with the development of vectors that allowed robust gene correction in HSPCs, while decreasing insertional mutation risk. Preclinical studies documented that lentiviral vectors (LVs) derived from the human immunodeficiency virus (HIV) were superior to the RVs, both in terms of safety and efficacy. From the safety point of view, LVs are based on a self-inactivating (SIN) configuration in order to minimize the risk of producing HIV replication-competent lentiviral (RCL) particles. They can be adapted to incorporate the use of a physiological gene promoter and are more likely to possess a safer integration profile as compared to RVs.

Several LV-based trials entered the clinic showing favorable safety profiles and treatment efficacy for blood-borne disorders such as immunodeficiencies and hemoglobinopathies. In addition, LV-based GT has been also applied to lysosomal storage diseases (LSDs), inherited metabolic disorders that are caused by enzyme deficiencies within the lysosome, resulting in accumulation of undegraded substrate. This storage process leads to a broad spectrum of clinical manifestations depending on the specific substrate and site of accumulation. Although the pathogenesis of inborn errors may be different, the exploitation of HSPCs is applied with a dual purpose, namely both the replacement of blood cells in the immunodeficiencies and hematological disorders and the sustained tissue release of biotherapeutics to reduce the accumulation of metabolites in LSDs.

Thanks to the positive safety and efficacy data from HSPC-GT clinical trials collected during more than 10 years, two advanced therapy medicinal products (ATMPs) based on engineered HSPCs have been approved for the EU market, and many other clinical trials are also in advanced stages in the US. In May 2016, the European Medicines Agency (EMA) approved, for the first time for licensure, an *ex vivo*

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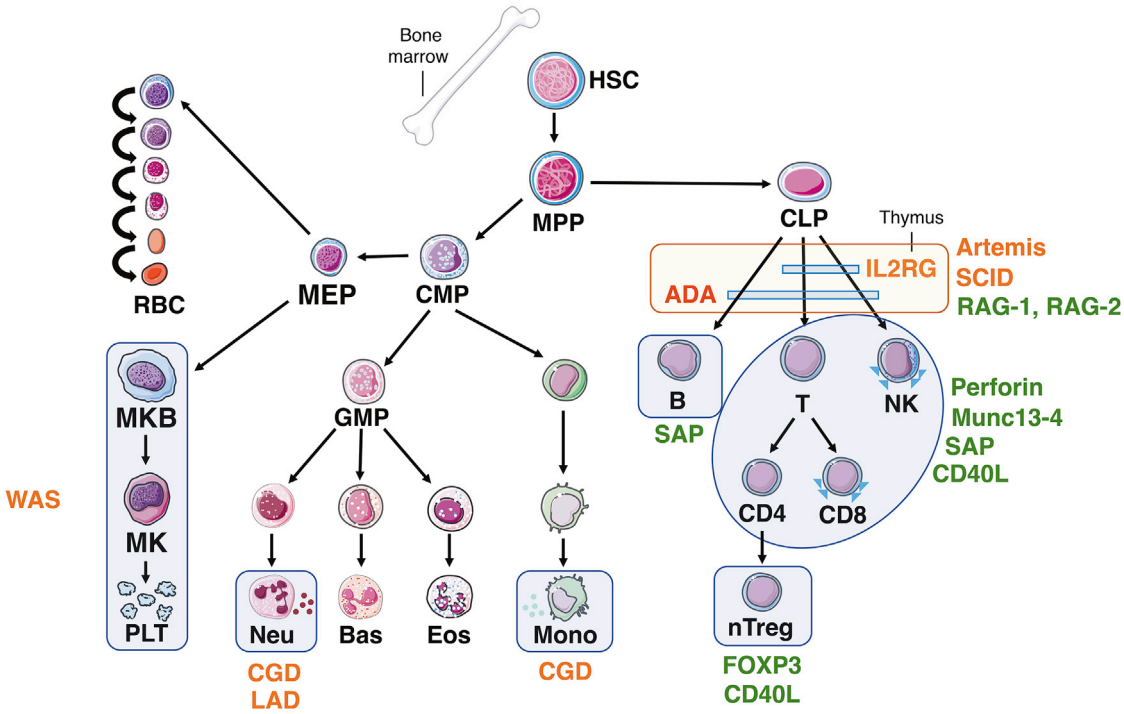

Figure 1. Gene Therapy for Primary Immunodeficiencies

Schematic representations of each hematopoietic cell lineage. Blue boxes denote the cell type(s) most affected in the various primary immunodeficiencies. The color of genes indicates approved gene products (red), products under clinical trial (orange), and products in preclinical studies (green). HSC, hematopoietic stem cell; MPP, multilineage progenitor; CMP, common lymphoid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte-erythroid progenitor; GMP, granulocyte-monocyte progenitor; MKB, megakaryoblast; MK, megakaryocyte; RBC, red blood cell; PLT, platelet; Neu, neutrophil; Bas, basophil; Eos, eosinophil; Mono, monocyte; B, B cell; T, T cell; NK, NK cell; nTreg, natural T regulatory cell.

HSPC-GT for the treatment of ADA-SCID under the commercial name of Strimvelis.[7–10] Following this, in May 2019, Zynteglo became the first GT product for transfusion-dependent (non-β^0/β^0) β-thalassemia patients more than 12 years old and given marketing authorization by the EMA.[11] Another HSPC-GT-based medicine, Libmeldy, recently received EMA positive opinion for marketing authorization to treat metachromatic leukodystrophy (MLD).[12] However, several challenges remain, including the long-term monitoring of the efficiency and safety in patients receiving RV- and LV-based GT products as they are exposed to the risk of insertional mutagenesis, and the possibility of expanding HSPC-GT to new diseases.

In this review, we discuss the clinical progress made during more than two decades and the challenges that HSPC-GT based on integrating vector gene addition strategies will face in the future.

PIDs

PIDs are a large group of rare hereditary disorders that affect one or more components of the immune system.[13] Patients with PIDs commonly have an increased vulnerability to infections, autoimmunity, and inflammation. When PIDs are left undiagnosed or are misdiagnosed, the immune system remains defective, and patients suffer from severe illness, disability, permanent organ damage, or even death. At present, newborn screening, progress in transplantation techniques and the development of GT have gradually improved the diagnosis and survival outcomes of patients with PIDs. We outline here the recent clinical advances in the field of HSPC-GT as a treatment strategy for four of the most extensively investigated PIDs (SCID-X1, ADA-SCID, Wiskott-Aldrich syndrome [WAS], and chronic granulomatous disease [CGD]) (Figure 1). GT for Artemis and CD18 deficiencies have recently entered the clinical arena (Figure 1). Moreover, several other PIDs, including disorders due to genes affecting the innate and/or adaptive immune system, are currently under investigation at the preclinical level with promising results (Figure 1).

SCID-X1

SCID-X1, the most common form of SCID in humans, is caused by inactivating mutations in the cytokine receptor common gamma chain (γ_c) gene. As a result, T cells fail to develop, and patients have no or very low T lymphocyte and natural killer (NK) cell counts, with present but impaired B cells. Following up on the successful outcomes of allogeneic HSCT, HSPC-GT for SCID-X1 has been developed using a gamma-RV encoding the common γ_c transgene to transduce patients' bone marrow (BM) $CD34^+$ cells.[14–16] The selective advantage conferred by common γ_c expression in lymphoid

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progenitors led to a rapid and sustained expansion of T lymphocytes in the peripheral blood of patients already at 30 days after infusion. Gene-corrected B and NK cells were detected in the patients' blood, and humoral immunity was also restored, albeit suboptimally, due to the low level of transduced B cell engraftment achieved in the absence of a pre-conditioning.[14–16]

Despite the substantial improvements in clinical and immunological features, serious genotoxic adverse events occurred.[5] Six of the 20 patients with SCID-X1 undergoing RV-based HSPC-GT developed an uncontrolled clonal T-cell proliferation as early as 2.5 years and up to 15 years after GT. Lymphoproliferation was linked predominantly to an RV integration mapped in the proximity of the distal promoter of the LIM domain only-2 (LMO2) locus, which caused an aberrant LMO2 expression, thereby promoting the uncontrolled proliferation of the targeted clones. After chemotherapy, acute lymphoblastic leukemia underwent remission in five of the six patients, while one patient died.

The successful outcomes obtained in other clinical trials using SIN LVs[17,18] encouraged the development of a similar approach for SCID-X1. In the first LV-based HSPC-GT trial[19], five pediatric/young adults (median age, 16 years) with a history of unsuccessful haploidentical HSCT received, after low-dose busulfan conditioning, BM $CD34^+$ cells transduced with a codon-optimized SIN LV (Cl20-i4-EF1a-hgcOPT). This vector encodes the common γ_c gene/cDNA under the transcriptional control of the elongation factor 1a core promoter and containing a chromatin insulator element derived from chicken β-globin (cHS4) as an additional safety feature (Table 1). The 36-month-long follow-up reported for two patients showed substantial multilineage gene marking, reconstitution of humoral immunity, response to vaccination, and overall clinical improvement after GT. In a subsequent dual-center, phase I/II safety and efficacy trial, eight infants (median age, 3.5 months) treated with the same drug product were monitored for up to 16.4 months (Table 1).[20] After treatment, the number of $CD3^+$ lymphocytes and NK cells normalized in all but one infant. Expansion of gene-corrected T, B, and NK cells was associated with infection clearance and immunoglobulin normalization. None of the patients showed signs of lymphoproliferation, and integration site analysis performed in lineage-sorted blood cells revealed an overall polyclonal pattern without clonal dominance. Some of the identified integration sites were consistent with previous studies with patients receiving LV-based HSPC-GT.[21]

More recently, pre-clinical approaches of gene editing for SCID-X1 that could potentially correct most known pathogenic mutations have been reported.[34,35] The advantage of gene editing over a classical gene addition approach is that it preserves normal copy number and upstream and downstream non-coding elements that regulate expression. The codon-optimized IL2RG cDNA efficiently and precisely integrated into the endogenous start codon in $CD34^+$ HSPCs of healthy male donors or SCID-X1 patients at high frequency. Multi-lineage hematopoiesis derived from edited $CD34^+$ cells was demonstrated using serial transplantation in immunodeficient mice.[34,35] No evidence of significant genotoxicity was identified. Further studies need to be conducted to address the safety and efficacy of gene editing in humans.

ADA-SCID

ADA-SCID is an autosomal recessive monogenic disorder of purine metabolism.[36] In patients, the accumulation of the toxic ADA by-products deoxyadenosine and deoxyadenosine triphosphate (dAXP) leads to defects in the differentiation and function of T, B, and NK cells, leading to severe lymphocytopenia. Non-immunological manifestations, including cognitive impairment and auditory defects, are also observed.[37] If left untreated, most patients with ADA-SCID die from opportunistic infections within the first year of life.

Enzyme replacement therapy (ERT) with polyethylene glycol-modified bovine ADA (PEG-ADA) is used as the initial therapy for most patients with ADA-SCID until they are able to undergo allogeneic HSCT or HSPC-GT.[38,39] ERT ameliorates immune function, thereby decreasing the incidence of severe infections and supporting patients' growth. However, in the long term, PEG-ADA therapy loses its efficacy in a substantial proportion of patients, probably due to the development of anti-ADA neutralizing antibodies, thereby causing a decrease of lymphocyte counts and leaving the patients susceptible to infection, autoimmunity, and malignancies.[40,41] HSCT from a matched sibling donor is the preferred treatment, but it is available for fewer than 20% of patients with ADA-SCID.[13,42]

The high medical need endorsed ADA-SCID as a compelling candidate for treatment with HSPC-GT. The first successful phase I/II HSPC-GT trial for ADA-SCID consisted of an infusion of autologous BM-derived $CD34^+$ cells transduced with a gamma-RV encoding for the human ADA cDNA sequence following low-dose conditioning (Table 1). Busulfan was chosen as a chemotherapy agent because of its wide use in allogeneic HSCT. The busulfan dose corresponded approximately to 25% of the total dose normally used in full-intensity myeloablative protocols (Figure 2).[4,43,44] Before HSPC-GT, all patients had received either unsuccessful haploidentical HSCT or ERT with PEG-ADA.

The most recently reported clinical update of 18 patients[44] showed that the ADA gene transfer into HSPCs led to a sustained immune reconstitution, ranging from partial to full restoration of functional thymopoiesis, correction of T cell functions, and adequate systemic detoxification.[44] In the first 3 years of follow-up, the proportion of transduced cells was 1%–10% of the total BM $CD34^+$ cells. Consistently with a selective advantage of corrected immune cells, after 1 year, T, B, and NK cells became the majority of lymphocytes in the peripheral blood progressively and remained stable throughout the follow-up. The expansion of corrected cells expressing the ADA enzyme was paralleled by an increase of activity in the peripheral blood lymphocytes, which led to a substantial reduction of toxic dAXP to levels within the range of those found in patients successfully transplanted with allogeneic HSCT.[44] The levels of genetic correction were sufficient to provide a significant clinical benefit. Most patients

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Table 1. Most recent HSPC Gene Therapy Available on ClinicalTrials.gov (Recruiting or Completed)

Disease (Gene)	Trial Phase	Vector	Conditioning	Preliminary Outcomes	Clinical Trial Registry Number (Reference): ClinicalTrials.gov:
SCID-X1 (*IL2RG*)	I/II	G2SCID LV	low-dose busulfan	still recruiting	NCT03311503
SCID-X1 (*IL2RG*)	I/II	G2SCID LV	low-dose busulfan	still recruiting	NCT03601286
SCID-X1 (*IL2RG*)	I/II	TYF-IL-2Rg self-inactivating LV (TYF-IL-2Rg)	not known	still recruiting	NCT03217617
SCID-X1 (*IL2RG*)	I/II	LV VSV-G pseudotyped CL20-4i-EF1a-hyc-OPT	low-dose busulfan	sustained marking levels and restoration of humoral responses to immunization	NCT01306019 (De Ravin et al.[19])
ADA-SCID (*ADA*)	I/II	EFS-ADA LV	low-dose busulfan	sustained engraftment of genetically modified HSPCs in all of the patients with long-term metabolic detoxification from deoxyadenosine nucleotides after stopping ERT	NCT02999984 (Kohn et al.[22])
ADA-SCID (*ADA*)	I/II	EFS-ADA LV	low-dose busulfan	sustained engraftment of genetically modified HSPCs in 9 of 10 patients	NCT01852071 (Kohn et al.[22])
ADA-SCID (*ADA*)	I/II	EFS-ADA LV	low-dose busulfan	still recruiting	NCT03765632
ADA-SCID (*ADA*)	II/III	EFS-ADA LV	low-dose busulfan	suspended (recruitment on hold for business reasons)	NCT04140539
ADA-SCID (*ADA*)	not applicable[a]	self-inactivating LV TYF-ADA	not known	still recruiting	NCT03645460
WAS (*WAS*)	I/II	w1.6_hWASP_WPRE (VSVg) LV	reduced-intensity conditioning regimen with busulfan and fludarabine	sustained engraftment of genetically modified HSPCs with reduction of bleeding events and restoration of WASP expression in lymphocytes and plateles,	NCT01515462 (Ferrua et al.[17])
WAS (*WAS*)	I/II	w1.6_hWASP_WPRE (VSVg) LV[b]	myeloablative conditioning regimen with busulfan and fludarabine	sustained multi-lineage vector gene marking over time; all subjects had improvement or resolution of eczema and none had intercurrent severe infectious events	NCT01410825 (Labrosse et al.[23])
WAS (*WAS*)	II	w1.6_hWASP_WPRE (VSVg) LV[b]	reduced-intensity conditioning regimen of busulfan and fludarabine	no results available	NCT03837483
WAS (*WAS*)	I/II	w1.6_hWASP_WPRE (VSVg) LV[b]	myeloablative conditioning regimen with busulfan and fludarabine	stable engraftment of genetically and functionally corrected lymphoid and myeloid cells in all patients with lack of severe adverse events or clonal expansion	NCT02333760 (Magnani et al., 2020[24])
X-CGD (*gp91phox*)	I/II	G1XCGD LV	myeloablative conditioning regimen with busulfan	stable vector copy numbers and persistence of oxidase-positive neutrophils in six of seven surviving patients; no new CGD-related infections	NCT01855685 and NCT02234934 (Kohn et al.[25])
X-CGD (*gp91phox*)	I/II	G1XCGD LV	myeloablative conditioning regimen with busulfan	two of four patients showed clinical and biological benefits	NCT02757911 (Magnani et al., 2020[26])
Transfusion-dependent β-thalassemia (*HBB*)	I/II	GLOBE LV	myeloablative conditioning with treosulfan and thiotepa	robust and persistent engraftment of genetically modified HSPCs in seven of nine patients and achievement of transfusion independence in four of six children	NCT02453477 (Scaramuzza et al., 2020[27])

(*Continued on next page*)

Table 1. *Continued*

Disease (Gene)	Trial Phase	Vector	Conditioning	Preliminary Outcomes	Clinical Trial Registry Number (Reference): ClinicalTrials.gov:
Transfusion-dependent β-thalassemia (*HBB*)	III	LV βA-T87Q-globin	myeloablative conditioning regimen with busulfan	transfusion independence was observed in most of the patients; HbAT87Q stabilized approximately 6 months after treatment, and patients who stopped RBC transfusions had improved erythropoiesis	NCT02906202 (Thompson et al.[28])
Transfusion-dependent β-thalassemia (*HBB*)	III	LV βA-T87Q-globin	myeloablative conditioning regimen with busulfan	in three of four patients with ≥6 months follow-up have stopped transfusions and one patient has achieved transfusion independence	NCT03207009 (Lal et al.[29])
SCD (*HBB*)	I/II	LV βA-T87Q-globin	myeloablative conditioning with busulfan	improvement in hematologic parameters and disease-related symptoms	NCT02151526 (Magrin et al.[30])
SCD (*HBB*)	I/II	LV βA-T87Q-globin	myeloablative conditioning with busulfan	reduction in the annualized rate of disease-related symptoms; patients maintained HbAT87Q production, demonstrating the durability of gene therapy-derived β-globin gene expression	NCT02140554 (Walters et al.[31])
SCD (*HBB*)	I/II	γ-globin LV	reduced intensity conditioning with melphalan	still recruiting	NCT02186418
SCD (*HBB*)	I/II	GLOBE1 LV Expressing the βAS3 Globin Gene	myeloablative conditioning with busulfan	still recruiting	NCT03964792
SCD (*HBB*)	I/II	Lenti/G-βAS3-FB LV	myeloablative conditioning with busulfan	still recruiting	NCT02247843
SCD (*HBB*)	I	LV encoding human γ-globin G16D and short-hairpin RNA734 for selection of hypoxanthine guanine phosphoribosyltransferase	reduced intensity conditioning with melphalan	still recruiting	NCT04091737
MLD (*ARSA*)	I/II	LV ARSA	myeloablative conditioning with busulfan	sustained multilineage engraftment of genetically modified HSPCs and clinical improvement compared to natural history patients	NCT01560182 (Fumagalli et al., 2020, 16th Annual WORLD Symposium, conference)
MLD (*ARSA*)	II	LV ARSA	myeloablative conditioning with busulfan	preliminary results showed multilineage engraftment of genetically modified HSPCs and restoration of ARSA activity	NCT03392987 (Fumagalli et al., 2020, 16th Annual WORLD Symposium, conference)
X-ALD (*ABCD1*)	II/III	SIN LV MNDprom-ABCD1 (Lenti-D) encoding human adrenoleukodystrophy protein	myeloablative conditioning with busulfan and cyclophosphamide	gene marked cells after engraftment and measurable ALD protein in all of the patients	NCT01896102 (Eichler et al.[32])
X-ALD (*ABCD1*)	III	autologous $CD34^+$ cells transduced with SIN LV MNDprom-ABCD1 (Lenti-D) encoding human adrenoleukodystrophy protein	myeloablative conditioning with busulfan and fludarabine	still recruiting	NCT03852498
MPS I (*IDUA*)	I/II	LV IDUA	myeloablative conditioning with busulfan and fludarabine	preliminary results showed multilineage engraftment of genetically modified HSPCs and restoration of IDUA activity	NCT03488394 (Gentner et al., 2020, 16th Annual WORLD Symposium, conference)

(*Continued on next page*)

Table 1. *Continued*

Disease (Gene)	Trial Phase	Vector	Conditioning	Preliminary Outcomes	Clinical Trial Registry Number (Reference): ClinicalTrials.gov:
MPS III (*SGSH*)	I/II	CD11b LV vector encoding for human SGSH	myeloablative conditioning with busulfan	preliminary results showed multilineage engraftment of gene-modified cells and sustained vector copy number	NCT04201405 (Kinsella et al., 2020[33])
Fabry disease (*GLA*)	I	LV AVR-RD-01	myeloablative conditioning	durable engraftment; sustained plasma and leukocyte enzyme activity	NCT02800070 (AvroBio data update, ASGCT 2020)
Fabry disease (*GLA*)	I/II	LV AVR-RD-01	myeloablative conditioning	durable engraftment; sustained plasma and leukocyte enzyme activity	NCT03454893 (AvroBio data update, ASGCT 2020)

[a]Not applicable is used to describe trials without US Food and Drug Administration (FDA)-defined phases.
[b]Same vector design but performed in different manufacturing sites and with different transduction protocols.

attend school, and the rate of severe infections significantly dropped.[44] The initial cohort was extended to a total of 22 subjects who were part of the clinical development program, including clinical trial pilot studies and a compassionate use program. A 100% survival was reported, with the longest follow-up at 18 years after GT (F. Barzaghi, 2020, 19th Biennial Meeting of The European Society for Immunodeficiencies Online Meeting, conference).[39] Five of these patients required additional intervention (allogeneic HSCT or ERT) due to treatment failure.

Full approval of the medicinal product in the EU was granted in April 2016 after 10 months of review under a centralized procedure. The initial price in Italy was in the range of the cost of 2 years of ERT. As of June 2020, 12 patients have received the commercial product at San Raffaele Hospital, the only treatment center due to the short shelf-life of the product prepared fresh after BM harvest. All patients are alive, and 11 are intervention-free without HSCT or PEG-ADA after GT (M. Migliavacca, 2020, 19th Biennial Meeting of The European Society for Immunodeficiencies Online Meeting, conference).[39]

In terms of safety, adverse events were consistent with those reported for patients with ADA-SCID undergoing low-dose myeloablative busulfan conditioning, including non-serious opportunistic infections of the respiratory, gastrointestinal, and urinary tract, as well as autoimmune manifestations. At the time of the latest communications (F. Barzaghi, 2020, 19th Biennial Meeting of The European Society for Immunodeficiencies Online Meeting, conference; M. Migliavacca, 2020, 19th Biennial Meeting of The European Society for Immunodeficiencies Online Meeting, conference) no events of clinically manifesting mutagenesis were reported, and patients continued long-term monitoring.[4,44] Recently, an event of lymphoid T cell leukemia was reported in one patient, and its relationship with GT is currently under investigation.[45]

Clinical trials conducted in the UK and US demonstrated a similar clinical efficacy of gamma-RV HSPC-GT combined with mild chemotherapy (Table 1).[3,46] Most of the 28 patients treated in these studies recovered immune functions, showed normal ADA activity in peripheral blood mononuclear cells (PBMCs), remained off ERT, and discontinued immunoglobulin replacement therapy.[39] No serious adverse events attributable to the medicinal product were recorded. Although some clones harboring vector integrations near *MECOM* or *LMO2* loci persisted in the long term, there was no evidence of leukoproliferative events throughout the follow-up.[46]

In recent years, HSPC-GT using SIN LVs have also been developed. Multi-centric non-randomized phase I/II trials (ClinicalTrials.gov: NCT01852071, NCT02999984, and NCT01380990) are ongoing using EFS LVs encoding for a codon-optimized human ADA cDNA, following non-myeloablative conditioning with busulfan in infants/children and adults upon ERT discontinuation (Table 1). Overall, 53 patients were reported to have received treatment with LV HSPC-GT, with two treatment failures.[39,47,48] Preliminary data showed that all subjects survived, and engraftment of gene-corrected cells was achieved and remained stable over 24 months. Peripheral blood leukocyte counts improved, and patients no longer experienced severe opportunistic infections.

It is of paramount importance to continue the follow-up of patients to gain valuable information on the long-term efficacy and safety outcomes of the gamma-RV HSPC-GT while also monitoring the outcome of LV HSPC-GT.

WAS

WAS is a rare and complex X-linked immunodeficiency characterized by microthrombocytopenia, eczema, and recurrent infections.[49] Patients are also at a high risk of autoimmunity and lymphoproliferative disorders. Loss-of-function mutations in the gene encoding the WAS protein (WASp) cause actin polymerization defects, which compromise many critical immunological processes, including immune synapsis formation, cell migration, and cytotoxicity.[50] Depending on the severity, WAS patients treated merely with symptomatic treatments die within their third decade. As for other PIDs, allogeneic HSCT using related or unrelated human leukocyte antigen (HLA)-matched donors can be curative for WAS patients, especially if transplanted at a young age.[51] However, a substantial

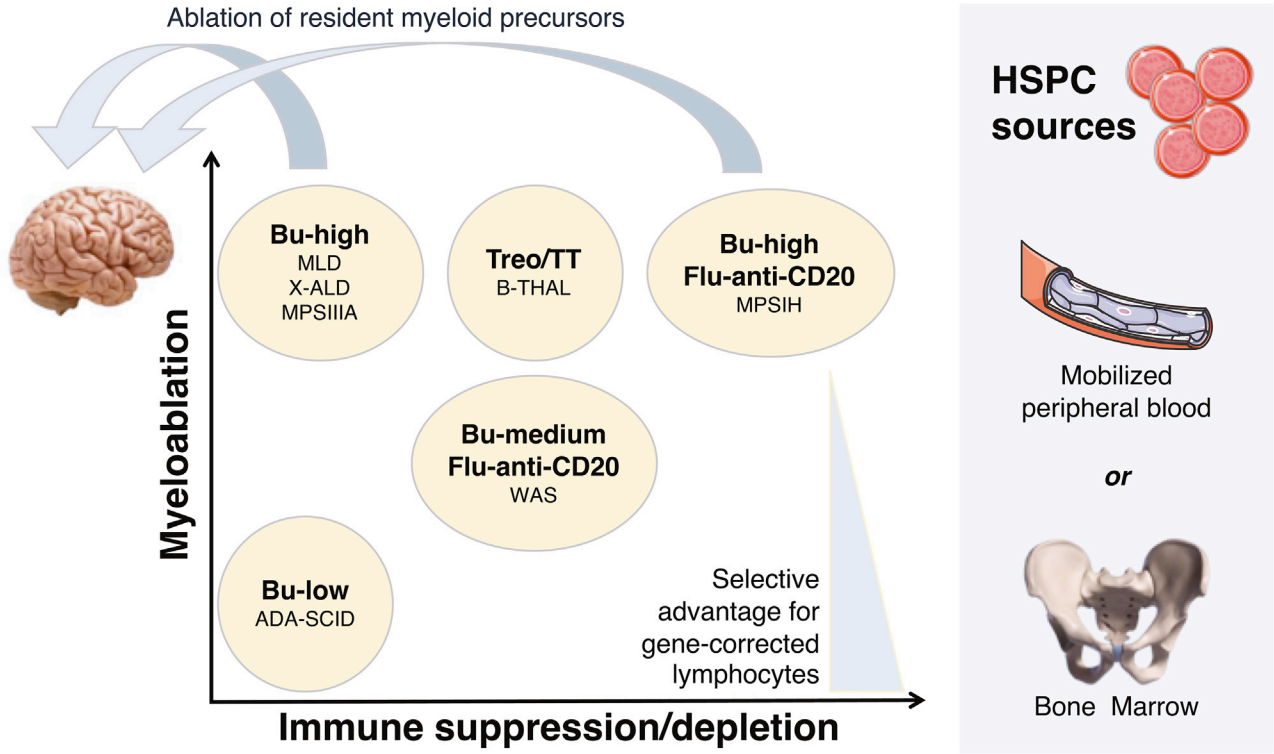

Figure 2. Summary of Representative Conditioning Regimens Preparatory for Gene Therapy and the HSPC Sources

Conditioning regimens are designed based on the target disease and gene-corrected chimerism, presence of selective advantage, need for immune suppression, or ablation of resident brain myeloid cells. The first ADA-SCID HSPC-GT trials resulted in low or absent engraftment due to no preconditioning. In the subsequent trials, a minimal dose intensity conditioning with busulfan intravenously (i.v.), corresponding to ~25% of the standard dose used in allogeneic HSCT (Bu-low), was introduced, allowing good levels of multilineage engraftment of corrected cells. The rationale of combining busulfan at a reduced intensity dose (Bu-medium) and fludarabine (Flu) in the WAS trials was to minimize toxicity and fully exploit the selective growth advantage of gene-corrected cells (role of busulfan), deplete the lymphoid compartment of potentially autoreactive lymphocytes (role of Flu and anti-CD20), and prevent lymphoproliferative disorders due to EBV reactivation (role of anti-CD20). For the LSDs and hemoglobinopathies, a myeloablative dose of alkylating agents (Bu-high or Treo/TT) is required to cross the BBB and obtain high engraftment levels, which is necessary due to the absence of a natural selective advantage for the gene-corrected cells. In the MPS IH trial Flu and anti-CD20 were foreseen to achieve a level of immunosuppression able to reduce or abolish an anti-IDUA B and T cell response that may jeopardize the survival of the gene-modified cells. HSPC collection can be performed through a BM harvest or apheresis from a peripheral vein after mobilization with G-CSF alone or in combination with plerixafor. BBB, brain-blood barrier; BM, bone marrow; Bu, busulfan; Flu, fludarabine; HSCT, hematopoietic stem cell transplantation; LSD, lysosomal storage disease; Treo, treosulfan; TT, thiotepa.

fraction of patients (~10%) rejected the graft and showed a mixed donor cell chimerism.

Autologous HSPC-GT, combined with reduced-intensity preconditioning, represents a suitable therapeutic option for patients with WAS. Initial studies using a gamma-RV carrying a functional WAS gene under the control of a strong viral promoter showed the feasibility of the approach and revealed the selective advantage of gene-corrected cells in all patients.[52,53] However, 9 of the 10 treated patients developed acute leukemia associated with insertional mutagenesis, which activated several well-known proto-oncogenes (i.e., *LMO2*, *MDS1*, and *MN1*), highlighting that the use of gamma-RV in this disease is associated with a high risk of leukemogenic events.[52,54]

In 2010, a clinical trial of HSPC-GT for WAS was initiated in Milan using SIN LVs encoding the human WASp under the control of a 1.6-kb proximal fragment of the endogenous promoter to ensure physiological transgene expression.[17,21,55] Since then, 34 patients have been treated in four centers using the same vector backbone but different manufacturing sites and protocols, as well as preparatory conditioning regimens (Table 1). In the pivotal study conducted at the San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), eight pediatric subjects exhibiting severe WAS clinical manifestations were enrolled, following a reduced-intensity conditioning regimen (Figure 2). Patients received autologous $CD34^+$ cells isolated either from BM (five patients) or mobilized peripheral blood (mPB) (two patients) or a combination of both (one patient) and transduced with the WASp-encoding LV. The documented median follow-up was 3.6 years (range, 0.5–5.6 years).[17]

Starting from 1 year, all eight patients displayed stable long-term engraftment of transduced cells with polyclonal hematopoietic reconstitution (granulocytes, megakaryocytic precursors, erythroid cells, B cells, and NK cells). Albeit more slowly compared with other lineages,

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transduced T cells started appearing 4 months after GT. Except for one patient, T, B, and NK cell counts were normal, and the fraction of WASp-expressing lymphocytes substantially increased from a median of 3.9% pre-GT to 66.7% (range, 55.7%–98.6%) at 1 year after HSPC-GT. The restoration of WASp expression resulted in the correction of T cell proliferation defects in response to T cell receptor (TCR) activation. Eczema scores improved considerably in all patients at 3 years of follow-up. WASp expression in most of the platelets led to a substantial increase of platelet counts and correction of mean platelet volume and function, contributing to substantially reduce bleeding events.[56] The adverse events occurred in the first 6 months of follow-up and were mostly due to severe infections, but the reconstitution of a normal cellular immune response led to a progressive and significant decline of infectious sequelae.

A positive risk-benefit profile was reported also for the other patients treated with HSPC-GT in an early access program in Milan or in the open-label trials carried out in Paris, London, and Boston.[54,57,58] Two patients died of preexisting conditions (refractory infectious disease and neurological disorder) and one due to post-splenectomy sepsis after influenza.[54,57] All of the other patients were alive and showed significant clinical improvements. Eczema and susceptibility to infections had resolved. Evidence of renewed thymopoiesis was capable of quantitatively restoring normal levels of $CD4^+$ T cells, which displayed a polyclonal T cell receptor repertoire. Response to microbial antigens was detected in some patients. After GT, patients' dendritic cells showed normal podosome formation,[57] indicating that WASp correction restored cytoskeleton rearrangement.

In all LV-based HSPC-GT studies,[17,21,57,58] the integration site analysis revealed a preferential vector distribution within transcription units, which is typical for LVs, with no evidence of clustering within proto-oncogenes (i.e., *LMO2*, *CCND2*, and *MECOM*), as previously reported in gamma-RV clinical trials.[59] Studies on clonal dynamics based on vector insertions allowed answering crucial questions on the real-life behavior of human HSPCs, complementing and expanding the data derived from animal models.[59,60] Moreover, longitudinal clonal tracking studies contributed to reveal the lymphoid and myeloid hierarchies of human hematopoiesis, the short- and long-term fates of transduced HSPCs, and the return to dormancy of HSPCs after transplantation. Long-term evaluation of the clinical outcomes and safety in larger cohorts will provide a much more comprehensive and definitive assessment of the potential of LV-based HSPC-GT for WAS.

CGD

CGD is a PID disorder of phagocytes caused by loss-of-function mutations in the genes encoding the components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Most of the mutations responsible for X-linked CGD (X-CGD) are identified in the CYBB gene encoding the $Gp91^{phox}$ subunit of NADPH. Autosomal recessive forms also exist ($p22^{phox}$, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$), accounting collectively for one-third of the CGD cases. Patients' phagocytes are unable to generate normal amounts of reactive oxygen species (ROS) and, therefore, patients are highly susceptible to recurrent life-threatening infections and experience a chronic inflammatory response. Lifelong antibacterial and antifungal prophylaxis and IFNγ treatment are used to prevent and treat infections, while HSCT is a curative treatment for patients with CGD.[61] HSPC-GT represents a new therapeutic option for patients who cannot be considered for allogeneic HSCT.

In early GT clinical studies for X-CGD, patients received an infusion of autologous $CD34^+$ HSPCs transduced *ex vivo* with a gamma-RV vector encoding $Gp91^{phox}$ in the absence of BM conditioning.[62] The proportion of corrected granulocytes declined soon after GT due to poor HSPC engraftment. Later studies introduced myeloablative conditioning with busulfan prior to the infusion of $CD34^+$ cells transduced with a gamma-RV encoding $Gp91^{phox}$ expression under the control of the Friend mink cell spleen focus-forming virus (SFFV) long terminal repeat (LTR).[63–67] However, although vector-positive cells were present in the peripheral blood, only a small fraction of circulating neutrophils exhibited a functional NADPH oxidase. The partial reconstitution of ROS generation in neutrophils was sufficient to resolve or ameliorate the life-threatening infections. However, most patients developed a severe myelodysplastic syndrome caused by the expansion of clones harboring monosomy 7 and transactivation of MDS-EVI1 and PRDM16 proto-oncogenes.[65]

Following the appearance of these serious genotoxic events, gamma-RVs were put aside, and SIN LVs were developed to allow myeloid-specific expression of $Gp91^{phox}$ in phagocytes. Pre-clinical mouse and human studies provided evidence of the efficacy of HSPC-GT mediated by LVs encoding $Gp91^{phox}$ under chimeric myeloid promoters.[68–72] In one of these studies the emergence of tumors in XCGD mice suggests that a chronic inflammatory XCGD background may contribute to oncogenesis.[72]

A multicentric phase I/II study using one of these LVs (G1XCGD) is currently ongoing in Europe and the United States (Table 1).[25] Nine patients with severe XCGD (age range, 2–27 years) received mPB or BM $CD34^+$ cells transduced with a clinical grade G1XCGD preparation after busulfan preconditioning at a myeloablative dose. The reported minimum and maximum follow-ups are 12 and 36 months, respectively. Two patients died within 3 months of treatment for pre-existing comorbidities (hyperacute sterile pneumonitis, fatal intracranial bleed post-transplant associated with refractory autoimmune platelet destruction). Corrected circulating neutrophils were detectable in the peripheral blood within 1 month of GT. NADPH activity was corrected in >15% of neutrophils in all patients at 1 month after GT and persisted in six of seven surviving patients, reaching 16%–46% at 12 months. The viral copy number (VCN) in blood neutrophils remained stable in six out of seven patients during 24 months. Longitudinal analysis of vector integration site distributions showed highly polyclonal populations of gene-modified cells. The patients with stable NADPH activity in neutrophils remained clinically well and did

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not experience new bacterial and fungal infections. Long-term follow-up results are needed to validate the clinical outcome and safety of the HSPC-GT approach for CGD.

Red Blood Cell Disorders

Since the early days of GT, red blood cell monogenic disorders were considered ideal targets for genetic correction, but their clinical application came only following more than a decade of improvement in vector design and extensive preclinical work. GT is a potential alternative to allogeneic HSCT for the treatment of β-thalassemia and sickle-cell disease (SCD), representing the most frequent monogenic disorder worldwide.[73,74] Both diseases are caused by mutations in the HBB gene, encoding for β-globin. β-Thalassemia is characterized by mutations that completely abolish (β^0/β^0 genotype) or reduce the expression of hemoglobin β chain, leading to an imbalance between the α and β chains. The clinical phenotype varies due to different genetic defects and is characterized by anemia and ineffective erythropoiesis accompanied by concomitant complications, such as extramedullary hematopoiesis and iron overload.[75] The main treatment in thalassemia is red blood cell transfusion, which affects patients' life quality and expectancy. Allogeneic stem cell transplantation is strongly recommended for patients with an HLA-matched donor,[76,77] but this option is only available for the minority of patients.

SCD is caused by a single mutation resulting in valine-glutamic acid residue substitution at position 6 of β globin (HbS), which induces polymerization of deoxygenated hemoglobin tetramers and induces the typical sickle-shaped erythrocyte formation, causing hemolytic anemia and stroke.[78]

The experience in allogeneic HSCT suggests that a 10%–30% chimerism in BM mononuclear cells may be sufficient to improve clinical conditions in thalassemic patients.[79] In addition, preclinical studies conducted in disease and immunodeficient mouse models, as well as *ex vivo* studies using patients' HSPCs, showed that GT could be a valid therapeutic option for β-thalassemia and SCD.[80–83]

However, several critical aspects had to be assessed in the development of a GT approach. First, globin gene expression is tightly regulated in red blood cells and requires the presence of large-sized locus control region (LCR) elements and gene sequences, potentially affecting the design and manufacture of clinically usable vectors.[84] Moreover, the BM microenvironment in β-thalassemia is characterized by chronic stress erythropoiesis and inflammation. In SCD, the abnormal vascular network could have a detrimental impact on the clinical outcome due to impaired HSC function.[78,85–87] In addition, a marked erythroid expansion and the lack of a selective advantage required myeloablative conditioning to facilitate the engraftment of gene-corrected HSPCs.[88]

The first attempt to treat β-thalassemia with GT started in 2007 in France using a SIN LV encoding a mutated β-globin gene (β^{T87Q}) and insulated with two copies of the 250-bp core of the chicken cHS4 chromatin insulator (BGI vector). Clinical efficacy with transfusion independence in the treated patients was achieved, but there was some initial concern due to the clonal dominance of an LV vector integration in the HGMA2 gene, which turned out to be benign and self-limiting.[89] More recently, two different phase I/II clinical trials (ClinicalTrials.gov: NCT01745120 and NCT02151526) were started using a vector similar to the previous one but modified to remove the insulators (LentiGlobin BB305) (Table 1). The presence of the β^{T87Q} mutation allowed the quantification of the vector-derived hemoglobin A^{T87Q} from the endogenous one. Variable HbA^{T87Q} expression was observed, which reached at least 8 g per deciliter in 7 out of 22 treated patients. At more than two years of median follow-up, transfusion independence was reached in most of non-β^0/β^0 phenotype and 38% of patients with the most severe β^0/β^0 genotype. Remaining patients with β^0 or more severe β^+ phenotypes achieved different degrees of reduction in transfusion requirements. Notably, a safe integration profile with no clonal dominance was shown.[90] These data reveal the need to increase gene correction efficiency in HSPCs to improve the clinical outcome, particularly in those patients presenting the most severe phenotypes. Based on these results, two phase III trials (ClinicalTrials.gov: NCT02906202 and NCT03207009) commenced, adopting a new optimized transduction protocol. In 2019, the EMA gave marketing authorization to the first *ex vivo* GT product for patients with a non-β^0/β^0 genotype. The product was first launched in Germany in January 2020 on a model of outcome-based payment agreement split into five installments.

Another trial (ClinicalTrials.gov: NCT02453477) was conducted in Italy using HSPCs transduced with the GLOBE LV harboring the human β-globin gene under the control of the minimal endogenous promoter and two elements from the LCR vector (Table 1). Transduced $CD34^+$ cells were infused in patients by intrabone injection after myeloablative conditioning with treosulfan and thiotepa. Three adults and six minors with different severe genotype (β^0/β^0, β^+/β^+, and β^0/β^+) have been treated. A more significant clinical benefit was observed in younger subjects with four out of six pediatric patients who discontinued transfusion shortly after GT, while the three adult patients had a reduction of transfusion requirement.[91] The superior outcome in younger subjects could be related to biological differences in HSPCs, BM niche, and comorbidities in adult patients.

The BB305 LV encoding a β-globin transgene (βT87Q globin) with antisickling properties was used to treat the first SCD patient showing proper gene correction levels and improved clinical condition.[92] These promising results were not confirmed in other multicenter studies, probably due to low transduction levels and poor engraftment of gene-corrected cells. To overcome these issues, a different source of HSPC (plerixafor-mobilized rather than BM-derived HSPCs) and optimized transduction protocol were introduced.[31,93]

Moreover, in January 2020, a phase I/II trial based on the use of LV encoding for a β-globin transgene carrying three different anti-sickling mutations (βAS3) started. Since the persistence of fetal hemoglobin (HbF) expression in erythrocytes of adult SCD patients was

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known to result in a better clinical outcome,[94] another attempt to ameliorate the treatment of SCD is based on transplantation HSPCs transduced with HbF encoding vector after reduced-intensity conditioning.[95]

Clinical trials for β-thalassemia and SCD based on induction of endogenous HbF by CRISPR-Cas9 modification of $CD34^+$ cells have recently started, with initial evidence of efficacy (S. Corbacioglu, 2020, Eur. Hematol. Assoc., conference).

Inherited Metabolic Disorders

Inherited monogenic metabolic disorders affecting the lysosomal and peroxisomal metabolic activity primarily impair the central nervous system (CNS) functions, leading to oxidative stress, local inflammation, microglial activation, progressive demyelination, and axonal degeneration. For some of these diseases, the standard of care is mainly based on ERT and allogeneic HSCT, but the capacity of a "neurocorrection" remains difficult to reach due to the enzyme inability to cross the blood-brain barrier (BBB) and to achieve corrective and stable levels of the missing enzyme in the brain.[96,97]

To overcome these limitations, HSPC-GT, supported by pre-clinical data,[98,99] has been shown to be a more effective source of functional enzyme than wild-type (WT) cells, inducing enzyme overexpression in HSPC progeny, including in microglia derived from stem cells. Moreover, microglia cells, which have been involved in the pathogenesis of many neurodegenerative conditions, including LSDs,[100–103] serve as cellular vehicles to deliver the enzyme to other brain-resident cells, eliminating the accumulated metabolites and preventing damage. Also, defective microglia cells are thought to be involved in the pathogenesis of neurological manifestations in some metabolic disorders,[104] and therefore GT approaches are also aimed at their replacement with gene-corrected precursors of microglia-like cells.

The first clinical application of LV-based GT was carried out in the context of X-linked adrenoleukodystrophy (X-ALD), followed by MLD, with the common aim of correcting the neurological phenotype. More recently, encouraging preliminary results were observed in studies started in Europe for the treatment of mucopolysaccharidosis (MPS) type I and III. Phase I and II clinical trials for Fabry disease are also ongoing (AVR-RD-01, Avrobio) (ClinicalTrials.gov: NCT02800070 and NCT03454893) (Table 1).

X-ALD

X-ALD is a rare X-linked disorder of peroxisomal oxidation due to mutations in the ABCD1 gene, which encodes the peroxisomal membrane ALD protein (ALDP).[105] ALDP deficiency leads to impaired transmembrane transport of very-long-chain fatty acids into peroxisomes, where they are degraded.[105,106] The cerebral variant of ALD (CALD), which occurs in one-third of patients, affects boys under the age of 12 and entails disability and death without treatment.[105] To date, the only curative therapy for CALD is represented by allogeneic HSCT, which is effective in stabilizing disease progression but only when performed at an early phase of neurodegeneration.[107,108] However, the survival and functional outcomes remain challenging due to the possible onset of graft failure, infections, and GvHD, especially in the context of an HLA-mismatch unrelated donor.[109] To overcome these limitations, in 2009, *ex vivo* HSPC-GT was applied for the first time using LVs for treatment of this monogenic disease, showing to be effective in two patients with CALD.[110] Since then, the cohort was enlarged with two additional patients. In a 5- to 10-year follow-up report, despite a marked decrease in VCN and ALDP expression in circulating cells, patients showed an overall clinical stabilization, positive effects on brain demyelination, stable engraftment, and long-term survival of transduced HSPCs.[111,112] Following these results, from 2013 onward, an additional 17 patients were treated in the ALD-102 study, a larger multicenter phase II/III clinical trial (ClinicalTrials.gov: NCT01896102) starting in 2013 (Table 1).[32] Fifteen patients are alive without significant functional disability at 24 months after GT upon the administration of a myeloablative conditioning regimen with busulfan and cyclophosphamide. One death was related to a rapid neurologic deterioration while another was due to HSCT-related complications. Polyclonal reconstitution with multilineage gene marking and no molecular evidence of clonal dominance were observed in all the patients. Encouragingly, a phase III clinical trial (ClinicalTrials.gov: NCT03852498), the ALD-104 study, was initiated to recruit up to 35 CALD male subjects ($\leq$ 17 years of age) who will be infused with Lenti-D drug product after myeloablative conditioning with busulfan and fludarabine. Lenti-D drug product is the same in both ALD-102 and ALD-104 studies and consists of autologous $CD34^+$ cell-enriched cells transduced with Lenti-D LV encoding human ALDP protein, suspended in a cryopreservative solution.

Altogether, these preliminary results confirm HSPC-GT as a preferable treatment option for ALD that would avoid the morbidity and complications associated with the allogeneic HSCT.

MLD

MLD is an autosomal recessive LSD caused by the inherited deficiency of arylsulfatase A (ARSA). It determines the accumulation of sulfatides and causes demyelination and neurodegeneration.[113] Three distinct clinical subtypes of MLD are recognized based on the age of disease onset: late infantile (LI-MLD, around 2 years of age), juvenile (J-MLD, ages 3–16 years), and adult (A-MLD, ages >16 years). LI-MLD patients have the most severe neurologic evolution and die within a few years from symptom onset. Their neurological manifestations are namely motor related, including weakness, gait abnormalities, quadriparesis, dysarthria, and incontinence. In the past two decades, significant limitations have emerged using allogeneic HSCT particularly with regard to early onset (LI and early juvenile [EJ] children) MLD patients, even when children are healed prior to the onset of overt symptoms.[114]

In an attempt to treat this fatal disease, a huge step forward was made starting in 2010 when a clinical trial (ClinicalTrials.gov: NCT01560182) with HSPC-GT started at the SR-Tiget in Milan (Table 1).[18] The rationale of this trial, supported by preclinical data,

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was based on restoring ARSA production and secretion in the CNS by the cells derived from the transplant. Indeed, it has been shown that, through delivering supraphysiological levels of ARSA enzyme, HSPC-GT, but not HSCT, could prevent and correct the disease manifestations in the mouse model.[98,115] This HSPC-GT approach is based on the transplantation of autologous HSPCs that differentiate into macrophages and microglia in the CNS, providing the necessary ARSA for cross-correction of the affected tissue. Thirty-three asymptomatic or presymptomatic patients (18 LI and 15 EJ children) were followed up to 7.5 years after receiving a BM fresh formulation of the product in 29 patients, while 4 have been treated with a cryopreserved formulation. Thirty out of 33 patients are alive, showing stable engraftment of gene-corrected $CD34^+$ cells with the restoration of ARSA activity in cerebrospinal fluid (CSF) and peripheral blood, without any selective advantage.[116,117] These data confirm that HSPC-GT is well tolerated, and presymptomatic LI patients show a sustained clinical benefit ameliorating neurocognitive function and lifespan relative to their untreated siblings. Prolonged follow-up is ongoing to confirm the long-term safety and clinical efficacy, and to investigate predictable factors on outcome after HSPC-GT. These results provide the first clinical proof that HSPC-GT could represent a valuable and efficacious therapeutic option for MLD, also in regard to the limitations of treating with allogeneic HSCT. In October 2020, the Committee for Medicinal Products for Human Use (CHMP) released a positive opinion recommending the granting of a marketing authorization for this advanced therapy medicinal product, named Libmeldy, for the treatment of children with the LI and the EJ forms of MLD.

MPS

MPS I is a rare genetic disease caused by mutation of the gene coding for the α-L-iduronidase (IDUA) enzyme involved in glycosaminoglycan (GAG) degradation. The lack of the IDUA enzyme causes accumulation of two GAGs, heparan sulfate and dermatan sulfate, responsible for irreversible damage in many organs and tissues, particularly in the connective tissue and the CNS.[118] Consequently, heterogeneous clinical manifestations ensue, leading to the classification of disease into three variants, according to disease severity. The severe variant, or Hurler syndrome (MPSIH), becomes evident by 2 years of age and is characterized by organomegaly, growth impairment, skeletal deformities, and intellectual disability.

ERT and HSCT, the only available approved therapeutic options, show limited efficacy, as proven by many studies. ERT can improve visceral organ damages but cannot correct bone abnormalities or neurological deficits, as it cannot cross the BBB. At the same time, HSCT can stabilize disease progression, lengthening life expectancy and improving quality of life, but it is less efficient in treating bone deformities and cognitive defects.[119–122]

Taking advantage of the information obtained in the context of the MLD-GT experience, SR-Tiget researchers tested LV-mediated HSC-GT in the mouse model of MPS I by challenging mice with the transplantation of WT and LV-transduced $Idua^{-/-}$ HSPCs. Enzymatic activity was restored to supra-normal levels, allowing delivery of the functional IDUA enzyme to organs, including the CNS, and metabolic correction of the affected tissues, as shown by the clearance of accumulated GAGs within hematopoietic and non-hematopoietic cells.[123] Based on these preclinical data, a phase I/II clinical trial of HSPC-GT in MPSIH patients is now ongoing at SR-Tiget (Table 1). Following a busulfan/fludarabine-based myeloablative conditioning, eight patients received autologous HSPCs transduced *ex vivo* with a 36-h transduction protocol with prostaglandin E2 as a transduction enhancer.

Encouragingly, in terms of safety and biological efficacy, these data compare favorably with allogeneic HSCT where CNS enzyme delivery relies only on microglia replacement by donor-derived cells, a slowly progressing phenomenon, while the relative avascularity of the ground substance of the musculoskeletal tissues prevents/affects enzyme and donor-derived cell penetration into these tissues. Conversely, the therapeutic potential of HSPC-GT for the treatment of MPSIH from the preliminary biological results indicate the rapid achievement of supraphysiological levels of IDUA and reductions of GAGs, especially toward CNS and bone as the main target organs with the initial evidence of clinical improvement.[124] Delivering higher quantities of the enzyme rather than WT cells in the bone might favor the cross-correction of bone-resident cells, such as mesenchymal stromal cells and osteoclasts, which have been reported to play a role in the skeletal manifestations of the disease, while the infiltration of the CNS by myeloid cells producing supra-normal enzyme quantities might convey therapeutic levels of the protein, potentially translating into a better long-term clinical outcome.

A similar *ex vivo* GT strategy was developed for MPSIIIA, an LSD caused by loss-of-function mutations in the SGSH gene coding for the lysosomal*N*-sulfoglucosamine sulfohydrolase. The main difference with the MPSIH-GT approach is based on the use of myeloid-specific promoter CD11b to control the expression of the SGSH gene to permit cell-specific gene expression in the myeloid cells repopulating the brain after cell transplantation.[125] Initial preclinical studies showed neurological improvement in the mouse model using autologous myeloid-driven LV-HSPC-corrected cells together with transplantation of WT cells. Based on these results, Orchard Therapeutics announced the dosing of the first MPSIIIA patient in the context of a phase I/II study (ClinicalTrials.gov: NCT04201405) at Manchester University NHS Foundation Trust in the UK (Table 1).

Similarly to this protocol, the same researchers also developed a clinical trial for MPSIIIB, which is caused by the deficiency of the enzyme α-*N*-acetylglucosaminidase coded by the NAGLU gene.[126] In the murine model, the LV expressing the human NAGLU cDNA under the transcriptional control of myeloid CD11b promoter was used at high titer with or without prednisolone, an anti-inflammatory steroid agent to check the importance of inflammation on behavior. The results showed that the supraphysiological enzyme levels in the brain, together with the normalization of heparan sulfate and prednisolone treatment, contributed to the correction of astrocytosis and microgliosis and finally to

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behavioral correction. The clinical development of this HSCT-GT trial is currently ongoing at the University of Manchester.

In this scenario, where early intervention is imperative to prevent irreversible neurological deficits, neonatal screening is essential to ensure timely treatment. The other critical issue is related to the necessity of developing an efficient strategy to overcome the BBB obstacle using *ex vivo* GT together with direct intra-brain delivery of the viral vector[127] or normal or gene-corrected hematopoietic progenitors.[128]

CONCLUSIONS

At present, HSPC-GT represents an effective approach for treating patients suffering from PIDs, hereditary red blood cells, and metabolic disorders. Substantial disease correction was observed in most treated patients, indicative of sustained engraftment of gene-corrected HSPCs. Decades of preclinical and clinical experimentations have led to crucial improvements in vector engineering, transduction procedure, and the pre-transplant conditioning regimen to deplete hematopoietic progenitors and promote homing and engraftment of gene-corrected HSCs to the BM. The advent of third-generation SIN LVs improved the transduction of long-term engrafting HSPCs, which allowed enhanced immune recovery and a significant decrease in the potential for insertional mutagenesis. Non-myeloablative conditioning regimens reduced morbidity and mortality associated with HSPC-GT and have demonstrated to be effective and safe applications in diseases only when gene-corrected cells have an advantage over uncorrected cells. Alternatively, when gene-modified cells do not exhibit a selective advantage, more intensive conditioning is needed to support the engraftment of a substantial amount of HSCs and reduce the risk of graft failure.

The long-term monitoring of patients remains of paramount importance to ensure that HSPC-GT remains safe with a durable effect over time. In point of fact, regulatory authorities recommend sponsors to follow-up study subjects for 15 years. Various approaches have been proposed to monitor long-term treated subjects, including product-specific registries, disease registries, minimally interventional studies, and observational studies.[129,130] Some of these studies present significant regulatory and logistical challenges due to the very long follow-up, different requirements in countries of origin where patients are followed, and lack of a consensus on the "standard of care" procedures for HSPC-GT.

Despite the clinical success of HSPC-GT, some challenges remain for the future. Incremental advances in allogeneic HSCT for some diseases, particularly in pediatric patients, may reduce the HSPC-GT gap. The preparation of GT products requires dedicated GMP facilities for manufacturing. Initially, phase I/II single-center trials were carried out in qualified centers, limiting drug availability. Clinical trials are now based mainly on cryopreserved formulations of the drug product, which would allow monitoring of its quality before administration and global distribution. However, the risk-benefit analysis should consider the time demanded for the release of the drug product for rapidly progressing diseases that require rapid treatment. Although some of these medicinal products have shown the potential to cure the diseases for at least 10–15 years and possibly permanently, their cost has led to an intense global debate. Strimvelis is available for all patients in the EU. It entered the market in 2016 and in Italy was included in the list of innovative drugs with a dedicated budget and reimbursed through a management entry access agreement under pay-by-results conditions. The high cost of new medicinal products, including Zynteglo for patients with transfusion-dependent β-thalassemia marketed at 1.57 million euros ($1.77 million) to be paid over 5 years, and Zolgensma, approved for children with spinal muscular atrophy (SMA) at a price tag of $2.125 million, has forced a public discussion on the affordability of these costs.

Advanced therapies for rare or ultra-rare diseases such as those described in this review require rethinking the pricing and reimbursement and access models. In the context of the EU market, it is certainly unthinkable, given the complexity, to equip every country with a center capable of administering them, but it is also necessary to guarantee access to all patients who need it throughout the European territories, when they reach registration, regardless of the country of origin. It is therefore desirable to review the production and regulatory mechanisms, and to shorten times and costs in order to make their production and market access sustainable, while guaranteeing the same high-quality standard required for traditional drugs.

With genome editing entering the clinical arena, this will add another important armamentarium to *ex vivo* GT. Gene editing has the advantage of correcting or eliminating mutations that lead to genetically driven diseases, preserving physiological regulation of gene expression and virtually eliminating the risk of insertional mutagenesis. Nevertheless, the positive clinical experience gained from HSPC-GT trials has paved the way for the translation of genome editing toward a clinical application.

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AUTHOR CONTRIBUTIONS

All authors have contributed to the concept design and writing of the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) is a joint venture between Fondazione Telethon and Ospedale San Raffaele (OSR). Gene therapies for ADA-SCID, Wiskott-Aldrich

syndrome (WAS), metachromatic leukodystrophy (MLD), β-thalassemia (BTHAL), and mucopolysaccharidosis I (MPS I) developed at SR-Tiget were licensed to Orchard Therapeutics (OTL) in 2018 and 2019. A.A. is the Principal Investigator of the above clinical trials.

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