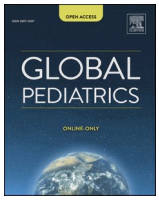
Global Pediatrics 9 (2024) 100196

Contents lists available at ScienceDirect

Global Pediatrics

journal homepage: www.elsevier.com/locate/gpeds



Gene therapy for rare haematological and neurometabolic paediatric diseases

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ARTICLE INFO

Keywords:
Gene therapy
Primary immunodeficiencies
Haemoglobinopathies
Lysosomal storage disorders

ABSTRACT

Nowadays, gene therapy hast the potential to cure an increasingly greater number of monogenic inherited disorders with absent or limited treatment options, and radically change their natural history. Hematopoietic stem cells (HSCs) represent one of the preferred targets for gene therapy, as genetic modification of multipotent cells ensures a permanent correction of the progeny. Gene-corrected HSCs and their progeny can also be used as cell vehicles to deliver molecules into the circulation and tissues, including the central nervous system or the skeleton. Major successes of this approach have been achieved in the field of monogenic blood disorders and neurometabolic diseases and several medicinal products have recently reached the stage of marketing approval by the EMA based on safety and efficacy data collected over more than 10 years of clinical trials. Gene therapy for these severe pathologies offers undeniable advantages over the sole alternative therapy of allogeneic transplantation because it can be applied to every patient, even when no matched HLA donor is available, reducing mortality and complications related to allogeneic transplantation, such as graft-versus-host disease, graft rejection, organ toxicity, and infections. Additionally, in neurometabolic diseases, gene therapy allows supra-physiological expression of the transgene, consequently producing supra-normal levels of the missing enzyme, providing a greater clinical benefit compared to allogeneic transplantation. Despite these remarkable achievements, several challenges remain for HSPC gene therapy regarding access to treatment and its sustainability for the future.

Introduction

Gene therapy has significantly evolved since the second half of the 20th century, thanks to enormous advances in understanding the molecular basis of many human diseases and the development of efficient molecular techniques for gene transfer. Currently, it represents a safe and effective therapeutic reality for several clinical conditions, such as monogenic inherited diseases and cancers, which have limited or absent treatment options. It falls within the group of so-called advanced therapies, technically referred to as ATMPs (Advanced Therapy Medicinal Products), which are innovative therapies or drugs consisting of recombinant nucleic acid (DNA or RNA), cells, and tissues. The goal of gene therapy is the correction of the defective gene through the transfer of the healthy gene or through gene editing directly within the patient's cells in the case of inherited diseases, or to provide a new preventive or therapeutic function to modified cells in the case of acquired diseases.

The genetic material typically can be transferred in two ways, *ex vivo* or *in vivo* (Fig. 1). In the *ex vivo* approach, the transfer of the therapeutic gene occurs during the in vitro culture of cells harvested from the patient, so the medicinal product consists of autologous genetically modified cells, which are re-administered to the patient fresh or cryopreserved after chemotherapy. Hematopoietic stem cells (HSCs) represent one of the preferred targets for *ex vivo* gene therapy (HSC-GT), as genetic modification of multipotent cells guarantees permanent and long-lasting correction of progeny[1]. In the *in vivo* approach, the gene is delivered directly into the target cells or tissue systemically or locally (e.g., intracerebral or intraocular), and the medicinal product is represented by the integrating vector. Most vectors used for gene transfer are of viral origin, obtained through genetic modifications that, on one hand, eliminate viral genes essential for pathogenicity and viral replication, making them harmless, and on the other hand, insert the genetic material necessary for correction. The most commonly used viral vectors

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https://doi.org/10.1016/j.gpeds.2024.100196
Received 17 April 2024; Accepted 1 June 2024
Available online 9 June 2024

for *ex vivo* gene therapy derive from γ-retroviruses (γ-RVs) or lentiviruses (LVs). The insertion of the therapeutic gene into the genome occurs randomly, but LVs have a safer and more controlled integration profile compared to γ-RVs[2]. For *in vivo* gene therapy, vectors derived from adenoviruses, adeno-associated viruses (AAVs), herpes simplex virus type 1, and LVs are used.

Up to date, 10 gene therapy products have been approved by the European Medicines Agency (EMA) for rare genetic disorders (Table 1). In this review, we will focus on the progress of clinical development of gene therapy medicinal products for the treatment of monogenic blood disorders, including primary immunodeficiencies (PIDs) and haemoglobinopathies, and neurometabolic diseases.

Gene therapy for haematological disorders

Primary immunodeficiencies

PIDs represent a growing group of inherited disorders (up to 500) due to altered development/function of immune system, leading to increased susceptibility to infections, autoimmunity, inflammatory disease, allergy and malignancies. Conventional treatment represented by hematopoietic stem cell transplantation (HSCT) is a definitive cure, however not always available and, despite continuous progress in the field, still characterized by significant morbidity and mortality risks. Since the 1990s, gene therapy has emerged as a revolutionary treatment strategy, able to restoring the functionality of the immune system through the correction of the genetic defect, first in the lymphocytes, responsible for defences against viruses, bacteria and fungi, and then in HSCs, progenitors of all the immune system cells[3,4]. Severe combined immunodeficiency due to defect of adenosine deaminase (ADA-SCID) is an autosomal recessive monogenic disorder of purine metabolism, characterized by profound lymphocytopenia and non-immunological manifestations involving other organs likely central nervous system, liver and kidney. Gene therapy for this severe immunodeficiency consists of an infusion of autologous bone marrow-derived stem cells transduced with a γ-RV vector encoding for the human ADA cDNA sequence following busulfan low-dose conditioning. Engraftment of gene corrected stem cells led to a sustained immune reconstitution, ranging from partial to full restoration of functional thymopoiesis, correction of lymphocytes functions, and adequate systemic detoxification. In May 2016, EMA approved *ex vivo* HSC-GT for the treatment of ADA-SCID under the commercial name of Strimvelis, the first example of an HSC-based gene therapy medicine to enter the market. Up to date, 45 children with this condition coming from over 20 countries around the world, have been treated at San Raffaele Hospital, the only treatment center due to the short shelf-life of the product prepared fresh after BM harvest. Thanks to the treatment all patients are alive and most have developed a functional immune system, finally allowing community life and did not require secondary interventions[5].

Another primary immune deficiency for which gene therapy has been proven to be safe and effective therapy in clinical trials is Wiskott-Aldrich syndrome (WAS), an X-linked PID with microthrombocytopenia, recurrent infections, severe eczema and increased risks of autoimmunity and lymphoid malignancies. Gene therapy with HSC-transduced with LV is able to restore WAS protein expression leading to substantially improved immune function with increased platelet counts that reflect in meaningful and durable improvement in disease manifestations. Long term results demonstrate no increased risk of malignancies and of genotoxic events to date[6].

Overall, over 200 patients with primary immunodeficiencies have been treated with gene therapy, including SCID due to IL2RG, ARTEMIS defects, chronic granulomatous disease and leukocyte adhesion defect. Moreover, preclinical studies with promising results are also ongoing for other PIDs including CD40L, SAP and PRF1 deficiencies[7].

Haemoglobinopathies

Beta-thalassemia and sickle cell anemia are hereditary diseases characterized by defects in the production of haemoglobin beta-chain and represent the most frequent monogenic disorder worldwide. In thalassemia, conventional therapies, such as blood transfusions and chelation treatments, have improved patients' quality of life, but lead to long-term dependency and potential complications. Furthermore, in sickle cell anemia current therapies are often unable to control episodes of blood vessel blockages, causing chronic pain and serious complications. In recent years, a growing number of clinical trials have been started to evaluate gene therapy approaches for the treatment of these disorders. The first approaches were carried out thanks to the infusion of

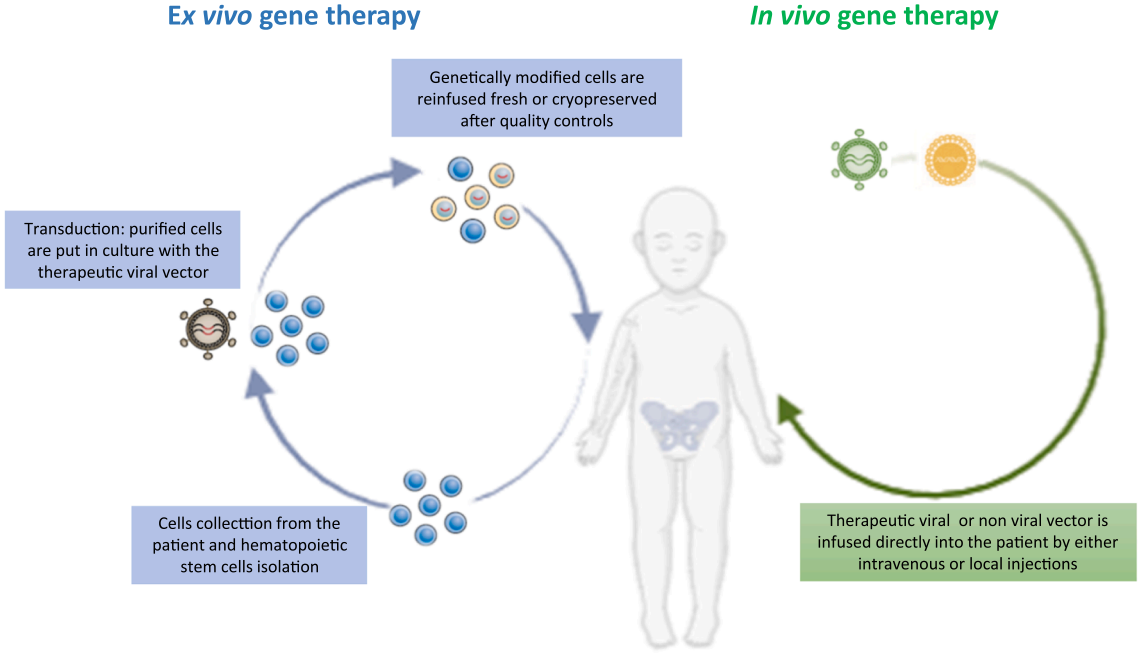

Fig. 1. Schematic representation of *ex vivo* and *in vivo* gene therapy

Table 1
EMA approved gene therapies for rare genetic disorders

Disease	Indication	Target cells	Vector/ approach	Therapeutic Gene/ Target	Commercial name
ADA-SCID	Patients without HLA identical familiar donor	Autologous CD34+ cells	γ-RV (*ex vivo*)	*Adenosine deaminase*	Strimvelis
Beta-thalassemia transfusion dependent	Patients ≥12 ys with genotype non–β^0/β^0 and without matched donor	Autologous CD34+ cells	LV (*ex vivo)*	*Beta globin* ($\beta^{A\text{-}T87Q}$)	Zynteglo (betibeglogene autotemcel) *
Metachromatic leukodystrophy	Pre-symptomatic patients (late infantile or early juvenile forms) or early symptomatic (early juvenile form)	Autologous CD34+ cells	LV (*ex vivo*)	*Arylsulfatase A (ARSA)*	Libmeldy (atidarsagene autotemcel)
Cerebral adrenoleukodystrophy	Early onset pediatric patients without familiar donor	Autologous CD34+ cells	LV (*ex vivo*)	*ABCD1*	Skysona (elivaldogene autotemcel)*
Inherited retinal dystrophy	Patient with biallelic RPE65 mutations with vital retinal cells and blindness	Photoreceptors cells	AAV2 (*in vivo*)	*RPE65*	Luxturna (voretigene neparvovec)
Spinal muscular atrophy (SMA)	Patients with SMA 5q and SMN1 gene biallelic mutations and a) clinical diagnosis of SMA type 1, or b) up to 3 copies of SMN2gene	Motor neuron	AAV9 (*in vivo)*	*SMN1*	Zolgensma (onasemnogene abeparvovec)
Aromatic L-amino acid decarboxylase deficiency (AADC)	Patients ≥18 months with clinical, molecular and genetic diagnosis of AADC and severe phenotype	Neuron	AAV2 *(in vivo intra putamen)*	*DDC*	Upstaza (ladocagene exuparvovec)
Hemophilia A	Adult patients with severe hemophilia A with no history of FVIII inhibitors and negative antibodies anti AAV5	Hepatocyte	AAV5 *(in vivo)*	*FVIII*	Roctavian (valoctocogene roxaparvovec)
Hemophilia B	Adult patients with mild or severe forms of hemophilia B with no history of FIX inhibitors	Hepatocyte	AAV5 *(in vivo)*	*FIX*	Hemgenix (etranacogene dezaparvovec)
Beta-thalassemia transfusion dependent Sickle cells anemia	Patients ≥12 ys with B-thal transfusion dependent or sickle cells anemia without HLA identical donor	Autologous CD34+ cells	Gene editing *(ex vivo)*	*BCL11A inactivation to produce fetal hemoglobin*	Casgevy (exagamglogene autotemcel)

* withdrawn by the sponsor for commercial reasons. For complete information refer to Summary of Product Characteristics (SmPC).

HSCs into which the beta globin gene was stably inserted using LV vectors[8]. Subsequently, the gene editing approach was used in order to inactivate a gene called *BCL11A*, to induce fetal hemoglobin production at levels that counterbalance the lack of normal hemoglobin[9]. Both gene therapy approaches have proven effective. In fact, clinical studies have demonstrated that a single administration of HSC-GT, corrected with a lentiviral vector or through gene editing, allows for a drastic reduction in dependence on transfusions in patients with thalassemia and in episodes of painful crises in patients with sickle cell anemia[10,11]. The drug based on lentiviral vectors that had obtained authorization by EMA and FDA was then withdrawn by the company in Europe for commercial reasons. A first drug based on *ex vivo* gene editing was recently approved in the United States and Europe for the treatment of transfusion dependent thalassemia and sickle cell anemia.

Gene therapy for genetic neurometabolic diseases

Hereditary metabolic disease represent a group of genetic disorders caused by mutations in genes coding for enzymes involved in lipids and protein metabolism. The enzymatic defect lead to accumulation of toxic metabolites and lack of essential molecular substrates, causing subsequent cytotoxicity and cellular death. Clinical pictures are heterogeneous and involve many organs and tissues, with central nervous system often involved, and if severely compromised the prognosis is usually unfavourable. Gene therapy for inherited neurometabolic disorders has particularly focused on lysosomal and peroxisomal storage disorders (LSD and PSD, respectively) with severe neurological involvement. Overall, these conditions represent the leading cause of neurodegeneration in paediatric age and are mostly devoid of effective treatments. Because genetically corrected HSPCs can cross the blood–brain-barrier and differentiate into microglia able to deliver the enzyme to other brain-resident cells, eliminating the accumulated metabolites and preventing damage, HSPC GT has been investigated as possible effective treatment for these neurodegenerative diseases.

Adrenoleukodystrophy

Adrenoleukodystrophy (X-ALD) is a rare X-linked PSD due to mutations in the *ABCD1* gene, which encodes the peroxisomal membrane ALD protein, involved in the transport of very-long-chain fatty acids into peroxisomes for degradation. The clinical spectrum of X-ALD is variable, and in the most severe form, the disease is characterized by an infantile cerebral onset with rapid and progressive degeneration of cerebral white matter (*childhood cerebral*, ccALD). Allogeneic HSCT has represented the first treatment capable of favourably modifying the disease, but it is associated with significant morbidity and mortality, and is not always feasible due to the absence of HLA-matched donors[12]. Clinical trials of HSC-GT based on autologous transplantation of HSCs genetically modified with a LV encoding normal human ABDC1 cDNA have been initiated since the 1990s, showing similar efficacy of allogeneic transplantation in stabilization of the disease in the central nervous system in patients with ccALD when performed at an early phase of neurodegeneration[13,14]. The therapy, which was approved for marketing by the FDA in 2022 under the name elivaldogene autotemcel (Skysona), represents a valid opportunity especially for patients without a compatible donor, and in general a preferable treatment option for early-stage ccALD patients due to reduced mortality and complications of autologous transplantation compared to allogeneic transplantation. However, it should be mentioned that among the more than 30 patients treated with this drug in the experimental phases, at least 3 cases developed a myelodysplastic syndrome associated with a clonal proliferation due activation of a proto-oncogene, which is likely associated with the design of the vector including a strong viral promoter. Importantly, no cases of genotoxicity related to LV have been reported, when the vector contains a cellular promoter, confirming the safety of this design[2].

Metachromatic leukodystrophy

Metachromatic leukodystrophy (MLD) is a rare LSD caused by

mutations in the *ARSA* gene encoding the lysosomal enzyme arylsulfatase A (ARSA). In patients affected, lysosomal accumulation of sulfatides due to enzymatic defect results in progressive demyelination of the central and peripheral nervous system, leading to severe deterioration of motor and neurocognitive functions. The infantile form of the disease is the most severe, characterized by onset before 2.5 years of age, very rapid progression and fatal outcome within a few years of symptom onset. Until very recently, MLD had no effective treatments. HSCT from a healthy donor has been extensively tested in infantile and juvenile MLD over the last two decades with highly variable and overall poor results. *Ex vivo* gene therapy with autologous HSCs engineered with lentiviral vectors was developed at SR-Tiget with the intention of restoring and increasing the production and secretion of ARSA in the nervous system by the progeny derived from transplantation. Based on preclinical data obtained in the animal model of the disease, the strategy was tested in a phase I/II clinical trial for patients with infantile and early juvenile MLD in a pre-symptomatic or early symptomatic phase with clear evidence of benefit: early treatment was able to prevent the onset of the disease or arrest its progression, preserving motor performance and development similar to healthy children of the same age[15,16]. Moreover, most patients displayed normal cognitive development and prevention or delay of central and peripheral demyelination and brain atrophy throughout follow-up. Thus, the drug was approved for marketing as atidarsagene autotemcel (arsa cel) by the EMA in 2020 (Libmeldy), by the UK's drug agency (MHRA) in 2022, and in US by the FDA in 2024 (Lenmeldy), for the treatment of pre-symptomatic late infantile, pre-symptomatic early juvenile or early symptomatic early juvenile MLD patients.

Mucopolysaccharidoses

Mucopolysaccharidoses (MPSs) are a group of ten rare multisystemic LSDs caused by deficiency of enzymes involved in degradation of glycosaminoglycans (GAGs), characterized by the involvement of both the central nervous system (CNS) and visceral organs with hepatosplenomegaly, cardiac valve disease, respiratory disease, and skeletal abnormalities. Enzyme replacement therapy and allogeneic HSCT, aiming at delivering the deficient enzyme and reducing GAG accumulation, are standard treatments for several MPSs, but have limitations including lack of efficacy on brain and skeletal manifestations, high costs, and safety issues. Gene therapy approaches aiming at correction of the genetic defect have been investigated over the last years and represent an attractive and potentially more effective alternative strategy to overcome these limitations. Phase I/II clinical trials of *ex vivo* HSPC-GT have been developed for MPSIH and MPSIIIA, for which preliminary results have shown promising biological and clinical outcomes[17,18]. Despite the limited follow-up, MPSIH patients showed progressive gain of cognitive and motor skills and early skeletal response, suggesting that IDUA enzyme expression at supraphysiological levels leads to efficient cross-correction of non-hematopoietic cells, including neurons and possibly skeletal cells. *In vivo* gene therapy by local brain delivery or systemic intravenous injections of viral vectors resulted in biochemical and clinical improvements in preclinical models, and early clinical trials of various MPSs are ongoing.

Conclusion

Currently, gene therapy represents an effective and safe treatment approach for patients suffering from PIDs, hereditary red blood cells, and metabolic disorders, especially when performed before the onset of clinical manifestations or organ damage. Despite this clinical success, several critical issues and challenges have emerged in recent years and remain for the future, regarding access to treatment and its sustainability, due to high production and logistic costs, long term patient follow up, pricing and reimbursement processes. Indeed, companies have already disinvested in some gene therapies. Therefore, drastic changes are needed in order to shorten times of development, costs and make sure that access to these life-saving innovative treatments is sustainable and at the same time available for rare disease patients[19].

CRediT authorship contribution statement

Vera Gallo: Conceptualization, Writing – original draft. **Alessandro Aiuti:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A. A. and V.G. are investigators of clinical trials sponsored by Orchard Therapeutics or Fondazione Telethon. Fondazione Telethon is the current market authorization holder of Strimvelis.

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