Review Article

Therapeutics in paediatric genetic diseases: current and future landscape

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Abstract

There are more than 7,000 paediatric genetic diseases (PGDs) but less than 5% have treatment options. Treatment strategies targeting different levels of the biological process of the disease have led to optimal health outcomes in a subset of patients with PGDs, where treatment is available. In the past 3 decades, there has been rapid advancement in the development of novel therapies, including gene therapy, for many PGDs. The therapeutic success of treatment relies heavily on knowledge of the genetic basis and the disease mechanism. Specifically, gene therapy has been shown to be effective in various clinical trials, and indeed, these trials have led to regulatory approvals, paving the way for gene therapies for other types of PGDs. In this review, we provide an overview of the treatment strategies and focus on some of the recent advancements in therapeutics for PGDs.

Keywords: Antisense oligonucleotide, gene therapy, paediatric genetic diseases, therapeutics

INTRODUCTION

Paediatric genetic diseases (PGDs) are reported to affect approximately 2% to 3% of all live births.[1] There are more than 7,000 different PGDs, and although individually they are rare, affecting less than 1 in 2,000 individuals, cumulatively they affect more than 350 million people globally. The estimated cost of hospitalisations related to PGDs reached US$ 22.9 billion in the United States in 2013, and it is anticipated that it will continue to rise further.[2]

The unprecedented rapid advances in genomic technologies in the past decade have exponentially improved our understanding of PGDs at the molecular and biochemical levels, enabling diagnosis in 25% to 50% of previously undiagnosed patients. While understanding the underlying aetiology enables the family to be counselled regarding risks in future pregnancies, understanding the biological pathway allows targeted treatments for some of these patients. This has led to a fundamental shift in the management of patients with PGDs — from looking for a diagnosis, to seeking a treatment.

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In this review, we provide an overview of the treatment strategies and focus on some of the recent advancements in therapeutics for PGDs.

CURRENT STATE OF THERAPEUTICS IN PAEDIATRIC GENETIC DISEASES

While there are more than 7,000 PGDs, treatment is available only for 5% of these diseases.[3] It is estimated that 1 in 3 children admitted to the intensive care unit has an underlying genetic disease, and in the absence of treatment, 30% of these children die before the age of 5 years. To incentivise pharmaceutical industries to develop drugs for

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these disorders, the Orphan Drug Act was passed in the United States in 1983, and since then, it has led to the approval of more than 600 drugs in this category. These include enzyme replacement therapy (ERT), such as Myozyme for Pompe disease, Aldurazyme for mucopolysaccharidosis type I and Fabrazyme for Fabry disease, among others.

Despite this, the number of treatment options remain limited. There are numerous factors that contribute to this:

1. Despite improvements in diagnostics, the genetic basis of disease in about 50% of patients remains unknown. Even in patients where the genetic basis is identified, there is insufficient understanding of the pathophysiological mechanisms.
2. There is early onset of protein dysfunction, especially during foetal development, which is not amenable to intervention by the time of diagnosis, usually in the postnatal period, because of irreversible pathological changes that have occurred resulting from protein dysfunction during the foetal developmental stage.
3. The majority of newly reported disorders are diagnosed in severely affected individuals in whom the protein function would either be absent or significantly reduced and, hence, not amenable to rescue with therapeutic modalities.

Treatment strategies

Currently, treatment of PGDs is directed at treating the clinical phenotype, including the individual medical and surgical interventions that may be common across many disorders. Indeed, supportive and symptomatic treatments are the mainstay of management for most of these disorders. However, treatment options targeting different aspects of the biological process starting from the gene, to the messenger RNA (mRNA), then to the protein or the metabolic or biochemical abnormality have led to successes in a subset of patients [Table 1].

Treatment of metabolic or biochemical abnormality

Inborn errors of metabolism (IEMs), a subset of PGDs, present with biochemical abnormality suggesting an enzymatic defect in the breakdown of specific metabolites, commonly leading to either toxic accumulation of metabolites upstream of the block or deficiency of metabolites downstream of the block. For example, phenylketonuria (PKU) is associated with accumulation of phenylalanine — resulting from deficiency of phenylalanine hydroxylase — which is toxic to the developing brain. Identification of this biochemical abnormality led to the initiation of phenylalanine-restricted diet in PKU patients, which has been shown to improve neurocognitive outcomes.[4] A challenge, however, with such dietary manipulation is the need for lifelong dietary adherence, which may lead to non-compliance, resulting in poor control of the disease.[5]

Another strategy to treat IEMs is to use pharmacological agents to bind the toxic compound so that it can be excreted. Examples include sodium benzoate therapy for urea cycle defects and penicillamine for Wilson disease, among others.[6,7] In some cases, replacement of the deficient metabolite, such as thyroxine in congenital hypothyroidism and use of alternative sources of glucose in patients with disorders of gluconeogenesis, has been met with reasonable success.[8,9] Finally, supplementation with cofactors, such as tetrahydrobiopterin, has been shown to increase the residual enzyme activity in patients with hypomorphic (or partially functioning) alleles related to PKU.[10]

Treatment of dysfunctional protein

ERT is an established treatment for many lysosomal storage diseases (LSDs), including Gaucher disease, Pompe disease, Fabry disease and mucopolysaccharidosis.[11-14] The treatment is based on the provision of adequate exogenous enzyme to overcome the block in the metabolic pathway and thus clearance of the stored toxic substrate. ERTs are proven to be efficacious, but a few challenges remain. ERTs are generally safe and well tolerated in patients, but there are associated risks of allergic reactions or anaphylaxis.[15] Patients may develop neutralising antibodies that reduce the treatment efficacy of ERTs. For example, patients with Pompe disease who are CRIM (cross-reactive immunologic material)-negative with no residual enzyme activity have high titres of IgG antibodies against the recombinant enzymes, which leads to poor clinical response to ERTs.[16]

In Gaucher disease, ERT is efficacious in the reversal of lysosomal storage and hence reduces haematopoietic, visceral (liver and spleen) and bone involvement. However, ERT does not improve the neurological outcomes in Gaucher disease type 3 with neurodegenerative symptoms because the macromolecular enzyme is unable to cross the blood–brain barrier (BBB) even at high doses.[17] Similarly, early clinical studies showed that a therapeutic approach using ERTs was not feasible in primary neurological conditions such as Tay-Sachs and Niemann-Pick A disease because the intravenously administered enzymes did not cross the BBB.[18] In addition, because of the short half-life of the enzymes, patients on ERT will need regular infusions for optimal outcomes because substrate re-accumulation occurs if ERT is interrupted. Lifelong treatment with ERT places a high cost burden on patients, which will in turn reduce patient accessibility to receiving this form of treatment.[19-21]

Treatment of abnormal RNA

Antisense oligonucleotides (ASOs) are short oligonucleotides (10–30 nucleotides) that bind to cellular RNAs via complementary base pairing and can be engineered to alter pre-mRNA splicing, mRNA stability, transcription or RNA–protein interaction.[22] ASO therapy can be personalised to target patient-specific pathogenic variants because of high sequence specificity of ASO binding.[23] This therapeutic approach has been successfully used in the treatment of

Table 1. Overview and examples of therapeutic strategies in managing patients with genetic disorders.

Treatment target	Treatment strategy	Example of diseases	Examples of treatment
Metabolic abnormality			
Accumulation of toxic metabolite	Reduction in substrate upstream	Phenylketonuria	Phenylalanine-restricted diet
	Binding toxic by-product	Urea cycle defects	Sodium benzoate to bind and remove toxic ammonia
		Wilson disease	Penicillamine to bind excess copper
Inadequate substrate	Replacement of substrate	Hypothyroidism	Thyroxine
		Glycogen storage disease	Cornstarch during overnight fasting
Reduced enzymatic activity	Supplementation with cofactors	Phenylketonuria	Tetrahydrobiopterin
		Homocystinuria	Pyridoxine
	Avoidance	G6PD deficiency	Anti-malarial drugs
Dysfunctional protein	Enzyme replacement therapy	Lysosomal storage disorders	Enzyme replacement therapy (e.g., Fabrazyme for Fabry disease, Myozyme for Pompe disease)
	Replacement of extracellular protein	Haemophilia A	Factor VIII infusion
Abnormal mRNA	Bind and block aberrant mRNA using antisense oligonucleotides	Spinal muscular atrophy	Spinraza (Nusinersen)
		Duchenne muscular dystrophy	Exondys 51 (eteplirsen), Vyondys 53 (golodirsen)
		Batten disease	Milasen
		Hereditary transthyretin-mediated amyloidosis	Tegsedi (inotersen)
	Small interfering RNAs harness the RNA interference pathway to degrade disease-associated mRNA	Acute hepatic porphyria	Givlaari (Givosiran)
		Primary hyperoxaluria type 1	Oxlumo (lumasiran)
		Hereditary transthyretin-mediated amyloidosis	Onpattro (patisiran)
Mutation-containing gene	Modulation of gene expression	Sickle cell disease	Decitabine to stimulate HbF production
	Somatic modification with transplantation	Lysosomal storage disorders (e.g., Krabbe, Gaucher, X-linked adrenoleukodystrophy)	Haematopoetic stem cell transplantation
		Maple syrup urine disease	Liver transplantation
	Somatic modification with gene transfer	Severe combined immunodeficiency	Strimvelis (GSK2696273)
		Spinal muscular atrophy	Zolgensma (onasemnogene abeparvovec-xioi)
		RPE65-related retinal disease	Luxturna (voretigene neparvovec-rzyl)

HbF: foetal haemoglobin, mRNA: messenger RNA

several neurological conditions, such as spinal muscular atrophy (SMA), Duchenne muscular dystrophy, Huntington disease and Batten disease.[23,24]

Batten disease is a group of neurodegenerative neuronal ceroid lipofuscinosis with onset of neurological symptoms in early childhood and is associated with high morbidity and mortality. In 2019, Milasen, a patient-customised ASO, was developed for a patient with Batten disease with a unique mutation in the *MFSD8* gene.[23] The patient was found to be compound heterozygous for a missense variant c.1102G>C and a SVA (SINE-VNTR-Alu) insertion in the *MFSD8* gene that caused a splicing defect and led to premature translational termination. The 22-nucleotide ASO was designed to correct the splicing defect and restore the MFSD8 protein expression in this patient. No serious adverse events were reported in the patient, and treatment led to a reduction in frequency and duration of seizures.[23]

Treatment of the mutation-containing gene

Haematopoietic stem cell transplant (HSCT) has been used for LSDs, including Hunter syndrome, Hurler syndrome, Gaucher disease, Krabbe disease and X-linked adrenoleukodystrophy, among others.[25-28] The principle of HSCT in LSDs is based on the cross-correction mechanism, whereby patients who undergo HSCT will receive a continuous supply of the enzyme produced by the donor-derived myeloid cells, which are then taken up by enzyme-deficient host cells. Early intervention with HSCT is essential to prevent disease progression and leads to better disease outcomes in patients.[29] Liver transplantation is another established therapy for selected groups of IEMs, such as α-1 antitrypsin deficiency, urea cycle defects, maple syrup urine disorder and glycogen storage disorders, among others.[30-32] Both treatment modalities improve the clinical course of the diseases and quality of life in patients and are potentially curative in patients with IEMs treated

with liver transplantation.[33-35] However, transplantation is associated with a high risk of morbidity, particularly a high risk of infection because of immunosuppression and risk of graft-versus-host disease in HSCT, and significant risk of mortality.[36] Besides that, the scarcity of donor organs limits the number of eligible patients who can undergo liver transplant.

Gene therapy

Background

Gene therapy is a technique with huge potential in the development of novel and potentially curative therapies for human genetic diseases by genetic modification of cells. The concept of gene therapy by using an exogenous DNA to replace the defective DNA in human genetic conditions was first introduced by Friedmann and Roblin in 1972.[37] It was predicted that gene engineering had enormous potential in therapeutics to treat human genetic diseases. However, the authors concluded that further attempts at gene therapy in human patients should be opposed because of insufficient understanding of human genetics in human diseases at that time.[37] But because the outlook for gene therapy in the improvement of human health is promising, researchers still evince substantial interest in optimising gene modification technology.

The first human clinical trial of gene therapy in 2 patients with adenosine deaminase (ADA) deficiency was related to severe combined immunodeficiency (SCID); it was conducted at the National Institutes of Health in Bethesda, Maryland, in 1990 and was considered a major scientific breakthrough at that time.[38] This clinical trial involved *ex vivo* transfer of a functional gene for ADA using retroviral vectors to the autologous T-cells of 2 patients[38] that were subsequently reinfused back into the patients. Even though the result of the trial was confounded by the simultaneous ERT received by the patients, this success story became an important catalyst for development in the field of gene therapy.

Initial setbacks

However, there were 2 major setbacks in the history of gene therapy: the first involved a patient with ornithine transcarbamylase (OTC) deficiency who died after a severe adverse immune reaction to the adenoviral vector used to carry a functional copy of the *OTC* gene[39]; the second involved development of acute leukaemia resulting from insertional mutagenesis in 5 of 20 patients (including 1 death) with X-linked SCID (SCID-X1) who underwent γ-retrovirus–mediated *ex vivo* gene therapy.[40,41] These incidents raised significant safety concerns, and the latter incident resulted in a temporary suspension of gene therapy trials using γ-retroviral vectors in transduction of bone marrow stem cells.[42] This highlighted the importance of careful regulation and monitoring of gene therapy in humans in subsequent clinical trials.

Current landscape

Depending on the target cells, gene therapy can be divided into 2 main types: somatic and germline. Somatic gene therapy refers to gene modification restricted to somatic cells, where changes cannot be passed on to the next generation, whereas germline gene therapy refers to gene modification on reproductive cells, where changes made are heritable. Germline genome editing remains technically and ethically challenging, and most countries ban germline genetic modification either by law or by guidelines.[43] Somatic gene therapy, on the other hand, led to significant breakthroughs in many different clinical applications in PGDs. This review will focus on somatic gene therapy, with a discussion of gene therapy strategies and the associated risks, recent advances in gene therapy for paediatric genetic disorders and challenges of gene therapy in precision medicine [Table 2].

Gene therapy strategies

The therapeutic success of gene therapy in genetic diseases relies heavily on the knowledge of the specific characteristics and function of the relevant gene in humans, the genetic changes that lead to disease manifestation and the presence of a regulatory system that affects the gene expression that potentially could be manipulated to reverse disease phenotype.

Gene therapy strategies are highly disease dependent. For monogenic recessive conditions with a nonfunctional gene that lead to a protein defect or deficiency, gene augmentation using the gene replacement method could revert the disease phenotype by transferring a normal wild-type copy of the gene to produce therapeutic protein levels. This is the most commonly used strategy in most gene therapy trials.[44] In certain complex, multigenic diseases, single gene replacement may not be effective to revert the disease phenotype. In such cases, an alternative approach using gene addition to transfer other therapeutic genes may improve cellular function and homeostasis to modulate the disease course.[44] A gene silencing strategy is applied in certain dominant diseases, whereby suppression of the dysfunctional gene expression is required to ameliorate disease progression.[45] The 2 main methods for silencing gene expression are ASO and RNA interference (RNAi). ASO binds to the target RNA as a single strand and inhibits mRNA translation. RNAi, an endogenous cellular mechanism, regulates gene expression by activating ribonucleases to degrade target mRNA, along with other enzyme complexes.[46] Gene editing tools with higher target gene specificity include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and more recently developed programmable clustered regularly interspaced short palindromic repeat (CRISPR)-associated systems, CRISPR-Cas9; these offer more precise genetic modification as compared to gene augmentation and gene silencing strategies.[47] ZFNs, TALENs and CRISPR-Cas9 all deliver a site-specific DNA double-strand break and subsequent

Table 2. Overview of gene therapy strategies and FDA-/EMA-approved treatment.

Gene Therapy Strategies	Mechanism	FDA-/EMA-Approved treatment
Gene augmentation		
Gene replacement	To transfer a functional copy of the gene to replace the nonfunctional copy, so as to produce therapeutic levels of protein to revert disease phenotype. This approach is used in monogenic diseases because of the single gene defect.	*Luxturna (voretigene neparvovec-rzyl) Strimvelis (GSK2696273) Zolgensma (onasemnogene abeparvovec-xioi) Zynteglo (betibeglogene autotemcel) Roctavian (valoctocogene roxaparvovec)
Gene addition	To introduce an additional protein-coding gene to improve cellular function and homeostasis of cells, to improve disease phenotype. This approach is used in complex disorders because of the combined effects of multiple genes and environmental factors, such as cancer and heart diseases.	*Kymriah (tisagenlecleucel) Yescarta (axicabtagene ciloleucel) Tecartus (brexucabtagene autoleucel) Carvykti (ciltacabtagene autoleucel) Breyanzi (lisocabtagene maraleucel) Abecma (idecabtagene vicleucel)
Gene silencing		
Antisense oligonucleotide	Directly binds to the target mRNA via complementary base pairing and alters pre-mRNA splicing, mRNA stability, transcription or RNA-protein interaction to reduce deleterious protein levels.	Spinraza (nusinersen) Exondys 51 (eteplirsen) Vyondys 53 (golodirsen) Milasen
RNA interference	Small interfering RNA to repress gene expression by triggering RNA degradation and/or inhibiting protein synthesis.	Onpattro (patisiran) Givlaari (givosiran) Oxlumo (lumasiran)
Gene editing	Programmable sequence-specific nucleases that deliver genetic modification via a double-strand break in the specific genomic target sequence, followed by generation of desired modifications during subsequent DNA break repair using HR or NHEJ. The gene editing tools that are currently widely used are ZFNs, TALENs and the CRISPR/Cas9 system.	
ZFNs	First endonuclease assembled by fusing a site-specific DNA-binding domain on zinc-finger protein to a non-sequence-specific DNA cleavage domain on Fok1 endonuclease. Fok1 endonuclease works as a dimer in which double-strand DNA cleavage occurs only at the sites of binding of 2 ZFNs to the opposite DNA strands.	Clinical trials in various diseases
TALENs	TALENs are assembled by fusing a Fok1 cleavage domain to a DNA-binding domain consisting of a highly conserved repeat sequence from TALE that can associate with single or longer-sequence nucleotides. TALENs act as dimers for DNA cleavage to occur.	Clinical trials in various diseases
CRISPR/Cas9 system	An RNA-guided DNA cleavage module comprising a single-stranded guide RNA and Cas9 endonuclease. The single-strand RNA binds to the target sequence and Cas9 cleaves the target DNA to generate a double-strand break, and with DNA repair through HR or NHEJ, targeted genomic modification can be made.	Clinical trials in various diseases

*Approved under Cell, Tissue, or Gene Therapy Products (CTGTP) regulatory framework in Singapore.CRISPR: clustered regularly interspaced short palindromic repeat, EMA: European Medicines Agency, FDA: United States Food and Drug Administration, HR: homologous recombination, mRNA: messenger RNA, NHEJ: non-homologous end joining, TALE: transcription activator-like effector, TALENs: transcription activator-like effector nucleases, ZFN: zinc-finger nucleases

DNA repair by homologous recombination or non-homologous end joining to generate the desired genetic modifications.[48]

The therapeutic target determines the administration routes and gene delivery system when designing a gene therapy.[49] Gene therapy can be delivered to the affected individual either via *in vivo* or *ex vivo* therapy.[50] Vector-based *in vivo* therapy offers direct delivery of the therapeutic gene. However, there may be more off-target effects, and certain target cells or tissues, such as the central nervous system, may require an invasive administration route such as an intrathecal or intraparenchymal route to cross the BBB.[50] On the other hand, cell-based *ex vivo* therapy is a technically challenging multi-step process because it requires a dedicated cell therapy facility for gene modification on the cells, and these modified cells then need to be transplanted into the individuals.[49] Most of the Food and Drug Administration (FDA)-approved *in vivo* gene therapies use viral vectors, rather than non-viral systems, for their highly efficient ability to transduce cells with the therapeutic gene sequence. The selection of an ideal vector is dependent on the expected effect, size of the transgene and the safety profile.[51]

After successful insertion of the therapeutic gene sequence, tight regulation of transgene expression to achieve

physiological levels is desirable using either endogenously regulated or exogenously controlled systems, to avoid toxicity.[52] Strategies to improve therapeutic efficacy of the gene therapy include the following: increasing target-cell specificity of modified cells or vectors, reducing immunogenicity of viral and non-viral vectors and planning of immunosuppression schedule, formation of allogenic cells with regulated expression of the transgene, avoidance of expression of the transgene in antigen-presenting cells, and delivery of the transgene to immune privileged sites (e.g., brain, eye, liver).[53]

Over the past 3 decades, advancement in gene therapy has faced numerous challenges because of several high-profile adverse events, including insertional mutagenesis with integrating retroviral or lentiviral vectors and deaths of patients in clinical gene therapy trials.[41,42] Despite all these setbacks, the research efforts to improve on gene therapy technologies with minimal adverse event risks continued. In 2012, the approval of the first gene therapy product Glybera (alipogene tiparvovec), an adeno-associated virus (AAV)-mediated gene therapy for lipoprotein lipase deficiency by the European Medicines Agency had catalysed the emergence of gene therapy trials in various monogenic diseases, including inherited retinal dystrophy, primary immunodeficiencies, primary neurological diseases, haemoglobinopathies, haemophilia and inherited metabolic disorders. Although Glybera was later withdrawn from the market in 2017[54] because of the ultra-rarity of the disease (1 in a million), high cost of therapy to the patient and hefty maintenance costs, because of the need to monitor patients over a long period of time, this did not prevent further research and development of novel gene therapies for various genetic diseases.

Currently, there are 11 commercially available gene therapy products. These include the following:

1. Luxturna (voretigene neparvovec-rzyl) — *in vivo* gene therapy for patients with confirmed biallelic RPE65 mutation–associated retinal dystrophy;
2. Zolgensma (onasemnogene abeparvovec-xioi) — *in vivo* gene therapy for paediatric patients less than 2 years old with SMA;
3. Roctavian (valoctocogene roxaparvovec) — *in vivo* gene therapy for adult patients with severe haemophilia A without a history of factor VIII inhibitors and without detectable antibodies to AAV serotype 5 (AAV5) in Europe;
4. Chimeric antigen receptor T-cell (CAR-T) therapies — Yescarta (axicabtagene ciloleucel), Kymriah (tisagenlecleucel), Tecartus (brexucabtagene autoleucel), Carvykti (ciltacabtagene autoleucel), Breyanzi (lisocabtagene maraleucel) and Abecma (idecabtagene vicleucel) — *ex vivo* gene therapies used to treat adult and/or paediatric patients with haematological malignancies;
5. Zynteglo (betibeglogene autotemcel) — *ex vivo* gene therapy for adult and paediatric patients with transfusion-dependent β-thalassemia (TDT); and
6. Strimvelis® (GSK2696273) — an *ex vivo* stem cell gene therapy used in the treatment of ADA-SCID in Europe.

Kymriah and Luxturna are currently approved for use in Singapore under the Cell, Tissue and Gene Therapy Products (CTGTP) regulatory framework, which came into effect on 1 March 2021.

We highlight therapeutic advances with gene selective therapies in ADA-SCID, TDT and SMA.

Gene therapy in primary immunodeficiency disorder

ADA is an enzyme that is responsible for the clearance of toxic purine metabolites from the body. ADA deficiency is associated with ADA-SCID, which is a life-threatening primary immunodeficiency with impaired T-, B-, and NK-cell development, with a high risk of opportunistic infection.[55] Early trials for ADA-SCID used γ-retroviral vectors to carry a functional copy of the *ADA* gene, and improvement in T-lymphocyte count was observed in treated patients.[56,57] However, these patients also received ERT during the trial period, and hence, the direct effect of the gene transfer was not measurable. In subsequent trials, patients were preconditioned with a non-myeloablative regimen, and ERT was discontinued during the trial.[58-61] Self-inactivating lentiviral vectors, instead of γ-retroviral vectors, were used in subsequent clinical studies because of safety concerns regarding the use of γ-retroviral vectors — namely, that they could potentially lead to leukaemic transformation in the recipients. However, multiple studies had shown that γ-retroviral vectors appeared to be safe in ADA-SCID gene therapy.[60,62] In 2016, Strimvelis became the first *ex vivo* stem cell gene therapy approved by the European Commission because of good safety and efficacy track records. The market approval of Strimvelis was based on data collected from a total of 18 ADA-SCID children treated from 2000 to 2011, with a median follow-up of about 7 years.[63] All the patients studied survived with no evidence of leukaemia. These patients had good clinical outcomes, with evidence of long-term gene correction in T lymphocytes and increase in lymphocyte counts; they also demonstrated increased immunity against infections over time and improved growth.[64]

Gene therapy in transfusion-dependent β-thalassemia

TDT is one of the most common inherited haemoglobinopathies worldwide and poses significant health and economic burdens to patients and families. Currently available symptomatic treatments include regular blood transfusions and iron chelation therapy as well as newer therapies such as luspatercept, which may reduce transfusion requirements in patients.[65] Allogenic bone marrow transplantation is curative, but an HLA (human leukocyte antigen)-matched donor is required. Gene transfer targeting haematopoietic stem cells offers an alternative treatment option that is potentially curative for patients who

cannot undergo bone marrow transplantation. Lentiviral vectors can stably transmit various regulatory elements and coding sequences of the β-globin gene and hence have become candidate vectors to be used in gene therapy design for β-thalassemia.

Cavazzana-Calvo *et al.* treated a patient who had HbE/β-thalassemia with haematopoietic stem cells transduced with a lentivirus vector–mediated functional copy of the β-globin gene.[66] The patient received a high dose of chemotherapy before re-infusion of the gene-corrected haematopoietic stem cells, which helped to eliminate most of the diseased haematopoietic stem cells. There was good clinical response in the patient, who remained transfusion free for almost 2 years.[66] The therapeutic effect was associated with a haematopoietic cell clone bearing a vector insertion in the *HMGA2* gene with significantly raised expression of *HMGA2* that was potentially oncogenic. However, there was no evidence of leukaemic transformation of the clone.[66] This highlighted the theoretical risk of insertional mutagenesis with randomly integrating viral vectors. Continual research efforts are needed to minimise these adverse consequences by improving on current vector design or developing novel vectors. Clinical trials are currently ongoing to assess the efficacy of this therapy in patients with TDT regardless of genotype.[67] In 2019, Zynteglo (betibeglogene autotemcel) was conditionally approved in the European Union by the European Commission for the treatment of patients older than 12 years with TDT who have non-β^0 mutations and who do not have a matched sibling donor.[68] The safety and effectiveness of Zynteglo were established in 2 multicentre clinical studies that included adult and paediatric patients with β-thalassemia requiring regular blood transfusions. Of 41 patients receiving Zynteglo, 89% achieved transfusion independence.[69] Based on these findings, in 2022, the FDA granted approval for Zynteglo to treat β-thalassemia in adults and the paediatric population and recommended that patients who received Zynteglo should have their blood monitored for at least 15 years to detect any evidence of cancer.[70]

Another approach to the treatment of TDT is based on the knowledge that elevated levels of foetal haemoglobin are associated with improved morbidity and mortality in patients with TDT.[71] Frangoul *et al.* used CRISPR-Cas9 gene editing technology to 'switch off' *BCL11A*, which is responsible for the repression of HbF expression after birth, in haematopoietic stem and progenitor cells (HSPCs) to reduce the expression of *BCL11A*.[72] This led to the reactivation of HbF production in the patient with TDT after receiving the genetically edited CD34+ HSPCs. The patient had stable haemoglobin levels at the fourth month after the infusion, with a long transfusion-free period within the 18-month follow-up period. This result showed that CRISPR-Cas9 editing of *BCL11A* in HSPCs in the long-term treatment of TDT was feasible.[72]

Gene-selective therapies in spinal muscular atrophy

SMA is a severe neuromuscular disorder with an early onset during infancy or childhood. It is caused by mutations in the *SMN1* gene, which leads to loss of *SMN1* gene expression. *SMN2*, on the other hand, differs from the *SMN1* by a single nucleotide polymorphism in exon 7 (c.840C>T) that affects the activity of an exon splice enhancer and excludes exon 7 in nearly 90% of *SMN2* transcripts, leading to the production of a truncated protein.[73,74] The disease severity in SMA decreases with increase in the number of paralogous *SMN2* genes.[75] If patients carry only 1 copy of *SMN2*, they will have the severe form of SMA type 0 or 1, whereby they will have significant neuromuscular weakness and a limited life expectancy of around 2 years.[76] For this reason, *SMN2* has become an attractive target of drug development.

Currently, there are 3 gene-targeting SMN replacement therapies that have received regulatory approval. Nusinersen (Spinraza) is an ASO that targets the intronic splicing silencer N1 (ISS-N1) in intron 7 of *SMN2*.[77] This drug manipulates the splicing pattern of *SMN2* to produce full-length and functional SMN protein by exon 7 inclusion. Early clinical trials showed that the treatment, which is given via an intrathecal injection, was safely tolerated by patients, with improvement in motor function.[78] Subsequent clinical trials showed that nusinersen is safe and efficacious in more varied patient groups,[79-81] with evidence of clinical improvement in motor function after the first year of treatment.[82] However, patients will need repeated intrathecal administration of nusinersen, which may be less acceptable. Risdiplam is a small-molecule drug that acts as an *SMN2* pre-mRNA splicing modifier to increase exon 7 inclusion in *SMN2* mRNA transcripts and full functional SMN protein production.[83] The oral administration of risdiplam offers an alternative treatment option, especially for patients who are unable to receive intrathecal injections. The phase II/III FIREFISH trial showed that higher tested doses of risdiplam improved motor function in 7 of 17 infants with infantile-onset SMA.[84] Further phase III trials are ongoing to assess the effectiveness of risdiplam in patients with different types of SMA and also in presymptomatic SMA in infants less than 6 weeks old.[85]

Onasemnogene abeparvovec (Zolgensma) is an *in vivo* recombinant AAV9-based gene therapy designed to deliver a functional copy of *SMN1* to encode for functional SMN protein to preserve motor neuron function.[86] Early preclinical studies in mouse and non-human primate models demonstrated that intravenous injections of AAV9-mediated gene transfer can cross the BBB and transduce target motor neurons in the spinal cord.[87,88] Further studies showed that AAV9-mediated SMN gene expression delivered on postnatal day 1 significantly improved the lifespan and motor symptoms in mice with SMA, and early treatment was associated with better outcomes.[88-90] The promising results

from these trials led to clinical trials of onasemnogene, which demonstrated significant improvement in motor function and reduced permanent ventilatory needs in symptomatic infants with SMA type 1.[86,91]

CONCLUSION

Advances in genomic technologies have heralded an unprecedented shift in the management of patients with PGDs, from diagnostics to therapeutics. The high cost of gene-selective treatment and the difficulty in obtaining coverage and reimbursement present a major challenge to patient accessibility to the treatment. To achieve the realisation of precision medicine for better patient-centric care in the near future, all stakeholders will need to work together to develop innovative reimbursement solutions, so all patients can obtain access to these therapies.

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There are no conflicts of interest.

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