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Therapeutic Approaches for Treatment of Genetic Disorders: Tradition Leading to Evolution

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Abstract

With more than 10,000 genetic disorders reported to date, the treatment offered to patients in the clinics is very limited. To develop a safe and efficacious treatment, it is important to understand the underlying pathophysiology and genetic etiology of the disease. This review aims to describe some available treatment approaches like conventional therapy, gene therapy, and stem cell therapy to treat these disorders. We also describe some of our clinical cases of different disease categories and their clinical management through these treatment approaches.

We structure this brief with an introduction followed by an overview of treatment modalities for genetic disorders available to date.

Keywords: Therapies, genetic disorders

Introduction

There are approximately 20,000 coding genes in the human genome that account for about 10,000 reported genetic disorders. These genetic diseases affect about 6-8% of the population worldwide. Although treatments have been in clinical trials for decades, very few, except inborn errors of metabolism, have successfully transitioned to the clinic (Barigga et al., 2021). To identify and develop potential therapies for patients, it is crucial to understand the pathophysiological cascade of the disorder and the molecular effect of the genetic mutation (Turnpenny et al., 2017). If the disease is recessive, providing the cell with a functional copy of the gene/protein will be enough to manage the disorder, however, if it is a dominant disorder with a gain of function mutation, blocking or disrupting the mutant gene will be necessary to correct the disorder at the gene level (Turnpenny et al., 2017). Despite vast challenges, the current era has witnessed a dramatic shift in successful treatments in the clinic, bringing hope to patients with genetic disorders. Within this context, we review the currently approved disease-modifying therapies and their mechanisms through specific patient case scenarios from the clinic.

Modalities of treatment for genetic disorders

Based on the etiopathophysiology, treatment approaches vary and can be divided into categories based on the level of intervention (**Figure 1**). These include symptomatic and supportive management; modifying the metabolic cassette by diet restrictions or special supplementation; providing deficient or mutant protein through protein, hormone, or enzyme replacement therapy; replacing abnormal

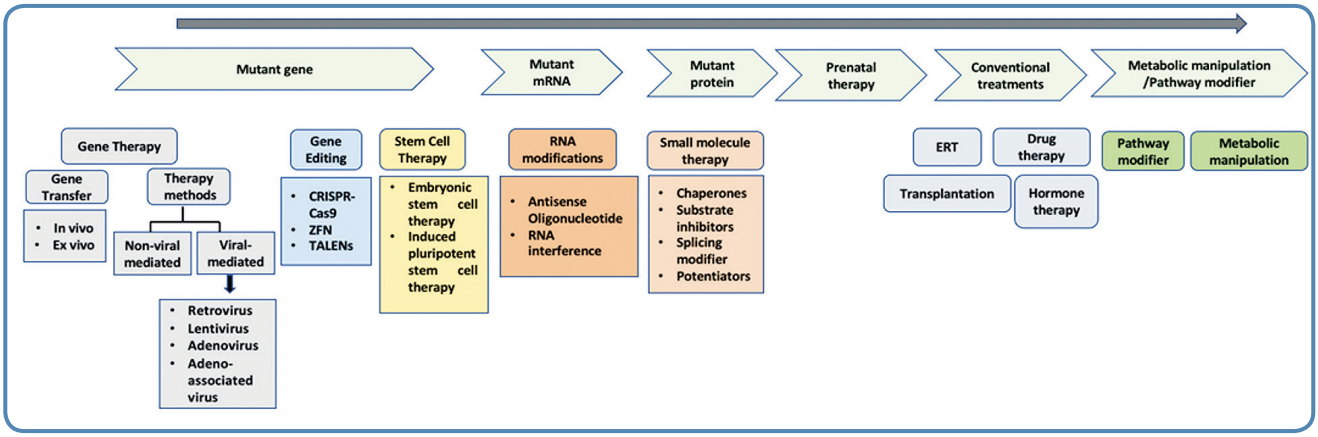

Figure 1 Different therapeutic approaches for the treatment of genetic disorders based on the level of intervention.

protein - stem cell therapy, organ transplantation; correcting the genetic defect - gene therapy; and RNA based therapies (Gambello et al., 2018; Barigga et al., 2021).

1. Dietary manipulation

Inborn errors of metabolism (IEMs) are disorders caused by mutations affecting enzyme expression and function, leading to impaired metabolism (Gambello et al., 2018). There are around 116 treatable inborn metabolic disorders where timely treatment can prevent intellectual disability. For these disorders, the manipulation does not correct the basic defect but ameliorates the phenotype through pharmacological therapies (Pyeritz et al., 2021). The modalities of dietary modifications include:

- Restriction of the offending metabolite, e.g., phenylalanine in phenylketonuria, branched-chain amino acids in maple syrup urine disease (MSUD), galactose in galactosemia.
- Supplementation of deficient product, e.g., L- carnitine in systemic primary carnitine deficiency, L- arginine in urea cycle disorders (UCD).
- Vitamin/coenzyme supplementation, e.g., vitamin B12 in methylmalonic aciduria and homocystinuria (Cblc type), biotin in biotinidase deficiency.
- Stimulate an alternative metabolic pathway, e.g., carnitine in organic acidurias, glycine in isovaleric acidemia, penicillamine in Wilson disease, sodium benzoate and sodium phenylbutyrate in urea cycle disorders.
- Metabolic pathway inhibition, e.g., 2-(2-nitro-4-tri-fluorometylbenzoyl)-1,3-cyclohexanedione (NTBC) prevents the production of toxic metabolites by blocking the tyrosine metabolic pathway.

Table 1 depicts the pharmacological therapeutic options for patients with metabolic disorders of amino acids, lipids, and carbohydrates. Traditionally, though some metabolic disorders are treated using dietary modulation, many remain challenging to treat by these methods. For some IEMs, high-dose vitamins can help partially restore the enzymatic activity by raising cellular concentrations of cofactor as listed in **Table 2**. Other therapeutic options including enzyme replacement therapy (ERT), tissue transplantation, and gene therapy are discussed below.

Case study

A 1-month-old male child, second born to non-consanguineous parents, presented with complaints of lethargy, vomiting, and progressive encephalopathy. He was completely well till this presentation. On investigation, his ammonia levels were raised, and he had metabolic acidosis with an increased anion gap. Tandem mass spectrometry (TMS) showed increased propionylcarnitine (C3) and gas chromatography mass spectrometry (GCMS) of urine identified elevated propionylglycine, methylcitrate and 3-hydroxypropionate, suggestive of propionic acidemia. After initial emergency management and stabilization, he was treated with a protein-restricted diet and supplementation with isoleucine, valine, methionine, and threonine-free formula along with carnitine supplementation and metronidazole administration. He is on long-term follow-up and doing well.

2. Pathway modification:

Porphyrias are inherited disorders due to defects in the heme biosynthetic pathway. Treatment includes avoidance of triggers like exposure to sunlight and reduction in the amount of porphyrin in the body. Panhematin (hemin), an enzyme inhibitor, helps reduce porphyrin levels and is used for the treatment of acute intermittent porphyria (Pyeritz et al., 2021).

Vosoritide (trade name Voxzogo) is a recombinant C-type natriuretic peptide analog. It stimulates endochondral ossification by decreasing fibroblast growth factor receptor 3 (FGFR3) activity and thereby preventing inhibition of chondrocyte mineralization. It was approved by the United States Food and Drug Administration (FDA) in 2021 for use in patients with achondroplasia with open epiphyses, aged 5 years or above.

3. Replacement of Deficient Product:

This includes thyroid replacement in congenital hypothyroidism, packed red blood cells (RBCs) in thalassemia major, factor VIII in hemophilia A, factor IX in hemophilia B, and adrenocortical hormones in congenital adrenal hyperplasia (Turnpenny et al., 2017). Enzyme replacement therapy for inborn errors of metabolism is elaborated below.

3a. Enzyme Replacement Therapy (ERT)

Lysosomal storage disorders (LSDs) have

been a paradigm for the treatment of genetic disorders with successful treatments spanning three decades. Replacement of the deficient lysosomal enzyme is the commonest therapeutic modality and currently, there are fourteen ERTs approved by regulatory authorities available for ten LSDs and many more are in clinical trials (**Table 3**) (Pogue et al., 2018). The enzyme requires efficient targeting to the lysosomes and over the years this has been achieved by enhancing the mannose -6 - phosphate receptors. The treatment is lifelong. Although ERT has been translational for the treatment of some disorders like Gaucher disease, there remain limitations to this treatment modality. The therapeutic enzyme may not reach specific regions like the central nervous system and skeletal system and have a limited impact on their disease process (Beck et al., 2018).

Beyond LSDs, ERT is used for adenosine deaminase (ADA)-deficient severe combined immunodeficiency. ERT with polyethylene glycol-conjugated adenosine deaminase (PEG-ADA) is one of the modalities of treatment that allows the reduction of the accumulated substrate. The main indications for use are stabilization prior to hematopoietic stem cell transplantation (HSCT) or if there are contraindications to HSCT (Turnpenny et al., 2017). Additional disorders included recently in the armamentarium are Pegvaliase for phenylketonuria and trials are ongoing for other disorders (Table 3).

Case Study

A three-year child presents with failure to thrive, abdominal distention, and extreme irritability. He has been symptomatic since the last 6 months with multiple hospital visits. His parents are non-consanguineously married and there is no significant family history. His developmental milestones are normal except for a mild motor delay attributed to the massive abdominal distension. Examination identifies massive splenohepatomegaly with decreased growth parameters. Laboratory testing reveals anemia, thrombocytopenia, and decreased bone density with Erlenmeyer flask deformity on the femur radiograph. Eye examination is normal. Hematological disorders and infections are excluded, and he is diagnosed to have Gaucherdisease type 1 by glucocerebrosidase enzyme testing that shows a deficiency of the enzyme on a blood sample. The biallelic mutation is identified in the GBA gene - c.1603C>T (p.R496C) in exon 11. Definitive therapy with enzyme replacement therapy is initiated with the recombinant enzyme, imiglucerase. It is given as an intravenous infusion over two hours once every two weeks. Improvement in growth, decrease in the organomegaly, improved hematological parameters, and chitotriosidase biomarker is seen on follow-up. This child is now 14 years old with significantly improved disease parameters and activities and abilities like any other child of his age.

4. Drug treatment

This includes statins for familial hypercholesterolemia that reduce plasma low-density lipoprotein (LDL) through upregulation of the LDL receptors by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and endogenous cholesterol biosynthesis. Oral or intravenous bisphosphonates inhibit osteoclastic bone resorption and are used to increase bone mineral density in children and adults with osteogenesis imperfecta (Turnpenny et al., 2017). Everolimus (Afinitor) a small molecule drug is approved for the treatment of tuberous sclerosis complex (TSC)- associated partial-onset seizures in patients aged 2 years or above. It is an mTOR inhibitor that helps in tumor cell apoptosis and reduction in cell proliferation and angiogenesis, thereby inhibiting tumor cell growth in affected patients.

Proprotein convertase subtilisin/kexin type 9(PCSK9) inhibitors are a new class of drugs used for the reduction of low-density lipid-cholesterol levels in patients with hyperlipidemia (Turnpenny et al., 2017). They target and inactivate PCSK9 protease which attaches to LDL receptors leading to destruction in lysosomes. Evolocumab and Alirocumab are FDA-approved monoclonal antibodies for the treatment of patients with heterozygous familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease that require LDL lowering. They are PCSK9 inhibitors that reduce LDL levels by 50-60% more than statins would do alone.

Hydroxyurea is an antineoplastic drug that is used for the treatment of patients with sickle cell anemia and thalassemia intermedia. It is known to reduce painful episodes and increase HbF levels. Through different mechanisms of action,

it provides various therapeutic benefits to these patients. It is a potent ribonucleotide reductase (RR) inhibitor that inhibits DNA synthesis, by inhibiting the conversion of ribonucleotides to deoxyribonucleotides, resulting in cellular cytotoxicity, decrease in WBC, and elevated HbF levels, but this is still not fully understood.

5. **Tissue transplantation:**

This includes hematopoietic stem cell transplantation (HSCT) for many disorders including betathalassemia, inherited bone marrow failure syndromes, primary immune deficiency, e.g., severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome, phagocytic cell defects (chronic granulomatous disease (CGD), leukocyte adhesion deficiency (LAD), severe congenital neutropenia), and familial hemophagocytic lymphohistiocytosis. Organ transplantation including liver, kidney, lung, and rarely heart transplantation is also performed. Liver transplantation can be done for the treatment of patients with urea cycle defects, tyrosinemia, Wilson disease, and MSUD (Turnpenny et al., 2017; Beck, 2018). A case synopsis of liver transplantation in our patient is described below.

Case Study

A 2.5 kg male neonate born at term presented with poor feeding and lethargy at day 5 of life. He developed seizures, dystonic posturing of limbs, encephalopathy, and respiratory distress. Urine ketones were positive with no metabolic acidosis. Investigations suggested the possibility of MSUD. The neonate was managed on a protein-restricted diet specific to MSUD and at the age of 22 months, India's first liver transplantation for MSUD was performed. The child is now 9 years of age and is doing well without any dietary restrictions.

6. **Prenatal treatment:**

Prenatal treatment of genetic disorders offers several benefits by treating inherited disorders early in life even before birth. Such early intervention reduces the period of irreversible damage to the affected organ (Turnpenny et al., 2017). An example is the treatment of a female fetus affected with congenital adrenal hyperplasia by prenatal administration of dexamethasone,in which virilization can be prevented by administration of dexamethasone, which suppresses fetal pituitary-adrenal axis, in small doses by the mother throughout pregnancy.

Lower urinary tract obstruction (LUTO) is a rare fetal condition that involves blockage in the urinary tract of the fetus which if left untreated can have severe complications and morbidity. Fetal interventions like vesicocentesis, vesico-amniotic shunting, and fetal cystoscopy are options of available treatment for LUTO.

Early fetal treatment interventions also include treatment of fetal arrhythmias like supraventricular tachyarrhythmia (SVT) and atrioventricular heart block. SVT is treated with antiarrhythmic medications like digoxin, flecainide, sotalol, and sometimes amiodarone. Depending on the etiology of the heart block involved, specific treatment including beta-sympathomimetics, immunoglobulin, apheresis, and/or fluorinated glucocorticoids can be incorporated. Percutaneous fetoscopy for the repair of open spina bifida has proven to be much more beneficial in comparison to postnatal treatment. Fetal therapy is reported for various other disorders like in utero stem cell transplantation in sickle cell disease, prenatal correction of X-linked hypohidrotic ectodermal dysplasia, and fetoscopic endotracheal occlusion (FETO) in fetuses with congenital diaphragmatic hernia.

7. **Small Molecule Therapy**

7a. Chaperone Therapy:

Genetic variations that impact protein structure and folding are degraded in the endoplasmic reticulum and proteosomes. Pharmacological chaperones are molecules that bind to the proteins and promote the correct folding of enzymes, thereby enhancing their function. Synthetic chaperones are used for the treatment of lysosomal storage disorders to correct target enzyme conformation. Though safe and efficacious, their use is limited due to their mutation specificity and undetermined optimal concentration on their inhibition activity (Beck, 2018). An example of chaperone therapy is Migalastat for amenable GLAvariants, in which the drug binds to the active site of enzyme alpha-galactosidase (the enzyme deficient in Fabry disease) leading to its stabilization and trafficking, increasing its catalytic activity (Beck, 2018). Small molecule modulators for cystic fibrosis include drugs that correct protein folding or enhance the cystic fibrosis transmembrane conductance regulator (CFTR) channel function.

Lumacaftor, Tizacaftor and Elexacaftor are used independently or in combination for patients with the common F508del mutation. It assists to correct the protein configuration to enable appropriate cell trafficking of water and chloride. Ivacaftor is a potentiator that improves the transport of chloride ions through the ion channel by binding to the defective protein and increasing the open probability of the channel. They are approved for use in CF patients with specific mutations in the CFTR gene that control the gating of chloride ions across the plasma membrane (Turnpenny et al., 2017; Beck, 2018; Gambello et al., 2018).

7b. Substrate reduction therapy:

The aim of substrate reduction therapy (SRT) is to reduce the synthesis of the substrate or substrate precursor which are accumulated due to the enzyme deficiency. Advantages include oral administration, the potential to cross the blood-brain barrier and less immunogenicity (Beck, 2018). Examples of substrate inhibitors include N-butyldeoxynojirimycin (Zavesca®, Miglustat) for Gaucher type 1 and Eliglustat (Cerdelga). These drugs inhibit glucosylceramide synthase and limit the accumulation of the precursor, glucosylceramide. This enables the residual enzyme activity in the cell to metabolize the decreased substrate. Miglustat is approved for mild neuronopathic (type 3) Gaucher and non-neuronopathic (type 1) Gaucher disease. The gastrointestinal side effects limit the use of Miglustat for the approved indications. The second sanctioned oral therapy for Gaucher disease, Eliglustat (Cerdelga), is approved for use in adult Gaucher disease type 1 [with known CYP2D6 genotype]. Genistein, a naturally occurring isoflavone, inhibits the synthesis of glycosaminoglycans and was proposed for the treatment of mucopolysaccharidosis (MPS) type III. However long-term data on efficacy is lacking for this drug (Beck, 2018; Gambello et al., 2018).

7c. Stop-codon read-through enhancers:

Small molecules which modulate splicing and enhance stop-codon read-through have become a popular approach for the treatment of genetic disorders like spinal muscular atrophy (SMA) (Beck et al., 2018). Risdiplam (brand name Evrysdi) is a small molecule SMN2 enhancer which is taken orally daily by SMA patients. This splicing modifier increases the SMN protein expression by preventing exon 7 of the SMN2 gene from getting spliced thereby causing a decrease in exon 7 deleted SMN protein and increasing the production of full-length SMN protein. Ataluren (brand name Translarna) enables read-through of the premature stop codon to produce full-length dystrophin protein or protein larger than the mutated version. It results in a Becker-type phenotype and is used for Duchenne muscular dystrophy (DMD) patients aged 5 years or above.

Case Study

A 15-month-old male child, first born to non-consanguineous parents, came to medical attention with complaints of being unable to hold neck or roll over, limited limb movements, and poor speech. On examination he had hypotonia, areflexia and tongue fasciculations. He was diagnosed with SMA type 1 and was started on Risdiplam (2.7 ml per day) and after 5 months he showed improvements in limb movements, swallowing and speech.

8. RNA modifications:

These therapies are based on mRNA modification with suppression of mRNA levels or correcting the function of mRNA and include antisense oligonucleotide-based therapy and RNA interference.

8a. Antisense Oligonucleotide (ASO):

ASOis a therapeutic strategy in which a short sequence-specific single-stranded antisense oligonucleotide (usually 8-30 bases in length) binds to the target mRNA inhibiting its gene expression at the protein level (**Figure 2**) (Turnpenny et al., 2017). **Table 4** shows some ASO-based treatments that are approved for use by the US FDA. Although advances made in developing ASO have considerably impacted patient management, a key challenge is that each ASO is mutation specific. Therefore, developing a therapy for a rare mutation can be expensive (Shahryariet al., 2019).

Case Study

A 5-year-old male child, born to non-consanguineous parents, was evaluated in our clinic with delayed motor milestones and subsequently was diagnosed with SMA type 2 at the age of 15 months. There was history of an episode of respiratory infection which was managed at home. The child was selected to

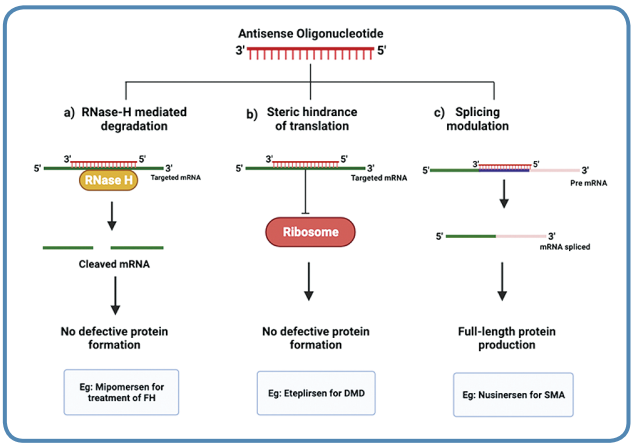

Figure 2 Mechanism of antisense oligonucleotide therapy. ASO therapy utilizes three different mechanisms for therapeutic effect. (a) The binding of ASO to the target mRNA leads to RNase-H mediated degradation of target mRNA. The ASO is again free to bind other mRNA molecules. (b) Other morpholino-based ASOs bind to target mRNA preventing the binding of ribosomes and limiting protein translation. (c) Splicing modifying ASO alters mRNA splicing in such a way that it results in the inclusion of the desired exon for increased full-length protein formation (O'Connor et al., 2006) (created using Biorender.com).

receive intrathecal Nusinersen through the humanitarian access program and has received 4 doses to date. He is currently showing improved neck and hand control as well as overall well-being.

8b. RNA interference (RNAi)

This therapy, unlike ASO (being bound to target mRNA) targets the gene and cleaves off the mRNA with resultant gene silencing. It is 1000-fold more efficacious than ASO therapy. The synthetic double-stranded RNA sequences called small interfering RNAs (siRNAs) bind to targeted mRNA resulting in their RISC-associated cleavage. This therapy is of special interest when gene knockdown at a specific target is desired (**Figure 3**). Examples of RNAi therapy are Patisiran for hereditary amyloidosis and Lumasiran for primary hyperoxaluria type 1 (PH1) (**Table 4**) (Turnpenny et al., 2017; Shahryari et al., 2019).

9. Stem Cell Therapy:

Stem cells are unspecialized cells that have

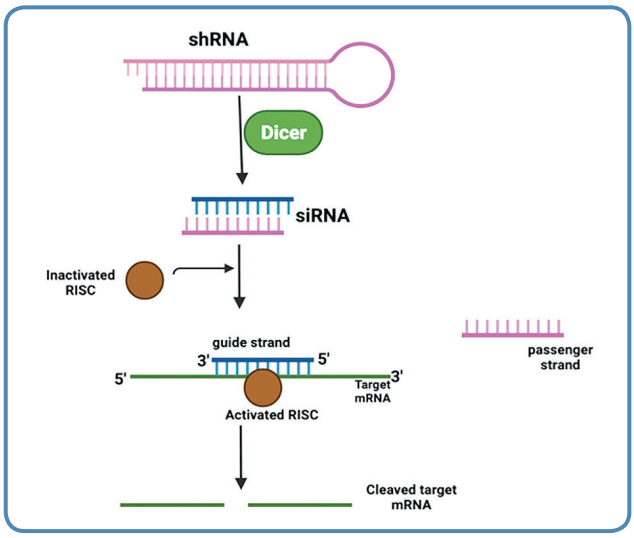

Figure 3 Mechanism of RNA inteference therapy for the treatment of genetic disorders. This therapy hijacks the endogenous RNAi pathway to target the specific degradation of RNA. When double-stranded shRNA enters the cell, it is cleaved by Dicer protein into short segments. The inactivated RNA-induced silencing complex (RISC) results in the separation of siRNA followed by RISC-activated binding of guide strand RNA to target mRNA and its degradation. Patisiran, an RNAi-based therapy, targets TTR (transthyretin) mRNA degrading both normal and mutant mRNA (O'Connor et al., 2006) (created using Biorender.com).

the capacity for self-renewal and the ability to differentiate into specialized cells of many lineages upon proper stimulation. Stem cells can be embryonic stem cells, which are pluripotent cells derived from the inner cell mass of the blastocyst and can differentiate into derivatives of all three germ layers i.e., ectoderm, endoderm, and mesoderm. Somatic stem cells, now called induced pluripotent stem cells, are cells capable of self-renewal and can differentiate into cell types of tissues from which they are derived (Turnpenny et al., 2017; Maldonado et al., 2021).

9a. Hematopoietic stem cell transplantation

HSCT is a promising treatment approach owing to the accessibility of the hematopoietic cells, their well-described behavior and ability to survive ex-vivo manipulation. The rationale of transplantation is the introduction of healthy cells that would be internalized in the donor

to allow enzyme production (Maldonado et al., 2021). The recipient hematopoietic system is repopulated with the healthy donor cells that can produce the deficient enzyme lifelong. Efficacy is proved for mucopolysaccharidosis (MPS) type I, metachromatic leukodystrophy (MLD), and X-linked adrenoleukodystrophy (ALD). Premedication with ERT prior to transplant in patients of MPS I is reported to improve transplant outcomes. The current hematopoietic stem cell transplantation (HSCT) strategies involve a healthy donor and allogenic transplant with its attending limitations. Genetic disorders where HSCT is an approved therapeutic modality include primary immune deficiencies, hemoglobinopathies, bone marrow failure syndromes, lysosomal disorders including MPS-1, alpha mannosidosis,presymptomatic, late-onset and slowly progressing MLD, and presymptomatic cerebral X-linked ALD (Beck, 2018; Gambello et al., 2018; Maldonado et al., 2021).

10. Gene therapy:

Gene therapy is a therapeutic approach to correct defective gene function by replacing or targeted editing of the defective gene. Gene editing through zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and CRISPR/Cas9 are the most used methods (Gupta et al., 2015). Sickle cell disease and Leber congenital amaurosis are some recent examples of use of gene editing for definitive treatment (Shahryari et al., 2019).

Gene therapy trials in humans began in 1990, but wide inroads have only been made in the last decade when the first human gene therapy, Luxturna for inherited retinal atrophy was approved by the US FDA. Despite immense therapeutic implications for the treatment of genetic disorders, there remain challenges that limit easy transition to the clinic (Turnpenny et al., 2017). These include safety and efficacy, product interaction with the host cells, need for prolonged clinical laboratory studies, high cost of therapeutic products, and immune response to transgene or associated vector (Shahryari et al., 2019). The two mechanisms for transgene transfer include ex vivo and in vivo therapy (**Figure 4**). Based on the genetic disorders to be treated, gene therapies are limited to different target organs involving specific gene transfer methods and suitable vectors. Type of gene therapy are subdivided into viral-mediated and non-viral mediated gene therapy (Turnpenny et al., 2017).

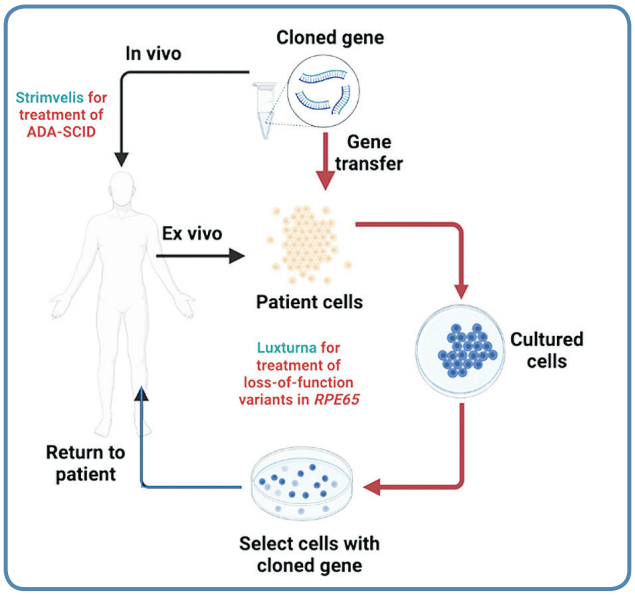

Figure 4 **Ex vivo and in vivo gene transfer methods.** Transfer of transgene for successful gene therapy can be done in two ways.Ex vivo therapy involves removing the cells from a patient, modifying them in vitro, and returning the modified cells to the patient. Luxturna is an AAV2-based ex vivo gene therapy approved for the treatment of patients with loss of function mutation in the *RPE65* gene. In vivo gene transfer involves the direct transfer of modified cells into the patient. A retroviral-based gene therapy Strimvelis involves in vivo gene transfer of the autologous HSC in patients with ADA-SCID (Turnpenny et al., 2017; Shahryari et al., 2019) (created using Biorender.com).

Gene therapy in autologous patient-derived progenitor cells and ex vivo gene therapy are alternative approaches that limit graft vs host disease (GVHD). The most recent gene therapy to be approved is Zynteglo [Betibeglogeneautotemcel (beti-cel)], a lentiviral associated βA(T87Q)-globin gene sequence for use in children 12 years or older with transfusion-dependent beta-thalassemia. This is an autologous transplant with ex vivo gene therapy via a lentiviral vector with genetic modification of the autologous hematopoietic stem cells (Beck, 2018; Shahryari et al., 2019). Recently, Roctavian™ (Valoctocogene roxaparvovec) has

been granted conditional marketing approval by the European Commission (EC) for treatment of severe hemophilia A in adult patients without a history of Factor VIII inhibitors and absence of detectable antibodies to adeno-associated virus sero type 5 (AAV5). This is the first approved single-time infusion therapy for hemophilia A. It delivers the functional copy of the gene enabling the body to produce Factor VIII.

10a. Viral vectors:

These are commonly used for gene therapy currently. Their benefits include high efficiency of transduction of transgene to the host, specific and targeted gene therapy, safety and efficacy, and reduced administration of doses. The common viral vectors used include retrovirus, lentivirus, adenovirus, and adeno-associated virus (**Table 5**) (Turnpenny et al., 2017).

10b. Non-viral vector therapy:

These are liposome-mediated DNA transfers. This is safer and unlikely to elicit an immune response. However, they have a low efficacy with transient expression of a transgene. However, they can incorporate a large sequence of DNA for delivery to the target cell. Nanoparticles are alternative synthetic vectors that offer similar benefits to liposome-mediated gene transfer. The efficacy and expression studies are still under investigation and a topic of debate (O'Connor et al., 2006; Turnpenny et al., 2017).

Case study

A 15-month-old male born to non-consanguineous parents presented with weakness in both lower limbs since infancy and an inability to stand independently. His social and language milestones were age appropriate. He was diagnosed with SMA type 1 having a homozygous deletion of exon 7 in the SMN1 gene. The child was enrolled in the AVXS-101 GMAP gene therapy study and meanwhile was given regular physiotherapy. He successfully received Zolgensma as an intravenous single-time dose. Post 8 months he is now able to feed himself and stand with support for some time.

Gene therapy possesses a relative advantage of attaining a tag of an orphan drug. However, it is important that the transgene must survive and proliferate in the host without eliciting any negative immune responses (Shahryari et al., 2019). **Table 6** shows few approved gene therapies for treatment of genetic disorders.

Conclusion

With advancements in medical science and the approval of various therapies, the treatment of various genetic diseases is now possible through various approaches (**Table 7**). With different gene editing and transfer methods available, we can practically rewrite the genetic code inside the cell with innumerable options. However, to develop a successful curative treatment, it is vital to further understand the cellular and genetic pathology of each disorder (Turnpenny et al., 2017). Additionally, it is important to address other technical and ethical issues while developing a genetic treatment to increase its safety and efficacy. The ratio of benefits to risks must always be acceptable and beneficial for patients. Previously untreatable disorders like Duchenne muscular dystrophy and spinal muscular atrophy now have efficacious therapies, indicating that in the coming days more therapies will follow. Despite the approval of many therapies for rare disorders, the impact on short and long-term outcomes requires follow-up and definite criteria to demonstrate a detailed impact on the natural history of the disorder.

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Table 1 **Treatment options for common inborn errors of metabolism**
(Pyeritz et al., 2021; Barigga et al., 2021)

Disorder	Enzyme deficiency/ Metabolic defect	Dietary management	Additional therapeutic options
Disorders of Amino Acid Metabolism			
Phenylketonuria	Phenylalanine hydroxylase (PAH) deficiency	Phenylalanine-restricted diet	Tetrahydrobiopterin, large neutral amino acids, phenylalanine ammonia lyase enzyme therapy(emerging treatment)
Tetrahydro-biopterin (BH4) deficiency	Six disorders of disturbance of BH4 biosynthesis or recycling	Phenylalanine-restricted diet	Sapropterin dihydrochloride, L-DOPA, 5 hydroxytryptophan, folinic acid
Tyrosinemia I	Fumaryl Acetoacetate hydrolase deficiency	Phenylalanine and tyrosine-restricted diet	Nitisinone (NTBC)
Classical homocystinuria	Cystathionine beta synthase deficiency	Methionine-restricted diet	Pyridoxine (B6), vitamin B12, folic acid, betaine
Glutaric acidemia type 1	Glutaryl CoA dehydrogenase deficiency	Low protein, lysine-free, tryptophan-reduced diet	Riboflavin, L-carnitine supplementation
Propionic acidemia and methylmalonic acidemia	Propionyl CoA carboxylase and methyl malonyl CoA-mutase deficiency	Methionine, isoleucine, threonine, and valine-restricted diet	Biotin, L-carnitine and vitamin B12 supplementation, hemodialysis, and hemofiltration
Maple syrup urine disease	Branched-chain alpha-keto acid dehydrogenase-complex deficiency	Dietary restriction of branched-chain amino acids	High dose thiamine
Urea cycle disorders	Deficiency of enzymes of the urea cycle (eight enzymes)	Protein-restricted diet except in type 2 citrullinemia where lactose-free, MCT-enriched diet is recommended	Sodium benzoate, phenylacetate, L-arginine (except in arginase deficiency), L-citrulline, carbamylglutamate
GLUT1 deficiency	Glucose transport 1 deficiency	Ketogenic diet, avoid valproic acid and carbohydrate sugars	

Glycogen storage disorder type I	Glucose 6-phosphatase deficiency	Frequent meals with complex carbohydrates; limit simple sugars in diet	Uncooked cornstarch
Fructose 1,6 bisphosphatase deficiency	Fructose 1,6 Bisphosphatase deficiency	Avoid fasting; limit use of fructose, sucrose and sorbitol	-
Disorders of Lipid Metabolism			
Very long-chain acyl CoA dehydrogenase deficiency	Very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency	Frequent feeds and avoid fasting; dietary mix - 10% natural fat - reduction of long-chain fat,essential fatty acids supplementation	Medium chain triglyceride supplementation
Urea cycle disorders	Deficiency of enzymes of the urea cycle (eight enzymes)	Protein-restricted diet except in type 2 citrullinemia where lactose-free, MCT-enriched diet is recommended	Sodium benzoate, phenylacetate, L-arginine (except in arginase deficiency), L-citrulline, carbamylglutamate
Disorders of Carbohydrate Metabolism			
Classic galactosemia	Galactose-1-phosphate uridyl transferase	Galactose and lactose-free diet	Calcium and Vitamin D supplementation

Table 2 **Vitamin-responsive genetic disorders**

Vitamin	Disorder
Pyridoxine	• Classic homocystinuria • Pyridoxine-dependent epilepsy • Pyridoxamine 5'-phosphate oxidase deficiency • Pyridoxal phosphate-binding protein deficiency • Gyrate atrophy of choroid and retina • Primary oxaluria type 1 • Adult hypophosphatasia • Early infantile epileptic encephalopathy • Hyperphosphatasia with mental retardation • Hyperprolinemia type 2
Cobalamin	• Combined methylmalonic acidemia and homocystinuria (cblC,cblD,cblF) • Isolated methylmalonic acidemia • Isolated homocystinemia • Intrinsic factor deficiency • Transcobalamin II deficiency • Imerslund- Grasbeck syndrome
Biotin	• Biotinidase deficiency • Holocarboxylase synthetase deficiency • Propionic acidemia • Biotin-thiamineresponsive encephalopathy • Pyruvate carboxylase deficiency • 3-methycrotonyl-CoA carboxylase 1 deficiency • Acetyl-CoA carboxylase deficiency
Thiamine	• Maple syrup urine disease • Pyruvate dehydrogenase deficiency • Biotin-thiamine responsive encephalopathy • Thiamine responsive megaloblastic anemia • Encephalopathy due to thiamine phosphokinase deficiency • Amish lethal microcephaly • Thiamine-responsive bilateral striatal degeneration and polyneuropathy
Riboflavin	• Riboflavin transporter deficiency • Multiple Acyl-CoA dehydrogenase deficiency • X-linked Charcot-Marie-Tooth disease type 4 • Acyl-CoA dehydrogenase 9 deficiency • Combined oxidative phosphorylation deficiency 6 • Mitochondrial complex 1 deficiency type 4 • Methemoglobinemia due to methemoglobin reductase deficiency • Metabolic encephalomyopathic crisis with rhabdomyolysis, neurodegeneration, arrhythmias
Folate	• Primary cerebral folate deficiency • Secondary cerebral folate deficiency • Combined immunodeficiency and megaloblastic anemia • Hereditary folate malabsorption • Glutamate formiminotransferase deficiency
Vitamin A	• Abetalipoproteinemia
Vitamin D	• Vitamin D dependent rickets type 1,2,3
Vitamin E	• Abetalipoproteinemia • Ataxia with vitamin E deficiency

Table 3 **Enzyme Replacement Therapies**
(Pogue et al., 2018; Barigga et al., 2021; Maldonado et al., 2021).

Disorder	Deficient enzyme	Approved ERT	Trade Name	Regulatory Approval
Gaucher disease	Glucocerebrosidase	Imiglucerase Velaglucerase alfa Taliglucerase alfa	Cerezyme® VPRIV® Elelyso®	DCGI & FDA DCGI & FDA FDA
Fabry disease	Alpha-galactosidase A	Agalsidase-beta Agalsidase alpha	Fabrazyme® Replagal®	DCGI & FDA EMA
MPS-I, Hurler/Scheie	Alpha-L-iduronidase	Laronidase	Aldurazyme®	FDA
MPS-II (Hunter syndrome)	Iduronate-2 sulfatase	Idursulfase, Idursulfase beta	Elaprase® Hunterase®	DGCI FDA
MPS-IV A(Morquio syndrome)	N-acetylgalac-tosamine-6-sulfatase	Elosulfase alfa	Vimizim®	FDA
MPS-VI (Maroteaux-Lamy syndrome)	N-acetylgalac-tosamine-4-sulfatase	Galsulfase	Naglazyme®	FDA
MPS-VII (Sly syndrome)	Beta-glucuronidase	Vestronidase alfa	Mepsevil®	FDA
Pompe disease	Acid alfa glucosidase	Alglucosidase alfa	Myozyme®	DGCI
Alpha-mannosidosis	Alpha-mannosidase	Velmanase alpha	Lamzede®	FDA
Hypophosphatasia	Tissue non-specific alkaline phosphatase	Asfotase alpha	Strensiq®	FDA
Lysosomal acid lipase deficiency	Lysosomal acid lipase	Sebelipase alfa	Kanuma®	FDA
Adenosine deami-nase deficiency	Adenosine deaminase	Pegademase bovine Elapegade-mase-lvlr	Adagen® Revcovi®	FDA
Phenylketonuria	Phenylalanine hydroxylase	Pegvaliase	Palynziq®	FDA
Neuronal ceroid lipofuscinosis type 2	Tripeptidyl peptidase 1	Cerliponase alpha	Brineura®	FDA

*MPS – Mucopolysaccharidosis, US FDA – United StatesFood and Drug Administration, EMA- European Medicines Agency, DCGI- Drugs Controller General of India

Table 4 **Therapeutic products based on RNA modification therapy**
(O'Connor et al., 2006; Turnpenny et al., 2017; Shahryari et al., 2019).

Therapeutic product	Approval authority & year	Disorder	RNA modification & Mechanism of action	Inclusion criteria	Effect of therapy	Approximate treatment cost
Exondys 51 (Eteplirsen)	USA FDA 2016	DMD	**Morpholino ASO** designed to cause skipping of exon 51 of the dystrophin gene.	Mutation in DMD gene amenable to exon 51 skipping	It causes exon skipping resulting in a short protein with greater functionality	$300,000 annually per patient
Vyondys 53 (Golodirsen)	US FDA 2019	DMD	**Morpholino ASO** designed to cause skipping of exon 53 of the dystrophin gene.	Mutation in DMD gene amenable to exon 53 skipping	It causes exon skipping resulting in a short protein with greater functionality	$300,000 annually per patient
Viltepso (Viltolarsen)	US FDA 2020	DMD	Second approved **morpholino ASO** designed to cause skipping of exon 53 of the dystrophin gene.	Mutation in DMD gene amenable to exon 53 skipping	It causes exon skipping resulting in a short protein with greater functionality	$1300 for 5ml vial
Amondys 45 (Casimersen)	US FDA (2021)	DMD	**Morpholino ASO** designed to cause depletion of exon 51 of the dystrophin gene. exon skipping of exon 45 and dystrophin synthesis	Mutation in DMD gene amenable to Exon 45 skipping	It causes exon skipping resulting in a short protein with greater functionality	$1680 for 2ml vial
Spinraza (Nusinersen)	US FDA 2016	SMA type-1	**ASO** which targets intron 7 on the SMN2 hnRNA modulating alternative splicing by increasing inclusion of exon 7 in the final processed RNA	SMA patients who have at least one copy of the SMN2 gene	Modify the expression of SMN2	$125000 per injection
Kinamro (Mipomersen)	US FDA 2013	Familial hypercholesterolemia (FH)	**ASO** that interferes with the synthesis of ApoBresulting in RNase H-mediated disruption of the mRNA molecule	Variants in the *LDLR, APOB, PCSK9* genes	Reduce the synthesis of ApoB in the hepatocytes	$6910 for 1ml vial

Tegsedi (Inotersen)	US FDA 2018	Familial amyloid polyneuropathy (FAP)	**ASO** that causes degradation of mutant and wild-type TTR mRNA through binding to the TTR mRNA	All FAP diagnosed patients	Reduction of serum TTR protein and TTR protein deposits in tissues	$420,000 annually per patient
Onpattro (Patisiran)	US FDA 2018	Familial amyloid polyneuropathy (FAP)	Lipid nanoparticle containing an **RNAi** targeting the transthyretin mRNA	All FAP diagnosed patients	Results in a reduction of mutant protein	$345000 per 2 mg/ml
Givlaari (Givosiran)	US FDA 2019	Acute hepatic porphyria (AHP)	Aminolevulinate synthase 1 (ALAS1) directed **RNAi**	All AHP diagnosed adult patients	Degradation of ALSA1 mRNA in hepatocytes reducing its elevated levels in liver	$575000 per year per patient
Oxlumo (Lumasiran)	US FDA 2020	Primary hyperoxaluria type 1 (PH1)	**RNAi** that reduces the levels of glycolate oxidase enzyme by targeting glycolate oxidase encoding mRNA	All PH1 diagnosed patients	Reduction of glycolate oxidase levels by silencing of gene encoding glycolate oxidase	$493000 per year per patient

US FDA – United States Food and Drug Administration, DMD – Duchenne muscular dystrophy, SMA – spinal muscular atrophy

Table 5 **A comparison of viral vectors for gene transfer**
(O'Connor et al., 2006; Turnpenny et al., 2017; Maldonado et al., 2021)

Vector	Retrovirus	Lentivirus	Adenovirus	Adeno-associated virus
Viral genome	RNA	RNA	dsDNA	ssDNA
Transfection capacity	<8kb	8-10kb	8-30kb	4.5-8kb
Genome integration	Yes	Yes	No	No
Long-term expression	Yes	Yes	No	Yes
Immune response to vector	Few	Few	Yes	No
Cell division requirement for target cell	Yes	G1 phase	No	No
Limitations	Risk of insertional mutagenesis; only infects dividing cells	Risk of insertional mutagenesis	Contains genes involved in the process of malignant transformation, so there is a potential risk of induced malignancy	Can be activated by any adenovirus infection; causes immune response

Advantages	Persistent gene transfer in dividing cells	Can be integrated into non-dividing cells, useful in the treatment of neurological conditions	Infect a wide variety of cell types, stable, can infect non-dividing cells, they have a greater capacity to infect different tissues	Infect a wide variety of cell types and non-pathogenic
Treatment for	ADA-SCID	Neurological conditions, beta-thalassemia	Cystic fibrosis	Retinal dystrophy caused by bi-allelic loss of function *RPE65* mutations

RNA – ribonucleic acid, DNA – deoxyribonucleic acid, ADA-SCID – adenosine deaminase deficient severe combined immunodeficiency, ss single stranded, ds double stranded.

Table 6 **Approved gene therapy products for the treatment of genetic disorders** (Shahryari et al., 2019; Maldonado et al., 2021).

Product name	Approval authority	Disorder	Vector and mechanism of action	Limitation	Price
Luxturna (VoretigeneNeparvovec-rzyl)	US FDA 2017	Retinal dystrophy caused by bi-allelic loss of function *RPE65* mutations	AAV2 carrying a normal copy of the *RPE65* gene	Conjunctival hyperemia, cataract, increased intraocular pressure and retinal tear, holes, and inflammation	$850,000 per patient, $425,000 per eye
Zolgensma (OnasemnogeneAbeparvovec)	US FDA 2019	SMA 1 patients <2 years of age	Non-replicating recombinant AAV9 containing a functional copy of human SMN1 gene under the control of CMV enhancer/chicken-β-actin-hybrid promoter	Benefits of the drug in patients with advanced SMA not recorded	$2.125 million for a one-time treatment
Strimvelis (GSK-2696273)	EMA 2016	ADA-SCID	Retroviral vector transduced autologous HSC expressing ADA	HCV infected patients (> 15 IU/ ml nucleic acid test)	$648000 per patient
Zynteglo (Betibeglogene-autotemcel)	EMA 2019	Beta-thalassemia (transfusion dependent patients aged 12 years or above)	Lentiviral associated βA(T87Q)-globin gene sequence	Thrombocytopenia, not suitable for pregnant or breastfeeding women	$1.8 million for a one-time treatment
Roctavian™ (Valoctocogene roxaparvovec)	EC 2022 (conditional approval)	Severe hemophilia A	An AAV5- encoding human B domain-deleted factor VIII	Transient infusion associated reactions and mild to moderate rise in liver enzymes with no long-lasting clinical sequelae	$2.5 million for one-time treatment

US FDA – United States Food and Drug Administration, EMA- European Medicines Agency, ADA-SCID – adenosine deaminase deficient severe combined immunodeficiency, SMA – spinal muscular atrophy, AAV – adeno-associated virus, HSC – hematopoietic stem cells, HCV – hepatitis C virus

Table 7 **Summary of different therapeutic approaches**

Therapeutic Option	Disorders	Advantages	Disadvantages
Dietary management	Small molecular disorders e.g.,phenylketonuria, tyrosinemia, homocystinuria	Easy intervention, cheaper treatment approach which can be modified specific to patients' need	Lifelong therapy; does not correct the gene defect
Enzyme replacement therapy	Lysosomal storage disorders, phenylketonuria	Fewer side effects, longer drug history, and wider availability	Lifelong intravenous therapy; does not cross the blood-brain barrier; immune response to therapy
Substrate reduction therapy	Gaucher type 1, Mucopolysaccharidosis-III	Oral therapy, crosses blood-brain barrier, pharmacodynamic response generally complementary to ERTs, does not elicit immune response	Not widely available
Chaperone therapy	Fabry disease, cystic fibrosis	Oral therapy, wide tissue distribution, fewer immunogenicity reactions	Lifelong intervention and does not cure the disorder
HSCT	Severe combined immunodeficiency, lysosomal storage disorders, X-linked adrenoleukodystrophy	Improves the neuronopathic phenotype	Difficulty to identify a human leukocyte antigen (HLA)-matched donor; procedure regimen related morbidity and mortality; GVHD; limited impact on CNS and skeletal manifestation
Liver transplant	Urea cycle disorder, tyrosinemia, Wilson disease,maple syrup urine disease	Provides relief and better lifestyle quality with no dietary restrictions	Need of donor match and risk of immune rejection
Kidney transplant	Polycystic kidney disease, primary hyperoxaluria	Improved lifestyle, prevention of renal failure and recurrent stone formation	Need of donor match and risk of immune rejection
RNA based therapies	Spinal muscular atrophy, familial amyloid polyneuropathy, familial hypercholesterolemia	Personalized treatment, rapid development, target specific	Expensive treatment which requires regular administration
Gene therapy	Spinal muscular atrophy, beta-thalassemia, ADA-SCID, retinal dystrophy	One time dosage which cures the disorder at gene level	Have some associated side effects, very expensive with many therapies still under research
Prenatal therapy	Congenital adrenal hyperplasia, heart block, LUTO	Earliest intervention even before the onset of disease	Only available for a very few disorders that are diagnosed during fetal life

Hormonal therapy	Turner syndrome, Prader-Willi syndrome, Noonan syndrome, idiopathic short stature, congenital hypothyroidism	Easy availability, wide distribution	Only a management approach, not curative

ERT – enzyme replacement therapy, ADA-SCID – adenosine deaminase-deficient severe combined immunodeficiency, HSCT – hematopoietic stem cell transplantation, GVHD – graft versus host disease, CNS – central nervous system, LUTO – lower urinary tract obstruction