Genetic Treatment Approaches in Rare Pediatric Diseases

Abstract
Gene and cell therapies have been developed and approved for a growing number of pediatric diseases. Researchers are working on developing new therapies for additional diseases, but their number is still limited. While genetic and cell-based treatments are often grouped under the umbrella term gene and cell therapy, there is no uniform gene and cell therapy, just as there is no uniform surgery. Each therapy is tailored to the specific disease being treated and makes a specific genetic alteration or replenishes the population of a specific type of cell that is not functioning properly in the patient's body.The diseases targeted by gene and cell therapies are often complex and difficult to treat. Due to this complexity and because each therapy is specifically tailored to the disease, the development of new therapies is a lengthy process. The development and approval process is strictly regulated at regional and European levels.The research and development of cell and gene therapies is a lengthy process that begins with basic research in the laboratory and ideally ends with a safe, effective therapy. Various studies in Europe and around the world are at different stages of research. With the advancement of research techniques, scientists are gaining new insights into the fundamentals of cell biology and genetics. In the laboratory, scientists are researching novel therapies and using human cells and tissues to create models of specific pediatric diseases. Clinical researchers conduct clinical trials in which patients receive novel therapies under strict medical supervision to determine their effectiveness. Medical and clinical researchers are also investigating whether approved therapies can be repurposed, whether they could be useful for treating other diseases or other elements of the same disease.

Key Words
Gene therapy-gene addition-gene silencing-gene editing-pediatrics-child

Introduction
Gene Therapy involves using genetic material to treat or prevent diseases. Three common effects of gene therapies in cells include*gene addition*, whereas it is a process introducing a functional gene that contains instructions for the cell to produce a specific protein. Vectors, often viruses, are utilized to deliver the functional gene to the cell's nucleus, where the DNA is housed. The introduced gene may reside in the nucleus permanently after a single administration. Depending on the design, the new gene may integrate into the main DNA or remain adjacent to it, providing additional instructions. *Gene silencing* is an approach, where the delivered genetic material hinders or suppresses the activity of an existing gene in a cell, leading to a reduction in the production of a particular protein. A third variant, *gene editing*, is a technique, which involves correcting segments of DNA by modifying or deleting information within an individual's affected gene. Genetic material is directly delivered to edit or alter specific DNA segments within a cell to rectify the protein production. Gene editing employs precise technology to make these targeted modifications. There are many more gene therapies mentioned in the manuscript and especially found in table 1.

Different Gene Therapy Approaches

Gene addition
Gene-addition tools involve the direct introduction of a functional gene, also known as a therapeutic gene or transgene, into the nucleus of target cells using a vector as a delivery vehicle. When adding a new gene, viral-based vectors are commonly used, either delivered ex vivo or in vivo. Vectors play a crucial role in gene addition techniques. Viruses are utilized as vectors due to their efficient cell entry capabilities, with various types of viral vectors employed for gene addition. Before transporting a transgene into a patient's cells, the vectors are modified to eliminate viral disease-causing genes and their replication ability.After a vector delivers the transgene into the nucleus, where DNA is housed in chromosomes, the cell initiates the production of new proteins to enhance functionality. The gene can either persist as an additional DNA segment in the cell or integrate into the chromosomes and become part of the cell's DNA, both leading to functional protein synthesis.

Gene silencing
Gene silencing is a natural process in genetics where gene expression is suppressed, either by inhibiting transcription or translation of genetic information. Transcriptional gene silencing may involve epigenetic changes to DNA or the binding of repressors to a silencer. Post-transcriptional gene silencing involves processes that occur after transcription of genetic information.

Gene editing
Gene editing aims to modify genetic material directly within a cell by delivering genetic material that can edit pieces of DNA. This alteration changes the instructions encoded in the DNA to correct the protein produced and restore proper cell function.There are various gene editing approaches currently under research, each with its own unique mechanisms. For instance, CRISPR Cas9 utilizes two main components, a guide RNA that locates the DNA sequence to be edited and a Cas enzyme or nuclease that cuts and edits the DNA at the specified location. Following this, the cell's natural DNA repair process takes place, resulting in a permanent desired change. Other gene editing techniques, such as TALENs and Zinc Finger Nucleases, may not necessarily involve the use of guide RNA or the scissor-like feature of the Cas9 enzyme.

CRISPR gene editing

CRISPR–CAS-associated protein (Cas) systems (CRISPR–Cas systems) originate from RNA-based bacterial defense systems (22). They recognize and eliminate foreign DNA from invading plasmids and bacteriophage and are thus considered a type of bacterial immune system. The system detects and cuts bacteriophage genomes, saving fragments of them into repetitive CRISPR arrays as a record of previous infection so they can be targeted again in the future if they meet the same invader. This array is passed from generation to generation, growing with every new encounter.Diagram of a bacterial CRISPR locus indicating the genes included and CRISPR array. The CRISPR array of repeats and spacers is transcribed as a single pre-CRISPR transcript and the repeat regions bind the trans-activating CRISPR RNA (tracrRNA) in the locus. RNAse III then binds these double stranded sections and cleaves them, separating all the spacer and repeat pairs from the original single transcript, producing lots of CRISPR RNA–tracerRNA fragments in which the spacer sequence is now known as a protospacer. These fragments bind Cas9, an RNA guided DNA endonuclease, activating and programing it to search the genome for protospacer adjacent motifs, which are often only three bases long. If it finds a PAM, Cas9 then unwinds the DNA and if there's a match between the protospacer in its CRISPR-RNA and the genomic DNA, Cas9 continues unwinding the DNA and cleaves the target.Most Cas9 systems used in labs create a blunt-ended DSB three bases away from the PAM sequence, offering predictable cleavage. While the CRISPR–Cas system is a natural process, in 2012, Professors Emmanuelle Charpentier and Jennifer Doudna were able to show that it was a programable system,18 for which they received the Nobel Prize in 2020. Since then, scientists have been able to adapt it, using short guide RNAs (sgRNAs) to make DSBs at the very specific locations they require, and take advantage of NHEJ or HDR to repair the break and introduce mutations or make desired alterations.Cas9 can be programed to make a DSB wherever the user wishes in the genome. To do this, the Cas9 endonuclease is only supplied with a sgRNA that combines a tracr and protospacer sequence and is typically 98–100 bases long, 20 bases of which is the protospacer. Rather than being a phage derived sequence, the protospacer sequence is chosen by the scientist to target their desired genome location for cleavage.Cleavage of a desired genomic sequence by supplying Cas9 with a sgRNA. The enzymes involved in strand cleavage are indicated. Unlike TALENs, this system can easily be multiplexed, cutting many targets at once. The different guide RNAs just need to be delivered into cells at the same time as Cas9. These can be delivered to cells in a number of ways, including transfection of plasmids encoding Cas9 and the sgRNAs, transfection of Cas9 messenger RNA (mRNA) and synthesized sgRNA, transfection of recombinant Cas9 protein and synthesized sgRNAs and lentivirus transduction to deliver Cas9 and sgRNA expression vectors. Each has its own pros and cons, and the choice of which to use will depend on factors such as cost, time, cell type and whether you wish to target a single point or are performing a whole genome screen.While CRISPR is very flexible, easy to use and good for multiplexing, it does make mistakes; this is useful in the setting of an adaptive immune system where bacteriophage may mutate to keep up with the arms race, but is not as helpful in genome modification. Cas9 will bind and sometimes cleave off-target sites that don't perfectly match the protospacer, generating unwanted mutations. Scientists, however, have come up with a number of approaches to limit this activity including Cas9 mutants with enhanced specificity and dual nickase strategies.

Further therapeutic genetic approaches are RNA interference, ZFNs and TALENs, like described in detail in Table 1.

Gene Therapy Strategies.	**Mechanism**
Gene Replacement	Transfer a functional copy of the gene to replace the nonfunctional copy, producing therapeutic levels of protein to reverse disease phenotype. Used in monogenic diseases due to single gene defect. *Luxturna (voretigeneneparvovec-rzyl) Strimvelis (GSK2696273) Zolgensma (onasemnogeneabeparvovec-xioi) Zynteglo (betibeglogeneautotemcel) Roctavian (valoctocogeneroxaparvovec)
Gene Addition	Introduce an additional protein-coding gene to improve cellular function and homeostasis, improving disease phenotype. Used in complex disorders due to combined effects of multiple genes and environmental factors like cancer and heart diseases.*Kymriah (tisagenlecleucel) Yescarta (axicabtageneciloleucel) Tecartus (brexucabtageneautoleucel) Carvykti (ciltacabtageneautoleucel) Breyanzi (lisocabtagenemaraleucel) Abecma (idecabtagenevicleucel).
Gene Silencing	Antisense Oligonucleotide; binds to target mRNA via complementary base pairing, altering 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 represses gene expression by triggering RNA degradation and/or inhibiting protein synthesis. Onpattro (patisiran); Givlaari (givosiran); Oxlumo (lumasiran)
Gene Editing	Programmable sequence-specific nucleases deliver genetic modification via double-strand break in specific genomic target sequence, followed by desired modifications during subsequent DNA break repair using HR or NHEJ. Widely used gene editing tools are ZFNs, TALENs, and CRISPR/Cas9 system.
ZFNs	First endonuclease fusing site-specific DNA-binding domain on zinc-finger protein to non-s sequence-specific DNA cleavage domain on Fok1 endonuclease. Fok1 endonuclease works as dimer for double-strand DNA cleavage at sites of binding of 2 ZFNs to opposite DNA strands. Clinical trials in various diseases
TALENs	Assembled by fusing Fok1 cleavage domain to DNA-binding domain consisting of highly conserved repeat sequence from TALE that can associate with single or longer-sequence nucleotides. TALENs act as dimers for DNA cleavage.Clinical trials in various diseases

CRISPR/Cas9 System	RNA-guided DNA cleavage module comprising single-stranded guide RNA and Cas9 endonuclease. Single-strand RNA binds to target sequence and Cas9 cleaves target DNA to generate double-strand break, with DNA repair through HR or NHEJ for targeted genomic modification.Clinical trials in various diseases

Table 1:Overview of gene therapy strategies and FDA-/EMA-approved treatments

Different Common Genetic Pediatric Diseases with the Option of Gene Therapy

Leukemia and Lymphoma and Chimeric Antigen Receptor T-cell therapy
is caused by an overproduction of specific cells in the blood system. The type of cancer depends on the type of cell that is overproduced.Blood stem cell transplants are used to treat leukemia, lymphomas, myeloma, myelodysplastic syndrome, and myeloproliferative disorders. The patient's stem cells, which produce faulty cell types, are removed through chemotherapy. (This also removes the healthy, non-cancerous cells; patients are susceptible to infections and other health complications at this stage of therapy). They are then replaced either by healthy donor cells or by their own cells modified in the laboratory. This is commonly referred to as a bone marrow transplant, bone marrow stem cell transplant, or hematopoietic stem cell transplant. Read our information sheet on blood stem cell transplants here.A hybrid gene and cell therapy called *Chimeric Antigen Receptor T-cell therapy (CAR-T therapy)* is used to treat certain aggressive blood cancers. This is a personalized treatment where the patient's own immune cells are extracted, genetically reprogrammed in the laboratory to target cancer, and reintroduced into the body. In children and adolescents, CAR-T therapy is used to treat certain leukemias (acute B-cell lymphoblastic leukemia), and in adults, it is used to treat certain lymphomas (diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma) and multiple myeloma. Since this is a highly complex procedure, it is usually only used when other standard treatments have not been effective.

Beta-thalassemias and Hybrid Gene Cell Therapy
Beta-thalassemias are a group of blood disorders caused by abnormal production of hemoglobin, the molecule in red blood cells that carries oxygen. This can lead to anemia, fragile bones, and delayed growth.There are three main forms of beta-thalassemia. Beta-thalassemia major is the most severe form, with symptoms such as severe anemia, delayed growth, and abnormal skeletal development typically appearing within the first two years of life. Beta-thalassemia intermedia presents symptoms later in life, with the most common and severe symptom being mild to moderate anemia. Beta-thalassemia minor may manifest as mild anemia or even be asymptomatic.Individuals with severe beta-thalassemia may require regular blood transfusions. These transfusions or the disease itself can lead to an excess of iron in the blood, resulting in heart, liver, and hormonal complications. These complications can be severe and even life-threatening, and severe beta-thalassemia should be managed by a multidisciplinary healthcare team.Beta-thalassemia is typically treated with blood transfusions and medications. It can also be treated through cell therapy (blood stem cell transplantation) or *hybrid gene cell therapy*.

Melanoma in Childhood and Gene Therapy (Imlygic)
Melanomas are a common form of skin cancer. It is believed that they are caused by exposure to ultraviolet light from the sun, especially from unusually intense sun exposure.Melanomas are typically removed through surgery. If surgery is not an option, advanced stage melanomas are treated with BRAF inhibitors. These medications target a protein that promotes the uncontrolled division of cancer cells.If a melanoma grows down into deeper layers of skin tissue, there is a risk that it may spread to other parts of the body and form cancerous tissue. Depending on where the cancer has spread, this can be treated with *gene therapy since 2015* (*Imlygic*), which attacks cancer cells and stimulates the body's immune system to recognize and destroy cancer cells.

Cerebral Adrenoleukodystrophy and Hybrid Gene Cell Therapy (Skysona)
Cerebral Adrenoleukodystrophy (CALD) is a genetically inherited condition in which the body is unable to break down fatty acids, known as very long-chain fatty acids (VLCFAs). As a result, these VLCFAs accumulate in the brain, nervous system, and adrenal glands. It is believed that this accumulation causes inflammation, damaging the outer insulating layer of nerve cells (the myelin sheath) so that they no longer function.Neurological symptoms of CALD include learning difficulties, vision and hearing loss, as well as seizures. People with CALD may also have difficulty swallowing and coordinating their movements and balance. Damage to the adrenal glands means that some people with CALD produce insufficient amounts of certain steroid hormones (cortisol and aldosterone). These individuals are at risk of an adrenal crisis, caused by a sudden drop in cortisol levels. These crises can lead to dizziness, nausea, and unconsciousness and can be fatal if left untreated.CALD is traditionally treated with medications and physiotherapy to prevent the progression of symptoms. If a suitable donor is found, a blood stem cell transplant can also be performed. Read our information sheet on blood stem cell transplants here. If no suitable donor is found, patients may be offered a hybrid gene cell therapy (*Skysona,* approved by the EMA in 2021 for use in Europe). In this therapy, the patient's own cells are taken, modified in the laboratory to break down the accumulation of VLCFAs, and reintroduced into the body.

Metachromatic Leukodystrophy (MLD) and Hybrid Gene Cell Therapy (Libmeldy)
Metachromatic leukodystrophy (MLD) is a genetic disorder in which fatty molecules called sulfatides cannot be broken down. These sulfatides accumulate in cells, particularly in the brain, spinal cord, and peripheral nerves. These deposits are toxic and damage the body's ability to form an insulating layer (myelin sheath) for nerve cells in the brain, spinal cord, and throughout the body.There are three forms of MLD: the late infantile form, which manifests before the age of two and is the most common form; the juvenile form, which develops between the ages of three and sixteen; and the adult form, which occurs after the age of sixteen and is the least common form. The initial signs and symptoms as well as the rate of progression vary depending on the age at which the disease first appears.People with MLD may develop sensory problems, such as blindness, hearing loss, or the loss of sensations like touch, pain, or warmth. They often experience mobility issues such as difficulty walking or coordinating movements, stiffness and rigidity, or paralysis, and have difficulty speaking or swallowing. Cognitive and psychological problems include loss of memory function, decreased cognitive ability, and emotional instability or personality changes. Seizures and psychoses are also common in MLD.

Treatment options for MLD depend on the stage at which the disease is recognized and diagnosed. In most cases, it involves supportive care, with a multidisciplinary team helping to manage symptoms. In asymptomatic or mildly symptomatic cases, a blood stem cell transplant may be performed to slow the progression of symptoms. If a suitable donor is not found, patients may be offered a *hybrid gene cell therapy (Libmeldy*) since 2020, in which the patient's own blood stem cells are taken, modified in the laboratory to break down sulfatides, and then injected back into the body.

Spinal Muscular Atrophy (SMA) and Gene Therapy (Zolgensma)

Spinal muscular atrophy is a genetically inherited neuromuscular disorder. It leads to the death of motor neurons (the nerve cells that transmit signals from the central nervous system to the muscles, triggering movements) and progressive muscle wasting (3-8,10,14,15). There are five subtypes of SMA (SMA 0-5), depending on when the first symptoms occur and what symptoms are present. Infants with SMA Type 0/1 typically do not reach the age of four if appropriate treatment is not found, while individuals with SMA Type 4 (adult-onset SMA) have a normal life expectancy.Common symptoms in severe SMA include general muscle weakness and "floppiness," feeding and swallowing difficulties, and breathing problems. Infants may have difficulty reaching developmental milestones such as rolling, sitting, or standing; in some cases, a baby or child may lose a skill they had previously mastered, such as independent sitting.Traditional treatment for SMA focuses on managing symptoms and complaints through physical therapy and occupational therapy. Spinal fusion surgery may be performed to reduce pressure on the lungs, and ventilation can support breathing (3-8,10,14,15). In Europe, children under 24 months may be eligible for *gene therapy (Zolgensma*) since 2020, where a functional copy of the mutated gene causing the disease is inserted, restoring nerve function.Some private clinics offer blood stem cell transplants for SMA treatment; however, there is no scientific or clinical evidence that this is an effective approach (3-8,14,15).

Retinal dystrophy and gene therapy (Luxturna)

Retinal dystrophy is the term for a group of genetic diseases that lead to damage to the retina - the light-sensitive membrane on the back of the eye. The light-sensitive cells are damaged, so that visual information is not transmitted to the brain. These diseases often progress over time and lead to a progressive loss of vision.Gene therapy can be used to treat two specific retinal dystrophies - retinitis pigmentosa and Leber congenital amaurosis. This gene therapy (*Luxturna*, approved by the EMA in Europe in 2018) can only be used if there is still a sufficient number of healthy cells in the retina and if the mutation causing the disorder is in a specific gene (RPE65).

Gene Therapy for Hereditary Spastic Paraplegia Type 50

There are more than 10,000 individual rare diseases, most of which do not have a therapy. Personalized genetic therapy is a promising approach for treating these diseases. A roadmap for individualized treatment of an ultra-rare disease by developing a gene replacement therapy for a single patient with hereditary spastic paraplegia type 50 (SPG50) was performed (2). Through a collaborative effort, a gene therapy product carrying the AP4M1 gene was created and successfully administered to a 4-year-old patient within 3 years of diagnosis as part of a single-patient phase 1 trial (2). The primary endpoints were safety and tolerability, with secondary endpoints evaluating efficacy. At 12 months after dosing, the therapy was well tolerated, with no serious adverse events observed. Minor events such as transient neutropenia and Clostridioides difficile gastroenteritis were experienced but resolved. Preliminary efficacy measures suggest a stabilization of the disease course. Longer follow-up is needed to confirm safety and provide further insights on the therapy's efficacy. This report supports the safety of gene therapy for SPG50 and offers insights into precision therapy development for rare diseases (2).

Gene Therapy for Niemann Pick Disease Type C1 (NPC1)

Niemann-Pick disease, type C1 (NPC1) is a rare, fatal neurodegenerative disorder caused by mutations in the NPC1 gene, which codes for a protein involved in cholesterol transport within lysosomes (18). Currently, there are no approved treatments for this condition. Studies in mouse models of NPC1 have shown that systemic and central nervous system delivery of AAV9-hNPC1 can significantly improve disease symptoms (18). In this study, researchers investigated the effects of different doses of AAV9-hNPC1 in Npc1 m1N mice at various stages of disease progression (18). Higher doses of the viral vector and treatment at an earlier age were associated with better transduction in the nervous system and increased lifespan. Similar positive outcomes were observed in Npc1 I1061T mice, which mimic a common human NPC1 variant (18). These results provide valuable insights into the optimal dosing, timing of treatment, and efficacy of gene therapy in severe and milder forms of NPC1 deficiency, suggesting that early intervention before symptoms appear may offer the best results for individuals with NPC1 (18).

Gene Therapy forGiant Axonal Neuropathy

Giant axonal neuropathy is a rare, autosomal recessive, pediatric, polysymptomatic, neurodegenerative disorder caused by biallelic loss-of-function variants in GAN, the gene encoding gigaxonin.In a recent study, researchers conducted an intrathecal dose-escalation trial of scAAV9/JeT-GAN (a self-complementary adeno-associated virus-based gene therapy containing the GAN transgene) in children with giant axonal neuropathy (19). The primary focus was on safety, with the main secondary clinical goal being to achieve at least a 95% posterior probability of slowing the rate of change in the 32-item Motor Function Measure total percent score at 1-year post-treatment compared to pre-treatment (19).Four differentintrathecal doses of scAAV9/JeT-GAN were administered to a total of 14 participants: 3.5×1013 total vector genomes (vg) in 2 participants, 1.2×1014 vg in 4 participants, 1.8×1014 vg in 5 participants, and 3.5×1014 vg in 3 participants (19). Over a median observation period of 68.7 months (range, 8.6 to 90.5), there were 48 serious adverse events, with only one (fever) possibly related to treatment, and 129 of 682 adverse events possibly linked to treatment. The mean pretreatment slope in the overall cohort was -7.17 percentage points per year (19).At the 1-year mark post-treatment, the changes in slope were as follows: -0.54 percentage points with the 3.5×1013-vg dose, 3.23 percentage points with the 1.2×1014-vg dose, 5.32 percentage points with the 1.8×1014-vg dose, and 3.43 percentage points with the 3.5×1014-vg dose. The corresponding posterior probabilities for slowing the slope were 44%, 92%, 99% (above the efficacy threshold), and 90%, respectively. Between 6 and 24 months after gene transfer, sensory-nerve action potential amplitudes either increased, stopped declining, or became recordable after being absent in 6 participants but remained absent in 8.Intrathecal gene transfer with scAAV9/JeT-GAN for giant axonal neuropathy showed adverse events and

potential benefits in motor function scores and other measures at certain vector doses over a year. Further research is needed to assess the safety and effectiveness of intrathecal AAV-mediated gene therapy for this condition.

Gene Therapy for Pompe Disease

Pompe disease is a neuromuscular disorder caused by a deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA), which breaks down glycogen into glucose. The accumulation of glycogen in cells disrupts their function, particularly in muscle cells, leading to cardiac, motor, and respiratory issues. There are two main forms of Pompe disease: classical infantile-onset (IOPD) and late-onset (LOPD). IOPD, caused by a severe GAA deficiency, presents at birth and is fatal by age 2 without treatment. LOPD, which is less severe due to partial GAA activity, can manifest in childhood, adolescence, or adulthood with muscle weakness and breathing difficulties. Enzyme replacement therapy (ERT) has been available since 2006, providing clinical benefits but not a cure. Recent advancements in early diagnosis, improved treatments, and newborn screening are improving care for Pompe disease. Gene therapy using adeno-associated virus (AAV) vectors is in clinical trials for both LOPD and IOPD, showing promising results in LOPD trials where participants were able to discontinue ERT after gene therapy (9,13,20,21). While there are still obstacles to overcome, the development of curative therapies for Pompe disease is advancing, with potential for transformative treatments.

Discussion

Gene therapy in pediatrics is an innovative treatment approach for addressing various genetic disorders that present in childhood (1,11,12,16,17). It entails altering or fixing a defective gene or inserting a healthy gene into the cells of a patient.There are over 7,000 paediatric genetic diseases (PGDs), with less than 5% having treatment options. Treatment approaches targeting different aspects of the disease's biological process have resulted in positive health outcomes for some patients with PGDs. Over the past 30 years, significant progress has been made in developing new therapies, including gene therapy, for numerous PGDs. Successful treatment outcomes depend on a thorough understanding of the genetic basis and disease mechanism. Gene therapy, in particular, has demonstrated effectiveness in various clinical trials, leading to regulatory approvals and opening the door for gene therapies for other PGDs.Different diseases present different challenges in the development of new therapies. Developing a gene therapy for a disease caused by a single gene mutation is less complex than developing a gene therapy for a disease caused by multiple genes or a combination of genetic and lifestyle factors. Some tissues and organs are also more accessible for cell extraction or therapy administration (e.g., blood or the eye). In gene and cell therapy, millions of cells need to be modified to achieve a successful effect, making the question of how to effectively administer the therapy an important aspect of development.Gene and cell therapies must undergo rigorous scientific, ethical, and regulatory scrutiny in the research and clinical trial phases, as well as potentially in the marketing phase. The journey from the lab to the bedside - from developing a treatment in the lab to its regular use in the clinic - takes many years. A study that shows promise in clinical trials may take several years to receive full approval from authorities, and there may be further delays between approval of a treatment and its availability through a national public health service.In recent years, gene therapy has made significant progress. More than 4000 protein-coding genes have been linked to over 6000 genetic diseases, and the use of next-generation sequencing has greatly improved the diagnosis of genetic disorders. While most genetic diseases are considered rare or very rare, with fewer than 1:100,000 cases, only one of the 12 approved gene therapies (excluding RNA therapies) targets an ultrarare disease. This article examines three gene supplementation therapy approaches that can be used for various rare genetic diseases: lentiviral vector-modified autologous CD34+ hematopoietic stem cell transplantation, systemic delivery of adeno-associated virus (AAV) vectors to the liver, and local AAV delivery to the cerebrospinal fluid and brain (23,24,25).The success of gene therapy in treating genetic diseases depends on understanding the specific characteristics and function of the relevant gene, the genetic changes that cause disease, and the regulatory systems that affect gene expression. This knowledge can be used to develop strategies tailored to different types of diseases.For monogenic recessive conditions where a nonfunctional gene leads to a protein defect, gene augmentation through gene replacement can be used to restore normal protein levels and reverse the disease phenotype. In more complex diseases involving multiple genes, gene addition may be necessary to improve cellular function and modulate the disease course. In dominant diseases, gene silencing strategies like ASO and RNA interference can be employed to suppress dysfunctional gene expression.Advanced gene editing tools like ZFNs, TALENs, and CRISPR-Cas9 offer precise genetic modifications compared to traditional gene therapy methods. The choice of gene therapy approach depends on the therapeutic target and may involve in vivo or ex vivo delivery methods using viral vectors or non-viral systems.Regulating transgene expression is crucial to avoid toxicity, and strategies to enhance therapeutic efficacy include increasing target-cell specificity, reducing immunogenicity, and delivering the transgene to immune privileged sites. Despite challenges and setbacks in the field, ongoing research aims to improve gene therapy technologies with minimal risks.The approval of Glybera in 2012 marked a milestone in gene therapy, leading to trials for various monogenic diseases. While Glybera was later withdrawn due to cost and rarity of the disease it treated, research and development of novel gene therapies for genetic diseases continue to progress.There are currently 11 gene therapy products available commercially. These include Luxturna, Zolgensma, Roctavian, CAR-T therapies (Yescarta, Kymriah, Tecartus, Carvykti, Breyanzi, and Abecma), Zynteglo, and Strimvelis. Kymriah and Luxturna are approved for use in Singapore under the CTGTP regulatory framework. Therapeutic advances have been madein ADA-SCID, TDT, and SMA with gene-selective therapies.

Conclusion

Gene and cell therapies undergo rigorous scientific, ethical, and regulatory scrutiny during the phases of research and clinical testing and potentially during the marketing phase. The journey from the laboratory to the patient's bedside - from the development of a treatment in the lab to its regular use in the clinic takes many years. Studies have shown promise in clinical trials may take several years to be fully approved by regulatory authorities, and there may be further delays between the approval of a treatment and its availability through a national public health service.The development of genomic technologies has brought about a significant change in how patients with PGDs are treated, moving from diagnosis to treatment. The high expense of gene-specific therapies and the challenges in securing coverage and reimbursement pose a significant barrier to patients seeking these treatments. In order to make precision medicine a reality for improved patient-focused care in the coming years, collaboration among all involved parties is essential to create new reimbursement strategies that ensure all patients can benefit from these therapies.

Rare pediatric neurogenetic diseases typically manifest early in life, lack specific treatment options, have high mortality rates, and present a significant threat to children's health and survival. Adeno-associated virus (AAV)-mediated gene

therapy, a form of disease-modifying treatment, offers a novel approach to addressing these conditions and represents a major breakthrough in the field. Currently, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved AAV-mediated gene therapy products for spinal muscular atrophy, aromatic L-amino acid decarboxylase deficiency, and Duchenne muscular dystrophy. Recent preclinical and clinical trial data suggest that AAV-mediated gene therapy holds great promise for the treatment of genetic disorders. The expedited approval process for rare disease treatments may offer hope for children with rare neurogenetic conditions. However, AAV-mediated gene therapy comes with inherent risks and challenges, underscoring the need for standardized regulatory oversight and robust long-term monitoring to assess its effectiveness and safety.Additive gene therapies in research are focus of interest, which are clearly divided in table 1. A one-time genetic approach is desirable in many rare pediatric diseases and established only in few pediatric diseases to date. Research in this field is running fast by biopharmaceutical companies to develop prize-intensive therapies for all these rare or ultrarare pediatric populations.

References

(1) Brdička R, Macek M Jr, Brdičková KV. Gene therapy - once just a dream, now a reality. Cas Lek Cesk. 2024;163(3):87-93. English. PMID: 38981729.

(2) Dowling JJ, Pirovolakis T, Devakandan K, Stosic A, Pidsadny M, Nigro E, Sahin M, Ebrahimi-Fakhari D, Messahel S, Varadarajan G, Greenberg BM, Chen X, Minassian BA, Cohn R, Bonnemann CG, Gray SJ. AAV gene therapy for hereditary spastic paraplegia type 50: a phase 1 trial in a single patient. Nat Med. 2024 Jul;30(7):1882-1887. doi: 10.1038/s41591-024-03078-4. Epub 2024 Jun 28. PMID: 38942994.

(3) Farrar MA, Calotes-Castillo L, De Silva R, Barclay P, Attwood L, Cini J, Ferrie M, Kariyawasam DS. Gene therapy-based strategies for spinal muscular atrophy-an Asia-Pacific perspective. Mol Cell Pediatr. 2023 Nov 15;10(1):17. doi: 10.1186/s40348-023-00171-5. PMID: 37964159; PMCID: PMC10645685.

(4) Stettner GM, Hasselmann O, Tscherter A, Galiart E, Jacquier D, Klein A. Treatment of spinal muscular atrophy with OnasemnogeneAbeparvovec in Switzerland: a prospective observational case series study. BMC Neurol. 2023 Feb 28;23(1):88. doi: 10.1186/s12883-023-03133-6. PMID: 36855136; PMCID: PMC9971686.

(5) Leon-Astudillo C, Wagner M, Salabarria SM, Lammers J, Berthy J, Zingariello CD, Byrne BJ, Smith BK. Polysomnography findings in children with spinal muscular atrophy after onasemnogene-abeparvovec. Sleep Med. 2023 Jan;101:234-237. doi: 10.1016/j.sleep.2022.11.006. Epub 2022 Nov 10. PMID: 36442421.

(6) Galletta F, Cucinotta U, Marseglia L, Cacciola A, Gallizzi R, Cuzzocrea S, Messina S, Toscano A, Gitto E. Hemophagocytic lymphohistiocytosis following gene replacement therapy in a child with type 1 spinal muscular atrophy. J Clin Pharm Ther. 2022 Sep;47(9):1478-1481. doi: 10.1111/jcpt.13733. Epub 2022 Aug 4. PMID: 35924856.

(7) Weiß C, Ziegler A, Becker LL, Johannsen J, Brennenstuhl H, Schreiber G, Flotats-Bastardas M, Stoltenburg C, Hartmann H, Illsinger S, Denecke J, Pechmann A, Müller-Felber W, Vill K, Blaschek A, Smitka M, van der Stam L, Weiss K, Winter B, Goldhahn K, Plecko B, Horber V, Bernert G, Husain RA, Rauscher C, Trollmann R, Garbade SF, Hahn A, von der Hagen M, Kaindl AM. Gene replacement therapy with onasemnogeneabeparvovec in children with spinal muscular atrophy aged 24 months or younger and bodyweight up to 15 kg: an observational cohort study. Lancet Child Adolesc Health. 2022 Jan;6(1):17-27. doi: 10.1016/S2352-4642(21)00287-X. Epub 2021 Oct 29. PMID: 34756190.

(8) Kirschner J, Butoianu N, Goemans N, Haberlova J, Kostera-Pruszczyk A, Mercuri E, van der Pol WL, Quijano-Roy S, Sejersen T, Tizzano EF, Ziegler A, Servais L, Muntoni F. European ad-hoc consensus statement on gene replacement therapy for spinal muscular atrophy. Eur J Paediatr Neurol. 2020 Sep;28:38-43. doi: 10.1016/j.ejpn.2020.07.001. Epub 2020 Jul 9. PMID: 32763124; PMCID: PMC7347351.

(9) Mendell JR, Al-Zaidy SA, Rodino-Klapac LR, Goodspeed K, Gray SJ, Kay CN, Boye SL, Boye SE, George LA, Salabarria S, Corti M, Byrne BJ, Tremblay JP. Current Clinical Applications of In Vivo Gene Therapy with AAVs. Mol Ther. 2021 Feb 3;29(2):464-488. doi: 10.1016/j.ymthe.2020.12.007. Epub 2020 Dec 10. PMID: 33309881; PMCID: PMC7854298.

(10) Stolte B, Schreiber-Katz O, Günther R, Wurster CD, Petri S, Osmanovic A, Freigang M, Uzelac Z, Leo M, von Velsen O, Bayer W, Dittmer U, Kleinschnitz C, Hagenacker T. Prevalence of Anti-Adeno-Associated Virus Serotype 9 Antibodies in Adult Patients with Spinal Muscular Atrophy. Hum Gene Ther. 2022 Sep;33(17-18):968-976. doi: 10.1089/hum.2022.054. Epub 2022 Sep 7. PMID: 35943879.

(11) Hwu WL. Gene therapy for ultrarare diseases: a geneticist's perspective. J Biomed Sci. 2024 Aug 13;31(1):79. doi: 10.1186/s12929-024-01070-1. PMID: 39138523.

(12) Sadjadi R, Picher-Martel V, Morrow JM, Thedens D, DiCamillo PA, McCray BA, Pareyson D, Herrmann DN, Reilly MM, Li J, Castro D, Shy ME; Inherited Neuropathy Consortium. Clinical Characteristics of Charcot-Marie-Tooth Disease Type 4J. Neurology. 2024 Sep 10;103(5):e209763. doi: 10.1212/WNL.0000000000209763. Epub 2024 Aug 12. PMID: 39133880.

(13) Vrellaku B, Sethw Hassan I, Howitt R, Webster CP, Harriss E, McBlane F, Betts C, Schettini J, Lion M, Mindur JE, Duerr M, Shaw PJ, Kirby J, Azzouz M, Servais L. A systematic review of immunosuppressive protocols used in AAV gene therapy for monogenic disorders. Mol Ther. 2024 Jul 22:S1525-0016(24)00471-4. doi: 10.1016/j.ymthe.2024.07.016. Epub ahead of print. PMID: 39044426.

(14) Muntoni F, Signorovitch J, Sajeev G, Done N, Yao Z, Goemans N, McDonald C, Mercuri E, Niks EH, Wong B, Vandenborne K, Straub V, de Groot IJM, Tian C, Manzur A, Dieye I, Lane H, Ward SJ, Servais L; PRO-DMD-01 study investigators; Association Française contre les Myopathies; UK NorthStar Clinical Network; ImagingDMD investigators; cTAP. Meaningful changes in motor function in Duchenne muscular dystrophy (DMD): A multi-center study. PLoS One. 2024 Jul 10;19(7):e0304984. doi: 10.1371/journal.pone.0304984. PMID: 38985784; PMCID: PMC11236155.

(15) Iff J, Carmichael C, McKee S, Sehinovych I, McNeill C, Tesi-Rocha C, Henricson E, Muntoni F, Kitchen H. Eteplirsen Treatment for Duchenne Muscular Dystrophy: A Qualitative Patient Experience Study. Adv Ther. 2024 Aug;41(8):3278-3298. doi: 10.1007/s12325-024-02915-9. Epub 2024 Jul 3. PMID: 38958840; PMCID: PMC11263411.

(16) Lopes FM, Grenier C, Jarvis BW, Al Mahdy S, Lène-McKay A, Gurney AM, Newman WG, Waddington SN, Woolf AS, Roberts NA. Human *HPSE2* gene transfer ameliorates bladder pathophysiology in a mutant mouse

model of urofacial syndrome. Elife. 2024 Jul 11;13:RP91828. doi: 10.7554/eLife.91828. PMID: 38990208; PMCID: PMC11239176.
(17) Audouard E, Khefif N, Gillet-Legrand B, Nobilleau F, Bouazizi O, Stanga S, Despres G, Alves S, Lamazière A, Cartier N, Piguet F. Modulation of Brain Cholesterol Metabolism through CYP46A1 Overexpression for Rett Syndrome. Pharmaceutics. 2024 Jun 3;16(6):756. doi: 10.3390/pharmaceutics16060756. PMID: 38931878; PMCID: PMC11207948.
(18) Mylvara AV, Gibson AL, Gu T, Davidson CD, Incao AA, Melnyk K, Pierre-Jacques D, Cologna SM, Venditti CP, Porter FD, Pavan WJ. Optimization of systemic AAV9 gene therapy in Niemann-Pick disease type C1 mice. bioRxiv [Preprint]. 2024 Jun 8:2024.06.07.597901. doi: 10.1101/2024.06.07.597901. PMID: 38895471; PMCID: PMC11185674.
(19) Bharucha-Goebel DX, Todd JJ, Saade D, Norato G, Jain M, Lehky T, Bailey RM, Chichester JA, Calcedo R, Armao D, Foley AR, Mohassel P, Tesfaye E, Carlin BP, Seremula B, Waite M, Zein WM, Huryn LA, Crawford TO, Sumner CJ, Hoke A, Heiss JD, Charnas L, Hooper JE, Bouldin TW, Kang EM, Rybin D, Gray SJ, Bönnemann CG; GAN Trial Team. Intrathecal Gene Therapy for Giant Axonal Neuropathy. N Engl J Med. 2024 Mar 21;390(12):1092-1104. doi: 10.1056/NEJMoa2307952. PMID: 38507752.
(20) Minskaia E, Galieva A, Egorov AD, Ivanov R, Karabelsky A. Viral Vectors in Gene Replacement Therapy. Biochemistry (Mosc). 2023 Dec;88(12):2157-2178. doi: 10.1134/S0006297923120179. PMID: 38462459.
(21) Blind JE, Ghosh S, Niese TD, Gardner JC, Stack-Simone S, Dean A, Washam M. A comprehensive literature scoping review of infection prevention and control methods for viral-mediated gene therapies. Antimicrob Steward Healthc Epidemiol. 2024 Jan 31;4(1):e15. doi: 10.1017/ash.2024.1. PMID: 38415097; PMCID: PMC10897728.
(22) Vaidyanathan S, Kerschner JL, Paranjapye A, Sinha V, Lin B, Bedrosian TA, Thrasher AJ, Turchiano G, Harris A, Porteus MH. Investigating adverse genomic and regulatory changes caused by replacement of the full-length *CFTR*cDNA using Cas9 and AAV. Mol Ther Nucleic Acids. 2024 Feb 2;35(1):102134. doi: 10.1016/j.omtn.2024.102134. PMID: 38384445; PMCID: PMC10879780.
(23) Wilton-Clark H, Yokota T. Safety concerns surrounding AAV and CRISPR therapies in neuromuscular treatment. Med. 2023 Dec 8;4(12):855-856. doi: 10.1016/j.medj.2023.11.008. PMID: 38070478.
(24) Wang X, Lin X, He H, Peng J. Adeno-associated virus-mediated gene therapy for rare pediatric neurogenetic diseases: Current status and outlook. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2023 Sept 28;48(9):1388-1396. English, Chinese. doi: 10.11817/j.issn.1672-7347.2023.220639. PMID: 38044650; PMCID: PMC10929874.
(25) Gardin A, Ronzitti G. Current limitations of gene therapy for rare pediatric diseases: Lessons learned from clinical experience with AAV vectors. Arch Pediatr. 2023 Nov;30(8S1):8S46-8S52. doi: 10.1016/S0929-693X(23)00227-0. PMID: 38043983.