Review Article

Gene therapies for Spinal muscular atrophy and Duchenne muscular dystrophy : a pathbreaking moment in therapeutics

Abstract

Gene therapy has been proven to be a boon for several neuromuscular diseases. A concept introduced early 1960's, has been put into clinical practice in the past 5-10 years. The process by which a healthy gene replaces a defective gene in a human body through vectors is truly pathbreaking. Patients with inherited degenerative neuromuscular disorders such as spinal muscular atrophy, and muscular dystrophies undergo progressive deterioration and eventually death. Gene therapy and other adjunctive clinical approaches have provided such patients with a second chance to truly live their life. The nusinersen, risdiplam, zolgensma, eteplirsen, golodirsen, viltolarsen, casimersen and ataluren are developed as gene therapies. This review focuses on the different gene therapies currently developed for spinal muscular atrophy and Duchenne muscular dystrophy

Keywords: gene therapy, spinal muscular atrophy, muscular dystrophies, SMN-1 gene, gene splicing, viral vectors, dystrophin gene, AAV-9 vectors, ASOs, exon skipping, CRISPR-Cas9

Introduction

The emergence of gene therapy is one of the revolutionary watershed moments in therapeutics. Gene therapy is a technique by which a defective or missing gene/allele is added/replaced or substituted by a copy of a healthy gene/allele using delivery methods such as oligonucleotides or viral and non-viral vectors. Ever since the term "gene" has been coined by Wilhelm Johannsen and the double-stranded DNA double helix model was discovered by Watson and Crick, the ability to manipulate and alter genetic sequences was being explored. The initial step to modify genetic mammalian cells through delivery of coding DNA from healthy cells was performed by Wacław and Elisabeth Szy-balski. In 1989, retroviruses were introduced as vectors for the delivery of foreign genetic material into mammalian cells. The first ever successful trial of

administration of genetically modified lymphocytes was done in 1991, on a then 4-year-old girl, who was born with the autosomal recessive disorder, adenosine deaminase (ADA) deficiency.[1] Gene therapy can be either *ex-vivo* or *in-vivo*. In *ex-vivo*, targeted cells from the patient are extracted, cultured, a vector carrying a healthy copy of the gene is infused into the target cells and transduced cells are returned to the patient. In *in-vivo*, a vector carrying a healthy copy of the gene is directly injected into a patient's bloodstream.[2] Vectors are the vehicles used in delivering nucleic acids into either somatic or germline cells. Vectors can be segregated into viral and non-viral. Amongst viral vectors, retrovirus and adenovirus vectors are most commonly used whereas, in non-viral vectors, plasmids, DNA, and RNA are the most common types. The majority of the clinical trials in gene therapy are conducted for genetic diseases, cancers, and some infectious diseases such as HIV-AIDS, HBV/HCV, malaria, and Ebola.

Spinal muscular atrophy (SMA) and muscular dystrophies

Spinal muscular atrophy (SMA) is a devastating, autosomal recessive neurodegenerative disease that causes progressive muscle weakness and atrophy. It can cause death in infants less than 2 years of age if left untreated. SMA affects 1 in 10,000 live births worldwide. High incidence rates of SMA were found in small countries of the European continent such as Iceland and Slovakia (13.7 and 17.8 per 100,000 live births) and much lower incidence in Cuba (5 per 100,000 live births).[3] The same study conducted in Cuba also compared incidence based on ethnicities where the Caucasians had a higher prevalence for SMA as compared to African Americans and Hispanics probably due to a low carrier frequency amongst the latter.[4] Nevertheless, different studies show variable prevalence and incidence as these studies were conducted in much smaller populations (countries) and usually infants die before enrolment. The genetic defect is a homozygous deletion mutation in the Survival motor neuron-1 gene (*SMN-1*) situated in the q-arm of chromosome-5 encoding for a Survival motor neuron protein (SMN) which is crucial for the development of lower motor neurons in the spinal cord and brain. Lack of SMN protein results in degeneration of motor neurons leading to muscle wasting and weakness. SMN can be further divided into 5 subtypes based on the age of onset and motor milestones attained: from the severe SMA-0 to milder SMA-IV.[5]

SMA-0, also known as congenital SMA affects infants in-utero resulting in the death of infants before or after birth due to hypotonia, severe joint deformities, muscle paralysis, and respiratory distress. SMA-I is the most common type of all SMAs' and affects 45-60% of the total SMA population. It is found in infants less than 6 months with dysphagia, and respiratory distress. SMA-II occurs in infants of 6-18 months of age, capable of sitting without assistance but unable to walk. SMA-III has a later onset of age > 18 months until adulthood, with patients able to walk but lose their ambulation in early childhood. SMA-IV, the milder phenotype, diagnosed in patients more than 30 years of age, able to walk well with very few motor disabilities and a normal lifespan.[3,6]

The disease severity is inversely proportional to the *SMN-2* copies present. Two forms of SMN genes are identified: *SMN-1* which encodes for full-length SMN protein and *SMN-2*, a backup gene. *SMN-2* is a pseudogene that differs from *SMN-1* in 5 base pairs but a specific single base substitution of nucleotide "C" with "T" in the 6th position of exon-7 often leads to exclusion of

exon-7 itself in SMN mRNA transcript. However, by alternative splicing, two kinds of proteins are synthesized: 90% of unstable and easily degradable SMN protein and the remaining 10% of functional SMN protein.[5] This 10% functional SMN protein is what provides some compensation for the loss of SMN protein not produced by the SMN-1. Most SMA patients have around 2-4 SMA-2 copies. Hence, greater the SMN-2 copies, milder the severeness of SMA.[7]

The X-linked recessive neurodegenerative illness Duchenne muscular dystrophy (DMD) causes gradual muscle weakening. It is seen in 1 in 5000 live births frequently affecting boys. Females are said to be asymptomatic carriers although some manifest clinical features of this disease due to inactivation of the healthy X chromosome and expression of the mutated X chromosome or "lyonization".[8] A deletion mutation in the dystrophin gene (*DMD*), on the short arm (p) of the X chromosome, the largest human gene known, consisting of a total of 79 exons, encoding for dystrophin protein gives rise to the disease. Due to its large number of exons, the gene is prone to multiple mistakes during the DNA replication process leading to mutations. Most of the mutations are large deletions.[9,10]

Diseases caused due to mutations in the dystrophin gene are grouped as "dystrophinopathies" which include DMD (Duchenne muscular dystrophy) and Becker's muscular dystrophy (BMD). In DMD, there is complete loss of dystrophin protein whereas, in BMD, there is partial loss or unstable dystrophin protein produced. Genomic deletion of exons 17-48, was linked with Becker's muscular dystrophy (BMD), a milder manifestation of the disease.[8] Dystrophin is a sarcolemma protein, that constitutes within dystrophin-associated glycoprotein complex (DAGC) which anchors the cytoskeleton of myocytes to the extracellular matrix. Hence, a lack of dystrophin disrupts the integrity and permeability of the sarcolemma resulting in muscle damage and necrosis. Therefore, gene therapy replacement of dystrophin necessitates the development of a shortened or full-length protein capable of reassembling the DAGC and supporting a robust mechanical connection between the extracellular matrix (ECM) and the cytoskeleton.[10]

Diagnosis of DMD is usually done by muscle biopsy or genetic testing along with the manifestation of clinical features. Through muscle biopsy dystrophin immunostaining, the deficiency of dystrophin can be detected. However, DNA testing is the gold standard for diagnostic confirmation. The process of DNA testing screens for deletions through multiplex ligation-dependent probe amplification (MLPA) or chromosomal microarray analysis (CMA). All 79 exons are sequenced to look for missense, nonsense, splice site, and minor indel variants if the deletions' results are absent. The drawback of this test is that the intronic mutations are not identified, hence RNA sequencing may be required to detect them.[9]

Diagnosis of SMA in a suspected patient is made based on clinical features such as hypotonia, muscle atrophy, respiratory distress ("bell-chest" deformity) lack of motor milestones achieved. Genetic testing is a highly definitive method of diagnosis for any mutations in the *SMN-1*/*SMN-2* gene. Methods such as quantitative PCR and MLPA are used. These methods should also aid in the quantification of the number of SMN-1/2 copies.[11]
Apart from genetic testing in parental carriers, newborns must also be screened for neuromuscular disorders as sooner the diagnosis, better will be treatment outcome. Hence,

screening for SMA was added to the Recommended Uniform Screening Panel in 2017 along with Pompe's disease.[12] DMD is yet to be added; however, Food and Drug Administration (FDA) has approved a test called GSP Neonatal Creatine Kinase-MM which detects levels of creatine kinase muscle type isoform (CK-MM) in blood indicative of muscle damage although not used for confirmational diagnosis of DMD.[13]

Multidisciplinary approach and supportive care along with psychological support provided to patients and their families were the only ways to manage SMA patients earlier. But in December 2016, nusinersen (spinraza) became the first ever drug to be approved for SMA and provided a glimmer of hope. Slowly, several other therapeutic drugs such as zolgesma and risdiplam were approved by FDA in 2019 and 2020 respectively.[3] Multiple studies and clinical trials are being conducted to discover new treatments for SMA. This review summarizes clinically approved drugs for SMA and DMD, their mechanism and adverse effects along with new therapeutic targets currently in development.

Mechanistic approaches for gene therapies in SMA

Splicing of SMN2 pre-mRNA

SMA patients have at least one copy of the *SMN2* gene. By understanding their genetic causes, it can be inferred that *SMN2* must be targeted to produce the functional protein. This is the goal of treating SMA.[14] The pre-mRNA splicing involves deleting the intronic (non-coding) sequences and joining the exonic (coding) sequences to form the functional mRNA in eukaryotes. The recognition of 5' and 3' splice sites (5'ss and 3'ss) identifies the length of intron.[11] In pre-mRNA splicing *cis*-elements and transacting factors work together to govern whether an exon should be included or not in splicing.[15] *SMN2* gene undergoes a silent mutation in exon 7 that causes defective splicing of the mRNA which produces either a highly unstable protein or none.[16] So, gene therapy should involve blocking the mutation to promote inclusion of exon 7.[15,16] Several approaches have been designed to uphold the exon 7 function such as small molecules to enhance the rate of transcription, and functional antisense oligonucleotides (ASOs).[17] The process of alternate splicing depends on the regulation and interaction between many *cis*- elements and *trans*- factors.[14,15]

Antisense oligonucleotides (ASOs)

Discovery of antisense oligonucleotides boasts a huge success for the treatment of SMA and other inherited disorders. Oligonucleotides are primers that consist of 18-30 base pairs. They occupy a position in the target mRNA, which changes the expression of the gene by either splicing or by incorporation of the cellular enzyme RNase H leading to degradation.[18] These molecules act and disable the intronic splicing silencer N1 (ISS-N1) which is present in the 7th position of the intron of *SMN2*.[14,19] These ASOs are adept at exon 7 inclusion in SMN2 transcripts and increasing levels of SMN protein both *in vitro* and *in vivo*. Thus, ASOs inhibit the ISS-N1 in intron 7 for exon inclusion.[19]

Small molecules

This approach was designed for promotion and upregulation of *SMN* gene expression.[14,16] Risdiplam (Evrysdi™) is the first orally available drug available for the treatment which was approved by FDA in 2020.[20] Like the ASOs, these small molecules are responsible for *SMN2* gene splicing modification which leads to the restoration of functional protein synthesis.[16] These small molecules are easily penetrable and cause less systemic complications.[20,21]

SMN1 gene substitution

This approach of treatment focuses on replacing the *SMN1* gene completely to produce functional and highly stable SMN protein in motor neurons.[22] This therapy uses viral vectors to convert the mutated or unstable gene into a stable gene.[23] Viral vectors are researched extensively and are one of the safest and effective carriers.[23–25]

Adeno-associated vectors (AAV)

AAVs (25 nm in size) belonging to the *Parvoviridae* family, non-enveloped and very small that are genetically modified for carrying the genetic material.[23,24] It contains only a single-stranded genome which carries three genes namely: *rep* which code for proteins involved in replication, *aap* genes needed for capsid protein assemblage and *cap* for the structural proteins of capsid.[23] These genes produce various products which are coding sequences. Later, these sequences are flanked by the *cis*-acting elements called inverted terminal repeats (ITRs). These repeats are important for packaging and replication of virus.[23] These vectors have absence of viral DNA so that it behaves as a nanoparticle for easy transfer and delivery of DNA to the nucleus.[24] In general, rAAV is used as a vector for gene transfer because it lacks the genes which encode for structural proteins and other components of the virus. It is less immunogenic than the traditional AAV so there will be no multiplication of immune response and genetic expression.[24] Based on the serotypes of AAV which is from 1 to 13, AAV9 has the highest capability for reaching the CNS.23 Recombinant AAV9 serotype vector based onasemnogene abeparvovec (zolgensma) was approved by FDA in May 2019 for the treatment of SMA.[25]

One of the major drawbacks of this platform is the single stranded genome. For expression of the gene, AAV must be converted into double-stranded for maximum efficacy. To overcome this limitation, self-complementary AAV is employed in which the single-stranded genome divides itself into the double-stranded genome. This saves time and causes gene expression at a faster rate but the packaging capacity of the vector is diminished.[24]

Mechanistic approaches for gene therapies in DMD

The mechanistic approaches evaluated to restore dystrophin consists of 1) nonsense mutation suppression 2) exon skipping using synthetic antisense oligonucleotides (ASO) and 3) Gene therapy via Adeno-Associated Virus Vector(AAV).[26]

Nonsense suppression to restore dystrophin

An example of small-molecule nonsense suppressors is Translational read through inducing drugs (TRIDs). Ataluren through this mechanism promotes full-length dystrophin production in cell culture by suppressing nonsense mutations. The European Medicines Agency (EMA) has granted approval of Ataluren for DMD treatment.[27]

Exon skipping for restoration of dystrophin

Nearly, 13-14% of people with DMD have an out-of-frame type of deletion that could be rectified through the skipping of exon 51, which is higher than the number of people who can be rectified through the skipping of any other exons.[28,9] Skipping of exon denotes the application of synthetic antisense oligonucleotides (ASO) to prohibit a certain exon from participating in splicing by inhibiting a splice enhancer site. The deletion mutations change the reading frame and produces a protein that is shortened or unstable. The reading frame may be rectified in some individuals with deletion mutation through skipping of an extra exon proximal to the deletion. Thus this new shortened transcript might allow for the synthesis of a steady, functioning protein because this truncated coding region would not be containing a disturbing reading frame.[26] FDA's rapid eteplirsen approval in the year 2016 in September was based on the ability to raise dystrophin levels in patients, becoming the first approved medication to treat DMD.[29]

Similarly, attempts were undertaken to produce ASOs that facilitate the skipping of certain exons of dystrophin protein. Another ASO clinically studied includes drisapersen which also induces exon 51 skipping.[28] The reading frame of dystrophin RNA is restored by skipping this exon and allows for the translation of a shortened dystrophin rather than none at all. As a result, the extreme DMD phenotype may be converted to a lesser BMD phenotype. Significantly, exon 51 skipping could improve a vast number of people with DMD or roughly 14% of the patients.[28]

Gene therapy via Adeno-Associated Virus (AAV) Vector

The pathogenesis of DMD is rectified by swapping out the mutant gene for a regular one. Due to the huge gene size and the dispersion of muscle across the body, however, this effort has proven to be difficult. The former makes viral vector packing difficult, whereas the latter calls for whole-body therapy. To overcome these challenges, the shortened micro-dystrophin gene as well as gene transfer systemically using adeno-associated virus (AAV) are developed.[30] Several laboratories have investigated various micro-gene sequences and AAV serotypes in animal models. Preclinical evidence proposes that AAV micro-dystrophin administration through the intravascular route improves muscle pathology, boosts muscular force, and reduces dystrophic cardiomyopathy in animal models.[30]

Infusion of large doses of vector, integration of functional expression cassettes expressed in striated muscles, and minimum immune activation are all required for efficient use of this approach. Vector extravasation for systemic delivery through the vasculature requires a critical threshold of vector particle concentration. While the exact dose required in people varies based on the AAV serotype utilized as a reservoir of capsid protein, the route of delivery, the robustness of the gene regulatory cassette (RCs) utilized to promote dystrophin protein

production and the specific micro-dystrophin cDNA sequence. Animal experiments utilizing vectors obtained from AAV serotypes 6, 8, and 9 have recommended dosing within the range of 1014 vector genomes per kilogram. By using muscle-specific regulatory cassettes (RCs) created from the MCK (Muscle creatine kinase) and desmin genes, off-target consequences like dystrophin production in the liver could be reduced. Stronger RCs as well as the usage of codon-optimized, then functionally improved cDNAs can increase gene expression on a per genome basis. RC optimization is presently focused on building compact, but potent cassettes that can work in all types of muscle fibers in the AAV delivery method.[10]

Currently approved gene therapies

Nusinersen

Nusinersen (Spinraza) is an ASO that promotes exon 7 inclusion by inhibiting the intronic splicing silencer N1 (ISS-N1) in SMN2 intron 7.[15] ISS-N1 silencing is one of the most reliable mechanisms for promoting exon 7 inclusion.[18] When Nusinersen crosses the ISS-N1, it obstructs hnRNP recruitment, which results in exon 7 production in mRNA and amplifies the formation of SMN2 protein.[17] (Figure 1) Nusinersen is the first drug available for use in SMA among both children and adult patients. Nusinersen is given intrathecally, so it is limited to CNS to not experience systemic side effects. With their great target specificity, oligonucleotides can access therapeutic sites that were previously inaccessible, with less systemic exposure and toxicity and extended half-life dosing.[17]

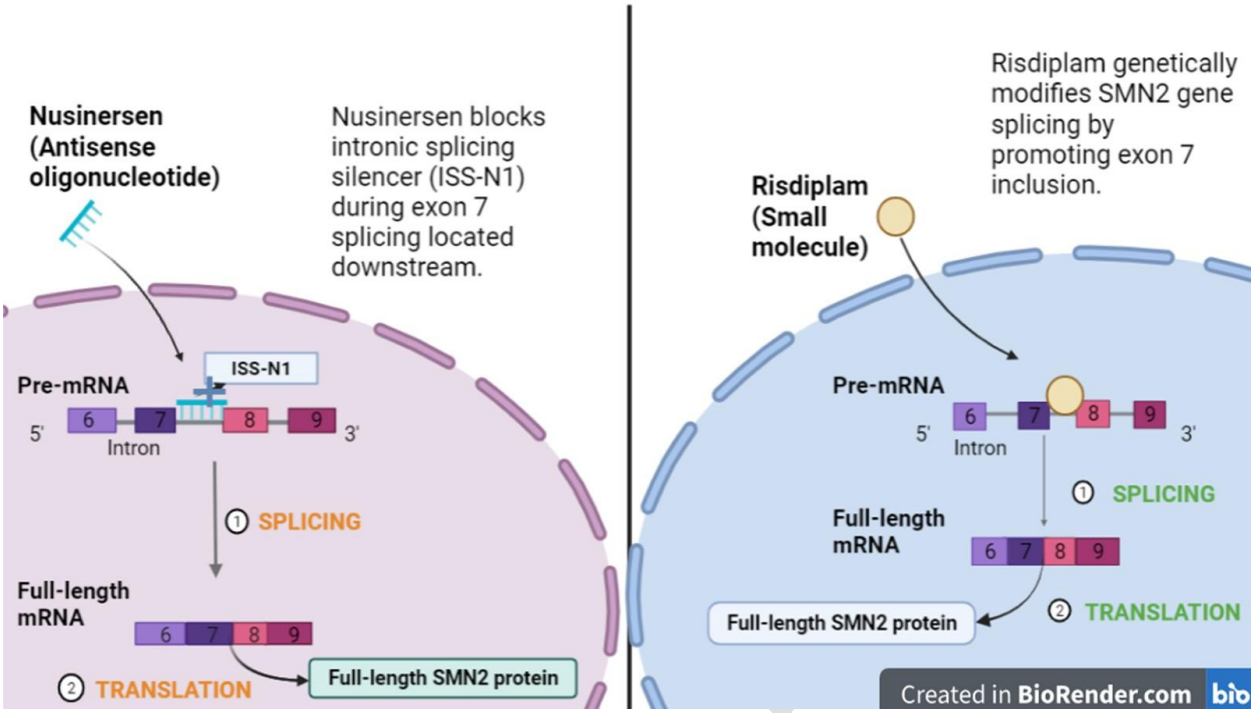

Figure 1: Contrasting mechanisms of nusinersen and risdiplam in spinal muscular atrophy

Nusinersen has undergone two important phase 3 trials namely CHERISH and ENDEAR trials. **(Table 1)** It involved the administration of intrathecal nusinersen in patients with SMA. ENDEAR and CHERISH trials dealt with SMA type 1 and type 2 patients. These trials showed beneficial results and improvement in motor function. The selection criteria for participation in CHERISH or ENDEAR trials is determined by age and *SMN2* copies detected. EMBRACE trials enrolled 21 patients excluded from the CHERISH or ENDEAR trials. So, EMBRACE trials were designed to evaluate the effectiveness of nusinersen in poorly prognosed individuals. The findings were positive and coincided with the previous trials and nusinersen was established effective and safe even for poorly prognosed patients and infants.[31] Since nusinersen is administered intrathecally, it can pose a problem for patients with SMA type 2 who also present with scoliosis, spinal disc fusion etc. However, image-guided intrathecal administration has been designed for these patients to receive the drug.[21] The effects of pharmacokinetic and pharmacodynamic properties of oligonucleotides has not yet been extensively studied. In the ENDEAR trial, 53% participants in the nusinersen group had a risk of death or permanent ventilation (hazard ratio=0.53).[32] The common adverse reactions are respiratory tract infections and distress.[31,32]

Risdiplam

Risdiplam is a splicing modifier used for patients of ages 2 months or older. By interacting with two locations in the SMN2 pre-mRNA: the exonic splicing enhancer 2 (ESE2) of exon 7 and the 50-splice site (50 ss) of intron 7, risdiplam alters the splicing of the SMN2 gene. By interacting with two locations on the SMN2 pre-mRNA, this mechanism raises levels of full-length SMN2

protein and therefore, lessens the impact of splicing and chances of off-target impacts.[6] (Figure 1) Risdiplam is a small molecule given orally immediately after meals at the exact time every day.[21] Oral route is an important advantage because SMA is a multisystem disease and risdiplam can reach other systemic tissues as well.[21] The agent showed beneficent results with regard to the pharmacokinetics and the pharmacodynamics along with an increase in body distribution and consistent plasma levels. Risdiplam is not a substrate for multi-drug resistant protein 1 which would ordinarily block this drug from entering the CNS due to energy efflux from the neurons.[20,21] After 4 weeks of risdiplam use, it has been reported a 2-fold increase from the threshold levels of SMN proteins and these findings were fixed for a year minimally. (Table1) In preclinical studies, risdiplam showed an increase in the number of motor neurons and widening of the extensor digitorum longus muscle length.33 Also, the transportation and storage measures as well as the cost are the additional advantages which makes risdiplam a suitable therapeutic agent.[20,21]

The common adverse events of risdiplam are respiratory tract infections, mouth ulcers, urinary tract infections, fever, rash and joint pain.[20,21] This drug is contraindicated in patients with liver abnormalities or diseases and also in pregnant women due to risk of fetal adversity. Hence, pregnancy testing is advised for women of reproductive age before beginning the use of this drug. Risdiplam has been reported to affect the reproductive organs.[21]

Zolgensma (Onasemnogene abeparvovec)

Zolgensma is a gene therapy which contains non-replicated rAAV9 viral vectors designed to treat SMA under the age of 2. Zolgensma adeno-associated virus serotype 9 vector, non-replicating and recombinant with cDNA of *SMN1* gene delivered to motor neurons. **(Figure 2)** Zolgensma was studied in SMA type 1 patients up to 8 months of age.[34] Also, it has been shown that AAV9 effectively crosses blood-brain-barrier and neurons. The efficacy of this drug was also documented with a single-dose, systemic administration i.e., intravenously or intrathecally. Further, systemic route targets the motor neurons directly and widespread CNS biodistribution.[21,24,25] When given intravenously, it targets all regions of central nervous system's neurons including the alpha motor neurons. The cDNA does not add itself into the host-cell genome in neurons, instead it forms distinct episomes.[21] The distinctive structure of cDNA and its promoters make a rapid and persistent expression of SMN protein, which is not dependent of the host-cell mediated synthesis. There is a presumption of long-term therapeutic success because motor neurons do not replicate.[21]

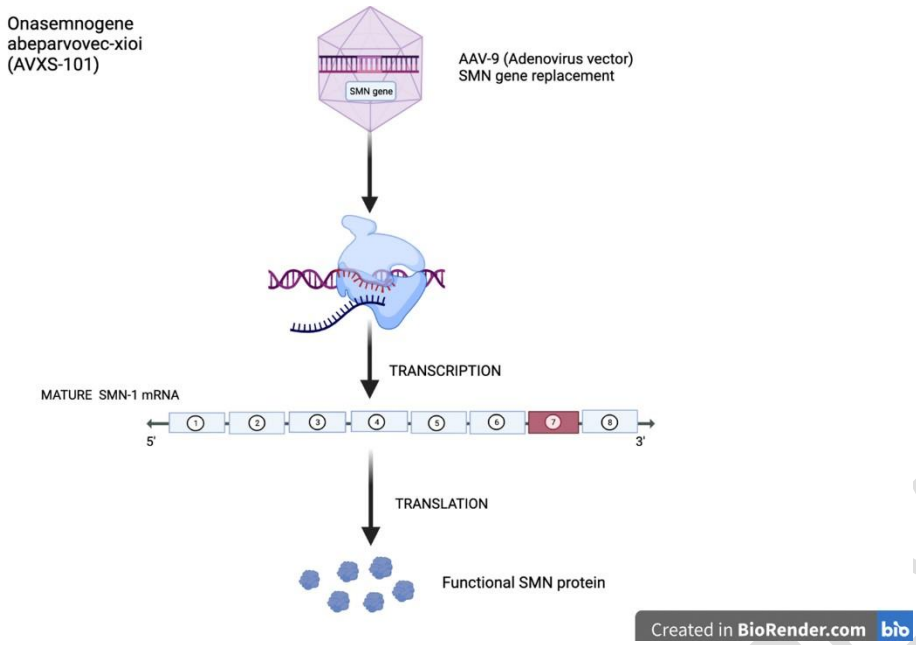

Figure 2: Mechanism of onasemnogene abeparvocec

Systemic administration also carries potential side effects. This route having a high ability to target motor neurons also causes adverse reactions involving hepatic, cardiac and immune cells. Corticosteroids are given to reduce the inflammatory adverse reactions. The most reported adverse reactions are elevated liver transaminases, acute liver injury, thrombotic microangiopathy, high troponin levels and thrombocytopenia. Regular monitoring of cardiac function, complete blood count and liver function are needed for 3 months of drug administration.[25] Prednisolone was used in the START trial, after the first patient reported an elevation of liver enzymes 16 times more than normal.[35] **(Table1)** In the STR1VE trial, all 22 patients experienced pyrexia and, some patients showed respiratory syncytial virus bronchiolitis, pneumonia and respiratory distress.[25]

Gene therapy for DMD

Treatment with oligonucleotides has been approved and used for managing DMD, along with corticosteroids being the backbone of the therapy. Exon skipping is the most popular treatment strategy, in which the splicing of the pre-mRNA dystrophin transcript is altered and thereby reading frame of translation as well as the dystrophin protein expression are restored. Thus, exon-skipping has been acknowledged as a reliable method for treatment in DMD patients.[36] **(Figure 3)**

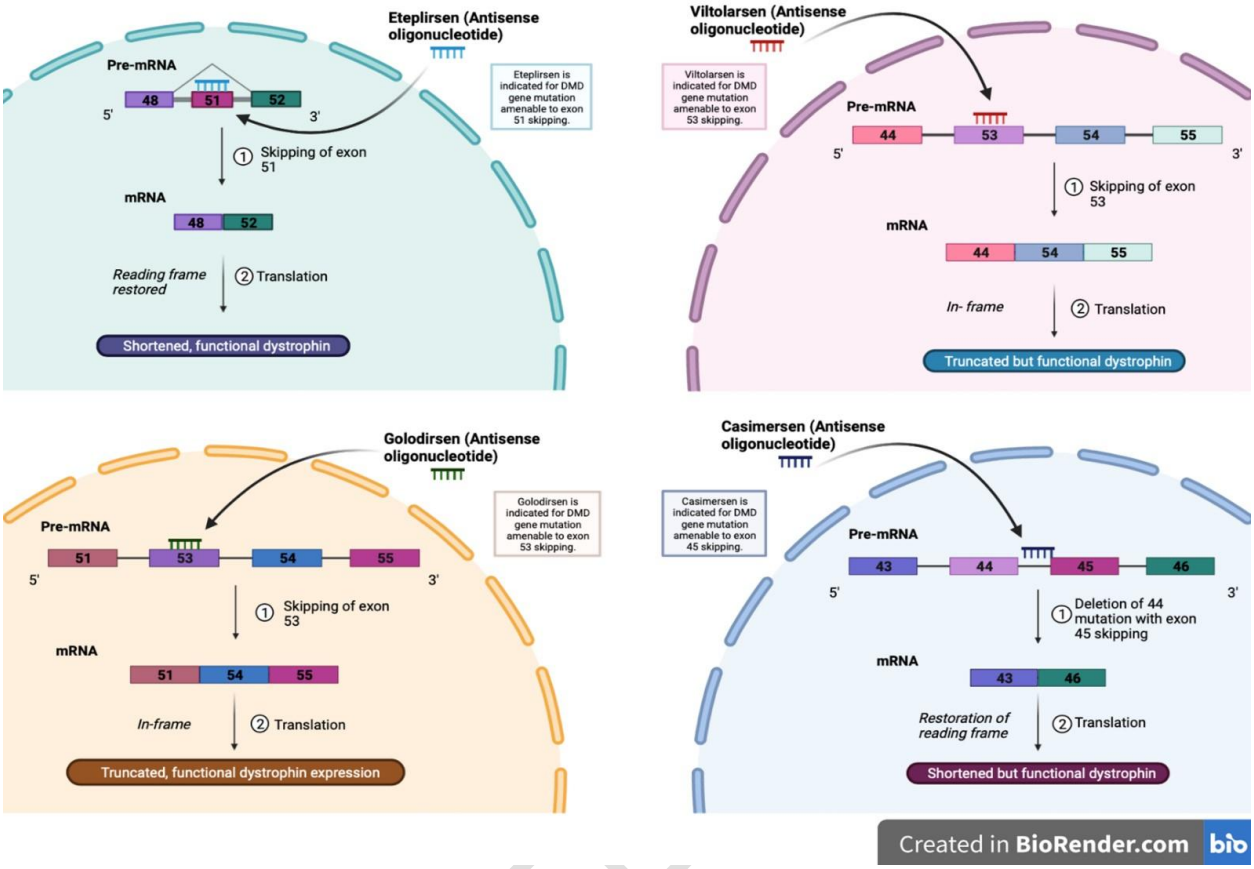

Figure 3: Mechanism of antisense oligonucleotides in Duchenne Muscular Dystrophy

The FDA authorized eteplirsen, a phosphorodiamidate morpholino oligomer (PMO), to treat DMD in 2016. Eteplirsen (Sarepta Therapeutics), via exon 51 skipping and reading frame restoration of DMD transcripts, can hypothetically treat up to 13% of DMD patients. The PROMOVI trial for eteplirsen showed increased expression of dystrophin, rising over 7.0-fold over baseline by the 96th week.[37] **(Table 1)** Vomiting, dermatillomania, joint pain, rash, catheter area soreness, and upper respiratory infection were all reported in at least 10% of persons who received eteplirsen during clinical studies.[38]

The PMOs specific to faulty reading frames are constantly being developed. The examples of PMO targeting exon 53 present on the main DMD transcript include golodirsen as well as viltolarsen. Treatment with golodirsen or viltolarsen could hypothetically assist 7.7% of DMD patients. Multiple exon skipping utilizing PMOs aiming both exons 51 as well as exon 53 might potentially re-establish the mRNA reading frame, allowing for treatment of an extra 8.1% of patients with DMD.[36]

Twenty-five boys (aged 6–15 years) having DMD responsive to exon 53 skipping were given 30 mg/kg of golodirsen (Vyondys 53) in stage 1/2 clinical experiment. Substantial boost in exon 53 skipping led to expression of dystrophin at forty eighth week, with a ~16 fold increase above the baseline.[39] (Table 1) In the viltolarsen (Viltepso) dose-finding phase 2 randomized clinical

study, sixteen boys (aged 4–9) having ambulant DMD responsive to exon 53 skipping were given 40 mg/kg or 80 mg/kg of viltolarsen weekly one time for a period twenty four weeks, and fourteen boys achieved dystrophin amounts greater than 5.3-5.4% of regular level.[36] Both golodirsen and viltolarsen were approved by the FDA in 2019 and 2020 respectively.[36] Headache, vomiting, abdominal discomfort, nausea, cough, flu symptoms, and fever were most frequently reported by the patients receiving golodirsen. Patients receiving golodirsen also reported hypersensitivity responses such as fever, rash, hives, itching, skin irritation, and skin exfoliation.[40] Upper respiratory infection, injection area sensitivity, cough, and fever are the most prevalent adverse effects in patients receiving viltolarsen.[41]

A mutation which is susceptible to exon 45 skipping is present in almost 8% of people with DMD. Casimersen (Amondys 45) (Sarepta Therapeutics) was tested in double-blind, placebo-controlled experiment in which 43 participants were randomly assigned to receive casimersen (30 mg/kg) or a placebo intravenously. Patients who got casimersen had a considerably higher rise in dystrophin protein amounts from baseline to week 48 of therapy than the participants who received placebo.[42,43] Casimersen was authorized by the FDA in 2021 for the therapy of people with DMD having a proven DMD gene mutation that is susceptible to exon 45 skipping.[43] Upper respiratory infections, cough, pyrexia, headache, arthralgia, and throat pain were the most prevalent adverse effects seen in DMD patients who were given Casimersen.[44] Thus, eteplirsen, golodirsen, viltolarsen, and casimersen are all authorized solely based on dystrophin levels, not muscular function.[36]

Table 1: The clinical studies evaluating the gene therapies for SMA and DMD

Study	Phase	Study design	Subjects	Study Arms		Primary Outcomes	Treatment duration	Results	References
				Experimental	Comparator				
ENDEAR	3	RDBSPC Randomized, Double-Blind, Sham-Procedure Controlled Study	SMA type 1 genetic diagnosis	IT nusinersen 12 mg on days 1, 15, 29, 64 and retention doses on 183, 302 (n=73)	Sham procedure: needle prick on lower back (n=37)	Improvement in motor function. Permanent ventilation or estimated deaths	2-27 months	51% achieved higher motor milestone response in the nusinersen group. 47% have lower occurrence of death or assisted ventilation in the nusinersen group.	[32]
CHERISH	3	Multicenter Randomized, Double-blind, Sham-Procedure	SMA diagnosis and symptoms from greater than 6 months of age	Nusinersen 12 mg solution via intrathecal (IT) injection on Days 1, 29, 85 and 274 (n=84)	Sham comparator on Days 1, 29, 85 and 274 (n=42)	Difference of Baseline in Hammersmith Functional Motor Scale Expanded (HFMSE) Score at month 15	15 months period	Difference from baseline in HFMSE: least squares mean 3.9 (3.0 to 4.9) vs -1.0 (-2.5 to 0.5) 57% patients in the nusinersen group reported an increase in HFMSE scores from the baseline compared to 26% in the sham group (at month 15)	[45]

EMBRACE	2	Randomized, double-blind, sham-procedure	5q SMA gene deletion or mutation. SMA with ≤6 months of age	IT nusinersen 12 mg on days 1, 15, 29, 64 then maintenance doses once in 4 months (n=14)	Sham procedure (n=7)	Safety and tolerability	14 months	Motor milestone responder rates higher in nusinersen (93%) vs sham procedure (29%) No nusinersen related adverse events were reported	[31]
SUNFISH (Part1)	2	Multicenter, double-blind, placebo-controlled study	SMA Type 2 and 3 of ages 2-25 years old	Oral Risdiplam for 12 months (n=35)	Placebo (n=16)	To establish the correct dose.	12 months	70% of the participants with increase (≥ 1 score) in the Motor Function Measure 32 (MFM32) High SMN protein level	[46]

SUNFISH (part2)	3	Randomised, double-blind, placebo-controlled study	SMA Type 2 and 3 of ages 2-25 years old	Oral risdiplam 500 mg (for subjects weighing ≥20 kg) or 0·25 mg/kg (for subjects weighing < 20 kg) (n=120)	Placebo (n=60)	32-item Motor Function Measure total score	12 months	Least squares mean difference from baseline 1·36 (95% CI 0·61 to 2·11) in the treatment group and –0·19 (–1·22 to 0·84) in the placebo group	[47]
STR1VE	3	Multicenter, open-label, single-arm, single-dose, trial	SMA Type 1 with 1 or 2 copies of SMN2 gene less than 6 months of age.	One-time Onasemnogene Abeparvovec-xioi via intravenously (n=22)		Erect sitting for 30s or longer at age 18th month Survival at 14th month	Until age of 18 months or early termination	13 patients accomplished erect sitting for 30s or longer. 20 patients did not require permanent ventilation.	[48]

START	1/2a	Open-label study, single arm-infusion, Single-center trial	Type 1 SMA of ≤ 6 months age	15 (13 in long term follow-up) Cohort 1: 3 patients received 6.7×1013 vg/kg (low-dose) of Onasemnogene abeparvovec Cohort 2: 12 patients received 1.1×1014 vg/kg (therapeutic dose) Cohort 1: All three received concomitant Nusinersen Cohort 2: 6 never received Nusinersen and the remaining received concomitant Nusinersen	24 months safety Assess the safety and efficacy in terms of Adverse Events (AEs).	Median of 5.2 years	All SAEs attributed for SMA included acute respiratory failure pneumonia, dehydration, respiratory distress No permanent ventilation required among cohort 2	[35]

Finkel *et al.*, 2013	2a	Open-label, sequential dose-ranging trial	Male DMD patients with nonsense mutation, 5 years and older.	Ataluren thrice a day for 28 days as per any of the three dosage regimens cohort 1 (n=6):4, 4, and 8 mg/kg; cohort 2 (n=20): 10, 10, and 20 mg/kg or cohort 3 (n=12): 20, 20, and 40 mg/kg	Biopsy taken of the muscle extensor digitorum brevis (EDB) on day 28 to assess dystrophin levels.	Till 28th day after treatment	Increased dystrophin levels in 61% of patients in a quantitative immunofluorescence assessing ratio of dystrophin.	[49]

PROMOVI	3	Open Label, Non-Randomized	Male patients with DMD of 7-16 years.	Weekly IV Eteplirsen given 30 mg/kg, for a period of 96 weeks (n=79)	Untreated Control Group (group were not susceptible to exon 51 skipping treatment) (n=30)	To assess 6-Minute Walk Test (6MWT) distance.	Eteplirsen's effectiveness and safety were monitored above a period of 96 weeks.	Treated group showed higher exon skipping and dystrophin protein as compared with baseline Decrease in mean 6MWT from 382.6m at baseline to 252.2 at the 96th week in untreated group. Decrease in mean 6MWT from 374.6m at baseline to 256.2m at the 96th week in eteplirsen treated group. 96 week may not be sufficient to show difference in 6MWT	[37]

Frank *et al.*, 2020	1/2	Part 1 : Randomized, double-blind, placebo controlled Part 2 : open label	Male DMD patients of age 6-15 years	Part 1: Golodirsen IV infusions, weekly with dose escalation for 12 weeks (n=8) Part 2: Golodirsen IV infusions 30 mg/kg weekly, for up to 168 weeks.	Part 1: Placebo comparator (n=4)	To assess dystrophin protein expression by the end of 48th week, using Western blot and immunohistochemistry (Part 2a).	Assessment of clinical and biological effectiveness at weeks 48 and 114. If adverse reactions happened between commencement of the initial dose and 28 days following the final dose they were regarded as treatment-emergent.	Increased exon 53 skipping with approx. ~16-fold dystrophin expression above the baseline at 48th week	[39]

IT intrathecal; IV intravenous;

Small molecules

In 2014, the EMA approved ataluren (PTC Therapeutics). It has been used as an oral therapeutic agent for people with DMD of age 5 years or older with ambulatory nonsense mutation.[50] Thirty-eight boys with DMD who had nonsense mutations underwent a phase 2a open-label, sequential dose-ranging study, following one of the following dosing schedules - 4 mg/kg, 4 mg/kg, and 8 mg/kg or 10 mg/kg, 10 mg/kg, and 20 mg/kg, or 20 mg/kg, 20 mg/kg, and 40 mg/kg, receiving ataluren for a period twenty eight days, thrice per day.[49] (Table 1) The synthesis of complete dystrophin protein in the extensor brevis muscle served as the study's key endpoint. In a quantifiable immunofluorescence analysis established on the dystrophin/spectrin proportion, over 61% of participants demonstrated elevated dystrophin protein levels as a result of ataluren therapy, with a mean change in dystrophin production of 11.0% from before the start of therapy to after the end therapy.[50] Headaches, gastrointestinal problems, and dizziness were noted in the clinical trials. Serum alanine transaminase (ALT) and aspartate transaminase (AST) were elevated asymptomatically.[50]

Cost and accessibility

Gene therapy has instilled a renewed sense of hope for patients diagnosed with SMA and DMD. The approved agents are under orphan drug designation and affordability is an important impediment for their usage.[51] Healthcare budgets are becoming unable to support the costs of these expensive therapies as more orphan medications are being produced. However, from the viewpoint of an investor, the costs of most orphan medications appear reasonable given the anticipated free cash flows and the necessary cost of capital.[51]

Spinal Muscular Atrophy

Nusinersen had regulatory approval pending so the pharmaceutical company made nusinersen accessible for treatment of infants with SMA type 1. The introduction of expanded access programs (EAP) employed intrathecal route of administration of nusinersen in infants diagnosed with SMA. However, several planning and management-related challenges are handled differently across nations. Nevertheless, "real-world data" supported that gene therapy of nusinersen showed improved motor function in SMA type 1.[52] Irish Health Service Executive in 2019 did not approve the use of nusinersen for treatment of SMA based on the enormous cost and limited therapeutic benefits. China has accepted and is giving insurance coverage starting from 2022.[53]

Currently regarded as the most expensive drug in the world, zolgensma single infusion costs €1.9 million ($2.125 million).[51,54] The cost of zolgensma has undergone price negotiations which is

now an integral part of the market access, where the entire cost and expenditure precedes over the patient's condition. This is morally and monetarily a hard task from all sides. In January 2021, a toddler received a one-time-infusion of zolgensma worth 16 crore INR through a lottery system free of cost from an US company.[55] There are conflicts regarding the market value of zolgensma where the total outflow of money is important than the patient's woe. But generally, the fixed research and development expenses are much higher for orphan drugs. This attributes to the high cost of an orphan drug.[51]

Duchenne Muscular Dystrophy

The cost of Eteplirsen is $300,000 annually as disclosed by Sarepta Therapeutics, with the price fluctuating according to the patient's body weight.[29] Golodirsen is also priced identically to eteplirsen i.e., several hundred thousand dollars annually per person, varying based on the person's body mass.[56] In a case of 25 kg child, the price of viltolarsen is estimated to be $587,000 per year.[57] A year's worth stock of casimersen to be given to a child of 30 kg is anticipated to be $748,800.[58] The accessibility to all these drugs depends on the insurance coverage and affordability of patients.[57]

Emerging therapeutic strategies

The above clinically approved/licensed therapies can't be the sole cure for SMA. Not all SMA patients receiving these drugs are capable of full-time relief or recovery. Patients and families must calculate the risk, cost and availability of such drugs as the long-term side effects of such drugs are still latent. SMN-restoration therapies focus on increasing SMN protein within the motor neurons of the brain and spinal cord. Recent studies show SMA as more of a multisystem disorder affecting the neurons in the muscle, lungs and liver.[6] Hence the new therapeutic targets for SMA focuses on treating SMA as a multi-system pathology and also covering patients of all ends of SMA spectrum. Also, insurance companies tend to restrict the purchase of such expensive drugs to such patients provoking a second thought on treating pre-symptomatic SMA infants with higher number of SMN-2 copies though they still develop motor imbalance.

Muscle enhancing therapies

Rapid irreversible loss of motor neurons has led to denervation muscle atrophy resulting in low muscle mass and extreme fatigue amongst SMA patients. Treatments which can increase muscle mass and improve muscle strength can provide better quality of life in patients.

Reldesemtiv (CK-2127107) is a fast skeletal muscle troponin activator, which increases the affinity of calcium to troponin C and sensitizes the sarcomere to the calcium release and hence, increases muscle contractility.[59] Reldesemtiv is highly selective for skeletal muscles with little or no effect on cardiac or smooth muscles. Analysis of patients administered with this drug showed improvement in the maximal expiratory pressure (MEP) and 6 Minute Walk Test (6MWT). This drug is currently under phase-II clinical trials.[6] The drug is also being evaluated for amyotrophic lateral sclerosis.[60]

Myostatin, a member of the TGF- beta family, is a small molecule expressed in all skeletal muscle cells. It's a negative regulator of muscle growth and differentiation. Inhibition of myostatin pathway provides promising results in improvement of muscle mass and function.[5] The common methods for myostatin inhibition developed are: 1- Direct antibodies against myostatin, 2-Ab's against its receptor- ActRIIB, 3-Follistatin, an endogenous myostatin inhibitor and 4-ActRIIB ligand traps.[61] The SRK-015 (Apitegromab) is a monoclonal antibody designed selectively to target and inhibits myostatin activation.[6] This drug is currently being evaluating in phase-2/3 trials after positive outcomes in initial trial which showed improvement in motor functioning.[62]

Autophagy and apoptosis

The concentration of autophagosomes elevated in cytoplasm of degrading motor neurons in SMA patients hinting at disruption in the autophagy pathway, can be involved in SMA pathophysiology. Intraventricular administration of *3 methyl-adenine(3MA*), inhibitor of enzymes regulating apoptotic pathways, showed improved motor function and increase of motor neuron lifespan in SMA pups.[6]

Apoptosis has also shown to be involved in SMA pathogenesis. JNKs or the c-Jun N-terminal kinases signaling pathways, regulate and promote processes such as apoptosis is found to be activated in several neuromuscular diseases including SMA. Hence, a pharmacological inhibition of these pathways can prevent death of motor neurons and be a future target of SMA independent approaches.[63]

RhoA/Rho Kinase inhibitors

RhoA/Rho Kinase (ROCK) signaling pathway is a regulator of cell integrity and actin cytoskeletal dynamics. Dysregulation of ROCK pathway has been associated with SMA pathogenesis. Rho Kinase is a kinase enzyme phosphorylated by Rho-A, a GTPase protein that results in activation and phosphorylation of p-LIMK. LIMK(LIM Kinases) suppresses maturation and development of NMJ's.[64] Hence, RhoA-Rho kinase pathway's inhibition, blocks activation of LIMK and therefore, allows for NMJ maturation and increased lifespan of SMA mice. Pharmacological inhibition of ROCK pathway by Y-27632 and fasudil, on SMA mouse models improved motor function, increase muscle fiber mass without compromise on healthy motor neurons or SMN expression.[65]

Stem cell therapy

Similarly, in SMA patients, neural stem cells are transplanted directly into CSF of the spinal cord or brain to replenish the degenerative motor neurons. Such therapies come with a high price tag, unknown long-term side effects and graft rejection. Hence, such approaches are still under experimental stage.[66,67]

CRISPR/Cas-9

Cas-9 protein acts as a pair of molecular scissors that cleaves or cuts viral DNA strands neutralizing and providing protection against the foreign entity.[68] CRISPR expanded as Clustered Regularly Interspaced Palindromic Repeats are specific DNA sequences located in the bacterial genome. During an immune response, bits of invading viral DNA is incorporated into the CRISPR loci as "spacers" to form a CRISPR array. This DNA sequence now undergoes transcription to form a long-stranded precursor CRISPR RNA or pre-crRNA. Cas-9 along with crRNA and trancrRNA form individual endonuclease effector complex. These effector complexes then recognize a previous incoming viral DNA strand and Cas-9 protein induces double strand breaks (DBS), removing a piece of viral genome which neutralizes the virus and hence, preventing further infection to the host cell.[69] The advent of CRISPR/Cas-9 system has provided hopes for restoration of dystrophin protein mainly by exon skipping at genomic DNA level.[70] In direct exon skipping, 2 guide RNA are engineered to cleave or cut genomic sequences encoding for the desired exons to synthesize a truncated but a partially functional and stable dystrophin protein. In classical exon skipping, a single guide RNA and SpCas9 protein were designed targeting the splice acceptor sites for DMD exons.[69] Other ways by which dystrophin expression can be restored includes reframing of out of frame exons and removal of duplicated exons.[71] CRISPR-Cas 9 has shown to provide permanent benefits as new therapeutic approaches in multiple diseases such as immunotherapy in cancer, treatment of HIV and even in monogenetic diseases.

Conclusion

Gene therapy has emerged as a potential tool to reduce or eradicate the occurrence of many rare diseases. Massive progress is being made in the field of gene therapy to curate new treatments for neuromuscular disorders. The mechanism of most gene therapies is to resolve the defect at its genetic level. This approach is being used in almost all the available therapies in the market. Along with its benefits, gene therapy includes several disadvantages. The most common drawbacks are the inaccessibility to the common public and the enormous cost involved in gene therapies.

Gene therapy has targeted the genetic cause of SMA and has to improve muscle activity. However future primary focus is on securing the SMN gene and protein synthesis in motor neurons for longer duration. Exon skipping through ASOs aimed to produce functional dystrophin are shown to be useful for DMD. In coming years, gene editing (e.g., CRISPR/Cas9) could further improve morbidity in SMA and DMD.

References

1. Arabi, F., Mansouri, V., & Ahmadbeigi, N. (2022). Gene therapy clinical trials, where do we go? An overview. *Biomedicine & Pharmacotherapy*, *153*, 113324. https://doi.org/10.1016/j.biopha.2022.113324

2. Ra, R. (2020). Gene therapy: An updated overview on the promising success stories. *Malays J Pathol*, 15.

3. Chong, L. C., Gandhi, G., Lee, J. M., Yeo, W. W. Y., & Choi, S.-B. (2021). Drug Discovery of Spinal Muscular Atrophy (SMA) from the Computational Perspective: A Comprehensive Review. *International Journal of Molecular Sciences*, *22*(16), 8962. https://doi.org/10.3390/ijms22168962

4. Verhaart, I. E. C., Robertson, A., Wilson, I. J., Aartsma-Rus, A., Cameron, S., Jones, C. C., Cook, S. F., & Lochmüller, H. (2017). Prevalence, incidence and carrier frequency of 5q–linked spinal muscular atrophy – a literature review. *Orphanet Journal of Rare Diseases*, *12*(1), 124. https://doi.org/10.1186/s13023-017-0671-8

5. Ojala, K. S., Reedich, E. J., DiDonato, C. J., & Meriney, S. D. (2021). In Search of a Cure: The Development of Therapeutics to Alter the Progression of Spinal Muscular Atrophy. *Brain Sciences*, *11*(2), 194. https://doi.org/10.3390/brainsci11020194

6. Messina, S., & Sframeli, M. (2020). New Treatments in Spinal Muscular Atrophy: Positive Results and New Challenges. *Journal of Clinical Medicine*, *9*(7), E2222. https://doi.org/10.3390/jcm9072222

7. Kolb, S. J., & Kissel, J. T. (2015). Spinal Muscular Atrophy. *Neurologic Clinics*, *33*(4), 831–846. https://doi.org/10.1016/j.ncl.2015.07.004

8. Steven Pavlakis, V. V. (n.d.). *Duchenne Muscular Dystrophy*.

9. Sun, C., Shen, L., Zhang, Z., & Xie, X. (2020). Therapeutic Strategies for Duchenne Muscular Dystrophy: An Update. *Genes*, *11*(8), 837. https://doi.org/10.3390/genes11080837

10. Ramos, J., & Chamberlain, J. S. (2015). Gene therapy for Duchenne muscular dystrophy. *Expert Opinion on Orphan Drugs*, *3*(11), 1255–1266. https://doi.org/10.1517/21678707.2015.1088780

11. Mercuri, E., Finkel, R. S., Muntoni, F., Wirth, B., Montes, J., Main, M., Mazzone, E. S., Vitale, M., Snyder, B., Quijano-Roy, S., Bertini, E., Davis, R. H., Meyer, O. H., Simonds, A. K., Schroth, M. K., Graham, R. J., Kirschner, J., Iannaccone, S. T., Crawford, T. O., … SMA Care Group. (2018). Diagnosis and management of spinal muscular atrophy: Part 1: Recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. *Neuromuscular Disorders: NMD*, *28*(2), 103–115. https://doi.org/10.1016/j.nmd.2017.11.005

12. Bharucha-Goebel, D., & Kaufmann, P. (2017). Treatment Advances in Spinal Muscular Atrophy. *Current Neurology and Neuroscience Reports*, *17*(11), 91. https://doi.org/10.1007/s11910-017-0798-y

13. *FDA authorizes first test to aid in newborn screening for Duchenne Muscular Dystrophy | FDA*. (n.d.). Retrieved September 18, 2022, from https://www.fda.gov/news-events/press-announcements/fda-authorizes-first-test-aid-newborn-screening-duchenne-muscular-dystrophy

14. Tisdale, S., & Pellizzoni, L. (2015). Disease mechanisms and therapeutic approaches in spinal muscular atrophy. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *35*(23), 8691–8700. https://doi.org/10.1523/JNEUROSCI.0417-15.2015

15. Singh, R. N., & Singh, N. N. (2018). Mechanism of Splicing Regulation of Spinal Muscular Atrophy Genes. *Advances in Neurobiology*, *20*, 31–61. https://doi.org/10.1007/978-3-319-89689-2_2

16. Vita, G., Vita, G. L., Musumeci, O., Rodolico, C., & Messina, S. (2019). Genetic neuromuscular disorders: Living the era of a therapeutic revolution. Part 2: diseases of motor neuron and skeletal muscle. *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *40*(4), 671–681. https://doi.org/10.1007/s10072-019-03764-z

17. Li, Q. (2020). Nusinersen as a Therapeutic Agent for Spinal Muscular Atrophy. *Yonsei Medical Journal*, *61*(4), 273–283. https://doi.org/10.3349/ymj.2020.61.4.273

18. Scoles, D. R., Minikel, E. V., & Pulst, S. M. (2019). Antisense oligonucleotides: A primer. *Neurology. Genetics*, *5*(2), e323. https://doi.org/10.1212/NXG.0000000000000323

19. Singh, N. K., Singh, N. N., Androphy, E. J., & Singh, R. N. (2006). Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. *Molecular and Cellular Biology*, *26*(4), 1333–1346. https://doi.org/10.1128/MCB.26.4.1333-1346.2006

20. Singh, R. N., Ottesen, E. W., & Singh, N. N. (2020). The First Orally Deliverable Small Molecule for the Treatment of Spinal Muscular Atrophy. *Neuroscience Insights*, *15*, 2633105520973985. https://doi.org/10.1177/2633105520973985

21. Kakazu, J., Walker, N. L., Babin, K. C., Trettin, K. A., Lee, C., Sutker, P. B., Kaye, A. M., & Kaye, A. D. (2021). Risdiplam for the Use of Spinal Muscular Atrophy. *Orthopedic Reviews*, *13*(2), 25579. https://doi.org/10.52965/001c.25579

22. Al-Zaidy, S. A., & Mendell, J. R. (2019). From Clinical Trials to Clinical Practice: Practical Considerations for Gene Replacement Therapy in SMA Type 1. *Pediatric Neurology*, *100*, 3–11. https://doi.org/10.1016/j.pediatrneurol.2019.06.007

23. Pattali, R., Mou, Y., & Li, X.-J. (2019). AAV9 Vector: A Novel modality in gene therapy for spinal muscular atrophy. *Gene Therapy*, *26*(7–8), 287–295. https://doi.org/10.1038/s41434-019-0085-4

24. Naso, M. F., Tomkowicz, B., Perry, W. L., & Strohl, W. R. (2017). Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs: Clinical Immunotherapeutics, Biopharmaceuticals and Gene Therapy*, *31*(4), 317–334. https://doi.org/10.1007/s40259-017-0234-5

25. Kotulska, K., Fattal-Valevski, A., & Haberlova, J. (2021). Recombinant Adeno-Associated Virus Serotype 9 Gene Therapy in Spinal Muscular Atrophy. *Frontiers in Neurology*, *12*, 726468. https://doi.org/10.3389/fneur.2021.726468

26. Shieh, P. B. (2018). Emerging Strategies in the Treatment of Duchenne Muscular Dystrophy. *Neurotherapeutics*, *15*(4), 840–848. https://doi.org/10.1007/s13311-018-00687-z

27. Ng, M. Y., Li, H., Ghelfi, M. D., Goldman, Y. E., & Cooperman, B. S. (2021). Ataluren and aminoglycosides stimulate read-through of nonsense codons by orthogonal mechanisms.

Proceedings of the National Academy of Sciences, *118*(2), e2020599118. https://doi.org/10.1073/pnas.2020599118

28. Wurster, C. D., & Ludolph, A. C. (2018). Antisense oligonucleotides in neurological disorders. *Therapeutic Advances in Neurological Disorders*, *11*, 175628641877693. https://doi.org/10.1177/1756286418776932

29. Lim, K. R., Maruyama, R., & Yokota, T. (2017). Eteplirsen in the treatment of Duchenne muscular dystrophy. *Drug Design, Development and Therapy*, *Volume11*, 533–545. https://doi.org/10.2147/DDDT.S97635

30. Duan, D. (2018). Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy. *Molecular Therapy*, *26*(10), 2337–2356. https://doi.org/10.1016/j.ymthe.2018.07.011

31. Acsadi, G., Crawford, T. O., Müller-Felber, W., Shieh, P. B., Richardson, R., Natarajan, N., Castro, D., Ramirez-Schrempp, D., Gambino, G., Sun, P., & Farwell, W. (2021). Safety and efficacy of nusinersen in spinal muscular atrophy: The EMBRACE study. *Muscle & Nerve*, *63*(5), 668–677. https://doi.org/10.1002/mus.27187

32. Finkel, R. S., Mercuri, E., Darras, B. T., Connolly, A. M., Kuntz, N. L., Kirschner, J., Chiriboga, C. A., Saito, K., Servais, L., Tizzano, E., Topaloglu, H., Tulinius, M., Montes, J., Glanzman, A. M., Bishop, K., Zhong, Z. J., Gheuens, S., Bennett, C. F., Schneider, E., … ENDEAR Study Group. (2017). Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *The New England Journal of Medicine*, *377*(18), 1723–1732. https://doi.org/10.1056/NEJMoa1702752

33. Poirier, A., Weetall, M., Heinig, K., Bucheli, F., Schoenlein, K., Alsenz, J., Bassett, S., Ullah, M., Senn, C., Ratni, H., Naryshkin, N., Paushkin, S., & Mueller, L. (2018). Risdiplam distributes and increases SMN protein in both the central nervous system and peripheral organs. *Pharmacology Research & Perspectives*, *6*(6), e00447. https://doi.org/10.1002/prp2.447

34. Friese, J., Geitmann, S., Holzwarth, D., Müller, N., Sassen, R., Baur, U., Adler, K., & Kirschner, J. (2021). Safety Monitoring of Gene Therapy for Spinal Muscular Atrophy with Onasemnogene

Abeparvovec -A Single Centre Experience. *Journal of Neuromuscular Diseases*, *8*(2), 209–216. https://doi.org/10.3233/JND-200593

35. Mendell, J. R., Al-Zaidy, S. A., Lehman, K. J., McColly, M., Lowes, L. P., Alfano, L. N., Reash, N. F., Iammarino, M. A., Church, K. R., Kleyn, A., Meriggioli, M. N., & Shell, R. (2021). Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Abeparvovec in Spinal Muscular Atrophy. *JAMA Neurology*, *78*(7), 834–841. https://doi.org/10.1001/jamaneurol.2021.1272

36. Aoki, Y., & Wood, M. J. A. (2021). Emerging Oligonucleotide Therapeutics for Rare Neuromuscular Diseases. *Journal of Neuromuscular Diseases*, *8*(6), 869–884. https://doi.org/10.3233/JND-200560

37. McDonald, C. M., Shieh, P. B., Abdel-Hamid, H. Z., Connolly, A. M., Ciafaloni, E., Wagner, K. R., Goemans, N., Mercuri, E., Khan, N., Koenig, E., Malhotra, J., Zhang, W., Han, B., & Mendell, J. R. (2021). Open-Label Evaluation of Eteplirsen in Patients with Duchenne Muscular Dystrophy Amenable to Exon 51 Skipping: PROMOVI Trial. *Journal of Neuromuscular Diseases*, *8*(6), 989–1001. https://doi.org/10.3233/JND-210643

38. FDA. (2016). *Eteplirsen—Prescribing information*. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206488lbl.pdf

39. Frank, D. E., Schnell, F. J., Akana, C., El-Husayni, S. H., Desjardins, C. A., Morgan, J., Charleston, J. S., Sardone, V., Domingos, J., Dickson, G., Straub, V., Guglieri, M., Mercuri, E., Servais, L., Muntoni, F., & SKIP-NMD Study Group. (2020). Increased dystrophin production with golodirsen in patients with Duchenne muscular dystrophy. *Neurology*, *94*(21), e2270–e2282. https://doi.org/10.1212/WNL.0000000000009233

40. FDA. (2019, December 12). *FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation*. https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation

41. FDA. (2020, August 12). *FDA Approves Targeted Treatment for Rare Duchenne Muscular Dystrophy Mutation*. https://www.fda.gov/news-events/press-announcements/fda-approves-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation

42. FDA, U. (2021). *Amondys 45 (casimersen) injection, for intravenous use: US prescribing information.* https://www.accessdata. fda.gov/drugsatfda_docs/label/2021/213026lbl.pdf.

43. Clark, & Yokota. (2021). *Casimersen for Duchenne muscular dystrophy*. https://doi.org/10.1358/dot.2021.57.12.3352740

44. FDA. (2021, February 25). *FDA Approves Targeted Treatment for Rare Duchenne Muscular Dystrophy Mutation*. https://www.fda.gov/news-events/press-announcements/fda-approves-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation-0

45. Mercuri, E., Darras, B. T., Chiriboga, C. A., Day, J. W., Campbell, C., Connolly, A. M., Iannaccone, S. T., Kirschner, J., Kuntz, N. L., Saito, K., Shieh, P. B., Tulinius, M., Mazzone, E. S., Montes, J., Bishop, K. M., Yang, Q., Foster, R., Gheuens, S., Bennett, C. F., … Finkel, R. S. (2018). Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. *New England Journal of Medicine*, *378*(7), 625–635. https://doi.org/10.1056/NEJMoa1710504

46. Mercuri, E., Baranello, G., Kirschner, J., Servais, L., Goemans, N., Pera, M. C., Buchbjerg, J., Yeung, W. Y., Kletzl, H., Gerber, M., Czech, C., Cleary, Y., Gorni, K., & Khwaja, O. (2019). Update from SUNFISH Part 1: Safety, Tolerability and PK/PD from the Dose-Finding Study, Including Exploratory Efficacy Data in Patients with Type 2 or 3 Spinal Muscular Atrophy (SMA) Treated with Risdiplam (RG7916) (S25.007). *Neurology*, *92*(15 Supplement). https://n.neurology.org/content/92/15_Supplement/S25.007

47. Mercuri, E., Deconinck, N., Mazzone, E. S., Nascimento, A., Oskoui, M., Saito, K., Vuillerot, C., Baranello, G., Boespflug-Tanguy, O., Goemans, N., Kirschner, J., Kostera-Pruszczyk, A., Servais, L., Gerber, M., Gorni, K., Khwaja, O., Kletzl, H., Scalco, R. S., Staunton, H., … SUNFISH Study Group. (2022). Safety and efficacy of once-daily risdiplam in type 2 and non-ambulant type 3 spinal muscular atrophy (SUNFISH part 2): A phase 3, double-blind,

randomised, placebo-controlled trial. *The Lancet. Neurology*, *21*(1), 42–52. https://doi.org/10.1016/S1474-4422(21)00367-7

48. Day, J. W., Finkel, R. S., Chiriboga, C. A., Connolly, A. M., Crawford, T. O., Darras, B. T., Iannaccone, S. T., Kuntz, N. L., Peña, L. D. M., Shieh, P. B., Smith, E. C., Kwon, J. M., Zaidman, C. M., Schultz, M., Feltner, D. E., Tauscher-Wisniewski, S., Ouyang, H., Chand, D. H., Sproule, D. M., … Mendell, J. R. (2021). Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): An open-label, single-arm, multicentre, phase 3 trial. *The Lancet Neurology*, *20*(4), 284–293. https://doi.org/10.1016/S1474-4422(21)00001-6
49. Finkel, R. S., Flanigan, K. M., Wong, B., Bönnemann, C., Sampson, J., Sweeney, H. L., Reha, A., Northcutt, V. J., Elfring, G., Barth, J., & Peltz, S. W. (2013). Phase 2a Study of Ataluren-Mediated Dystrophin Production in Patients with Nonsense Mutation Duchenne Muscular Dystrophy. *PLoS ONE*, *8*(12), e81302. https://doi.org/10.1371/journal.pone.0081302
50. Michorowska, S. (2021). Ataluren—Promising Therapeutic Premature Termination Codon Readthrough Frontrunner. *Pharmaceuticals*, *14*(8), 785. https://doi.org/10.3390/ph14080785
51. Nuijten, M. (2022). Pricing Zolgensma – the world's most expensive drug. *Journal of Market Access & Health Policy*, *10*(1), 2022353. https://doi.org/10.1080/20016689.2021.2022353
52. Messina, S., & Sframeli, M. (2020). New Treatments in Spinal Muscular Atrophy: Positive Results and New Challenges. *Journal of Clinical Medicine*, *9*(7), E2222. https://doi.org/10.3390/jcm9072222
53. Liu, A. (2021, December 3). *Biogen's Spinraza, Fosun Kite's Yescarta and a controversial Alzheimer's drug: All you need to know about China's new state coverage*. Biogen's Spinraza, Fosun Kite's Yescarta and a controversial Alzheimer's drug: All you need to know about China's new state coverage
54. Barkats, M. (2020). [SMA: From gene discovery to gene therapy]. *Medecine Sciences: M/S*, *36*(2), 137–140. https://doi.org/10.1051/medsci/2020010

55. Ray, M. (2021, August 3). *Toddler with rare genetic disorder gets ₹16 cr injection free from US firm*. https://www.hindustantimes.com/india-news/toddler-with-rare-genetic-disorder-gets-rs-16-cr-injection-free-from-us-firm-101627951512872.html

56. Aartsma-Rus, A., & Corey, D. R. (2020). The 10th Oligonucleotide Therapy Approved: Golodirsen for Duchenne Muscular Dystrophy. *Nucleic Acid Therapeutics*, *30*(2), 67–70. https://doi.org/10.1089/nat.2020.0845

57. Roshmi, R. R., & Yokota, T. (2021). Pharmacological Profile of Viltolarsen for the Treatment of Duchenne Muscular Dystrophy: A Japanese Experience. *Clinical Pharmacology: Advances and Applications*, *Volume 13*, 235–242. https://doi.org/10.2147/CPAA.S288842

58. Conduent Business Services. (2021). Amondys New Drug Fact Blast. *Missouri Department of Social Services*. https://dss.mo.gov/mhd/cs/advisory/rdac/pdf/amondys-45-casimersen-ndfb_mo.pdf

59. Andrews, J. A., Miller, T. M., Vijayakumar, V., Stoltz, R., James, J. K., Meng, L., Wolff, A. A., & Malik, F. I. (2018). CK-2127107 amplifies skeletal muscle response to nerve activation in humans. *Muscle & Nerve*, *57*(5), 729–734. https://doi.org/10.1002/mus.26017

60. Collibee, S. E., Bergnes, G., Chuang, C., Ashcraft, L., Gardina, J., Garard, M., Jamison, C. R., Lu, K., Lu, P.-P., Muci, A., Romero, A., Valkevich, E., Wang, W., Warrington, J., Yao, B., Durham, N., Hartman, J., Marquez, A., Hinken, A., … Morgan, B. P. (2021). Discovery of Reldesemtiv, a Fast Skeletal Muscle Troponin Activator for the Treatment of Impaired Muscle Function. *Journal of Medicinal Chemistry*, *64*(20), 14930–14941. https://doi.org/10.1021/acs.jmedchem.1c01067

61. Long, K. K., O'Shea, K. M., Khairallah, R. J., Howell, K., Paushkin, S., Chen, K. S., Cote, S. M., Webster, M. T., Stains, J. P., Treece, E., Buckler, A., & Donovan, A. (2019). Specific inhibition of myostatin activation is beneficial in mouse models of SMA therapy. *Human Molecular Genetics*, *28*(7), 1076–1089. https://doi.org/10.1093/hmg/ddy382

62. Chaytow, H., Faller, K. M. E., Huang, Y.-T., & Gillingwater, T. H. (2021). Spinal muscular atrophy: From approved therapies to future therapeutic targets for personalized medicine. *Cell Reports. Medicine*, *2*(7), 100346. https://doi.org/10.1016/j.xcrm.2021.100346

63. Schellino, R., Boido, M., Borsello, T., & Vercelli, A. (2018). Pharmacological c-Jun NH2-Terminal Kinase (JNK) Pathway Inhibition Reduces Severity of Spinal Muscular Atrophy Disease in Mice. *Frontiers in Molecular Neuroscience*, *11*. https://www.frontiersin.org/article/10.3389/fnmol.2018.00308

64. Bowerman, M., Beauvais, A., Anderson, C. L., & Kothary, R. (2010). Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. *Human Molecular Genetics*, *19*(8), 1468–1478. https://doi.org/10.1093/hmg/ddq021

65. Bowerman, M., Murray, L. M., Boyer, J. G., Anderson, C. L., & Kothary, R. (2012). Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy. *BMC Medicine*, *10*, 24. https://doi.org/10.1186/1741-7015-10-24

66. Sumner, C. J. (2006). Therapeutics development for spinal muscular atrophy. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics*, *3*(2), 235–245. https://doi.org/10.1016/j.nurx.2006.01.010

67. Parente, V., & Corti, S. (2018). Advances in spinal muscular atrophy therapeutics. *Therapeutic Advances in Neurological Disorders*, *11*, 1756285618754501. https://doi.org/10.1177/1756285618754501

68. Ma, Y., Zhang, L., & Huang, X. (2014). Genome modification by CRISPR/Cas9. *The FEBS Journal*, *281*(23), 5186–5193. https://doi.org/10.1111/febs.13110

69. Lim, K. R. Q., Yoon, C., & Yokota, T. (2018). Applications of CRISPR/Cas9 for the Treatment of Duchenne Muscular Dystrophy. *Journal of Personalized Medicine*, *8*(4), E38. https://doi.org/10.3390/jpm8040038

70. Himič, V., & Davies, K. E. (2021). Evaluating the potential of novel genetic approaches for the treatment of Duchenne muscular dystrophy. *European Journal of Human Genetics: EJHG*, *29*(9), 1369–1376. https://doi.org/10.1038/s41431-021-00811-2

71. Mollanoori, H., Rahmati, Y., Hassani, B., Havasi Mehr, M., & Teimourian, S. (2021). Promising therapeutic approaches using CRISPR/Cas9 genome editing technology in the treatment of

Duchenne muscular dystrophy. *Genes & Diseases*, *8*(2), 146–156.

https://doi.org/10.1016/j.gendis.2019.12.007