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Gene Therapies for Spinal Muscular Atrophy and Duchenne Muscular Dystrophy: A Pathbreaking Moment in Therapeutics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background: Gene therapy has proved to be a boon for neuromuscular diseases. A concept introduced in the 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. This review is centered on the different gene therapies

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currently developed for spinal muscular atrophy and Duchenne muscular dystrophy and also sheds light on the emerging therapies.

Methods: Literature search was done in Medline, Embase, and Google Scholar. The relevant and important articles were included for developing the narrative review.

Results and Discussion: The nusinersen, risdiplam, zolgensma, eteplirsen, golodirsen, viltolarsen, casimersen and ataluren are developed as gene therapies for Spinal muscular atrophy and Duchenne muscular dystrophy. The therapies approved under orphan drug designation have shown muscle strength improvement in clinical trials. However, long-term data on safety and efficacy is to be determined. The cost of the therapies acts as an impediment in most cases.

Conclusion: The development of gene therapies for inherited genetic disorders would be a prelude for several other genetic diseases. The studies in this arena would provide a prototype for translational research from bench to bedside. Further efforts are mandated towards long term safety, efficacy, and affordability of therapies.

Keywords: Gene therapy; spinal muscular atrophy; muscular dystrophies; SMN-1 gene; gene splicing; viral vectors; dystrophin gene; Adeno-Associated Virus (AAV)-9 vectors; antisense oligonucleotides (ASOs); exon skipping; CRISPR-Cas9.

1. 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 the 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 the delivery of coding DNA from healthy cells was performed by Wacław and Elisabeth Szy-Balski. In 1989, retroviruses were introduced as vectors in 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 and 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 virus disease.

2. 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 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 in the development of lower motor neurons in the spinal cord and brain. Lack of SMN protein results in the 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 greater than 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 severity of the SMA [7].

The X-linked recessive neurodegenerative illness, Duchenne muscular dystrophy (DMD) causes gradual muscle weakening due to muscle cell degeneration. 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]. In early years of life, there is wasting with weakening of the muscles. During teen years, the ambulation is lost. If patients are not aided by intervention measures such as non-invasive ventilation, the condition results in death in late teen years due to respiratory insufficiency or cardiac complications. 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 and Becker's muscular dystrophy (BMD). In DMD, there is a 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 (ECM). 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 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) and 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 polymerase chain reaction (PCR) and MLPA are used. These methods should also aid in the quantification of the number of SMN-1/SMN-2 copies [11].

Apart from genetic testing in parental carriers, newborns must also be screened for neuromuscular disorders because sooner the diagnosis, better will be the 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].

A 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.

3. METHODS

We searched the databases such as Medline, Embase, and Google Scholar for all relevant articles. The search terms used were 'gene therapy', 'spinal muscular atrophy', 'Duchenne muscular dystrophy', 'nusinersen', 'risdiplam', 'zolgensma', 'eteplirsen', 'golodirsen', 'viltolarsen', 'casimersen' and 'ataluren'. Some articles were also obtained from references of selected articles. The summarized narrative review was done from the important articles.

4. RESULTS AND DISCUSSION

The therapeutics for SMA and DMD has come from supportive care to gene therapies. The process of therapeutic development has been a synergy of *in-vitro* studies and clinical trials.

Splicing of SMN2 pre-mRNA, *SMN1* gene substitution, nonsense suppression to restore dystrophin, exon skipping for restoration of dystrophin, AAV mediated micro-dystrophin transfer are the prime mechanistic approaches for available gene therapies [14,15]. Currently, 'nusinersen', 'risdiplam', 'zolgensma', 'eteplirsen', 'golodirsen', 'viltolarsen', 'casimersen' and 'ataluren' are approved under orphan drug designation. The important clinical trials on safety and efficacy are summarized in Table 1. The present article should be viewed as a narrative review, which has its limitations of being a summarized overview of therapies in SMA and DMD.

5. MECHANISTIC APPROACHES FOR GENE THERAPIES IN SMA

5.1 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 [15]. 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 [16]. *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 [17]. So, gene therapy should involve blocking the mutation to promote the inclusion of exon 7 [16,17]. 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) [18]. The process of alternate splicing depends on the regulation and interaction between many *cis-elements* and *trans-factors* [15,16].

5.1.1 Antisense oligonucleotides

The discovery of antisense oligonucleotides (ASOs) boasts a huge success in 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 [19]. These molecules act and disable the intronic splicing silencer N1 (ISS-N1) which is present in the 7th position of the intron of *SMN2* [15,20]. These ASOs are adept at exon 7 inclusion in SMN2 transcripts and increase levels of SMN protein both *in vitro* and *in vivo*. Thus, ASOs inhibit the ISS-N1 in intron 7 for exon 7 inclusion [20].

5.1.2 Small molecules

This approach was designed for the promotion and upregulation of *SMN* gene expression [15,17]. Risdiplam (Evrysdi™) is the first oral drug available for treatment, which was approved by FDA in 2020 [21]. Like the ASOs, these small molecules are responsible for *SMN2* gene splicing modification which leads to the restoration of functional protein synthesis [17]. These small molecules are easily penetrable and cause less systemic complications [21,22].

5.2 SMN1 Gene Substitution

This approach of the treatment focus on replacing the *SMN1* gene completely to produce functional and highly stable SMN protein in motor neurons [23]. This therapy uses viral vectors to convert the mutated or unstable gene into a stable gene [24]. Viral vectors are researched extensively and are one of the safest and most effective carriers [24–26].

5.2.1 Adeno-Associated Vectors (AAV)

AAVs are small viruses (25 nm) belonging to the Parvoviridae family, non-enveloped and are modified for carrying the genetic material [24,25]. 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 a *cap* for the structural proteins of capsid [24]. 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 the packaging and replication of virus [24]. These vectors have an absence of the viral DNA so it behaves as a nanoparticle for easy transfer and the delivery of DNA to the nucleus [25]. In general, recombinant AAV (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 [25]. Based on the serotypes of AAV which are from 1 to 13, AAV9 has the highest capability for reaching the CNS [24]. Recombinant AAV9 serotype vector based onasemnogene abeparvovec (zolgensma) was approved by FDA in May 2019 in the treatment of SMA [26].

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 to the double-stranded genome. This saves time and causes gene expression at a faster rate but the packaging capacity of the vector is diminished [25].

6. MECHANISTIC APPROACHES FOR GENE THERAPIES IN DMD

6.1 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 [14,27].

6.2 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 ASO to prohibit a certain exon from participating in splicing by inhibiting a splice enhancer site. The deletion mutations change the reading frame and produce 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 in the synthesis of a steady, functioning protein because this truncated coding region would not be containing a disturbing reading frame [14]. 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 patients [28].

6.3 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 AAV is 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 the systemic delivery through the vasculature requires a critical threshold of vector particle concentration. While the exact dose required in people varies, it is based on the AAV serotype utilized as a reservoir of capsid protein, the route of delivery, the robustness of the gene regulatory cassette (RCs) used to promote dystrophin 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 RCs created from the muscle creatine kinase (MCK) 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].

7. CURRENTLY APPROVED GENE THERAPIES

7.1 Nusinersen

Nusinersen (Spinraza) is an ASO that promotes the inclusion of exon 7 by inhibiting the intronic splicing silencer N1 (ISS-N1) in SMN2 intron 7 [16]. ISS-N1 silencing is one of the most reliable mechanisms for promoting exon 7 inclusion [19]. When Nusinersen crosses the ISS-N1, it obstructs heterogenous nuclear ribonucleoproteins (hnRNP) recruitment, which results in exon 7 production in mRNA and amplifies the formation of SMN2 protein [18]. (Fig. 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 central nervous system (CNS) to not experience the 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 [18].

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 the improvement in motor function. The selection criteria for participation in CHERISH or ENDEAR trials are 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 [22]. The effects of pharmacokinetic and pharmacodynamic properties of oligonucleotides have not yet been extensively studied. In the ENDEAR trial, 53% of participants in the nusinersen group had a risk of death or permanent ventilation (hazard ratio=0.53) [32]. The serious and common adverse reactions are headache, vomiting, back pain, epistaxis, pyrexia, cough, pneumonia, influenza, respiratory tract infections and distress [31,32].

7.2 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]. (Fig. 1) Risdiplam is a small molecule given orally immediately after meals at the exact time every day [22]. The oral route is an important advantage because SMA is a multisystem disease and risdiplam can reach other systemic tissues as well [22]. 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 in the neurons [21,22]. After 4 weeks of risdiplam use, it has been reported a 2-fold increase in the threshold levels of SMN protein and these findings were fixed for a year minimally. (Table 1) In preclinical studies, risdiplam showed an increase in the number of motor neurons and a 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 make risdiplam a suitable therapeutic agent [21,22].

The common adverse events of risdiplam of infantile onset are respiratory tract infections, pneumonia, mouth ulcers, constipation, vomiting, diarrhea, urinary tract infections, fever, rash and joint pain [21,22]. This drug is contraindicated in patients with liver abnormalities or diseases and also in pregnant women due to the 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 [22].

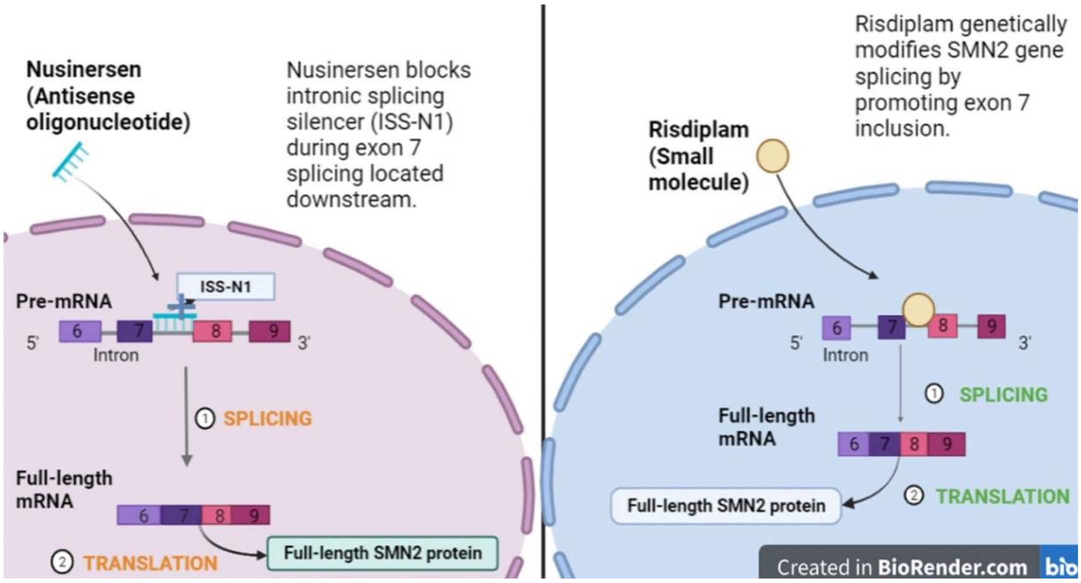

Fig. 1. Contrasting mechanisms of nusinersen and risdiplam in spinal muscular atrophy: Nusinersen employs exon 7 inclusion by inhibiting intronic splicing silencer N1 (ISS N1) whereas risdiplam alters splicing of gene in intron 7 to increase exon 7 inclusion. Ultimately, both nusinersen and Risdiplam increase levels of full-length SMN-2 protein

7.3 Zolgensma (Onasemnogene Abeparvovec)

Zolgensma is a gene therapy which contains non-replicated rAAV9 viral vectors designed to deliver cDNA *SMN1* gene to motor neurons and used to treat SMA under the age of 2. (Fig. 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 the blood-brain-barrier and neurons. The efficacy of this drug was also documented with a single-dose, the systemic administration i.e., intravenously or intrathecally. Further, the systemic route targets the motor neurons directly and widespread CNS biodistribution [22,25,26]. When given intravenously, it targets all regions of the central nervous system's neurons including the alpha motor neurons. The cDNA does not add itself to the host-cell genome in neurons, instead, it forms distinct episomes [22]. 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 [22].

The systemic administration also carries potential adverse reactions involving hepatic, cardiac and immune system. Corticosteroids are given to reduce 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 [26]. Prednisolone was used in the START trial, after the first patient reported an elevation of liver enzymes 16 times more than normal [35] (Table 1). In the STR1VE trial, all 22 patients experienced pyrexia and most reported adverse effects are bronchiolitis respiratory syncytial virus bronchiolitis, pneumonia and respiratory distress. Also, there were 3 patients which experienced drug-related adverse events out of which two had high liver enzymes (aminotransferases) and one with hydrocephalus [26].

7.4 Gene Therapy for DMD

The treatment with oligonucleotides have 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 the treatment in DMD patients [36] (Fig, 3 and Fig. 4).

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 the 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 exons skipping utilizing PMOs aiming both exons 51 as well as exon 53 might potentially re-establish the mRNA reading frame, allowing for the 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 a stage 1/2 clinical experiment. A substantial boost in exon 53 skipping led to the expression of dystrophin in 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 of twenty-four weeks, and fourteen boys achieved dystrophin amounts to greater than 5.3-5.4% of a 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 a double-blind, a 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 considerably a higher rise in dystrophin protein amounts from the baseline to week 48 of therapy than the participants who received a 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].

7.5 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 an 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 of 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].

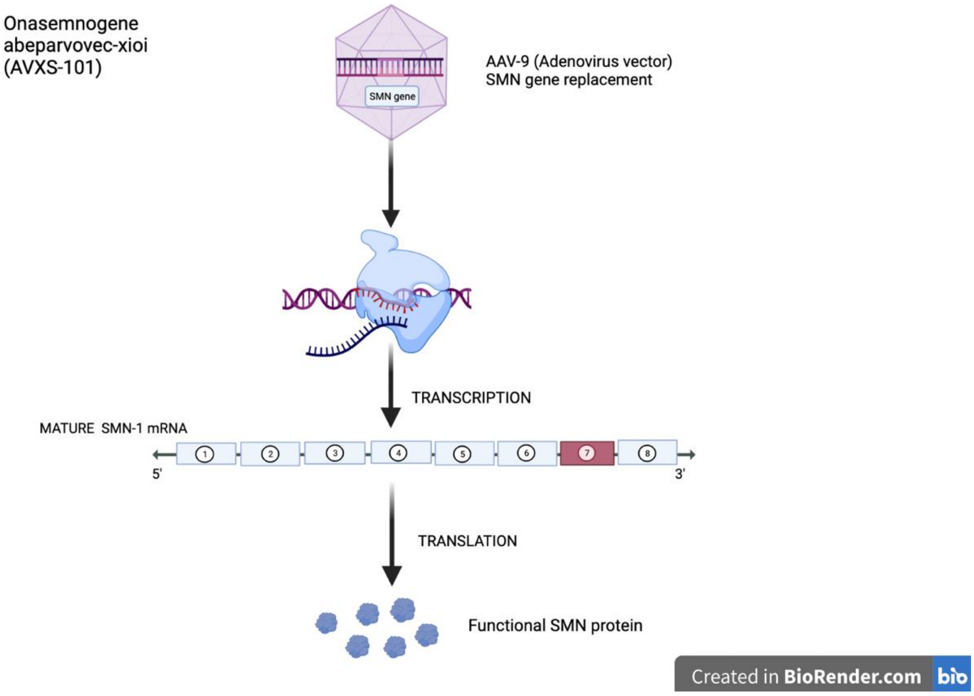

Fig. 2. Mechanism of onasemnogene abeparvovec; it carries AAV-9 viral vector containing *SMN-1* gene, to be delivered to motor neurons. The gene integrates with the normal human genome, undergoes transcription and translation to produce more of the functional SMN-1 protein

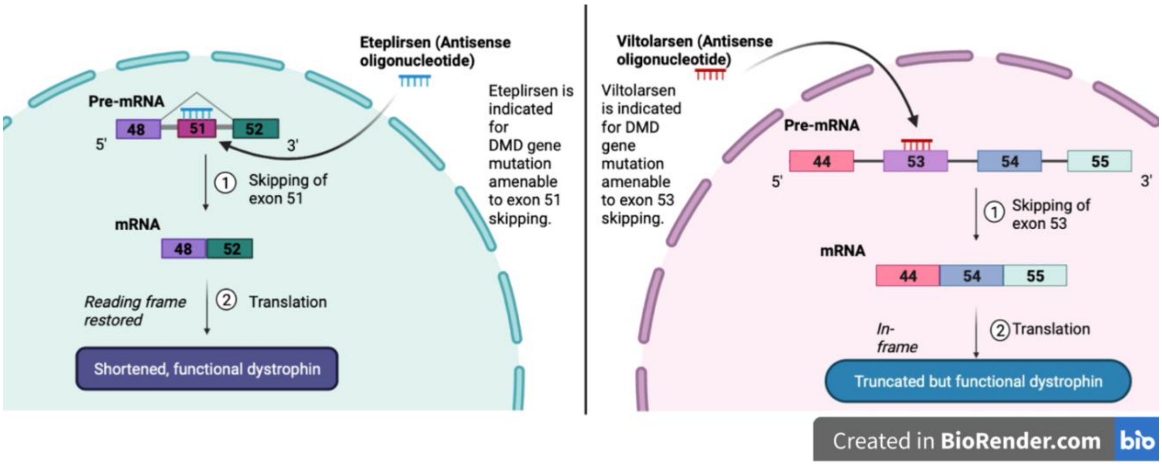

Fig. 3. Mechanisms of Eteplirsen and Viltolarsen in Duchenne Muscular Dystrophy. Eteplirsen binds to exon 51, resulting in its exclusion, producing shortened, however functional dystrophin protein. Viltolarsen targets exon 53 skipping, resulting in truncated but functional, dystrophin protein

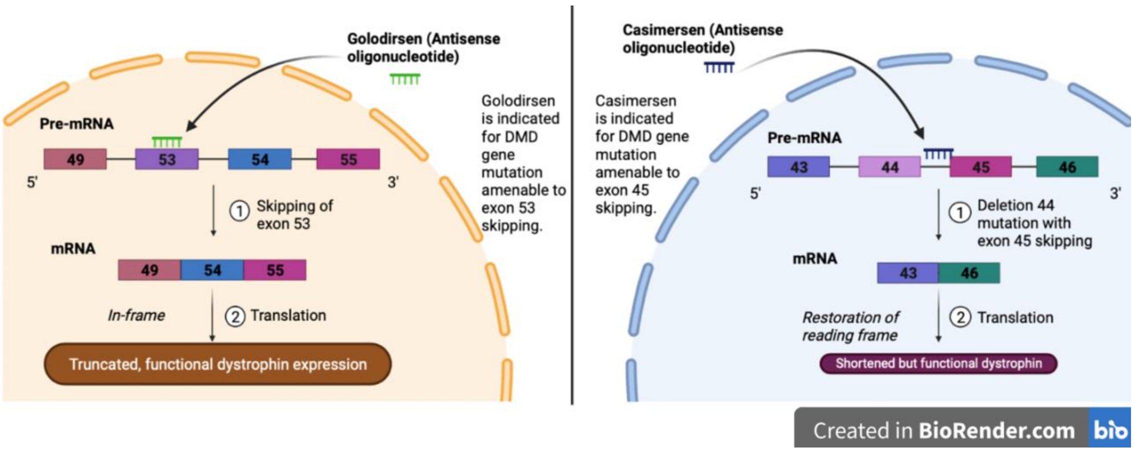

Fig. 4. Mechanism of Golodirsen and Casimersen in Duchenne Muscular Dystrophy. Golodirsen binds and excludes exon 53, resulting in shortened but functional dystrophin protein. Deletion mutation 44 with casimersen-mediated exon 45 exclusion, resulting in shortened but functional dystrophin protein

8. COST AND ACCESSIBILITY

Gene therapy has instilled a renewed sense of hope in patients diagnosed with SMA and DMD. The approved agents are under orphan drugs designation and affordability is an important impediment to their usage [51]. Healthcare budgets are becoming unable to support the costs of these expensive therapies as more orphan medications are being produced. However, in 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].

Nusinersen had regulatory approval pending so the pharmaceutical company made nusinersen accessible for the 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 the treatment of SMA based on the enormous cost and limited therapeutic benefits. China has accepted and is giving insurance coverage starting in 2022 [53].

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	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 the lower back (n=37)	Improvement in motor function. Permanent ventilation or estimated deaths	2-27 months	51% achieved a higher motor milestone response in the nusinersen group. 47% have a lower occurrence of death or assisted ventilation in the nusinersen group.	[32]
CHERISH	3	Multicenter randomized, a double-blind, sham-procedure	SMA diagnosis and symptoms ≥ 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	The difference from the baseline in HFMSE: least squares mean 3.9 (3.0 to 4.9) vs -1.0 (-2.5 to 0.5) 57% of 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	A randomized, a double-blind, sham-procedure	5q SMN 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 (Part 1)	2	Multicenter, a double-blind, a 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 an increase (≥ 1 score) in the Motor Function Measure 32 (MFM32) High SMN protein level demonstrated	[46]

Study	**Phase**	**Study design**	**Subjects**	**Study Arms**		**Primary Outcomes**	**Treatment**	**Results**	**References**
SUNFISH (Part 2)	3	A randomized, a double-blind, a placebo-controlled study	SMA-I and III of ages 2-25 years	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 the difference from the 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, the open-label, single-arm, single-dose, trial	SMA-I with 1 or 2 copies of *SMN2* gene < 6 months of age	One-time Onasemnogene Abeparvovec-xioi via intravenously (n=22)		Erect sitting for 30s or longer at age of 18 months Survival in the 14th month	Until the age of 18 months or early termination	13 patients accomplished erect sitting for the 30s or longer. 20 patients did not require permanent ventilation.	[48]
START	1/2a	The open-label study, single arm-infusion, single-center trial	SMA-I 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 is required among cohort 2	[35]
Finkel et al., 2013	2a	The open-label, sequential dose-ranging trial	Male DMD patients with the 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 was taken off the muscle extensor digitorum brevis (EDB) on day 28 to assess dystrophin levels.	Till the 28th day after the treatment	Increased dystrophin levels in 61% of patients in quantitative immunofluorescence assessing the 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	To assess the 6-minute walk test (6 MWT) distance.	Eteplirsen's effectiveness and safety were monitored over a period of 96 weeks.	The treated group showed a higher exon skipping and dystrophin protein as compared with the baseline	[37]

Study	Phase	Study design	Subjects	Study Arms		Primary Outcomes	Treatment	Results	References
					skipping treatment) (n=30)			Decrease in mean 6MWT from 382.6m at the baseline to 252.2 at the 96th week in the untreated group. Decrease in mean 6MWT from 374.6m at the baseline to 256.2m at the 96th week in the eteplirsen treated group. 96 weeks may not be sufficient to show the difference in 6MWT	
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 the 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 the commencement of the initial dose and 28 days following the final dose they were regarded as the treatment emergent.	Increased exon 53 skipping with approx. ~16-fold dystrophin expression above the baseline at the 48th week	[39]

IT intrathecal; IV intravenous; SAE serious adverse event

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 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 crores INR through a lottery system free of cost from a US company [55]. There are conflicts regarding the market value of zolgensma where the total outflow of money is more 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 orphan drugs [51].

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 the case of a 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].

9. ADJUVANT THERAPEUTIC STRATEGIES UNDER DEVELOPMENT

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 focus on treating SMA as a multi-system pathology and also covering patients of all ends of the SMA spectrum. Also, insurance companies tend to restrict the purchase of such expensive drugs to such patients provoking second thoughts on treating pre-symptomatic SMA infants with a higher number of SMN-2 copies though they still develop a motor imbalance.

9.1 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 a 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 this drug showed the 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].

The myostatin, a member of the transforming growth factor-beta (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 the myostatin pathway provides promising results in the improvement of muscle mass and function [5]. The common methods for myostatin inhibition developed are: 1- Direct antibodies(Ab's) 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 inhibit the myostatin activation [6]. This drug is currently being evaluated in phase-2/3 trials after positive outcomes in an initial trial which showed the improvement in motor functioning [62].

9.2 Therapies Targeting Sensory Neural Defects

Chronic pain is reported in most of adolescent SMA [63]. Further, mouse models of SMA have demonstrated pain hypersensitivity, due to hyperexcitability of dorsal root ganglia neurons [64]. However, basic pathology is still elusive between neuropathy and inflammation. Nevertheless, norepinephrine levels are shown to be increased in mouse models of SMA [64]. It remains to be evaluated the role of antioxidants, and antiapoptotic agents such as co-enzyme Q, alpha tocopherol, genistein, and alpha lipoic acid which have shown neuroprotection [65–67].

9.3 Autophagy and Apoptosis

The concentration of autophagosomes elevated in the 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), an inhibitor of enzymes regulating apoptotic pathways, showed improved motor function and an increase in motor neuron lifespan in SMA pups.[6]

Apoptosis has also been 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, pharmacological inhibition of these pathways can prevent the death of motor neurons and be a future target of SMA-independent approaches [68].

9.4 RhoA/Rho Kinase Inhibitors

RhoA/Rho Kinase (ROCK) signaling pathway is a regulator of cell integrity and actin cytoskeletal dynamics. Dysregulation of the ROCK pathway has been associated with SMA pathogenesis. Rho Kinase is a kinase enzyme phosphorylated by Rho-A, a GTPase protein that results in the activation and phosphorylation of p-LIMK. LIM Kinases (LIMK) suppresses the maturation and development of neuromuscular junctions (NMJ) [69]. Hence, the RhoA-Rho kinase pathway's inhibition, blocks the activation of LIMK and therefore, allows for NMJ maturation and increased lifespan of the SMA mice. Pharmacological inhibition of ROCK pathway by Y-27632 and fasudil, on SMA mouse models improved motor function, increase muscle fiber mass without compromising on healthy motor neurons or SMN expression [70].

9.5 Stem Cell Therapy

Similarly, in SMA patients, neural stem cells are transplanted directly into the cerebrospinal fluid (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 in under the experimental stage [71,72].

9.6 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 [73]. 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 the viral genome which neutralizes the virus and hence, preventing further infection to the host cell [74]. The advent of the CRISPR/Cas-9 system has provided hopes for the restoration of dystrophin protein mainly by exon skipping at the genomic DNA level [75]. 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 partially functional and stable dystrophin protein. In classical exon skipping, a single guide RNA and SpCas9 protein were designed to target the splice acceptor sites for DMD exons [74]. Other ways by which dystrophin expression can be restored includes reframing of out of frame exons and removal of duplicated exons [76].

10. 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. Nevertheless, long term data is yet to be determined. 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. Continued efforts are required to improve long term safety, efficacy and affordability.

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

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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