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Spinal muscular atrophy and current new generation treatment

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ABSTRACT

Spinal muscular atrophy (SMA) is an uncommon neuromuscular disorder characterized by the gradual degeneration and weakening of muscles. The condition often manifests in the pediatric and teenage populations, however, it may also impact adults. It is characterized by the manifestation of muscle weakness and muscle wasting, leading to a range of consequences such as diminished strength, respiratory impairment, and potentially fatal outcomes. In recent times, there has been a notable emergence of novel therapeutic interventions aimed at addressing this particular ailment. Spinal muscular atrophy is caused by low levels of survival motor neuron (SMN) protein resulting from SMN1 gene mutations or deletions. Until 2016, no treatment for SMA was available. Treatment prospects were added with the recent approval of two groundbreaking SMN-targeted therapies using antisense oligonucleotides or virus-mediated gene therapy. These two new therapeutics have a common goal: To increase the production of SMN protein in motor neurons and improve survival by revitalizing motor function. The present review endeavors to go into an in-analysis of various therapy alternatives.

Keywords: Antisense oligonucleotides, gene therapy, ribonucleic acid, spinal muscular atrophy, viral vector.

Motor neuron diseases are neurological conditions marked by skeletal muscle atrophy, degeneration of spinal motor neurons, and often lethal motor dysfunction.[1] The aforementioned condition is an uncommon hereditary ailment that has the potential to present itself throughout the early stages of life, adolescence, or maturity.[2] The existing therapeutic interventions for spinal muscular atrophy (SMA) include disease-modifying modalities, such as gene replacement therapy and small-molecule enhancers.[3] The use of gene therapy, particularly in the context of onasemnogene abeparvovec, has shown encouraging outcomes in enhancing motor milestones and reducing the likelihood of mortality or the need for long-term assisted breathing.[4]

Furthermore, the potential therapeutic applications of thyrotropin-releasing hormone analogs and phenylbutyrate in the treatment of SMA have been examined in clinical studies.[5] The establishment of standardized protocols for the provision of medical treatment to individuals with SMA is of utmost importance in order to facilitate comprehensive, interdisciplinary care and enhance patient outcomes.[6]

In general, the progress made in comprehending the molecular pathophysiology of SMA and the subsequent emergence of novel therapeutic interventions has instilled optimism among those afflicted by this incapacitating disease. Many clinical trials for the treatment of SMA are still ongoing and offer hope, and this review describes the current pipeline of next-generation therapies and trials.

FEATURES AND CLASSIFICATION OF SPINAL MUSCULAR ATROPHY DISEASE

Spinal muscular atrophy is an inherited disease that causes the degeneration of cells in the spinal cord and the destruction of associated alpha motor cells.[7] It causes dysfunction and degeneration of alpha-motor neurons in the spinal cord and

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brainstem, as well as progressive muscle atrophy and weakness of the limbs, trunk, ampullary (controlling swallowing), and respiratory muscles. Caused by mutations in survival motor neuron 1 (SMN1) on chromosome 5 (5q13), it is responsible for 95% of SMA cases, with an average incidence of 1 in 6000 to 1 in 11000, varying according to ethnicity.[8] Spinal muscular atrophy is classified according to five main phenotypes depending on the age of onset of symptoms and the highest motor function achieved.[9]

Type 0- Presents in the neonatal period with early respiratory failure with hypotonia, severe weakness and progressively decreasing fetal movements typically associated with arthrogryposis. Death occurs at birth or in the first month of life; this is a rare phenotype.[10]

Type 1- Presents in the first six months of life with limited head control, hypotonia, and areflexia. Type 1, Weakness of the inter-rib muscles and preservation of diaphragm function leads to a paradoxical breathing pattern and a bell-shaped chest. Swallowing difficulties are typical of tongue fasciculations and associated complications such as failure to thrive and aspiration. Without respiratory support, most patients die before the age of two years.[11]

Type 2 (intermediate spinal muscular atrophy)- Occurs between six and 18 months of age; can sit but has hypotonia, areflexia, and progressive proximal weakness disproportionately affecting arms and legs. If not prevented, it causes progressive scoliosis, and intercostal muscle weakness, and lung disease. With good care and treatment, about 70 percent of patients survive to the age of 25. Respiratory distress is the main cause of death.[12]

Type 3 (mild spinal muscular atrophy)- It is characterized by progressive proximal weakness affecting the legs and arms. Patients are ambulatory but may need a wheelchair as the disease progresses. Lung disorders that may occur in patients are mild compared to other groups and may be almost invisible.[12,13]

Type 4 (also known as adult spinal muscular atrophy)- It occurs in adults after the age of 21. This type is the mildest phenotype for patients. They present with mild leg weakness. Generally, quality of life is not highly affected.[13]

THE SURVIVAL MOTOR NEURON GENE AND ITS STRUCTURE

In 1990, as a result of genetic scans; all three types of SMA were mapped to chromosome 5q13.2. Over time, as these scans improved, the numbers increased. The region contains a large 500 kilobase (kb) copy of the telomeric SMN1 gene (encoding SMN) and its paralogue centromeric SMN2. Both SMN genes consist of nine exons (including exons 1, 2a, 2b, 3, 4, 5, 6, 7, and 8 [encoding the 3' untranslated region (UTR)].[14]

The SMN1 pre-messenger ribonucleic acids (mRNAs) are usually spliced to maintain all exons. The SMN1 gene is the important gene for this disease. Mutation in SMN1 has a significant effect on the disease. SMN1 and SMN are identical in everything and they differ in a single point. The SMN2 differs from SMN1 by a single nucleotide variant (C→T) in exon 7. A single functional, coding variant c.840C>T in exon 7 of SMN2 inactivates an exonic splicing enhancer and simultaneously creates an exonic splicing silencer. This change is translationally silent but affects splicing so that exon 7 is excluded from most of it. The SMN2 transcripts result in truncated, degraded SMN protein. As exon 7 is sometimes conserved, each copy of SMN2 produces ~10% full-length, functional SMN.

Clinical manifestations of SMA, including impaired alpha-motor neuron development and degeneration: Loss of the SMN1 gene leads to SMA, the severity of which is partially modified by various copies of SMN2.[10,15]

CURRENT TREATMENTS AND STUDIES IN SPINAL MUSCULAR ATROPHY

Novel treatment strategies for SMA are using cutting-edge technical advancements that provide a more thorough comprehension of the fundamental genetic cause of the condition. Several treatment methods, including gene transfer, have been used to mitigate or ameliorate muscle weakness in persons afflicted with SMA.

In addition, current research endeavors have been undertaken to examine the effectiveness of gene transfer therapy as a prospective preventative intervention for SMA. There exists

a diverse array of therapy modalities that may be used for the effective management of SMA. The conventional therapeutic method entails the use of physical therapy and respiratory assistance as means to mitigate symptoms and impede the advancement of the ailment. An innovative treatment strategy entails the use of a pharmaceutical substance that incorporates a replica of the genetic sequence accountable for encoding the SMN1 protein. Extensive study has been undertaken to investigate novel approaches in the field of treatment methodologies.[16]

Antisense oligonucleotide therapies

The use of antisense oligonucleotide (ASO) gene therapy has considerable potential as a viable strategy for addressing neuromuscular problems. They are artificially synthesized, unpaired deoxyribonucleic acid (DNA) molecules that possess the ability to selectively bind to certain mRNA sequences, hence modulating the production of proteins,[17] as shown in Figure 1.

According to a study, the modulation of gene expression and mRNA splicing may occur inside the neurological system.[18] Antisense oligonucleotides are specifically engineered to interact with target RNA molecules by Watson-Crick base pairing, hence influencing their functionality.[19] Exon skipping is a method via which ASOs might exert their effects. This process involves the omission of certain exons during the RNA splicing process, leading to the synthesis of a shortened but biologically active protein.

Antisense oligonucleotides have shown effectiveness in both preclinical animal models and clinical studies conducted on individuals with neuromuscular illnesses, namely SMA.[20] In general, gene therapy targeting the ASO gene has significant potential in the treatment of neuromuscular illnesses and is now the subject of extensive investigation.

In general, the method by which antisense therapy operates entails the selective binding of ASOs to RNA molecules of interest. This binding event results in the suppression of translation, the initiation of RNA changes, the manipulation of splicing, and the silence of genes. The pathways might exhibit variability based on the particular target and cellular environment.

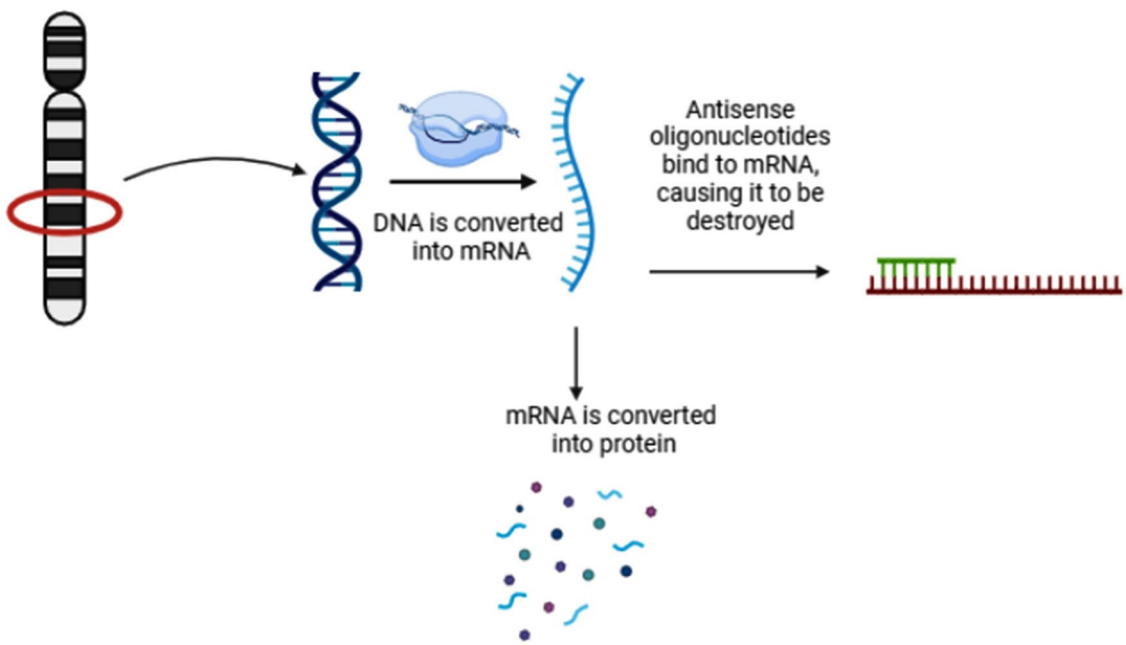

Figure 1. The mechanism of action of antisense treatment.
mRNA: Messenger ribonucleic acid; DNA: Deoxyribonucleic acid.
Figure 1 was created with BioRender (BioRender.com).

mRNA and siRNA treatment options

To make up for the loss of the SMN1 gene in SMA patients, mRNA treatments send modified mRNA molecules that may create useful proteins. The SMN protein, which is insufficient in SMA, is the target of these treatments. The mRNA treatments may increase motor neuron function and restore levels of the SMN protein by administering exogenous mRNA encoding the SMN protein.[21]

Contrarily, small interfering RNA (siRNA) treatments use tiny interfering RNA molecules to precisely target and degrade the SMN2 gene's mRNA, which results in a shortened and less useful version of the SMN protein. The siRNA treatments may boost the relative expression of the SMN1 gene and encourage the creation of functional SMN protein by decreasing the levels of the SMN2 mRNA.[21,22]

In preclinical and clinical investigations, mRNA and siRNA treatments have both shown encouraging outcomes for the treatment of SMA. By increasing SMN protein levels, these treatments have the potential to benefit SMA patients' motor function and quality of life.

MicroRNAs (miRNA) as a biomarker in spinal muscular atrophy

The use of miRNA therapies has shown promise in the therapy of SMA. Numerous studies have been conducted to examine the involvement of distinct miRNAs in the pathophysiology of SMA and their potential as therapeutic targets.

The possibility of miR-133a, miR-133b, miR-206, and miR-1 as biomarkers for monitoring the response to nusinersen therapy in juvenile patients with SMA has been the subject of investigation. The potential of these circulating miRNAs to serve as indicators of disease progression and therapy efficacy has been shown.[23]

In general, it can be said that miRNA-based therapeutic approaches possess considerable promise in the context of treating SMA due to their ability to selectively target and modulate particular molecular pathways implicated in the pathogenesis of the illness. Additional investigation is required in order to comprehensively comprehend the mechanics and therapeutic ramifications of miRNAs in SMA.

The inhibition of myostatin

The prospective therapy technique for SMA involves the exploration of inhibiting myostatin, which is a negative regulator of muscle development. Numerous research has been conducted to examine the impact of myostatin inhibition on mice models of SMA.

According to a study, a study has shown that the targeted suppression of myostatin activation by the use of a monoclonal antibody has shown to be an efficient method in enhancing muscle mass and improving functionality in mice models of SMA. The results of this study indicate that the suppression of myostatin might potentially be used as a therapeutic approach for patients with SMA, independent of the timing of therapy initiation with SMN upregulation.[24]

Nevertheless, previous research has shown inconclusive findings. The study conducted by another study revealed that the suppression of myostatin did not effectively alleviate the disease characteristics seen in mice with severe SMA.[25] In a different study, it was shown that the administration of myostatin inhibitors to mice with SMA resulted in only marginal enhancements in motor function and did not lead to an increase in overall survival.[26]

Despite the inconsistent findings, the inhibition of myostatin continues to be a subject of study in the context of SMA treatment. There exists a hypothesis suggesting that the suppression of myostatin may have the capacity to enhance muscle pathology and perhaps maintain proprioceptive connections onto motor neurons that are depleted in SMA.[27] Additional investigation is required in order to comprehensively comprehend the impacts of myostatin inhibition in SMA and its viability as a therapeutic focal point.

VIRAL VECTOR TYPES

Viral vector is a virus-mediated method used to deliver and transfer genetic information to the target cell. Viruses are widely used in gene therapy due to their evolutionary mechanisms such as replication and expression for efficient gene delivery.[28] The idea of viral vectors was first introduced in 1970 by Martine Cline,

who worked on retrovirus RNAs. When Cline analyzed the transformation mechanisms of viruses, he discovered that viruses transfer their genetic material to the host cell genome. By the early 1980s, she proved that genes could be transferred *in vitro* and *in vivo* with high efficiency. In 1982, Cline[29] was the first to perform human gene therapy for "Thalassaemia". In 1990, Blaese et al.[30] successfully treated two children with retrovirus vectors carrying the ADA gene in severe combined immunodeficiency disease.

Vectors can be divided into two delivery systems viral and non-viral. Viral vector systems have been developed for *in vitro*, *ex vivo*, and *in vivo* gene transfer. The most commonly used viral vectors are retrovirus, adenovirus, and adeno-associated virus (AAV).[31]

Other less commonly used viral vectors are derived from herpes simplex virus 1, vaccinia virus, or baculovirus. Non-viral vectors can be plasmid DNA, oligodeoxynucleotides or chemically synthesized compounds resembling them, a double-stranded DNA strand that replicates in bacteria.[32] Important points to be considered in determining the optimum vector and delivery system are: *(i)* the target cells and their characteristics, i.e. the ability to be virally transformed *ex vivo* and re-injected into the patient, *(ii)* the amount of expression required and *(iii)* the size of the genetic material to be transferred.[33]

For these reasons, they continue to be used as the most effective gene transfer tools today. DNA and RNA viruses are used in gene therapy and cancer diseases by modifying their genomes. Thus, viruses used as vectors have been made safer.[34]

Lentiviral vectors from the retrovirus family

Retroviruses are a family of capsid-containing enveloped viruses with a 7-11 kb positive-stranded, single-stranded RNA genome in two copies. The retroviral genome contains two long terminal repeats regions at the 5' and 3' ends and three large reading regions named gag, pol, env. Long terminal repeats act as promoters and regulators of the expression of gag, pol, and env, which in turn transcribe capsid proteins, replication enzymes, and envelope glycoproteins. Regarding the viral life cycle, once the capsid enters the host cell, the viral RNA genome is converted into double-stranded DNA by the reverse transcriptase enzyme.[35]

Lentiviruses are classified as one of the seven genera of the Retroviridae family. Lentiviruses are approximately 80-120 nm in diameter and have hereditary material containing two positively charged single-stranded RNAs. The genome of the lentivirus is approximately 9-10 kb. Lentiviral vectors have the ability to integrate into the genomes of dividing and non-dividing cells under *in vivo* or *in vitro* conditions. At both ends of the lentiviruses, there are 600-900 nucleotide-long homologous regions (long terminal repeats) required for virus replication, integration, and expression of related genes.[36,37]

Adenoviral vectors

It is a non-enveloped, linear double-stranded DNA virus of approximately 36 kb with a 100 nm icosahedral capsid. There are more than 50 serotypes.[38] Adenoviral vectors have a gene-carrying capacity of 7.5 kb and the ability to infect dividing and non-dividing cells. These features provide an advantage *in vivo* gene therapy. This viral vector cannot integrate into the host genome.[39]

Adeno-associated viral vectors

Adeno-associated viruses are enveloped, single-stranded DNA viruses belonging to the Parvovirus family.[40] They are similar to adenoviral vectors in terms of their properties, but AAVs are safer since adenoviral vectors have some deficiencies in replication and pathogenicity. The AAV genome consists of approximately 5 Kbp. There are only two genes in the genome structure. These are Rep (replicase, required for viral genome replication) and Cap (encodes structural proteins). Rep and cap genes contain short inverted terminal repeats.[41]

CURRENT THERAPIES

To date, therapeutic strategies in SMA have been divided into SMN gene-dependent therapies or SMN-independent therapies. Deletion of the SMN1 gene or a new mutation that may occur,

treatment studies that can tolerate copies of the SMN2 gene are ongoing. For this purpose, the limited expression of the SMN protein produced by SMN2 copies is partially compensated by the limited expression of SMN2 protein.[13,42]

Nusinersen first approved gene replacement therapy for spinal muscular atrophy

The researchers first discovered an intronic splicing silencer N1 (ISS-N1) sequence in intron 7 of SMN2, which is involved in mRNA skipping exon 7. Inhibition of the ISS-N1 molecule by ASOs has been shown to increase SMN2-mRNA exon 7 recruitment and improve SMA phenotypes.[43]

In 2011, a phase 1 trial of nusinersen, one of the ASOs with the greatest potential was given via cerebrospinal fluid transfusion in SMA patients. A subsequent phase 3 placebo-controlled trial showed significant favourability in motor function and survival in infants treated with type 1 SMA. Nusinersen was approved by the Food and Drug Administration (FDA) in late December 2016 and by the European Medicines Agency in June 2017.[44]

Viral vector-based survival motor neuron1 gene replacement

In this study targeting SMN2, adeno-associated virus serotype 9 (AAV9) viral vector was used. The aim here is to transfer the SMN1 gene into nerve cells with another therapeutic approach, as opposed to increasing SMN production. Among various gene delivery vectors, self-complementary AAV9 has been a promising study as it can also cross the brain barrier.[45]

The first AAV9-SMN1 gene therapy, Zolgensma (AVXS-101 or onasemnogene abeparvovec), was administered to 15 type 1 SMA patients and showed a significant improvement in motor and survival in a phase 1/2a trial. A follow-up study also confirmed the significant efficacy of early treatment. It is also applied for the treatment of type 2 SMA. However, the FDA recently partially stopped the intrathecal administration of Zolgensma in SMA patients over certain ages (≥2 years and <5 years) due to safety concerns.[13,46]

Treatment of spinal muscular atrophy with myostatin inhibitors

Since muscle weakness is always at the forefront of SMA studies, most of the treatments from SMN have focussed on muscle. Myostatin is a growth factor produced primarily in skeletal muscle cells to inhibit muscle growth. The aim of the studies is to block the myostatin signaling pathway, which may lead to an increase in muscle mass. As a result, it may improve muscle strength and motor function.[47]

Follistatin acts as an endogenous antagonist of myostatin. In SMA studies in mice, overexpression of recombinant follistatin led to survival and increased skeletal muscle mass. This study suggests that it is a possible treatment for neuromuscular disorders including SMA. SRK-015 (Scholar Rock), another myostatin inhibitor, is a human monoclonal antibody. SRK-015 has been proven by studies to increase muscle mass. A phase 2 trial is ongoing in type 2 and type 3 SMA patients via monthly intravenous administration.[48]

In conclusion, the medicines outlined above have the potential to greatly improve the quality of life for persons with SMA by specifically addressing the underlying cause of the disorder and increasing the production of SMN protein. However, it is essential to recognize that the advancement and evaluation of both SMN-dependent and SMN-independent treatments are now in their early stages of research and clinical testing. Additional research is necessary in order to have a full comprehension of the safety and efficacy of the aforementioned subject matter. With the increasing awareness of SMA in our country, studies in which new combinations of different therapeutics can maximize the benefits of SMA treatment are of interest and ongoing. Zolgensma and nusinersen therapies have recently been investigated in a small group of patients, but the long-term benefit is still uncertain. Since Zolgensma and nusinersen have different mechanisms of action, a drug-drug interaction is less likely. Nusinersen works by targeting an intron sequence to increase the inclusion of exon 7. The Zolgensma drug AAV9 remains a concern in gene therapy due to the low transmission of viral particles within the body and possible immune response. Each of the therapies

mentioned above is promising in the treatment of SMA. As these studies progress, there will be room for a variety of therapies with the addition of new therapeutic perspectives based on a better understanding of SMA pathomechanisms.

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