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Introduction of Spinal Muscular Atrophy Disease and the Latest Treatment Approaches based on Gene Therapy

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Abstract:

Spinal muscular atrophy (SMA) is a prevalent autosomal recessive disorder characterized by gradual weakening of the skeletal and respiratory muscles, resulting in substantial impairment. The illness is a result of genetic abnormalities in the survival motor neuron 1 (*SMN1*) gene, which leads to a reduction in the SMN protein and subsequently causes the degeneration of lower motor neurons. Gene therapy is a method that has the potential to cure or prevent uncommon monogenic illnesses by substituting a defective gene with a functional one. Gene therapy is particularly suitable for monogenic illnesses since it has the ability to correct abnormalities in a single gene. Currently, Nusinersen, risdiplam, and onasemnogene abeparvovec are the only officially sanctioned treatments for SMA that have the ability to influence the course of the illness. The purpose of this analysis is to examine and analyze their mechanisms of action, impacts, and potential safety issues. Nusinersen and risdiplam function by altering the *SMN2* gene product, whereas onasemnogene abeparvovec operates by introducing copies of the *SMN1* gene into cells. In this article, we briefly describe the pathogenesis and treatment strate, gies of SMA.

INTRODUCTION

Spinal muscular atrophy (SMA) is a genetic disorder characterized by the degeneration of neurons in the spinal cord, resulting from a lack of survival of motoneuron protein (SMN). The reduction in the synthesis of the whole SMN protein occurs due to an insertion or deletion of the *SMN1* gene situated on the 5q13.2 chromosome. The SMN protein is crucial in the development of SMA (1). It has a role in preserving cellular homeostasis. SMN is accountable for the accurate assembly of the spliceosome and the production of ribonucleoproteins. It has the potential to facilitate the formation of RNA complexes with the diverse proteins necessary for effective transportation or localized protein synthesis. SMN regulates the production of several components that participate in DNA repair or possess anti-apoptotic properties. Deficiency of the SMN protein results in the deterioration of -motoneurons located in the anterior horns of the spinal cord, which in turn leads to progressive muscle wasting (2).

Furthermore, in more severe manifestations of SMA, there is evidence of involvement of other cell and tissue types, leading to the emergence of symptoms that are not directly connected to motor neurons. There is a notable decrease in the quantity of chondroblasts in the hypertrophic zone of the growth plate, which leads to hindered bone growth. Angiogenesis and vascular maturation abnormalities caused by SMN deficiency exacerbate motor neuron hypoxia, therefore playing a role in the development of SMA. SMA is a degenerative and diverse condition. Irrespective of the specific kind, SMA has a substantial impact on patients and impacts them in intricate ways. In the absence of proper treatment, it results in muscular debility, paralysis, and ultimately, in extreme instances, fatality due to respiratory insufficiency (2).

The SMA phenotype is classified into four classes of severity (SMA I, SMA II, SMA III, SMA IV) according to the age at which symptoms appear and the level

of motor function attained. There are four types of SMA. Type 1 is the most severe, characterized by the patient's inability to sit. Type 2 involves the patient being unable to walk without assistance. Type 3 allows for some movement capabilities. Type 4 refers to adult-onset SMA (3).

The clinical significance of different kinds of SMA is mostly determined by the quantity of *SMN2* copies, while other genetic or environmental factors have a minimal impact. As the number of *SMN2* copy mutations increases, the likelihood of having a severe phenotype also increases. Therefore, when the number of *SMN2* types increases, the clinical severity also increases. Untreated, this condition will lead to significant impairments in motor function, like the inability to walk; a heightened likelihood of respiratory problems requiring some level of ventilatory assistance; a greater susceptibility to orthopedic issues such as painful contractures and scoliosis; and a decreased lifespan. SMA Type 1 accounts for 45% to 60% of all cases of SMA, making it the predominant form of the disease. Individuals diagnosed with SMA Type 1 who possess two copies of the *SMN2* gene have a very unfavorable outlook. Typically, these patients exhibit symptoms of SMA before reaching six months of age, which is characterized by their inability to sit. Regrettably, these infants generally do not live beyond the age of two without substantial reliance on mechanical ventilation and nutritional assistance. The *SMN2* gene is an extremely similar duplicate of the *SMN1* gene and is therefore regarded as a phenotypic modulator of the SMA disease. A higher quantity of *SMN2* gene copies corresponds to a less severe manifestation of SMA in clinical presentation (3).

Pathogenesis of SMA

The gene responsible for causing SMA is located in the q13 area of chromosome 5. SMA is a condition that occurs due to a mutation or deletion in the survival of *SMN1*gene, leading to the inability to produce the survival motor neuron (SMN) protein. SMN is a ubiquitously expressed protein that is involved in the formation of the U-rich small nuclear ribonucleoprotein (U snRNP) and aids in the transportation of axonal mRNAs (4). A recent study has provided a detailed summary of the activities of the SMN protein. The 5q13 region is characterized by a virtually symmetrical structure resulting from the duplication of a long stretch by inversion. *SMN1* is situated on one side of the telomere within this area. On the centriole side of this area, there is a parallel counterpart of *SMN1* called survival of motor neuron gene 2 (*SMN2*) (5). *SMN2* only produces a small quantity of the functional SMN protein, which is insufficient to make up for the decrease in SMN induced by the deletion of *SMN1*. *SMN1* and *SMN2* exhibit a high degree of similarity, with just 16 known variations in their base sequences. The main distinction is in the sixth nucleotide of exon 7; SMN1 has a cytosine (C), while *SMN2* possesses a thymine (T) (6). While this variation in a single DNA nucleotide does not impact the encoding of amino acids, it does influence the process of exon 7 splicing. Consequently, the majority of the gene products produced by the *SMN1* gene consist of complete messenger RNA molecules that include exon 7. In contrast, the gene products of *SMN2* are messenger RNA molecules that lack exon 7 (Δ7). The Δ7 mRNA experiences a displacement of its stop codon, leading to the production of a shortened SMN protein that lacks functionality and is very unstable. Thus, enhancing the incorporation of exon 7 of *SMN2* is a viable approach for treating SMA (6).

The degree of SMA is directly influenced by the quantity of SMN protein present. In general, individuals with moderate SMA possess a greater number of *SMN2* gene copies compared to those with severe SMA. This is because a larger copy number of *SMN2* results in elevated quantities of SMN protein. Furthermore, many regulations, including point mutations, might impact the severity of SMA patients by modifying the incorporation of exon 7. The precursor mRNA of *SMN2* contains several cis-acting splicing regulatory elements, including exonic splice silencer (ESS), exonic splicing enhancer (ESE), intronic splicing silencer (ISS), and intronic splicing enhancer (ISE). These components bind to trans-acting factors and regulate the inclusion of exon 7 (7).

Options for the treatment of SMA

Throughout history, there have been several therapy methods available for SMA; however, only a limited number of them are applicable to patient treatment. However, they are practical and can be transformed into innovative medications in the future. Our approach to developing medications against SMA follows the sequence of "Gene-RNA-Protein-Cell-Tissue." Numerous exemplary evaluations have provided detailed descriptions of several of these tactics.

Gene-Targeted Therapy: This treatment involves introducing intact SMN-encoding genes into cells to induce their expression, resulting in a substantial synthesis of SMN protein. This procedure effectively transferred the *SMN1* gene to motor neurons in several animal models by using self-complementary adeno-associated virus 9 (scAAV9), resulting in a significant extension of the lifespan in mice models of SMA. The medicine abeparvovec-xioi, developed by Novartis' AveXis, gained approval in the US in 2019 and was introduced to the EU in 2020 using this approach. Another approach is to enhance the transcriptional expression of *SMN2*, for instance by using the histone deacetylase inhibitor SAHA. This method has successfully resulted in elevated levels of SMN protein in animals. Nevertheless, this method has not achieved success in clinical studies so far (8, 9).

Therapy aimed at modifying splicing: This treatment approach seeks to enhance the production of the functional SMN protein by increasing the incorporation of *SMN2* exon 7. There are now two medications that have been authorized for sale. The first drug is called nusinersen, while the second drug, which was introduced more recently, is called risdiplam (10).

Protein stabilisation therapy: Cellular proteins maintain

a state of dynamic equilibrium, where their synthesis and degradation are balanced. This approach maintains the protein abundance of SMN by either inhibiting protein breakdown or facilitating the continuation of protein synthesis in spite of the existence of stop codons. The main drugs utilized in this therapeutic strategy are indoprofen and aminoglycosides. Specific small-molecule medicines have similar effects and possess the capacity to greatly augment the production of the SMN protein in cells. Nevertheless, their influence on the lifespan of SMA mice is only slightly enhanced (11, 12).

Substitution of cells: This method seeks to replace healthy neurons and restore the nerve supply to muscles. An alternative is to prompt the differentiation of embryonic stem cells or induced pluripotent stem cells (iPSCs) into neurons or neural stem cells in a controlled laboratory environment, with the intention of later transplanting these cells into the body. An alternative approach involves reprogramming fibroblasts from patients with SMA into iPSCs, and then genetically correcting them in a laboratory setting. These iPSCs may then be further induced to differentiate into motor neurons, which can be transplanted back into the same patient. This strategy is suitable for nearly all neurological diseases; however, present innovation has not sufficiently progressed for the management of SMA (13, 14).

Neuroprotective therapy: This technique is designed to provide nutrients and safeguard neurons. Riluzole is the primary medicine used in this treatment. Riluzole can enhance the survival of neurons and the development of axons by reducing the harmful effects of glutamate and increasing the production of neurotrophic factors. It has been approved for the management of amyotrophic lateral sclerosis, which is another type of neurodegenerative disease (15). Nevertheless, riluzole had little impact on the average lifespan of SMA animals and did not provide any defense against the loss of proximal axons. Despite the positive outcome of a clinical study with a limited number of participants, demonstrating potential benefits of riluzole for children with SMA, there is a lack of published findings from subsequent trials. In summary, this treatment approach lacks specificity and is unable to enhance SMN protein levels near their origin, thereby limiting its effectiveness in treating the condition (15).

FDA-approved treatments for SMA

Nusinersen

Nusinersen was authorized by the Food and Drug Administration (FDA) and the European Medical Agency (EMA) in December 2016 and June 2017, respectively, as one of the first medications for the treatment of SMA. This medication is classified as an antisense oligonucleotide (ASO) and works by suppressing the splicing process in the intron 7 of the *SMN2* gene, resulting in the incorporation of exon 7 into the *SMN2* mRNA transcripts (16). The ISS-N1 sequence is situated in exon 7, after the 5'ss. This segment hinders the inclusion of exon 7 by attaching to positions 10-24 of intron 7. Evidence demonstrates that the use of nusinersen may enhance the concentration of the SMN protein by inhibiting ISS-N1 and, as a consequence, inhibiting hnRNP. This leads to the inclusion of exon 7 in *SMN2* transcription. The progressive nature of the illness is decelerated by the augmented presence of functioning SMN protein. The medication is given intrathecally, meaning it is injected into the spinal canal. During the initial loading phase, it is supplied four times over two-month. In the maintenance period, it is given every four months. The dosages are specifically designed to affect the central nervous system. It is important to note that if the medication is given subcutaneously (under the skin) or intravenously (via a vein), it does not pass across the blood-brain barrier. An extensive study was required to establish the efficacy of nusinersen (17).

The safety of nusinersen has been assessed in several trials involving persons with SMA. Based on a thorough analysis of the data, no particular safety issues were found that could be directly linked to the medicine. The primary adverse effects associated with nusinersen are upper and lower respiratory tract infections, atelectasis, constipation, headache, back discomfort, and post-lumbar puncture syndrome. Two issues have been noted while using other experimental antisense oligonucleotides, and the FDA has cautioned about the potential occurrence of thrombocytopenia and renal toxicity with nusinersen. If there is a clinical need, it is suggested to do platelet count, prothrombin time, and spot urine protein tests before administering the treatment. This drug does not have any documented significant interactions. Due to the very short length of several clinical trials (less than 18 months), continuing investigations are being conducted to assess the long-term implications of drug interactions and safety hazards associated with the treatment. Nevertheless, nusinersen must be administered at intervals of 4 months, with an initial cost of $750,000 for the first year, followed by an annual cost of $350,000 thereafter (18, 19).

Clinical trials with nusinersen for SMA

In 2016, the first human clinical trials of nusinersen in children with SMA types 2 and 3 and type 1 (CS3a study were reported. The promising outcomes provided the basis for three Phase 3 trials: ENDEAR, CHERISH, and NURTURE. The approval of nusinersen as the first disease-modifying medication for SMA was mostly based on the outcomes of the first two trials (20, 21).

The ENDEAR (CS3B) trial was a multicenter worldwide research that used a randomized, double-blind, sham-controlled design. The study focused on babies diagnosed with SMA type 1 who had two copies of the *SMN2* gene and were under 7 months of age at the time of enrollment. A total of 121 babies with symptoms participated in the trial, including 80 in the nusinersen group and 41 in the control group (which did not receive any medication) (22). The trial was halted prematurely due to a prespecified interim analysis that revealed a much greater proportion of motor-milestone responders in the treatment group

compared to the control group, with percentages of 41% and 0% respectively. Ultimately, the treatment group exhibited a percentage of 51%, whilst the control group maintained a percentage of 0%. Multiple participants achieved important motor milestones with therapeutic implications, including acquiring head control, the ability to turn over, and attaining unsupported sit. During the research period, those who received therapy had significantly better overall survival and also had more time before needing permanent assisted breathing. Infants with a shorter duration of the illness had more favorable results compared to those with longer periods of illness, suggesting that initiating therapy soon is essential in SMA type 1 (22).

The CHERISH (CS4) study was a multinational, double-blind, sham-controlled clinical trial conducted in children between the ages of 2 and 12 who have SMA type 2. The trial comprised a total of 126 children who exhibited symptoms, with 84 children randomized to the nusinersen group and 42 children assigned to the control group. In addition, they were classified according to their age at the time of screening, either as under 6 years old or 6 years old and above (23). Just 16% of the population were above the age of 6. Every person exhibited the capacity to sit autonomously and attained notably high initial ratings on both the HFMSE and RULM evaluations. The experiment was terminated early due to ethical considerations, namely because of an interim analysis. The change in HFMSE score from the initial measurement was 5.9 points, as calculated using the least- squares mean approach. In the end, there was a difference of 4.9 points between the two groups. The nusinersen group had an average rise of 3.9 points, while the control group had an average drop of 1.0 points. A clinical significance is indicated by suggesting a minimum improvement of 3 points on the HFMSE scale (23).

In December 2016, the US FDA approved nusinersen as the initial drug for treating SMA, making it the first of its kind (24). In August 2021, nusinersen is accessible in 22 European countries. It is available without any limitations in 14 countries, while in 7 countries it is only accessible to particular forms of spinal muscular atrophy (SMA) and/or with some limits. Additionally, in 1 country it may be accessed via an early access program. Nusinersen has obtained regulatory authorization for its utilization in all types of SMA, encompassing all age cohorts and illness phases. As of March 2021, Biogen reported that over 11,000 patients worldwide have undergone nusinersen therapy (24).

The research observed individuals diagnosed with spinal muscular atrophy (SMA) types 2 and 3, ranging in age from 2 to 15 years at the beginning of the trial. These individuals were first included in the CS2 Phase 1/2 study and then continued their participation in the CS12 Phase 2 open-label extension study. The findings demonstrated sustained enhancements in motor abilities and steady disease activity over a span of roughly 3 years (25). Similarly, the CS3A study concludes that a significant fraction of the SMA type 1 therapy group saw a long-lasting clinical improvement, which is similar to the findings of the ENDEAR research, for a median follow-up period of 36.2 months. Upon completion of the trial, 75% of the subjects were still living. EMBRACE was a Phase 2 research that included 21 symptomatic babies and children who were not eligible for the ENDEAR or CHERISH investigations. The duration of the blinded 14-month portion was reduced according to the interim findings of the ENDEAR study. Phase 2 of the study had a total of 20 participants who were enrolled in an open-label research trial that spanned a duration of 28 months. All participants, with the exception of one, who were administered nusinersen in all phases of the study, met the HINE-2 motor-milestone response criteria, irrespective of their age at the onset of SMA (26).

Zolgensma

Zolgensma (AVXS-101, Onasemnogene Abeparvovec) is the second medicine that has been authorized for the treatment of SMA. The FDA granted approval to Zolgensma in 2019 for the therapeutic intervention of genetic testing-diagnosed SMA in children below the age of 2. Gene replacement therapy (GRT) was developed as a result of understanding the genetic cause of the condition (27). Onasemnogene abeparvovec is a gene therapy that employs an adeno-associated virus as the vector to transport a fully functioning copy of the human *SMN* gene to targeted motor neuron cells. Administering the SMN protein intravenously once leads to its expression in the motor neurons of a kid. This expression improves muscular mobility, function, and the child's overall survival in the case of SMA. The ongoing and completed clinical trial of onasemnogene abeparvovec has shown that it is safe and effective. The experiment had 36 pediatric infants diagnosed with infantile-onset SMA, ranging in age from around 2 weeks to 8 months at the beginning of the study. It is important to mention that, unlike nusinersen, Zolgensma can pass across the blood-brain barrier. Additionally, a single injection of Zolgensma during a 1-hour intravenous infusion is enough to achieve widespread expression of the SMN protein throughout the body. In contrast to the typical disease progression seen in kids with infantile-onset SMA, those who received treatment with onasemnogene abeparvovec showed a notable enhancement in their capacity to achieve developmental motor milestones, including improved head control and the ability to sit unassisted. Onasemnogene abeparvovec often causes increased levels of liver enzymes and vomiting as adverse effects. Therefore, it is necessary to closely monitor the liver function of patients for a minimum of 3 months after the administration of onasemnogene abeparvovec (28-30).

Currently, more than 12 unique serotypes of adeno-associated viruses (AAVs) have been discovered. Their cell tropism varies, and this variance is governed by the kind of viral surface proteins they possess. Furthermore, they vary in terms of their transduction efficiency and capacity to elicit an immunological response. AAVs have the capacity to infect both

actively dividing and non-dividing cells, while also showing low immunogenicity and decreased toxicity. The aforementioned traits render AAVs well-suited for the sustained expression of transgenes in clinical environments. Furthermore, a considerable proportion of AAVs have shown the capacity to efficiently deliver genetic material into both neurons and glial cells. This capacity has facilitated the creation of vectors obtained from AAVs for the treatment of neurodegenerative diseases (29).

Clinical trials with Zolgensma

The START clinical study was done in the United States to evaluate the effects of a single dose of onasemnogene abeparvovec on newborns diagnosed with SMA type 1. The research included a sample of 15 newborns, with a mean age of 6.3 months, all of whom have both copies of *SMN2*. Out of these newborns, 12 were given a high dosage of onasemnogene abeparvovec, whereas 3 got a low dosage. The experiment was conducted as a Phase 1 trial and the findings were compared to those of a historical cohort. Following a gene transfer period of 20 months, 11 out of the 12 toddlers who were administered a substantial amount of onasemnogene abeparvovec were capable of sitting independently and feeding themselves autonomously. The treatment resulted in the individual's survival and achievement of motor milestones and motor abilities that deviates from the expected development of the disease (28).

An open-label Phase 3 research, known as CL-303 (STR1VE-US), was conducted in the United States. The research included 22 infants aged below 6 months who were diagnosed with SMA type 1 and had 2 copies of the *SMN2* gene. The research was a single-arm trial conducted over a period of 18 months, including the injection of a single dosage of Zolgensma by intravenous route. At 14 months old, 20 out of 22 patients were alive and did not need permanent ventilation, while 18 out of 22 patients were fully free from the need for ventilatory assistance. By the age of 18 months, 59% of the individuals included in the research demonstrated the ability to sit alone for a minimum of thirty seconds. In comparison, none of the participants in the control group achieved this milestone. Additionally, 68% of the participants did not need assistance with feeding. After one month of treatment, the participants' CHOP INTEND scores had increased, with an average improvement of 6.9 points from the initial score (31).

The STR1VE-EU (CL-302) trial was conducted in Europe to investigate persons diagnosed with spinal muscular atrophy type 1 and possessing either 1 or 2 copies of the *SMN2* gene. The study consisted of 32 patients who had a more serious pattern at the start of the trial, in contrast to the START and STR1VE-US investigations. The patients' survival rate was similar to that of the STRIVE-US research, with 97% (31/32) of kids surviving without the need for permanent ventilatory support at 14 months of age, and 39% of patients not needing any daily ventilatory assistance. After the research, 44% of participants were able to sit independently for at least 10 seconds. The observed impact is less pronounced compared to the STR1VE-US trial, perhaps due to starting disparities in the study populations. Nevertheless, there was a comparable rise in CHOP INTEND scores, exhibiting an average shift of 6.0 points from the first measurement (32).

Risks of Zolgensma

Safety concerns include potential complications such as impaired liver function, low platelet count, clotting disorders affecting small blood vessels, and increased levels of troponin-I. Thrombotic microangiopathy is an uncommon, sudden, and potentially fatal illness, marked by low platelet count and the destruction of red blood cells in small blood vessels, with low platelet count being a prominent characteristic. Due to the potential for liver damage and other immune-related negative effects after AAV-based gene therapy, it is advised to provide preventative systemic corticosteroids before and after giving onasemnogene abeparvovec (33, 34). The intravenous injection of onasemnogene abeparvovec has the potential to influence several kinds of cells. Side effects have been recorded in multiple cell types and tissues, such as thrombocytes in the blood, as well as the liver, kidneys, and heart. Typical adverse effects include of emesis and increased levels of liver enzymes. While typically temporary and not medically significant, it is crucial to acknowledge the possibility of liver failure due to hepatotoxicity resulting from a hyperinflammatory response. It is recommended to monitor liver enzymes for a minimum of 3 months after receiving the onasemnogene abeparvovec infusion. No central nervous system (CNS) side effects have been seen in people. However, toxicity in the dorsal root ganglion has been seen in nonhuman primates when administered intrathecally. Hence, it is important to closely observe this as a potential unfavorable occurrence. Inpatient therapy may be required for these immune-mediated side effects after medication, along with the administration of intravenous steroids and other immunosuppressants (35).

Small-molecule compounds

Risdiplam is a small compound that, similar to nusinersen, alters the process of *SMN2* pre-mRNA splicing. This molecule easily passes across the blood-brain barrier and is taken orally once a day, allowing it to be distributed and absorbed effectively in both central and peripheral tissues. Risdiplam, marketed as Evrysdi®, is the first orally administered medication specifically designed for the treatment of SMA. It has received approval in several countries throughout the globe. It is authorized for treating spinal muscular atrophy (SMA) in children who are at least 2 months old in the United States and the European Union. In the European Union, this authorization is specifically for treating a kind of SMA called 5q-autosomal recessive SMA, which includes SMA types 1, 2, or 3, or SMA with one to four copies of the survival motor neuron 2 (*SMN2*) gene. Risdiplam functions as a regulator of *SMN2* pre-mRNA splicing, resulting in an augmentation of the production of intact SMN protein. The absence of this protein is responsible for

the development of SMA's pathological processes. Risdiplam shown a substantial improvement in motor function for newborns diagnosed with SMA type 1 and for people aged 2-25 years with SMA type 2 or 3 throughout phase 2/3 clinical studies. The motor enhancements were sustained for duration of up to 2 years by the administration of risdiplam. Risdiplam had overall good tolerability, demonstrating a favorable balance between benefits and risks. Risdiplam, being an oral medication, offers a simple and beneficial therapy choice for a wide spectrum of patients of different ages and subtypes of SMA (36, 37).

Clinical trials with Risdiplam

The FIREFISH trial is a continuing study that consists of two parts. It is an open-label multicenter research conducted in newborns that have SMA type 1 and 2 *SMN2* copies. Part 1, which has been finished, was an initial phase aimed at determining the appropriate dosage, assessing safety, and studying the pharmacokinetics and pharmacodynamics. A total of twenty-one babies, ranging in age from 1 to 7 months, and with a condition that started at a median age of 2 months, were included in the study. Initially, all participants had low baseline scores on the CHOP INTEND and HINE-2 evaluations, and none of them were able to sit with no help. During the trial period, most of the adverse events (AEs) encountered were mostly linked to decreased respiratory function resulting from the underlying disease, rather than the study medicine. These included respiratory tract infections, acute respiratory failure, and respiratory distress. Four individuals succumbed, all due to complications associated with SMA (38). The pharmacokinetic findings indicated a 2.1-fold increase in the concentration of SMN protein in the blood, compared to the initial level, four weeks after initiating the high-dose treatment. The provided data was used to establish the treatment dose of 0.2 mg per kilogram for the subsequent phase of the investigation. Further investigations on the effectiveness of risdiplam revealed a favorable clinical outcome. By the time they were 12 months old, 41% (7 out of 17) of the participants exhibited the capacity to sit alone for at least 5 seconds (38).

SUNFISH consists of two separate phases. The first phase, with 51 participants, focused on determining the appropriate dosage. The second phase assesses the efficacy and security of risdiplam in a heterogeneous population of individuals diagnosed with SMA types 2 and 3. Each segment of the research consisted of separate cohorts of patients, and part 2 is now in process. The findings from Part 1 demonstrated a significant improvement in motor performance in comparison to a control group that did not receive any kind of therapy. The research design for part 2 is a Phase 3 trial, which is randomized and double-blind (39). The study spans a duration of twelve months, during which patients are given either 2 doses of risdiplam or a placebo. This continues by an additional 12-month period when every participant receive risdiplam in an open-label fashion. Afterwards, all individuals will be eligible for a three-year open-label extension. The second phase of the research had a total of 180 participants, with 71% diagnosed with SMA type 2 and the remaining 29% diagnosed with SMA type 3. The participants ranged in age from 2 to 25 years and had limited mobility, being unable to walk. However, they were capable of sitting alone for a minimum of 5 seconds. Notably, there were no explicit criteria for eliminating persons with scoliosis, contractures, or those who needed nutritional or respiratory care (39).

Risks of Risdiplam

Like nusinersen and onasemnogene abeparvovec, most adverse effects (AEs), including substantial AEs, seem to be associated with the progression of the underlying disease or related complications, rather than the medicine itself. Consequently, persons with SMA type 1 have a higher frequency of serious AEs compared to those with types 2 or 3. The most often reported AEs linked to risdiplam in the FIREFISH and SUNFISH experiments are fever, diarrhea, mouth and aphthous ulcers, arthralgia, urinary tract infection, constipation, and skin rash. There have been instances of skin-related issues perhaps linked to risdiplam that have resolved on their own. Initial studies done on risdiplam in cynomolgus monkeys, which were exposed to high amounts of the drug, showed retinal impairment after being treated for a period of 5-6 months (39, 40).

SMA gene therapy challenges

The main difficulties related to gene therapy are the handling and delivery of the complex therapeutic material (AAV9 vector with transcription-competent SMN cDNA) and the effective distribution of the introduced gene throughout the body, including the central nervous system (CNS). Gene therapy raises additional issues about the overexpression of the introduced gene and the immune response triggered by the AAV9 vector. Neuronal tissues are very vulnerable to low levels of SMN in severe SMA. However, there is increasing data that suggests there are broad developmental abnormalities throughout the embryonic phases of severe SMA (41). It is premature to draw any conclusive judgments on the enduring impacts of gene therapy. Nevertheless, it is informative to consider a prior investigation involving non-human primates and piglets who received intravenous infusion of a modified AAV9 carrying the SMN gene, as well as more recent research done on mice with a severe form of SMA. These studies provide information on the potential long-term impacts of AAV9-based gene therapy for SMA. The mice research specifically identified synapse loss and motor neurodegeneration as a result of the toxicity caused by the overexpression of human SMN using AAV9-mediated gene therapy (42). The majority of individuals with SMA saw statistically significant gains from onasemnogene therapy. Significantly, those who had previously had nusinersen treatment also saw positive effects from gene therapy. Nevertheless, the results uncovered several treatment-induced adverse events. For example, pyrexia was the common adverse event seen in all three clinical studies with onasemnogene. Other negative occurrences seen

Table1. Names and frequencies of genes involved in the occurrence of lung cancer (2).

Drug	Type	Function	Clinical Trials	Status
Nusinersen (Spinraza)	Antisense oligonucleotide	Modifying splicing to enhance the creation of SMN protein by binding *SMN2* mRNA	ENDEAR (Phase III) NURTURE (Phase II) CHERISH (Phase III) DEVOTE (Phase II/III) ONWARD (Phase III)	Approved by FDA in 2016
Onasemnogene abeparvovec (Zolgensma)	Gene replacement therapy	Adenovirus vector (AAV9)-mediated delivery of the *SMN1* gene	START (Phase I) STR1VE (Phase III) NCT05089656 (Phase III)	Approved by FDA in 2019
Risdiplam (Evrysdi)	Small molecule	Directly binding to ESE2 of the *SMN2* transcript	FIREFISH (Phase II, III) SUNFISH (Phase II, III) JEWELFISH (Phase II) RAINBOWFISH (Phase II)	Approved by FDA in 2020
Olesoxime	Cholesterol-like compound	Reducing neuronal degeneration and death	Phase 2 OLEOS trial	Development stopped
Reldesemtiv **CK-2127107**	Fast skeletal muscle troponin activator (FSTA)	Increase muscle strength and cytokinetics	Phase 2 trial (NCT02644668)	Phase III trail is planned
SK-015	Monoclonal antibody	Promote muscle cells growth and division	TOPAZ (PhaseII)	Ongoing clinical trials
Pyridostigmine	Acetylcholine esterase inhibitor	Improved muscle strength and fatigue	SPACE Trial	Phase II

in younger children with SMA included bronchiolitis, pneumonia, respiratory distress, and respiratory syncytial virus bronchiolitis. Previous findings indicate that individuals treated with onasemnogene had significantly elevated levels of SMN expression in the liver, which may be attributed to the hepatotropism of the AAV9 vector (31, 43).

Although Zolgensma has shown favorable outcomes, there are still existing hazards. This medication has been approved for two specific groups of patients: those who have been diagnosed with 5q SMA and have a mutation in both copies of the *SMN1* gene, as well as a clinical manifestation of *SMA1*, and those who have been identified with 5q SMA and has a mutation in both copies of the *SMN1* gene, along with up to 3 copies of the *SMN2* gene. Currently, there is a lack of evidence addressing age or weight limits for these specific patient groups. In theory, a substantial population of SMA patients would have the potential to undergo Zolgensma therapy. Nevertheless, the existing clinical trial results only pertain to infants under six months old and weighing less than 8.4 kg. Limited information is accessible regarding the safety and efficacy of Zolgensma in older or heavier patients (44). A significant issue that might arise is a reduction or absence of treatment effectiveness owing to the existence of pre-existing antibodies against AAV9 in the community of individuals with SMA (45). Despite the confirmation of all the limits, a substantial issue will persist—the exorbitant cost of this medication, which amounts to almost $2 million for a single dosage of Zolgensma. It is among the most costly medications available for purchase (44).

Nucinersen is now the only licensed therapy medication for children and adults with SMA, a group of neurodegenerative illnesses. It shows promise as an ASO (antisense oligonucleotide) treatment for many neurodegenerative diseases. While the central nervous system has a long-lasting half-life, a significant disadvantage is the requirement of providing 4 initial doses and then three annual maintenance doses, resulting in patients having repeated intrathecal administrations. If the single genotype correction of Zolgensma does not achieve the expected outcomes, Spinraza treatment remains a feasible option for these patients (22). Another issue associated with medications

that control pre-mRNA splicing is the potential for unintended effects on non-target molecules or processes. Risdiplam and Branaplam have shown the ability to cause significant disruptions in the process of splicing, resulting in the inclusion of unintended exons, skipping of exons, retention of introns, elimination of introns, and the use of alternative splice sites. Hence, it is essential to discover more efficient dosage schedules to properly use existing therapeutic medicines that target splicing modulation (46).

CONCLUSIONS

SMA is a hereditary condition that leads to progressive muscle degeneration and loss of function due to issues with the *SMN1* gene. It is an autosomal recessive condition, meaning that both copies of the gene must be affected for the sickness to occur. In all patients, there is a second gene called *SMN2* located near the centromere. This gene is not damaged, but it does have a variant in exon 7 where a C nucleotide is replaced by a T nucleotide. This variation affects a splice enhancer, causing exon 7 to be excluded in most of the transcript produced by this gene. As a result, the protein produced by *SMN2* is unstable and cannot replace the mutant SMN1 protein. After conducting effective research on illness models and doing in-depth investigations on the roles of SMN during the last decade, it has been proposed that targeting SMN2 for overexpression might be a potential therapeutic strategy. Thanks to the introduction of three recently developed treatments, namely nusinersen (Spinraza), onasemnogene abeparvovec (Zolgensma), and risdiplam (Evrysdi), patients now have increased survival rates and better overall results. Nevertheless, patients and their families still encounter several obstacles related to the use of these treatments, such as inadequate treatment outcomes and a fluctuation in the advantages experienced by those who do react. This indicates that the pursuit of finding a cure for SMA is ongoing. In recent times, we have seen a significant milestone when the first medication that modifies diseases has obtained permission from the Food and Drug Administration. This treatment is now accessible to patients outside the confines of the clinical study. This medication is a novel antisense oligonucleotide that, when given by intrathecal administration, has the ability to enhance the inclusion of exon 7 in most of the *SMN2* mRNA and boost the creation of fully functional SMN protein. Despite facing significant criticism about its price, Zolgensma is a very successful single-dose therapy for SMA. It offers a higher cost-efficiency compared to similar medicines, Evrysdi and Spinraza, and necessitates shorter treatment duration. The concise characteristics of Zolgensma are very likely to contribute to the future popularity of this drug among doctors. Administering Zolgensma to children less than two years of age early on has been supported by significant research, showing superior results. Due to the effectiveness of this medication, clinicians may have more confidence in diagnosing SMA early, leading to improved survival rates and quality of life for their patients. Adverse effects linked to this gene therapy include of localized injection responses, feelings of nausea, increased levels of ALT (alanine aminotransferase), and hypersensitivity reactions. Newborn screening and prompt therapy with these innovative medications will soon be established as the customary approach for managing SMA. Nevertheless, it is important to note that the advantages of newborn screening and prompt intervention for SMA are only applicable to infants, and there are still several unsolved concerns for older children and adults who have SMA. Despite being first deemed incurable, the advent of novel treatments in the early 21st century has resulted in a tremendous improvement in the standard of life for those afflicted with SMA. When we contemplate the history of the SMA, we are amazed by the remarkable accomplishments of our predecessors and are motivated by the extraordinary scientists of our era. This essay aims to enhance the understanding of physicians and young researchers on the introduction of SMA illness and its treatment approaches.

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Conflict of Interest

Authors declared no conflict of interest.

Consent for publication

Not Applicable

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