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Spinal muscular atrophy: Where are we now? Current challenges and high hopes

Review

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Abstract

Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by muscle weakness. It causes movement issues and severe physical disability. SMA is classified into four types based on the level of function achieved, age of onset, and maximum function achieved. The deletion or point mutation in the Survival of Motor Neuron 1 (*SMN1*) gene causes SMA. As a result, no full-length protein is produced. A nearly identical paralog, *SMN2*, provides enough stable protein to prevent death but not enough to compensate for SMN1's loss. The difference between *SMN1* and *SMN2* is due to different exon 7 alternative splicing patterns. SMA molecular therapies currently focus on restoring functional SMN protein by splicing modification of SMN2 exon 7 or elevated SMN protein levels. Nusinersen, an antisense oligonucleotide targeting the ISS-N1 sequence in SMN2 intron 7, was the first drug approved by the Food and Drug Administration. Risdiplam, a novel therapeutic that acts as an SMN2 exon 7 splicing modifier, was recently approved. All of these drugs result in the inclusion of SMN2 exon 7, and thus the production of functional SMN protein. Onasemnogene abeparvovec is a gene therapy that uses a recombinant adeno-associated virus that encodes the SMN protein. There are also experimental therapies available, such as reldesemtiv and apitegromab (SRK-015), which focus on improving muscle function or increasing muscle tissue growth, respectively. Although approved therapies have been shown to be effective, not all SMA patients can benefit from them due to age or weight, but primarily due to their high cost. This demonstrates the significance of continuous treatment improvement in today's medical challenges.

Keywords

spinal muscular atrophy • drug treatment • nusinersen • AAV9 • risdiplam • experimental therapy

1. Introduction

Spinal muscular atrophy (SMA) is one of the biggest challenges in today's medicine. This disease affects 1 in every 6,000–10,000 live births [1]. The carrier frequency varies (1:38–1:50), with Caucasian and Asian populations having the highest [2]. It is a neurodegenerative disease of the spinal cord that causes progressive muscle wasting, weakness, respiratory distress, and paralysis [1, 2]. Furthermore, due to its severity, it has been demonstrated that it is the most common genetic cause of childhood death [3]. SMA is a neuromuscular disorder that is caused by a homozygous deletion or point mutation in the Survival of Motor Neuron 1 (*SMN1*) gene, which is located on the long arm of chromosome 5 (5q13). A mutation or deletion in the *SMN1* gene prevents the SMN protein from being produced. The SMN protein is widely expressed and primarily found in the cytoplasm. The SMN protein in humans is encoded by two genes, *SMN1* and *SMN2*. Both genes are found on chromosome 5 and are nearly identical (with the exception of 11 compared to SMN1), with one in non-coding exon 8 [4]. The transition in SMN2 exon 7 results in the significant exclusion of this exon from the mature transcript, contributing to the production of a predominantly nonfunctional and unstable SMNΔ7 protein [5]. However, a small number of *SMN2* transcripts contain exon 7 and encode full-length, functional SMN, so when *SMN1* is mutated or deleted – it provides enough SMN to prevent death but not enough to fully compensate for the loss of *SMN1* expression (Fig.1) [6]. The number of SMN2 copies in the genome ranges from 0 to 8. According to research, the more SMN2 genes a patient has, the more functional protein is produced and the milder the SMA phenotype [7]. Exon 7 SMN1 contains three exonic splicing enhancers (ESEs) that can promote exon 7 inclusion into the mature transcript. The most important appears to be ESE2, to which trans factors, such as tra2-1, attach, interact

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with the exon, and are required for exon 7 inclusion [8]. The C6T transition eliminates an exonic splicing enhancer (ESE) that is dependent on SF2/ASF. This motif is found in *SMN1* exon 7 and displaces the inhibitory effect of the heterogeneous nuclear ribonucleoprotein (hnRNP) A1, contributing to the formation of the protein SMNΔ7 [9].

The disease is characterized by symmetrical muscle weakness, which can cause movement problems and severe physical disability. SMA is classified into four clinical types based on the age of symptom onset and the severity of the disease. According to some sources, SMA type 0 is the most severe form of muscular atrophy, and symptoms appear during prenatal development [10]. SMA type I (also known as Werdnig-Hoffmann disease) manifests itself in the first weeks or months of a child's life as muscle weakness and an apparent lack of movement progress. Children's health rapidly deteriorates, and mechanical airway ventilation may be required. Children with SMA type II (also known as Dubowitz disease) can develop normally up to the age of six months and learn to sit without assistance, but as they get older, they develop symmetrical muscle weakness, swallowing difficulties, and respiratory problems [11]. Symptoms of SMA type III (also known as Kugelberg-Welander disease) appear when children are able to walk. However, the disease causes walking difficulties or difficulties climbing stairs. SMA type IV can occur in adults, but it is extremely rare. Symptoms do not manifest themselves until the age of 20 or 30. They include walking problems, difficulty rising from a squatting position, walking upstairs, and frequent falls. Patients in this group have significantly reduced mobility, but they can move around independently for many years [12].

Some medications are currently available to alleviate the symptoms of this disease, but they are prohibitively expensive. The first, nusinersen, marketed as Spinraza by Biogen (Cambridge, MA, USA), was approved by the FDA in December 2016 and the European Medicines Agency (EMA) in June 2017. The first year's cost ranged from $516,896 to $907,665, and the second year's cost ranged from $258,448 to $457,889. The price differs between the United States and Europe, and it fluctuated over a three-year period between 2017 and 2020. The second medication, onasemnogene abeparvovec, marketed by Novartis (Basel, Switzerland) as Zolgensma, was approved by the FDA in May 2019 and by the EMA in August 2020. It is known as the most ex-

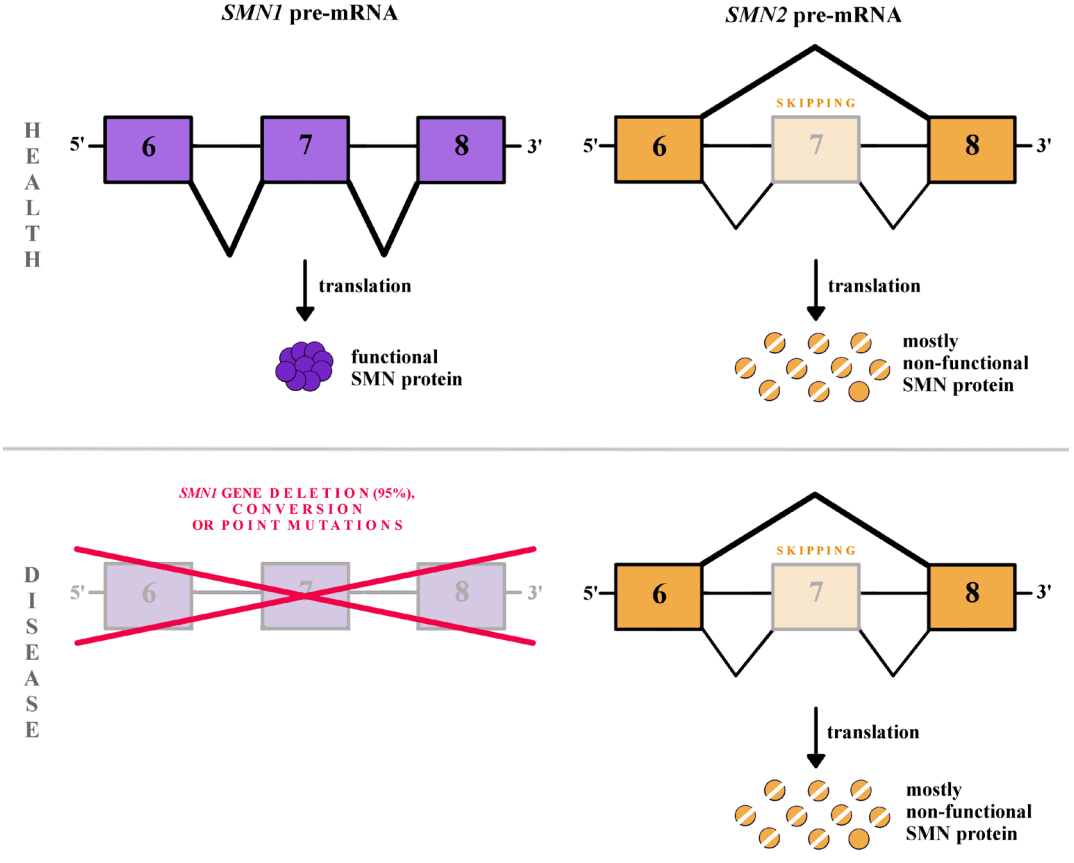


Fig. 1. ***SMN1*** *produces all the functional protein and is the gene affected in spinal muscular atrophy (SMA).* ***SMN2*** *produces non-functional and unstable protein nevertheless it makes only 10% functional protein. Mutation or deletion causes that SMN1 is not produced.*
SMN1- survival of motor neuron; SMN2- survival of motor neuron 2

pensive medication in the world, with its price of $2.1 million in the USA for a single injection. A third drug is risdiplam, marketed as Evrysdi by Roche, which was approved by the FDA in August 2020 and by EMA in March 2021 [3]. Although the FDA has approved these three drugs for use, clinical trials are still ongoing. Current SMA treatment methods are expensive and frequently insufficient. Because of the limited window, treatment time is a critical component for successful therapy. There is currently no fully successful SMA cure, and more solutions should be developed. This review describes various types of drugs, treatment-related issues, and ongoing research.

2. Nusinersen (Spinraza)

There was no efficient method of SMA therapy for a very long time. One of the most promising methods today is a treatment based on SMN protein level restoration. One of the ideas is to use oligonucleotides to modify the splicing of *SMN2* exon 7. Oligonucleotides that are delivered directly to the central nervous system (CNS) show high target specificity, but also low toxicity and systemic exposure. Oligonucleotide therapy differs from other gene therapy because it is reversible, and there is no problem with permanent alteration [13]. However, only 1% of administrated oligonucleotides are capable of crossing the blood-brain barrier, which is a huge problem to resolve. Intrathecal or intracerebroventricular injections can ameliorate the transport of oligonucleotides to their destinations in the CNS, but these methods are very invasive. One of the solutions avoiding this obstacle is to administer oligonucleotides via an intranasal or oral application. The other problem is that the pharmacodynamic and pharmacokinetic properties of this treatment are not well known [14]. In 2006, the intronic splicing silencer N1 (ISS-N1) region was discovered by Singh Laboratory. It is an intronic-cis element, located in the *SMN2* intron 7 downstream to the 5′ splicing site. It plays a major role in modulating alternative splicing of *SMN2* exon 7 in a regulatory network connected with the pathogenesis of SMA. It recruits spliced repressors hnRNP A1 and A2 that inhibit exon inclusion [14, 15].

2.1. Mechanisms of action

Nusinersen is an orphan drug (antisense oligonucleotide) that disrupts the function of ISS-N1 in intron 7 of the *SMN2* gene, which allows production of the functional SMN protein (Fig. 3B) [16]. Chemically, nusinersen is a 2′-O-methoxyethyl (2′MOE) modified antisense oligonucleotide (AO) [16] (Fig. 2A) that differs from RNA nucleotides with the addition of a methoxy group at the 2′-hydroxyl position and a phosphorothioate backbone, which increases its stability and RNA hybridization affinity by improving resistance to nuclease activity. Nusinersen boosted exon 7 inclusion by binding to a particular region in the intron downstream exon 7 of the *SMN2*. It is possible to convert *SMN2* mRNA with exon 7 into a functional, full-length SMN protein [16]. The dosage recommended by FDA is 12 mg administrated intrathecally. Three doses are administered every 14 days, the next one 30 days after the third dose, and then one dose every four months (probably for the rest of the patient's life) [14].

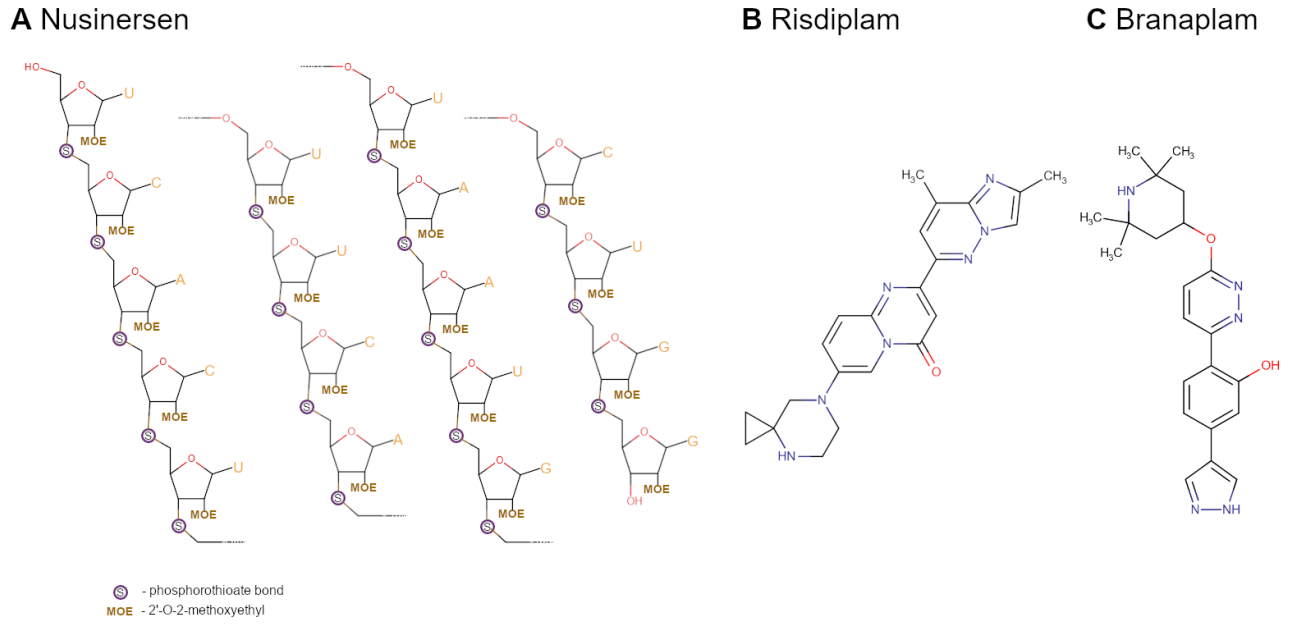


Fig. 2. Chemical structural formulas of **(A)** *Nusinersen (Spinraza) as sodium salt;* $C_{234}H_{340}N_{61}O_{128}P_{17}S_{17}$, **(B)** *Risdiplam (Evrysdi);* $C_{22}H_{23}N_7O$. **(C)** *Branaplam;* $C_{22}H_{27}N_5O_2$.

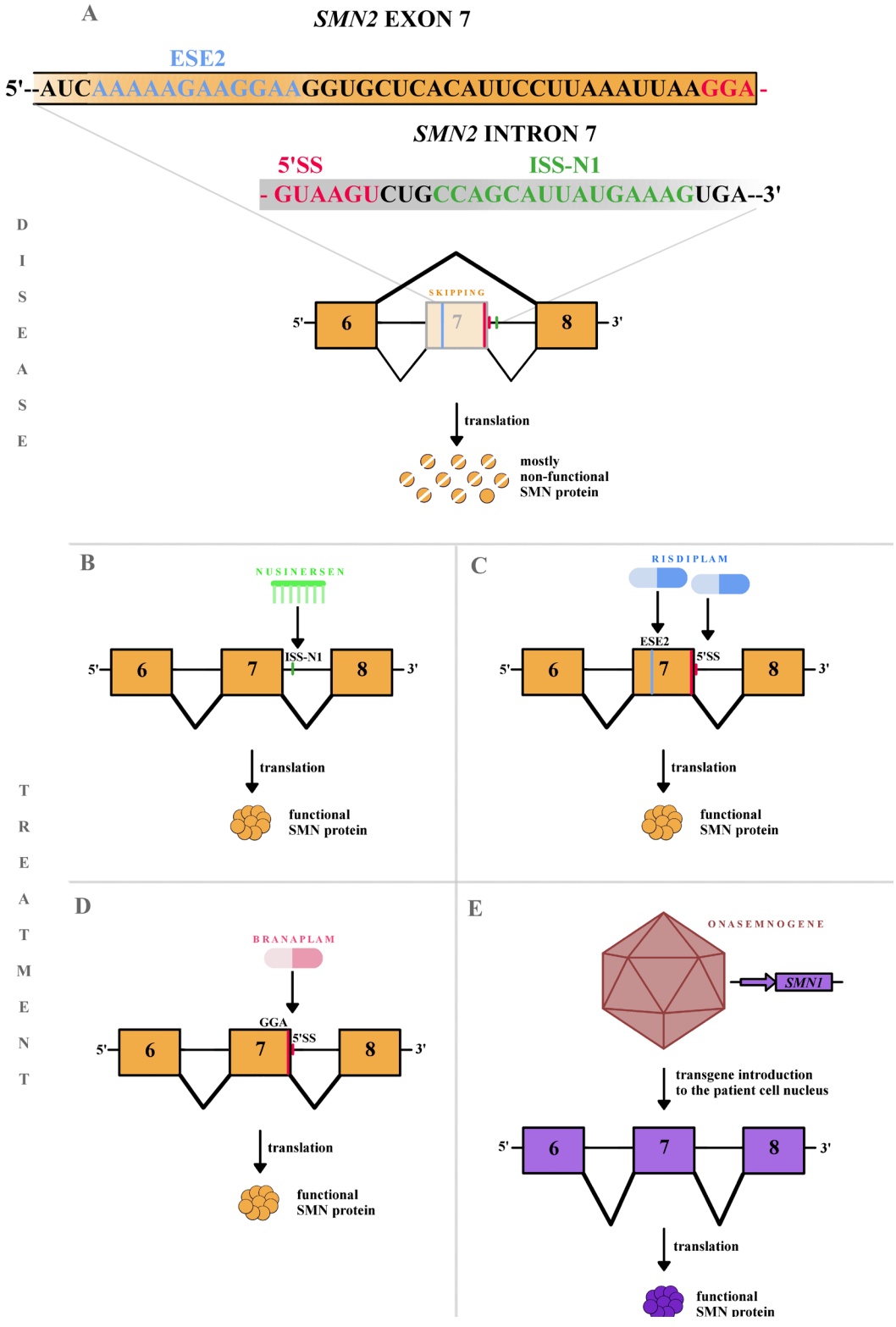


Fig. 3. Mechanisms of SMA treatment using different types of therapy. **(A)** *Mutations in* ***SMN1*** *gene result in SMN protein deficiency.* **(B)** *Nusinersen (Spinraza) targets the ISS-N1 region in the* ***SMN2*** *gene allowing the inclusion of exon 7 in the mRNA, which leads to high full-length SMN protein production. This treatment can bypass the loss of function in the* ***SMN1*** *gene.* **(C)** *Risdiplam (Evrysdi) is an* ***SMN2*** *splicing modifier designed to increase the level of SMN protein by affecting* ***SMN2*** *gene.* **(D)** *Branaplam stabilizes duplex U1:5'ss at the 5'ss* ***SMN2*** *exon 7 consequently contributing to enhancing exon 7 inclusion.* **(E)** *Onasemnogene abeparvovec (Zolgensma) is a gene therapy based on using adeno-associated virus subtype 9 (AAV9). AAV9 delivers a functional copy of the* ***SMN1*** *gene which results in increasing the level of SMN protein. N1- survival of motor neuron; SMN2- survival of motor neuron 2; ISS-N1- intronic splicing silencer N1*

2.2. Pharmacokinetics

Autopsy of the three individuals showed that nusinersen after intrathecal injection was transported from cerebrospinal fluid (CFS) to motor neurons, including vascular endothelial cells and glial cells via the central nervous system. The quantity of nusinersen in cerebrospinal fluid (CSF) was still detectable 168 days after dosing, which means that the exposure to this treatment was long-lasting. The maximum plasma concentration was observed between 1.7 and 6.0 h after drug administration, depending on the dosage. Nusinersen was also detected in peripheral tissues, such as kidneys, liver, and skeletal muscle, which supports the observation of its migration from the CSF. Nusinersen is metabolized by an exonuclease 3'- or 5'-mediated hydrolysis, and its terminal elimination half-life is assessed as 135–177 and 63–87 days, respectively. Finally, it is secreted by the urinary tract [17].

2.3. Clinical and therapeutic trials

2.3.1. Phase I

In the first phase, the doses 1, 3, 6, and 9 mg of nusinersen were administrated intrathecally to 28 children aged 2–14. Briefly, in patients treated with 1 and 3 mg, no difference was observed, but in those treated with 6 and 9 mg of nusinersen, there was twice the quantity of SMN protein 9–14 months after injection compared to the baseline. The visible symptomatic effect of this treatment was shown, however, only in patients treated with 9 mg of nusinersen 85 days after injection. The improvement was examined by Hammersmith Functional Motor Scale-Expanded score [15]. Also, the mortality was much lower – 16 of 20 patients lived longer than 18 months, while previously the average survival in a patient with SMA type I was 10 months. What is more, 10 of 16 patients did not need respiratory support [18].

2.3.2. Phase II

Success in the first phase has led to the second phase performed on 20 participants, aged 3 weeks to 7 months, who obtained doses of 6 or 12 mg of nusinersen. In this phase, patients had homozygous gene deletion or mutations or compound heterozygous mutation of *SMN1* and SMA originated from infancy. Significant amelioration of developmental motor milestones compared to the baseline was shown, confirmed by higher Hammersmith Infant Neurological Exam-Part 2 (HINE-2) and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) scores. The highest scores were observed a year after the injection of the first dose in the group that obtained 12 mg of nusinersen [19]. In the NURTURE study (NCT02386553), 25 patients with proven 2 or 3 copies of *SMN2* had achieved the ability to sit independently, 23/25 (92%) achieved walking with assistance, and 22/25 (88%) achieved walking independently [20].

2.3.3. Phase III (ENDEAR)

In the ENDEAR study (NCT02865109), 121 participants aged <7 months were examined after obtaining a dose equivalent to 12 mg of nusinersen in a 2-year-old child. A year after the first dose, there was a significant amelioration in achieving the motor-milestone response in comparison to the control group. In addition, the risk of death was reduced by 63% compared with the control group, as was mortality, which was reduced by 23%. CHOP-INTEND and HFMSE scores were also much higher [21].

2.4. Side effects

Side effects were very similar in every phase. The most common were procedural headache (21.4% of participants), and back pain (17.9% of participants). Other side effects were procedural nausea, puncture syndrome, post-lumbar pain, nausea, headache, fluid leakage, vomiting, and cerebrospinal leakage. There was no significant difference in side effects between the control group and group treated with nusinersen. An immunogenic response to nusinersen was not detectable 9–14 months after a single intrathecal dose of nusinersen [15, 19, 21].

3. Onasemnogene abeparvovec (Zolgensma)

On May 24, 2019, FDA approved onasemnogene abeparvovec (Zolgensma) for treating pediatric patients younger than 2 years suffering from spinal muscular atrophy with bi-allelic mutations in the *SMN1* gene [22]. Onasemnogene abeparvovec is a gene therapy based on using the recombinant adeno-associated virus subtype 9 (AAV9) which is designed to deliver a functional copy of the *SMN1* gene encoding human SMN protein (Fig. 3E) [22, 23].

3.1. Mechanism of action

AAV9 vector technology can cross the blood-brain barrier and target central nervous system neurons at all regions of the spinal cord. AAV9 virus vector contains a hybrid cytomegalovirus enhancer-chicken beta-actin promoter which permits long-lasting production of SMN [22, 24]. Onasemnogene abeparvovec is a one-dose intravenous infusion which is a hopeful alternative to more commonly used chronic treatment with nusinersen. The dosage is determined by patients' body weight. According to the FDA the recommended dose for pediatric patients is 1.1×10^{14} vector genomes per kilogram (vg/kg) [22, 25].

3.2. Pharmacokinetics

Studies of saliva, urine, and stool were taken at different amounts of time after the infusion of oasemnogene abeparvovec. This shows a higher concentration of vector in stool than in either saliva or urine – after 1 day, the level of AAV9 vector in saliva and urine was low; in saliva, the concentration was undetectable within 3 weeks and in urine within 1 to 2 weeks. In stool, the amount of vector was significantly higher after 1 and 2 weeks than in saliva and urine and also was undetectable in 1

to 2 months after the infusion. The autopsy of two patients who died after the infusion of nasemnogene abeparvovec shows the highest level of this therapeutic in the liver, but vector was also detected in the spleen, heart, pancreas, inguinal lymph node, skeletal muscles, peripheral nerves, kidney, lung, intestines, spinal cord, brain, and thymus. The immunostaining experiments of SMN protein show expression in spinal motor neurons, neuronal and glial cells of the brain, but also in the heart, liver, and skeletal muscles [22].

3.3. Clinical and therapeutical trials

In the NCT02122952 clinical trial, Mendell et al. studied functional replacement of the mutated gene *SMN1*. 15 pediatric patients who suffer from SMA type I (homozygous *SMN1* exon 7 deletions, and two copies of *SMN2*) were divided into two cohorts. Patients in the first cohort received a low dose of onasemnogene abeparvovec and those in the second cohort got a higher dose. The main purpose here was to examine if there is any difference in the length of life between groups treated with onasemnogene and the control group. The outcome was compared to the historical cohorts (studies of the natural history of the disease) using the CHOP – INTEND scale of motor function (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders, ranging from 0 to 64, with higher scores indicating better function) [24]. The results showed better survival of patients – all of them reached an age of at least 20 months without the need for permanent mechanical ventilation. In comparison, only 8% of the historical cohort did not require permanent mechanical ventilation. What is more, most of the patients who received a higher dose of onasemnogene abeparvovec (second cohort) were able to sit unassisted at least 5 seconds and could roll over, all of them acquired head control, and two patients achieved standing and walking independently. They also were able to crawl and pull to stand. These results clearly proved that onasemnogene abeparvovec has a potential for treating patients with SMN type I – a higher dose of AAV9 vector effected an extended survival, and increased scores on the CHOP INTEND scale, which indicated better motor functions [4]. The results of this clinical study (NCT02122952) were extended by Lowes et al. al. where they investigate the impact of age and baseline motor function on the motor function improvement in the second cohort which received a higher dose of onasemnogene abeparvovec. Patients from this cohort were split into groups based on the combination of age at dosing (< three months vs. > three months) and baseline CHOP-INTEND scores (< 20 vs. ≥ 20) [26]. The results one month after the injection showed a faster increase in the Early Dosing/Low Motor group than in the Late Dosing group and in the Early Dosing/High Motor group. After 24 months Early Dosing/Low Motor group gained a higher mean total CHOP-INTEND score than patients from the Late Dosing group. However, patients in the Early Dosing/Low Motor group reached a similar final score to the Late Dosing group despite having a lower mean baseline motor function at the beginning. These studies demonstrate that age of pediatric patients plays a significant role in achieving motor function improvements regardless of baseline motor function at the beginning [26].

The cost-effectiveness of onasemnogene abeparvovec in comparison to the chronic treatment for SMA type I with nusinersen was also studied by Malone et al. They created a Markov model which simulates the experience of pediatric patients diagnosed with SMA I and two copies of *SMN2* before the age of six months and treated with Zolgensma or Spinraza but also with SMA-related supportive care [25]. The model was present as cost/quality-adjusted life years ($/QALY) of AAV9 vector treatment versus nusinersen over a lifetime. The results prove that life expectancy for patients treating with onasemnogene abeparvovec is more effective and affordable than with nusinersen [25].

3.4. Side effects

The infusion of onasemnogene abeparvovec can provide aminotransferase elevations like the significantly higher level of rate of aminotransferase (AST) and alanine aminotransferase (ALT). Moreover, one patient in the clinical trial had a serious liver injury – prior to the infusion this patient had increased AST and ALT (unknown reason). Due to that, patients are receiving an oral corticosteroid therapy before and after the infusion. Besides aminotransferase elevations, treating with onasemnogene abeparvovec can thrombocytopenia, increased troponin-I level, vomiting, and thrombotic microangiopathy [22]. In the NCT02122952 clinical study, 14 patients (14/15) had respiratory illnesses, which can be very dangerous for children suffering from SMA type I and can result in death or tracheostomy [24].

4. Risdiplam (Evrysdi)

Risdiplam (Evrysdi) is a *SMN2* splicing modifier administered to patients at least 2 months old suffering from SMA caused by mutations in chromosome 5q that conduce to SMN protein deficiency [27]. It is a small-molecule drug with the chemical formula $C_{22}H_{23}N_7O$ (Fig. 2B). Risdiplam is designed to increase the level of SMN protein and targets exonic splicing enhancer 2 (ESE2) located in *SMN2* exon 7 and 5′ splicing site of *SMN2* intron 7, causing inclusion of exon 7 and production of fully functional SMN protein in the CNS and in peripheral tissues of the body (Fig. 3C) [26, 27]. Risdiplam is taken orally, and the dosage is determined by patient age and body weight [27].

4.1. Pharmacokinetics

Pharmacokinetics has been studied in healthy adult individuals and patients with SMA. Doses of risdiplam reached levels between 0.6 and 18 mg in a single-ascending-dose study in healthy adults and between 0.02 and 0.25 mg/kg once daily in a multiple-ascending-dose study in patients suffering from SMA [27]. After

taking risdiplam orally the maximum concentration of this therapeutic in plasma (T_{max}) was achieved between 1 and 4 hours. Risdiplam can bind to serum albumin, but there was no binding to alpha-1 acid glycoprotein. Risdiplam has an elimination half-life from healthy adults of 50 hours, and this drug is primarily metabolized by flavin monooxygenase 1 and 3 (FMO1, FMO3) and also by cytochromes 450 (CYPs) [27].

4.2. Clinical trials

Currently, there are four still-open clinical trials investigating Risdiplam. The first trial is named FIREFISH (NCT02913482) and this is a two-part open-label study of infants aged 1–7 months with SMA type I and two *SMN2* gene copies [26, 28]. Part one of this study was focused on safety, tolerability, pharmacokinetics, and pharmacodynamics of different dose levels of risdiplam and the results showed survival rate of 90.5% (19/21). None of the surviving infants require tracheostomy, reach permanent ventilation, or lose the ability to swallow. The FIREFISH part 1 outcome indicates the improvement of survival rates compared with patients at the same age in natural history studies [28].

The aim for the second part was to investigate the efficacy of risdiplam at the dose selected in the first part of the trial. Effectiveness here means the proportion of infants sitting without support for 5 seconds after 12 months of treatment (based on the Gross Motor Scales of Infant and Toddler Development, third edition [BSID-III]). The study met its primary endpoint [26, 29].

Another two-part trial is SUNFISH (NCT02908685) which is a double-blind, placebo-controlled study of patients aged 2–25 years with SMA types II or III. Part 1 of this study was focused on dose escalation and in the second part the efficacy of the risdiplam dose selected in the first part was compared with placebo patients [26, 30]. The analysis of the results after 2 years showed an improvement of motor function compared to natural history data. The results were measured using the Motor Function Measure scale (MFM) which is a validated scale used to calculate motor function, and the change from a baseline was higher than in the historical cohort [26].

JEWELFISH (NCT03032172) is a clinical trial which finished recruitment and is focusing on people aged between 6–60 years who had been treated with other SMA-directed therapies in the past. The analysis of safety data showed no drug-related safety finding [26, 31].

RAINBOWFISH (NCT03779334) is a single-arm, multicenter study which is currently in phase 2 and is focused on analyzing the efficacy, safety, pharmacokinetics, and pharmacodynamics in babies who are not yet presenting any symptoms. No results have yet been published [26].

4.3. Side effects

According to the SUNFISH clinical study the most common side effects observed were fever, cough, vomiting, upper respiratory tract infections, cold and sore throat. However, the most dangerous illness was pneumonia [26]. Impairment of fertility was also studied on rat models – oral administration to rats for 4 or 26 weeks resulted in histopathological changes in the testis (and epididymis at mid/high doses of risdiplam). Retinal abnormalities were observed in a 39-week toxicity study in monkeys – risdiplam taken orally induced functional abnormalities on the electroretinogram in all mid and high-dose animals earliest examination time. On week 22 retinal degeneration was detected by optical coherence tomography [27].

Table 1. Summary of risdiplam clinical trials Roche. Roche announces 2-year risdiplam data from SUNFISH and new data from JEWELFISH in infants, children and adults with spinal muscular atrophy (SMA) [29, 30, 31, 32, 33] SMN1- survival of motor neuron; SMN2- survival of motor neuron 2; MFM- Motor Function Measure

Clinical trial	Identification number	Characteristics	Patients profile	Main purpose of study	Current status of study
FIREFISH	NCT02913482	Two-part, open label study.	Infants aged 1-7 months of age with SMA type I and two SMN2 gene copies.	Part one: dose-escalation study Part two: investigation of efficiency at the dose selected in the first part.	The study met its primary endpoint.
SUNFISH	NCT02908685	Two-part, double blind, placebo-controlled study.	People between 2-25 years old with SMA type II or III.	Part one: dose- escalation study Part two: motor function evaluation using total score of MFM.	The study met its primary endpoint.
JEWELFISH	NCT03032172	Open-label exploratory trial.	People between 6 months – 60 years, previously treated with SMA-directed therapies	Safety and tolerability of daily risdiplam dose in non-naïve patients who have taken nusinersen, olesoxime or onasemnogene abeparvovec-xioi.	The study has completed recruitment.
RAINBOWFISH	NCT03779334	Single-arm, multicentre study.	Babies from birth to six weeks of age (at first dose) with genetically diagnosed SMA, without symptoms.	Efficacy, safety, pharmacokinetics and pharmacodynamics.	The study is currently in phase 2. No results were published yet.

5. Experimental therapies

The Food and Drug Administration and the European Medicines Agency have authorized three therapies thus far: Spinraza, Zolgensma, and Evrysdi are all SMN-enhancing medications, although other medicines are being tested in clinical studies as well. Currently, therapies in clinical trials are aimed at treating the disease's symptoms rather than the cause, which in SMA are neuromuscular function, muscle weakness, and muscle fatigue. Reldesemtiv (previously CK-2127107, CK-107) is a fast skeletal muscle troponin activator of the next generation (FSMT). Nerve impulses are diminished in numerous disorders, including SMA, and this adds to muscle weakness. Damage to motor neurons reduces calcium ion release, resulting in less effective muscle activation to produce a movement [34]. The calcium sensitivity of the troponin-tropomyosin complex is increased by reldesemtiv. The rate of calcium release from troponin slows, resulting in an increase in the amount of calcium in the body. The drug is currently in phase II clinical trials for people with SMA types II, III, and IV (NCT02644668) [35].

Scholar Rock's Apitegromab (SRK-015) is a myostatin inhibitor that allows the body's muscle mass to grow. Myostatin (GDF-8, growth differentiation factor 8) is a non-active polypeptide proMyostatin that inhibits muscle cell growth and differentiation. The mature growth factor is released from the precursor in two stages. Proprotein convertases cleave proMyostatin, resulting in the production of inactive, latent myostatin. Second, predomains are cleaved by a BMP/Tolloid protease, such as Tolloid-like protein 2 (TLL-2) or bone morphogenetic protein 1 (BMP1), releasing the mature growth factor and allowing it to interact with its receptor [36]. The conformational flexibility of known latency-related structural elements, as well as regions adjacent to the tolloid and furin of the proteolytic cleavage site, is affected by highly specific binding to the arm region in the prodomain of pro/latent myostatin, according to structural studies on the binding of apitegromab to proMyostatin and the latent form of the protein [37]. Positive results from a phase 2 clinical trial (TOPAZ, NCT03921528) contributed to Scholar Rock's announcement of a phase 3 study named SAPPHIRE (NCT05156320), which might be the final step for approval of SRK-015 as an addition therapy to conventional SMN treatment [38].

6. High hopes

Many different drugs have been attempted since the genetic basis of the disease was discovered, but the majority of them have failed. For instance, olesoxime (cholest-4-en-3-one, oxime) is a cholesterol-like compound that was first developed by Trophos [39, 40]. The drug was found to be safe and well-tolerated in phase 2 clinical studies in 3- to 25-year-olds with confirmed SMA type II or non-ambulatory SMA type III. However, there was no improvement in motor function when compared to those receiving placebo [41, 42]. The goal of the open-label extension study (OLEOS, NCT02628743) was to examine the safety, tolerability, and efficacy of the drug over a longer period of time. It was discovered that motor function looked to remain steady for 52 weeks, but then began to deteriorate [40, 43]. Roche decided to stop developing olesoxime in May 2018 because of technological challenges and lower-than-expected efficacy.

Novartis's Branaplam (LMI070, NVS-SM1) was in a phase II clinical trial in children with SMA type I (NCT02268552). It is a small molecule that is taken orally once a week. It was discovered during a high-throughput screening of pyridazine 2 and refined using multi-parameter lead optimization (Fig. 2C) [32]. It works by modifying SMN2 exon 7 splicing to increase the amount of functional SMN protein. Branaplam stabilizes the duplex U1:5′ splice site at the 5′ splice site SMN2 exon 7, thereby improving exon 7 inclusion (Fig. 3D). The drug has been shown in studies to improve the survival rate of a mouse model of SMA [33]. Novartis announced in July 2021 that it would no longer pursue branaplam as a treatment for SMA but would instead promote the molecule as a potential treatment for Huntington's disease.

PTC Pharmaceuticals, Roche, and the SMA Foundation collaborated to develop RG7800, a small molecule. An oral small-molecule compound was discovered through chemical screening and optimization to modify the splicing of SMN2 exon 7. A compound from the pyrazolopyrazine subclass has begun clinical trials in adults with SMA type II or type III, and the study is known as MOONFISH [42]. Initial results were promising as SMN protein levels doubled [43]. However, further studies were halted because it was discovered that the drug accumulates in the cornea of the experimental animals and is not removed quickly enough, which could lead to toxicity. The focus was on creating another chemical, RG7916, and participants in the MOONFISH (NCT02240355) research were invited to join in the JEWELFISH clinical trial of risdiplam (NCT03032172) [44].

The quinazoline derivative RG3039 was developed as an oral experimental medication to block a scavenger mRNA decapping enzyme (DcpS) and so enhance protein levels. The medication penetrated the blood-brain barrier into CNS tissues and inhibited the enzyme DcpS, however, SMN levels were low in early tests. DcpS enzyme activity is significantly suppressed in tissues from SMA mice, suggesting that enzyme activity could be used as a pharmacodynamic measure of medication activity in SMA patients' clinical trials [45]. RG3039 has also been proven to extend life and improve SMN protein levels in mice with varied degrees of illness severity [46]. Repligen, which created the drug, has secured a deal with Pfizer to continue developing RG3039. However, the contract was unexpectedly terminated in 2015, and progress on the drug was suspended.

Valproic acid (VPA) is a short-chain carboxylic acid, a drug with anticonvulsant properties and a histone deacetylase (HDAC) inhibitor with a terminal half-life ($t_{1/2}$) of 8–10 hours in human serum. The function of HDAC is deacetylation, which

can alter gene transcription selectively and thus promote chromatin condensation. It was able to conclude that histone deacetylase inhibitor drugs increase *SMN2* promoter activity by up-regulation of *SMN2* expression via inhibition of HDAC2 [47]. Initial studies have shown the acid to be safe, well-tolerated with no apparent hepatotoxicity, although two people developed carnitine deficiency [48]. However, while VPA enhances motor abilities in 2- to 3-year-olds, side effects such as increased body development and gastrointestinal difficulties have been reported in the treatment group in subsequent studies [49]. A study from genetically confirmed SMA type III [50]. All these data and the results of a study in adults (ages 17-25) confirm that VPA is a promising potential treatment [51].

Patients had high hopes when research on each of these drugs began; however, none of these drugs have been brought to market for various reasons.

7. Limitations and future directions for SMA therapy

The current treatment for SMA is insufficient. Several children were not cured after receiving nusinersen injections during the ENDEAR clinical study; several still need breathing support, assistance with feeding, and other daily routine tasks. In rare cases, motor development was interrupted, and some individuals died as a result [18]. Furthermore, the medication's intrathecal injection may cause tissue damage, infections, weakness, and malaise [14]. A large number of patients have severe scoliosis, making intrathecal injections extremely difficult, if not impossible [52]. Chronic treatment, on the other hand, can be a major challenge to conquer. Intranasal or oral administration is one technique to tackle this problem, although it is insufficient due to the blood-brain barrier. Because of the preservation of nusinersen in peripheral tissues, which can cause substantial damage, pharmacodynamics and pharmacokinetics must also be investigated [14]. Treatment with nusinersen did not result in significant improvement in adult patients. The compound muscle action potential (CMAP) was measured, and while there was an improvement in terms of higher CMAP amplitudes, there was a decrease in CMAP upon repetitive motor stimulation. Neuromuscular defects persist after treatment, implying that nusinersen treatment is ineffective in adult patients. Furthermore, there was a link between CMAP results and motor function (as measured by the 6-minute walk), fatigue, elbow extension, shoulder abduction, and revised upper limb module. This could imply that this disease is more complex in adults, and that in their case, all disabilities are secondary effects, and that restoring the SMN protein is insufficient. There is also no correlation between CMAP decrement and disease severity, disease duration, or patient age, implying that this disease is unique in each case and that there are individual differences. Examining these differences can lead to the best treatment method for each individual, but it will also be a difficult and time-consuming process. More clinical research is needed to confirm the short- and long-term tolerability, safety, effectiveness, and peripheral administration of all SMA therapies [52].

Another significant limitation is time, which is crucial in the treatment. To begin with, the disease is very aggressive, and the sooner treatment begins, the better the chances of survival and better results. The age of the patient at the start of therapy has a high correlation with treatment success. Early SMA therapy appears to be critical for enhancing therapeutic results. Elevating the SMN protein level in a patient with advanced SMA, who has essentially no motor neurons, is ineffective [18]. Second, the cost of drugs rises in tandem with the weight of the child, which rises rapidly as the child grows. Because of the extremely high cost of this treatment, it may be discontinued. Hopefully, the FDA re-

Table 2. Summary of approved therapeutics and substances under clinical trials for SMA [20, 23, 24, 28, 35, 36, 38]

Therapeutic	Management company	Characteristics	Patient's profile	Dosage and administration
Spinraza**	Biogen	Survival motor neuron 2 (*SMN2*) splicing modifier	All ages and types	Administered by intrathecal injection at an equivalent dose of 12 mg (4–5 ml based on age)
Zolgensma**	Novartis Gene Therapies*	Gene therapy based on using recombinant adeno-associated virus subtype 9 (AAV9) to overexpression *SMN1* gene	Less than two years old paediatric patients with spinal muscular atrophy and bi-allelic mutations in the *SMN1* gene	One intravenous infusion; dosage based on patient's body weight
Evrysdi**	Roche, Genetech Inc.	Survival motor neuron 2 (*SMN2*) splicing modifier	Patients 2 months of age and older suffering from SMA	Administrated orally once a day; dosage is determined by patient age and body weight
Reldesemtiv	Cytokinetics/Astellas	Muscle drug (non-*SMN*)	Patients with SMA types 2, 3 and 4 who are age 12 or older	Administrated orally; phase 2 trial completed
Apitegromab	Scholar Rock	Muscle drug (non-*SMN*)	Patient between 2 and 21 who have SMA type 2 or 3.	Administrated by intravenous infusion; phase 2 trial ongoing

**previously the name of the company was AveXis Inc.*
*** therapeutics approved by EMA and FDA*

cently accepted the lower pricing of risdiplam, which may result in other cost reductions [52].

It is also critical to reduce the cost of all treatments. Because of the extremely high cost, the treatment is not covered by all insurance companies in the United States. They added some restrictions to the treatment's availability, such as if the disease is caused by biallelic SMN1 mutations. Other restrictions include age, symptom manifestation, and disease stage. Furthermore, the high cost of the drug is not the only financial issue; there are numerous other expenses such as rehabilitation, the purchase of specialized equipment, administration costs, and the inability to work caused by the disease in the case of adult patients or parents who have children with SMA and must care for them full-time [53, 54, 55]. As a result, access to the treatment may be severely restricted.

Despite the fact that there are several approved medicines on the market and compounds in clinical trials, researchers are always looking for new drugs to help SMA sufferers. The future direction in which neuromuscular junction (NMJ) instability can be studied is worth emphasizing. It may be able to increase NMJ protection in adults by using phenotypic modifiers, and it may also be possible to undertake alterations in embryos that result in an improved postnatal phenotype. Mice have been used in such research with great success [56].

The goals of future research will then be to solve all of the previously mentioned problems. Because SMN-based therapies will be sufficient for the majority of SMA patients, it is crucial to enhance SMN restoration to improve motor function and to carefully examine all drug action and metabolism [52].

8. Conclusions

The discovery of genetic causes of SMA has allowed for the development of disease-treating therapies. Each of these therapies, however, has advantages and disadvantages. Nusinersen has transformed the lives of SMA children and their families. In 51% of patients, the treatment reduces the risk of death while also improving the patients' quality of life [18]. According to the findings of Sansone and colleagues (2020), the use of nusinersen aids in the treatment of respiratory problems by lowering mortality and the need for respiratory support [57]. However, there are some significant obstacles to overcome and better investigate in this treatment. Treating patients with spinal muscular atrophy who have bi-allelic mutations in the *SMN1* gene with onasemnogene abeparvovec is a fantastic opportunity as well as a hopeful alternative to long-term treatment with nusinersen. The results of various medical trials demonstrated that this therapeutic has the potential to improve overall survival as well as life quality by providing better motor functions. The disadvantage of this gene therapy is the patient age limit (less than 2 years old), which prevents onasemnogene abeparvovec from being available to dozens of people. It is possible that future research will change this. Despite the fact that clinical trials are still ongoing, the results of treating SMA patients with risdiplam show promising potential. In comparison to natural history studies, patients who received risdiplam had higher survival rates and improved motor functions. Risdiplam and onasemnogene abeparvovec do not have any dangerous side effects. Clinical trials are being conducted to test various drugs that increase SMN levels or with muscle-enhancing therapies. It is also important to note that, despite all of the risks and side effects associated with this therapy, patients and their families are adamant about completing the treatment at any cost. Simple things like preparing breakfast or sitting independently are sufficient milestones for participants. They admit that they are willing to go to any length for these simple things, demonstrating the importance of full participation in the treatment and its ongoing improvement [58].

Abbreviations

AAV9 - adeno-associated virus 9; **AO** - antisense oligonucleotide; **CMAP** -compound muscle action potential; **CSF** - cerebrospinal fluid; **ESE** - exonic splicing enhancer; **HINE** - The Hammersmith Infant Neurological Examination; **ISS** - intronic splicing silencer; **SMA** - spinal muscular atrophy; **SMN** - survival of motor neuron.

Authors' Contribution

M.P.: supervising the project, writing– original draft preparation, writing – review and editing, literature review, final proofreading, and approval of the version for publication; **M.G.**: funding acquisition, writing – original draft preparation, writing – review and editing, literature review; **J.W.**: writing – original draft preparation, writing – review and editing, literature review; **K.M.**: visualization, funding acquisition; **A.K.**: supervising the project, writing – review and editing, final proofreading and approval of the version for publication.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

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