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REVIEW ARTICLE

Wider perspective on Spinal Muscular Atrophy – pathogenesis, diagnosis, treatment

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Abstract

Introduction and Objective. Spinal muscular atrophy (SMA) is an autosomal recessive inherited disease that leads to the atrophy or weakening of skeletal muscles and changes in the brainstem. It is the second most common cause of infant mortality worldwide, with a prevalence ranging from about 1 in 6,000 to 1 in 10,000 live births. The cause of the disease is the presence of mutations in the survival motor neuron 1 (SMN1) gene on chromosome 5q11.2 to 13.3. The aim of this review is to summarize and compile the current state of knowledge on SMA, including its pathogenesis, diagnosis and treatment.
Review Methods. The review is based on scientific publications found in the PubMed, Scopus, and Google Scholar databases, published between 2018–2024.
Brief description of the state of knowledge. Genetic testing for SMA is the most accurate method, demonstrating a 100% positive predictive value. To determine disease severity, the number of SMN2 gene copies is analyzed, while carrier detection involves analyzing copies of the SMN1 gene. Clinical trials evaluated using the HFMSE scale demonstrated significant improvements in motor function with risdiplam, apitegromab, and nusinersen. Onasemnogene abeparvovec was assessed using the CHOP INTEND scale, also showed improvements in motor function.
Summary. Treating SMA presents a significant challenge for doctors, as selecting the appropriate therapy and timing its introduction are crucial. Currently, the FDA-approved drugs include nusinersen, onasemnogene abeparvovec, and risdiplam.

Key words

SMA, spinal muscular atrophy, SMA pathogenesis, MA diagnostics, SMA pharmacological treatment

INTRODUCTION

Spinal muscular atrophy (SMA) is the second leading cause of infant mortality worldwide. This autosomal recessive neurodegenerative disease involves progressive motor neuron degeneration in the anterior horn of the spinal cord, leading to skeletal muscle weakness, atrophy, and structural changes in brainstem regions [1, 2].The main cause of the disease is the presence of mutations in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q11.2–13.3. Prevalence and carrier frequency vary by ethnicity. The disease occurs in 1 in 6,000 – 1 in 10,000 live births, with higher rates in Caucasians and lower in Hispanics. Carrier frequency ranges from 1 in 25 – 1 in 209 [3]. Both *SMN1* and survival motor neuron 2 (*SMN2)* genes encode the survival motor neuron (SMN) protein, but transcripts derived from *SMN1* are full-length and generate functional protein, whereas the vast majority of transcripts derived from *SMN2* are truncated, generating a less stable version of the SMN protein. *SMN2* cannot fully replace *SMN1*, but its copy number inversely correlates with disease severity. SMN protein is vital for DNA repair, transcription, splicing, translation, stress granule formation, transport, cytoskeletal dynamics, and signalling [3]. Research on SMA patients and mouse models shows that SMN protein is crucial in all tissues, including the brain, spinal cord, liver, lungs, heart, skeletal muscles, ovaries, and testes. Literature indicates a gender-specific impact on SMN function and SMA pathogenesis. SMN is vital for male reproductive health, addressing global fertility concerns. The milder SMA phenotype in females may result from gender-dependent factors, such as X chromosome-linked modifiers, mitochondrial effects, and sex hormones [3].

SMA impacts the entire family and is categorized into five types based on symptom onset and motor function [4–6] (Tab. l).

Pathogenesis of SMA. Spinal muscular atrophy (SMA) is an autosomal recessive inherited disease caused by mutations in the *Survival Motor Neuron 1* (*SMN1*) gene [7]. Approximately 95% of SMA patients have a homozygous deletion of exon 7 of the *SMN1* gene located on chromosome 5q13. SMA can manifest with various phenotypes due to the existence of two versions in the human body: *SMN1* – the telomeric version, and *SMN2* – the centromeric version [5]. *SMN2* differs from *SMN1* by only 1% – it contains thymine instead of cytosine at codon 280 and exon 7 [8]. This subtle codon change disrupts splicing [9].

Transcription of the *SMN1* gene allows for the production of complete mRNA, from which functional SMN1 Protein can be synthesized. In contrast, transcription of the *SMN2* gene provides only 10–15% coverage for generating complete and functional mRNA. 85–90% of SMN1 Protein from *SMN2* transcription is non-functional, meaning that the *SMN2* gene results in the production of significantly less SMN1

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Table 1. Clinical classification of SMA [4,5,6]

SMA TYPE	SMA 0	SMA 1	SMA 2	SMA 3	SMA 4
Other names	Pre-natal, congenital SMA	Werding-Hoffmans disease, severe SMA, 'non-sitters' SMA	Intermediate SMA, 'sitters' SMA, Dubowitz disease	Kugelberg-Welander disease, mild SMA, 'walkers' SMA	Adult SMA
Age of onset	Prenatal	<6 months	6–18 months	>18 months	>21 years
Life expectancy	<6 months	<2 years without respiratory support	>2 years, 70% alive at 25-years-old	Adult, almost normal	Adult, normal
Highest Motor milestones	Unable to achieve any motor milestones	Some head movement control, sometimes can sit supported	Sits, never stands or walks	Stands and walks	All normal motor functions
SMN2 copy numbers	1	2	2–3	3–4	≥4
Other features	Areflexia Neonatal weakness Hypotonia Reduced prenatal movements Facial diplegia Atrial septal defects, Joint contractures, Respiratory failure	Poor head control Areflexia/ Hyporeflexia Swallowing difficulties Hypotonia Paradoxal breathing 'Frog leg' posture Tongue fasciculations Respiratory failure	Proximal muscle weakness Hyporeflexia Postural tremor Developmental delay Loss of motor skill Scoliosis	Fatigue Proximal muscle weakness Postural tremor Loss of patellar reflexes Resembles muscular dystrophy	Fatigue Mild and progressive muscle weakness

Protein [10]. Therefore, the deficiency of *SMN1* gene function can be compensated by *SMN2* function – the more copies of the *SMN2* gene, the less severe the disease, with milder phenotypes associated with the presence of 3 or more copies of *SMN2*. In a mouse model, expression of 8–16 copies of the *SMN2* gene completely alleviated disease symptoms [9].

The remaining 5% of cases consist of complex heterozygotes and carriers of intragenic mutations in *SMN1* – missense, nonsense, insertions, duplications, deletions, or splicing site mutations, with the missense mutation in exon 6 p.Ty272Cys being the most frequently described [11]. Complete absence of SMN1 Protein production is lethal [5].

Regarding SMN Protein itself plays a role in mRNA transport through axons and in the transport of beta-actin complexes with ribonucleoproteins. Another hypothesis suggests a role for SMN Protein in Small Nuclear RNA (snRNA) synthesis, thus in spliceosome formation, which excises introns from pre-mRNA. Importantly, motor neurons are sensitive to defective spliceosome function, leading to faulty mRNA splicing and the translation of abnormal proteins essential for motor neuron function [12]. In other words, SMN Protein forms a complex with Geminis 2–8 in the cytoplasm and in nuclear gems – bodies. This complex is responsible for the formation of Small Nuclear Ribonucleoproteins (snRNPs) and pre-mRNA splicing. In SMA patients, this complex is disrupted, leading to significant splicing disturbances [13].

Diagnostics. The initial SMA diagnosis involves a physical examination for such symptoms as muscle weakness, hypotonia, reduced tendon reflexes, and motor function loss, followed by a detailed family history. Genetic testing is crucial, with 95% of SMA patients showing homozygous deletion of exon 7 in the *SMN1* gene. Thus, genetic diagnosis usually focuses on exon 7 screening. This diagnostic method has shown a 100% positive predictive value, with no reported false positives to date. *SMN1* copy number analysis is essential for carrier identification, with new mutations linked to carrier status now being recognized. Quantifying *SMN2* copy number is critical for clinical classification, assessing disease severity, and prognosis. The C -> T nucleotide change in exon 7 underpins various molecular tests for detecting *SMN1/2* copy number alterations. The remaining 5% of patients may have pathogenic variants that can be missed in initial diagnostics [14]. The majority of these remaining patients will exhibit deletion in one allele and a point mutation (intragenic missense mutation, nonsense mutation, or reading frame shift) in *SMN1* on the second allele, resulting in compound heterozygosity [14, 15].

Early disease detection and appropriate interventions can significantly improve the clinical course of the disease; therefore, newborn screening is essential. Pre-symptomatic identification is crucial, thus rapid and reliable diagnostics are of paramount importance. Since the identification of the SMA gene in 1995, numerous genetic tests have been developed for SMA diagnosis, focusing on *SMN1/2* copy number determination. Current methods include single nucleotide conformation polymorphism, restriction fragment length polymorphism, real-time PCR (RT-PCR), denaturing high-performance liquid chromatography, multiplex ligation-dependent probe amplification (MLPA), quantitative PCR (qPCR), competitive PCR, high-resolution melting analysis, and liquid microarray analysis [2, 6].

Among the primary methods gaining superiority over others are qPCR and MLPA [2].

qPCR and similar PCR-based methods are limited to a few loci at a time and cannot detect gene conversions, novel variants, or copy number changes above 4 without a normal reference sample. In contrast, MLPA assays up to 40 targets simultaneously, distinguishing *SMN1* deletions from conversions and accurately measuring *SMN1/2* copy numbers. MLPA uses a single pair of primers for amplification and quantification of up to 20 genomic loci in one reaction with as little as 20 ng of DNA, allowing precise detection of *SMN1* and *SMN2* copy numbers. However, MLPA has limitations: DNA variants at probe binding sites may cause false positives, reactions are sensitive to contamination, it cannot analyze single cells or detect unknown point mutations, and it requires a CE analyzer, which is costlier than flat gel electrophoresis. The advantages and disadvantages of multiplex qPCR and MLPA as molecular genetic tests for diagnosing SMA are summarized in Table 2 [2, 6].

Whole-genome sequencing can offer insights into novel structural variants and mutations, aiding in understanding their role as disease modifiers and informing about therapeutic options. Recently developed saliva-based tests enable rapid, non-invasive SMA diagnosis, with new strip

Table 2. Advantages and limitations of qPCR and MLPA as molecular genetic tests for SMA diagnosis [2,6]

	Multiplex qPCR	MLPA
Advantages	• Low Cost • <4h work time • High accuracy • Can detect polymorphism in *SMN2*	• Can detect all copy numbers in all SMN exons • Biological material may be blood but also prenatal materials • High precision in detecting *SMN1* copies
Limitations	• Genetic material obtained from peripheral whole blood via EDTA-containing tubes or buccal swabs (quantities ranging from 10–40 nanograms per microliter). • Absence of identification of nonsense mutation, reading frame alteration, or amino acid substitution mutations. • Identification limited to asymptomatic carriers solely through the genetic makeup characterized by duplications in specific gene loci among particular ethnic groups. • Inability to ascertain the duplication levels across all exons of the survival motor neuron *SMN* gene. • Single nucleotide variances situated within the regions complementary to the primers might potentially influence the precise determination of *SMN1* and *SMN2* gene copy numbers	• Genetic variations within the binding regions of probes targeting *SMN1* alleles can impede the process of probe hybridization. • Assays are susceptible to the presence of contaminants, affecting the accuracy and reliability of reactions. • Current methodologies lack the capability to analyze individual cells, a crucial aspect for pre-implantation genetic screening. • Absence of identification of specific nucleotide alterations. • Methodology exhibits high sensitivity towards detecting minor genetic alterations such as small deletions, insertions, and nucleotide mismatches. • MLPA necessitates the use of a Capillary Electrophoresis analyzer, which represents a more expensive alternative, compared to conventional slab gel electrophoresis employed for Restriction Fragment Length Polymorphism analysis. • Inability to differentiate individuals carrying silent mutations in the genetic sequence. • Incapability to identify the presence polymorphism within the *SMN2* gene. • Extended duration of processing, requiring a time frame of 48 hours.

tests offering high sensitivity, portability, and lower cost for point-of-care use [16].

Sequencing offers comprehensive detection of affected individuals, but it has a lengthy implementation time. A challenge is identifying variants of uncertain significance, especially distinguishing between *SMN1* and *SMN2* due to their homology. Laboratories may also face difficulties if the variant is rare or unreported in other affected family members. Clinical evaluation and verifying the presence of the variant in other affected individuals are essential for accurate diagnosis [17].

Parental testing can clarify inherited sequence variants, aiding genetic counselling. Carrier screening is crucial for at-risk families. In March 2017, the American College of Obstetricians and Gynecologists recommended offering SMA carrier screening to all women planning to be or are currently pregnant. SMA carrier testing is usually included in a commercial panel for various autosomal recessive genetic disorders. A residual risk remains even if SMA is not previously recorded in the family, due to high carrier frequency and *de novo* mutations [14]. A limitation of carrier testing is determining whether *SMN1* copies are in cis or trans cis carriers or 2+0 carriers, comprise 3.7% of SMA carriers. These are individuals who have 2 copies of *SMN1* in cis, and current technologies are unable to detect them because they cannot identify the haplotype phase. Therefore, the residual risk for carrier status is higher upon a negative carrier screen or a carrier screening result identifying 2 copies of *SMN1*. 39 *SMN1* copies in cis are more common among individuals of Ashkenazi Jewish descent and African descent. Prenatal testing can proceed if both parents are carriers or if one parent is a carrier and the other has elevated risk. Two methods are available: chorionic villus sampling at 10–13 weeks gestation, and amniocentesis at around 15 weeks. Maternal contamination checks are performed on both sample types to ensure results reflect foetal, not maternal, genetic information [14].

Pre-implantation genetic testing for monogenic/single gene disorders can be employed pre-conception if carrier parents have been identified. To conduct pre-implantation genetic testing for monogenic/single gene disorders, *in vitro* fertilization must first occur.

Laboratories lack consensus on the optimal SMA carrier testing method. Most use next-generation sequencing to assess *SMN1* and *SMN2*, often supplemented by Sanger sequencing and multiplex ligation-dependent probe amplification for variant confirmation. Physical examination, risk assessment, and family history are key in choosing the testing method. Research is ongoing to develop accessible diagnostic technologies for earlier detection, especially in low-resource settings [2, 6, 14, 18].

Tests such as electromyography and muscle biopsy are feasible, and biochemical tests for muscle creatine kinase, released by deteriorating muscles, are sometimes performed, though not usually necessary. Biomarkers for SMA are urgently needed for treatment guidance and therapy assessment. Despite extensive research, *SMN* expression levels have not emerged as the primary SMA biomarker. However, recent studies suggest that full-length SMN transcript levels in blood extracellular vesicles may be a promising SMA biomarker. Potential biomarkers are described in Table 3 [19–23].

Pharmacological treatment. SMA therapy is a challenge for clinicians due to the diverse, often severe course of the disease, and the emerging market for treating the condition and gene therapy. In addition, clinicians must select the most appropriate method and the right time to start treatment. Currently, three drugs are approved by the American Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of SMA: nusinersen, onasemnogen abeparwowek and risdiplam [24]. Ongoing research on further potential drugs that could be used in SMA therapy include Apitegromab [25]. In addition, attention is being paid to a potential gene therapy mechanism based on the *ZPR1* and *PLS3* genes [26].

Nusinersen. The first approved drug for targeted therapy of SMA was nusinersen (Spinraza), approved by FDA in December 2016 and by EMA in May 2017 [27]. It was invented in 2010, and belongs to synthetic antisense oligonucleotides. The substance is 18-mer 2′-O-(2-methoxyethyl) (MOE) phosphonothioate oligodeoxyribonucleotides (ODN), also known as ASO-10–27 5′-TCACTTTCATAATGCTGG-3′ [28].

Table 3. Potential future candidates for SMA biomarkers [19,20,21,22,23]

Biomolecular candidates: • SMN protein • Neurofilament • Muscle indicators	• Diminished concentrations of circulating survival motor neuron protein exhibit a positive correlation with the progression and severity of the ailment. • Elevated neurofilament levels signify an escalated extent of axonal degeneration and denote a more advanced stage of the disease. • Various indicators of muscular integrity such as creatinine, creatine kinase, and other markers indicative of muscle damage demonstrate elevated levels in individuals with advanced stages of spinal muscular atrophy.
Genetic candidates: • SMN2 gene copy number or polymorphisms • Modifier genes	• A reduced number of copies of the *SMN2* gene exhibit a positive correlation with decreased quantities of SMN protein, and are indicative of a more advanced and severe stage of the disease. • Modifier genes, which encompass certain *non-SMN* genes, have the potential to alter the phenotypic expression of spinal muscular atrophy.
Gene transcription and splicing regulators: • Micro RNAs • Methylation factors • Long non-coding RNAs	• Research suggests that different microRNAs may vary in expression based on the severity of SMA. • Methylation of *SMN2* impacts its expression and could be used to measure survival motor neuron protein production. Genome-wide methylation patterns, involving genes beyond *SMN1* and *SMN2*, might also indicate disease severity. • Long non-coding RNAs can regulate gene expression, including the activation of *SMN2*.
Imaging candidates: • Muscle imaging approaches • Electrical impedance myography (EIM)	• MRI serves as a valuable tool for assessing spinal muscular atrophy severity, muscle atrophy, and treatment efficacy. • EIM detects muscle action potentials elicited by stimulation, demonstrating sensitivity to subtle changes in disease progression.
Electrophysiological parameters: • Compound muscle action potential (CMAP) • Motor unit number estimation (MUNE) • Repetitive nerve stimulation (RNS)	• CMAP measurements reflect the responsiveness of muscles to motor nerve stimulation, with decline observed at disease onset. • Motor unit number estimation techniques, achieved through various means including incremental stimulation, estimate the remaining motor units. • Repetitive nerve stimulation assesses neuromuscular junction function and serves as an exploratory marker of SMA disease progression.

It modulates the information splicing of *SMN 2* precursor RNA by promoting the incorporation of exon 7 into *SMN 2* mRNA transcripts. Through this mechanism, there is an increase in the synthesis of the complete SMN protein [27].

Patients with SMA are administered 12 mg of nusinersen intrathecally through a lumbar puncture. The schedule of substance intake is based on a day model: 0, 14, 28 or 63. Thereafter, booster doses are given every 4 months [29]. Through such an intake schedule, the drug is accumulated in an effective concentration in the fluid surrounding the spinal cord and brain. The drug is approved for the treatment of newborns, children and adults suffering from SMA with a confirmed 5q genetic mutation [30].

Nusinersen is absorbed into the central nervous system (CNS). Its highest concentration is determined in CNS tissues, plasma, skeletal muscle, liver and kidneys. Bioavailability after intrathecal administration is 100%. However, the time of maximum drug concentration in plasma is found after 2–6 hours [31]. It is excreted primarily via the renal-urinary route in the form of pharmacologically inactive truncated oligonucleotides [28]. It is estimated that the half-life of the drug in the final elimination phase in cerebrospinal fluid is 135–177 days, and in plasma – 63–87 days [30].

The study involved 323 infants and children with SMA, who were divided into 2 groups: 83 underwent sham treatment, and 240 received nusinursen. At least one adverse symptom in the study group occurred in 96% of the subjects, and in the control group in 99%. The most common symptoms in those taking nusinursen were fever (48%), upper respiratory tract infection (39%), nasopharyngitis (25%), vomiting (24%), headache (22%), back pain (17%), scoliosis (13%), and post-lumbar puncture syndrome (11%). In the control group, symptoms were found in: 47%, 34%, 23%, 16%, 4%, 0%, 6%, 0%. Severe side-effects were reported by 7% of subjects [32].

The efficacy study of nusinersen involved 21 paediatric patients, 14 of whom were taking the drug, and 7 belonged to the control group. After 16 months of therapy, the patients were evaluated using the Hammersmith Infant Neurological Examination – 2 (HINE-2) [33]. This scale serves as a motor milestone test. It assesses the achievement of milestones: sitting, voluntary grasp, head control, ability to kick in supine, rolling, crawling on all fours or bottom shuffling, walking and standing. The results showed that milestone response and an increase in HINE-2 scores were seen in 29% in the control group, and 79% in the research group [33].

Table 4. Improvement in assessed milestones on the Hammersmith Infant Neurological Examination – 2 (HINE – 2) scale in a study on paediatric patients [33]

Milestones:	Research group	Control group
Rolling	64%	0%
Sitting	64%	14%
Head control	36%	0%
Crawling on all fours or bottom shuffling	21%	14%
Walking	7%	0%

Onasemnogen abeparvovek. Onasemnogen abeparvovek (Zolgensma) is among the most expensive drugs in the world, withthe cost of a single dose ranging from $3 million – $6 million. The drug was approved by the FDA in May 2019 and conditionally at the EMA in May 2020, but received full marketing authorization in May 2022 [34]. Zolgensma is the first gene therapy for the treatment of SMA, during which direct delivery of the *SMN1* gene and production of SMN protein occurs [35]. The therapy is based on adeno-associated virus (AAV) vectors (an adeno-associated virus (AAV) vector-based therapy). The AAV9 capsid delivers a fully functional copy of the *SMN* gene after control of the cytomegalovirus enhancer/chicken-β-actin hybrid promoter [36].

Zolgensma crosses the blood-brain barrier and can therefore be administered intravenously. In the US, it is indicated in the treatment of patients with SMA under 2 years of age, and in Europe in patients with SMA 1 or no more than 3 copies of *SMN 2,* regardless of disease form, age and weight [37]. Patients receive the drug in a single

intravenous infusion lasting about 1 hour at a dose of 1.1 × 10 14 vg/kg. Patients receive 1 mg/kg of prednisolone orally 24 hours before receiving Zolgensma. After receiving gene therapy, patients take 1 mg/kg prednisolone orally for the next 30 days [38].

In 90% of patients taking onasemnogen, an increase in aminotransferase activity is found, therefore the drug is potentially hepatotoxic. The mechanism of liver damage involves an immune response triggered by the expression of the viral vector, or *SMN* gene products in the organ. Prophylactic corticosteroids are administered to prevent this Other side-effects observed during this gene therapy are thrombocytopenia, cardiac abnormalities, vomiting, and fever. Because of the risk of complications, troponin levels, platelet counts, and liver enzymes should be monitored [39].

After intravenous administration of Zolgensma, the DNA vector is quantifiable at low concentrations in saliva, declines to undetectable levels within 3 weeks and at very low concentrations in urine, and disappears after 1–2 weeks. The drug is excreted primarily in faeces [36].

Twenty-two patients under the age of 6 months with SMA 1 were included in the study, each of whom was given onasemnogen abeparvovide intravenously, of whom 19 completed the study as 2 patients dropped out before the age of 14 months, and 1 died due to respiratory failure (not caused by the drug). Analysis of the results showed that 41% of the patients maintained normal developmental ability after taking the drug, 86% took exclusively oral feedings, and 64% maintained age-appropriate weight. In terms of motor skills, 86% of the children saw an improvement of at least one milestone. At 18 months of age, as many as 18 patients did not require ventilatory support. In addition, patients were evaluated using the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) scale, which is used to assess motor function. The score range is 0–64, where the higher the score, the better the patient's development. Analysis showed that 95% of subjects scored above 40, 64% scored above 50, and 22% scored above 60 [40].

An analysis of the adverse effects occurring was also performed. A minimum of 1 adverse symptom was observed in each patient. It was observed that 55% experienced fever, 50% upper respiratory tract infection, 41% constipation and scoliosis, 32% cough, and increased aspartate aminotransferase activity in 27%, and alanine aminotransferase activity in 23% [40].

Table 5. Improvements in motor function based on the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) scale in paediatric patients taking Zolgensma are shown [40]

Time after taking the drug	Average increase in points on the scale CHOP INTEND
1 month after taking the drug	6.9
3 month after taking the drug	11.7
6 month after taking the drug	14.6

The study ultimately included 14 children with biallelic *SMN1* deletions and 2 copies of *SMN2* who were administered Zolgensme. The patients' results were compared with 23 naturally ill patients (Pediatric Neuromuscular Clinical Research PNCR). Analysis of the therapy patients showed that 100% of them achieved the ability to sit independently for at least 30 seconds before the age of 18 months. In the group of untreated SMA 1 patients, none of the 23 children achieved this ability. The ability to sit independently by 18 months of age was achieved by 12 patients; 64% achieved the ability to walk independently by taking a minimum of 5 steps, thus achieving the ability of coordination and balance. None of the patients required artificial respiratory ventilation, whereas in the untreated cohort, 26% used it. Body weight at or above the 3rd percentile was found in 93% of patients [41].

Risdiplam. Risdiplam (Evrysdi) is the first oral SMA drug to be approved by the FDA in August 2020 and at the EMA in March 2021 [42]. In the US, the drug was qualified for the treatment of SMA in patients over 2 months of age, while in 2022, the patient pools were expanded to include those who were younger. In Europe, indications have been narrowed to SMA types 1, 2 or 3 and in patients with up to 4 copies of *SMN2*. The drug is classified as an *SMN2*-directed pre-mRNA splicing modifier, resulting in an increase in *SMN2*'s ability to produce a complete and functional SMN protein [43].

Risdiplam dosage depends on the patient's age and weight. Patients under 2 months of age take 0.15 mg/kg [44]. Children between 2 months and 2 years of age take 0.2 mg/kg, while those over 2 years of age and under 20 kg take 0.25 mg/kg, and over 20 kg the dose is 5 mg. Patients take the drug orally after a meal, once a day [45].

Risdiplam has the ability to penetrate the blood-brain barrier. The half-life of the drug is about 50 hours [46]. Maximum plasma concentration is reached after 1–4 hours. The substance is metabolized primarily using flavin monooxygenase 1 and 3 (FMO1 and FMO3) and by CYP1A1, CYP2J2, CYP3A4 and CYP3A7 [43]. The unchanged form of the drug in circulation accounts for 83%, and the main circulating metabolite is pharmacologically inactive. Risdiplam is excreted mainly in faeces (53%, where 14% is the unchanged form) and urine (28%, with 8 in the unchanged form) [47].

The study included 21 infants between the ages of 1–7 months. Each of them took risdiplam, but 4 of them at a low dose (last dose – 0.08 mg/kg) and 17 at a high dose (last dose – 0.2 mg/kg). The patients had symptomatic and genetically confirmed SMA, and had 2 copies of *SMN2*. Patients who had not previously received any *SMN2*-targeted therapies or gene therapy were eligible fot the study. After 12 months of therapy, significant improvements in function were seen in patients taking the higher dose of risdiplam. In 33% of the infants, they had the ability to sit unsupported for a minimum of 5 seconds. 9 patients kept their heads upright consistently, and 1 was able to maintain a standing position. The infants were also assessed for swallowing skills. None of them lost this ability and were able to take food by mouth [48].

Non-dependent patients between the ages of 2–25 years with clinical symptoms characteristic of SMA 2 or 3, were eligible for the study. 180 patients were eventually included, who were divided into 2 groups; 120 took risdiplam,and 60

Table 6. Mean values of the survival motor neuron (SMN) levels in patients taking risdiplam [48]

Average SMN concentration	Group using low dose of risdiplam	Group using high dose of risdiplam
Initial	1.31 ng/ml (0,58–4,82).	2.54 ng/ml (1.1–6.4)
After 4 weeks of therapy	4.49 ng/ml (2,61–5,55)	5.87 ng/ml (2.84–8.76)
After 12 months of therapy	3.05 ng/ml (1,75–5,51)	5.66 ng/ml (2.66–8.6)

placebo for 12 months, followed by risdiplam. Four patients (3 from the study group and 1 from the control group) did not complete the study. Doses of the drug were 0.25mg/kg in patients weighing less than 20 kg and 5 mg in those weighing more than 20 kg. Patients were evaluated using the HFMSE scale, and it was deduced that after 2 years of therapy there was an improvement in scores of at least 2 points in 45% of the subjects. In addition, the Revised Upper Limb Modul (RULM) scale was used, with an improvement of at least 2 points in 52% of patients. Over the course of the study, adverse symptoms were noted in 91.7% of patients taking the drug alone. While taking placebo, they were found in 91.7%, while after switching to risdiplam they were found in 80%. The most common adverse reactions in the first year of taking the drug were reported: upper respiratory tract infection (31.7%), nasopharyngitis (25.8%), fever (20.8%), headache (20%), diarrhea (16.7%), vomiting (14.2%), and pneumonia (7.5%). The same symptoms were observed in the group of patients taking placebo in: 30%, 25%, 16.7%, 16.7%, 8.3%, 23.3% and 1.7% [49].

Apitegromab. Apitegromab is a human monoclonal antibody that binds to proformins of myostatin, promyostatin and latent myostatin, so it has the ability to inhibit the function of this protein. It belongs to transforming growth factor beta (TGF-β). Through expression primarily in skeletal muscle cells, it leads to inhibition of muscle growth via activin receptors [25].

The study included 58 patients between the ages of 2–21 who had SMA type 2 or 3. The patients were divided into 3 groups and followed for 12 months. The first contained 23 ambulatory patients between the ages of 5–21, 11 of whom were taking 20mg/kg of apitegromab and 12 were additionally still receiving nusinersen. The second included 15 outpatients between the ages of 5–21, who were taking 20mg/kg of apitegromab and nusinersen. The third group, on the other hand, included 20 non-inferior patients over the age of 2, all of whom were taking nusinersen, and 10 of whom were additionally receiving apitegromab at a dose of 2 mg/kg, with the rest receiving 20 mg/kg. After 12 months, patients were evaluated on the Revised Hammersmith Scale (RHS) or HFMSE. After analyzing the results using the RHS in Group 1, it was noted that after taking the antibody, an increase of 3 or 5 points occurred in 27.3% and 9.1%, but in patients receiving nusinersen at the same time, the percentages were 16.7% and 0%, respectively. In Group 2, the HFMSE scales were used and it was found that a minimum 2-point increase occurred in 35.7% of patients, where a 3- or 5-point increase was seen in 28.6% and 14.3%. In Group 3, it was found that there was an increase in HFMSE scores by an average of 6.2. In addition, an improvement in the number of milestones was noted. Two patients taking 20 mg/kg showed an improvement in achieving milestones (1 by 3, the other by 1). In contrast, 1 new milestone was noted in 1 patient taking 5 mg/kg [50].

CONCLUSIONS

SMA is an autosomal recessive inherited disease that leads to the atrophy and weakening of skeletal muscles and changes in the brainstem. There are 5 types of the disease, with type 0 being the most severe, with the result that most of the afflicted infants do not survive beyond 6 months. A homozygous deletion in exon 7 of the *SMN1* gene located on chromosome 5q13 is characteristic of 95% of patients with SMA. SMA occurs in 2 variants: telomeric *SMN1* and centromeric *SMN2*. Importantly, the function of the *SMN1* gene can be compensated by the *SMN2* gene, resulting in a milder clinical course. The SMN protein, as a key component of motor neurons, is essential for their proper function. Motor neurons of the spinal cord are most vulnerable to SMN protein deficiency, as *SMN* expression in the spinal cord remains consistently high throughout life.

Genetic testing for SMA is the most accurate method, demonstrating a 100% positive predictive value. These tests detect deletions in exon 7 of the *SMN1* gene. To determine disease severity, the number of *SMN2* copies is analyzed, while carrier detection involves analyzing copies of *SMN1*. Notably, the methods gaining an advantage in diagnostics are MLPA and qPCR. An important aspect of diagnostics is the detection of carrier status in parents before pregnancy through preimplantation genetic testing or during the prenatal period.

Treating SMA presents a significant challenge for doctors, as selecting the appropriate therapy and timing its introduction are crucial. Currently, the FDA-approved drugs include nusinersen, onasemnogene abeparvovec, and risdiplam. Nusinersen is administered intrathecally via lumbar puncture. Patients undergoing this therapy must receive maintenance doses every 4 months after the initial dose. In contrast, onasemnogene abeparvovec is administered as a single intravenous dose. Therefore, from an economic standpoint, it appears more cost-effective to administer onasemnogene abeparvovec just once. Risdiplam, on the other hand, is the first oral medication for SMA. Clinical trials evaluated using the HFMSE scale demonstrated significant improvements in motor function with risdiplam, apitegromab, and nusinersen. Onasemnogene abeparvovec was assessed using the CHOP INTEND scale, also showing improvements in motor function.

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