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New prospects fot the treatment of Spinal Muscular Atrophy

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Abstract

Introduction: Spinal muscular atrophy (SMA) is one of the most common genetically determined causes of infant and young child death.

The aim of the study: Review of medical literature on therapeutic strategies used in the treatment of SMA.

Material and method: Standard criteria were used to review the literature data. The search of articles in the PubMed database was carried out using the following keywords:… ..
Description: Therapeutic mechanisms in SMA are directed to: 1) modification of pre-mRNA splicing of the SMN2 gene; 2) gene therapy; 3) neuroprotection; 4) improving skeletal muscle function.
Summary: The gigantic advances in the development of therapeutical strategies resulted in the approval of two drugs for the treatment of SMA and the commencement of clinical studies on numerous with bundles.

Keywords: nusinersen, zolgensma, reldesemtiv

1. INTRODUCTION

Spinal muscular atrophy (SMA) is a rare neuromuscular disease which is one of the most common genetically determined causes of infant and young child death [1]. About 1 in 6.000 to 10.000 live births occur in the disease. The essence of the disease is the degeneration of the motor neurons of the spinal cord, which results in damage to the neuromuscular junctions (NMJ), progressive impairment of skeletal muscles, as well as their atrophy [2, 3]. Ultimately, the disease leads to death due to respiratory failure [4].

The clinical picture of SMA is heterogeneous, therefore the disease has been divided into five types depending on: 1) the age of onset of symptoms; 2) the highest achieved level of motor development; 3) life expectancy (table 1).

Table 1. Classification of types of spinal muscular atrophy [5, 6]

type of SMA	**The most common age of of onset of symptoms**	**The highest level motor development**	**The age of natural death**
type 0	before birth	very complex motor and nerve deficits, respiratory failure	before or within a few weeks after birth
type I (Werdnig-Hoffmann disease)	during 6 months old	flaccid baby, unable to lift the head, not sitting without support	death in infancy
type II (Dubowitz disease)	from 6 to 12 months old	possibility of staying in a sitting position, no independent walking	survival to adulthood
type III (Kugelberg-Welander disease)	after 12 months old	takes steps without support	normal life expectancy
type IV	over 30 years old	normal, mild damage to motor functions	normal life expectancy

People with type I SMA require the most intensified treatment, because the child's condition deteriorates very quickly. Generalized flaccidity, hyporeflections or areflexions, difficulty sucking and swallowing, and weakness of screaming and breathing begin to appear [7]. The infant is unable to lift the head and maintain a sitting position without support. The muscles of the extremities, torso, lungs and esophagus are rapidly weakened, which usually

leads to loss of swallowing ability and respiratory failure. The risk of death is very high, and life expectancy in the absence of assisted breathing is less than two years [7].

In types II and III, highly specialized care is also needed, but the risk of premature death is much lower - these people have the opportunity to achieve a normal life expectancy, but with significant physical disability [8].

2. GENETIC SUBSTRATE OF SMA

The cause of neuromuscular disorders in spinal muscular atrophy is the deficiency of the survival motor neuron (SMN) protein, which results from the deletion or mutation of the SMN1 gene [9]. The SMN protein is part of the multi-protein complex, in which there are at least six other proteins called Gemins2-7 [10]. The entire protein complex plays several key regulatory functions in neuronal cells, including: 1) the assembly of small nuclear ribonucleoproteins (snRNP) 2) actin dynamics; 3) transport of mRNP; 4) ubiquitin homeostasis; 5) biogenesis of fg5 telomerase and 6) the release of synaptic vesicles [11, 12, 13].

The spare SMN2 gene, present in all individuals, is an almost identical copy of the SMN1 gene [14]. However, the protein resulting from the SMN2 gene is not able to compensate for the SMN1 mutation. This is probably due to a change in position 6 of exon 7 SMN2 (c6t), which leads to the exclusion of exon 7 during pre-mRNA assembly and consequently, to the formation of a shorter, unstable SMN2Δ7 protein, which does not function as a full-length protein formed from SMN1 (SMN-FL) [15]. Research conducted in laboratories at the University of Massachusetts Medical School in the USA has shown that the main inhibitory element in the regulation of exon 7 SMN2 splicing is Intronic Splicing Silencer N1 (ISS-N1) [16].

Due to the fact that patients with SMA have a different number of copies of the SMN2 gene, it has been proved that a higher number of copies of this gene causes a less acute phenotype of the disease [17]. Thus, the variation in clinical severity observed in SMA is explained by different levels of SMN protein derived from the SMN2 gene [18].

3. MECHANISMS USED IN THE TREATMENT OF SMA

The identification of the gene responsible for spinal muscular atrophy in 1995 allowed the search for an effective drug to be started (table 2) [19].

3.1. CORRECTION OF SMN2 PRE-MRNA SPLICIN

3.1.1. Nusinersen

Nusinersen (Spinraza) discovered in 2004, was first approved for the treatment of all forms of spinal muscular atrophy in infants and adults in the USA in December 2016 [20]. From May 2017, the drug was also approved for SMA treatment throughout the European Union. The drug belongs to the group of antisense oligonucleotides (ASO) and its action is based on the modification of SMN2 gene splicing in such a way as to increase the production of fully functional SMN protein [21]. ASO by binding to the intronic splicing silencer N1 (ISS-N1) causes suppression of splicing. This leads to the inclusion of exon 7 in SMN mRNA, thus forming a full-length SMN protein.

In the years 2015–2016 two phase III studies were carried out for nusinersen: the ENDEAR study (NCT02193074), in which 127 infants with SMA type I participated, and the CHERISH study (NCT02292537) with 126 children with SMA type II [22].

Table 2. New Therapeutic Approaches in Spinal Muscular Atrophy: Current Clinical Trials

Mechanism	Drug	ClinicalTrials.gov Identifier:	Type of SMA	Clinical stage
increases the production of SMN protein from the SMN2 gene	**Nusinersen (Spinraza)**	NCT01494701	I, II, III, IV	approved drug
		NCT01703988	I, II, III, IV	
		NCT01839656	II	
		NCT02193074	I	
		NCT02292537	II	
		NCT02386553	pre-symptomatic	
		NCT02462759	I	
		NCT02865109	I	
		NCT03709784	II, III	
	RG3039	-	-	suspended
	RG7800	NCT02240355	I, II, III	suspended
	RG7916 (Risdiplam)	NCT02633709	healthy people	phase 1, completed
		NCT02913482	I	phase 2 and 3, active
		NCT02908685	II, III	phase 2 and 3, active
		NCT03032172	II, III	phase 2, recruting
		NCT03779334	pre-symptomatic	phase 2, recruting
		NCT03988907	healthy people	phase 1, recruting
	LMI070 (NVS-SM1; Branaplam)	NCT02268552	I	phase 1 and 2, active
SMN1 gene therapy	**AVXS-101 (Zolgensma)**	NCT02122952	I	approved drug
		NCT03306277	I	
		NCT03381729	II	
		NCT03505099	pre-symptomatic	
		NCT04042025	I, II, III	
improvement of muscle contractility	**CK-2127107 (CK107; Reldesemtiv)**	NCT02644668	II, III, IV	phase 2, completed
	SRK-015	NCT03921528	II, III	phase 2, recruiting
neuroprotection	**TRO-19622 (Olesoxime)**	NCT02628743	II, III	suspended

Both studies showed that the drug improved muscle performance measured on the Hammersmith Functional Motor Scale (HFMS) by an average of 4 points compared to baseline and 5.9 points compared to the control group. Another study has been conducted since 2015 to assess the effect of nusinersen in preventing the onset of SMA symptoms in 25 newborns with genetically confirmed disease who have not yet had their first symptoms

(NURTURE - NCT02386553) [23]. Recent, partial results of this study have shown that all children participating in the study did not require chronic ventilation and reached the stages of motor development expected for their age. The average index in test called Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), which is a measure of general motor activity in infants, was 58.4 points [24].

3.1.2. Small molecules

In the last decade, many companies and academic laboratories have focused on the small molecules, which, like nusinersen, are directed to the SMN2 gene and by modifying pre-mRNA splicing, increase the levels of functional SMN protein.

One of the first small molecules studied for the treatment of SMA was a quinazoline derivative called RG3039. It was assumed that the substance, by inhibiting the DcpS protein, would increase the production of full-length SMN protein [25]. DcpS protein is a scavenger mRNA-decapping enzyme, which removes of short mRNA fragments containing the 5 ′ mRNA cap structure, which appear in the 3′ → 5′ mRNA decay pathway, following deadenylation and exosome-mediated turnover [26]. Thus, scientists believed that RG3039 by blocking DcpS should increase levels of mRNA which could produce functional SMN protein. The results of two preclinical studies on SMA mouse models were published in 2013 [27, 28]. On their basis, it was found that RG3039 crosses the blood-brain barrier and reaches motor neurons, resulting in a significant improvement in the motor function of mouses. Researchers used these studies to estimate the dose of RG3039 to test the drug in human clinical trials. The blinded phase 1a study examined a single increasing dose of RG3039 in 32 healthy volunteers, while the phase 1b study evaluated the effect of multiple increasing doses of the drug in 32 other healthy volunteers. Researchers presented the results of this study at the Cure SMA conference in 2014 - the drug was shown to inhibit more than 90% of DcpS, but SMN protein levels did not change. Due to the fact that increased levels of SMN protein were considered necessary in helping patients, RG3039 found that it would be ineffective in patients with SMA and suspended further work on the drug [29].

Another small molecule which brought hope in the treatment of SMA was a substance called RG7800. The drug proved to be well tolerated in a study in healthy volunteers, so at the end of 2014, the MOONFISH clinical trial (NCT02240355) was started, in which RG7800 was administered to adults with SMA type II and III [30]. However, after three months, the study was suspended due to observation of corneal accumulation in experimental animals.

Researchers from Roche have developed another small molecule which modifying splicing SMN2 called RG7916 (risdiplam). Currently the drugi is at the stage of advanced clinical trials [31]. In August 2016, a phase 1 study (NCT02633709) was completed to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of increasing doses of risdiplam in healthy volunteers [32]. Thanks to this study, the optimal dose was determined for people with SMA and four phase II clinical trials with SMA patients were started.

FIREFISH (NCT02913482) is a two-part study in 21 infants aged 1 to 7 months with SMA type I [33]. This is an open study, which means that all children in the study receive the medicine and there is no placebo group. The aim of the first part of the study was to assess safety and determine the optimal dose of the drug for the second part of the study. Participants received increasing doses of risdiplam once a day for 4 weeks before switching to the extension phase. Part 1 of the study has been completed. In the second part of the study, participants will receive risdiplam at the dose set out in part 1 of the study, with treatments going to continue maximum up to 24-months. The main analysis will be based on how many children can sit unassisted after 12 months of treatment. In May 2019, during the American Academy of Neurology conference in the USA, partial results of part 1 of the FIREFISH

clinical trial were revealed [34]. The overwhelming majority of children have achieved major milestones of development according to the Hammersmith Infant Neuromuscular Examination (HINE-2) scale and showed improvement of motor functions on the CHOP-INTEND scale. The median improvement on the CHOP-INTEND scale was 16 points, while 57% of children achieved at least 40 points on the CHOP-INTEND scale. No child required tracheotomy or permanent ventilation, or lost swallowing skills. Two infants died during the study for reasons not related to the study preparation. The most common adverse events are fever (52.4%), upper respiratory tract infection (42.9%), diarrhea (28.6%), vomiting (23.8%), cough (23.8%) pneumonia (19 , 0%) and constipation (19.0%). The results of part 2 of the study are likely to appear at the end of 2023.

SUNFISH (NCT02908685) assesses the safety, tolerability and efficacy of risdiplam in 51 people with type II or type III SMA [35]. There are two groups in the study, which have also been divided into two parts. The first group includes young people and adults (between 12 and 25 years old), while the second group consists of children between 2 and 11 years old. In each group, participants receive risdiplam or placebo for 12 weeks to determine the optimal dose (part 1). In part 2, participants are treated for 24 months at the dose set in part 1 of the study. At the end of the 24-month period, participants are offered the opportunity to participate in an open extension study. The study began in autumn 2016, while in March 2019 on the Myology Congress in Bordeaux partial results of the study was presented [36]. After 4 weeks, risdiplam doubled the SMN protein level in the participants on average compared to the level before administration. In addition, after 12 months of taking the drug, all participants experienced an improvement in muscle function measured on the Motor Function Measure (MFM) scale, with improvement being greater in the group of younger participants (age 2-11 years) than in the older group (12-25 years). Part 2 of the SUNFISH study is currently underway. The results are expected at the end of 2020.

Another test using risdiplam is the JEWELFISH test (NCT03032172). It aims to assess the effectiveness of risdiplam in adolescents and adults aged 2-60 years who have previously taken RG7800 as part of clinical trials [37]. The study was launched in January 2017 and is currently being expanded. The qualification process was also opened for patients with SMA receiving nusinersen as part of standard treatment or olesoxime as part of a clinical trial. The RAINBOWFISH study (NCT03779334) concerns newborns up to 6 weeks whose genetic testing indicates SMA, but the first symptoms of the disease have not yet appeared [38]. The study will test the effectiveness of risdiplam in preventing the symptoms of the disease. The program is currently at the stage of recruitment of participants. The latest clinical trial of risdiplam is the open two-part, non-randomized trial NCT03988907 [39]. It aims to assess the effect of risdiplam on the pharmacokinetics of midazolam after oral administration to healthy adult men and women.

A promising small molecule drug which is also at the stage of experiments is the compound LMI070 (branaplam). It was discovered by Novartis after testing about 1.4 million compounds that could increase SMN protein production [40]. After identifying branaplam, scientists tested it on mouse SMA models and found that it increases the amount of SMN protein, which leads to improved body weight and animal survival [41]. At the end of 2014, Novartis began the clinical trial program with LMI070. In clinical centers in Belgium, Denmark and Germany, the preparation was started for 13 infants with SMA type I [42]. Initial observations showed an amazingly large improvement in motor function in most children participating in the study. In May 2016, further enrollment was halted and drug doses were discontinued for those already participating in the study, because animal studies during ongoing human studies showed serious side effects such as peripheral nerve, spinal cord and blood vessel damage. After two months, it was found that the toxic effect applies only to this specific species of animal and in 2018 confirmation of the safety of the preparation was

obtained. Therefore, the possibility of conducting clinical trials with branaplam was restored and extended to other countries.

3.2. REPLACEMENT OF THE SMN1 GENE

Gene therapy is focused on compensating for genetic defects by introducing the right copy of the desired DNA into human cells, which usually occurs through the use of viral vectors [43]. This method is an extremely promising treatment option for monogenic diseases.

In May 2019, the American Food and Drug Administration (FDA) approved the first gene therapy for the treatment of SMA [44]. AVXS-101 (zolgensma) is approved for the treatment of all types of SMA in children up to 2 years of age, including pre-symptomatic infants. The idea of gene therapy in SMA is to provide copies of the SMN1 gene to motor neurons [45]. The DNA sequence is introduced using a prepared virus from the AAV9 family. When the SMN1 transgene reaches the patient's cells, it completes the production of SMN protein by these cells. Since the neuronal cells in the spinal cord are not divided, it is assumed that a single administration of gene therapy should be enough for the patient's whole life.

The decision regarding FDA approval was based on the positive results of the completed phase 1 START study and the ongoing phase 3 STR1VE study. The START study (NCT02122952) was conducted to evaluate the safety, tolerability and efficacy of the drug in 15 infants with SMA type I [46]. The effects of low or high dose treatment after 24 months of treatment were assessed. At the end of the study, each of the 12 patients receiving the high dose did not require constant ventilation. 11 patients achieved and retained the ability to swallow on their own. 11 children were able to keep their heads straight. 11 tested children achieved the ability to sit independently for at least 5 seconds, 10 for at least 10 seconds, and 9 for at least 30 seconds. In addition, 3 patients were able to stand and walk on their own. The STR1VE study (NCT03306277) is an ongoing 3 phase study that includes 20 infants with type I SMA [47]. Preliminary results showed that in six children who received Zolgensma, the average CHOP-INTEND score increased by an average of 7.8 points after a month of treatment [48].

Other clinical trials using zolgensma are also currently underway. In the STRONG phase 1 clinical trial (NCT03381729), which is currently in the recruitment phase, different doses of AVXS-101 are tested in children with type II SMA up to the age of 5 [49]. The drug is administered once in a small or high dose to the spinal cord. The planned completion date of the study is June 2021. A phase 3 clinical trial called SPR1NT (NCT03505099) is currently recruiting infants with SMA up to 6 weeks of age who are not yet showing any symptoms of the disease [50]. The end of the study is planned for 2023. The planned study called REACH (NCT04042025) is a study covering all patients, involving patients with type I-III SMA, aged 6 months to 18 years who do not qualify for other studies [51].

3.3. IMPROVEMENT OF MUSCULAR CRISIS

Strategies that do not deal with genetics associated with SMN are also being investigated in SMA therapy. Such a therapeutic approach is to increase the muscle's ability to contract, which slows down the disease. Compounds that target such mechanisms are being put into clinical research. One of them is Reldesemtiv (CK-2127107, CK-107). The compound belongs to the group of fast skeletal muscle troponin activators and its goal is to increase the muscle contraction response in the event of a reduced nerve signal [52]. The drug will achieve its goal by slowing the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibers. By slowing down the release rate of calcium, reldesemtiv sensitizes the sarcomer to calcium, which leads to increased muscle contractility.

The results of one phase 1 study conducted in healthy volunteers showed that reldesemtiv was well tolerated without any adverse effects and led to an increase in muscle contraction strength measured by nerve stimulation [53].

The drug also underwent a phase 2 clinical trial in patients with type II, III and IV SMA (NCT02644668) [54]. It was found that after 4 and 8 weeks of treatment, the distance that patients could walk in six minutes increased. In addition, the study showed that the time until muscle fatigue depended on the dose taken by the participants. Patients after 8 weeks of taking the 150 mg dose walked over an average of 7.72 meters more than participants in the placebo group in 6 minutes; while participants taking the 450 mg dose at the same time (6 minutes) walked 24.89 m more than the placebo group [55].

SRK-015 is another drug currently undergoing research for the treatment of SMA. It is a selective inhibitor of myostatin activation factor [56]. Combines with the inactive form of myostatin and limits its ability to activate and break down muscle mass. As a consequence, it increases muscle mass and muscle strength. Preclinical studies in a mouse model showed that SRK-015 helped improve capacity and strength [57]. In May 2019, Scholar Rock began a phase 2 TOPAZ study (NCT03921528) involving 55 patients with type II and III SMA [58]. It is estimated that the study will be completed in April 2021.

3.4. NEUROPROTECTION

Since SMA in particular affects the lower motor neurons in the spinal cord, leading to muscle atrophy, it can be assumed that neuroprotection will allow the preservation of motor neuron function and prevent the disease symptoms from getting worse. It is suspected that this approach has significant clinical benefit in patients with SMA and may be an effective therapeutic approach in combination with genetic therapies for patients with SMA.

TRO-19622 (olesoxime) has a neuroprotective effect, enabling the functioning of neurons at low levels of SMN protein [59]. By affecting the processes of cellular respiration, olesoxime allows nerve cells to function even in a situation of reduced levels of SMN protein. In the years 2010-2013, clinical studies of olesoxime were carried out in Europe in patients with type II and III SMA. [60]. The results showed a clear improvement in the functioning of people taking the drug, manifested, among others, in a reduced number of infections and a slowdown in the scoliosis process. Better muscle strength preservation was also observed, although the effect was not equally pronounced at all time points. The FDA and European Medicines Agency (EMA) have asked for additional evidence of effectiveness to determine if this is a clinically significant effect. In May 2018, in the face of increasing technological difficulties and lower than expected effectiveness observed after 18 months of the OLEOS study, Roche decided to complete the development of olesoksim.

4. CONCLUSION

Over the past ten years there has been enormous progress in developing therapeutic strategies for patients with SMA. These advances resulted in the launch of two drugs for the treatment of SMA: Nusinersen (Spinraza) - approved in 2016 and Zolgensma (AVXS-101) - approved in 2019. Despite the successes in developing therapies in SMA, researchers are still researching other compounds that would prove effective in SMA.

Small molecules, which specifically target the SMN2 gene and modulate its splicing, give rise to the greatest hopes. Oral administration makes these drugs more tolerable than ASO because they eliminate the need for repeated lumbar puncture. However, because small molecules are not completely specific, there is a risk that these drugs can affect the expression of other genes and cause numerous side effects.

Gene therapy is another strategy for treating SMA. The most important advantage of therapy using modified AAV9 viruses is the possibility of achieving significant clinical

benefits only by a single intravenous infusion. Gene therapy has been found to significantly improve SMA, but researchers are not sure that the positive effect will last in the long term.

Research is currently underway on the effectiveness of neuroprotective compounds and compounds that improve skeletal muscle function. Dynamic research on SMA seems to bring more therapeutic options for this group of patients in the coming years.

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