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Therapeutic advances in 5q-linked spinal muscular atrophy

Avanços terapêuticos na atrofia muscular espinhal ligada ao cromossomo 5q

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ABSTRACT

Spinal muscular atrophy (SMA) is a severe and clinically-heterogeneous motor neuron disease caused, in most cases, by a homozygous mutation in the *SMN1* gene. Regarding the age of onset and motor involvement, at least four distinct clinical phenotypes have been recognized. This clinical variability is, in part, related to the *SMN2* copy number. By now, only supportive therapies have been available. However, promising specific therapies are currently being developed based on different mechanisms to increase the level of SMN protein; in particular, intrathecal antisense oligonucleotides that prevent the skipping of exon 7 during *SMN2* transcription, and intravenous *SMN1* insertion using viral vector. These therapeutic perspectives open a new era in the natural history of the disease. In this review, we intend to discuss the most recent and promising therapeutic strategies, with special consideration to the pathogenesis of the disease and the mechanisms of action of such therapies.

Keywords: spinal muscular atrophy; motor neuron disease; antisense oligonucleotides; genetic therapy

RESUMO

A atrofia muscular espinhal (AME) é uma grave doença dos neurônios motores, de grande variabilidade clínica e causada na maioria dos casos por mutação em homozigose no gene *SMN1*. Pelo menos quatro fenótipos clínicos distintos são reconhecidos com base na idade de início e no grau de envolvimento motor. Tal variabilidade clínica é em parte relacionada com o número de cópias do gene *SMN2*. Até recentemente, apenas terapias de suporte estavam disponíveis. Atualmente, terapias especificas estão sendo desenvolvidas com base em diferentes mecanismos para aumentar o nível de proteína SMN; em particular oligonucleotídeos antissenso por via intratecal e inserção de cópia do gene *SMN1*, via endovenosa, usando vetor viral. Nesta revisão, objetivamos discutir as mais recentes e promissoras estratégias terapêuticas, com consideração especial aos aspectos patogênicos da doença e aos mecanismos de ação de tais terapias.

Palavras-chave: atrofia muscular espinhal; doença do neurônio motor; oligonucleotídeos antissenso; terapia genética.

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by loss of lower motor neurons in the spinal cord and somatic motor nuclei in the brainstem, causing progressive proximal weakness, atrophy of skeletal muscles and variable grades of ventilatory insufficiency. The most common form of SMA is caused by mutations in the survival motor neuron 1 (*SMN1*) gene (5q11.2) and is transmitted in an autosomal recessive fashion[1]. It is the most-commonly inherited childhood neuromuscular disease with an incidence rate of 1 in 6,000 to 11,000 live births[2,3] and with a high frequency of carriers (1:40 to 1:67)[2,3]. Spinal muscular atrophy has been classified clinically into at least four types based on the age of onset and the maximal function attained[4]. Type 1 SMA is characterized by an onset that occurs within the first six months of age, and the children never achieve the ability to sit without support. Type 2 SMA is characterized by an onset of muscle weakness and hypotonia usually after six months of age; the children can sit but are unable to walk unaided. Type 3 SMA manifests in the second year of life or later, and the affected patients achieve the ability to walk. Type 4 SMA is a mild form of adult onset muscle weakness. There is also a type 0 form, in which the manifestation starts during pregnancy, and the child is extremely affected early in the first days of life[5]. In the last 20 years, many researchers have developed studies aimed at finding effective therapies. Presently, this field is being enriched with new, exciting perspectives. The present text will focus on the most recent and promising therapeutic strategies. Published literature on therapies for SMA was identified through PubMed searches. Search terms were 'spinal muscular atrophy' OR 'Werdnig Hoffmann' OR 'Kugelberg Welander'

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AND 'therapies' OR 'perspectives'. Available clinical trial studies in the last five years were used in this review. Additional data presented in posters from international congresses and medical conferences were also considered.

Pathogenesis

Approximately 95%–98% of individuals with a clinical diagnosis of SMA lack exon 7 in both copies of the *SMN1* gene, while approximately 2%–5% are compound heterozygotes for the deletion of *SMN1*'s exon 7 in one allele and an intragenic mutation of *SMN1* in another allele[1,6]. The human *SMN* gene is located at chromosome 5 (5q) and exists in two copies, *SMN1* and *SMN2*. The *SMN1* gene is the telomeric copy and produces a full-length survival motor neuron (SMN) protein necessary for normal lower motor neuron function[1]. In normal individuals, most of the complete SMN protein is encoded by the *SMN1* gene, because during the transcription of the *SMN2* gene, the exchange of cytosine for thymine in exon 7 creates a splicing suppressor site for the pre-mRNA, which skips the exon 7 and results in a truncated and rapidly-degradable protein (Figure 1A)[1,6,7]. The *SMN2* pre-mRNA splicing is abnormal 75%–90% of the time, so the amount of functional SMN protein produced by *SMN2* copy is not sufficient to prevent the progressive degeneration of motor neuron when the *SMN1* gene is absent (Figure 1B). The *SMN2* gene copy number varies, ranging from zero to five. Several studies have demonstrated a strong inverse correlation between the number of *SMN2* copies and SMA severity[7,8,9,10]. Most type 1 SMA patients carry two *SMN2* copies, whereas type 2 SMA patients carry three and type 3 SMA patients carry three or four *SMN2* copies. Patients with type 0 usually have only one copy of the *SMN2*[5]. Individuals with a lack of *SMN1* gene and five copies of *SMN2* can be asymptomatic[10]. The number of *SMN2* copies is the only biomarker indicator, and clinical trials consider this number to get a more homogeneous cohort. However, in isolated cases, the number of copies is not an absolute indicator of severity, and numerous studies seek to identify other factors involved that could explain the intrafamilial variability occasionally observed among affected siblings with the same number of copies[11,12,13]. Since the finding that the rare c.859G > C *SMN2* variant can be associated with a milder SMA phenotype[11], much research on the *SMN2* role as phenotype modifier has been done[12,13]. The evaluation of other exons besides exon 7

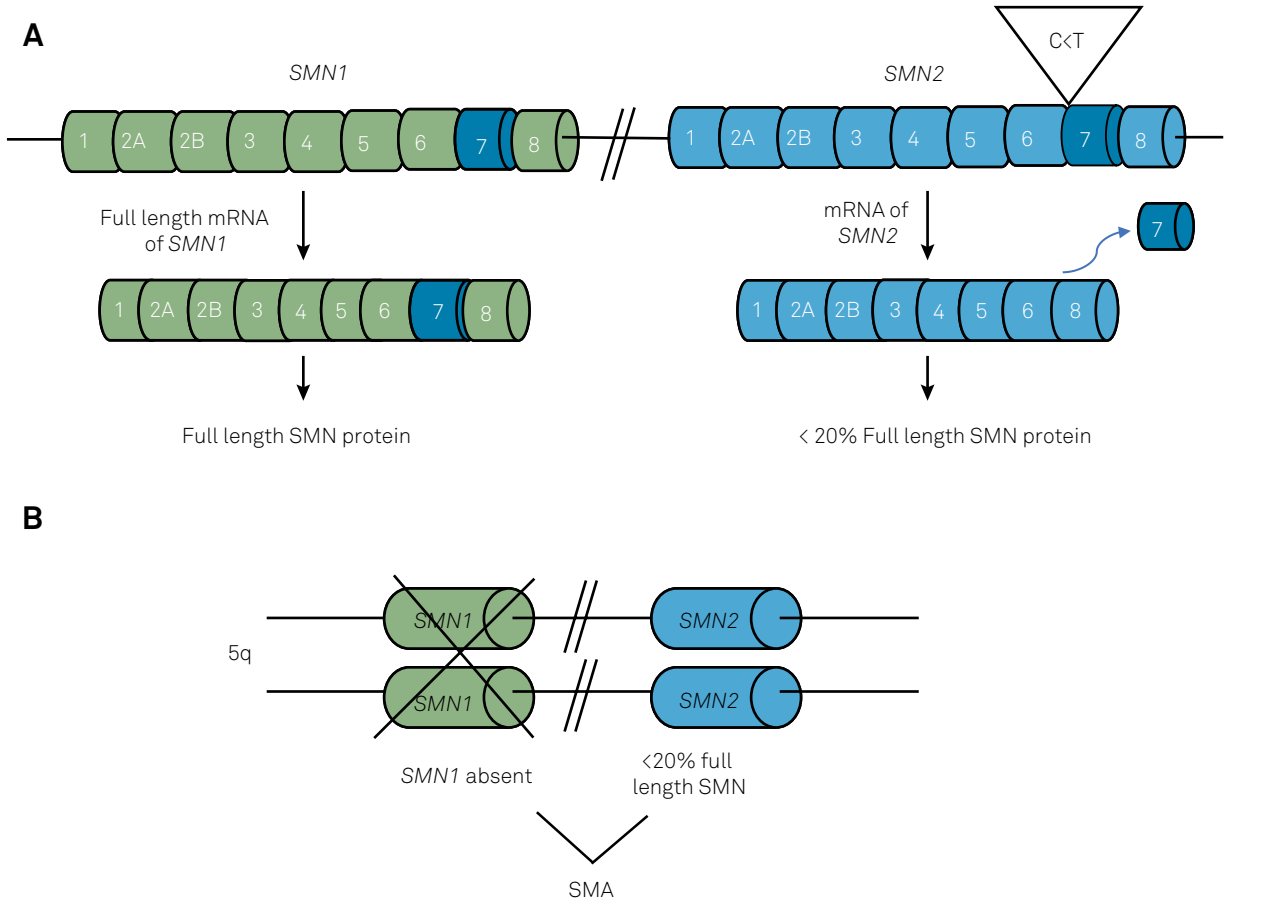

Figure 1. Genetic cause of spinal muscular atrophy (SMA). A) In normal individuals, *SMN1* produces 100% of full-length SMN protein. A homologous gene (*SMN2*) produces less than 20% of full-length SMN protein because the exchange of cytosine for thymine in exon 7 creates a splicing suppressor site of the pre-mRNA that skips the exon 7, resulting in a truncated and rapidly degradable protein. B) The absence (mutation) of two *SMN1* copies causes SMA because the low amount of SMN produced by the *SMN2* copies is not sufficient to prevent the motor neuron death.

and other possible *SMN2* variants that could modify the level of SMN protein expression would be useful.

The functional protein encoded by the *SMN* gene forms a macromolecular complex whose main function is to assemble small nuclear ribonucleic proteins (RNP), which are essential to the nuclear pre-mRNA splicing mechanism[14,15,16]. A SMN protein deficiency reduces the availability of small nuclear RNP causing splicing alteration and motor neuron damage[14,15,16]. In addition to neuronal degeneration, immaturity of proximal portions of the axons, Schwann cells, dorsal roots and myoneural junction, as well as decreased muscle fiber fusion capacity and skeletal muscle atrophy must be considered[16,17]. Defective axonal transport of mRNA, axonal growth and budding, neurotransmission and postnatal muscle maturation are probably involved in the selective vulnerability of the motor neuron during the early stages of the disease[18]. Several lines of research on the pathogenesis of SMA have focused on the interactions between these different structures and cells and the different functions of the SMN protein to elucidate the reasons for the vulnerability of the spinal motor neuron[17,18].

Genetic diagnosis

For the majority of SMA patients, multiplex ligation dependent amplification and quantitative polymerase chain reaction are currently used for accurate determination of *SMN1* homozygous deletion and the number of *SMN1* and *SMN2* copies, thus permitting highly accurate carrier detection and detection of *SMN2* copy number[12,19]. In the much rarer cases with point mutation in one or two *SMN1* copies, whole gene sequencing is required.

Multidisciplinary approach

Until recently, the treatment of SMA has only been supportive in nature. A multidisciplinary team comprising physiotherapists, orthopedists and nutritionists, among others, aim to prevent and delay contractures, kyphoscoliosis and respiratory complications, and to provide adequate nutritional support[12,20]. The guidelines for the multidisciplinary treatment of SMA were published in 2007[21] and were recently reviewed[22] to standardize supportive treatment and establish parameters for evaluating the results of new therapies.

Specific therapies in SMA

From the clarification of the molecular defect and the understanding of SMN protein functions, numerous studies have emerged in animal models and cell cultures that seek to find a specific treatment for SMA[20,23,24,25]. In the last 20 years, these studies have used different strategies[20,23,24,25]: 1) increasing the level of the SMN protein by activating *SMN2* gene expression or by preventing exon 7 exclusion in the *SMN2* gene; 2) genetic therapy to introduce an exogenous and normal *SMN1* gene; 3) promoting anti-apoptosis neuroprotection; 4) targeted improvements of skeletal muscle strength and function; and 5) stem cell therapy to replace affected motor neurons. Presently, the most concrete and promising long-term therapeutic strategies are preventing exon 7 exclusion in the *SMN2* gene through different compounds such as antisense oligonucleotides (ASOs) and adeno-associated virus genetic therapy (Table).

Antisense oligonucleotides are therapeutic short fragments of nucleic acid (RNA molecules) that bind to their complementary sequences in a specific mRNA. The binding can be directed to a targeted intron or exon and can influence the targeted splicing event in different ways[26]. Regarding SMA, ASOs have been synthesized against natural splicing inhibitory sequences of exon 7 at pre-mRNA intron 7 of *SMN2* gene. Therefore, ASOs are splicing modulators that prevent the binding of *SMN2* pre-mRNA with splicing inhibitors. They can identify exonic, intronic or intron 7-exon 8 junction elements, which silence the inclusion of exon 7. Consequently, ASOs promote the inclusion of exon 7 during the splicing, thus increasing full-length SMN2 protein production[27,28,29].

In 2004, researchers at the Massachusetts Medical School identified the Intronic Splicing Silencer N1 (ISS-N1) site located downstream of *SMN2* exon 7. Subsequently, an ASO was engineered to displace the hnRNP protein from the ISS-N1 site on the *SMN2* pre-mRNA, facilitating exon 7 inclusion at the *SMN2* mRNA (Figure 2A)[27,29,30]. Later, this ASO was named nusinersen [29-O-(2-methoxyethyl)] and marketed by Biogen after receiving approval from the Food and Drug Administration in 2016, and the European Medicines Agency in 2017. The intrathecal route was considered the best option to deliver the ASO to the spinal motor neurons, and a first study demonstrated safety, tolerability, adequate cerebrospinal fluid levels and, as a side effect, post-puncture syndrome, which is routinely found during treatments for other conditions when using this pathway[31,32]. An open-label phase 2 study of 20 patients with type 1 SMA demonstrated functional improvement assessed by the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) functional scale and increased amplitude of the composed action potential of the ulnar and peroneal nerve[33]. The survival and the age of dependence on permanent ventilation increased in comparison to the disease's natural history[33].

The phase 3, randomized, double-blind, sham-procedure controlled, study ENDEAR (ClinicalTrials.gov Identifier: NCT02193074) included 121 infants with SMA type 1 (with two *SMN2* copies) with onset of symptoms at six months of age or younger who received intrathecal nusinersen (12 mg scale dose) for 13 months with a 2:1 distribution (drug vs. placebo)[34]. The primary endpoints were a motor milestone response (defined according to results on the Hammersmith Infant Neurological Examination [HINE]) and event-free survival (time to death or the use of permanent assisted ventilation). The prespecified interim analysis of the study showed that a significantly larger proportion of infants in the nusinersen group than infants in the control group achieved motor

Table. Main aspects of clinical trials for the most recent and promising SMA drug therapies.

Molecule / study	Target population /age / SMN2 copies	Sample size / study phase	Endpoints	Preliminary results
Nusinersen / ENDEAR[34]	SMA1 / ≤ 6 months / 2 copies	121 infants / placebo-controlled / phase 3	HINE, CHOP-INTEND	51% of the patients had HINE improvement; major efficacy when illness duration < 12 weeks
Nusinersen / CHERISH[35]	SMA2 and 3 / 2 to 12 years / 2 or 3 copies	126 patients / placebo-controlled / phase 3	HFMSE	57.3% of the patients had at least 3 points higher score in HFMSE
Nusinersen / NURTURE[36]	Presymptomatic / 2 or 3 copies	20 infants / phase 2	HINE, WHO motor milestones	No patient died or needed ventilatory support, all achieved some of the expected HINE motor milestones for healthy infants based on age;
Nusinersen / SHINE, NCT02594124	SMA patients from other nusinersen studies	Open label / extension study		Ongoing
AVXS-101[42]	SMA1 / < 6 months / 2 copies	15 infants / open label / phase 1-2	Safety and tolerability; death or permanent ventilation; ability to sit; CHOP-INTEND	Positive impact on the survival and on motor function; 11/12 patients achieved motor milestones not seen in this population; results depended on age at onset and basal motor function
AVXS-101 / STR1VE, NCT03306277	SMA 1 / < 6 months / 1 or 2 copies	Open label / phase 3	Achievement of independent sitting; event-free survival	Ongoing
RO7034067 / FIREFISH, NCT02913482	SMA1 / 1-7months / 2 copies	Open label / phase 2	Safety; sitting without support (BSID-III)	Ongoing
RO7034067 / SUNFISH, NCT02908685	SMA2 and 3 / 2 to 25 years	Placebo-controlled / phase 2	Safety; MFM-32	Ongoing
Branaplam / LMI070X2201, NCT02268552	SMA1 / 1-7 months	Phase 1/2		Ongoing
Olesoxime / TRO19622[44]	SMA2 or 3 / 3 to 25 years	165 patients / controlled / Phase 2	MFM	Primary endpoint not met. Patients were stable compared to placebo.
Olesoxime / NCT02628743	SMA 2 or 3 / 3 to 25 years	Open label / Phase 2	MFM	Ongoing
CK-2127107 / NCT02644668	SMA 2 to 4 / > 12years	Placebo-controlled / phase 2	HFMS-E, 6MWT, FVC	Ongoing

BSID-III: Bayley Scales of Infant and Toddler Development - third edition; CHOP-INTEND: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; FVC: forced vital capacity; HFMSE: Hammersmith Functional Motor Scale Expanded; HINE: Hammersmith Infant Neurological Examination; MFM: motor function measurement; SMA: spinal muscular atrophy; WHO: World Health Organization; 6MWT: six-minute walk test.

milestones (41% vs. 0%), and the trial was terminated[34]. At the end-of-trial visits (80 in the nusinersen group and 41 in the control group), the proportion of infants who achieved motor milestones increased to 51% in the nusinersen group. In addition, 22% of the infants achieved full head control, 10% were able to roll over, 8% were able to sit independently, and 1% were able to stand in the nusinersen group, in comparison to the control group, in which no infant achieved these milestones[34]. With regard to the CHOP-INTEND functional scale evaluation, a higher percentage of infants in the nusinersen group showed improvement in comparison to the control group (71% vs. 3%, $p < 0.001$)[34]. In addition, at the end of the analysis, 39% of the infants in the nusinersen group had died or received permanent assisted ventilation compared with 68% of the infants in the control group[34]. The best results were observed in patients who started treatment within 13 weeks after the onset of disease[34].The risk of death was 63% lower in the nusinersen group than in the control group. The incidence and severity of adverse events were similar in the two groups (nusinersen vs. control)[34].

For nonambulant patients with SMA type 2 and 3, most of them with three copies of *SMN2*, intrathecal nusinersen (12 mg dose) was administered in a placebo-controlled phase 3 study named CHERISH (ClinicalTrials.gov Identifier: NCT02292537) that was conducted over 15 months[35]. A preliminary analysis of 126 patients (84 treated vs. 42 untreated) was presented at the World Muscle Society conference in October 2017[35] and showed that the scores on the Hammersmith Functional Motor Scale Expanded were significantly higher in treated patients compared with the untreated patients. In addition, 57.3% of the treated patients had a score that increased at least 3 points or higher compared with 20.5% of the untreated group. Ten treated patients acquired the ability to roll, and one was able to stand. Also, in this study, the treatment was extended to all patients. Children from CHERISH and other trials of nusinersen are being transitioned into the SHINE (ClinicalTrials.gov Identifier: NCT02594124) open-label extension study.

Finally, another clinical trial administered nusinersen to 20 presymptomatic children with SMA (2 or 3 *SMN2* copies)

(NURTURE – ClinicalTrials.gov Identifier: NCT02386553). Data presented at the World Muscle Society conference in October 2017[36] showed that after one year of treatment, no infant died or required ventilatory intervention. Among nine patients who were evaluated after 365 days of treatment, all achieved the expected HINE motor milestones for healthy infants based on age in the categories of head control and kicking, seven in rolling, six in sitting, five in crawling, five in cruising and three in standing unaided.

These patients had a previously incurable disease that caused serious limitations; the positive results displayed in these studies have globally brought hope globally to the medical community, multidisciplinary teams and families. Antisense oligonucleotide-nusinersen is the only effective, approved and marketed treatment, and the proposed administration consists of three starting doses over a 14-day interval, a fourth dose 30 days after the third and a maintenance dose every four months. The need to maintain treatment over a long period of time is due to the fact that ASOs are directed to mRNA and not to DNA targets. A limitation to the global distribution of the drug is its very high cost ($125,000 per dose). In Brazil, the drug has just been approved by Agência Nacional de Vigilância Sanitária (ANVISA, http://portal.anvisa.gov.br/), and the price has just been defined (R$ 290,928,80 per dose) (http://portal.anvisa.gov.br/documents/374947/2829072). By now, the drug has also been approved in the European Union, Japan and Canada.

Besides treatment efficacy and safety, it is also essential to know at which period inside the natural history of the disease therapies might start to produce beneficial results[37]. In addition, it has been considered that SMA may not be a disease exclusively dependent on spinal motor neuron involvement, and the involvement of other nervous system structures and other organs needs to be evaluated[37,38,39]. Thus, considering the restricted effect of an intrathecal drug delivery, restoration of SMN production also in peripheral tissues seems essential for optimal outcome.

Another highly-promising treatment is gene therapy utilizing a nonreplicating self-complementary adeno-associated viral serotype 9 capsid to deliver a functional copy of a human *SMN* gene (Figure 2B)[40]. After several studies in animal models, and once researchers overcame the technical difficulties inherent to the gene therapy[41], a phase 1/2 open-label study (AVXS-101; ClinicalTrials.gov Identifier: NCT02122952) was conducted[42]. Only SMA type 1 infants with onset of the disease before six months of age, with two copies of *SMN2* and without the variant c.859 < C in exon 7 of *SMN2,* since this genetic modifier predicts a milder phenotype of the disease[43], were included. From the 15 patients included, three received a single intravenous low dose of gene therapy (6.7×1013 vg per kilogram of body weight), and 12 received a single intravenous high dose (2×1014 vg per kilogram). The primary outcome was safety, and the secondary outcome was the time until death or the need for permanent ventilatory assistance. In exploratory analyses, the authors compared scores on the CHOP-INTEND scale of motor function with scores in studies of the natural history of the disease[42]. As of the data cutoff date of August 7, 2017, all 15 patients were alive and event-free at 20 months of age, compared with a rate of survival of 8% in an historical cohort. In the high-dose cohort, a rapid increase from baseline in the score on the CHOP-INTEND scale followed gene delivery, with an increase of 9.8 points at one month and 15.4 points at three months, compared with a decline in this score in an historical cohort. Of the 12 patients who had received the high dose, 11 sat unassisted, nine rolled over, 11 fed orally and could speak, and two walked independently[42]. In addition, most of the patients who did not require supportive care at enrollment were free of nutritional support (6/7 patients) and ventilatory support (7/10 patients) at the last follow-up visit. The treatment outcomes seem to be influenced by the age of onset of the treatment and the baseline functional status[42]. Elevated serum aminotransferase levels occurred in four patients and were attenuated by prednisolone[42]. The authors highlighted the fact that the presence of antibodies to the virus in the general population may be a limitation of adeno-associated virus gene-replacement therapy in some children. An open-label, phase 3 gene therapy study (AVXS-101), including infants with type 1 SMA under six months of age, is ongoing (STR1VE; ClinicalTrials.gov Identifier: NCT03306277).

In addition to nusinersen and gene therapy, research on other forms of therapies are under way. In Europe, treatment with olesoxime (trophos 19622) has shown improvements to neuronal survival, preventing apoptosis by means of a mitochondrial stabilization against altered mitochondrial permeability[44]. In a phase 2 placebo-controlled study, 165 patients with SMA types 2 and 3 and ages 3–25 years, received the drug by oral administration (10mg/kg/day) for 104 weeks[44]. Good safety and tolerability were observed, and motor function was measured by the Motor Function Measurement scale, which showed an improvement of 0.18 points in treated patients vs. a worsening of 1.82 points in the untreated. This apparent stabilizing effect needs new studies for confirmation.

Orally administered small molecules that can modulate exon 7 splicing in *SMN2* transcripts are currently being used in clinical trials sponsored by Novartis Pharmaceuticals (Branaplam; LMI070X2201) and Hoffmann-La Roche (RG7916). In animal models (mice), after the oral administration of these small molecules, an increase in full-length SMN2 protein was observed, which resulted in improvements to body weight and extended lifespans[45,46,47]. Roche has conducted studies in infants with SMA type 1 (FIREFISH; ClinicalTrials.gov Identifier: NCT02913482) and types 2 and 3 (SUNFISH; ClinicalTrials.gov Identifier: NCT02908685) to investigate safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of the compound RG7916 and now is

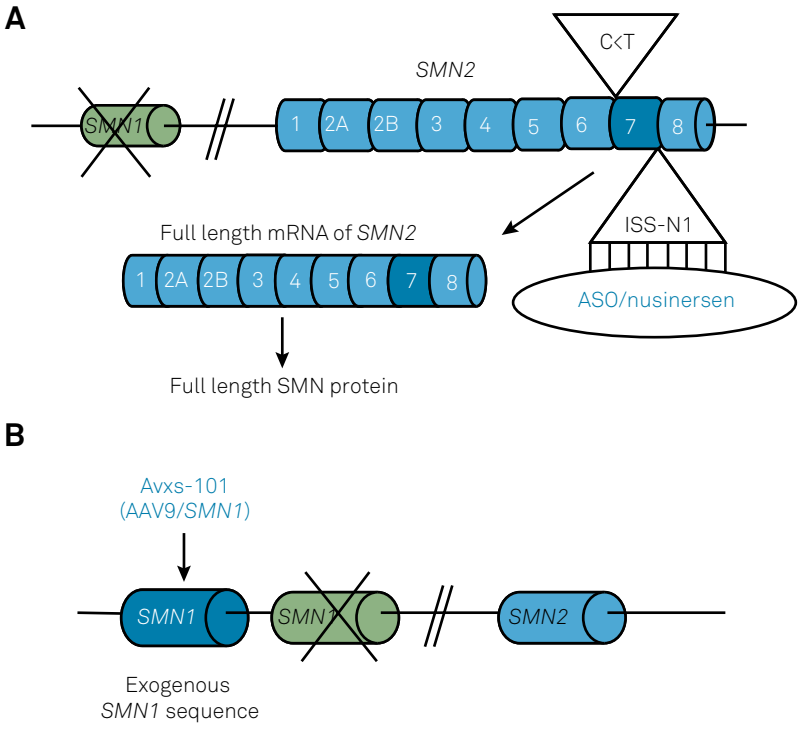

Figure 2. Therapeutic strategies for spinal muscular atrophy (SMA). A) ASO/nusinersen binds to the ISS-N1 (intronic splicing silencer N1) in the intron downstream of exon 7 of the *SMN2* transcript increasing exon 7 inclusion in *SMN2*-mRNA and consequently the full-length SMN2 protein. B) Exogenous *SMN1* (AVXS-101), identical to wild type *SMN1* gene, is inserted on the patient DNA using an AAV9 virus.

recruiting patients for a phase 2 trial in different countries[48]. In an open-label phase 1/2 study by Novartis (LMI070X2201; ClinicalTrials.gov Identifier: NCT02268552) the enrolled patients were closely monitored, because unexpected injuries to the peripheral nerves, spinal cord, testes and kidney vascularization have been observed in parallel chronic preclinical toxicology studies[49]. One of the ongoing studies on the possible therapy with the small molecule RG7916 includes a branch named JEWELFISH (ClinicalTrials.gov Identifier: NCT03032172) that will accept patients already submitted to other therapies.

In collaboration with Astellas, Cytokinetics is developing CK-2127107 (CK-107), a next-generation fast skeletal muscle troponin activator that can slow the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibers, thus increasing skeletal muscle contractility and therefore physical performance. A phase 2, double-blind, placebo-controlled, multiple dose study to demonstrate the potential pharmacodynamics effects and the effect on the skeletal muscle function and fatigability for patients with type 2, type 3, and type 4 SMA is ongoing (ClinicalTrials.gov Identifier: NCT02644668).

Final considerations

In summary, the results of the clinical studies using nusinersen and, preliminarily, AVXS-101, expose the success of treatment for this devastating disease. However, many questions still need to be answered[20,50]. 1) Do the treatments slow down the rate of motor neuron degeneration or stabilize the disease? 2) Do the treatments recover already lost motor function? 3) How much SMN is needed to achieve functional improvement? 4) Will it be possible to combine ASOs, gene therapy and neuroprotection?

To expand the reliability of clinical trials, it is urgent, internationally, to provide accurate and uniform methods of evaluating respiratory function (survival and time of dependence on the ventilator), muscle strength and motor function, nutritional status, quality of life questionnaires, and neurophysiological effects as well as other types of laboratory biomarkers[12]. It is also important to maintain and update local and national patient registries and to interact with family associations.

Finally, nusinersen is already approved in the US, Europe and Brazil, but the high cost requires public health attitudes to make this treatment available to the general population. In patients with type 1 SMA, the new therapies should be made available within a supposed therapeutic window, which ranges from weeks to six months of life. There should also be a broad intervention regarding the urgent inclusion of SMA in neonatal screenings[51]. A marked aspect of the near future of SMA will be the change of the landscape for diagnostics, clinical management and therapeutic trials[50], considering that the new therapies may result in changing phenotypes and, consequently, supportive care.

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