

Therapeutic advances in 5q-linked spinal muscular atrophy

Avanços terapêuticos na atrofia muscular espinhal ligada ao cromossomo 5q

Umbertina Conti Reed¹, Edmar Zanoteli¹

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 específicas 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 fashion¹. 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 attained⁴. 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 life⁵. 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'

¹Universidade de São Paulo, Faculdade de Medicina, Departamento de Neurologia, São Paulo SP, Brasil.

Correspondence: Umbertina Conti Reed; Av. Dr. Enéas de Carvalho Aguiar, 255 / 5º andar / sala 5131; 05403-900 São Paulo SP, Brasil; E-mail: umbertina.reed@hc.fm.usp.br

Conflict of interest: EZ has received financial support for travel/talks from BioMarin, Biogen, Sanofi-Genzyme, Roche and PTC. EZ is principal investigator for clinical trials sponsored by Roche. These do not influence the content of this work.

Received 19 September 2017; Received in final form 15 December 2017.

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 function¹. 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 asymptomatic¹⁰. 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 phenotype¹¹, much research on the *SMN2* role as phenotype modifier has been done^{12,13}. The evaluation of other exons besides exon 7

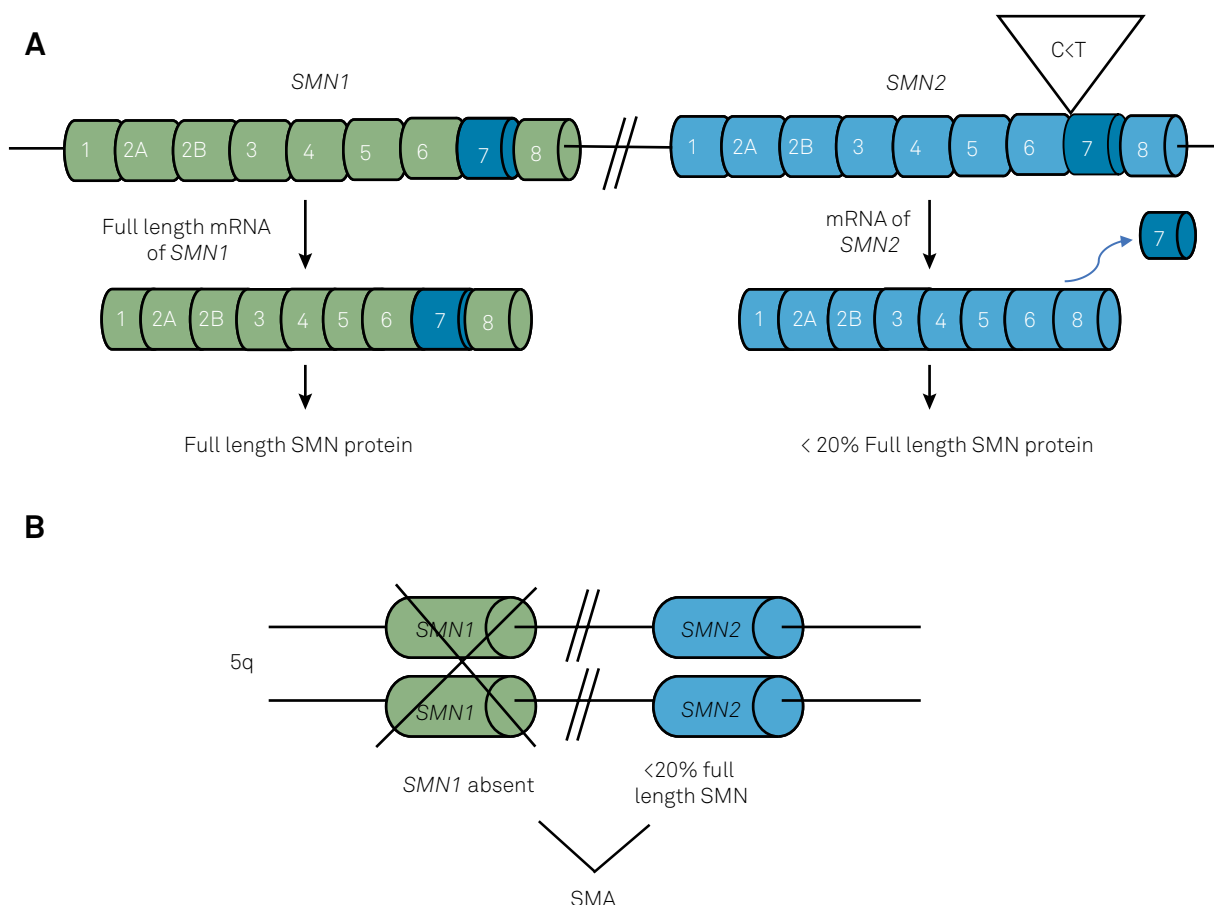


Figure 1. Genetic cause of spinal muscular atrophy (SMA). A) In normal individuals, *SMN1* produces 100% of full-length SMN protein. A homologous gene (*SMN2*) produces less than 20% of full-length SMN protein because the exchange of cytosine for thymine in exon 7 creates a splicing suppressor site of 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 disease¹⁸. 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 2007²¹ and were recently reviewed²² 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 ways²⁶. 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 nerve³³. The survival and the age of dependence on permanent ventilation increased in comparison to the disease's natural history³³.

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 / STRIVE, 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 / 1-7 months / 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 / > 12 years	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 terminated³⁴. 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 milestones³⁴. 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 group³⁴. The best results were observed in patients who started treatment within 13 weeks after the onset of disease³⁴. 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 months³⁵. A preliminary analysis of 126 patients (84 treated vs. 42 untreated) was presented at the World Muscle Society conference in October 2017³⁵ 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 2017³⁶ 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 results³⁷. 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 therapy⁴¹, a phase 1/2 open-label study (AVXS-101; ClinicalTrials.gov Identifier: NCT02122952) was conducted⁴². 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 disease⁴³, 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 disease⁴². 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 independently⁴². 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 status⁴². Elevated serum aminotransferase levels occurred in four patients and were attenuated by prednisolone⁴². 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 (STRIVE; 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 permeability⁴⁴. 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 weeks⁴⁴. 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

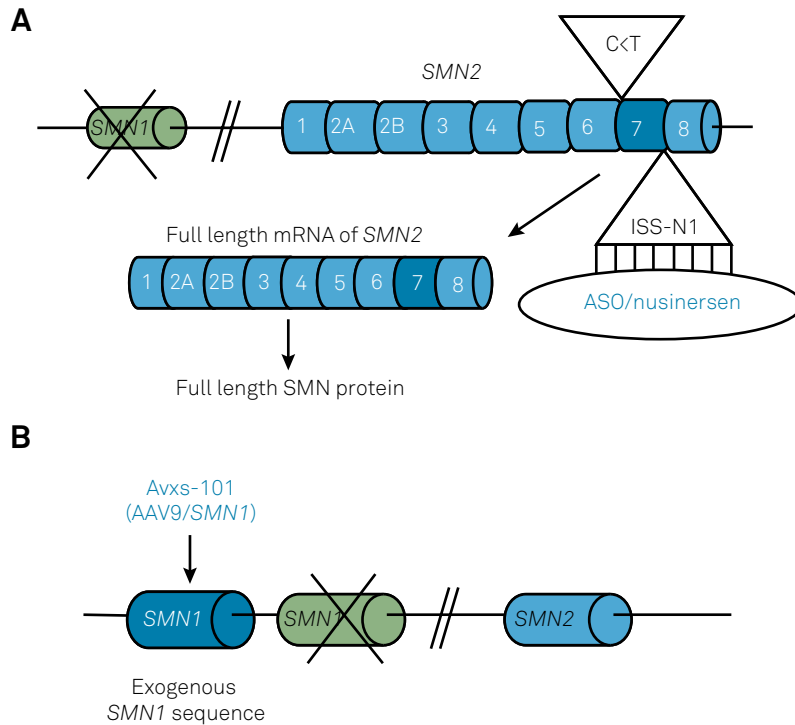


Figure 2. Therapeutic strategies for spinal muscular atrophy (SMA). A) ASO/nusinersen binds to the ISS-N1 (intrinsic splicing silencer N1) in the intron downstream of exon 7 of the SMN2 transcript increasing exon 7 inclusion in SMN2-mRNA and consequently the full-length SMN protein. B) Exogenous SMN1 (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 countries⁴⁸. 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 pre-clinical toxicology studies⁴⁹. 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 biomarkers¹². 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 screenings⁵¹. A marked aspect of the near future of SMA will be the change of the landscape for diagnostics, clinical management and therapeutic trials⁵⁰, considering that the new therapies may result in changing phenotypes and, consequently, supportive care.

References

- Lefebvre S, Bürglen L, Reboullet S, Clermont O, Bulet P, Viollet L et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*. 1995 Jan;80(1):155-65. [https://doi.org/10.1016/0092-8674\(95\)90460-3](https://doi.org/10.1016/0092-8674(95)90460-3)
- Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlfis EM et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. *Eur J Hum Genet*. 2012 Jan;20(1):27-32. <https://doi.org/10.1038/ejhg.2011.134>
- Verhaart IE, Robertson A, Leary R, McMacken G, König K, Kirschner J et al. A multi-source approach to determine SMA incidence and research ready population. *J Neurol*. 2017 Jul;264(7):1465-73. <https://doi.org/10.1007/s00415-017-8549-1>
- Munsat TL, Davies KE. International SMA consortium meeting. (26-28 June 1992, Bonn, Germany). *Neuromuscul Disord*. 1992;2(5-6):423-8. [https://doi.org/10.1016/S0960-8966\(06\)80015-5](https://doi.org/10.1016/S0960-8966(06)80015-5)
- Grotto S, Cuisset JM, Marret S, Drunat S, Faure P, Audebert-Bellanger S et al. Type 0 Spinal Muscular Atrophy: Further Delineation of Prenatal and Postnatal Features in 16 Patients. *J Neuromuscul Dis*. 2016 Nov;3(4):487-95. <https://doi.org/10.3233/JND-160177>
- Parsons DW, McAndrew PE, Iannaccone ST, Mendell JR, Burghes AH, Prior TW. Intragenic telSMN mutations: frequency, distribution, evidence of a founder effect, and modification of the spinal muscular atrophy phenotype by cenSMN copy number. *Am J Hum Genet*. 1998 Dec;63(6):1712-23. <https://doi.org/10.1086/302160>
- Lefebvre S, Bulet P, Liu Q, Bertrand S, Clermont O, Munnich A et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet*. 1997 Jul;16(3):265-9. <https://doi.org/10.1038/ng0797-265>
- McAndrew PE, Parsons DW, Simard LR, Rochette C, Ray PN, Mendell JR et al. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. *Am J Hum Genet*. 1997 Jun;60(6):1411-22. <https://doi.org/10.1086/515465>
- Wirth B, Herz M, Wetter A, Moskau S, Hahnen E, Rudnik-Schöneborn S et al. Quantitative analysis of survival motor neuron copies: identification of subtle SMN1 mutations in patients with spinal muscular atrophy, genotype-phenotype correlation, and implications for genetic counseling. *Am J Hum Genet*. 1999 May;64(5):1340-56. <https://doi.org/10.1086/302369>
- Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *Am J Med Genet A*. 2004 Oct;130A(3):307-10. <https://doi.org/10.1002/ajmg.a.30251>
- Vezain M, Saugier-Verber P, Goïna E, Touraine R, Manel V, Toutain A et al. A rare SMN2 variant in a previously unrecognized composite splicing regulatory element induces exon 7 inclusion and reduces the clinical severity of spinal muscular atrophy. *Hum Mutat*. 2010 Jan;31(1):E1110-25. <https://doi.org/10.1002/humu.21173>
- Finkel R, Bertini E, Muntoni F, Mercuri E. 209th ENMC International Workshop: Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy 7-9 November 2014, Heemskerk, The Netherlands. *Neuromuscul Disord*. 2015 Jul;25(7):593-602. <https://doi.org/10.1016/j.nmd.2015.04.009>
- Wu X, Wang SH, Sun J, Krainer AR, Hua Y, Prior TW. A-44G transition in SMN2 intron 6 protects patients with spinal muscular atrophy. *Hum Mol Genet*. 2017 Jul;26(14):2768-80. <https://doi.org/10.1093/hmg/ddx166>
- Gubitz AK, Feng W, Dreyfuss G. The SMN complex. *Exp Cell Res*. 2004 May;296(1):51-6. <https://doi.org/10.1016/j.yexcr.2004.03.022>
- Feng W, Gubitz AK, Wan L, Battle DJ, Dostie J, Golembe TJ et al. Gemins modulate the expression and activity of the SMN complex. *Hum Mol Genet*. 2005 Jun;14(12):1605-11. <https://doi.org/10.1093/hmg/ddi168>
- Donlin-Asp PG, Bassell GJ, Rossoll W. A role for the survival of motor neuron protein in mRNP assembly and transport. *Curr Opin Neurobiol*. 2016 Aug;39:53-61. <https://doi.org/10.1016/j.conb.2016.04.004>
- lascione DM, Henderson CE, Lee JC. Spinal muscular atrophy: from tissue specificity to therapeutic strategies. *F1000Prime Rep*. 2015;7:04. <https://doi.org/10.12703/P7-04>
- Tu WY, Simpson JE, Highley JR, Heath PR. Spinal muscular atrophy: factors that modulate motor neurone vulnerability. *Neurobiol Dis*. 2017 Jun;102:11-20. <https://doi.org/10.1016/j.nbd.2017.01.011>
- Alías L, Bernal S, Barceló MJ, Also-Rallo E, Martínez-Hernández R, Rodríguez-Alvarez FJ et al. Accuracy of marker analysis, quantitative real-time polymerase chain reaction, and multiple ligation-dependent probe amplification to determine SMN2 copy number in patients with spinal muscular atrophy. *Genet Test Mol Biomarkers*. 2011 Sep;15(9):587-94. <https://doi.org/10.1089/gtmb.2010.0253>
- Farrar MA, Park SB, Vucic S, Carey KA, Turner BJ, Gillingwater TH et al. Emerging therapies and challenges in spinal muscular atrophy. *Ann Neurol*. 2017 Mar;81(3):355-68. <https://doi.org/10.1002/ana.24864>
- Wang CH, Finkel RS, Bertini ES, Schroth M, Simonds A, Wong B et al. Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol*. 2007 Aug;22(8):1027-49. <https://doi.org/10.1177/0883073807305788>
- Finkel RS, Sejersen T, Mercuri E, Bertini E, Chen K, Crawford TO et al. 218th ENMC International Workshop: revisiting the consensus on standards of care in SMA Naarden, The Netherlands, 19-21 February 2016. *Neuromuscul Disord*. 2017 Jun;27(6):596-605. <https://doi.org/10.1016/j.nmd.2017.02.014>
- Zanoteli E, Maximino JR, Conti Reed U, Chadi G. Spinal muscular atrophy: from animal model to clinical trial. *Funct Neurol*. 2010 Apr-Jun;25(2):73-9.
- Ydewalle C, Sumner CJ. Spinal muscular atrophy therapeutics: where do we stand? *Neurotherapeutics*. 2015 Apr;12(2):303-16. <https://doi.org/10.1007/s13311-015-0337-y>
- Sumner CJ. Therapeutics development for spinal muscular atrophy. *NeuroRx*. 2006 Apr;3(2):235-45. <https://doi.org/10.1016/j.nurx.2006.01.010>
- Bennett CF, Baker BF, Pham N, Swayze E, Geary RS. Pharmacology of antisense drugs. *Annu Rev Pharmacol Toxicol*. 2017 Jan;57(1):81-105. <https://doi.org/10.1146/annurev-pharmtox-010716-104846>
- Singh NK, Singh NN, Androphy EJ, Singh RN. Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. *Mol Cell Biol*. 2006 Feb;26(4):1333-46. <https://doi.org/10.1128/MCB.26.4.1333-1346.2006>
- Sivanesan S, Howell MD, DiDonato CJ, Singh RN. Antisense oligonucleotide mediated therapy of spinal muscular atrophy. *Transl Neurosci*. 2013 Mar;4(1):1-7. <https://doi.org/10.2478/s13380-013-0109-2>
- Singh NN, Lee BM, DiDonato CJ, Singh RN. Mechanistic principles of antisense targets for the treatment of spinal muscular atrophy. *Future Med Chem*. 2015;7(13):1793-808. <https://doi.org/10.4155/fmc.15.101>
- Ottesen EW. ISS-N1 makes the First FDA-approved drug for spinal muscular atrophy. *Transl Neurosci*. 2017 Jan;8(1):1-6. <https://doi.org/10.1515/tnsci-2017-0001>
- Chiriboga CA, Swoboda KJ, Darras BT, Iannaccone ST, Montes J, De Vivo DC et al. Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. *Neurology*. 2016 Mar;86(10):890-7. <https://doi.org/10.1212/WNL.0000000000002445>
- Haché M, Swoboda KJ, Sethna N, Farrow-Gillespie A, Khandji A, Xia S et al. Intrathecal Injections in Children With Spinal Muscular Atrophy: Nusinersen Clinical Trial Experience. *J Child Neurol*. 2016 Jun;31(7):899-906. <https://doi.org/10.1177/0883073815627882>

33. Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet*. 2016 Dec;388(10063):3017-26. [https://doi.org/10.1016/S0140-6736\(16\)31408-8](https://doi.org/10.1016/S0140-6736(16)31408-8)
34. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med*. 2017 Nov;377(18):1723-32. <https://doi.org/10.1056/NEJMoa1702752>
35. Mercuri E, Finkel RS, Kirschner J, Chiriboga C, Kuntz N, Sun P et al. Efficacy and safety of nusinersen in children with later-onset spinal muscular atrophy (SMA): end of study results from the phase 3 CHERISH study. *Neuromuscul Disord*. 2017;27 Suppl. 2:S210. <https://doi.org/10.1016/j.nmd.2017.06.418>
36. Hwu WL, De Vivo DC, Bertini E, Foster R, Gheuens S, Farwell W et al. Outcomes after 1-year in presymptomatic infants with genetically diagnosed spinal muscular atrophy (SMA) treated with nusinersen: interim results from the NURTURE study. *Neuromuscul Disord*. 2017;27 Suppl. 2:S212. <https://doi.org/10.1016/j.nmd.2017.06.424>
37. Jablonka S, Sendtner M. Developmental regulation of SMN expression: pathophysiological implications and perspectives for therapy development in spinal muscular atrophy. *Gene Ther*. 2017 Sep;24(9):506-13. <https://doi.org/10.1038/gt.2017.46>
38. Hamilton G, Gillingwater TH. Spinal muscular atrophy: going beyond the motor neuron. *Trends Mol Med*. 2013 Jan;19(1):40-50. <https://doi.org/10.1016/j.molmed.2012.11.002>
39. Nash LA, Burns JK, Chardon JW, Kothary R, Parks RJ. Spinal muscular atrophy: more than a disease of motor neurons? *Curr Mol Med*. 2016;16(9):779-92. <https://doi.org/10.2174/1566524016666161128113338>
40. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac L, Kissel JT et al. Gene therapy for spinal muscular atrophy type 1 shows potential to improve survival and motor functional outcomes. *Mol Ther*. 2016;24:S190. [https://doi.org/10.1016/S1525-0016\(16\)33289-0](https://doi.org/10.1016/S1525-0016(16)33289-0)
41. Meyer K, Ferraiuolo L, Schmelzer L, Braun L, McGovern V, Likhite S et al. Improving single injection CSF delivery of AAV9-mediated gene therapy for SMA: a dose-response study in mice and nonhuman primates. *Mol Ther*. 2015 Mar;23(3):477-87. <https://doi.org/10.1038/mt.2014.210>
42. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW et al. Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N Engl J Med*. 2017 Nov;377(18):1713-22. <https://doi.org/10.1056/NEJMoa1706198>
43. Prior TW, Krainer AR, Hua Y, Swoboda KJ, Snyder PC, Bridgeman SJ et al. A positive modifier of spinal muscular atrophy in the SMN2 gene. *Am J Hum Genet*. 2009 Sep;85(3):408-13. <https://doi.org/10.1016/j.ajhg.2009.08.002>
44. Bertini E, Dessaud E, Mercuri E, Muntoni F, Kirschner J, Reid C et al. Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular atrophy: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*. 2017 Jul;16(7):513-22. [https://doi.org/10.1016/S1474-4422\(17\)30085-6](https://doi.org/10.1016/S1474-4422(17)30085-6)
45. Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z et al. Motorneurondisease: SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science*. 2014;345(6197):688-93. <https://doi.org/10.1126/science.1250127>
46. Palacino J, Swalley SE, Song C, Cheung AK, Shu L, Zhang X et al. SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. *Nat Chem Biol*. 2015 Jul;11(7):511-7. <https://doi.org/10.1038/nchembio.1837>
47. Ratni H, Karp GM, Weetall M, Naryshkin NA, Paushkin SV, Chen KS et al. Specific correction of alternative survival motor neuron 2 splicing by small molecules: discovery of a potential novel medicine to treat spinal muscular atrophy. *J Med Chem*. 2016 Jul;59(13):6086-100. <https://doi.org/10.1021/acs.jmedchem.6b00459>
48. Mercuri E, Kirschner J, Baranello G, Servais L, Goemans N, Pera M et al. Clinical studies of RG 7916 in patients with spinal muscular atrophy: SUNFISH part 1 study update. *Neuromuscul Disord*. 2017;27 Suppl. 2:S209. <https://doi.org/10.1016/j.nmd.2017.06.415>
49. Charnas L, Voltz E, Pfister C, Peters T, Hartmann A, Berghs-Clairmont C et al. Safety and efficacy findings in the first-in-human trial (FIH) of the oral splice modulator branaplam in type 1 spinal muscular atrophy (SMA): interim results. *Neuromuscul Disord*. 2017;27 Suppl. 2:S207-8. <https://doi.org/10.1016/j.nmd.2017.06.411>
50. Tizzano EF, Finkel RS. Spinal muscular atrophy: A changing phenotype beyond the clinical trials. *Neuromuscul Disord*. 2017 Oct;27(10):883-9. <https://doi.org/10.1016/j.nmd.2017.05.011>
51. Burns JK, Kothary R, Parks RJ. Opening the window: the case for carrier and perinatal screening for spinal muscular atrophy. *Neuromuscul Disord*. 2016 Sep;26(9):551-9. <https://doi.org/10.1016/j.nmd.2016.06.459>