

Recent therapeutic developments in spinal muscular atrophy

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Abstract: Proximal spinal muscular atrophy (SMA) is an inherited neurodegenerative disease with a heterogeneous clinical phenotype. Although there is no cure for SMA, several strategies are currently being developed. In this review, we summarize the ongoing clinical trials and molecular mechanisms of successful approaches to SMA treatment.

Key words: Spinal muscular atrophy, therapeutic approaches, clinical trials

1. Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disease and leading genetic cause of infant mortality (1). Regardless of ethnicity, its incidence is 1 in 11,000 live births, with 1 in 40–60 carrier frequency, although it is estimated to be higher in countries where consanguineous marriages are common, such as Turkey (21%) (2). SMA is characterized by the loss of alpha motor neurons in the anterior horn cells of the spinal cord and brain stem motor nuclei, progressive muscle weakness, and atrophy. The clinical severity of patients differs from very severe to mild. SMA is classified into 5 groups (Types 0–IV), based on age of disease onset and achieved motor functions. The most severe form (Type 0) has a prenatal onset, and death occurs at birth or within the first few weeks of life. In contrast, in the mildest form (Type IV), the symptoms start between 10 and 30 years or after 30 years of age with mild motor impairments in a normal life span (3,4).

SMA is inherited in an autosomal recessive manner. Mutations in the survival of motor neuron 1 (*SMN1*) gene, located at the 5q13 chromosomal region, are the cause of SMA disease, regardless of clinical phenotype (5). Homozygous loss of exons 7 and 8 of *SMN1*, or exon 7 only, is detected in the majority of patients (90%–94%); however, subtle intragenic mutations are also found in 3%–4% of SMA patients (6–8). *SMN1* loss occurs due to either deletion or conversion to *SMN2*, the homologue copy of *SMN1* (6,9). *SMN2* has a nearly identical sequence to *SMN1*, except for 5 nucleotides, which do not have any

effect on the amino acid sequence of the encoded SMN protein. However, C to T transition in exon 7 of *SMN2* disrupts splicing of *SMN2* pre-mRNA that leads to the exclusion of exon 7 from the transcripts (10,11). As a result, almost 90% of the proteins synthesized from *SMN2* mRNA are truncated, and 10% of full-length protein is not adequate to compensate for the loss of SMN function (12,13) (Figure 1). *SMN2* has a variable number of copies in the human genome (14). Mostly, higher copies of *SMN2* are correlated with mild phenotypes due to an increased amount of full-length SMN protein; therefore, it is defined as a prognostic factor (15). However, severity is not always predictable from copy number. For instance, patients with three copies of *SMN2* may either have severe (Type Ic) or mild (Type III) phenotype. Similarly, patients with two copies of *SMN2* may have mild phenotype due to a rare G278R variant within the *SMN2* exon 7 (16–18).

SMN is a developmentally regulated ubiquitous protein, expressed in all cells. In addition to its housekeeping function in splicing, SMN is involved in multiple cellular processes such as actin dynamics (19–22), axonal transport (23), endocytosis (24), and neuromuscular junction maturation (reviewed in 25–28). Although SMN deficiency primarily affects motor neurons, SMN-dependent perturbations have also been reported in nonneuronal tissues, including in the muscle, heart, liver, and pancreas of SMA animal models as well as patients (29–32). Therefore, increasing SMN protein level is the most straightforward approach for SMA therapy,

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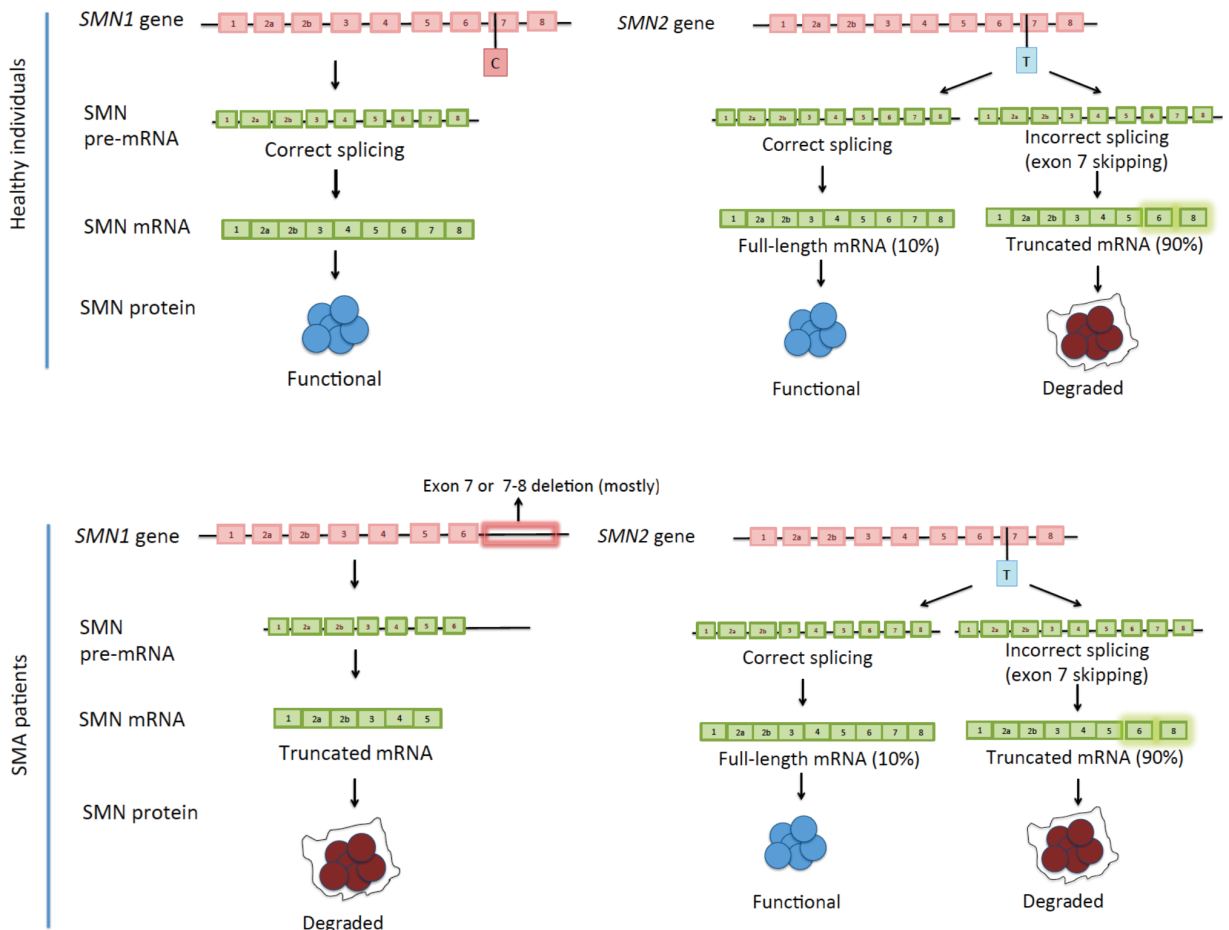


Figure 1. Schematic illustration of SMN genes, mRNA, and proteins in healthy individuals and SMA patients.

and has been studied for years with different strategies such as correction of SMN2 splicing, modulation of SMN gene expression, prevention of protein degradation, and replacement of *SMN1* (reviewed in 3,33–37). Significant efforts have been made to develop or identify effective small molecules (38–44), antisense oligonucleotides (45–47), and viral vectors (48–50) in order to increase SMN in preclinical research. The efficacy of some of the above has been tested in clinical trials. Additionally, SMN-independent therapeutic approaches have been investigated to protect the neuron and improve muscle survival/function via small molecules (51) and exercise (52,53). In this review, we aim to summarize genetic (*SMN1* gene replacement or upregulation/modification of *SMN2*) and nongenetic (neuroprotection or alteration of downstream motor unit function) therapeutic approaches to SMA (Table).

2. Antisense oligonucleotides

Antisense oligonucleotides (ASO) are synthetic and short nucleic acid molecules that can bind to RNA and modulate

its function (54). At present, the most successful ASO in SMA clinical trials is Nusinersen, formerly known as ISIS-SMN_{rx} or IONIS 396443. Nusinersen is an 18-mer ASO with 2'-O-methoxyethyl phosphorothioate (2'MOE)-modified chemical structure (46). It was designed to a specific sequence of SMN2 pre-mRNA, called intronic splicing silencer (ISS-N1), to promote exon 7 inclusion, which is mostly defective due to C to T transition at the sixth position of exon 7 (c.840C > T) (Figure 2). ASO binding to this sequence in intron 7 modifies splicing of SMN2 pre-mRNA with a complex molecular mechanism (16,46,55). Briefly, binding ASO to ISS-N1 abolishes the interaction of heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and A2 proteins, which play a role in the negative regulation of splicing. In addition, ASO binding promotes the recruitment of the cytotoxic granule associated with RNA binding protein (TIA1) to the downstream sequences of ISS-N1. TIA1 protein promotes U1 small nuclear ribonucleoprotein particle (U1snRNP) recruitment to 5' splice site of exon 7 and leads to the inclusion of exon 7 into the transcript. In summary, cis-

Table. Current therapeutic approaches in clinical trials for SMA (modified from Scoto et al.) (36).

Approach	Compound/Company	SMN dependency	Clinical trials			FDA approval	To patients
			Phase I	Phase II	Phase III		
Antisense oligonucleotide	Nusinersen (Spinraza)/Ionis Pharmaceuticals & Biogen	Dependent	—————→				→
Small molecule	Olexisome/Roche	Independent	—————→				
	CK-2127107/ Cytokinetics & Astellas	Independent	————→				
	LMI070/Novartis	Dependent	————→				
	RG7916/Roche	Dependent	————→				
	Celecoxib*	Dependent	————→				
	Pyridostigmine*	Independent	————→				
Gene therapy	AVXS-101/Avexis	Dependent	————→		————→		

* Marketed drugs for repositioning.

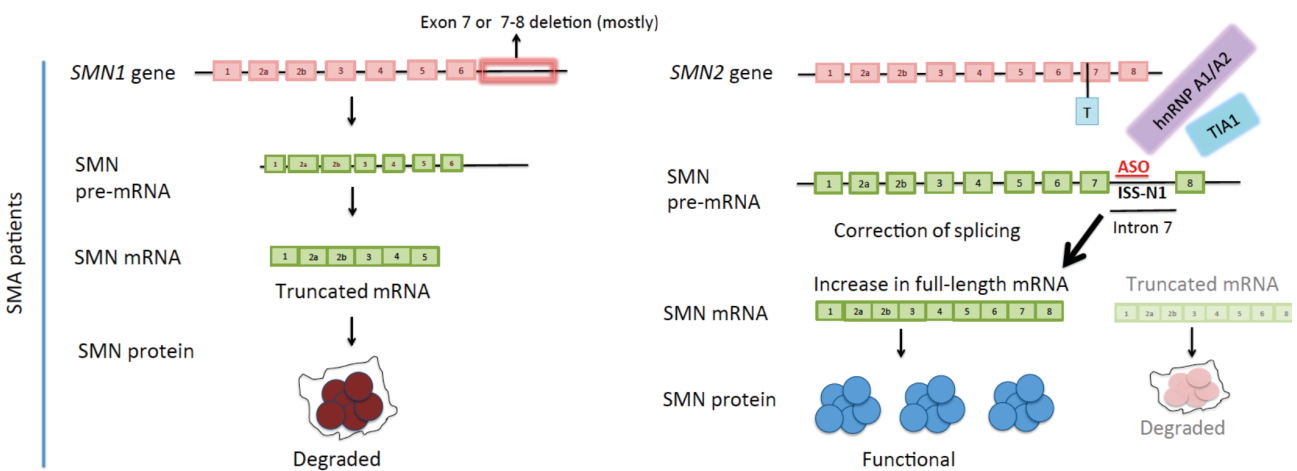


Figure 2. Binding region of ASO (nusinersen) in SMN2 pre-mRNA.

acting flanking sequences of exon 7 and transacting proteins are collectively important for exon 7 inclusion by ASO treatment (46,55,56).

Nusinersen (Spinraza, Biogen, Cambridge, MA, USA) has shown promising results in preclinical, early phase human, and clinical trials (47,57,58). The route of administration is intrathecal due to its poor transport across the blood-brain barrier. In SMA Type I infants, standardized outcome measures, including electrophysiological parameters, have shown an increase in muscle function scores and a favorable improvement in life span compared to natural history. Currently, there is an ongoing open label extension study (58). Based on significant interim results from two Phase 3 studies on infants and later onset SMA, the study subjects

switched to active treatment (59). In the final analysis, nusinersen-treated infants were more likely to be alive and have improved motor functions compared to the control group (57). Furthermore, the results indicated that early treatment is necessary to maximize the effect of the nusinersen treatment (57). A Phase 3 trial of nusinersen in later-onset patients (NCT02292537) was recently completed, whereas a Phase 2 trial is ongoing in presymptomatic (NCT02386553) patients. In addition, to evaluate the long-term efficacy of nusinersen, a Phase 3 open-label extension study is currently enrolling patients (SHINE, NCT02594124) (47, 58). Nusinersen (Spinraza) was approved as the first drug for the treatment of all types of SMA patients by the United States Food and Drug Administration (FDA) in December 2016, and by the

European Commission in June 2017 (36). In July 2017, the Social Security Institution of Turkey announced the administration conditions of nusinersen to SMA Type I patients.

Despite promising results, there are important questions left to be answered, including the therapeutic window for the disease and ethical issues (33). There seems to be a pathophysiologic developmental stage of motor neuron loss in animal models, and once motor neuron loss has progressed beyond a critical point it is unlikely to rescue the disease process (59,60). On the other hand, there is a differential sensitivity due to deficiency in SMN protein, motor neurons being vulnerable. Treatments that partially restore the levels of SMN protein may unmask the effects of chronic SMN deficiency in other tissues. It is worth noting that patients may not respond to nusinersen equally due to clinical severity, stage of the disease, *SMN2* copy number, possible effects of positive and/or negative modifier genes, and time and route of drug administration. Thus, there is a clear need to evaluate the evolution of the disease phenotype under treatment (61).

3. Small molecules

The effects of small molecules on SMA therapy have been investigated for more than a decade. Many synthetic and natural compounds have been tested, which may act on either SMN expression or independent targets that play a role in neuroprotection and/or muscle function (Figure 3) (44). The mechanism of SMN-dependent small molecules could be either increasing full-length SMN expression, by inducing promoter and/or modifying splicing of *SMN2* or increasing SMN protein stability (62). Among small molecules, histone deacetylase (HDAC) inhibitors were the most promising group in preclinical studies; however, clinical trials with sodium phenylbutyrate, valproic acid, and hydroxyurea were not as beneficial as expected (36). Nonetheless, research on small molecules is ongoing due to the following advantages: (1) possibility of oral or intravenous delivery, which may help to restore/support nonneuronal cells; (2) small molecules are cheaper than ASO and viral vectors; and (3) wider accessibility of patients. Additionally, the drug repositioning approach has been performed to investigate the effects of certain marketed drugs on SMA. Current clinical trials with small molecules are presented in the Table.

3.1. Olesoxime (TRO19622)

Olesoxime is a neuroprotective compound initially identified within 40,000 compounds using in vitro screening (63,64). The chemical structure of the compound is cholesterol-like and can cross the blood-brain barrier. It has been shown that olesoxime targets mitochondrial voltage-dependent anion channel (VDAC) and translocator protein 18 kDa (TSPO) proteins within

mitochondrial permeability transition pore complex, which regulates the permeability of the inner membrane. Although the mechanism of the action is not clear, olesoxime prevents the opening of the pore and maintains mitochondrial integrity, which probably has an effect on apoptotic factor release (44,64). Phase 2 of the clinical trial of olesoxime with Type 2 or 3 nonambulant SMA patients between the ages of 3 and 25 was recently completed, and it was found to be safe and generally well-tolerated (NCT01302600) (51). To evaluate long-term tolerability, safety, and efficacy, a Phase 2 trial is being conducted with patients that participated in previous olesoxime studies (NCT02628743). Due to its neuroprotective nature, its therapeutic potential has been investigated for other neurodegenerative diseases.

3.2. CK-2127107

CK-2127107 is an activator of fast skeletal troponin (44). Troponins are regulatory proteins associated with the actin filaments of sarcomere, a fundamental unit of muscle contraction (65). Muscle contraction is regulated by calcium, which is released from sarcoplasmic reticulum upon neuromuscular input. In the presence of intracellular calcium, CK-2127107 binds to troponin and disrupts its interaction with actin. Therefore, tropomyosin, another regulatory protein, can no longer block actin-myosin interaction, and triggers muscle contraction (65,66). Through slowing calcium release from troponin in fast skeletal muscle fibers, CK-2127107 sensitizes the sarcomere to calcium and increases muscle contractility (44). Thereby, it improves the muscle and physical functions of patients. Patients for Phase 2 of the clinical trial with CK-2127107 are currently being recruited (NCT02644668). Furthermore, since CK-2127107 targets muscle and not SMN protein, clinical trials are ongoing with elderly adults or patients suffering from other diseases (amyotrophic lateral sclerosis, chronic obstructive pulmonary disease), in which muscle weakness occurs.

3.3. RG7916 (RO7034067)

RG7916 is an SMN-dependent small molecule that aims to modulate *SMN2* splicing to increase full-length protein levels. Its chemical structure is not publically available. Three Phase 2 clinical trials with RG7916 are currently recruiting participants at the time of preparation of this review: Firefish (NCT02913482), Sunfish (NCT02908685), and Jewelfish (NCT03032172). RG7916 will be administered to cohorts that are infantile onset (Type I) in Firefish, Type II/III in Sunfish, and Type II/III in Jewelfish, which have been previously treated with *SMN2*-targeting therapy (36). The results of these studies will indicate the safety, tolerability, pharmacodynamics, pharmacokinetics, and efficacy of the molecule in the near future.

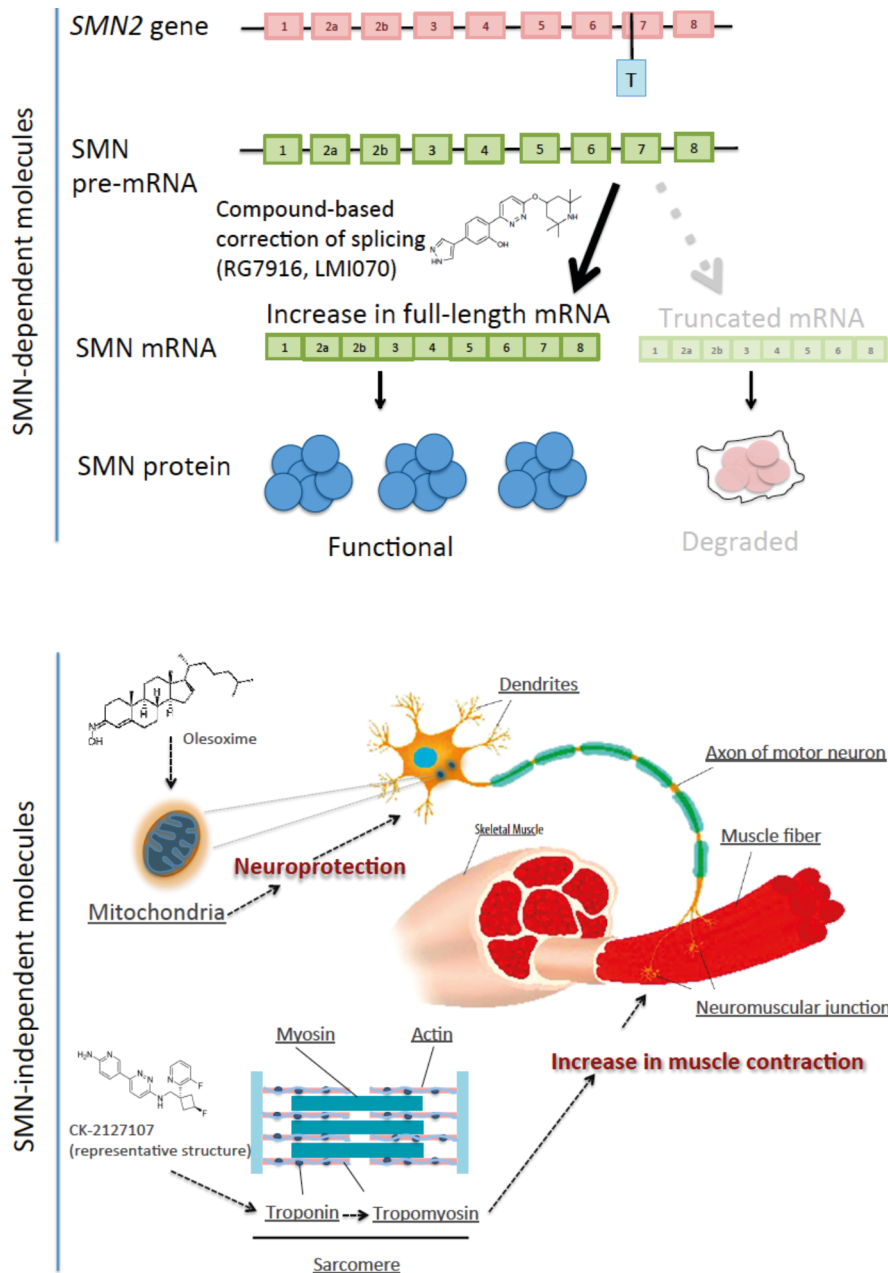


Figure 3. SMN-dependent and independent small molecules for SMA therapy (modified from Yeşbek-Kaymaz et al.) (44, 71).

3.4. LMI070 (NVS-SM1)

Another SMN2 splice modulator molecule is LMI070. This pyridazine-class molecule had been identified among 1.4×10^6 compounds with an in vitro high-throughput screening (67). It has been reported that LMI070 binds to 5' splice site of SMN2 exon 7; enhances the interaction of U1 snRNP, a spliceosomal RNA-protein complex; and induces the inclusion of exon 7 into the SMN transcript. Furthermore, it has been shown that it crosses the blood–

brain barrier and increases full-length SMN protein level in the brain and spinal cord of SMA mice (67). Due to unexpected findings in animal studies, Phase 1/2 open label trial with Type I patients was paused temporarily. It recently resumed recruiting participants (NCT02268552).

3.5. Celecoxib and pyridostigmine

Drug repositioning aims to identify new uses for existing drugs in conditions other than those originally developed for. Since the drugs had been previously studied in

clinical trials, safety tests were already completed, which accelerated the approval process. Additionally, this approach has been used for SMA, such as valproic acid, an antiepileptic drug with HDAC inhibition activity (43,68). Currently, two drugs are being clinically investigated for SMA therapy. One is pyridostigmine, an acetylcholinesterase inhibitor. Pyridostigmine increases neuronal transmission in the neuromuscular junction and decreases muscle weakness in myasthenia gravis patients (69). Since it affects the neuromuscular junction, the effects and efficacy of pyridostigmine are clinically investigated for SMA Type II, III, and IV patients in a placebo-controlled, Phase 2 trial (NCT02941328). The other drug is celecoxib, a nonsteroidal antiinflammatory drug inhibiting cyclooxygenase 2 (COX-2) selectively. Celecoxib is used as a pain reliever for arthritis, rheumatoid arthritis, and short-term pain. It has been shown that celecoxib activates p38 MAPK pathway, and increases SMN mRNA and protein levels in human and mice neuronal cell lines and in SMA mice models (70). A Phase 2 pilot, dose–response study of celecoxib will start with Type II or III patients (NCT02876094, not recruiting yet).

4. Gene therapy

Gene therapy is a promising approach for single gene disorders such as SMA (48). The main strategies of gene therapy are altering genetic material or delivering a healthy gene into cells. For SMA, this approach has been developed by either replacing the defective *SMN1* gene or modifying the *SMN2* gene to increase full-length

protein levels in cells. Several preclinical studies with different SMA model organisms have been performed by using adeno-associated viruses (AAV9) as a vector for delivering the correct *SMN1* gene into the cells (Figure 4). AAV9 can cross the blood–brain barrier and transduce nondividing cells, such as motor neurons, without integration of the host cell's genome (48). To increase transduction efficacy and expression of SMN, double stranded self-complementary AAV9 (scAAV9) vectors have been used and several parameters (life span, motor functions, safety, and delivery route) have been tested using mice, pig, and nonhuman primates as model organisms (50,72–75). Owing to encouraging preclinical results, FDA has been approved for the first clinical trials in humans with scAAV9-*SMN1*, under the name AVXS-101. AVXS-101 carries the full-length *SMN1* gene, having constitutive hybrid cytomegalovirus enhanced chicken- β -actin promoter. The results of the Phase 1 clinical trial were recently published (NCT02122952). AVXS-101 was delivered to 15 SMA Type I infants once and intravenously to evaluate safety, tolerability, and efficacy in an open-labelled, dose-escalating study. According to the results, this gene replacement therapy improved the motor functions of the patients and extended their survival, compared to the historical cohort used as controls. The authors emphasized the necessity of future studies for the long-term safety and efficacy of gene therapy (76). Currently, the gene replacement therapy Phase 3 pivotal trial with AVXS-101 is recruiting patients (STRIVE, NCT03306277).

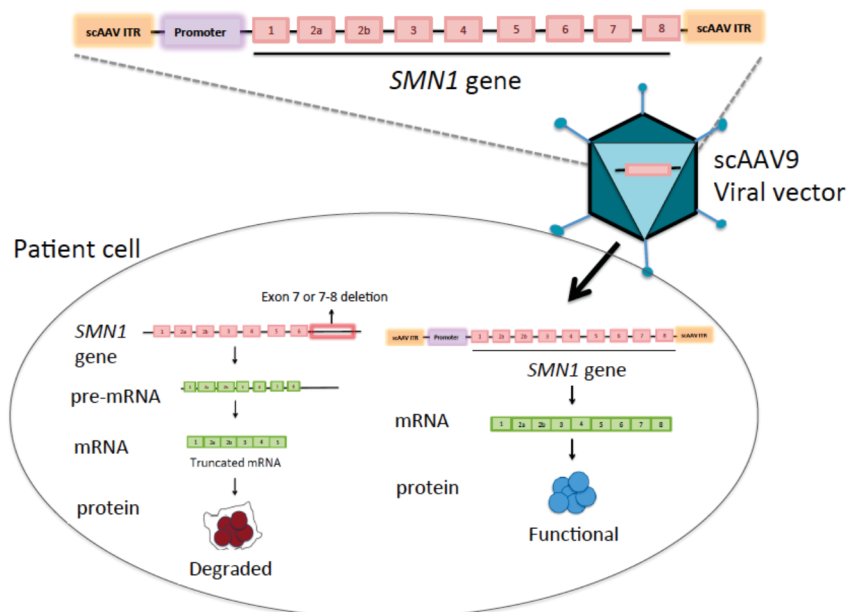


Figure 4. Representation of gene therapy approach for SMA with scAAV9 vector.

5. Conclusion

There is a change in the SMA landscape with the currently approved orphan treatment approaches. Standard care and follow-up of patients is even more important, with an expected evolution of the disease phenotype under

treatment. Progress in newborn and carrier screening programs will help to identify presymptomatic patients and provide appropriate genetic and prenatal diagnosis and genetic counseling.

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