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Therapeutic interventions for spinal muscular atrophy: preclinical and early clinical development opportunities

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Abstract

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative neuromuscular disease that presents primarily in children. Abnormalities in the *SMN1* gene cause reduced levels of the survival motor neuron (SMN) protein, while a second gene, *SMN2*, produces low levels of functional SMN protein. Currently available drugs do not cure, so a significant unmet need remains for patients treated after symptom onset.

Areas covered

Drugs available in the clinic, investigational agents and key questions for researchers are discussed. A pragmatic search of the literature was performed to identify therapies in late stages of preclinical, or in early stages of clinical development. This list was compared to the CureSMA pipeline for completeness. Drugs approved for indications that have potential for impact for SMA were included. These drugs target the primary deficiency in SMN protein or other pathways involved in SMA pathophysiology that are not SMN-protein dependent.

Expert opinion

Children treated after the onset of symptoms continue to have significant disability. Given the heterogeneity of the population phenotype evidenced by variable response to initial therapy, age at treatment onset and the need to demonstrate added value beyond approved therapeutics, the clinical development of new drugs will be challenging.

Keywords : antimyostatin, drug repositioning, gene therapy, SMN1 gene, Spinal Muscular atrophy, nusinersen, risdiplam, onasemnogene abeparvovec, clinical trials

Article Highlights

- Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative neuromuscular disease. Abnormalities in the SMN1 gene cause reduced levels of the survival motor neuron (SMN) protein. Moreover, a second gene, SMN2, produces low levels of functional SMN protein.

- SMA presents primarily in children. There is no cure and hence a significant unmet need remains.

- Three disease-modifying drugs (nusinersen, risdiplam and onasemnogene abeparvovec) are approved by the FDA for treatment of SMA. The purpose of these therapies is to restore the levels of SMN protein; they significantly improve outcomes for affected children.

- The number of surviving motoneurons may constitute a limiting factor of current treatment effects. Patients treated after the onset of symptoms have not exhibited normal motor development. New therapies targeting non-SMN pathways have potential for combination with currently approved drugs for enhanced impact.

- Several candidate drugs are in various stages of development [from academic proof of concept to early phase of clinical development]. These constitute a pipeline for monotherapy or combination therapy.

- These candidate drugs correct for the lack of a functional *SMN* gene through gene transfer, induce alternative splicing of the *SMN2 pre*-mRNA to optimize full-length protein expression, improve neuromuscular junction (NMJ) transmission, improve muscle contraction and size, or act by other molecular mechanisms in the motoneurons.

- Given the heterogeneity of the population phenotype evidenced by variable response to initial therapy and age at treatment onset and the need to demonstrate added value beyond approved therapeutics, the clinical development of these new drugs will be challenging.

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disease affecting around 1 in 9000 newborns. SMA is caused by abnormalities in the *Survival Motor Neuron 1* (*SMN1*) gene located on chromosome 5q11.2-13.2. A vast majority of patients have a homozygous deletion in the *SMN1* gene; a heterozygous deletion and a point mutation are observed in only 5% cases. Humans also have one or more copies of the *SMN2* gene. Measurement of the number of copies of *SMN2* is not yet standardized, and variations in the copy number may occur from lab to lab, especially when subjects have multiple copies of the gene (1). *SMN2* differs from *SMN1* by only five nucleotides. One is a critical C to T substitution in exon 7 that results in alternative splicing of exon 7, which is excluded from most, but not all, *SMN2* mRNA transcripts (2,3). The truncated protein produced from *SMN2* mRNA isoform lacking exon 7 is non-functional and rapidly degraded.

SMN is ubiquitously expressed in all cells and is highly conserved across species. SMN is concentrated in the Cajal bodies, a nuclear structure essential in spliceosomal snRNP biogenesis, and is also present in the cytoplasm. The SMN protein is critical for motoneuron development and maintenance, with highest levels of expression during fetal development and early postnatal period (4). A lower level of expression of SMN is needed throughout life (4). Its complete absence is embryonically lethal across all species. Spinal and bulbar motoneurons are especially sensitive to the lack of SMN protein, leading to the characteristic motor features SMA. SMN functions in spliceosome assembly and ribonucleoprotein biogenesis in all cells (2) and is potentially involved in autophagy, endocytosis, mRNA trafficking and local translation, cytoskeletal dynamics, mitochondria and bioenergetic pathways, and ubiquitin-proteasome system (5), leading to additional systemic effects mainly observed in humans in the most severe cases (6,7). There is accumulating evidence from animal models of SMA for the involvement of muscle (8), NMJs (9), liver (10), heart (11), and vascular endothelium (12) in the pathology of SMA, but clear evidence of clinical involvement of these organs in humans is limited to the most severe SMA cases.

The amount of SMN protein produced by SMA patients is driven by the number of *SMN2* copies, and the number of copies of *SMN2* is one of the main predictors of disease severity (3), yet several other factors modify the phenotype (13). SMA encompasses a broad spectrum

of severity, historically classified into five types according to age of onset of symptoms and highest motor milestone achieved in the untreated course of the disease (14). This clinical classification into types has contributed to a better nosology, but the phenotypic spectrum of the disease should be considered a continuum. On the most severe end of the spectrum (SMA0) are patients with symptom onset during fetal development or at birth, with contractures, respiratory insufficiency, and a very limited life expectancy. They typically harbor one or two copies of *SMN2*. The most frequent form, SMA1, is characterized by symptom onset before the age of 6 months with subsequent development of dysphagia and respiratory insufficiency. In absence of treatment, these infants never sit independently and die a median age of 12 months. Most have either two or three copies of *SMN2*. Patients who develop symptoms after the age of 6 months, can sit independently, but never acquire autonomous ambulation are diagnosed with SMA2 and may harbor two, three, or four copies of *SMN2*. Patients with SMA3 are able to achieve independent ambulation, though many will subsequently lose this function over time. A very limited number of patients first have symptoms in adulthood and are referred as SMA4; most of these individuals have four or more copies of *SMN2*. Across the spectrum of disease severity, SMA is associated with a significant burden and cost (15,16), and motor function continuously declines with time (17).

The historical classification will probably evolve in the age of new treatments. Indeed, with three drugs recently approved by the FDA and either approved or awaiting approval by the EMA, some SMA1 patients acquire the sitting position (18) and some SMA2 patients acquire autonomous ambulation (19). However, none of the patients treated after the onset of symptoms have had normal motor development. New therapies targeting non-SMN pathways have potential for combination with currently approved drugs for higher impact.

Approved therapies aim to restore the levels of SMN protein, but several therapies targeting other pathways are currently in development (Tables 1, Figure 1). Therapies for SMA that do not influence SMN expression, target pathways that responsible for the chronic systemic effects of SMA. A common feature of all treatments is that the earlier they are administered, the higher the efficacy (20). This has prompted several newborn screening programs across the world with the goal of enabling presymptomatic or very early symptomatic treatment initiation (21-23).

Treatments approved or in late-phase development have been recently reviewed (24). The aim of this review is to focus on treatments in late phase pre-clinical development or in the

early phases of clinical development. We conducted a pragmatic review of the literature to identify these treatments, ensuring completeness using the CureSMA drug pipeline list (https://www.curesma.org/wp-content/uploads/2020/11/2020_Sept-Graphic-Pipeline_v5.pdf) and analysis of trials in SMA registered with clinicaltrials.gov trials. We also discuss drugs currently approved for other indications that have potential for impact in SMA that have not yet been evaluated in a well-conducted clinical trial. The *SMN1* and *SMN2* pre-mRNAs and mechanisms of the drugs discussed are shown schematically in Figure 1.

2. Therapies designed to increase levels of SMN

2.1 *SMN1* gene replacement therapy

Gene replacement therapy offers the most intuitive approach for expression of SMN. Onasemnogene abeparvovec (originally known as AVX-101; sold under the brand name Zolgensma) is a recombinant self-complimentary adeno-associated virus serotype 9 (scAAV9) vector carrying cDNA that encodes a fully functional human SMN protein under control of the cytomegalovirus enhancer/chicken-β-actin-hybrid promoter (26). This vector is engineered from a non-replicating virus, and the human *SMN1* DNA cassette replaces most of the viral DNA, ensuring that the vector does not integrate into the host DNA. Some regions of the viral genome, such as the inverted terminal repeats that are crucial for the formation of the complementary nuclear *SMN1* episome, are retained. Once the vector is transferred into cells and translocated into the nucleus, SMN protein is highly expressed. After intravenous injection, AAV9 can cross the blood-brain barrier into the central nervous system and enter the motoneurons. Motoneurons are very adversely affected by low SMN protein levels, but SMN is ubiquitously expressed, and systemic delivery offers the advantage of increasing the SMN protein expression throughout the body.

Onasemnogene abeparvovec is FDA-approved for treatment of SMA in patients under 2 years of age. In Europe, the label covers patients up to 21 Kg. All clinical trials of onasemnogene abeparvovec to date (NCT02122952, NCT03461289, NCT03837184, NCT03306277, and NCT03505099) have been open label, single-dose studies, and required screening for AAV9 antibody titers prior to dosing. The main adverse effects reported in the clinical trials with the intravenous delivery of onasemnogene abeparvovec were transient elevation in serum transaminases and potential hepatotoxicity, as well as a transient

thrombocytopenia (26). Treatment with a glucocorticosteroid 24 hours prior to infusion and for the following month is needed to reduce these effects.

Jim Wilson's lab demonstrated elevation in transaminases in all three non-human primates but none of the three piglets treated with an AAV9 variant (AAVhu68) vector carrying the *SMN1* cDNA given intravenously (27). This elevation self-resolved in two of the non-human primates but in one led to acute liver failure and shock necessitating euthanasia. Degeneration of the dorsal root ganglia was noted in all of the six of the treated animals, and in the three piglets this led to associated proprioceptive deficit and sensory ataxia with significant impairment in ambulation necessitating euthanasia. These effects were thought to be a direct consequence of AAV transduction independent of an immune response to the capsid or transgene product, highlighting safety concerns with high-dose intravenous delivery. The pre-clinical development of this variant was halted by Biogen.

To enable dosing in older children and adults needing higher doses due to weight, where both safety and logistical issues in production are limiting factors, intrathecal delivery of a lower viral load is being evaluated in an early-phase clinical trial. Intrathecal scAAV9 is able to transduce motoneurons, muscle, and vascular endothelium, all potential treatment targets in SMA, while reducing peripheral exposure. In an ongoing phase 1 multicenter trial (NCT03381729, AVXS-101-CL-102), children 6 to 60 months of age with SMA2 have received three different doses of onasemnogene abeparvovec. There is currently an FDA hold on this trial based on preclinical findings of dorsal root ganglia mononuclear cell inflammation, sometimes accompanied by neuronal cell body degeneration or loss.

According to AveXis, two other trials of intrathecal onasemnogene abeparvovec delivery are in planning stages, pending the resolution of the current FDA hold on the AVXS-101-CL-102 study. One is a phase 3 open-label trial (AVXS-101-CL-305) in children with SMA1 up to 18 years of age and also including patients with SMA2 up to 40 years of age and SMA3 up to 60 years of age. The next phase 3 trial will be the first of onasemnogene abeparvovec to use a control arm (AVXS-101-CL-308). This will be a randomized (2:1), double-blind, sham-controlled, multicenter study to compare the efficacy and safety of intrathecally-administered AVXS-101 versus a sham procedure in children and adults with SMA3 up to 60 years of age. Onasemnogene abeparvovec is the only gene replacement therapy that has reached market for SMA. Other AAV-mediated gene therapies for treatment of SMA are reportedly in the

pipeline or under the scrutiny by other drug developers, but little information is available on these potential therapies (28,29).

2.2 *SMN2*- and *SMN2* transcript directed therapies

2.2.1 Approved therapies

Nusinersen was the first disease-modifying therapy approved for clinical use. It is an antisense oligonucleotide chemically modified with 2′-*O*-methoxyethyl sugar residues and phosphorothioate backbone linkages that alters the splicing of the *SMN2* pre-mRNA resulting in production of more full-length SMN protein. Nusinersen does not cross an intact blood-brain barrier when delivered systemically and is therefore delivered by intrathecal injection. The efficacy of nusinersen has been demonstrated in two double-blind sham-controlled randomized trials children with SMA1 and SMA2 who were carefully selected (30,31). Observational data has supported the efficacy across a broader spectrum of patients (18,19,32-35). The most striking impact has been demonstrated in infants treated before the onset of symptoms. Among these, normal development to age 4 years has been reported for those who harbor three copies of *SMN2*. For those with two copies of *SMN2*, about half developed mild motor impairment and about a quarter developed bulbar dysfunction, but this is considerably better prognosis than expected for untreated patients (36).

Risdiplam, also known as RO703406 and RG7916 is a small molecule that, like nusinersen, modifies *SMN2* pre-mRNA splicing. This small molecule crosses the blood brain barrier and is administered orally once daily with homogenous bioavailability in both central and peripheral tissues. (24,37-39). Risdiplam has been approved by FDA for use in children older than 2 months of age and is under review by EMA after recent completion of two phase 3 trials across a broad spectrum of patients up to 25 years of age.

2.2.2 Therapies in pre-clinical development

In recent years several high-throughput screening efforts have identified candidate drugs that modulate the inclusion of exon 7 of *SMN2*. These screens included an *in vivo SMN2* minigene reporter system in *Drosophila* motoneurons (40) and *SMN2* minigene-luciferase reporter system for the quantitative assessment of *SMN2* splicing with or without subsequent validation of candidate drugs using a patient-specific induced pluripotent stem cell-based assay (41,42).

Celecoxib is an FDA-approved selective non-steroidal anti-inflammatory COX-2 inhibitor that can cross the blood-brain barrier. Celecoxib acts by activating the p38 pathway, which is involved in regulating *SMN* transcript stability. Celecoxib treatment led to increased SMN protein levels, improved motor function, and survival in mouse model of severe SMA and is currently being investigated in a pilot, open-label, dose-response study in patients with SMA2 or 3 (NCT02876094).

Moxifloxacin is a synthetic fluoroquinolone antibiotic that inhibits the activity of the enzyme topoisomerase II, thus affecting the splicing of various genes including *SMN2* (40). Moxifloxacin causes a dose-dependent increase in SMN protein levels by promoting the inclusion of *SMN2* exon 7. The numbers and sizes of Cajal bodies in HeLa cells were almost doubled after moxifloxacin treatment, consistent with the increase in the numbers of Cajal bodies after SMN overexpression in HeLa cells. The increased numbers and sizes of Cajal bodies were interpreted as an effect of increased abundance of snRNPs secondary to the higher levels of SMN protein. Moxifloxacin treatment alters the expression levels of various splicing factors. Thus, this compound is expected to modulate splicing other transcripts in addition to *SMN2* pre-mRNA.

Securinine has been shown to promote *SMN2* exon 7 inclusion and to increase full-length *SMN2* mRNA and SMN protein expression in SMA patient-derived lymphoid cell lines (41). Securinine impacts on protein levels of certain splicing factors. For example, it downregulates hnRNP A1 and upregulates Tra2-β1. Chen et al. found that in an SMA mouse model, securinine administered through intraperitoneal injection caused an increase in *SMN2* exon 7 inclusion and SMN protein expression in both brain and spinal cord. Securinine is an herbal medicine product extracted from *Securinega suffruticosa* that has been shown to act as a gamma-aminobutyric acid (GABA) receptor antagonist. However, its effect on the splicing of *SMN2* pre-mRNA does not appear to be mediated through its GABA receptor antagonist function.

Rigosertib is a well-known polo-like kinase (PLK) inhibitor (42) that can selectively modulate *SMN2* splicing and increase SMN protein levels. Rigosertib is an orally bioavailable compound that inhibits the activities of several kinases, including PLK1, to influence multiple signaling cascades. It has been evaluated clinically for treatment of

hematological malignancies and solid tumors. Treatment with rigosertib increased the levels of SMN and ameliorated *SMN1* gene deletion, phenotypes observed in a human cell-based model of SMA. The activity of rigosertib in modulating *SMN2* splicing does not appear to result from inhibition of PLK1 or from effects on splicing of *SMN2* or stability of SMN. Treatment with rigosertib led to a reduction of mitochondrial oxidative stress, to the rescue of motoneuron progenitors differentiated from patient-derived induced pluripotent stem cells, and to increased levels of SMN protein in the spinal cord of the SMNΔ7 SMA mouse model. Future studies are needed to understand its mechanism of action and effects on the disease phenotype.

E1v1.11 is a phosphorodiamidate morpholino oligomer that is complementary to a region in the *SMN2* pre-mRNA. It blocks the intronic repressor element 1 in *SMN2* pre-mRNA to enhance exon 7 inclusion resulting in an increase in SMN protein levels. In a mouse model of severe SMA, E1v1.11 caused a dramatic dose-dependent improvement in muscle and NMJ pathology as well as in survival and growth of the mice (43).

Flunarizine is an EMA-approved calcium channel blocker used in Europe for the treatment of migraine or as an add on treatment of epilepsy. Flunarizine is not FDA-approved, as it may worsen Parkinsonism. Flunarizine has been shown to increase SMN protein abundance in the Cajal body, improve synaptic connection, increase the survival of motoneurons, and decrease muscle cell atrophy (44). In a mouse model of severe SMA, it resulted in a moderate increase (about 40%) in lifespan and increases in strength and bodyweight. The mode of action resides in an increase in abundance of a subset of components of the SMN-Gemins complex, Gemins2, 3, and 4. Flunarizine exposure also decreases the expression of thioredoxin-interacting protein TXNIP, which is a pro-oxidant encoded by an mRNA that is targeted by Gemin5 (45).

2.2.3 Therapies in early phase of clinical development

Branaplam (previously known as LMI070) is a pyridazine derivative that interacts with *SMN2* pre-mRNA and enhances exon 7 inclusion. It increases the level of functional SMN protein (46). Branaplam is currently being evaluated in a two-part phase 1b trial in infants with SMA1 (NCT02268552). A phase 2 trial is ongoing, intermediary results on 25 patients followed up for a mean duration of 7.6 months (0.03-11.83 months) have been presented, but not yet published (24,47). Outside of the usual adverse events reported in a SMA1 population

(pneumonia, constipation) thrombocytosis was noticed in 9/25 patients, but this was manageable and did not lead to treatment arrest. There were no deaths or the need for permanent ventilation, and 21/25 patients remained exclusively orally fed. These data must be interpreted in the context of a short follow-up. Regarding motor function, 15/25 patients who reached 218 days presented a mean CHOP intend increase of 14 points (47).

Salbutamol is a β_2-adrenoreceptor agonist. It has been shown to modulate *SMN2* gene splicing, promoting inclusion of exon 7. Salbutamol acts also on NMJs and is a broadly used treatment for congenital myasthenic syndrome (48). A pilot open-label study in patients with SMA2 and 3 indicate that the drug is well tolerated and improves motor function. A 1-year randomized double-blind, placebo-controlled study with salbutamol in 45 adult patients with SMA (37 completed the study) showed a significant and progressive increase in levels of full-length *SMN2* in peripheral blood. The majority of patients treated with salbutamol experienced an improvement in exploratory motor assessments (49).

3. Non-SMN protein-dependent therapies

3.1 Therapies designed to enhance neuromuscular junction function

Amifampridine (marketed as Firdapse) is currently approved for the treatment of Lambert-Eaton myasthenic syndrome, an autoimmune disorder in which antibodies target voltage-gated Ca^{2+} channels, resulting in a pre-synaptic pathology of NMJs. Amifampridine acts by blocking pre-synaptic K^+ channels thus increasing duration of acetylcholine release in the NMJ cleft (50). Amifampridine has also been successfully used to treat the post-synaptic NMJ disorder muscle-specific kinase myasthenia gravis and thus appears to be effective in treating both pre- and post-synaptic defects. NMJ defects are a key component in the SMA pathology, as demonstrated both in preclinical and in clinical studies (51-53). A phase 2 randomized cross-over study to evaluate the safety, tolerability, and efficacy of amifampridine in ambulatory SMA3 patients is currently ongoing (NCT03781479). A cross-over study in adult ambulant SMA3 patients using a similar compound, an extended-release formulation of 4-aminopyridine, that blocks voltage-sensitive potassium channels in the central and peripheral nervous system is now completed and results are pending (NCT01645787).

Pyridostigmine is an acetylcholinesterase inhibitor approved by the FDA and EMA as a first-line treatment of the postsynaptic NMJ disorder myasthenia gravis. Pyridostigmine inhibits the enzymatic breakdown of acetylcholine by acetylcholesterase thus increasing its bioavailability at NMJs and enhancing neuromuscular transmission. A phase 2, single center, double-blind, placebo-controlled, cross-over trial is ongoing to assess efficacy of pyridostigmine in patients with SMA2, 3, or 4 (54; NCT02941328).

3.2 Therapies directed at the muscle

3.2.1 Antimyostatin

Myostatin inhibition is a relatively recent therapeutic approach for treatment of SMA that is based on the possibility that motor function in SMA patients could be additionally improved by targeting skeletal muscle to reduce atrophy and improve muscle strength. Myostatin (also known as GDF-8), a member of the TGFβ superfamily, is a negative regulator of muscle mass. Genetic loss of myostatin results in significantly increased muscle mass in multiple species including one case reported in human (55-58). In preclinical models, pharmacologic inhibition of myostatin increases muscle mass and prevents muscle atrophy (59-63).

Different approaches to myostatin inhibition, including antibodies that bind to and inhibit the growth factor or antibodies directed against the myostatin receptor ActRIIB, have been tested in a broad range of neuromuscular disorders (64-68). The results have been disappointing, possibly due to the fact that myostatin is broadly down regulated in neuromuscular diseases including in SMA (69). Long and colleagues demonstrated that muSRK-015P, a monoclonal antibody that specifically inhibits myostatin activation, effectively increased muscle mass and function in two variants of the pharmacological mouse model of SMA in which pharmacologic restoration of SMN was induced either 1 or 24 days after birth to reflect early or later therapeutic intervention (60). Data from this model indicate that preventing myostatin activation has therapeutic potential as it blocked development of muscle and bone deficiencies. An optimized variant of SRK-015P, SRK-015, is currently in clinical development for treatment of milder forms of SMA. SRK-015 is a selective and local inhibitor of the activation of myostatin developed by Scholar Rock. A phase 2 proof-of-concept clinical trial in SMA2 and SMA3 patients is ongoing (NCT03921528). Patients enrolled in the trial receive SRK-015 intravenously once every 4 weeks either as a monotherapy or in conjunction with an approved SMN upregulator treatment over a 12-

month treatment period using the Hammersmith Functional Motor Scale Expanded as a primary outcome measure. Encouraging preliminary results have been recently released publicly (70). The trial is expected to be completed in January 2021.

A combination treatment of SMN-restoring therapy mediated by an antisense oligonucleotide with myostatin inhibition has been recently investigated in a Taiwanese SMA mouse model (71). In this study, the potential clinical benefit of myostatin inhibition on mice treated with a range of doses of the oligonucleotide agent was also explored, aimed at mimicking the situation of patients with the chronic forms of SMA2 and SMA3 and those in whom treatment is insufficient to restore necessary levels of SMN. The study showed that myostatin inhibition can improve survival, body weight gain, and righting reflex in mice with severe SMA treated with a low-dose of SMN-restoring antisense therapy. This study also characterized the effect of myostatin inhibition outside skeletal muscle, including on NMJs, dorsal root ganglia, and proprioceptive synapses in the spinal cord, and these results provide additional support for development of a combination muscle-enhancing and SMN-restoring therapy for SMA.

Recently Biogen has acquired ALG-801 (now known as BIIB110), which is a recombinant protein that acts as an ActRIIA/B ligand trap. It has effects on both myostatin and activins and is a novel way to inhibit myostatin-mediated signaling. BIIB110 is currently being tested in a phase 1A study (72)

3.2.2 Troponin activator

Reldesemtiv (also known as CY 5021 and CK-2127107) is an orally active, selective small molecule that slows the rate of calcium release from troponin complex of fast skeletal muscle fibers. It enhances muscle contractility by sensitizing sarcomeres to calcium. Exercise tolerance and performance are improved by reldesemtiv as demonstrated by preclinical studies on rat skeletal muscle *in vivo* (73). A double-blind placebo-controlled phase 2 study (NCT02644668) showed a good safety profile regardless of the dose (74). The design of the study could not prove efficacy of the drug; however, in the higher dose cohort, performance on the 6 minute-walk-test was improved. The development of troponin activators is thought be relevant for other diseases associated with muscle weakness such as amyotrophic lateral sclerosis.

4.Conclusion

SMA patients are currently benefiting from approaches that replace the *SMN1* gene or that increase SMN production from *SMN2* gene. Various agents that act on motoneurons outside *SMN* pathways or that stimulate muscle contraction or growth are currently in development. Although these approaches are promising, it is likely that their clinical development will be challenging in the current therapeutic landscape where an increasing number of patients are already on a disease-modifying treatment. The increasing number of cases detected through newborn screening (23) and treated at birth will certainly add a layer of complexity for future therapeutic development.

5. Expert opinion

Despite approval of three drugs in the USA, a significant unmet need remains in SMA, fueling academic and industrial research. SMA patients treated after the onset of symptoms, regardless of the treatment they received, have significant disability from motor, respiratory. and bulbar points of view. Some SMA1 patients treated after symptom onset may acquire sitting position (18, 19, 24, 26, 32, 39), mainly depending on the disease duration and baseline motor function level before treatment initiation, but autonomous ambulation is rarely achieved. There are anecdotical cases of treated SMA2 patients who acquire ambulation, but this milestone remains out of reach for most of patients irrespective of the drug used in treatment. In adults, increasing real-world data support the clinical benefits of nusinersen; however, these patients continue to have significant disability and intrathecal administration, the route used in nusinersen treatment, is challenging in this population. In patients treated pre-symptomatically either with nusinersen or onasemnogene abeparvovec, those with two copies of *SMN2* may achieve normal motor milestones by the age of 18 months, but some develop bulbar symptoms and others clinical signs of continued motoneuron degeneration or both. In this context, the development of next-generation drugs is very meaningful.

Nevertheless, important questions should be considered. So far, nusinersen, and risdiplam have excellent safety profiles. On the other hand, onasemnogene abeparvovec is generally safe, but serious adverse reactions including hepatotoxicity (75) and microangiopathy (76) have been reported, which questions the benefit/risk in older/heavier patients (77). This further reinforces the need of intrathecal development in older patients. For drugs in development, proving efficacy relative to these agents will be the biggest challenge. There are several difficulties of conducting clinical development in the context of a rare disease population that is already treated by one or more approaches. Future pivotal trials will have to

be conducted against a comparator in a non-inferiority trial or as a combination therapy against the approved drug plus placebo. Both types of trials will require a large number of participants unless the drug effect is considerable. Innovative outcomes able to capture a minimal change (15,17,78) may to a certain extend help to overcome this issue, but the clinical importance of minimal changes and the willingness of payers to pay for therapies that lead to minimal changes are important considerations. A difficulty in conducting combination trials is that innovative therapies have led to the emergence of new phenotypes, and this makes the design of new clinical trials even more challenging given the lack of a natural history comparison group. For example, pre-symptomatic patients with two *SMN2* copies treated very early with nusinersen present mainly with bulbar dysfunction. This phenotype was not previously observed in any of the clinical stages in the natural course of the disease.

The three approved drugs either replace *SMN1* using gene therapy or regulate alternative splicing of *SMN2* pre-mRNA to result in increased abundance of SMN protein. Nevertheless, a recent research conducted on human tissues has demonstrated that SMN protein expression in infants not affected by SMA mainly occurs in the prenatal and early postnatal period, with a rapid decline afterwards (4). This observation, coupled with the consistent observation of that outcomes are better when treatments are administered early (20), raises the question of further treatment development relevance after the neonatal or early infancy period. It is very likely that the progress of patients will be limited by the number of motoneurons available at the time of treatment initiation. Some of these cells could be in a reversible status of metabolic distress, explaining the rapid improvement in some patients upon treatment. The decline of motoneuron numbers before symptoms onset is such that the number of residual motoneuron will be at some point the limiting factor in patient progress. At this point, there is no evidence that patients treated with one of the approved drugs will further improve if shifted to a different approved or experimental therapy or upon an additional treatment. This risk, combined with the cost of a large combination trial, will likely limit the return on investment in future developments. At the current cost, the drugs for SMA are largely overpriced in comparison with global benchmark (16), and it is likely that many payers will be reluctant to pay for two different drugs at the current price.

Interestingly, the question of the very high price could be a rationale for investigation of drugs approved for other indications that can be repositioned or repurposed. At the current cost, the currently approved innovative medications are unaffordable in many countries,

whilst drugs such as moxifloxacin, pyridoxine, or flunarizine can be purchased for a very low price. Nevertheless, the benchmark for efficacy and safety evaluation of such drugs should not be lowered. Valproic acid, broadly prescribed off-label in several countries despite inconclusive trials (79), has significant adverse effects. Similar use of drugs with uncertain efficacy or unfavorable efficacy/safety ratios on the sole rationale of their low cost should be avoided.

Despite the methodological difficulties associated with development of an add-on therapy, there is rationale for drugs that target non-SMN-related mechanisms. For example, preliminary data have shown an increase in myostatin in patients treated with anti-myostatin and intermediate results of the phase 2 trial are encouraging. Drugs that target the NMJ may also prove useful if NMJ abnormalities remain after disease-modifying treatment. Whether NMJs become fully functional in patients treated with SMN-related therapies is unknown. Treated patients report improvement in fatigue and fatigue perception, but the involvement of NMJs in this improvement remains to be demonstrated. Interestingly, several other approaches, which to our knowledge have not yet entered formal pre-clinical development, have demonstrated significant benefit in mouse models subsequent to nusinersen treatment. One example is an antisense therapy that reduces neurocalcitonin expression (80). Neurocalcitonin is expressed in motoneurons and is independent of the SMN pathway.

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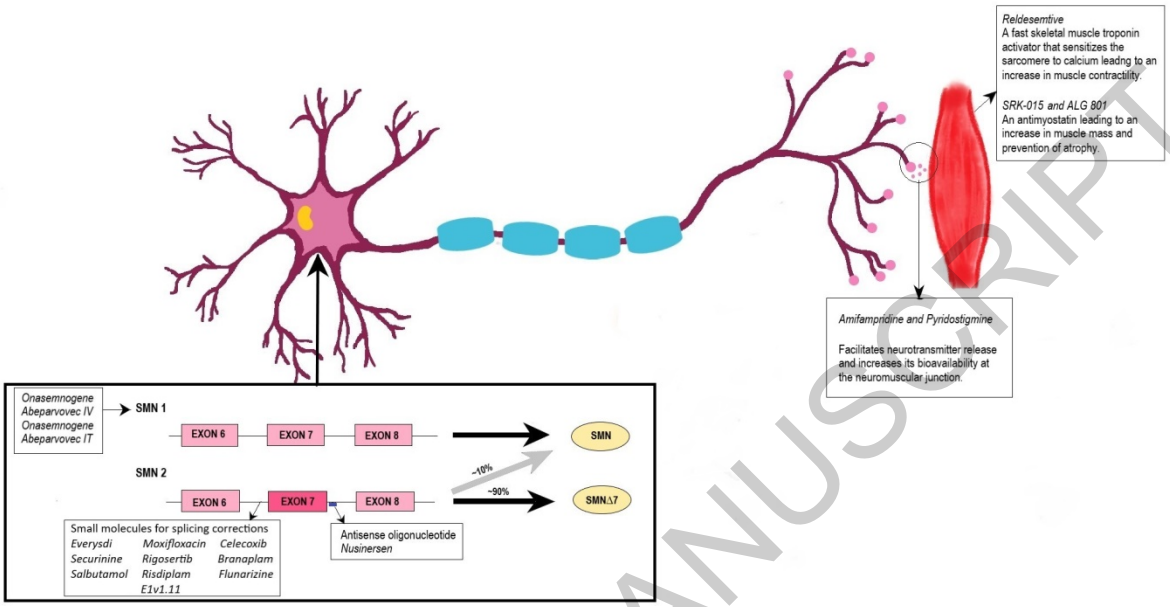
Table 1 Pipeline of potential SMA treatments, clinical phases, and targets

Light gray indicates drugs that are approved in another indication by either the FDA or the EMA

Dark gray indicates drugs that are approved for treatment of SMA by the FDA

Figure 1. Therapeutic targets of treatments in the late phase of pre-clinical development or in the early phase of clinical development.



SMN directed therapies		Phase of development	Mechanism of action	Reference
Motoneurons	**SMN1 directed**			
	Onasemnogene Abeparvovec IV	Approved by FDA, EMA, HC	SMN1	25
	Onasemnogene Abeparvovec IT	Phase III-FDA HOLD	SMN1	NCT 03381729
	SMN2 directed (Alternative splicing of SMN2 pre-mRNA)			
	Nusinersen	Approved by FDA, EMA HC	Antisense oligonucleotide that decrease the splicing of exon 7 of SMN2 RNA	29-35
	Risdiplam	Approved by FDA, under review by EMA and HC	Small molecule that interacts with SMN2 pre-mRNA and enhances exon 7 inclusion	36-38
	Branaplam	Phase1-2	Small molecule that interacts with SMN2 pre-mRNA and enhances exon 7 inclusion	45,46
	Celecoxib	Phase 1	Non-steroidal anti-inflammatory COX-2 inhibitor. Acivates the p38 pathway which is involved in regulating SMN transcript stability	NCT02876094
	Moxifloxacin	Preclinical	Synthetic fluoroquinolone antibiotic that inhibits the activity of topoisomerase II (TOPII) thus affecting the splicing process of *SMN2*	39
	Securinine	Preclinical	Impacts on protein levels of relevant splicing factors, including downregulation of hnRNP A1 and upregulation of Tra2-β1	40
	Rigosertib	Preclinical	Polo-like kinase (PLK) inhibitor that modulates SMN2 splicing	41
	Flunarizine	Preclinical	Calcium Channel Blocker that increase SMN protein in Cajal bodies	43,44
	Salbutamol	Phase II	b2-adrenoreceptor agonist	47,48
	E1v1.11	Preclinical	Antisense oligonucleotide optimised with a phosphorodiamidate morpholino oligomer.	42
	Non-SMN directed therapies			
NMJ	Amifampridine	Phase II	Blocks pre-synaptic K+ channels thus increasing duration of acetylcholine release in the NMJ cleft	49
	Pyridostigmine	Phase II	Acetylcholinesterase inhibitor, improves neuromuscualr transmission	53
Muscle	SRK-015	Phase II	Antimyostatin, increases muscle mass and prevents atrophy	NCT03921528
	ALG 801	Phase 1	Antimyostatin, increases muscle mass and prevents atrophy	
	Reldesemtive	Phase II	Selective small-molecule fast skeletal muscle troponin activator, reduces muscle fatigability	70,71