Pre-Marketing Evidence on SMA Pharmacological Therapies

Subjects: Orthopedics

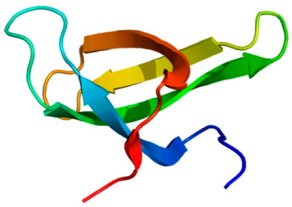
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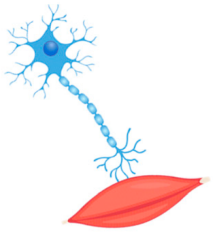
Spinal muscular atrophy (SMA) is a rare neuromuscular disease, with an estimated incidence of about 1 in 10,000 live births. To date, three orphan drugs have been approved for the treatment of SMA: nusinersen, onasemnogene abeparvovec, and risdiplam.

spinal muscular atrophy nusinersen onasemnogene abeparvovec

1. Preclinical Studies

The deeper knowledge of the molecular mechanisms underlying SMA's onset and progression has represented a significant milestone for the development of specific therapeutic strategies (**Figure 1**).





SMN-DEPENDENT THERAPEUTIC STRATEGIES

Therapeutic target	Mechanism of action	Treatments
SMN1 mutation	Replacement of *SMN1* through a viral vector	• Gene therapy
SMN2 splicing modulation	Modification of splicing to include exon 7	• Antisense oligonucleotides • Small molecules • Tetracycline
SMN deficiency	Stabilization of SMN protein	• Indoprofen • Proteasome inhibitors • Polyphenols
	Increase in SMN transcripts	• HDAC inhibitors • Hydroxyurea • Quinazolones • Aminoglycosides

SMN-INDEPENDENT THERAPEUTIC STRATEGIES

Therapeutic target	Mechanism of action	Treatments
Motor neuron loss	Neuroprotection	• Riluzole • Neurotrophic factors
	Cell therapy	• Stem cells
Muscular atrophy	Muscle enhancement	• Myostatin inhibitors • Fast troponin activators
Neuronal function	Neuronal transmission enhancement	• Salbutamol • Pyridostigmine

Figure 1. Possible therapeutic strategies for spinal muscular atrophy. Legend: HDAC = histone deacetylase; and SMN = survival motor neuron.

In this frame, animal testing provided a crucial help: several SMA animal models (*Caenorhabditis elegans*, zebrafish, and Drosophila) were indeed developed in the past. Nonetheless, only the setting up of a murine model able to accurately reproduce the human disease phenotype represented the actual cornerstone for preclinical studies [1]. Firstly, it must be considered that mice have only one SMN gene encoding for the SMN protein, instead of two as in humans. Moreover, homozygous mutations in the SMN gene induce early embryonic death in mice, whereas heterozygous SMN mice (SMN±) display a normal phenotype [2]. To overcome these issues, a novel transgenic murine model was developed, introducing the human *SMN2* gene in the SMN-null mice. This strategy made the murine disease phenotype more similar to the human one, also considering that *SMN2* manipulation allows for the introduction of specific patients' mutations. This was also paralleled by the adoption of reliable and highly reproducible experimental protocols. The Translational Research in Europe for the Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD) network [3] played a key role in this approach through the provision of internationally validated, standard operating procedures, useful to optimize protocols and accelerate the research process [4]. The timeline of the most important preclinical studies in the SMA field is summarized in **Table 1**.

Table 1. Timeline of preclinical studies of orphan drugs approved for spinal muscular atrophy treatment.

Drug	Mechanism of Action	Timeline	Detected Issues
Nusinersen	Inclusion of exon 7 in *SMN2* transcript	• 2006: Identification of ISS-N1 sequence located within *SMN2* intron 7 and synthesis of the first complementary ASO [5] • 2008: Synthesis of ASO 10-27 with high affinity to ISS-N1; first preclinical studies on SMA animal models (rodents) [6] • 2010–2011: The administration of ASO 10-27 via intrathecal or intracerebroventricular injection in SMA mice improved the expression levels of the SMN protein and ameliorated the disease phenotype; no increase in mice lifespan was detected [7] • 2011: Subcutaneous injection of ASO 10-27 in SMA mice induced an amelioration of peripheral symptoms and improved lifespan by more than 25 times [8]. Intrathecal injection in non-human primates ensured a proper	• Not able to cross the blood–brain barrier. • Administered only via intrathecal injection.

Drug	Mechanism of Action	Timeline	Detected Issues
		distribution at spinal cord level, without significant side effects [7]	
Onasemnogene abeparvovec	Replacement of *SMN1* gene	• 2009–2010: construction of a recombinant adeno-associated viral vector serotype 9 (scAAV9) including the *SMN1* gene [9] • 2010–2013: several preclinical studies conducted on the SMA murine model revealed that the drug had a high efficiency in improving lifespan and reducing the severity of symptoms [10]	• Uncertainties regarding treatment response and long-term outcomes. • Late administration not useful.
Risdiplam	Inclusion of exon 7 in *SMN2* transcript	• 2014: An orally administered coumarin derivative with a similar mechanism of action to nusinersen's was identified through drug design techniques in combination with high-throughput screening methods. In vitro test on patient-derived fibroblasts showed an increase in SMA protein levels and the oral administration in SMA mice improved motor function and lifespan [11] • 2016: Drug optimization techniques led to the synthesis of the compound RG7800 which passed toxicity and mutagenicity tests. The molecule did not exhibit phototoxicity issues. The administration in SMA mice confirmed the encouraging results obtained with the lead compound. Nonetheless, retinal toxicity was detected after the administration in non-human primates [12] • 2018: Further modifications of the RG7800 structure led to the synthesis of risdiplam, which displayed a higher affinity to the target and an excellent systemic bioavailability. The	• The systemic action could induce yet unknown side effects.

1.1. Nusinersen

Drug	Mechanism of Action	Timeline	Detected Issues
		drug resulted safe and well-tolerated after oral administration in SMA animal models	

Abbreviations: ASO = antisense oligonucleotide; SMA = spinal muscular atrophy; and SMN = survival motor neuron.

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skipping of exon 7 in the *SMN2* transcript and the subsequent synthesis of a truncated, non-functional protein (SMNΔ7). Nusinersen prevents the binding of specific splicing repressors, hnRNP-A1 and hnRNP-A2, to ISS-N1, allowing the integration of exon 7 into the final transcript and the synthesis of a full-length SMN protein. Since 2006, systematic screenings have been conducted to find the most effective ASO. Several ASOs were tested in murine models and in patient-derived fibroblasts, shifting along the ISS-N1, one base at a time. Once the strongest sequence had been identified, the specific ASO was administered in SMA mice to evaluate the effects of the compound on in vivo and ex vivo disease readouts. These treatments markedly increased the SMN protein levels and ameliorated the disease phenotype, extending the median lifespan (severely reduced in the SMA mice) by 25-fold and leading to the significant recovery of motor functions (including α-motor neuron count, mean area of muscle fibers, heart weight, thickness of the interventricular septum, left ventricular wall, and integrity of neuromuscular junctions), which were all relevant effects from a clinical perspective [8]. Moreover, the pharmacokinetic analysis performed on murine models and non-human primates highlighted a good bioavailability of the compound at the central nervous system level if administered by intrathecal infusion. These promising data provided a solid base for the following successful clinical trials, ending with nusinersen's approval for SMA treatment in children and adults. The main drawback of nusinersen use is represented by its inability to cross the blood–brain barrier (BBB) and the subsequent need for recurrent lumbar punctures for intrathecal administration. This considerably reduces therapeutic compliance, particularly for patients with severe scoliosis.

1.2. Onasemnogene Abeparvovec

The replacement of the *SMN1* defective gene is an alternative therapeutic approach that has been recently explored. Onasemnogene abeparvovec was approved in 2020 as the first, and currently unique, gene therapy for SMA, and it was indicated for SMA patients aged <2 years with bi-allelic mutations in the *SMN1* gene and three or fewer copies of the *SMN2* gene or infantile-onset SMA. The small dimension of the *SMN1* gene facilitated its delivery via a recombinant self-complementary adeno-associated viral vector serotype 9 (scAAV9). Preclinical studies in mice and non-human primates demonstrated the ability of the drug to cross the BBB after intravenous injection. Furthermore, this gene therapy significantly improved lifespan (median of 282 days for treated mice vs. 17.5 days for untreated SMAΔ7 mice) and ameliorated motor functions, particularly in early-treated animals [13][14]. Toxicology studies confirmed the safety and good tolerability of the treatment after intravenous injection, opening for the translatability of the drug in humans using this specific route of administration. In addition, it must be underlined that preclinical studies were performed on juvenile animals: the very encouraging obtained results supported again the importance of early administration. In fact, intravenous injection in mice within 24 h of birth showed greater efficacy compared to a later administration (within 5 or 10 days of birth), thus further reinforcing the relevance of neonatal screenings for early treatment.

1.3. Risdiplam

The success of nusinersen considerably boosted research for further therapies, in particular for those administered through a less invasive route. Innovative drug design techniques in combination with high-throughput screening methods permitted the identification of novel, small molecules that shared nusinersen's mechanism of action, promoting the inclusion of exon 7. The first molecule of interest was an orally administered coumarin derivative, subsequently modified to overcome potential in vitro mutagenicity issues and the phototoxicity typically associated with this drug class. Further compound optimization ameliorated the selectivity and affinity to the target, allowing a reduction of the dose required to achieve the therapeutic goals while limiting the side effects. Finally, after these implementations, the pyrido-pyrimidinone derivative risdiplam was synthesized and successively approved by the FDA and EMA as the first oral drug for SMA treatment in 2020 and 2021, respectively [15]. Preclinical studies performed in mice and non-human primates confirmed the drug's efficacy in restoring proper SMN protein levels and demonstrated a strong correlation between plasma and tissue concentration, overcoming the bioavailability issues of nusinersen [16]. In fact, several preclinical studies demonstrated that the SMN protein carries out a wide variety of actions at multiple levels and in different tissues, explaining the non-motor symptoms related to the pathology. Pharmacokinetic analyses documented the excellent distribution of risdiplam in several tissues, paving the way for an innovative therapy which combined an easier method of administration with the possibility to treat also peripheral symptoms. Real-world studies will help evaluate whether this systemic distribution could determine off-target effects, affecting the risk–benefit ratio of risdiplam.

2. Pivotal Clinical Trials

The characteristics of the pivotal clinical trials supporting the marketing approval of the three pharmacological therapies for the treatment of SMA are summarized in **Table 2**.

Table 2. Pivotal clinical trials leading to the marketing authorization of nusinersen, onasemnogene abeparvovec, and risdiplam.

Study	Study Design	Status	Eligibility Criteria	Exposure		Outcome	Results
				Treatment Group	Control Group		
				Nusinersen			
ENDEAR [17]	Phase III double-blind, controlled, randomized clinical trial.	Completed	Patients with homozygous deletions or mutations in the *SMN1* gene and with two copies of the *SMN2* gene and younger than 7 months at the screening date	80 patients treated with nusinersen at a dose adjusted by cerebrospinal fluid volume, such that the patients 2 years old or older received a dose	41 patients treated with a sham procedure	• Achievement of a motor milestone (defined based on the Hammersmith Infant Neurological	• 51% (37/73) of the patients in the treatment arm achieved at least one motor milestone, compared with 0 out of the 37

Study	Study Design	Status	Eligibility Criteria	Exposure		Outcome	Results
				Treatment Group	Control Group		
				equivalent to 12 mg.		Examination results) • Event-free survival (i.e., death or lifelong use of assisted ventilation)	patients in the control arm. • 61% (49/80) of the patients did not die or did not require permanent ventilation (compared to 32% [13/41] of the patients in the control group; p = 0.005) at the end-of-trial visit (i.e., day 183, 302, or 394).
Onasemnogene abeparvovec							
STR1VE-US [18]	Phase III open-label, single-arm clinical trial	Completed	Patients with SMA type 1, with biallelic *SMN1* mutations, with one or two copies of *SMN2*, and younger than 6 months of age at the date of onasemnogene abeparvovec administration	22 patients treated with a single intravenous administration of onasemnogene abeparvovec, over approximately 60 min: 1.1 × 10^{14} vg/kg	23 untreated children, aged less than 6 months and with SMA type 1 registered in the Pediatric Neuromuscular Clinical Research dataset (historical controls)	• Proportion of patients able to sit unassisted for at least 30 s at the 18 months follow-up visit • Survival to the age of 14 months (i.e., no death or permanent ventilation)	• 13 out of the 22 patients (59%) were able to sit unassisted for 30 s or more at 18 months of age (compared with 0 out of the 23 untreated patients in the Pediatric Neuromuscular Clinical Research

Study	Study Design	Status	Eligibility Criteria	Exposure		Outcome	Results
				Treatment Group	Control Group		
							cohort; $p < 0.0001$). • 20 out of the 22 patients (91%) survived without a need for permanent ventilation use for up to 14 months (vs. 6/23 [26.1%] of the untreated patients in the Pediatric Neuromuscular Clinical Research cohort; $p < 0.0001$).
Risdiplam							
SUNFISH—Part 2 [19]	Phase III double-blind, placebo-controlled, randomized clinical trial	Ongoing	Patients between 2 and 25 years of age, with type 2 or type 3 SMA	120 patients treated with oral-administered risdiplam (5 mg/day for patients weighing ≥ 20 kg or 0.25 mg/kg/day for patients weighing < 20 kg)	60 placebo-treated patients	Variation from the baseline in the 32-item Motor Function Measure's total score for patients at the 12th month of treatment compared to the placebo group	At month 12, the mean change from baseline in the MFM32 score was 1.36 (95% CI: 0.61 to 2.11) in the treatment group and −0.19 (95% CI: −1.22 to 0.84), with a difference of 1.55 (95% CI: 0.30–2.81; $p = 0.016$) in favor of the treatment with risdiplam.
FIREFISH—Part 2 [20]	Phase II/III open-label, single-arm clinical trial	Completed	Patients aged [17] 1 to 7 months with a type 1 SMA diagnosis	41 patients treated with risdiplam. The patients older	16 historical controls derived from the	Proportion of patients able to sit without assistance for at	12 out of the 41 patients (29%; 95% CI: 16 to 46) were able to sit

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Examination and the event-free survival (i.e., time to death or requiring permanent assisted ventilation). The results of the per protocol analysis showed that 51% of the patients in the treatment group and no patients in the control group achieved a motor milestone response and that the likelihood of event-free survival was higher in the treatment group than in the control group (hazard ratio for death or the use of permanent assisted ventilation, 0.53; $p = 0.005$). However, the ENDEAR trial was terminated early due to the results of the interim analysis and to the ethical consideration for the patients in the control group, and this led to the loss of data and a short time period for the assessment of the safety and the efficacy of nusinersen.

Study	Study Design	Status	Eligibility Criteria	Exposure		Outcome	Results
				Treatment Group	Control Group		
[18]			and two copies of the *SMN2* gene	than 5 months received a dose of 0.2 mg/kg/day; the patients younger than 5 months received a dose of 0.04 or 0.008 mg/kg/day, adjusted to 0.2 mg/kg/day within 1–3 months from starting treatment. [21]	NeuroNEXT study, and 24 historical controls	least 5 s after 12 months of treatment, based on the 3rd edition of the Bayley Scales of Infant and Toddler Development	without assistance for at least 5 s after 12 months of treatment; the percentage was significantly higher than in the historical controls ($p < 0.001$).

Abbreviations: CI = confidence interval; MFM32 = 32-item Motor Function Measure; SMA = spinal muscular atrophy; SMN = spinal motor neuron; and vg = vector genomes.

The marketing approval of risdiplam was based on two pivotal clinical trials: the FIREFISH trial in infantile-onset SMA [20] and the SUNFISH trial in late-onset SMA [19]. The FIREFISH study was an open-label, single-arm study including 41 infants aged between 1 and 7 months, with genetically confirmed Type 1 SMA. The primary endpoint was the ability to sit without support for at least 5 s after 12 months of treatment, a milestone that is never achieved in untreated patients affected by Type 1 SMA. After 12 months of treatment, 12 patients (29%) met this endpoint [20]. The internal validity of the FIREFISH trial was affected by the single-arm design, precluding a precise estimation of the magnitude of the benefit.

The SUNFISH study was a phase 3, randomized, double-blind, placebo-controlled clinical trial in patients aged between 2 and 25 years with confirmed type 2 or type 3 SMA. Patients were randomized, in a 2:1 ratio, to receive daily oral risdiplam (treatment group, N = 120) or daily oral placebo (control group, N = 60), and they were followed-up for 12 months. The primary endpoint was the change from the baseline in the 32-item Motor Function Measure (MFM32)'s total score at month 12. After one year, the least squares mean change from the baseline was 1.36 (95% confidence interval 0.61 to 2.11) in the risdiplam group and −0.19 (−1.22 to 0.84) in the placebo group, with a treatment difference of 1.55 (0.30 to 2.81, $p = 0.016$) in favor of risdiplam. However, the improvement in motor function was mainly detected in the younger patients (i.e., children aged between 2 and 5 years) rather than in the adolescent and adult patients, thus leaving unanswered questions for risdiplam use in adults with SMA when considering efficacy and long-term expectations [22].

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