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Research Article

Spinal muscular atrophy: Molecular genetics, Pathogenesis, and Therapeutic Approaches

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ABSTRACT
The mutation of ubiquitously expressed genes is regarded as one of the causes of many neurogenic disorders. Some of such disorders include Spinal muscular atrophy (SMA), Amyotrophic lateral sclerosis, Huntington's disease, etc. Spinal muscular atrophy is the leading genetic cause of infant death caused by the disruption of Survival motor neuron 1(SMN1) by deletion, mutation, or conversion. In human two isoforms of SMN exists SMN1, which are the disease-causing gene, and its paralog SMN2 that are retained. No effective medical treatment was known until the discovery of this disease-causing gene. The molecular understanding of SMN gene regulation greatly contributes to the development of treatment options. In the past few decades, there has been an intriguing development in the treatment of SMA, including antisense oligonucleotide therapy (Nusinersen), gene therapy (Onasemnogene abeparvovec), and small-molecule pre-mRNA modifiers (Risdipalm). The range of current strategies to impede the pathological mechanism in neurodegeneration can be expanded by better understanding these new medications and gene-related therapies for SMA. Thus, we briefly discuss our understanding of SMA and the disease-causing genes and outline some important therapeutic strategies that have been developed.

Keywords: Spinal muscular atrophy, Survival motor neuron, Neurogenic disorder, SMN targeting therapy, Gene therapy.

INTRODUCTION
Spinal muscular atrophy the leading genetic cause of infant death, first described by Werding and Hoffman in 1890 is an inherited autosomal recessive neurodegenerative disease. The disease is characterized by the degeneration of spinal cord motor neurons, and atrophy of skeletal muscle, which results in hypotonia and generalized weakness. It is mainly caused by the homozygous disruption of survival motor neurons 1(SMN1) by deletion, mutation, or conversion, generally showing the absence of SMN 1 exon 7 and retention of at least one copy of hypomorphic gene paralog SMN2 [1,2]. Thus, therapeutic strategies have been centered on restoring SMN expression by inhibiting alternative exon splicing and by gene therapy that would increase SMN protein expression in the motor neuron. It is crucial to fully understand the opportunities presented by these therapeutic approaches since they may also influence the emergence of treatment protocols for other neurogenic disorders [26]. Our review focuses on spinal muscular atrophy and the disease-causing gene SMN, and also discusses the critical understanding of treatment modalities that may be important for the future development of the medical intervention.

Spinal muscular atrophy and clinical manifestations
The incidence of the disease is estimated to be 1 in 10,000 live birth with a carrier frequency of 1 in 50, making SMA the leading cause of infant death and the second most common autosomal recessive disorder after cystic fibrosis [1, 2].
The SMA is classified into four types based on the age of onset and motor function achieved, which are summarized in Table no. 1. The clinical manifestation of different types of SMA are illustrated in Figure no. 1 [3,4].

Molecular genetics and pathogenesis
The SMN gene locus maps to the 5q13 region of the human chromosome and has two isoforms on each allele of the chromosome: the telomeric SMN1 gene, which gets to decide SMA, and the centromeric SMN2 gene, which determines disease severity [5]. Both isoforms are 99.9% identical and have identical promoters, also expressing RNA and protein ubiquitously. Transcription of the SMN1 gene results in the production of full-length mRNA that encodes the SMN protein. In the coding sequence, the SMN2 gene differs from the SMN1 gene by a single C-T

nucleotide transition at position six of exon 7 (Ex7+6), which does not change the amino acid sequence but causes alternative splicing of exon 7[6]. During the C-T transition, the hnRNP (heterogeneous nuclear ribonuclear protein), a known splicing repressor protein, antagonizes the exonic splice enhancer effect of SF2 (splicing factor 2) to promote exon 7 exclusion, particularly in SMN2. As a direct consequence of alternative splicing, the SMN2 gene produces perhaps less full-length transcript and protein and a variable amount of truncated mRNA lacking exon 7. Exon 7 deletion disrupts SMN's ability to oligomerize, resulting in an unstable and non-functional SMN protein that is rapidly degraded [7, 8].

Only around 10% of the SMN2 pre-mRNA is suitably spliced and translated into full-length SMN protein. This low level of protein may allow embryonic development, but it is insufficient to sustain the survival of spinal cord motor neurons [9].

Approximately 95% of SMA patients have homozygous SMN1 disruption, and 5% are compound heterozygous for deletion of one SMN1 allele and subtle intragenic mutation. All patients, however, have at least one copy of the SMN2 gene, which is inversely correlated to the severity of the disease [10]. For instance, individuals with homozygous SMN1 deletion and 5 copies of SMN2 were identified with a lack of SMA symptoms. Also a variant of SMN2 containing single nucleotide changes that can cause the inclusion of exon 7 has been identified in SMA patients carrying 2 copies of SMN2. Thus SMA is a result of deficiency but not a complete absence of SMN protein [11].

The motor neuron in the spinal cord contains a significantly greater amount of the SMN protein, which is encoded by the SMN gene. Within many cell types, the SMN protein is seen to be localized to several small discrete nuclear foci associated with coiled bodies named Gems [12].

To explain the pathogenesis of SMA, two main theories have been put forth: [13, 14, 15].

a. **SMN are involved in the biogenesis of small nuclear ribonucleoprotein and in mRNA splicing**: The primary component of the cellular organelle known as the spliceosome, which is responsible for post-transcriptional pre-mRNA splicing, is Uridine rich snRNPs. Exons are ligated by the U-snRNPs, which recognize the highly conserved sequence. As a component of a stable complex, SMN proteins are essential for the proper assembly of Smith class core proteins on U-snRNP, which is a critical necessity in the biogenesis of this vital RNP component of the RNA splicing machinery. The processing of RNA may require a large number of snRNPs. Motor neuron degeneration can be halted by defective SMN proteins, which prevent snRNP biogenesis.
b. **SMN has a motor neuron-specific function, independent from snRNPs assembly, such as mRNA transport along axon**: The SMN protein supports motor neuron survival by enabling normal axonal transport and preserving the integrity of the neuromuscular junction. Low concentrations of SMN protein are detrimental to motor neurons due to the length of axons and their interaction with skeletal muscle. Furthermore, it is also suggested that SMN protein interacts with hnRNP R, which in turn can interact with the 3′ untranslated region of β actin mRNA. A low concentration of SMN protein causes a low titre of β actin mRNA, suggesting the involvement of the protein in the transportation of ribonucleoprotein complex containing β actin that results in axonal outgrowth. Actin-binding protein Profilin controls the sequestration and release of actin monomer. In neuritic-like extension and growth cones, profiling IIa is seen to be colocalized with SMN protein. Thus, the mutant or low concentration of SMN protein knockdown profilin alone inhibits neuritic outgrowth [16].

In addition to these, SMA pathology is distinguished by some other defect that happens both centrally at synapses on motor neuron somata and dendrites as well as at NMJ. Presynaptic neurofilament accumulation, decreased vesicle content, impaired synaptic transmission, defective post-synaptic acetylcholine receptor clustering, and defective motor endplate development are some of the neuromuscular junction defects [11].

The cellular roles of SMN are depicted in Figure No. 2 [17,18,19,20,21,22]

Therapy

Advanced therapies have been developed through a detailed understanding of the molecular genetics of SMA. As our understanding of the SMN gene's organization has expanded, therapeutic advances have focused on promoting SMN2; to function like the SMN1 gene that was disrupted in patients. This in turn led to the investigation that could improve exon7 inclusion in SMN2 mRNA transcripts, increase SMN2 transcription by activating promoters, modify SMN protein translation, and prevent SMN protein degradation. The current treatment for SMA are summarized in the Figure no.3 [7].

Gene therapy

One of the significant therapeutic developments in SMA is gene therapy. The relatively small size of the SMN cDNA makes it an excellent candidate for viral-based replacement in spinal muscular atrophy. It was reported that SMA mice that received an intramuscular injection of a pseudotyped lentivirus vector had a modestly extended lifespan. The SMN protein level in SMA type 1 fibroblast was restored by the lentiviral vector expressing SMN. Additionally, it was discovered that multiple single injections of lentiviral vectors expressing SMN restored SMN in motor neurons, decreased motor neuron death, and prolonged life in SMA mice [23].

Using the self-complementary adeno-associated virus (scAAV) to deliver SMN to SMA mice is another ground-breaking technique. Delivery of scAAV9 into host cell is depicted in Figure No. 4 [27]. Insertional mutagenesis was prevented by delivering the transgene as a non-integrated stable extranuclear episome using an AAV vector platform. The anti- AAV9 antibody level is an important safety and efficacy consideration since children have low anti-AAV9 antibody titer frequencies [24]. The small SMN cDNA can be delivered throughout the body due to its simple packing onto the scAAV9 vector. AAV can also cross the blood-brain barrier to reach the central nervous system (CNS) and the spinal motor neuron, as well as deliver the cDNA to muscle and peripheral tissue. Preclinical research using a mouse model of AAV-mediated gene transfer revealed increased SMN expression. The replacement of SMN through a scAAV-mediated gene improved the lifespan, neuromuscular physiology, and motor neuron function of SMA pups [25, 26].

A successful viral gene delivery system for SMN1 is Onasemnogene abeparvovec, also known as AVXS-101 (ZOLGENSMA). AVXS-101 is available to patients with an SMN1 gene deletion or mutation who are younger than 2 years old. Onasemnogene is a scAAV-9 carrying human full-length SMN cDNA under the control of a hybrid chicken β-actin promoter. By delivering a functional copy of the human SMN gene, preventing neuronal cell death, and halting the disease's progression, AVXS-101 addresses the genetic cause of SMA [27].

The clinical trials AVXS-101-CL-101 (START, NCT02122952) and CL-303 (STR1VE-US, NCT03306277; STR1VE-EU, NCT03461289; STR1VE AP, NCT03837184) were carried out on symptomatic SMA type 1 patient. 15 infants under the age of nine months participated in the open-label, dose-escalation START clinical trial. Three of the 15 were given a dose of $6.7x10^{13}$ vg/kg, while the other ten were given a higher dose of $2x10^{14}$ vg/kg as a single i.v infusion. Eleven of the twelve patients demonstrated the ability to sit without assistance, nine learned to roll over, and two were able to walk independently. STRIVE- US is an open-label, multicentric phase III trial. A dose of $1.1x10^{14}$ vg/kg was used and the results showed a similar safety profile and similar clinical result as that of the phase I study [26, 28]. The different clinical trials are summerized in Figure no. 5.

A. SMN2 targeted- Small molecules and splicing modifiers targeting SMN2 splicing

Small molecules

Small molecules have been identified to restore SMN protein level by increasing the inclusion of exon 7 in the SMN2 transcript. They can act on the processing of SMN2 gene transcript as well as can modulate SMN2 splicing. Therapy using small molecules is of great advantage since they can cross BBB [26]. Small molecule splicing modifiers have been previously described as having low specificity for a particular gene because they target the general splicing machinery. Several potent molecules correcting the splicing deficit of the SMN2 gene have been identified; these molecules are a potential therapeutic strategy. It was demonstrated that these molecules specifically bind to SMN2 over other genes by directly binding to two distinct sites on the SMN2 pre-mRNA and stabilizing the RNP complex [29]. With high selectivity, a small molecule that can be taken orally can influence the balance of SMN2 splicing in favor of the production of full-length SMN2 messenger RNA. In the SMA mouse model, these substances cause an increase in SMN protein levels, improved motor function, and protection of the neuromuscular circuit [30]. The main drawback of these small molecules is their propensity to bind to off-targets. Risdiplam (RO073406/RG7916) was presented as the ideal candidate to reduce this non-specific effect [26].

Risdiplam(Evrysdi) is an orally available SMN2-directed RNA splicing modifier developed by Roche, PTC therapeutic Inc. and the SMA foundation for the treatment of SMA. This most recent medication improves the capacity of altered SMN2 to generate full-length and functional SMN protein [31]. By anchoring to the exonic splice enhancer 2 of exon 7 and the 5' splice site (5'ss) of intron 7 in the SMN2 pre-mRNA, this SMN2 splice modifier promotes exon 7 inclusion and stabilizes the RNP complex. Accordingly, it is recognized by the FDA for all SMA patients who are 2 months or older [29, 32]. The safety, tolerability, and efficacy of the drug were tested by the following trials summarized in Table no.2.

The ongoing trial RAINBOWFISH (NCT03779334) focuses on pre-symptomatic SMA patients.

The treatment-related side effects of Risdiplam are rashes, diarrhea, and nausea. Risdiplam was related to two serious adverse events femoral neck fracture and URTI, both of which improved with continued risdiplam therapy. Laboratory results, vital signs, and electrocardiogram data did not show any negative trends [33, 31, 34].

Another orally available small molecule Branaplam is also being tested in an open-label, two-part, phase1/2 study (NCT02268552). It is a pyridazine derivative that interacts with SMN2 pre-mRNA and enhances exon 7 inclusion to increase SMN protein level [28, 32, 33].

Antisense Oligonucleotides

ASO technology can now be employed to effectively control pre-mRNA splicing. It pumps up the production of functional SMN and re-directs the splicing of SMN2. Because ASO is made to base pair with a particular nucleotide sequence, it presents a lower risk of off-target effects [35]. Exon 7 splicing correction is the ultimate aim of the ASO-based approach. The targets are as follows:

- Intronic splicing silencer N1(ISS-N1)
- 3′ splice site of exon 8
- GC-rich sequence
- ISS-N2

These days, ISS-N1 targeting ASO is an important research topic because it has the potential to affect mRNA splicing through any of the following channels.

- Transcriptional factor dispersion on pre-mRNA.
- Pre-mRNA structural re-modeling
- Enrolling a new transaction factor.
- Cotranscriptional splicing control and transcriptional stuttering [36].

Intrathecally injected ASO with the brand name Spinraze is intended to prevent the SMN2 transcript's exon 7 from being blanked. These short strands of nucleic acid interact with the target RNA through complementary base pairing to alter its stability, structure, and functionality.

One strategy for managing exon 7 splicing involves using antisense technology to shift the competition between exon 7 and exon 8 at the 3' splice site in favor of exon 7, which brought up the level of the SMN protein. Another strategy is to block the intronic splice repressor element 1 sequence upstream of exon 7, as exon 7 inclusion was increased by the deletion of ISS-E1. Another target was made available by the discovery of the ISS-N1 component of the 5' splice site in exon 7. Exon 7 inclusion was nearly 100% on the primary transcript after ISS-N1 was deleted, and this increased the level of SMN protein in the transfected cell line, the patient-derived cell line, and the mouse model. The binding of U1 small nuclear RNA to the 5' splicing site of exon 7 is hindered by the displacement of a trans-acting negative repressor and/or the winding down of a cis-acting RNA stem-loop caused by the hybridization of ASO to the ISS-N1 region [37].

Nusinersen is the first disease-modifying antisense oligonucleotide drug that can modulate the pre-mRNA splicing of the SMN2 gene [38]. Nusinersen is an 18- mer ASO modified by 2'-O-2-methoxyethyl phosphorothioate to prevent degradation loss. They inhibit hnRNPA1 from binding to the intron 7 ISS-N1 motif, which obstructs a splice inhibitor site and encourages the inclusion of exon 7 in the pre-mRNA. Due to their size, they cannot pass the blood-brain barrier and thus are given intrathecally. In clinical trials, it showed significant benefits and a good safety profile. It was approved by FDA in December 2016 and by EMA in June 2017 [26]. The clinical trials with Nusinersen are summarized in the Figure no. 6

B. SMN stabilization

Proteasome research has also been expanded upon as a means of stabilizing the exon-skipped SMN protein as well as the low intracellular level of SMN. Treatment with proteasome inhibitors can stabilize the low level of full-length SMN protein, increasing the pool of SMN. A proteasomal inhibitor called bortezomib primarily prevents chymotrypsin cleavage. Although it boosts SMN, it hasn't been able to prolong SMA mice's survival. Aminoglycosides have been demonstrated to stabilise the protein by causing the SMN protein to undergo translational read-through. By ICV injection, the novel aminoglycoside TC007 was able to prolong survival and lessen the severity of the disease in SMA mice [39].

CONCLUSION

The medical field has noticed a rapid expansion in the treatment options for SMA since the identification of the disease-causing SMN1 gene. The development of SMN targeting approaches that modulate pre-mRNA splicing was prompted by the search for therapeutic approaches that could address the disease's fundamental underlying cause, which is basically a lack of SMN protein. The treatment of this neurodegenerative disorder has also benefited from gene therapy. The currently approved medications do not completely restore patients' motor function or development, despite the fact that this SMN-dependent therapy is the best for improved lifespan and motor function. Thus, ongoing SMN-dependent and independent strategies are expected to improve SMA. The development of treatment plans for other neurogenic disorders may also benefit from

advancements in present therapeutic methods, but this will obviously necessitate a deeper comprehension of current treatment practices.

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CONFLICT OF INTEREST

I, declare that I have no conflicts of interest related to the manuscript under review, or any related work or publications.

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Table 1: Types of Spinal muscular atrophy [3,4]

	Age	Highest function achieved
Type I	0-6 months	Never sit
Type II	7- 18 months	Sit, never stand
Type III	>18 months	Stand and walk during adulthood
Type IV	Second or third decade	Walk unaided

Table 2: Clinical trials with Risdiplam [31, 32, 33].

	FIREFISH TRIAL (NCT02913482)	SUNFISH TRIAL (NCT02908685)	JEWELFISH TRIAL (NCT030321725)
Design	Two- part, phase 2/3, non-randomized, open-label study	Multi-centric, two-part, phase 2/3, randomized and placebo-controlled trial	Multi-centric, phase 2, open-label trial.
Study population	Part 1 n= 21patients Part 2 n= 41patients	Later onset SMA patients (2-25 yrs). Part 1 n= 51 patients	174 SMA type 2 and 3 patients with 3 or 4 SMN2 copies.

	Both with homozygous deletion of SMN1 gene and 2 copies of SMN2 GENE.	Part 2 n= 180 patient	
Outcomes	29% of patients are able to sit independently after 12 months of treatment. 42 % could live without permanent ventilation.	Increased motor function. No treatment-related adverse effects.	No serious adverse effects were reported.

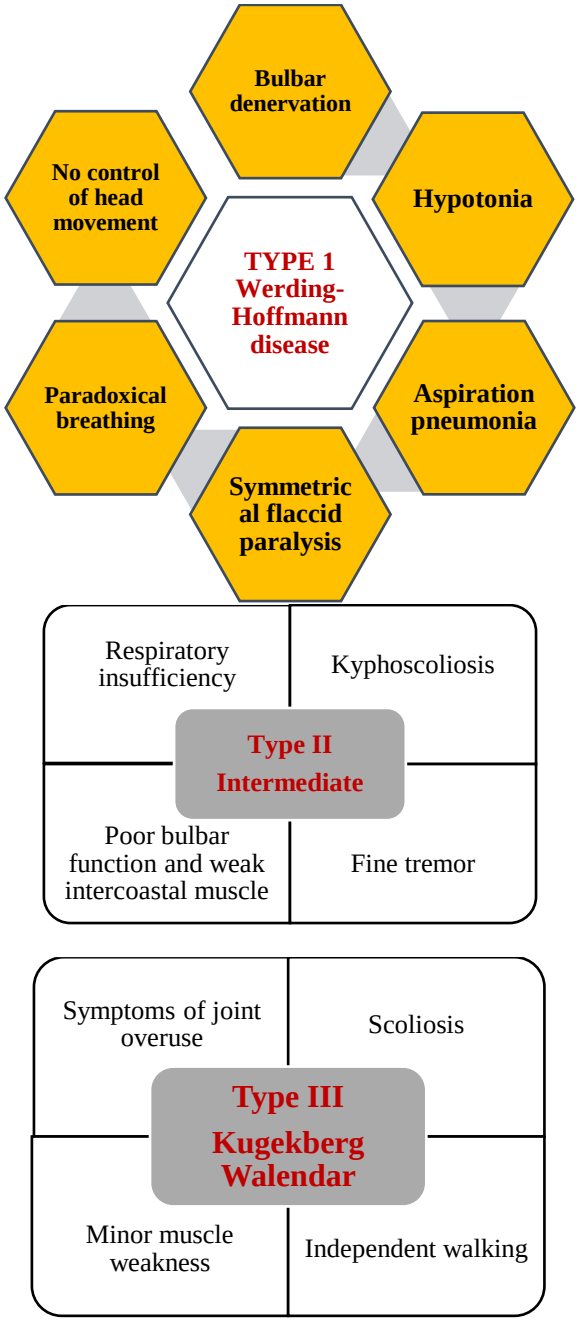

Figure 1: Illustration of various clinical features of SMA [3,4] .

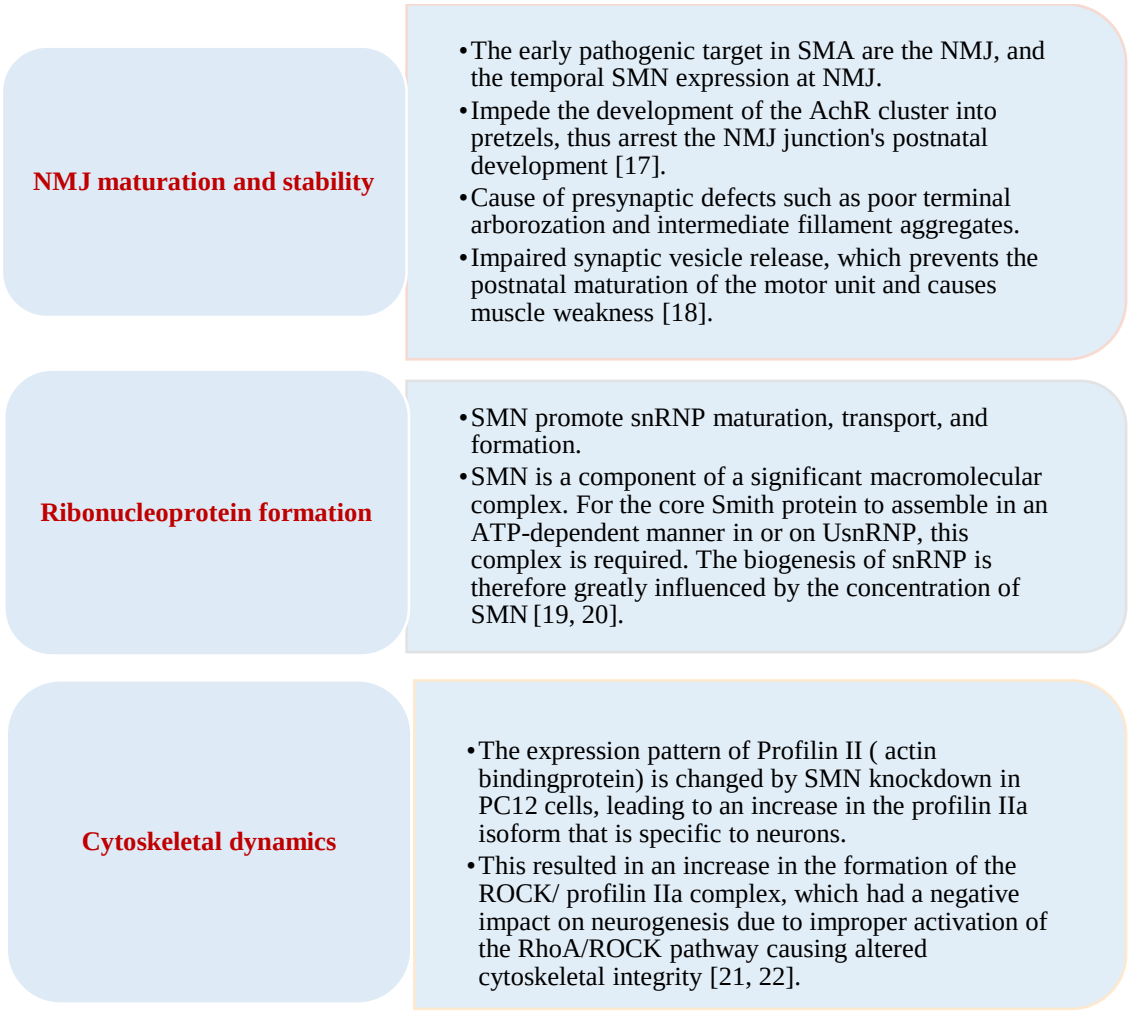

Figure 2: Illustration of different cellular roles of SMN

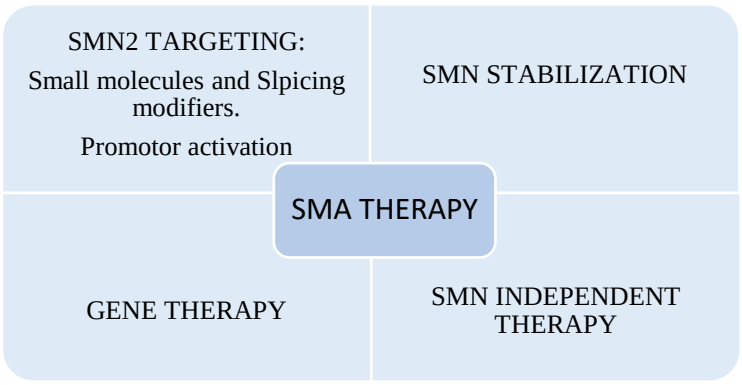

Figure 3: Illustration of different therapeutic approaches for SMA[7]

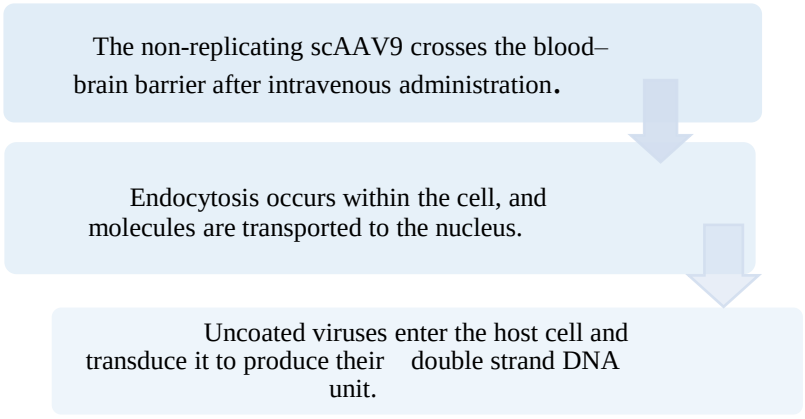

Figure 4: Illustration of delivery of AAV9 into host cell [27]

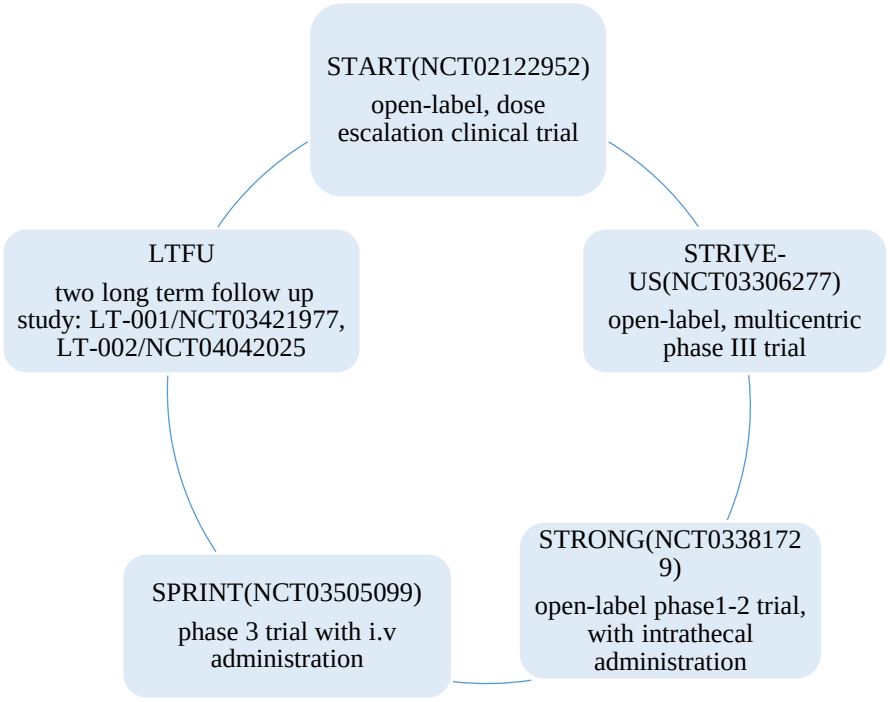

Figure 5: Illustration of the different clinical trials with Onasemnogene abeparvovec [26].

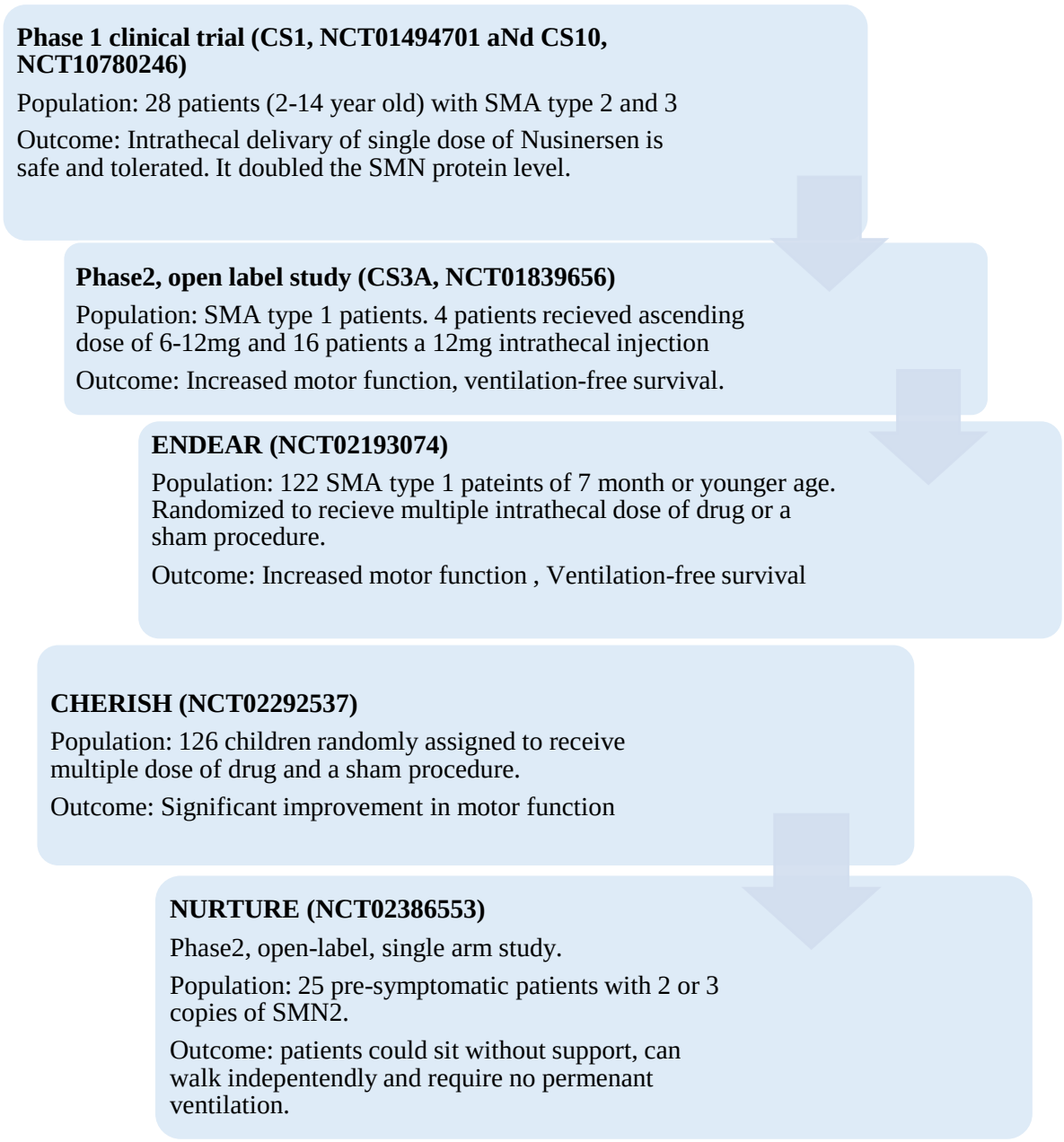

Figure 6: Illustration of the clinical trial with Nusinersen [26, 28, 33,39].