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PHARMACOLOGICAL TREATMENT OF SPINAL MUSCULAR ATROPHY TYPE 1

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Pharmacologic Treatment of Spinal Muscular Atrophy Type 1

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ABBREVIATION LIST

ASO – Antisense oligonucleotide

CHOP-INTEND – Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

CMAP – Compound muscle action potential

CSF – Cerebrospinal fluid

DNA – Deoxyribonucleic acid

EMA – European Medicines Agency

EMG – Electromyography

FDA – Food and Drug Administration

HDAC – Hystone deacetylase inhibitors

HFMSE – Hammersmith Functional Motor Scale Expanded

HINE-2 – The Hammersmith Infant Neurological Examination

hnRNPA1 – Heterogenous nuclear ribonucleoprotein A1

ICV – Intracerebroventriculary

ISS-N1 – Intronic splicing silencer N1

NIV – Non-invasive ventilation

RNA – Ribonucleic acid

SC – Subcutaneous

SMA – Spinal muscular atrophy

SMN – Survival motor neuron 2 gene

SMN – Survival motor neuron protein

SMN1 – Survival motor neuron 1 gene

VPA – Valproic acid

ABSTRACT

Spinal muscular atrophy type 1 is a neuromuscular genetic disorder that is characterized by infantile onset of muscle weakness and atrophy, leading to premature death in most cases.

It is caused by the loss of the survival motor neuron 1 (*SMN1*) gene and the retention of at least one copy of a highly homologous survival motor neuron 2 (*SMN2*) gene. This results in decreased survival motor neuron protein levels, leading to disease. A better understanding of the molecular and cellular pathology led to the development of several therapeutic targets.

Nusinersen, an antisense oligonucleotide, became the first approved drug for the treatment of spinal muscular atrophy type 1. Clinical studies were successful and assessed safety and efficacy of the drug. Although not a cure, nusinersen is able to hinder function impairments and increases survival. It is administered intrathecally at an equivalent dosage of 12 mg, with a 4-month dosing interval, after a loading period of 4 doses in 2 months, approximately.

Other alternative drugs for the treatment of the disease are currently being investigated, and preclinical and early clinical studies have shown some favorable evidence. Drugs such as olesoxime will likely have a key role in the future used in association to nusinersen, Identifying combinatorial therapeutic strategies is deemed as the future of spinal muscular atrophy type 1.

Key-words: Spinal muscular atrophy type 1; SMN1; SMN2; pharmacological treatment; nusinersen.

RESUMO

A atrofia muscular espinhal tipo 1 é uma doença neuromuscular de etiologia genética caracterizada por fraqueza e atrofia muscular de início até aos 6 meses de vida. A sobrevivência é limitada, com morte prematura na maioria dos casos.

A doença é causada por uma mutação homozigótica deletéria do gene 1 de sobrevivência do neurónio motor (*SMN1*), com retenção de pelo menos uma cópia do gene 2 de sobrevivência do neurónio motor (*SMN2*), um gene homólogo do gene 1. Consequentemente, os níveis da proteína de sobrevivência do neurónio motor tornam-se insuficientes, o que leva ao desenvolvimento da doença. Nos últimos anos, foi possível esclarecer melhor os mecanismos moleculares e celulares da doença, o que permitiu investigar possíveis tratamentos.

O nusinersen, um oligonucleotídeo antisense, foi o primeiro fármaco a ser aprovado para o tratamento da atrofia muscular espinhal tipo 1. Estudos pré-clínicos e clínicos validaram a segurança e eficácia do fármaco. A toma de nusinersen permite melhorar o fenótipo da doença e aumentar a sobrevivência, embora não seja uma cura. É de administração intratecal, com uma dose de 12 mg a cada 4 meses, depois de uma dose de carga constituída por 4 administrações em aproximadamente 2 meses.

Alternativas farmacológicas estão actualmente em investigação. Os primeiros estudos revelaram resultados favoráveis de alguns fármacos. Prevê-se que no futuro o tratamento farmacológico da doença envolva a toma do nusinersen associada a outro fármaco, como por exemplo o olesoxime. O futuro da investigação na atrofia muscular espinhal tipo 1 passa pelo estudo de possíveis combinações terapêuticas, que poderão ser a melhor opção farmacológica para o tratamento da doença.

Palavras-chave: Atrofia muscular espinhal tipo 1; SMN1; SMN2; tratamento farmacológico; nusinersen.

1. INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by degeneration of the anterior horn cells of the spinal cord, resulting in muscle atrophy and proximal muscle weakness (1).

SMA type 1, also called infantile onset spinal muscular atrophy, is the most severe form of the disease, with an estimated incidence of 1 in 6.000 to 11.000 live births (2). Onset of disease is within 6 months of age and infants are not able to achieve major motor milestones, such as rolling over or sitting independently (3-5). These infants require aggressive support to survive past 2 years of age, being dependent on respiratory and nutritional support (6,7).

The disease is caused by mutations in the survival motor neuron 1 (*SMN1*) gene, located on chromosome 5q13, resulting in reduced amount of functional full length survival motor neuron (SMN) protein (8). The discovery of the monogenetic cause of SMA led to further investigation on the genetic and cellular mechanisms of disease, aiming for the discovery of a possible treatment (9).

Throughout the last decades, several pharmacological strategies have been tested. Drugs attempting to affect SMN transcription have been widely studied, as well as drugs that could allow the improvement of phenotype without targeting the transcription of the protein. 2016 marked the year in which the first drug for the treatment of SMA was approved, an important milestone in neuromuscular diseases research. Nusinersen is an antisense oligonucleotide which had remarkable success on both preclinical and clinical studies (10).

In this study, the various pharmacological agents that have been studied for SMA type 1 will be reviewed. An overview of the clinical, genetic and pathogenic background will be

presented, highlighting how basic research of disease mechanisms led to the approval of nusinersen for the treatment of this condition.

Further major developments in the treatment of SMA type 1 are expected to take place in the upcoming years. Through this study it will be possible to understand the current state of drug research, and what should be expected in the near future.

2. METHODS

A systematic review of literature was conducted using the PubMed, Web of Science and Cochrane databases. Articles that were published between January 1994 and March 2018 were identified. The time frame applied was chosen because gene discovery of SMA was in 1994. Search terms used were: ("spinal muscular atrophy") AND ("type 1" OR "type I" OR "infantile") AND ("therapeutic" OR "pharmacological treatment" OR "drug" OR "nusinersen" OR "spinraza"). The reference lists of papers identified were also screened and added if met the inclusion criteria. Published peer-reviewed studies of any design were included if they investigated treatment strategies for SMA type 1, after a review of the titles and abstracts of the identified articles. Studies that were not in English and that were unrelated to the treatment of SMA were excluded.

The original literature search identified 344 studies, of which 77 studies fulfilled the search criteria and were included in the review.

3. RESULTS

3.1 UNDERSTANDING SPINAL MUSCULAR ATROPHY

SMA belongs to a wider group of disorders termed as the spinal muscular atrophies. Although the medical community uses the term "spinal muscular atrophy" when referring to the disease, the fact is that the correct designation of the disease should be "5qSMA" or "*SMN1*-related SMA". Nonetheless, "5qSMA" represents nearly 100% of the SMAs, being that the reason why the general term is accepted (1).

The SMAs are a heterogeneous group of genetic neuromuscular diseases characterized by motor neuron loss in the spinal cord and brainstem, causing muscle weakness and progressive atrophy. The clinical phenotype incorporates a wide clinical spectrum that is differentiated based on age of onset, pattern of muscle involvement and inheritance patterns, with detailed clinical and anatomical descriptions across severities (1).

SMAs are divided into proximal SMAs and distal SMAs. Proximal SMAs can then be divided into *SMN1*-related SMA or Non-*SMN1*-related SMA. The classic SMA belongs to the group of proximal SMAs, and is a *SMN1*-related SMA (8). It accounts for nearly 100% of the cases of SMAs, with an estimated incidence of 1 in 6.000 to 11.000 live births and a carrier frequency of 1 in 40-60 adults. It is the second most common autosomal recessive disorder of childhood after cystic fibrosis and is the leading genetic cause of infant mortality, which results from respiratory insufficiency (11,12). *SMN1*-related SMA is caused by a combination of homozygous deletions or mutations of the telomeric copy of the SMN gene (*SMN1*) on chromosome 5q13 and the presence of 1 or more copies of *SMN2*, an almost identical but partially functional centromeric copy, which is unique to humans. Defects in the *SMN1* gene result in abnormally low levels of the of the ubiquitous protein *survival of motor neuron*

(SMN), resulting in selective destruction of alpha motor neurons on the anterior horn cells of the spinal cord and brainstem (13,14).

The disease typically presents in infancy or childhood, and is clinically characterized by progressive muscle atrophy and weakness, loss of reflexes, tongue fasciculations, tremor, and denervation on electromyography. Weakness is symmetrical, more proximal than distal, lower limbs are more affected than upper limbs, and extraocular and facial muscles are relatively unaffected. Notably, the diaphragm is relatively spared, even though respiratory insufficiency is an important complication of SMA (1).

The phenotype is highly variable, and patients are classified into 1 of 5 types based on age of onset and the inability to achieve motor milestones. The main 3 types are type I (also called Werdnig-Hoffmann disease), type II and type III (also called Kugelberg Welander disease), and these various types are seen more as a continuum of severity rather than individual types. Type 0 is a very severe form with onset *in utero* and type IV is a mild late (adult) onset form that has normal life expectancy (5).

Other forms of proximal SMAs with different genetic background can be included in a group termed as Non-*SMN1*-related SMA. Among infantile SMA, the group of infantile SMA variants or SMA "plus" syndromes accounts for less than 5% of infantile forms (15). Infants are clinically similar to *SMN1*-related SMA patients, but additional clinical features may be evident, including arthrogryposis, abnormalities of extra-ocular movements or cardiomiopathy. Other infantile forms of Non-*SMN1*-related SMA include X-linked infantile SMA with arthrogryposis (XL-SMA), SMA due to mitochondrial dysfunction, SMA with pontocerebellar hypoplasia (SMA-PCH/PCH1) and SMA with respiratory distress (SMARD). Beyond infancy, several diseases are included in this group, including Kennedy's disease and autosomal dominant variants of SMA (15).

The other broad category of SMAs is distal spinal muscular atrophy (DSMA). Also known as distal hereditary motor neuropathies (dHMN), they are characterized by a slowly progressive symmetrical and predominantly distal limb weakness and atrophy (15).

3.2 CLINICAL CLASSIFICATION OF SMA

Given the clinical heterogeneity of SMA, in 1991 a committee of clinicians and geneticists suggested the introduction of types of the disease, based primarily on age of onset and highest level of motor function. This was done to promote collaborative studies between different centers and to identify the genes for SMA (16). Firstly, only 3 types were highlighted, and subsequent modifications added a type 4 for adult-onset cases and included a type 0 for patients with prenatal onset and death within weeks (17). There has been much debate about the appropriate classification of patients into these types, mainly because even within an individual type there are phenotypes of differing severities and about 25% of patients elude precise classification. However, even though this classification scheme has some shortcomings, this method of classification remains relevant in the genetic era and provides useful clinical and prognostic information (Table 1). A classification system based on continuous rather than discrete variables has been proposed, with the introduction of subtypes that subdivide patients into clinical subgroups (18,19). The classification subtypes will be detailed for type I.

3.2.1 TYPE 0

Type 0 patients are neonates who present with severe weakness and hypotonia, with a history of decreased fetal movements. It is assumed that in these cases the disease is probably of prenatal onset. Further examination reveals areflexia, facial diplegia, atrial septal defects and joint contractures, and respiratory failure is a major concern early on. Most patients are unable to survive beyond 6 months of age. Some authors do not consider this type as a separate one, rather considering these neonates as type 1 children at the most severe end of the spectrum (5).

3.2.2 TYPE 1

Infants with SMA type I (also known as Werdnig-Hoffmann disease) present with proximal weakness, affecting the legs more than the arms, prior to 6 months of age. By definition, they never achieve the ability to sit and remain seated unassisted ("nonsitters"). At the time of presentation infants typically show tongue fasciculations, generalized hypotonia and areflexia. Profound hypotonia manifests as poor head control and as a "frog-leg posture" in supine position. These infants slip through on vertical suspension and are unable to roll over independently. Weakness in intercostals muscles and with relative sparing of the diaphragm produces a bell-shaped chest and a pattern of paradoxical breathing sometimes referred as diaphragmatic breathing or "belly-breathing".

During the first year of life lower cranial nerves are involved due to progression of bulbar weakness, with increasing tongue and swallowing weakness. Due to increasing feeding difficulties, taking longer to feed, these infants are at risk of aspiration pneumonia and failure to thrive. Other cranial nerves are spared during early phases, but later in the course of the disease mild facial diplegia can develop and very rarely extra-ocular movements can become impaired.

Despite the profound weakness, cognition in these infants is normal and they are often alert, attentive and bright at the time of diagnosis. These patients typically die before the age of 2 years, although respiratory and nutritional support have prolonged life expectancy (3,5,20).

3.2.3 TYPE 2

Children with SMA type 2 (also known as intermediate SMA or Dubowitz disease) are able to sit unassisted and are never able to walk independently ("sitters"). This form of SMA tends to manifest as progressive proximal leg weakness affecting legs more than the arms,

hypotonia and areflexia, with onset between 6 and 18 months of age. Tongue fasciculations may be present and many have a fine postural tremor in their hands (polyminimyoclonus). Comorbidities in these children are mainly related to the orthopedic complications of bone and joint development in the setting of muscular weakness. Progressive scoliosis, joint contractures and ankylosis of the mandible may develop. They also develop progressive intercostals muscle weakness, which when associated with scoliosis results in restrictive lung disease, with need for respiratory support measures. Most maintain the ability to feed orally, although more severely affected children may eventually need nutritional support. Cognition is normal, and survival is into adulthood, but shortened in those with respiratory compromise. Widespread use of nutritional and respiratory support has increased survival well past their 25^{th} birthday (5).

3.2.4 TYPE 3

Children with type 3 (also known as Kugelberg-Welander disease) are able to walk independently at some point during their lifetime ("walkers"). Symptom onset is after 18 months of age. Children present with proximal muscle weakness affecting their legs more than their arms. Tendon reflexes are often preserved at the time of the diagnosis, but over time children lose proximal reflexes and afterwards distal reflexes. Polyminimyoclonus may also be exhibited over time. With the progression of leg muscle weakness, these children may lose ambulation. Respiratory lung disease and scoliosis are not as prevalent in this group when compared to children with type 2. Cognition is normal, and life expectancy is not significantly different compared to the normal population (5).

3.2.5 TYPE 4

Individuals with type 4 are at the mild end of the continuum. By definition symptom onset is after 21 years of age, but mean age of onset is mid-30s. Individuals present with proximal limb girdle weakness, and the clinical course is slowly progressive, with a small proportion losing independent ambulation within 20 years of symptom onset. Fasciculations are present in 75% of patients, with occasional muscle cramps. Scoliosis and respiratory lung disease are rare in this group. Cognition and life expectancy are normal, although this is the least prevalent type with little information published on the natural history (5).

Table 1: Classification and characteristics of SMA patients (6,21).

Type	**Age of onset**	**Highest function**	**Survival**	***SMN2* copies**
Type 0	Prenatal	Never sits or walks	< 6 months	1
Type 1	0 – 6 months	Never sit or walks	< 2 years	1 – 3
Type 2	7 – 18 months	Sits, but never walks	> 2 years/ adulthood	2 – 4
Type 3	18 months – 21 years	Stands and walks	Adulthood	3 – 4
Type 4	Over 21 years	Walks	Adulthood	> 4

SMN2 – Survival motor neuron 2 gene.

3.3 CLINICAL SUBGROUPS OF SMA TYPE 1

Clinicians and geneticists have made several attempts to subdivide type 1 patients into clinical subgroups, in order to reflect the extreme variability within the condition.

In 1995, a decimal classification was created. The decimal classification takes into account the notion that SMA is a continuum, and thus it goes from 1.1 to 3.9 according to clinical features. Type 1.1 can be seen as the equivalent to type 0 and type 3.9 can be seen as the equivalent to type 4.

Regarding type 1 patients, type 1.1 includes infants at the more severe end of the spectrum, with paralysis at birth and early respiratory and bulbar difficulties, with an overtly poor prognosis for survival. Type 1.5 identifies infants with features more typically observed in type 1. They are unable to raise the legs against gravity or maintain head posture, but have, at diagnosis, no difficulty with feeding and swallowing, no accumulation of pharyngeal secretions and no obvious distress. Type 1.9 includes infants at the other end of the spectrum, with some head control, almost achieving the ability to sit unaided, but not quite, and having a more reasonable respiratory reserve, although still somewhat compromised (18).

In 2012, a new classification scheme was proposed to subdivide type 1 patients. This classification overlaps with the decimal one into 1A, 1B and 1C. Different versions have been reported. One is based just on onset of symptoms, while another version uses a combination of onset and achievement of head control.

Purely based on onset, the classification is described as: 1A for infants who have clear clinical signs during the first 2 weeks of life; 1B for infants who have onset of weakness by 3 months of age; 1C for infants who have onset of weakness by 6 months of age. Using a combination of onset and achievement of head control the classification is: 1A when head control is never achieved, with signs at birth or in the neonatal period; 1B when head control

is never achieved, with onset after the neonatal period; 1C when head control is achieved and onset is after the neonatal period (6).

Recent studies have shown that there is a difference, both at baseline and at follow up, among type 1 infants classified according to onset and severity. Infants with severe phenotype, with early neonatal onset, had low motor scores at baseline and a rapid decline, and rarely survive beyond one year. While infants at the end of the spectrum, with mild phenotype, had significantly higher baseline scores and were overall more stable (19).

3.4 DIAGNOSIS OF SMA TYPE 1

The diagnostic process for SMA type 1 is generally prompted by clinical signs, unless there are previous familial cases. The clinician must suspect of SMA type 1 when an infant presents with hypotonia, progressive symmetric and proximal weakness affecting the legs more than the arms, weakness of the intercostals muscles with relative sparing of the diaphragm and bulbar muscle weakness with sparing of facial muscles (19,21).

As SMA type 1 is caused by an homozygous mutation in the *SMN1* gene, testing for the mutation should be undertaken (21).

Before the advent of genetic testing, the diagnosis of SMA type 1 was based on clinical features, neurophysiological studies with repetitive stimulation, muscle biopsy, measurement of creatinine kinase serum levels and exclusion of other causes. Nowadays, as genetic testing is readily available in most centers, other studies are rarely needed (21).

The gold standard of SMA genetic testing is a quantitative analysis of both *SMN1* and *SMN2* genes using multiplex ligation-dependent probe amplification (MLPA), quantitative polymerase chain reaction (qPCR) or next generation sequencing (NGS) (22).

Genetic testing for *SMN1*/*SMN2* is highly reliable and it is first line investigation if the disease is suspected (21).

The absence of both full *SMN1* copies diagnoses SMA type 1. If only 1 full copy is present and clinical phenotype is compatible with SMA type 1, the remaining *SMN1* gene should be sequenced looking for other mutations (15).

If both full *SMN1* copies are present, a diagnosis of SMA type 1 is unlikely. However, if there is a typical phenotype or consanguinity, then the *SMN1* gene should be sequenced. If sequence indicates an intact *SMN1* gene in the presence of a phenotype suggestive of SMA type 1, other motor neuron diseases should be considered (21).

The number of *SMN2* copies is not essential to diagnose SMA type 1. However, they should be routinely assessed, as it is an important factor influencing the severity of the phenotype. Moreover, the number of *SMN2* copies is currently used as a criterion for enrolment of patients into clinical trials (21).

Additional studies are usually unneeded in a typical presentation. The most common reason to perform additional studies is when genetic testing is negative for *SMN1* mutation. In this case, firstly the clinician must review clinical features and then these studies should be performed. EMG and nerve conduction tests are important for the differential diagnosis, because they help to distinguish between motor neuron disease, myopathy and neuromuscular junction disorders.

In more chronic forms of SMA type 1, in which the diagnosis might be less striking, valuable information can also be obtained through neurophysiological studies (20). Compound muscle action potentials (CMAPs) are typically reduced in SMA type 1, while motor conduction velocities and distal latencies are normal or only modestly reduced when the CMAP amplitude is substantially reduced. EMG shows chronic denervation with motor units of increased amplitude and duration, though in infants with severe SMA type 1 there may be normal-sized residual voluntary motor units (15).

3.5 THE STANDARDS OF CARE

Management of SMA type 1 is complex and multidisciplinary. There are many downstream complications of the disease, as weakness can lead to compromised respiration, impaired nutrition, skeletal deformity and other complications (23).

The standards of care help limiting the negative impact of these complications. Management can be divided by systems, including pulmonary, gastrointestinal, nutritional, orthopedic care and rehabilitation. For optimal care, these aspects should be dealt by a multidisciplinary team coordinated by a physician (21).

In 2007, a comprehensive consensus statement on SMA standard of care was published by Wang and a collaborating panel of experts. This document established guidelines for managing the multiple expected clinical problems that develop in patients with SMA as they age, and has been widely used throughout the world. In 2017, a two-part update of the topics covered in the previous recommendations was published (21,24). In this document, patients are divided according to a functional scale that classifies type 1 patients are indicated as “non-sitters”, while type 2, type 3 and type 4 are indicated as either “sitters” or “walkers” (considering that type 3 patients who lost ambulation share many aspects with type 2).

3.5.1 NUTRITIONAL AND GASTROINTESTINAL MANAGEMENT

Gastrointestinal and nutritional impairments are common in SMA type 1 patients. These infants have a higher risk of dysphagia, gastrointestinal dismotility, undernutrition and growth failure. (23) During each assessment, the clinician needs to pay attention to early signs of disease, mainly increased feeding time, oral pooling, cough, fatigue, aspiration and

recurrent infections. Weight gain is appropriate until during the first months of life, but reaches a plateau around 3 to 4 months of age, and without proper intervention weight loss occurs. Moreover, by one year of age, contracture of the masseter muscles often develops, which limits the opportunity for oral feeding (21).

Given the expected natural progression of the disease, shortly after diagnosis, a full-modified barium swallow fluoroscopic study is recommended. If the study reveals swallowing dysfunction, proactive care on feeding should be implemented. In the case of a failed swallow study or growth failure, a proactive approach is recommended. Long term gastrostomy tube feeding is the ideal method of feeding. Until gastrostomy tube can be placed, placement of short-term nasogastric or nasojejunal tube is recommended. Many experts recommend that Nissen fundoplication should be performed in conjunction with gastrostomy tube placement, even though there is no unanimous consensus. Although Nissen fundoplication is normally reserved for infants with gastro-esophageal reflux, it is known that a high percentage will develop reflux at some point, and so many experts recommend that Nissen should be opportunistically performed during the intervention for gastrostomy tube placement.

Additionally, regular assessment of growth is important. Since SMA-specific charts are not yet available, standardized growth charts are commonly used. Although standardized charts are inaccurate for SMA type 1 infants, they are a good tool to track growth trends. Optimally, they should be used with other body composition measurement tools.

An expert nutritionist should be involved to promote an appropriate diet, monitoring weight and intake of fluids, macronutrients and micronutrients. There is no consensus on the effect of the type of diet, but experts agree that diet type and administration should be based on individual tolerance. The Amino Acid diet is recommended by some experts (21,25,26).

3.5.2 RESPIRATORY MANAGEMENT

The leading cause of death in SMA type 1 is respiratory failure, so it is critical that a therapeutic relationship with an experienced pulmonary specialist familiar with pediatric neuromuscular disorders is established at the time of initial diagnosis. Formal pulmonary function tests are not possible due to their young age. As such, the focus of the clinical assessment is physical examination, evaluating respiratory rate, work of breathing, presence of paradoxical breathing, chest wall shape and skin color. Screening for respiratory failure should also include pulse oximetry and capnography or transcutaneous CO_2, and using sleep study or pneumogram with CO_2 recording if there is a minimal suspicion of hypoventilation. Intervention is based on a proactive approach, introducing therapies earlier in the disease process. A respiratory therapist should be involved to initiate and support assisted airway clearance and respiratory range of motion therapy.

The primary mode of airway clearance therapy should be manual chest physiotherapy combined with mechanical insufflation-exsufflation, which must be available for all infants. Oral suctioning with a mechanical suction pump and catheter is a critical part of airway clearance and should be used in any infant with ineffective cough. Non-invasive positive pressure ventilation (NIV) should be used in all symptomatic infants prior to signs of respiratory insufficiency. Early implementation of NIV has been shown to improve survival and quality of life. Tracheotomy ventilation is an option for selected patients in whom NIV is insufficient or fails. However, a commitment to a lifelong, full-time ventilatory support must be discussed with the family, and the decision should be focused individually on clinical status, prognosis and quality of life. This discussion ideally should involve a palliative care team (24,27).

3.5.3 NEUROMUSCULAR AND MUSCULOSKELETAL MANAGEMENT

Muscular impairments need to be routinely monitored. Evaluation of these infants includes assessments of strength and range of joint motion, relevant motor functional scales and timed tests to monitor those aspects of function that reflect activities of daily living. Regular monitoring of these aspects allows to identify changes over time and to recognize aspects requiring intervention, in addition to allowing evaluation of response to intervention (21).

3.5.4 REHABILITATION

For SMA type 1 the rehabilitation goals are mainly optimization of function, minimization of impairment and improvement of tolerance to various positions.

Rehabilitation in these infants is based on stretching exercises (through active-assistive and passive techniques) and on the use of adequate equipment to support function and to prevent or ameliorate contractures without limiting function. Thoracic bracing and cervical bracing are recommended for postural stabilization. Splints and orthoses are used to promote function and range of motion. Positioning recommendations are based on the use of seating systems, molded wheelchairs and custom sleeping systems (including the use of rolls, beanbags, molded pillows or wedges). Some infants can participate safely in aquatic therapy with proper head and neck support.

Chest physiotherapy with manual techniques is recommended as prophylaxis of pulmonary management to promote airway clearance and improve ventilation. Similarly, it is important to implement chest physiotherapy during illness or perioperative periods (21).

3.5.5 ORTHOPEDIC MANAGEMENT

Spinal deformity management is rarely discussed for infants with SMA1 due to their limited survival. If respiratory and nutritional functions are stable, specific rigid braces may be used, unless they compromise pulmonary function. Surgery is not recommended. Fragility fractures are common owing to disuse, osteoporosis and low vitamin D levels (21).

3.6 GENETIC BACKGROUND

The underlying genetic defect for SMA type 1 was only discovered a century after the first description of SMA (8). In 1995, a breakthrough study mapped the SMA genes to chromosome 5q13, in which two nearly identical genes encode for the same SMN protein. These genes were initially named as telomeric form and centromeric form of the *SMN* gene, but these genes are now more commonly referred to as *SMN1* and *SMN2*, respectively.

Being nearly identical, both genes encode the same 294 residue, 38-kDa SMN protein (28). Transcription of the *SMN1* gene produces full-length mRNA transcripts that encode the SMN protein. *SMN2* mRNAs are transcribed at similar levels in all cells. However, 85% of *SMN2* transcripts result in abnormal SMN protein due to a single nucleotide difference from cytosine to thymine in the sixth nucleotide of exon 7 (codon 280) of the *SMN2* gene. This single cytosine to thymine substitution in exon 7 weakens a binding site for a splicing activator, leading to exon skipping. The resulting *SMN2* mRNA is truncated, lacking the codons for 16 amino acids within exon 7 as well as the translational termination codon. The translated truncated protein is unstable and is rapidly degraded (29).

A small fraction of the total mRNA transcripts (10-15%) arising from the *SMN2* gene do contain exon 7, as the exclusion of exon 7 from *SMN2* mRNAs is not complete, producing the normal SMN protein. This justifies the fact that the number of SMN2 copies correlates well with a patient's clinical phenotype, as each copy produces low levels of full-length, functional SMN protein (30).

SMA type 1 is inherited as an autosomal recessive disease. It is caused by a deficiency of the SMN protein, which results from a combination of homozygous deletions or mutations of *SMN1* and the presence of 1 or more copies of *SMN2*. Complete absence of both *SMN1* and *SMN2* is incompatible with life, therefore affected infants have at least one copy of *SMN2*.

All patients are dependent upon their copy or copies of *SMN2* to produce the SMN protein necessary for survival, even though it is insufficient (1).

There are 3 types of *SMN1* mutations observed in patients. Firstly, a homozygous deletion of the totality of the *SMN1* gene, or of a part of the *SMN1* gene involving exon 7; secondly, a conversion of *SMN1* to an *SMN2*-like gene via a cytosine-to-thymine mutation, explained by the fact that this region of chromosome 5 is highly unstable; lastly, point mutations throughout the *SMN1* gene. The most common mutations are *SMN1* deletions and conversions, which occur in 95% of 5qSMA. The remaining 5% of cases have a deletion or gene conversion on one chromosome and a point mutation on the other chromosome; this difference has critical implications for diagnosis (8,31).

The mechanism of how SMN protein deficiency leads to the phenotype is still not entirely understood. SMN protein influences RNA processing functions in all cells. The fact that only motor neurons are severely affected with low SMN levels is a central question that is an area of active research (32).

3.7 RESEARCH FOR A DRUG TREATMENT

Prior to the 1990s, there were few clinical trials in SMA due to the fact that a clear molecular target was unknown. The few studies undertaken were based on pharmacological agents that had encouraging results in other diseases characterized by muscle weakness, such as amyotrophic lateral sclerosis or muscular dystrophy. Then, in 1995, the *SMN* gene was discovered and within 5 years it was possible to develop animal models of SMA that mimic many of the pathological and electrophysiological changes seen in patients, being the foundation for all therapeutic developments that followed (33).

Animal models provided "proof of principle" that increasing expression of full length SMN protein is protective. These models also established superb preclinical model systems for screening potential therapies, and permitted in-depth molecular and biochemical studies of disease pathology (34). With greater understanding of the genetic and molecular basis of SMA in the past 2 decades, several possible therapeutic approaches based on the general principle of increasing the expression of the SMN protein were suggested. Over the past 15 years, several therapeutic agents have been in various stages of development in clinical trials' pipeline for SMA.

To increase expression of full-length SMN protein, strategies include increasing the inclusion of exon 7 in SMN2 transcripts, enhancing SMN2 gene expression, stabilizing the SMN protein or replacing the SMN1 gene. These approaches include gene therapy for SMN replacement, antisense oligonucleotides (ASOs) to modify SMN2 splicing, and small molecules. Alternative attractive therapeutic strategies include neuroprotective agents and the use of targets downstream of SMN (once defined) (35,36).

3.8 NUSINERSEN

Nusinersen (Spinraza®, ISIS 396443) is an FDA-approved antisense oligonucleotide indicated for the treatment of SMA is pediatric and adult patients in Europe, Canada, Japan, Australia and in the United States of America (10,37). Nusinersen is a modified antisense oligonucleotide with molecular weight 7501 dalton, where the 2'-hydroxy groups of the ribofuranosyl rings are replaced with 2'-O-2-methoxyethyl groups and the phosphate linkages are replaced with phosphorothioate linkages. The molecular formula of Nusinersen is $C_{234}H_{323}N_{61}O_{128}P_{17}S_{17}Na_{17}$ (10,38).

Nusinersen modulates splicing of *SMN2* pre-mRNA. It works by binding to the ISS-N1 regulatory motif in the intron downstream of exon 7 on *SMN2* pre-mRNA, preventing the binding of factors such as heterogenous nuclear ribonucleoprotein A1 (hnRNPA1). This promotes the inclusion of exon 7, thereby increasing the amount of full length *SMN2* pre-mRNA and, subsequently, full-length SMN protein, partially compensating for deletions/mutations of *SMN1* (39,40).

Nusinersen is administered by intrathecal injection at an equivalent dose of 12 mg (4-5 mL based on age), with three initial loading doses at intervals of 14 days and a fourth loading dose 30 days later. The loading dose is followed by maintenance doses once every 4 months (41).

3.8.1 HOW NUSINERSEN WAS DISCOVERED

The mechanism of *SMN2* exon 7 splicing has been intensively studied. While several additional isoforms are generated by alternative splicing of both *SMN1* and *SMN2*, transcripts lacking exon 7 appear to be the major isoform produced by *SMN2* in all tissues, except in

testis. Given that *SMN2* has the potential to produce functional SMN protein, it is the main target for therapies to increase production of full length SMN protein (42).

Multiple potential methods to increase production of SMN protein from *SMN2* were explored. Approaches included increasing transcription, modulating *SMN2* exon 7 splicing, inducing translational read through SMNΔ7 transcript and increasing stability of SMN protein. One of the most promising methods was the redirection of *SMN2* splicing of exon 7 through antisense oligonucleotides (ASOs) (42). ASOs are single-strand, DNA-like molecules 15-35 nucleotides long designed to anneal to complementary sequences within a gene of interest. Through hybridization to the pre-mRNA, they can modulate target gene expression. Targets can be regulated by several different mechanisms, including restoration of the correct open reading frame, decreasing gene expression, alternative splicing, or, in the case of SMA, exon inclusion. Multiple different ASOs are being studied for the treatment of rare genetic diseases caused by aberrant mRNA splicing, including Duchenne muscular dystrophy, fibrodysplasia ossificans progressiva, Leber congenital amaurosis and tauopathies (43,44).

The pharmacological properties of ASOs can be altered by the chemical structure of the nucleotide backbone or sugar ring. Each modification of the backbone confers slightly different stability, toxicity, and function to the ASO. SMN is an attractive candidate for ASO therapy as patients have a functional copy of *SMN2* that is an ideal target for modulation (42). In 2004, Singh laboratory at University of Massachussetts Medical School discovered the most promising target for an ASO-based therapy of SMA: Intronic Splicing Silencer N1 (ISS-N1). ISS-N1 confers a very strong inhibitory effect on the inclusion of *SMN2* exon 7. Therefore, ISS-N1 is considered to be the master checkpoint of splicing regulation of *SMN2* exon 7, and sequestration of ISS-N1 by an ASO leads to full splicing correction in SMA patient cells. *In vivo* selection studies revealed that the 5'splice site of *SMN2* exon 7 is very weak, which prompted the discovery of ISS-N1. Subsequent studies revealed that ISS-N1 is a

complex regulatory element being affected by the presence of other regulatory elements upstream and downstream of ISS-N1 (39).

Adjacent to exon 7 5'-splice site, ISS-N1 contains a binding site for A1-dependent hnRNP-A1, which decreases exon 7 inclusion. Deletion of ISS-N1 promoted exon 7 inclusion, indicating that ISS-N1 was a very promising splice modulator target for *SMN2* (39). Upon its discovery, it was predicted that by targeting ISS-N1 it was possible to enhance *SMN2* exon 7 inclusion. Two mechanisms for targeting ISS-N1 were studied: blocking binding of hnRNP A1 to two target motifs in the region; or causing secondary structural rearrangements and preventing an inhibitory long-distance interaction with downstream sequences deep within intron 7 (40). Throughout the years, numerous studies demonstrated the efficacy of ASOs targeting ISS-N1 in both SMA patient cells and mouse models of SMA, using multiple ASO chemistries. ISS-N1 is likely to be the most studied antisense target for splicing correction for human disease (39).

3.8.2 PRECLINICAL STUDIES

Several studies indicated that ASOs targeting ISS-N1 dramatically promoted exon 7 inclusion to near 100% of the primary transcripts, and increased SMN protein levels in transfected cell lines, in patient-derived cells, and in mouse models of SMA (33).

Treatment of adult transgenic *SMN2* mice twice a week with these ASOs resulted in a significant increase in exon 7 inclusion in liver and kidney. However, exon 7 inclusion rate was not affected in spinal cord of these animals as the ASO did not penetrate the blood-brain barrier (33).

Another study using a different ASO targeting ISS-N1 (nucleotide range 13-33 downstream of exon 7) delivered intracerebroventricularly (ICV) to SMA mice increased SMN protein levels throughout the spinal cord and increased body weight and righting reflex

(45). To investigate the effect of ASO in the spinal cord, in another study ASO 10-27 was continuously infused in the right lateral ventricle at various doses for 7 days in adult SMA mice. ASO treatment resulted in an almost complete rescue of exon 7 exclusion and increased protein levels throughout all levels of the mouse spinal cord. Moreover, *in vivo* half-life of the ASO appeared to be very long, as the effect was still observable 6 months after completing the 7-day treatment (46).

Alternatively, a single ICV administration of morpholine-based oligomers targeting ISS-N1 in neonatal Δ7 mice extended the lifespan to over 100 days. The results indicated that increasing SMN levels in early stages is critical to improve the SMA phenotype (47,48).

The effectiveness of central versus systemic administration of the ASO in the SMA mice was approached in a different study. In this study, ASO was administered ICV on day 2, or subcutaneously (SC) on day 1 or day 3. Delivery of the ASO in the CNS efficiently corrected *SMN2* splicing in brain and spinal cord, leading to increased SMN protein levels but only modestly extended survival (10 vs 16 days). In marked contrast, systemic delivery of the ASO resulted in a median survival of over 100 days. Combining ICV and SC injections or 2 additional SC injections resulted in increased survival (173 and 137 days, respectively). Most rescued mice had nearly normal motor function. Since there was a difference between ICV and SC injections on the rescue of the phenotype, it was suggested that peripheral restoration of SMN expression is crucial for survival in this mouse model. On a histological level, motor neuron counts, muscle fiber size, and neuromuscular junction integrity were similar between treated mice as a factor contributing for the rescue of the SMA phenotype. These findings suggest that organs other that the CNS might contribute to SMA pathogenesis in mice. It is unclear if these findings are relevant to human disease, as the majority of patients with SMA do not have peripheral defects (49).

In 2010, Ionis Pharmaceuticals obtained license for exclusive use of ISS-N1-targeting ASOs. The company used their antisense technology, with their 2'-O-methoxyethyl phosphothioate chemistry, which had sulfur substituting for one of the non-bridging oxygen atoms in the phosphate backbone, and chemical modification of the sugar at the 2' position. These modifications helped to resist nuclease degradation and enhance cell penetration. Thus, they had created an excellent *in vivo* splicing modifier. The company screened over 500 different antisense oligonucleotides against various sites on *SMN2* exon 7 and its adjacent introns. The best results at splicing exon 7 into the *SMN2* pre-mRNA were from nusinersen, an 18-nucleotide antisense oligonucleotide that blocks the intronic binding site of the splicing repressor (ISS-N1) (40).

As nusinersen binded a unique sequence, off-target effects were not expected. Additionally, as the target is on an intron that is spliced out of the protein, the drug comes off and does not interfere with *SMN2* translation. In both *in vitro* and *in vivo* mouse models of SMA, nusinersen successfully targeted ISS-N1 and lead to an increase in about 90% of exon 7 inclusion (10).

3.8.3 CLINICAL STUDIES

Based on the strong preclinical data on the efficacy of nusinersen (ASO 396643), Ionis Pharmaceuticals partnered with Biogen and commenced clinical trials in humans (Table 2).

3.8.3.1 PHASE 1 STUDIES

ISIS 396443-CS1

In 2013, the first-in-human, open-label, single ascending dose study was completed. The goals of this first phase 1 study were to assess safety, tolerability, pharmacokinetics and preliminary efficacy of intrathecal nusinersen (50).

Given the neuromuscular nature of SMA, nusinersen was delivered intrathecally through a lumbar puncture. ASOs do not cross an intact blood-brain barrier when delivered systemically, while lumbar puncture effectively distributed nusinersen throughout the CNS and did not produce adverse effects beyond what had been previously reported for this procedure. Twenty eight patients aged from 2-14 years and diagnosed with SMA type 2 and type 3 received single escalating doses (1, 3, 6 and 9 mg) in cohorts of 6-10 participants (n=6 for doses 1, 3 and 6 mg; n=10 for 9 mg). CSF and plasma pharmacokinetics were measured, and patients were monitored for safety and tolerability. Efficacy of nusinersen was assessed using the Hammersmith Functional Motor Scale Expanded (HFMSE) and the Pediatric Quality of Life Inventory. Plasma and CSF levels of the drug were found to be dose-dependent. Extended CSF pharmacokinetics showed prolonged CSF half-life of the drug (4-6 months) after initial clearance. Intrathecal nusinersen was well tolerated with no safety concerns identified. In addition, the highest dose (9 mg) showed to produce significant increase in HFMSE score at 3 months post-dose, which was further increased 9-14 months post-dose during the extension study. Improvement in the Pediatric Quality of Life Inventory was not statistically significant. The most commonly reported adverse effects were headache

(39%), post-lumbar puncture syndrome (21%), back pain (18%), pyrexia (14%), constipation, upper respiratory tract infection, nausea and vomiting (11% each), although none of these events was considered to be related to nusinersen.

In this study, the injection procedure (through lumbar puncture) was well tolerated and was shown to be feasible in children with SMA, and incidence rate of post-lumbar puncture syndrome is consistent with the expected rate from published literature. However, the presence of hardware from scoliosis surgery in some patients may limit the feasibility of intrathecal injections.

This study also suggested that fixed doses are appropriate in this pediatric population, as no apparent correlations were observed between age or total body weight and CSF concentration. The prolonged half-life of nusinersen in CSF provided support for infrequent administration of the drug for maintenance levels following drug loading of the target tissue. Improvement in patients' motor function with the highest dose (9 mg), as evidenced by increases in HFMSE score, constitutes a clinically meaningful outcome in this endpoint, as improvement in motor function is not expected to occur as part of the predicted natural history of patients with SMA.

The favorable risk-benefit profile from this first-in-human clinical study of nusinersen provided encouragement for further developments of studies, and the fact that there were no dose-limiting safety issues suggested that doses higher than 9 mg should be considered.

The HFMSE score increase was particularly promising and led to the onset of larger phase 2 and phase 3 clinical trials (50).

Although this study was developed with type 2 and type 3 patients (there were no patients diagnosed with SMA type 1), it is a critical milestone for nusinersen and for the treatment of SMA, due to being the first study in humans.

CS2, CS10 and CS12

From the first phase 1 study, extension studies were designed. Patients randomized to the 1 mg cohort and completing study procedures were enrolled into the CS2 study, and patients randomized to the 3, 6 and 9 mg cohorts and completing study procedures were enrolled into the CS10 study (51).

CS2 was a phase 1/2 open label, multiple dose escalation study in patients aged 2 to 14 years diagnosed with SMA type 2 and 3. CS10 was a phase 1 or 1/2 open label, single dose study in patients aged 2 to 14 years diagnosed with SMA type 2 and 3. From these two studies, it was possible to design the study CS12, which is a phase 1, multi-centric, open label extension study to CS2 and CS10.

The focus of these studies was to evaluate the efficacy of nusinersen in terms of improvement in motor function. As such, primary endpoints were improvement in motor milestones. These studies showed that patients with SMA type 2 achieve motor milestones earlier when compared to patients with SMA type 3 (51).

3.8.3.2 PHASE 2 STUDIES

CS3A

The first phase 2 clinical trial was developed in 2014. Remarkably, this was the first study to include SMA type 1 patients (52).This was a phase 2, open-label, multiple-dose study

evaluating safety, tolerability, pharmacokinetics and efficacy of intrathecal nusinersen. Twenty SMA type 1 infants were enrolled into the study, aged between 3 weeks and 4 months, who had *SMN1* homozygous gene deletion or mutation. The infants were randomized to 1 of 2 independent treatment arms. Each arm had a loading dosing phase and then a maintenance-loading phase, and only the doses of the loading phase were different between each arm. The first 4 patients received 3 loading doses of 6 mg equivalent nusinersen on days 1, 15 and 85. The remaining 16 participants received 12 mg equivalent doses on the same schedule. All patients received multiple 12 mg equivalent doses starting on day 253 and then every 4 months. Safety assessments included adverse events, physical and neurological examinations, vital signs, clinical laboratory tests, CSF tests, and electrocardiograms. Clinical assessment included event-free survival and change from baseline of two assessments of motor function, namely the motor milestone portion of the Hammersmith Infant Neurological Exam – Part 2 (HINE-2) and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) motor test, and compound motor action potentials.

Standard intrathecal injections of nusinersen resulted in no safety concerns. All patients were reported to experience adverse effects (570 events), although the majority were mild (63%) or moderate (27%) in severity. A total of 77 were serious adverse effects, all of which were considered not or unlikely to be related to the study drug, with the most common being respiratory distress or failure or respiratory infections. There were no clinically significant changes in neurological examination findings, laboratory assessments, vital signs, electrocardiogram parameters or CSF safety patterns, with the exception of one mild event of transient neutropenia and one mild event of vomiting, both considered to be possibly related to nusinersen by the investigators.

CSF and plasma pharmacokinetics indicated that nusinersen cleared from the CSF into systemic circulation, consistent with normal CSF turnover. Likewise, dose dependent mean peak plasma concentrations were observed about 1 hour after dosing and declining over 24 hours. Nusinersen concentrations on CSF were quantifiable 15-168 days after dosing, which indicates prolonged exposure of the CSF and CNS tissues to the drug.

Spinal cord and brain tissues collected from three infants who died during the study allowed for pharmacokinetic and pharmacodynamic analyses. In all areas of the spinal cord, drug concentrations were greater than 10 µg/g, which is a concentration predicted to produce pharmacological effects.

Through immunohistochemical staining for nusinersen it was possible to confirm localization in neurons, vascular endothelial cells and glial cells throughout the CNS, with neurons being the cell type staining more intensively. Additionally, the drug was identified in peripheral tissues (such as liver and kidney), which was consistent with clearance from the CSF into systemic circulation. Analysis of thoracic spinal cord tissues showed that 50-69% of the *SMN2* transcripts contained exon 7, which corresponds to a 2.6 increase in full-length *SMN2* transcripts when compared to untreated infants. Likewise, comparing to untreated infants, those exposed to nusinersen had an apparent increase in SMN protein staining in thoracic cord motor neurons.

There was a promising clinical response in most infants regarding achievement of motor milestones and motor function, survival or permanent ventilation independence and neuromuscular electrophysiology. In the 12 mg dose group, incremental achievements of motor milestones, improvement in CHOP-INTEND score and increased compound muscle action potential of ulnar and peroneal nerves were observed, as compared with an expected decline based on natural history. Some of these infants achieved the ability to sit and roll over independently, and improved in head control, kicking, hand function, standing and walking,

changes which are beyond the expected for infants with SMA type 1, even though it should be noted that nusinersen did not restore normal age-appropriate function (52).

Overall, this phase 2 study in SMA type 1 patients showed that multiple doses of nusinersen are well tolerated, with mainly mild adverse effects, and that the drug is broadly distributed throughout the CNS, increasing full-length *SMN2* transcripts and SMN protein levels, leading to improved motor function, survival and electrophysiological function. Favourable safety and tolerability, pharmacokinetics, pharmacodynamics and an encouraging clinical response led to phase 3 clinical trials, allowing now the incorporation of a more frequent dosing regimen.

NURTURE (CS201)

NURTURE is another phase 2 study that focused on earlier treatment and was done alongside phase 3 studies. The aim of this study was to assess the effect of nusinersen treatment on presymptomatic individuals (53).

This was an open label single-arm study assessing the efficacy and safety of the drug in presymptomatic infants with genetically diagnosed SMA (*SMN1* deletion carriers with two or three copies of *SMN2*, SMA type 2/3 genotypes). The results were compared to a control group of affected siblings and natural history data. All patients were aged up to 6 weeks at the time of the first dose of nusinersen, in advance of the onset of overt disease symptoms. The primary endpoint was defined as time to respiratory intervention (invasise or non-invasive ventilation for over 6 hours per day continuously for more than 7 days straight, or tracheostomy) or death.

Initial results from interim analysis showed that there were neither deaths nor invasive respiratory interventions, and no infant required non-invasive ventilation 6 hours per day continuously for more than 7 days straight. Additionally, almost all patients were acquiring

motor milestones as measured using HINE-2 (such as sitting and crawling), and were generally achieved at age-appropriate time points. No severe adverse effects were attributable to nusinersen in this study.

These results support that there is a significantly greater effect of a presymptomatic treatment compared to treatment starting after symptom onset. Thus, early intervention should be preferred. Despite this, only three out of nine patients were standing unaided following 1 year of treatment, indicating that even presymptomatic treatment with nusinersen does not represent a complete cure (53).

EMBRACE

This is an ongoing phase 2 randomized, double-blind, sham controlled, multiple dose trial (NCT02462759) of intrathecally administered nusinersen in symptomatic infants/children with SMA not eligible to participate in the ENDEAR or CHERISH trials (phase 3 studies). The primary endpoint of this study is to assess safety and tolerability, and secondary endpoint is to examine pharmacokinetics of the drug. Patients could either have 2 or 3 copies of *SMN2*, and onset could either be before or after 6 months.

Part 1 of EMBRACE was early terminated because a phase 3 study of infants with SMA (ENDEAR) demonstrated a positive benefit/risk profile, and eligible patients were transferred to EMBRACE Part 2 to receive open label nusinersen. The trial is expected to be finished in April 2019 (54).

3.8.3.3 PHASE 3 STUDIES

ENDEAR

ENDEAR was a randomized, double-blind, sham-controlled phase 3 study to determine safety and efficacy of nusinersen in infants with SMA type 1 (55). In this study, 121 infants with symptomatic SMA, who had onset of symptoms before 6 months of age and were aged up to 7 months at the time of the first dose of study medication, were randomly assigned, in a 2:1 ratio, to undergo intrathecal administration of nusinersen or a sham procedure.

The dose of intrathecal nusinersen was adjusted according to the estimated volume of CSF for the patient's age on the day of dosing, so that each infant in the nusinersen group received a dose equivalent to a 12 mg dose in a person aged 2 years or older. Sham procedure consisted on a small needle prick to the skin over the lumbar spine, which was covered with a bandage to simulate the appearance of a lumbar puncture injection. Doses were administered on days 1, 15, 29 and 64 and maintenance doses on days 183 and 302. Sham procedures were performed on the same days in the control group.

Primary efficacy endpoints for this study were motor-milestone response (according to HINE-2) and event-free survival (time until death or the use of permanent assisted ventilation), while secondary endpoints included overall survival and subgroup analyses of event-free survival according to disease duration, using CHOP INTEND.

A prespecified interim analysis was performed when approximately 80 infants had been enrolled for at least 6 months. In the interim analysis, a significantly higher percentage of infants in the nusinersen group achieved improvements in motor milestones, 41% as compared to 0% in the sham control group. On CHOP INTEND, 63% of infants in the nusinersen group achieved a minimum of 4 points increase from baseline, compared to only 3% of the sham control group. The risk of death was reduced by 79% in the nusinersen group,

while overall reduction in risk of death or ventilation was found to be 29%. Furthermore, in the nusinersen group event-free survival was significantly higher (61%), as compared to the sham control group (32%).

The overall incidence of adverse effects was similar in both the nusinersen group (96%) and the control group (98%). The most frequent adverse effects in this study were respiratory infections and constipation. Comparing the nusinersen group to the control, a lower percentage of infants in the nusinersen group had a severe adverse effect, a serious adverse event or an adverse event that led to discontinuation of nusinersen. There was no meaningful between-group difference in the types of adverse effects reported, and there was not a specific pattern among infants who received nusinersen.

As the majority of adverse effects reported were consistent with those expected in the general population of infants with SMA type 1, and were similar to those reported in the previous phase 2 study, this trial supported that nusinersen is well tolerated and no safety concerns were identified.

The positive results of interim analysis and ethical considerations prompted early termination of the trial, and remaining patients were made eligible to receive nusinersen and transitioned onto SHINE, an open-label extension study designed to assess the effects of longer treatment duration on motor function and quality of life (55).

It is noted that even though the results were positive and endpoints were met early, several infants in the nusinersen group died, none achieved normal motor development and some needed continued feeding and ventilator support. These facts alone indicate that nusinersen is not a cure in symptomatic patients (55).

CHERISH

CHERISH is a phase 3, randomized, double-blind, sham-procedure control study of nusinersen in SMA type 2 and type 3 patients aged 2-12 years at screening and having onset

of disease at over 6 months of age (56). Primary endpoint was improvement of motor function from baseline (an increase of 3 or more points in HFMSE score). Interim analysis of 126 patients (84 on nusinersen arm, 42 on sham control group) enrolled in the study revealed a significant improvement of motor function at 15 months in nusinersen-treated patients, as compared to the control group. Fifty seven percent of patients achieved a 3-point or greater increase in HFMSE scores over baseline at 15 months, versus 21% of patients in the control group. Adverse events were largely related to the lumbar puncture procedure or were similar to those observed in the ENDEAR trial).

This study was stopped on the basis of results of interim analysis (significantly favoring nusinersen) and all the patients were transferred to an open label extension study (SHINE) in which they were all able to receive the drug (56).

SHINE

This is an ongoing phase 3 open label extension study for CS3B, CS4 and CS12. Patients who were enrolled in these studies were transitioned to receive nusinersen in SHINE extension trial, because of the results favoring the study drug. The goal of SHINE is to evaluate long-term safety, tolerability and efficacy of this drug in SMA patients. An estimated 289 patients will receive 12 mg of intrathecal nusinersen every 4 months. The projected completion date is 2022 (38).

Table 2: Clinical trials of nusinersen in spinal muscular atrophy. Adapted from Hoy S. (10)

Phase	Identifier	Status
1	Study CS1 (NCT01494701)	Completed
1	Study CS10 (NCT01780246)	Completed
1	Study CS12 (NCT02052791)	Completed
1/2	Study CS2 (NCT01703988)	Completed
2	NURTURE (NCT02386553)	Ongoing
2	EMBRACE (NCT02462759)	Ongoing
2	Study CS3A (NCT01839656)	Completed
3	CHERISH (NCT02292537)	Stopped
3	ENDEAR (NCT02193074)	Stopped
3	SHINE (NCT02594124)	Ongoing

3.8.4 APPROVAL OF NUSINERSEN

After being subject of multiple clinical trials, nusinersen was shown to be both safe and effective in raising SMN protein levels and reducing disease severity. Based on results mainly from ENDEAR, CHERISH and NURTURE studies, application for nusinersen was awarded fast-track designation and priority review by the US FDA (10).

On 23rd December 2016, nusinersen was approved by the US FDA for use in pediatric and adult patients with SMA. Hence, nusinersen became the first FDA approved drug for SMA, as well as the first antisense drug to treat the major population of a genetic disease through splicing correction. Following FDA's approval of nusinersen, on 20th May 2017, the drug received marketing authorization valid through the European Union by EMA (10).

In Portugal, there is an ongoing evaluation of nusinersen by INFARMED. Biogen applied a request for funding of nusinersen by the Portuguese public healthcare system

following marketing authorization. On the 27th March, INFARMED issued a communication stating that nusinersen will be available at every public hospital starting in early summer of 2018. However, five SMA type 1 patients in Portugal are already receiving treatment with intrathecal nusinersen within an Expanded Access Program (EAP).

Nusinersen is available as a clear, colorless, preservative free solution having ph 7.2, in strength of 12 mg/5 mL in a single-dose glass vial. Recommended dosing includes 3 loading doses at an interval of 14 days, followed by a fourth loading dose 30 days later. Maintenance dose is started after 4 months of the fourth loading dose and is administered every 4 months thereafter. Less frequent dosing may be effective for patients with milder forms of SMA, but there are no data yet available to support this possibility.

Each administration consists of 12 mg (5 mL) dose injected intrathecally over 1-3 minutes using spinal anesthesia needle. The nusinersen label carries warnings and precautions of increased risk for bleeding complications. Platelet count, prothrombin time, activated partial thromboplastine time and quantitative spot urine protein testing for renal toxicity is recommended at baseline and before each dose of this drug. The most common adverse reactions that occurred in at least 20% of nusinersen-treated patients and occurred at least 5% more frequently than in control groups were lower respiratory infection, upper respiratory infection, and constipation (10,57).

3.8.5 COST OF TREATMENT

In Europe, the cost of treatment with nusinersen is approximately 500.000€ per year for each patient. The price of nusinersen is expected to vary between country, taking into account differences at local level such as distribution model, tax policies, rebates and discounts, and certain aspects of the healthcare system (38).

In Portugal the price is yet to be established, as the ongoing process of negotiation between INFARMED and Biogen has not been concluded. In countries where nusinersen will not be provided freely for patients, such a high cost may limit the usage of the drug to only families with higher socioeconomic status, and may also affect compliance (58).

3.9 ALTERNATIVE DRUG TREATMENTS

Before the approval of nusinersen, early therapeutic efforts focused on repurposed drugs and small-molecule compounds for the treatment of SMA (35). A number of drugs that did not directly target SMN expression have been investigated. These therapies are aimed at modulating molecules and small pathways involved, such as neuroprotective mechanisms, cell survival, gene expression and axon outgrowth. Despite favorable results in mouse models, clinical trials with several of these agents have been disappointing, with no substantial clinical benefit demonstrated (42,59). Other compounds that increase SMN levels in assays have been tested, including histone deacetylase inhibitors, aminoglycosides and quinazolone derivatives. Despite favorable studies in animal models, clinical trials have been disappointing, with no substantial clinical benefit demonstrated (59).

More recently, many new small molecules have been discovered and developed specifically for their ability to affect the splicing of the *SMN2* gene to increase the amount of full-length SMN mRNA transcripts. Testing these compounds in clinical trials is an active area, holding promise for disease modifying activity (60).

3.9.1 HISTONE DEACETYLASE INHIBITORS

Earlier clinical trials aimed at increasing full-length SMN production from *SMN2* were focused on histone deacetylase (HDAC) inhibitors. HDAC inhibitors are a class of compounds that are epigenetic regulators of gene expression, and include valproic acid, sodium butyrate, phenylbutyrate, suberoylanilide hydroxamic acid and trichostatin A. Of these, valproic acid (VPA) has been studied in particularly great detail and is one of the only therapeutic strategies with published results from multiple clinical trials (42,61).

Consensus now is that VPA does not provide significant benefits for patients with SMA. However, trials that showed this preceded many current studies and have provided valuable insight into how to structure clinical trials for SMA patients, highlighting that studies should be conducted in SMA cohorts, with feasible outcome measures.

A phase 2 open label trial of VPA in SMA patients aged 2 to 31 years (SMA types 2 and 3) indicated that VPA was safe and well tolerated (46). While there was no significant change in full-length SMN transcript levels following treatment with VPA, there was a significant improvement in motor function in patients under 5 years of age (62). However, common adverse effects identified were weight gain and reductions in total or free plasma carnitine, which caused a worsening of gross motor function in two participants. This study concluded that further VPA clinical trials should be conducted on more restricted cohorts of patients and that carnitine should be supplemented to all subjects (62). Following this initial study, subsequent trials were then conducted.

The first one was the two-part CARNI-VAL trial, in which patients received VPA and a L-carnitine supplement. In both parts of the trial there was no significant improvement in motor function, even though some younger patients did show improvement. Seventy to 80% of patients had a weight gain of more than 20%, which was negatively related to motor function change (63).

A similar trial, VALIANT, was performed in ambulatory adults with SMA, and results were similar. VPA did not improve strength or motor function when compared to baseline or placebo controls (64). Although there was not a statistically significant difference between VPA-treated patients and the control group, the fact that some patients did improve motor function led to further investigation. In a study investigating the responsiveness to VPA in SMA patients, it was found that only one-third of patients responded to treatment and had increased *SMN2* transcript levels. Subsequent cellular analysis revealed that CD36 expression

levels were fivefold higher in non-responders, and it was concluded that CD36 overexpression prevented VPA-induced SMN expression. VPA may be useful for SMA patients who are ineligible for SMN-targeted therapies but have low levels of CD36 (65).

Other HDAC inhibitors have been intensively studied. Although some of them have had positive results, they are nowadays considered to be too toxic for chronic use to potentially treat SMA (42).

3.9.2 OLESOXIME

Olesoxime is a cholesterol-like molecule that was originally identified as a neuroprotective agent. The molecule appeared to act on neurons under cellular stress by preventing mitochondrial permeability transition pore openings. It was shown to inhibit cell death, rescue neurite outgrowth, prevent astrogliosis, and accelerate myelination in response to injury.

Although olesoxime did not show efficacy in amyotrophic lateral sclerosis (ALS) patients, there were no safety concerns and it is under study for patients with SMA (66,67).

In a two-year phase 2/3 clinical trial in SMA types 2/3, olesoxime seemed to be safe and well tolerated. It was administered daily at a dose of 10 mg/kg in an oral liquid suspension. The outcomes of the trial suggested that olesoxime supported the maintenance of motor function, particularly in a subgroup of patients aged 6 to 15 years and in patients who had high exposure to the drug (68).

An open label, single arm phase 2 study is currently ongoing to evaluate long-term safety, efficacy and tolerability in patients who had enrolled on the previous trial. Being a general neuroprotective agent, the outcomes of these studies are potentially of interest for the treatment of other neurodegenerative diseases.

3.9.3 QUINAZOLINES

Early research on quinazoline compounds demonstrated poor blood-brain barrier penetration, and further improvements led to development of a novel compound called RG3039. Animal studies were successful as it improved survival and motor function of tested mice, and led to modestly increased SMN levels. RG3039 was studied in a phase 1 clinical trial to study safety in healthy controls and was well tolerated. Further drug development into later phases has not been pursued to date (69).

3.9.4 HYDROXYUREA

Hydroxyurea in a compound used for the treatment of solid tumors and sickle cell anemia. Its ability to increase the expression of fetal hemoglobin suggested it might increase expression of *SMN2* gene, and thus it has been considered for the treatment of SMA. Early uncontrolled trials have shown positive trends. However, a subsequent controlled trial of hydroxyurea in 28 patients with SMA types 2 and 3 reported no benefit (70).

3.9.5 MUSCLE-ENHANCING DRUGS

Muscle-enhancing drugs are a group of therapies currently in development that is aimed at improving neuromuscular function, muscle weakness and muscle fatigue (42).

CK-107 is a troponin complex activator that slows calcium release from fast skeletal muscle troponin to sensitize the sarcomere to calcium. This leads to increased force output at submaximal frequencies of motor nerve stimulation (71). Preclinical studies met positive results. CK-107 reduced fatigability of rat skeletal muscle *in vivo*. Phase 1 clinical trials in healthy individuals assessed safety and had positive results (72). A phase 2 clinical trial with SMA type 2, type 3 and type 4 patients is currently taking place.

SRK-015 is a myostatin inhibitor which has been shown to increase muscle mass and force in healthy mice. The drug acts by binding to latent myostatin and hindering protease cleavage. This prevents latent myostatin activation, leading to increased muscle cell growth and differentiation (59). Preclinical studies had favorable results in improvement of motor function in animal models. SRK-015 is expected to enter clinical trials in the near future as both a standalone therapy and in combination with SMN-targeted therapies.

3.9.6 SMALL MOLECULES

Small molecule-based approaches to target *SMN2* splicing have been developed. Preclinical studies have indicated that orally available small molecules can efficiently increase the production of full-length *SMN2* mRNA and SMN protein. Improvement in motor function, protection of the neuromuscular system from degeneration and increased survival in SMA model mice were demonstrated in these studies (42).

Two independently developed drugs are being tested in phase 1 and phase 2 clinical trials. Branaplam (LMI070, NVS-SM1) is an analog of a molecule indentified in a high-throughput screening that increases exon 7 inclusion and SMN protein expression (73).
In vitro studies suggest that it enhances the binding of U1 snRNP to the 5'splice site of *SMN2* exon 7. Splicing alterations were observed in 35 genes in human fibroblasts, suggesting good selectivity of splicing modulation. The clinical development program was interrupted because data from animal toxicology studies that used daily dosing demonstrated unexpected injuries to the peripheral nerves, spinal cord, testes and renal blood vessels. In a phase 1 study, however, branaplam did not have the same effect. Branaplam is currently in phase 2 clinical testing in infants with SMA type 1 with exactly two copies of *SMN2* (73).

RG7916 is the second small molecule acting as *SMN2* splicing modifier that is in phase 2 clinical trials (74). The drug was shown to be safe, well tolerated and increased full-

length *SMN2* mRNA levels in a single ascending dose study in healthy volunteers.Two ongoing phase 2 studies are investigating RG7916 in SMA patients. FIREFISH (NCT029134482) is an open label trial in which the drug is administered to SMA type 1 patients, with expected completion date in 2020. SUNFISH (NCT02908685) is a randomized sham controlled study of patients with SMA type 2 and type 3, expected to be completed in 2020 as well. Initial reports from both trials suggested therapeutic benefit, with modest functional improvement.

Oral delivery of these drugs makes them more flexible and easier and safer to administer than ASOs. Hence, if and when formally approved, these drugs are likely to play a significant role in upcoming years.

3.9.7 OTHER DRUGS

A handful of trials have attempted to repurpose therapeutics approved for other indications for the treatment of SMA. Drugs such as gabapentin, thyrotropin releasing hormone, albuterol, riluzole, ceftriaxone and aclarubicine have been tested, mostly with disappointing results. However, agents such as riluzole and ceftriaxone require further studies, and may demonstrate benefit for SMA patients (42).

3.10 GENE THERAPY

Although not a pharmacological treatment for SMA, it is important to note that gene therapy is also showing promising results. AVXS-101 is a gene replacement therapy. It is a non-replicating, recombinant, adeno-associated virus serotype 9 carrying the complementary deoxyribonucleic acid (cDNA) of the human *SMN1* gene (scAAV9-SMN). Mice treated with scAAV9-SMN showed widespread transgene expression in spinal cord motor neurons, and there was complete rescue of the disease phenotype (75). Following these results, clinical trials in human subjects began. A phase 1 clinical trial (NCT02122952) evaluated safety and efficacy of a single intravenous delivery of AVXS-101 in infants with SMA type 1. Initial results showed promising preservation and improvement in motor function (75).

STR1VE (NCT033062777) is a phase 3, open label, single arm trial studying the efficacy and safety of a single intravenous dose of AVXS-101 in patients with SMA type 1 with one or two copies of *SMN2*. Completion of the trial is expected by 2020.

4. DISCUSSION

SMA type 1 is a devastating genetic neuromuscular disorder, leading to significant mortality and morbidity. Over the last two decades, significant advances in patient care and knowledge of the genetics and biology of SMA have led to the discovery of promising therapeutic strategies.

The approval of nusinersen is a major milestone for the treatment of SMA, and represents a key landmark in neuromuscular disease and translational research. However, it is important to note that infants treated with nusinersen do not achieve completely normal motor function, and face an uncertain future as they grow and develop from a baseline of established neuromuscular muscle weakness. Despite the positive results of nusinersen on motor function in SMA, some chief issues still need to be addressed and resolved in the future. SMA type 1 is a disease characterized by the rapidity of disease onset, and is believed to have a developmental pathogenesis. The timing of SMN-targeted therapies is essential for maximum efficacy, and accumulating evidence suggests that treatment should be initiated prior to symptom onset. It is important to discuss strategies such as newborn screening tests for SMA. This would minimize diagnostic delay, allowing for early initiation of treatment with nusinersen in presymptomatic infants. Newborn screening is currently under review in several countries, and is likely to be approved in the future (76,77).

Other issue is the fact that there is no practical alternative to periodic intrathecal delivery of nusinersen at the present time. Although pharmacokinetic studies indicate a prolonged CSF drug half-life, the current regime dosing regimen requires treatment every 3-4 months and intrathecal route therefore represents a barrier to the free availability of nusinersen in all health care settings due to the extra cost and facilities required.

Lastly, the fact that nusinersen is delivered directly into the CNS and not into systemic circulation is a concern as SMN upregulation may be required in other tissues. The effects of low SMN levels in other organ systems are yet to be completely understood, and it is unknown how will infants develop into adolescence and beyond living with low SMN levels peripherically. Careful monitoring by multispecialty teams is mandatory.

Numerous additional therapies, both SMN-dependent and SMN-independent, are currently in development and are likely to play a significant role in expanding therapeutic possibilities in coming years. Considering the results from preclinical studies and initial results from clinical trials, it is likely that SMN-targeted therapy on its own will not be sufficient to halt or restore all disease-associated phenotype. SMN restoration might not be a sufficient therapy for all patients. As such, the possibility of developing combinatorial therapeutic approaches holds great interest. Future research will focus on combining SMN-targeted therapy with other SMN-independent therapies, as the future of SMA therapy covering the whole life period of the infant may require a range of therapies in combination. However, it is expected that future trial design will be complex, as many patients will likely have already initiated treatment with nusinersen, and long term effects of the drug are still incompletely understood.

Given these many aspects that will be addressed in the near future, it is likely that the pharmacological treatment for SMA will go through noteworthy advances. The approval of nusinersen has wider implications for the treatment of other neurological diseases. It provided proof-of-principle that ASO therapy is a viable approach to other genetically determined diseases, such as ALS, Huntington's disease, Duchenne muscular dystrophy and myotonic dystrophy.

The history of SMA is a key example of how knowledge of the basic molecular genetic mechanism of a disease, coupled with detailed natural history studies, evidence-based management and standards of care combine to facilitate the translation of basic science into life-transforming therapies. Moreover, although nusinersen is not classified as gene therapy, several features of clinical development and approval process can serve as lessons to inform the development of gene therapies. The nusinersen pathway to marketing approval included rigorous clinical trials, flexibility in trial design and analysis, a collaborative effort with regular communication between the sponsor and FDA, and the use of FDA's expedited programs. Thus, nusinersen experience provides an excellent example for the development of gene therapies for the treatment of rare life-threatening diseases, as this experience can readily translate to gene therapy development programs.

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