



SPINAL MUSCULAR ATROPHY:

PATHOLOGY, DIAGNOSIS,
CLINICAL PRESENTATION,
THERAPEUTIC STRATEGIES & TREATMENTS



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SMA Europe is an umbrella organisation, founded in 2006, which includes spinal muscular atrophy (SMA) patient and research organisations from across Europe.

SMA Europe campaigns to improve the quality of life of people who live with SMA, to bring effective therapies to patients in a timely and sustainable way, and to encourage optimal patient care.

SMA Europe is a non-profit umbrella organisation that consists of 23 SMA patients and research organisations from 22 countries across Europe.

Authorship & Acknowledgements

This document was prepared by the Patient Community of SMA Europe, the principal author being Marija Krstić MD, who is the mother of a child with SMA and a patient advocate. Marija performed this work as a volunteer, assisted by Vanessa Christie-Brown (SMA Europe Coordinator). The design work was done by Danica Ilić, who has SMA. It is based on a document written Cure SMA, who we would like to thank for the reference.



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Introduction

The breadth and depth of the treatment landscape for spinal muscular atrophy (SMA) has expanded dramatically over the past decade, with a growing number of therapeutics with multiple targets and routes of administration being tested in clinical trials (CTs). As more of these experimental compounds move from the laboratory into clinical trials, there is an urgent need for clinical sites that are prepared to conduct clinical trials in SMA and that can take on patients from all over Europe.

Whilst this is very good news for the patient community, a concern is that clinical trial sites in Europe might not have the capacity or may not be equipped to take on additional trials. SMA Europe, as part of its mission to bring effective therapies to patients in a timely & sustainable way, has joined Cure SMA in the US and the Industry Consortium, in taking on a series of activities to alleviate these challenges and meet the needs of trial sponsors and the SMA patient community.

These activities include:

1. Identifying SMA clinical trial centres in Europe:
 - a. Mapping the spread of these centres across Europe
 - b. Assessing their readiness to conduct CTs on SMA
 - c. Assessing their capacity to undertake CTs on SMA
2. The provision of educational resources to help sites prepare for SMA Clinical trials, such as these information packs
3. The provision of training opportunities such as workshops, masterclasses and conferences.

This information pack forms part of SMA Europe's broader effort to optimise site readiness for SMA clinical trials. It is based on a document written by Cure SMA in the US, but which has been extensively adapted to fit the European situation and importantly, to reflect the patient's perspective. Its goal is to provide sites with a resource for research teams which addresses major aspects of preparing for and conducting clinical trials.

To this end, SMA Europe has written 3 booklets on the following topics:

1. Spinal Muscular Atrophy: Pathology, diagnosis, clinical presentation, therapeutic strategies & treatments
2. Standards of Care in spinal muscular atrophy
3. Conducting a clinical trial

These information packs will be updated to reflect changes in the science and the clinical trial landscape for SMA. Sites are encouraged to view it as a guide, recognising that it is one of many resources that can be helpful and that guidance from clinical trial sponsors, institutional review boards and the regulatory authorities should always take precedence when planning for, conducting and closing trials.



Spinal muscular atrophy

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1. Background

Spinal muscular atrophy (SMA) is a rare, genetically inherited, heterogeneous group of neuromuscular disorders. It is characterised by progressive skeletal muscle weakness and subsequent atrophy, due to abnormalities of the **motor neuron (MN)** in the **ventral horn** of the spinal cord. SMA is a debilitating disease that often takes away a person's ability to walk, sit, eat, speak or breathe and in the most severe cases, leads to paralysis at birth or soon after and premature death. In later onset SMA (childhood, adolescence or early adulthood), the affected individual loses the ability to walk (Kolb & Kissel, 2015).

SMA varies in severity, ranging from severe to mild, with an extremely variable age of onset linked to individual severity. Different clinical presentation of the disease may occur in the same family (Dubowitz, Infantile muscular atrophy - a broad spectrum, 1967). Although primary pathology in SMA affects the neuromuscular system, increasing evidence suggests that other tissues and organs are affected (Shababi, Lorson & Rudnik-Schoneborn, 2014; Bottai & Adami, 2013).

SMA is one of the most common **autosomal recessive** inherited disorders in humans after cystic fibrosis and is very often called "the number one genetic killer of children under the age of 2". With a range of effective therapies targeting the root of the disease, this definition tends to be historical. New therapies have significantly changed the course of the disease and survival, especially in those who have been treated presymptomatically. The responsible gene for SMA is a mutated gene called *survival motor neuron 1 (SMN1)* and which is located on **chromosome 5q**. This gene encodes for most of the body's survival motor neuron (SMN) protein, which is critical for the survival of lower motor neurons (LMNs), in contrast to other cell types (Melki, et al. 1990; Lefebvre, et al. 1995; Brzustowicz, et al. 1990; Monani, et al. 2000).

Humans have another gene, *SMN2*, which is located in the same region as *SMN1* and encodes a similar protein, however, the majority of these proteins are truncated and non-functional. The *SMN2* gene, however, produces some functional SMN protein, but it cannot fully compensate for the deficit of the *SMN1* gene (Monani, et al. 1999). The *SMN2* gene is often termed "the back-up" gene. Having more copies of the *SMN2*

gene is generally associated with less severe SMA symptoms, but this finding is not correlated in every case (Lefebvre, et al. 1997).

SMA can affect any race or gender. An estimation of the incidence of all types of SMA is 1 in 10 000, while the **prevalence** is 1-2 per 100 000 patients. SMA type 1 has the highest incidence (4-6 per 100,000) compared to other subtypes (1, 9 and 1, 7 for type II and type III respectively), but the lowest prevalence (0.004-0.28 per 100,000) due to a short life expectancy. Prevalence for both type II and type III is 1.5 per 100,000. Overall, the incidence for type I is 60% and 40% for both type II and type III. Because SMA is an **autosomal recessive** disease, both parents must be **carriers** for a child to inherit the condition. No signs of disease have been associated with being a **carrier**. The carrier frequency for the *SMN1* gene has been estimated to be 1 per 50 (Caucasian and Asian) to 1 per 100 (African Americans) (Verhaart, et al. 2017).

The first clinical presentation of SMA was described in 1891 by Guido Werdnig, from the Department of Pathological Anatomy, at the University of Graz in Austria, in two brothers with the onset of weakness at around 10 months of age. One died at age 3 and the other at 6 years of age. Although the names of Werdnig and Hoffmann have been associated with the severe subtype of SMA, they described the intermediate form. In addition, Johann Hoffmann, from the medical clinic in Heidelberg, described 7 patients from 3 families with an intermediate type (ability to sit unaided, joint laxity and progression of scoliosis). In 1899, Sylvestere presented at the paediatric society in Paris, a two-month-old infant with flaccid paralysis of all limbs and trunk since birth and sparing of the diaphragm. The first description of a severe form was by Beevor, in Brain, in 1902. The 5-week old child had an intercostal weakness with sparing the diaphragm and complete paralysis of the limbs and trunk. Degeneration of the anterior horns and posterior columns were found at autopsy (Beevor, 1902).

A detailed description of the intermediate form of SMA was described by Dubowitz in 1964, in the UK (Dubowitz, 1964). Through the examination of 12 patients, he described late infantile-onset after the children had achieved the ability to sit, but never achieved

the ability to stand independently. These children had severe weakness of the legs, were static over the years, and showed no significant deterioration.

The mild form of SMA, associated with Kugelberg and Welander, was described in 1956, through a series of 12 patients presenting with a limb-girdle muscular dystrophy with neurogenic electromyography (EMG) changes and muscle biopsy (Kugelberg & Welander, 1956).

Over the last century, the wide clinical spectrum of SMA was recognised with detailed descriptions across severities. Individuals with SMA often have difficulty performing the basic functions of life (breathing and swallowing). However, SMA does not affect a person's ability to think, learn nor their social skills. Their intellect is normal. Literature state that the unique feature of SMA is that it is the only neuromuscular disease to spare the diaphragm although there is intercostal weakness. Facial muscles are also spared. Some brainstem **motor neuron** groups, including oculomotor and trochlear (ocular), which innervate the muscles around the eyes, are for unknown reasons spared (Nichterwitz, et al. 2018), although, some ventilated individuals experience nearly complete paralysis of the diaphragm over time, along with the restricted movement of the eyes and facial muscle weakness.

Identification of a **gene locus**, the development of animal models of SMA in the early 2000s and the recognition of the role of *SMN2* in phenotype, have all opened a path to new therapies and a better understanding of the natural history of the disease. With two approved drugs for SMA (Spinraza™ and Zolgensma™) in Europe and, the third (Risdiplam), whose approval in the European Union is expected in the first half of 2021. with the increasing number of clinical trials with disease-modifying therapies, there is a reason for hope. The natural history of the disease will greatly change.

2. Genetics

The discovery of the *SMN* gene in 1995, as well as its localisation through studies of families with a history of SMA, had a major impact on the non-invasive diagnosis of SMA, **carrier** testing and therapeutic options. Localisation of the *SMN* gene to the 5q13.2 region was mapped across all SMA **phenotypes**.

The SMA region is a large duplicated region on **chromosome 5**. The **telomeric region** contains the *SMN1* gene, while the centromeric region contains the *SMN2* gene. These genes are almost identical in the 5q13 region. Both *SMN1* and *SMN2* contain 8 **exons** and 99% homology in sequencing. They differ only by five **nucleotides** and produce an almost identical protein, the *SMN* protein. The differences are in exons 7 and 8, **introns** 6 and 7. However, only one difference between the *SMN1* and *SMN2* protein is functionally important: a silent transition in **exon 7** (c.840C>T), on the *SMN2* gene, which disrupts an exonic splice enhancer (ESE) and creates a new exonic splice silencer (ESS). This substitution (C to T) causes exon 7 to be excluded from most of the *SMN2* transcripts (which do not change the amino acid sequence in the *SMN* protein), resulting in the production of a truncated *SMN* protein that

is shorter, unstable *in vivo* and rapidly degraded (Lefebvre, et al. 1995; Monani, et al. 1999; Lorson & Androphy, 2000). Only a small amount of *SMN2* transcripts are correctly spliced and produce full-length *SMN* protein. Thus, the *SMN2* gene produces about 5-10% of full-length functional protein, since exon 7 is on occasion not spliced out of the *SMN2* **mRNA**. In healthy **carriers**, with one *SMN1* copy and zero *SMN2* copies, 50% of functional full-length *SMN* protein is present, which is sufficient for normal functioning and **lower motor neurons** (LMN) survival. In SMA patients without a functional *SMN1* gene, the *SMN2* gene serves as a “back up” gene, with a range of 5-10% of functional protein production per *SMN2* gene. In patients with SMA, the most common *SMN2* copy numbers are 2-3, which gives an estimation of ~ 20-30% full-length *SMN* protein production. Milder **phenotypes**, with 4 copies of the *SMN2* gene, have about 40% of *SMN* protein, which is still below the 50% level seen in healthy **carriers**. Asymptomatic individuals with five copies (humans can have 1 to 7 copies) of the *SMN2* gene and zero copies of the *SMN1* gene have been described (Mailman, et al. 2002; Lefebvre, et al. 1997) (**Figure 1**)

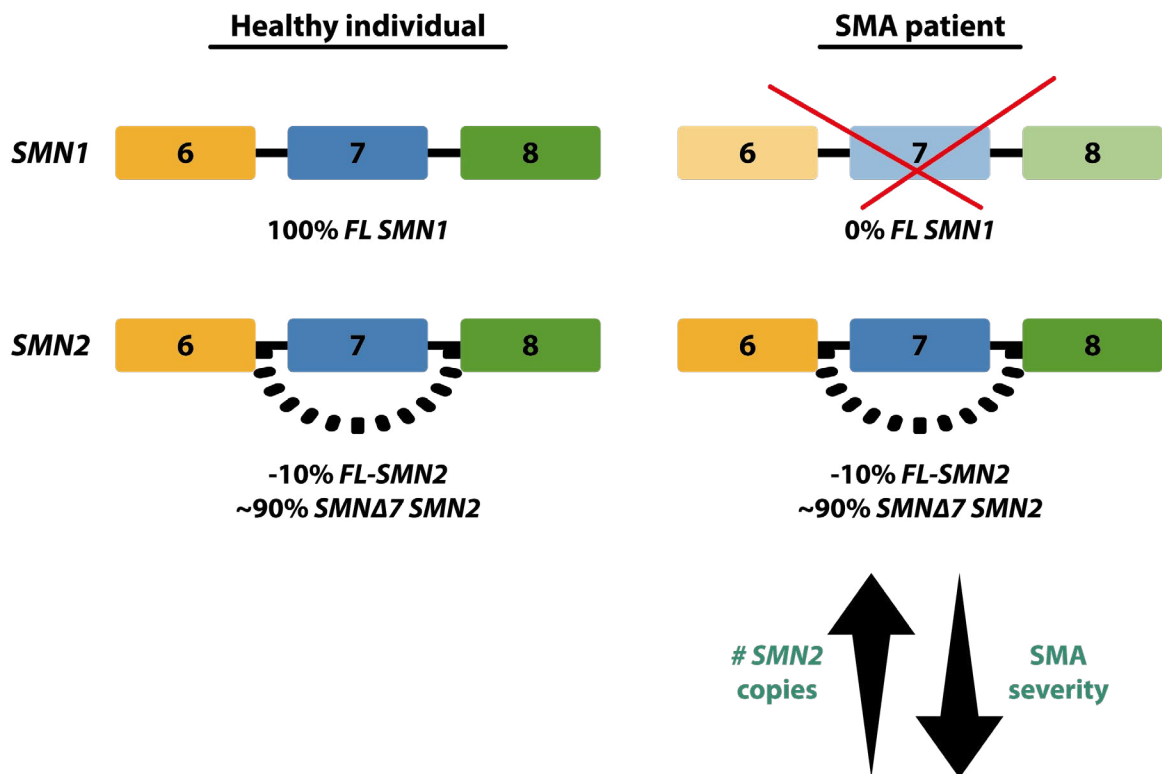


FIGURE 1. Diagram of *SMN1* and *SMN2* gene pathology in SMA

The genetics of SMA provides a unique opportunity for therapeutic development. *SMN2* provides attractive targets and the majority of drug development efforts in the field are focused on increasing *SMN* protein production from this gene (Iascone & Lee, 2015).

Individuals with SMA typically have inherited a faulty (mutant) *SMN1* gene from both parents. Most patients with 5qSMA have a homozygous deletion involving exon 7 and 8 or only exon 7 (95-98%). While the majority of the most severely affected SMA patients carry real homozygous deletions of *SMN1*, the majority of type II and III SMA patients show a homozygous absence of *SMN1* as a result of gene conversion of *SMN1* into *SMN2*, leading to an increase of 3 - 4 copies of *SMN2*. Four copies of this gene may produce enough functional protein to result in a mild phenotype (Wirth, 2000; Campbell, et al. 1997).

Di Donato *et al.* (1997) observed a strong association between the milder forms of SMA (types II and III) and partial gene-conversion events not involving exon 8 from the *SMN2* gene (Didonato, et al. 1997). Thus, SMA patients with either deletion of *SMN1* or a gene conversion of *SMN1* to *SMN2* will produce a smaller amount of functional *SMN* protein, being dependent

on the number of *SMN2* genes present, which is higher in gene conversion resulting in less severe phenotype.

In about 5% of SMA patients, subtle (point) mutations are present in the *SMN1* gene localised mostly in a region of exon 6 and 7. Those individuals are called “compound heterozygotes”, carrying a deletion/conversion on one chromosome and one subtle mutation on the other. There are 23 different subtle mutations published so far, which include nonsense, frameshift, missense and subtle splice site mutations. The phenotype and severity depend both on the type of subtle mutation and the number of *SMN2* copies in different combination (Wirth, et al. 1999).

De novo mutations occur at a rate of about 2%. This is because the *SMN* region on chromosome 5 is unstable, which leads to the deletion and conversion of the gene within the region. The high *de novo* mutation frequency is caused mainly by unequal crossing-over between the repeated units during paternal meiosis (Wirth, et al. 1997).

Spinal muscular atrophy modifiers

The disease-modifying role of higher *SMN2* gene copy number is described. It leads to later disease onset and better prognosis. There is a statistically significant correlation between 4 *SMN2* copies and SMA type IIIb or milder phenotypes. SMA type IV patients usually have 4 - 6 *SMN2* copies (Lefebvre, et al. 1997; Wirth, et al. 2006).

In populations with 2 *SMN1* copies, the number of *SMN2* copies varies, with 10-15% having no copies, 33% carrying 1 copy and 50% carrying 2 copies of *SMN2* (Mailman, et al. 2002). As the prevalence of 1-2 *SMN2* gene copies is the most prevalent genotype in the general population, the incidence of SMA type 1 is highest. In children with SMA, 80-96% of patients with type 1 carry 1-2 copies of the *SMN2* gene, while 4-20% with SMA type 2 or 3 carry 3 copies of the *SMN2* gene. About 96% of patient with SMA type 3 carry 3 or 4 *SMN2* copies.

SMN2 copy number is the main disease modifier, however, it does not predict severity accurately and there is no strict correlation. Other unknown factors must modify the SMA phenotype since affected and unaffected siblings with homozygous deletions of *SMN1* and identical haplotypes have been described in rare cases (Cobben, et al. 1995; Hahnen, et al. 1995). Intra-familial reports describe variation in phenotype despite carrying the same number of *SMN2* copies (Mailman, et al. 2002). Sometimes, less than five *SMN2* copies are found in asymptomatic carriers of a homozygous deletion of the *SMN1* gene and in some cases, six *SMN2* copies cannot rescue from SMA symptoms. Some SMA type I patients carry three *SMN2* copies and SMA type III patients harbour two copies of the *SMN2* gene (Cusco, et al. 2006). This shows that other modulators of *SMN2* gene splicing and other modifier genes exist, that alter the amount of full-length SMN protein it produces, influencing disease manifestation and high clinical heterogeneity in SMA as a monogenic disease. Study of these factors reveals other underlying SMA pathological mechanisms than in MNs and that can have an important clinical and therapeutic implication.

An inverse correlation has been observed between *SMN2* copy number and duration of survival and age of onset in adults (patients with 3 copies had significantly earlier onset compared to those with 4 copies). An inverse correlation was observed between *SMN2* copy numbers and brainstem involvement (facial weakness, chewing and swallowing problems, tongue fasciculation and dysphonic voice). *SMN2* copy number was associated with higher scores on the Hammersmith Functional Motor Scale (HFMS) (Elsheikh, et al. 2009). An influence of *SMN2* copy number on denervation was observed in SMA patients: the less *SMN2* copies, the less Motor Unit Number Estimation (MUNE) (Swoboda, et al. 2005) and maximum compound Motor Action Potential amplitude (CMAP). Heart defects were seen in those patients who carry one *SMN2* copy, but not in patients with increased *SMN2* copies (Rudnik-Sconeborn, et al. 2008).

The first candidate phenotypic modifiers for SMA besides the *SMN2* gene were 3 other genes localised in the SMA region (neuronal apoptosis inhibitory protein gene (NAIP); basal transcription factor subunit p44 (p44c) and H4F5 (also known as SERF1) genes. Deletion of those genes was observed more frequently in SMA type 1 patients than in milder forms (type 2 and 3). There is still no proof of these genes' involvement in the modification of SMA phenotypes since deletions of these genes in type 1 patients possibly reflect large deletions in *SMN1* and *SMN2* regions (Campbell, et al. 1997).

At the moment, *SMN2* gene copy number is the only accepted modifier of SMA phenotype in patients. A summary of other modifying factors and pathways connected with SMA pathology is given in Figure 2.

Modifying factors can be:

- *SMN* - dependent
- *SMN* - independent

***SMN* - dependent modifiers:**

Genomic integrity (refers to *SMN1*/*SMN2* copy numbers)

Sequence integrity (refers to gene conversion and point mutation)

Splicing factors (factors which alter *SMN2* pre-mRNA splicing). Upregulation of exon 7 inclusion in *SMN2* mRNA

can promote the amount of functional *SMN* protein produced). Splicing factors, namely TRA2- β 1 (SFRS10), SF2/ASF (SRSF1), SAM68(KHDRBS1), SRp30c (SRSF9), SRSF2, TIA1 (SFRS10), and hnRNP proteins (hnRNP-A1, hnRNP-G, hnRNP-M, hnRNP-U, hnRNP-Q) bind either directly or indirectly to cis-regulatory elements and facilitate the integration or skipping of exon 7. Besides the above-listed elements, **introns** adjacent to *SMN2* **exon 7** comprise other essential cis-elements, such as ISS-N1, A+100G, Element 1, that bind splicing repressors and represent compelling targets for SMA treatment. The principle of splicing correction is the basis of major approaches to SMA therapy with antisense oligonucleotides (ASO). Importantly, the first approved treatment for SMA, Spinraza™, is based on this principle: targeting the ISS-N1 negative element. Thus, factors altering *SMN2* pre-mRNA splicing may be considered as SMA severity modifiers through changing the amount of functional *SMN* protein produced.

Transcription regulation of *SMN2* gene - various signalling cascades induced by hormones (prolactin) or G proteins, including **DNA** methylation

Stabilisation of *SMN* mRNA

Posttranslational regulation and degradation of *SMN* protein (Survival Motor Neuron Protein phosphorylation, ubiquitination, sumoylation)

Exogenous factors: starvation, hypoxia, and oxidative stress. Starvation and hypoxia strongly reduce full-length *SMN2* levels.

Identification of key ***SMN*-independent pathways** is essential to understand the contribution of *SMN* to neuromuscular and systemic pathology. *SMN* independent factors are: **cytoskeleton** dynamics, **synaptic vesicle** (SV) trafficking and **neurotransmission**, **axonal transport** and local **translation**, and control of gene expression. **Motor neurons** are highly specialised cells whose cytoskeleton has a crucial role in numerous cellular functions, ranging from axonal growth and morphology maintenance to more specific functions such as **axonal transport**, SV trafficking and neurotransmission. These factors, to a certain extent, have been shown to affect and modify these processes in SMA.

For a more detailed description of modifying factor in SMA pathology, see the following references: (Maretina, et al. 2018; Oprea, et al. 2008; Wirth, Garbes & Riessland, 2013; Janzen, et al. 2018).

The apoptotic pathway plays a crucial role in **motor neuron** loss and an important one in the pathogenesis of other neurodegenerative diseases. Some **apoptosis** proteins are discussed as possible therapeutic targets. Some studies have reported an increase in autophagic features in SMA motor neurons, suggesting that **autophagy** dysregulation can represent a new pathogenetic hypothesis in SMA. Its pharmacological manipulation could influence the progression of the disease. Pharmacological and genetic inhibition of **autophagy** increases *SMN* levels, while induction of **autophagy** decreases these levels (Rodriguez-Muela, et al. 2018; Piras & Boido, 2018).

Identification of modifying factors and pathways, as well as molecules or drugs that upregulate full-length *SMN2* gene product or stabilise the *SMN* protein, are the most promising strategies for SMA therapy. In addition to *SMN*-dependent strategies, identification of *SMN*-independent modifiers unveils combined therapy approaches in a broad neurodegenerative context.

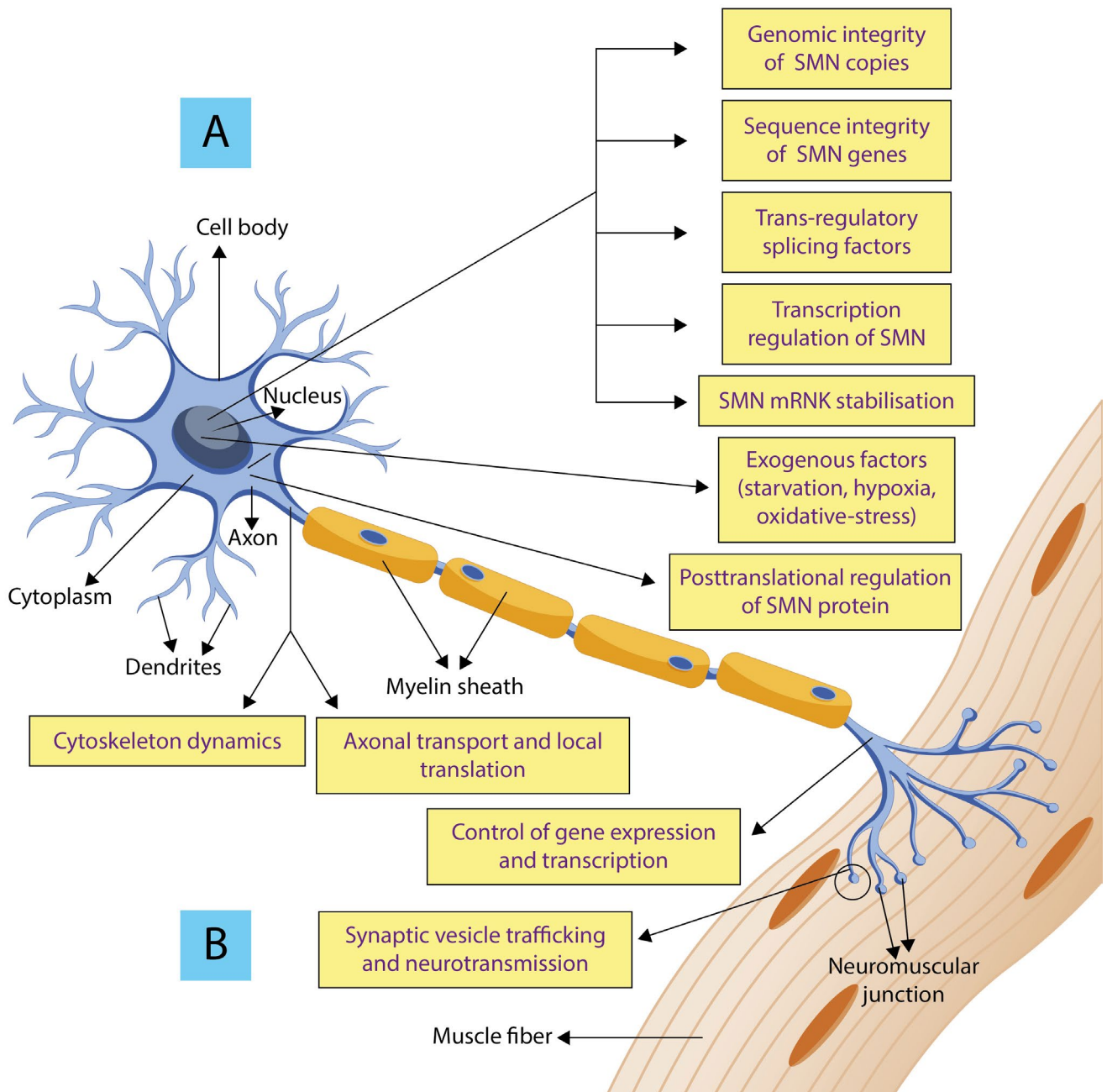


FIGURE 2. Overview of select SMA modifiers.

(A) depicts the SMN-dependent modifiers (B) depicts SMN-independent modifiers.

Carriers

A **carrier** is a healthy individual who is not at risk of developing the disease but has a risk of passing the gene mutation to his or her offspring. **Carriers** are unaffected **heterozygous** individuals, which means that they have one faulty and one functioning copy of the *SMN1* gene. **Carriers** of **autosomal recessive** disorders typically find out that they are both **carriers** by having an affected child. A child will only have SMA if both parents pass on the faulty copy of the *SMN1* gene, which occurs in 25% of cases (**Figure 3**). If only one parent is a **carrier**, the child is usually not at risk of SMA but does have a 50% risk of being a **carrier**.

Carriers fall into four main genotypic groups (**Figure 4**). The most common is the "1 + 0" **genotype** (one normal functional allele and an *SMN1*-deleted, disease allele). A much less common category is the "2 + 0" **genotype** with

two functional genes on one **chromosome** and none on the other. Furthermore, there are also “1 + 1D” and “2 + 1D” **genotypes**, which have one or two functional genes on one **chromosome** and a non-functional gene due to either a **point mutation** or a **microdeletion** on the other. These last two **genotypes** are very rare and are called compound heterozygotes. Four or even more copies of the *SMN1* gene have also been found, indicating a “2 + 2” or possibly a “3 + 1” **genotype**. This suggests “3 + 0” or “3 + 1D” **carrier genotypes** might also be possible however these are even rarer (Verhaart, et al. 2017).

A higher percentage of hidden **carriers** (“2 + 0”) with 2, even more copies of the *SMN1* gene is 3-8 times more prevalent in African American when compared to other ethnic groups, thereby decreasing the **sensitivity** of most **carrier** tests used (Verhaart, et al. 2017).

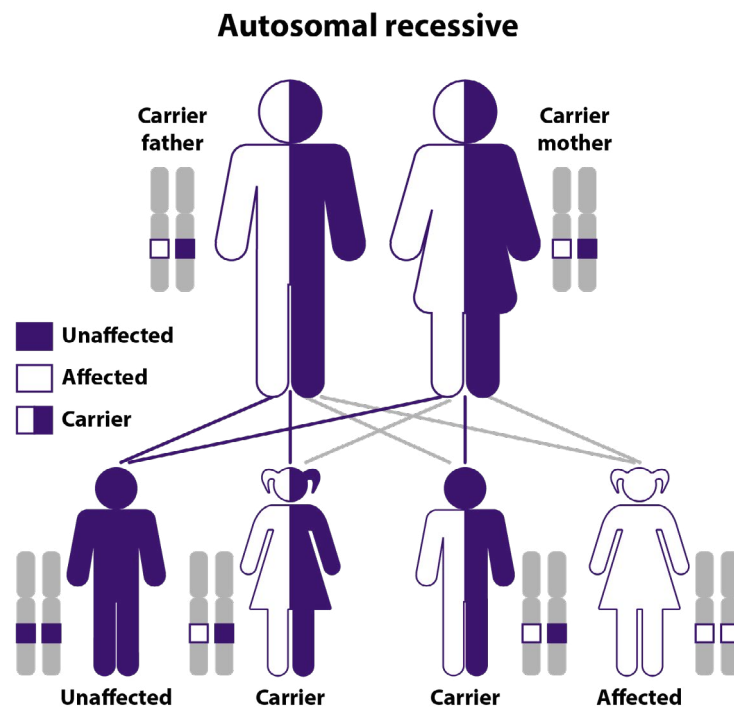
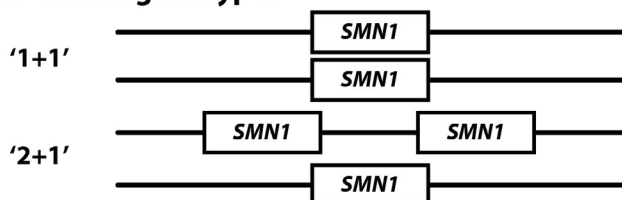
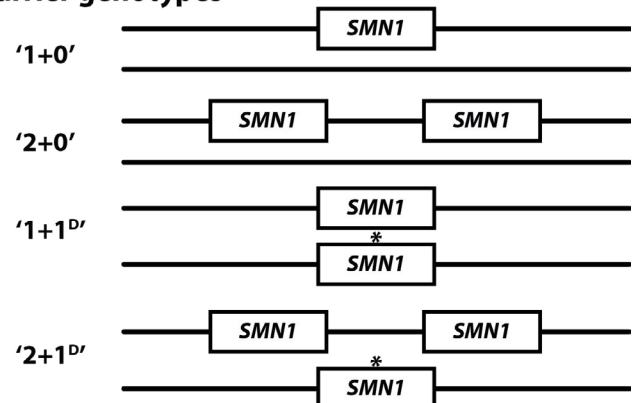


FIGURE 3. SMA is an autosomal recessive disease. Parents of an affected individual are typically **carriers** of one faulty copy. In families where both parents are **carriers**, there is a 25% chance that each of their children will have two faulty copies and have SMA, a 50% chance each that their children will be **carriers** and not have the disease, and a 25% chance that each of their children will have two normal *SMN1* genes and not have SMA, nor be **carriers**.

Non-carrier genotypes



Carrier genotypes



***Point mutation or microdeletion.**

FIGURE 4. Most common *SMN* genotypes among carriers and non-carriers. This image was published in Verhaart et al. Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy – a literature review. *Orphanet J Rare Dis.* 2017;12(1):124.

3. Neuromuscular pathology of spinal muscular atrophy

The main pathological feature of SMA, present in all human patients, is a specific loss of **lower motor neurons (MN)** ("spinal") and small myofibers ("muscular atrophy"). The name SMA was first used by Johann Hoffmann. It is now recognised that SMA is not just a disease of MNs but extends far beyond them. Loss of **motor neuron** cells from the spinal cord occurred subsequently to the development of other components of the neuromuscular system (axons and muscles) and specifically defects at the **neuromuscular junction (NMJ)**. **Motor neurons** are particularly sensitive to SMN protein paucity, but it is still uncertain if this is a selective vulnerability of motor neurons to depletion of *SMN* protein. These assumptions have been explored in numerous studies of animal models and, the results strongly indicate that there is a unique requirement for adequate levels of *SMN* protein in other cells except those in the central nervous system. Tissue-specific requirements for *SMN* protein was investigated in animal transgenic mice. To investigate the role of spinal neurons in the disease process and as therapeutic targets, researchers have restored *SMN* protein selectively to all CNS neurons in severe SMA model mice, using Nes-Cre recombinase transgene to activate the expression of an inducible *SMN* rescue allele (Lee A J-H, et al. 2012). Restoring the *SMN* proteins to the spinal neurons arrested the losses of motor neurons in transgenic mice. There was no significant difference in the number of motor neuron cells in transgenic mice compared to control at postnatal day 7. Restoring *SMN* protein to the spinal neurons of the mutant mice prevented the loss of proprioceptive synapses on the motor neuron cell bodies. SMA mice expressing neuronal *SMN* exhibited significantly fewer distal defects of the neuromuscular synapses. Accumulation of neurofilaments at pre-synapses was significantly reduced. The size and complexity of post-synapses were increased. Motor performances of transgenic mice improved as a four-fold increase in median survival. Nevertheless, mutants expressing neuronal *SMN* did not live beyond 3 weeks of birth. These effects were modest relative to the level of phenotypic rescue previously achieved following ubiquitous *SMN* expression with systemic delivery of self-complementary AAV9-mediated gene vector expressing *SMN1* cDNA to replace *SMN*

(scAAV9-SMN) in a mouse model of SMA (Foust KD, et al. 2010). AAV9 gene vector injected in neonatal mice at postnatal day 1 rescued neuromuscular physiology, motor function and life span.

This study implies that *SMN* protein delivered selectively to SMA motor neurons provide therapeutic benefit, but the mitigating effects on the overall disease and survival are modest. Restoring *SMN* protein in other cell types appears important, especially in severe SMA.

SMN is ubiquitously expressed and developmentally regulated. High levels of *SMN* expression occurs in most tissues during **embryogenesis** (skeletal muscles, heart, kidney, thymus, pancreas, brain and other neuronal cells, but not in lungs), followed by a significant reduction after birth in all tissues except in the brain and spinal cord, which remains relatively high until 2 weeks post-birth. These findings suggest that higher levels of *SMN* protein are required prenatally in comparison to the postnatal period and that most tissues require *SMN* protein for normal development (Burlet, et al. 2018). The contribution of *SMN2* to the level of full-length transcript in non-SMA fetuses in other tissues except the spinal cord was greater and virtually absent in the spinal cord. This suggests *SMN2* is a potentially important contributor to both the disease mechanism in SMA and in compensating for the pathology seen in tissues clinically unaffected by the disease. But, *SMN2* gene full-length transcripts become critical in the spinal cord upon homozygous loss of the *SMN1* gene (Soler-Botija, et al. 2005).

Motor neuron changes in spinal cord in humans

Prenatal findings

During embryological development, excess motoneurons undergo a phase of degeneration and death via a process known as programmed cell death (PCD) or **apoptosis**, allowing the final organisation of the nervous system. This process is very rapid and coincides with a stage of synaptogenesis. Prenatally, the normal apoptotic process is more pronounced in SMA, resulting in a prenatal deficiency in MN numbers in the spinal cord of SMA fetuses and the lumbar part of the spinal cord. **Motor neurons** are reduced by 15-35% compared to normal fetuses and by 50% in the early neonatal period (Simic, et al. 2000). The remaining SMA foetal MNs express normal levels of choline acetyltransferase (ChAT), the enzyme that catalyses the biosynthesis of acetylcholine (Ach), whose reduction is attributed to the loss of MNs, suggesting that the function of surviving MNs in this period may be adequate (Soler-Botija, et al. 2005).

Postnatal findings

The classical pathological features evident in SMA spinal cords are referred to as the neuropathological tetrad and include the loss of MNs, empty cell beds, **glial bundles** in ventral spinal roots (VRs) and heterotopic MNs.

Increased disease duration (increased patients age) is associated with higher MN losses. In severe cases, about 73% of MNs were lost in individuals aged 5-22 months (Simic, et al. 2000). Empty cell beds and glial bundles are frequently observed in the ventral horns (VHs) of spinal cords, representing a space where MNs previously resided. There are few post-mortem studies involving patients with intermediate or mild forms of SMA. MN loss was less severe in teenage SMA type II patients than in SMA type I patients of different ages (Ito, Shibata, Saito, Kobayashi & Osawa, 2011). In a limited number of patients, a severe reduction in MNs, as well as the hypoglossal and facial nucleus in the brainstem, were also reported in SMA type II (Araki, et al. 2003). MNs of the phrenic nerve are preserved. Many of the MNs remaining in SMA spinal cords are chromatolytic, have an accumulation of **neurofilaments** (Nf) and breakdown of the plasma membrane consistent with necrotic cell death. **Neurofilaments** are elevated in the cerebrospinal fluid (CSF) in infantile-onset SMA and decrease under Nusinersen (Spinraza™), the first approved treatment for SMA. This is used as an additional marker to monitor therapy efficacy in clinical trials (Winter, et al. 2019).

Heterotopic **MNs** are aberrantly localised in the white matter. They are presumed to be undifferentiated in the very early stage of development, without axons, dendrites and synaptic connections (Simic, et al. 2000). No human studies to date have examined **MNs** pathol-

ogy or quantity, based on relative susceptibility within different MNs subtypes within a spinal cord or caudal-rostral axis. Not all **motor neurons** and their **NMJs** are similarly susceptible to **SMN** protein deficiency. Patients with SMA have more weakness proximally than distally. They retain normal eye movements, external sphincter continence and diaphragm function (Martinez-Hernandez, Bernal, Alias & Tizzano, 2014). The precise origin of the selective vulnerability of some motor neurons and their **NMJs** remains unclear but it clearly demonstrates that not all cells respond to low levels of **SMN** in the same way. On the other hand, a study from 2012 investigated a range of morphological neuromuscular parameters and none conferred the risk to degeneration of different subtypes, suggesting that more subtle molecular differences are likely to underlie the relative susceptibility of some motor neuron pools (Thomson, et al. 2012)

Nerve pathology in humans

Reduced number of long myelinated axons with rare demyelination or degeneration are frequently observed in proximal VRs. An alternative hypothesis to explain the increased vulnerability of motor neurons to low *SMN* levels may be the consequence of disrupted **axonogenesis**. At post-symptomatic stages in severe SMA mice, only 20–35% of **motor neurons** are lost from the spinal cord, despite severe neuromuscular degeneration, indicating that **motor neuron** function is compromised prior to cell death (Monani, et al. 2000). This indicates that axonal pathology may precede **MNs** somal death.

There is also a degeneration of small unmyelinated axons in VRs and distal nerve terminals, as well as a reduced number of myelinated peripheral nerves and intramuscular nerves.

One of the hallmark pathological features in SMA are **glial** bundles in the VRs, which are present in all SMA types. They were first described in the lumbar segment of the spinal cord. They taper off with increasing distance from the spinal cord. **Glial** bundles are considered to be an astrocytic response to early-onset MN degeneration (Winter, et al. 2019). **Astrocytes** are an important source of **trophic factors** that provide essential signals for neuronal survival. In diseased and/or injured environments, **astrocytes** become reactive and undergo distinct morphological restructuring including the upregulation of glial fibrillary acidic protein (GFAP) and other neurofilament proteins. Reactive **astrocytes** secrete proinflammatory cytokines and, alter growth factor production, all of which promote neuron loss. **Microglia**, which are another important source of neurotrophic molecules, undergo similar morphological and functional changes when activated and serve as inflammatory cells in the CNS. In severe SMA mice models, astrocyte dysfunction and/or activation preceded **motor neuron** loss (Rindt, et al. 2015). **Astrocytes** switch away from their protective functions toward reactive and inflammatory cells and create a neural environment that exacerbates motor neuron malfunction. The phenotypic changes in SMA **astrocytes**, such as altered morphology, altered signalling and decreased growth factor production, could negatively impact **motor neuron** health (Sison, et al. 2017).

Neuromuscular Junction (NMJ) Pathology in humans

Prenatal findings point to early alteration of the motor unit in patients with severe SMA. **NMJ** pathology seems to start during the foetal period. As a result, initial muscle innervation cannot be maintained.

Disaggregated ACh receptors (AChRs) were found around the **myotubes** in SMA type 1 fetuses, but not in SMA type II. Accumulation of presynaptic vesicles, which were not in contact with muscle cells was observed both in humans (Martinez-Hernandez, et al. 2013) and mouse models (Kong, et al. 2009). Involvement of NMJ pathology in mice could be translated to human patients.

In postnatal findings, NMJ immaturity was documented in a form of simple motor terminal arborisations with no synaptic vesicles and persistent expression of immature forms of γ AChRs (Kong, et al. 2009). In mice models, neurofilament aggregation in axons ends, abnormally small and not developed motor endplates, loss of Schwann cells, delayed AChRs and axon maturation, were also documented (Kariya, et al. 2008). Potential cellular and molecular mechanisms underlying NMJ pathology are: Nf accumulation in motor nerve terminals, **axonal transport** and cytoskeletal abnormalities, altered calcium channels, mitochondrial dysfunction with decreased **ATP** levels and ubiquitin pathways.

Muscle pathology in humans

Motor neuron survival and skeletal muscle development are closely linked and depend upon continued cellular contact between these two cell types. Neuronal dysfunction can contribute significantly to muscle atrophy, but there is evidence to suggest that intrinsic abnormalities in SMA skeletal muscle, due to lack of *SMN* protein, could contribute directly to disease pathogenesis.

Presence of smaller and thinner **myotubes** in SMA patients was seen prenatally, with impairment in **myotubes** width, indicating a delay in muscle growth and maturation. These findings are consistent in the postnatal period (**Martinez-Hernandez, et al. 2009**). Expression of proteins required for myofiber development is altered, with higher expression of immature isoforms of **myosin** heavy chains, which persist postnatally in atrophic areas of more affected muscles. There is a correlation between myofiber maturity and innervational status (persistent expression of immature myofiber cell surface marker has been observed on denervated myofibers in contrast to large preserved innervated myofibers (**Walsh & Moore, 1986**).

Clinical diagnosis of SMA before genetic testing relied on muscle pathology from biopsy tissue acquired from quadriceps muscle. Large areas of small rounded myofibers of all types (hypotrophic) often involving all fascicles, interspersed by hypertrophic fibres were regularly seen. Hypotrophic fibres are evident in all SMA types, with a lesser percentage in less severe cases. There is a continuous growth of hypertrophic fibres type I (probably fibres which retained innervation or have been reinnervated from regenerative sprouts of surviving **MNs**) and arrest of growth of small fibres, due to early **denervation** which begins at ages 2 and above. With SMA disease progression, there is an increased replacement of muscles by **fibrosis** and fat.

Pathological changes in muscle are dependent on innervating **MNs**, but evidence showed that changes in muscles could occur independently of nerve degeneration, confirming that **SMN** has cell-autonomous roles in muscle (**Mutsaers, et al. 2011; Arnold, et al. 2004**). These findings suggest that treatments for SMA that target muscle, as well as **motor neurons**, are likely to be beneficial.

An early study that increased **SMN** in muscle did not find significant improvements in motor **phenotype** and restoring **SMN** in skeletal muscle alone is insufficient to correct disease pathology in SMA mice (**Gavrilina, et al. 2008**). On the other hand, a growing body of literature suggests that selective restoration of **SMN** levels by 50% in **muscle satellite cells** (regenerative cells) improved phenotype in SMA mice (**Nicole, et al. 2003**).

It is possible that postnatal muscle loss could secondarily trigger **MN** cell death as well as **NMJ** maturation defect and abnormal synapses.

SMN expression in non-SMA and SMA human tissues

There is a small number of data about baseline normal or disease-associated **SMN** levels in disease-relevant human tissues. There is even less understanding of **SMN** induction in the CNS tissues with treatment because **SMN** levels cannot be measured in the CNS tissues of living patients. To understand the dynamics of **SMN** expression in the human CNS and non-CNS tissues of unaffected and SMA patients (with 2 **SMN2** copies), researchers have recently quantified **SMN**-mRNA and **SMN** protein levels in human tissue (spinal cord, brain and muscle) during expedited autopsies (**Ramos, et al. 2019**) **SMN** protein levels showed a broad range in the spinal cord, cortex and muscle tissues isolated during fetal development in non-SMA controls and decreased during development. Postnatal control group less than 3 months of age had 2,3 fold decrease in SMA protein levels in spinal cord compared to prenatal levels. SMA affected spinal cords samples had a 6 fold decrease in the same age group (less than 3 months postnatally) compared to non-SMA spinal cord samples. There was only one fetal SMA affected

sample as spinal cord tissue examined. The highest **SMN** protein level of all SMA affected tissues was in this sample in 18 gestational week, but this level was approximately 5 fold reduced compared with median **SMN** levels of prenatal non SMA control samples. Compared to levels of SMA protein before 3 months of age and after 3 months of age there were no statistically significant differences between SMA and non-SMA samples. **SMN** protein become restricted at low levels postnatally, particularly after 3 months of age and remain low in cases 3 month-14 years. This study showed that the decrease in SMA protein levels in the spinal cord was most evident in samples spanning 3 months before and 3 months after birth (perinatal development). A similar result was observed in frontal cortex tissues and skeletal muscles. There was a 3 fold decrease in the early postnatal period compared to the prenatal period in non-SMA iliopsoas muscle, while approximately 2 fold decrease was found in postnatal samples of iliopsoas muscle and diaphragm in SMA compared to the same muscles in postnatal

non SMA controls. These findings imply a particular need for *SMN* protein in the CNS and muscles during gestational and early neonatal developmental stages. *SMN* protein may decrease rapidly in the last trimester and the first 3 months after birth, which highlights a possible optimal therapeutic window. Spinal cord *SMN* mRNA expression correlated moderately with *SMN* protein expression. The range of *SMN* transcript levels was restricted compared with that of protein. The decreases in median SMN1-FL or SMN2-FL mRNA levels between prenatal and postnatal samples (SMN1-FL, 25%; SMN2-FL, 29%) were modest compared with those of protein (79%). In non-SMA control samples, a significant drop of median SMN2-FL transcript occurred only between prenatal and early postnatal control samples, whereas median SMN1-FL transcript decreased significantly only between early and late postnatal samples. This may indicate an earlier developmental decrease in SMN2mRNA expression relative to that of SMN1mRNA in non-SMA spinal cord and brain samples. This earlier decrease of *SMN2* transcriptional activity could further contribute to earlier developmental reductions in *SMN* protein in SMA patients compared with controls. *SMN* protein expression only modestly correlated with a sum of *SMN1* and *SMN2* mRNA transcripts in prenatal control samples. In SMA postnatal samples *SMN* protein levels modestly correlated with SMN2-FL transcripts. The highest *SMN* protein expression and SMN2-FL mRNA expression was observed only in one fetal SMA sample. This may indicate that *SMN* protein independently decreased with age between prenatal and late postnatal samples and that additional, posttranscriptional mechanisms contribute to the decrease in *SMN* protein levels during perinatal development. This implies that other mechanisms, except SMN1 and SMN2 mRNA expression, could also account for variations of therapeutic response in patients with SMA. When controlled for copy number, SMN2-FL, SMN2-Δ7, and SMN2-FL/Δ7 transcript expression did not significantly differ between postnatal control and postnatal SMA with 2

SMN2 copies in spinal cord or cortex. *SMN2* transcript expression is unaltered by the loss of *SMN1* in the CNS. When controlled for *SMN2* copy number, postnatal control cases had median 2.1-fold more *SMN* protein than postnatal SMA cases.

Five patients who were treated with Nusinersen in this research showed ASO drug uptake in CNS samples (spinal cord and brain). ASO drug concentrations were 2 fold lower in the cervical spinal cord compared to the lumbar and thoracic spinal cord. Variable drug levels were seen in brain tissue. This pattern of drug concentration was associated with a 3-fold increase in SMN2-FL mRNA levels in cervical, thoracic, and lumbar/sacral spinal cord samples isolated from Nusinersen-treated cases. There was no change in SMN2-FL expression in brain tissues. Muscle and liver tissues also showed no increase in SMN-FL transcripts. Those with the highest level of SMN2 mRNA expression received Nusinersen prior to death or received multiple doses. ASO uptake and *SMN* protein expression were also assessed by immunostaining. ASO uptake were particularly evident in ventral horns and the staining was highest in the lumbo-sacral region and thoracic region. Less was observed in the cervical spinal cord and upper brain regions indicating caudal-to-rostral gradient in the CNS. The caudal-rostral gradient paralleled with SMN2-FL transcript expression. Immunostaining of *SMN* protein was evident in the ventral horn, macroglia and ependymal cells at all spinal levels and, the percentage of *SMN* positive cells in treated patients were either not significantly different or higher relative to those of unaffected control samples.

Other neuronal abnormalities in SMA

Brain

A correlation between neuronal pathology in other parts of the brain in relation to disease severity and *SMN2* copy numbers was reported. Morphologic analyses of post-mortem central nervous system in most severely affected SMA type I patients with one copy of *SMN2* gene, showed widespread neuronal degeneration in the motor cortex, nuclei pontis, reticular formation, substantia nigra, cerebellar structures and thalamus (Harding, et al. 2015). This indicates a potentially important role for *SMN* in regulating brain development.

Sensory pathways

Several cases of defective sensory neurons and thalamic lesions have been detected in genetically confirmed SMA. *SMN* levels are high in dorsal root ganglia and posterior horn of the spinal cord in the human foetus. Restoration of *SMN* in motor neurons of SMA mice repairs the NMJ defects and also restores synapses in the sensory neurons (Gogliotti, et al. 2012), suggesting that the sensory-motor circuit function is dependent on *SMN* levels in motor neurons.

Within spinal motor circuits, motor neurons (MNs) bridge the central and peripheral nervous systems by conveying central commands to the skeletal muscles. MNs receive synapses from sensory neurons, spinal interneurons and supraspinal pathways.

Whether synaptic dysfunction is responsible for the MN death or synaptic loss occurs in response to MN dysfunction is unresolved. The key role of excitatory synaptic drive in shaping the function of motor neurons during development was investigated by researchers from Columbia University in the United States (Fletcher, et al. 2017). *SMN* depleted motoneurons exhibited reduced monosynaptically - induced excitatory postsynaptic potential (EPSP). The modest overall reduction in a number of proprioceptive synapses on hyperexcitable motor neurons soma and dendrites were also found, indicating that synaptic dysfunction precedes synaptic loss and correlates with an increase in motor neurons sensory input resistance early in disease before motor neuron death.

Dysfunction of proprioceptive synapses caused increased input resistance of motor neurons and reduction in motor neuron firing in SMA. SMA MNs fire at significantly reduced frequencies after the blockade of proprioceptive transmission through expression of the tetanus toxin light chain subunit (TeNT), which inhibits neurotransmitter release (glutamate). Increasing neuronal activity pharmacologically by chronic exposure with kainate, a glutamate receptor agonist. *in vivo*, increased neuronal activity and improved righting time and led to the improvement in motor function.

Selective restoration of *SMN* protein in proprioceptive neurons rescued synaptic loss. The rescue of proprioceptive synapses or enhanced presynaptic function of the remaining synapses was demonstrated by improved EPSP. Selective restoration of *SMN* protein in motor neurons had no effect on the synaptic rescue.

SMN upregulation in proprioceptive neurons in SMA mice improved NMJ function and motor behaviour. Combination of *SMN* upregulation in both motoneurons and proprioceptive neurons significantly contributed to the improvement in motor deficit by improving righting time and increasing CMAP amplitude.

This study showed that synaptic mechanisms are also responsible for motor neuron dysfunction in spinal muscular atrophy.

Proprioception

In adults, proprioceptive afferent input contributes a significant amount of synaptic drive that continually modulates motor output, muscle stiffness and tone, which are critical for postural control. During development, motor neurons and muscles are dependent on each other, and their connection is essential to each component's growth and survival. This is evident in the clinical manifestations of SMA, which include hyporeflexia, whereby patients' reflexes are below normal, or absent and selective muscle weakness of proximal, axial, and intercostal muscle groups. Severely affected babies cannot sit without support. The stretch reflex is primary for movements. The circuitry involved in the stretch reflex comprises three different cell types: alpha motor neurons, muscle fibres, and proprioceptive sensory neurons. Upon muscle stretch, specialised sensory endings embedded in the muscle, called muscle spindles, lengthen and increase the firing frequency of primary proprioceptive sensory afferents. In SMA patients, muscle spindles are much thicker and cellular, capsules are thickened and denervated from sensory afferents. These observations suggest that spindle degeneration is a progressive process and that the loss of proprioceptive information from afferents may result from the inhibited contractile shortening of the spindle or complete sensory denervation. On electromyography (EMG) H (Hoffmann's)-reflex was absent in 86% of cases, while the M-wave was absent in 30% of cases, suggesting impaired sensory-motor function; H-reflexes were absent in 40% of SMA III patients, whereas milder abnormalities in M-wave responses were observed (Chiriboga, et al. 2015). These observations suggest that H-reflex responses may be a more sensitive method for detecting the initial stages of the disease.

4. SMA as a multi-organ disease

Although the primary pathology in SMA affects the neuromuscular system, evidence from clinical reports and animal studies have shown that other tissues and organs are involved. These patients have various complications and die prematurely, before damage to other organs occurs. Multi-organ dysfunction, including cardiac and vascular defects, is not a general feature of human SMA.

Cardiac abnormalities

Most of the cardiac abnormalities have been reported in SMA type I patients with one *SMN2* copy, while they are also present in patients with 2 *SMN2* copies. Congenital defects were most present in SMA type I patients. About 50% of SMA type I patients with structural cardiac defects had a single structural abnormality, atrial septal defect (ASD) as the most prevalent, followed by a ventricular septal defect (VSD). The other 50% had multiple structural abnormalities, ASD combined with VSD or hypoplastic left heart syndrome as the most frequent combination. Other congenital abnormalities were also reported: valvular aortic stenosis, hypoplastic aortic arch, coarctation of the aorta, tricuspid atresia and hypoplastic left heart syndrome. Patients with 1 *SMN2* copy had haemodynamically relevant congenital defects, which points to the role of *SMN* protein in cardiogenesis in SMA patients (Wijngaarde, et al., 2017). Ultrasonographic assessments of the heart in SMA fetuses with one *SMN2* copy revealed a high proportion of congenital abnormalities, associated with increased nuchal translucency (NT) (Parra, et al. 2012).

Abnormal electrocardiographic findings were artefacts due to baseline tremor from peripheral muscular fasciculations, which did not alter cardiac rhythm and function. Bradycardia (less than 40 beats per minute) was reported in severe cases, who survived many years due to assisted ventilation with signs of right ventricular overload. Bradycardia, fluctuation in blood pressure, irregular skin reaction to temperature changes and vascular abnormalities were interpreted as an autonomic nervous system dysfunction (nije nerve nego nervous)(ANS). Vascular abnormalities, like distal digital necrosis, were associated with cardiac abnormalities but also as an isolated feature. And dysfunction may lead to impaired regulation of vascular tone (Bottai & Adami, 2013; Parra, et al. 2012).

Interpretation of cardiac dysfunction in SMA type II and III patients should be taken with caution, as the normal population is also at risk to develop cardiac deficiency. Patients with long-standing respiratory dysfunction are prone to right heart overloading and pulmonary hypertension, with ECG pathological findings. Patients with atrioventricular disturbances (AV) have to be considered as having other types of dystrophy such as Emery - Dreifuss or myotonic, or autosomal dominant proximal SMA. Baseline tremor on ECG was reported in all SMA type II patients and in 50% of patients with SMA type III. A small number of patients had other rhythm abnormalities. Cardiac abnormalities are rare and, usually involve mitral valve prolapse. Complex cardiac malformations were reported only in 2 patients with later onset disease (Parra, et al. 2012).

Metabolic disorders

Metabolic defects on the level of lipid metabolism and glucose metabolism have been reported, implicating the liver and pancreas respectively.

Liver defects

Abnormal fatty acid metabolism is the most common metabolic defect exclusive to SMA type I patients with a few exceptions seen in milder patients. Mild to moderate ketonuria with elevated fatty acid levels in blood upon fasting was found, due to mitochondrial β oxidation abnormalities as well as elevated esterified carnitine (Crawford, Sladky, Hurko, Besner-Johnston & Kelley, 1999). Extreme muscle wasting in severe SMA patients could be explained by defects in fatty acid transport/oxidation as a major source of energy production during prolonged fasting (in



the absence of glucose), resulting in detrimental effects on the skeletal and cardiac muscle. The Amino Acid diet (AA), based on low levels of fatty acids, high carbohydrates and supplement proteins in the form of free amino acids, while controversial in the community, has not been scientifically evaluated. Other defects could be pinpointed directly to the liver. Liver-restricted *SMN* depletion leads to embryonic lethality. Mutant embryo livers showed abnormal development, function and iron overload associated with mis-regulated expression of genes involved in iron homeostasis. Severe SMA mice, which express low levels of *SMN*, exhibit lower reduced hepatic expression of insulin-like growth factor 1 (IGF1s, Somatomedin C). The importance of the liver in SMA pathology was indicated by experiments in which restoration of *SMN* and subsequent increase in insulin-like growth factor1 levels in the liver of SMA mice, through subcutaneous injection of therapeutic oligonucleotide (ASO), was an important factor for a high degree of rescue (Vitte, et al. 2004). IGF1 has an anabolic effect in adults and together with follistatin has an anabolic effect in muscles in SMA patients. In contrast, *myostatin*, inhibits myogenesis (muscle cell growth and differentiation). Modulating this pathway has been attempted to increase muscle mass and inhibit atrophy in the context of SMA disease and to prevent catabolic state.

Pancreatic defects

Glucose metabolism defects have been reported in the context of SMA. In SMA type II and III, hyperglycemia and glucose intolerance were reported, with hyperglucagonemia in the liver with resistance to insulin. Elevation in *glucagon* producing cells in the pancreas and loss of insulin β - producing cells were found. High-fat diets in patients' regimen induced glucose tolerance problems and obesity (Bowerman, et al. 2012). Hypoglycemia and *ketonuria* were observed in 2 patients (Bruce, Jacobsen, Dossing & Kondrup, 1995).

Gastrointestinal defects

Constipation, gastroesophageal reflux (GERB), abdominal distension, delayed gastric emptying and slow liquid transit in intestines and colon, were reported in SMA pathology. Impaired GI neuromuscular transmission could be responsible for some of these abnormalities as the *autonomic nervous system*. Whether impaired metabolism is linked to the GI system abnormalities remains to be determined (Wang, et al. 2007).

Collectively, it has become clear that SMA is more than a *motor neuron* disease and unravelling the complex pathology will only aid in the development of more potent therapeutics as SMA research continues to evolve.

5. Diagnosis and differential diagnosis —

Diagnosing SMA

The first step in diagnosing SMA is to suspect to SMA.

Parents or close relatives tend to identify the first symptoms (Lawton, Hickerton, Archibald, Mc Claren & Metcalfe, 2015) (e.g.: Increasing difficulty with breast feeding, gaining weight, moving limbs, rolling, sitting, walking (Lavie, Nisnkorn & Sagi Let Amirav, 2020) and sometimes breathing or respiratory infections which require hospitalisation).

Together with the patient's/carer's concerns and history, the physician may suspect SMA when a child is noticeably weak, floppy or delayed in meeting developmental milestones. This may include difficulties in feeding, breathing, an inability to achieve head control, roll-over, sit independently, stand or walk, or lose their ability to walk over time. Given that SMA is a rare disease and that its hallmark symptoms overlap with other neuromuscular diseases, SMA is often misdiagnosed or unrecognised, or both, so most parents undergo a prolonged and arduous diagnostic journey, if diagnosed at all.

This has serious implications on the psychological burden (worry and confusion (Lawton, Hickerton, Archibald, Mc Claren & Metcalfe, 2015) of families and relatives but also for care and treatment.

Various scientific studies around the world show that treating patients before the onset of the first symptoms or as soon as possible after that, could preserve the nervous system and prevent the onset of functional disabilities. Children detected and treated in the first week of life can benefit from normal functional development. The most important is early detection of first symptoms and referral for further diagnosis. Early diagnosis and prompt treatment will give the patient a higher chance of achieving a better motor milestone, social inclusion, work and productivity and what is most important, decrease the burden on the family and enable the patient to have a fulfilled life.

To address SMA diagnostic journey issues and to support the SMA community, SMA Europe partnered with Novartis Gene Therapy (previously known as AveXis), in hosting a workshop. This workshop, which gathered SMA Europe patient representatives and patients affected by SMA, aimed at identifying barriers to early diagnosis. The main question focused on how to shorten time to diagnosis.

Diagnostic delays were attributed to many factors:

- Lack of awareness in the general population and therefore families
- Lack of awareness of SMA symptoms in primary care/needs
- Some uncertainty around differential diagnosis of SMA and practical next steps
- Wide range in genetic testing standard practice, often not recognising the urgency of diagnosis for progressive conditions

In addition, limited information at diagnosis for patients/families, observation that HCPs sometimes lack expertise and empathy in discussions with patients/families and lack of clarity on treatment options/next steps for patients/families were also reported.

Recommendations to support early diagnosis and shortly after diagnosis are:

- Drive recognition of “referral triggers” through online education with PCP/paediatricians
- Support neurologists with a differential diagnosis tool
- Highlight genetic testing best practice when suspecting SMA
- Provide holistic SMA education, including medically validated tips for day-to-day care
- Support HCP’s dialogue with patients/caregivers
- Support families with counselling and coordinated visits
- Deliver decision making tools and guidance to help caregivers make choices quickly after diagnosis, taking into account their intuition and views and most importantly, joint decision-making

A physician may suspect a diagnosis of SMA when an infant or toddler displays one or more of the following clinical symptoms:

- Progressive muscle weakness (symmetrical, equally affecting both sides of the body) and usually more severe proximally (upper arms and legs) then distally (hands and feet). Symptoms are preceded by an asymptomatic period (except in the most severe of the types and SMA type 0)
- Fluttering of the chest wall when taking a breath and rapid diaphragmatic (belly) breathing
- Hypotonia with weak or absent reflexes
- Fine tremor of the fingers or **fasciculations** of the tongue
- Weak cry or cough
- Limited movement of limbs and trunk particularly antigravity movements (lower limbs)
- Floppy child
- Respiratory difficulties or failure
- Delayed or missed milestones (which are often interpreted as a sign of child being lazy because of the difficulty in distinguishing normal from abnormal development)
- When the family is claiming that they are suspicious

The only definitive way to confirm a diagnosis of SMA is via molecular (genetic) testing. Other methods, which were used in the era of pre-genetic testing, are muscle biopsy and electrophysiological studies such as EMG, performing specific tests that correlate with disease progression and severity such as compound muscle action potential (CMAP) and motor unit Count Estimate (MUNE). These tests are invasive, sometimes inconclusive and no longer used as diagnostic tools.

IMPORTANT: DATE IN WHICH THE GENE WAS DIAGNOSED (1995)

A diagnosis obtained before the gene was discovered can be incorrect. This means some people are without genetic confirmation etc. which has important consequences in accessing care and therapies.

Diagnostic genetic testing of spinal muscular atrophy

Genetic testing for SMA can be performed with deletion testing and point mutation testing.

Deletion testing

Homozygous deletion screening of *SMN1* gene represents the first tier in diagnostic testing. The absence of the *SMN1* gene can occur by deletion or by **gene conversion** to *SMN2* and is present in about 95-98% of the patients. Detection of the absence of **exon 7** of the *SMN1* gene can be performed with polymerase chain reaction (PCR) based targeted mutation analysis using a restriction enzyme (due to a mismatched primer). This test allows to distinguish *SMN1* from *SMN2* and is reliable as well as simple to set up in a clinical diagnostic laboratory. However, this test cannot determine **carrier** status or the number of *SMN2* copy. The multiplex ligation probe amplification test (MLPA) is a methodology which allows determination of both *SMN1* and *SMN2* copy number. MLPA is a quantitative **PCR test** and currently used in most **DNA** diagnostic laboratories as a diagnostic test, *SMN2* copy number test and to determine **carrier** status. The absence of undetectable *SMN1* in clinically suspected SMA is a reliable and powerful diagnostic test, extremely sensitive and specific, and the results can be obtained within a week.

Prenatal testing can be performed on chorionic villous sampling (CVS) specimens or amniotic fluid. The American College of Obstetricians and Gynaecologists Committee on Genetics recommended against prenatal screening in the general population (**ACOG Committee on Genetics, 2009**). Both tests carry a risk of miscarriage. The false-negative results can occur due to maternal contamination of the foetal specimen. Indications for prenatal diagnosis of SMA include:

- Family history and **carrier** status of both parents
- Presence of abnormal findings such as decreased foetal movements and contractures in utero or increased **nuchal translucency** on foetal ultrasound.

Point mutation testing

About 2-5% of affected patients are negative for the diagnostic **homozygous deletion** test. If a patient with an SMA **phenotype** possesses only one copy of the *SMN1* gene, it is likely that the remaining copy contains a more subtle mutation. Most of these patients are compound heterozygotes, with one *SMN1* allele with a point mutation and the other one missing. In these cases, the next diagnostic test performed would be direct exonic **DNA** - sequencing of PCR amplified genomic DNA.

Finally, there are patients that have one copy of the *SMN1* gene without point mutation. These patients may have a minor mutation in an **intron** sequence (affecting **splicing**) or in a regulatory part of the gene. In this case, RNA analysis would be required.

SMN2 testing

SMN2 copy number analysis may be of value within the settings of clinical trials and new-born screening in stratifying patients who are more likely to respond to therapy aimed at upregulating the levels of full-length *SMN* protein

from the *SMN2* gene. The results on *SMN2* copy numbers must be interpreted with caution, both in clinical trials or diagnostic testing, since the relationship with disease severity is not absolute. *SMN2* copy numbers are determined by the MLPA test.

IMPORTANT FOR PATIENTS: TIME TO RESULTS

Anxiety is enormous. Reliability of the information, refusal to accept the diagnosis, risk for siblings or for future children, genetic counselling, extended family members with SMA/risk of having it.

Impact of number of *SMN2* copies on access to treatments and care, reliability of copy number counting, modifying factors.

Carrier testing

Carrier testing is feasible in parents of patients with SMA or family history using quantitative PCR based dosage assay. It can also be performed using other techniques, like MLPA. PCR gene dosage analysis is required to distinguish carriers with one copy of the *SMN1* gene from normal individuals with 2 copy of the *SMN1* gene. There are however some limitations to the carrier test.

- About 2% of SMA cases arise as a result of de novo mutations in the affected individual. A large number of repeated sequences around the *SMN1* and *SMN2* gene loci, predispose this region to unequal crossovers and recombination and results in de novo mutations. Due to the occurrence of de novo mutations in SMA patients, one of the parents may not be a carrier.

- Copy number of the *SMN1* gene can vary on a **chromosome** allele. About 9% of the population possess 3 copies of *SMN1* gene (Foust, et al. 2010). The carrier status “2+0” with 2 *SMN1* copy on one allele and zero on another, will not be detected by carrier testing. SMA dosage carrier test will be false normal. These carriers have the same risk (25%) of having an affected child as carriers with one *SMN1* copy on one gene and zero on another (“1+0”).

In about 6% of parents with a child with a homozygous absence of *SMN1* gene, the carrier testing results will be normal due to de novo mutation or “2+0 carriers”. The findings of normal *SMN1* copy dosage test-significantly reduces the risk of being a **carrier**, although there is still a residual risk of being a carrier and a small recurrence risk of future affected offspring for individuals with 2 *SMN1* gene copy number.

The goal of SMA carrier testing is to identify couples at risk of having a child with SMA so as they can make their own informed reproductive choice. At present time, carrier testing in the general population is not recommended (Foust, et al. 2010). It is important that individuals understand the limitations of the test. The testing must be voluntary, informed consented to and confidential.

New-born Screening (NBS)

The purpose of NBS is to identify affected infants prior to the presentation of clinical symptoms. It is a successful programme which has improved the quality of life of many children with a variety of diseases.

SMA was recently recommended in the US to be added to the list of conditions tested at birth on the recommended uniform screening panel (RUSP). For a disorder to be included in a NBS program, it has to generally meet certain criteria: disease can be detected in the early newborn period when it would not be clinically detected, that the test has appropriate **sensitivity** and **specificity**, and there are clinical benefits for early therapeutic intervention.

The impact of NBS and benefits achieved could be:

- Direct benefits to the affected child (decrease early mortality and improvement of quality of life)
- Provide the SMA community with the best available options to tackle their disease
- Identification of **carriers** and prevention of additional cases through genetic counselling, which could have a major positive impact on close family members and other relatives in terms of adequate and self-decision making, especially about future pregnancies
 - To obtain proactive treatment earlier in disease progression with regard to respiratory care, physical therapy and nutrition and increase support for families and children
 - To allow patients to be enrolled in clinical trials at the earliest time period
 - To begin treatment before the potentially irreversible neuronal loss (in SMA type 1 the rapid loss of motor units occurs in the first 3 months, and severe **denervation** with loss of 95% of motor units within 6 months of age (Swoboda, et al. 2005). Starting the treatment at birth can significantly change the course of the disease and lead to a change in the **phenotype** in the pre-symptomatically treated child.
- Reduce in the economic and psychological burden of families and society related to the natural disease course
- Identification of milder, late onset cases of SMA and rare asymptomatic individuals with *SMN1* homozygous absence. The physicians and parent could monitor for motor problems and intervene more quickly.

The identification of SMA patients during NBS can only be done by **DNA** testing. There are more types of techniques; most of them require a DNA sample from dry blood spots. The clinical **sensitivity** of SMA NBS would be about 95% since it would not be able to detect compound heterozygotes. Thus, about 5% of cases will remain undiagnosed until manifestations of the disease occur and specific genetic tests are performed.

In addition to the benefits, there are some concerns about the impact of NBS on families of new-borns, as well as chronic patients with SMA. In the context of a NBS, the absence of clinical signs can make a diagnosis difficult to accept for parents and make a proper, decision about the future treatment, need for continuously scheduled visits and, care of the child through the lifespan. Families with children with SMA now have high expectations about treatment outcomes that sometimes overpass the actual treatment effect. It is therefore very important to communicate in the NBS process the diagnosis and treatment options, possible outcomes and limitations of treatments, as well as the importance of lifelong monitoring. This should be clear and transparent and reflect the current level of understanding of SMA.

NBS should not be viewed as a way to limit innovative medication to only pre-symptomatic patients. Data from NBS should not preclude access to therapy by patients who are symptomatic and chronic patients.

One of the major issues frequently discussed is the number of *SMN2* copies a child should have in order to be treated. The questions of whether or not patients with four copies of *SMN2* should be treated at birth is highly debated. The benefit of treating patients with more than three copies of *SMN2* at birth has not been determined. A treatment algorithm for infants diagnosed with spinal muscular atrophy through new-born screening was established by an SMA NBS Multidisciplinary Working Group supported by the patient advocacy group-CURE SMA in 2017 (Glascock, et al. 2018). The working group recommended immediate treatment for individuals predicted to manifest SMA with the qualifying **genotypes** of two or three copies of *SMN2*. In September 2019, the multidisciplinary working group reassessed the treatment algorithm for new-borns with SMA identified through new-born screening and gave a new recommendation - immediate treatment for infants diagnosed with SMA via NBS with four copies of the *SMN2* gene (Glascock, et al. 2020).

Patient advocates strongly support treatment of infants with 4 copies of SMN2 detected through NBS. This opinion is supported by the fact there are patients with 4 copies of the SMN2 gene who can sit but who have never walked independently and have severe disease progression.

Until this becomes a reality, standardised follow up of patients with four copies of *SMN2*, if no treatment is offered, has been recommended by physicians and patients advocates. The physician should present and explain the different approved and investigational treatments, clearly balancing expectations and unknown long-term effects and give parents time to make an informed decision.

In Europe, in May 2019, the European Neuromuscular Centre (ENMC) held a workshop, in the Netherlands, to gather the information currently available on NBS. Neuromuscular experts from Europe and the United States gave recommendations, in collaboration with patient representatives from SMA Europe. These recommendations were published in 2020 (Dangouloff, et al. 2020).

The key conclusion is that there is a high need for a working plan (WP), in order to provide the data for evidence-based and data-grounded decision making. Decision making should be a shared process which includes clinicians and the patient or patient advocates.

SMA NBS is not available anywhere in Europe at the moment, but there are NBS general trials for all babies born in a particular area: Germany (Munich area), Belgium (French-speaking area) and Italy (in two regions).

Some countries where NBS trials are ongoing or a full agreement will be reached:

- The Netherlands for all new-borns (full agreement included in the Guthrie test),
- France (Bordeaux and Strasbourg areas)
- Spain (Barcelona)

It is imperative that clinicians, patient advocacy groups, health care authorities and the community as a whole, work to promote:

- NBS in all European countries
- Education and awareness of SMA
- Reduction of diagnostic delays
- Enable proactive treatments for SMA patients

WHAT WILL THE IMPACT OF NBS BE?

On the community?

For the evolution of phenotypes?

For the parent?

How will they make their choices?

Is the choice not to treat acceptable?

Will they understand that there is no cure?

Will they have the adequate state of mind to make a decision?

How can they be helped?

How many visits?

Differential diagnosis

A number of inherited **motor neuron** diseases occur that are caused by mutations in genes other than the *SMN1* gene. They are referred to as non-5q-SMA diseases. As with 5q SMA, people affected also have early muscle weakness but with some features that differ from 5q SMA. They are heterogenous in distribution of weakness (predominantly proximal or distal, or bulbar) with other features like early contractures, diaphragmatic paralysis with respiratory failure, cerebellar degeneration, vocal paralysis, bulbar palsy and epilepsy. Most of these diseases can be diagnosed with **DNA testing**. Inheritance could be **autosomal dominant**, **autosomal recessive** or X-linked.

Several of these disorders need to be considered in the differential diagnosis of SMA.

SMA type I: Spinal muscular atrophy with respiratory distress type 1 (SMARD1) as a distal **autosomal recessive** disease, congenital SMA with contractures with lower limb predominance (SPSMA; HMSN2C), SMA with lower limbs predominance, early-onset (SMALED), Brown-Vialetto-van Laere syndrome (BVVLS), Fazio-Londe disease with bulbar palsy and all X-linked recessive diseases (Bulbo - SMA, Kennedy disease - SBMA/SMAX1, Infantile SMA with **arthrogryposis** - SMAX2 and Distal SMA, X-linked-SMAX3).

SMA type II and III: congenital neuropathies, congenital myopathies or muscular dystrophies, myasthenic syndromes, metabolic myopathies, SMA with progressive myoclonic epilepsy and juvenile amyotrophic lateral sclerosis (ALS).

6. Clinical presentation and classification

The clinical presentation, age of onset, degree of symptoms and rate of progression, varies greatly across SMA types and between patients. Differences are generally based on the number of copies of the *SMN2* back-up gene. Although this inverse correlation has been well established, there are exceptions. Three copies of the *SMN2* gene are occasionally found in type I patients, and two copies in some milder type II or type III patients. It depends on the **carrier** status and sequence variants in the *SMN2* gene, as well as on other modifying factors of disease severity previously described. There is huge variability in the clinical presentation of patients within the historical classification of SMA by types, depending on the age of onset of symptoms and the physical milestones reached and the time to diagnosis.

Patients are not comfortable with this classification and the consequential treatment decisions based on it. This is because the types do not reflect the severity of the disease in individuals.

SMA **phenotypes** vary within the same classification as do *SMN2* copy numbers. Although *SMN2* copy number correlates inversely with SMA phenotype severity, as stated in the literature, it is not true in every case and this correlation is far from predictive. SMA is a progressive disease across the spectrum of phenotypes and shortens life expectancy.

SMA is a spectrum disease. Disease classification should therefore be based on actual mobility (severity) status and not on artificial cuts or types assigned at the time of presentation, as it is in the current “historical” classification.

Treatment decisions should not be based on the current classification as there is no scientific evidence to suggest that a response to a treatment would be constant within and across types. Treatment decisions based on types would leave some pre-symptomatic populations without access, as it would be necessary to wait for symptoms to appear in order to classify them.

In SMA, muscle weakness can cause several complications ranging from respiratory, musculoskeletal and nutritional. As the disease progresses, patients can experience symptoms across systems as the degree of muscle weakness increases and the level of function decreases.

SMA type I (severe type) or Werding-Hoffmann disease

Infants with type I present symptoms in the first 6 months of life. They are usually normal at birth and the first symptoms are typically tongue **fasciculations**, generalised hypotonia, “frog-leg” posture in supine position, slip through on vertical suspension and lag of the head with attempts to pull infant in a sitting position and areflexia. These infants have no antigravity movements in their legs, with limited distal movements of the arms. They are unable to roll over and never achieve the ability to sit independently (“non-sitters”). They do not achieve motor skills beyond those which are present at the time of diagnosis. Jug-handle posture of the arms with flexion contractures and internal rotation could be frequently seen. Motor decline overall function is predictable, sometimes with a brief plateau phase. A case with sudden overnight paralysis with no concerns when put to sleep at the age of 6 weeks was reported in clinical practice by Viktor Dubowitz. The remarkable feature was acute, rapid onset followed by a fairly steady state with no obvious loss of any residual function. Marked deterioration at a time following an acute respiratory infection, leaves more severe and general weakness including in the face.

The term “non-sitter” also refers to the functional status of an individual throughout their life, not just to what motor function is achieved or present at the time of diagnosis. An individual’s functional status may change during the natural history and progression of the disease, so that a person who could sit independently (“sitter”) initially, might lose this ability and, become a “non-sitter”. This is of particular importance in the implementation of the standards of care, which should be tailored to each patient individually, according to their current functional status rather than the status at time of diagnosis.

Bulbar dysfunction, if not already present at the time of diagnosis (week suck), typically evolves within a few months after birth. In the first year of life, feeding difficulties appear with the involvement of the lower cranial nerves. The feeding is prolonged, there is difficulty in swallowing, failure to gain weight, leading to **aspiration** pneumonia. Supplemental feeding with a nasogastric tube or a gastrostomy is an option available to support infants with these limitations. Other cranial nerves are spared. Mild facial diplegia and rarely, late impairments in extraocular movements can be seen.

Respiratory problems, if not already present at birth or time of diagnosis, typically evolves after feeding becomes compromised. Abdominal breathing with “bell-shaped” chest with very little expansion, is a hallmark of severe types. The cough is weak. Pronounced sweating is present, especially during the night. Supportive care options for these symptoms include: nasal and oral suctioning, chest percussion, cough assist device to increase lung expansion (or AMBU-balloon) and mobilise secretion, and non-invasive ventilation. Severe cases have other organ involvement which are recognised as cardiac, vascular and metabolic defects being the most frequent ones.

Estimated life expectancy in severe cases is under the age of 2, but with more supportive care, the life span is longer (Oskoui, et al. 2007).

Type IA – sometimes referred to as type 0, occurs in infants with the onset of symptoms during the first week of life or noted prenatally. They die soon after birth, in less than 1 month. This is the most severe end of the spectrum of SMA, where patients typically have 1 copy of the *SMN2* gene. These cases need ventilator support at birth and show with facial weakness, general weakness and marked contractures of the limbs. In the most severe cases, death could happen *in utero*. This type may be characterised by diminished foetal movements in the third trimester, as reported by mothers. These cases have normal foetal movements in the first and second trimester on ultrasound, even in those who were born with *in utero* contractures and **arthrogryposis** due to poor in utero movements in the third trimester.

Type IB – patients develop symptoms between one week and three months of age. They do not need ventilatory support at birth but have severe weakness and a weak cry.

Type IC – for these patients, symptoms begin between 3 and 6 months of age. They may not need nutritional and respiratory support, but they never achieve the ability to sit independently.

Most infants with type IB and IC have 2 *SMN2* gene copies, but some patients with type 1C have 3 copies.

SMA type II (intermediate type) or Dubowitz disease

Symptoms onset is usually between 6 and 18 months of age, but some can develop symptoms before 6 months. These types of patients achieve the ability to sit without support but cannot get into a sitting position without assistance, roll, crawl or stay, but are never able to walk independently (“sitters”). These children present with proximal weakness affecting legs more than arms, hypotonia and areflexia. Some children have preserved distal reflexes (brachioradialis and Achilles tendons) in the early phase. A clinical feature frequently observed is a fine tremor of the hands. Tongue **fasciculations** may be present, but not universal. Clinical signs in the early phase also involve joints laxity, delay in meeting a motor milestone, loss of already achieved milestones or failing to meet milestones entirely. Many of the intermediate cases are static, with no obvious deterioration at a particular point. Pregnancy and respiratory infections could be the trigger for motor deterioration. The weakness of the muscles leads to the contractures, leading to deformity and rigidity of the joint and pain. **Ankylosis** of the mandible could develop. Problems with the spine (scoliosis) and hips (subluxation and dysplasia) are frequently present, are progressive and contribute to the further loss of functionality. Bones become porous and may break easily. Adults with type II often develop severe scoliosis and contracture with extremely limited mobility necessitating surgery, 24 hours help from caregivers, aids, and adaptive technology to perform basic daily activities.

The term “sitter” refers, not just to a person who could sit independently at the time of diagnosis, but also to a person who has been able to walk (“walker”) but lost this ability over time. Similarly, the functional status of an individual may be altered by a new therapy, so that a person who could not sit (“non-sitter”) independently, becomes able to sit independently (“sitter”). This is of particular importance in the implementation of standards of care, which should be tailored to each patient individually according to their current functional status rather than the one present at the time of diagnosis.

Most maintain the ability to feed orally, although more severely affected children have fatigue when chewing meals and some even need nutritional support. Bulbar function and cognitive functions are preserved.

They develop progressive intercostal weakness, resulting in restrictive lung disease. Some have difficulty breathing and coughing and may require respiratory support (airway clearance and non-invasive ventilation). **Body mass index** may be low, which represents reduced muscle mass and higher relative fat mass index. Those children are overweight as they are non-ambulatory patients.

In contrast to severe types, these patients have a survival rate of 98,5% at 5 years and 68,5% at 25 years, but many live longer due to improved supportive care (Zerres, et al. 1997). In patients with respiratory compromise, the life span is shortened.

Type IIA – those children could sit but lose the ability to sit independently over the time

Type IIB – those children maintain the ability to sit independently

Most of the children with SMA type II have 3 copies of the *SMN2* gene, but some with 2 copies or 4 copies have been seen.

SMA type III (mild type) or Kugelberg-Welander disease

Symptoms onset is usually between 18 months and adulthood. These patients can stand and walk independently, although many of them later lose these abilities. Children present with proximal muscle weakness, affecting their legs more than their arms. The first symptoms are usually abnormal gait when trying to compensate for weakness (particularly of the hips and thighs), climbing stairs, walking or running. Tendon reflexes are often preserved at the time of presentation, making the clinical diagnosis more challenging. Over time reflexes are lost and they also exhibit a fine tremor of the fingers, as seen in type II children. Progressive weakness can lead to loss of ambulation, while others may remain ambulatory into adolescence or adulthood. With the help of supportive devices, many are able to continue to walk despite their weakness.

As this group of patients initially had the ability to walk, they are called “walkers”. With the advent of new therapies and early diagnosis, a “walker” can also be a person who was not able to walk on his own but started walking due to the applied therapy. Likewise, a person who has lost the ability to walk due to progression of the disease becomes a “sitter”. This is of particular importance in the implementation of standards of care, which should be tailored to each patient individually according to their current functional status rather than the one present at the time of diagnosis.

Problems with the spine may occur at various rates and ages. Bones become weak, joint aches, fatigue sets in and foot deformity in ambulatory patients can be seen.

Swallowing and coughing problems, along with breathing difficulty at night, may occur later in the disease course, but are less commonly seen.

Sometimes the calves of these patients can be prominent and confused with Becker muscular dystrophy. Creatine kinase levels are often increased, no more than fivefold and may lead to wrong myopathy evaluation. EMG and nerve conduction testing show that the disorder is neurogenic versus myopathic (Munsat & Davies, 1992).

Type IIIA – symptoms onset occurs before 3 years of age

Type IIIB – symptoms onset occurs after 3 years of age

Most of the patients with SMA type III have 3 or 4 copies of the *SMN2* gene, but cases with 5 copies are also described.

SMA type IV (the mildest form)

Symptoms onset occurs after 21 years of age. The mean age is 30 years and, these patients present with proximal limb weakness. The clinical course is slowly progressive with a small proportion of patients losing ambulation within 20 years of symptoms, onset. **Fasciculations** and muscle cramps are present in some patients. Deformities, bulbar involvement and respiratory problems are rare. This is the least prevalent subtype with a normal life expectancy. Most of the patients have 3 or 4 copies of the *SMN2* gene.

Clinical Classification

The clinical classification most widely used is based on the age of symptoms, onset and the highest physical milestones achieved, with overlapping age of onset between groups. SMA is classified into three groups, based on the severity of the disease (severe, intermediate and mild), reflecting clinical criteria of disease that focus on the ability to sit, stand and walk. This classification system was established in 1992 at the International SMA consortium in Germany (Munsat & Davies, 1992)(**Table 1**).

Table 1. SMA Types

SMA TYPE	Age of Onset	Highest Motor Milestone	SMN2 Copy Number	Lifespan
IA	<1 week	Never sits	1	<1 month
IB	1 week - 3 months	Never sits	2,3	<2 years
IC	3 - 6 months	Never sits	2,3	<2 years
IIA	6 - 15 months	Sits independently Loses the ability to sit	2,3,4	>2 years
IIB	6 - 15 months	Sits independently Maintains the ability to sit	2,3,4	>2 years
IIIA	<3 years	Walks independently	3,4	Adult
IIIB	>3 years	Walks independently	3,4	Adult
IV	>21 years	Walks independently	4,5	Adult

This classification does not take into account a variety of manifestations within each of the groups, since there is a spectrum of severity in each of them. There are long-lived patients with type I who have never been able to sit on their own, but with better respiratory function and swallowing (milder form inside the type I group or borderline cases between type I and type II group). There has been much debate about the appropriate classification system for SMA. A classification system based on a continuous, rather than a discrete variable (e.g. 1,8) in cases of less severely affected type I patients has been proposed by Dubowitz, in order to better capture the clinical spectrum of disease (Dubowitz, 1995).

The approach is based on a numerical basis with decimals, to better reflect clinical presentation in each of the sub-groups. The Examiner needs to identify first the middle of the range and then complete the rest.

The proposed system has 4 types:

Type 1 - heavy with variations (1.1 - 1.9)

- 1.1 - Severe paralysis at birth with respiratory failure, bulbar paralysis and extremely negative prognosis of survival.
- 1.5 - Floppy baby from early infancy, mainly abdominal breathing but no spontaneous respiratory difficulty. No problem with feeding and breathing at the time of diagnosis.
- 1.9 - The child is almost able to sit independently but not quite, has no difficulty in feeding and has a better respiratory function at the time of diagnosis.

Type 2 - intermediate with variations (2.1 - 2.9)

- 2.1 - The child is barely able to maintain the sitting position.
- 2.5 - Sitting with stability and good posture, but cannot support his/her weight standing.
- 2.7 - Cannot stand or walk alone, but is good at sitting, and can develop the ability to walk with the aid of crutches.
- 2.9 - The patient has the ability to sit in a stable fashion or stand with the support, or independently if of a certain weight but not for a long time.

Type 3 - soft with variation (3.1 - 3.9)

3.1 - The patient can stand alone and take a few steps but is unstable.

3.5 - The patient has steady ambulation.

3.9 - The patient is on the verge of normal (although may not be able to walk fast, to hop on one leg or to run).

Type 4 - normal

This classification could be of use when conducting a clinical trial for assessing an actual improvement in function.

Before the advent of molecular diagnosis, SMA was classified into discrete subtypes, as described above; however, it is now apparent that the phenotype of SMA associated with *SMN1* pathogenic variants spans a broad continuum without clear delineation of subtypes. Newly approved treatment options are changing the natural history of SMA phenotypes and blurring the boundaries even further (Tizzano & Finkel, 2017).

Care for patients with SMA should be tailored according to their current functional status rather than the original classification of SMA types. Therefore, a classification of current functional level in the form of non-sitters, sitters, and walkers should be used. The non-sitters include the group of children who currently are not able to sit independently. The sitters include those who can sit independently but cannot walk independently. The walkers can walk independently. (Prior, Leach & Finanger, 2000; Standards of Care for Spinal Muscular Atrophy, 2018)

7. Therapeutic strategies and drug treatment

With significant advances in basic research and clinical development programmes in SMA, as well as with the FDA and EMA approved treatment for SMA - Nusinersen (Spinraza™, onasemnogene abeparvovec Zolgensma and Risdiplam - Evrysdi™) important insights into SMA pathophysiology have been obtained. New therapeutic targets, other than the *SMN2* genes have been evaluated. Despite this significant progress, there are still challenging aspects defining the window for optional therapeutic intervention, which cell types and organs need to be targeted other than MNs and what additional approaches can be used (regenerative therapy, stem cell therapy etc.)

Temporal requirements for SMN protein

For the successful treatment of SMA, it is very important to know when the *SMN* protein is needed to prevent disease development and what organs or systems need this protein for proper homeostasis. The realisation for the temporal requirements for *SMN* protein came from the fact that the onset of the disease occurs predominately during the paediatric age, that complete absence of *SMN1* and *SMN2* protein is embryonically lethal and that some aspects of SMA pathology begin prenatally with critical phases in the time of rapid neuromuscular development.

An early requirement for *SMN* protein was evaluated in severe SMA animal models. Restoring protein levels in the early postnatal period in a mice model (1-4 days postnatal) was effective at preventing the onset of the disease. The treated mutants survived beyond 250 days of age, about a 16-fold increase in survival. Augmenting the protein after 10 days postnatally, failed to deliver any benefit. Delaying for a day, greatly diminished benefit (Foust, et al. 2010; Robbins, Glascock, Osman, Miller & Lorson, 2014). Other studies investigated the temporal requirement for *SMN* protein in animal models. Restoring protein at day 4 postnatally proved remarkably effective, while at day 6 and after (post symptomatically), restoring *SMN* protein levels was not so effective. This was not so much a consequence of motor neuron loss, as a result of motor neuron dysfunction reflected in morphological defects of the NMJ (Lutz, et al. 2011). It seems that *SMN* protein deficit first impacts the distal end of the motor unit, which is dependent on SMA protein concentrations to develop its mature, adult state.

Studies also suggest that the requirement for the *SMN* protein wanes once the NMJs have fully developed and adulthood is reached (Kariya, et al. 2014). The results strongly indicate that the relative maturity of the neuromuscular synapse determines the temporal requirements for the *SMN* protein. If that is the case, of particular relevance is the fact that timely reinstatement of the *SMN* protein may halt the progression of the disease and serve as an effective post-symptomatic therapeutic strategy. In an intermediate SMA mice model, post-symptomatic treatment had effects as late as day 25 postnatally from the restoration of *SMN* protein levels (Feng, et al. 2016). This implies that patients symptomatic for mild forms of SMA will derive benefit from the timely restoration of *SMN* protein.

The major conclusions from animal studies are:

- Early requirements for *SMN* protein are crucial. Restoring the levels of *SMN* protein in the early postnatal period, even in the more severe cases results in optimal clinical outcome.
- It may be possible to reverse disease phenotype.
- if *SMN* is restored in a timely manner. The precise window of opportunity in humans still needs to be determined because of the relatively small number of studies on SMA type II and III patients.

Recent clinical trials of *SMN*-inducing drugs Nusinersen and scAAV9-*SMN* demonstrate a range of clinical efficacy, with the time of treatment initiation playing a critical role in the magnitude of clinical response. Temporal requirements and therapeutic benefit of early treatment, before symptom onset, were demonstrated in two clinical trials on presymptomatic babies with 2 and 3 *SMN2* copies. In data from presymptomatic SMA infants (15 with 2 *SMN2* copies and 10 *SMN2* copy number = 3), treatment with Nusinersen was initiated at less than 6 weeks of life, and after a median of 2.9 years of treatment, 100% of children sat and 88% walked independently (De Vivo, et al. 2019). In a phase III trial of Nusinersen, symptomatic infants with 2 copies of *SMN2* dosed starting at an average age of 5.4 months showed reduced mortality, and 51% demonstrated improvements in motor function compared with 0% of infants in the control group, with 8% sitting independently at study completion (Finkel, et al. 2017). Together, these trials powerfully illustrate that a delay of *SMN* induction of weeks or months can substantially reduce achievement of motor milestones.

Target cells for *SMN* protein restoration

The primary target cells in SMA therapy approaches are MNs, because they express markedly lower levels of full-length *SMN* protein from the *SMN2* gene (in the absence of the *SMN1* gene), than other cell populations in the spinal cord (Ruggiu, et al. 2012). *SMN* reduction in cells types other than MNs also contributes to SMA pathogenesis.

Increasing *SMN* protein levels improves deficits and loss of sensory-motor synapses, evaluated by electrophysiological measurements (H-reflex) (Martinez, et al. 2012). Studies on the potential contribution of intrinsic skeletal muscle abnormalities to the SMA phenotype are controversial (see Muscle pathology in SMA). Astrocyte dysfunction may also contribute to SMA pathology as well as intrinsic defects in Schwann cells (peripheral nerve pathology) (Hunter, et al. 2014).

Studies aimed at increasing IGF-1 through peripheral administration of a drug, have shown improvements in neuromuscular pathology in mice models and **phenotype** rescue. This occurred through both direct IGF1 AVV1 delivery (Tsai, et al. 2014) or by ASO administration (Hua, et al. 2011), in order to upregulate *SMN2* full-length protein production and increase liver production of IGF-1. On the other hand, AAV9-SMN1 vectors delivered intravenously led to widespread *SMN* expression in the CNS and the periphery (liver and muscles) with increase in median survival in mice from 15 to 100 days (Dominguez, et al. 2011), but CNS restricted *SMN* expression achieved comparable phenotypic rescue (Glascok, Shababi, Wetz, Krogman & Lorson, 2012).

For optimal clinical efficacy, therapeutic restoration should occur in the whole nervous system and perhaps other peripheral organs (especially in the muscles and the liver). Because of methodological and species differences in studies, more research is needed before organ and cell type specific requirements for *SMN* protein can be fully defined.

Therapeutic approaches

Beside *SMN*-dependent therapeutic strategies, SMA drug development programmes also attack SMA from multiple other angles. Other downstream targets are currently being explored, so if one approach fails, several others might come to light, combined therapies in particular (**Figure 5**).

This gives patients and families a great reason for hope, especially for patients whose disease progression is slow but who retain significant motor deficits.

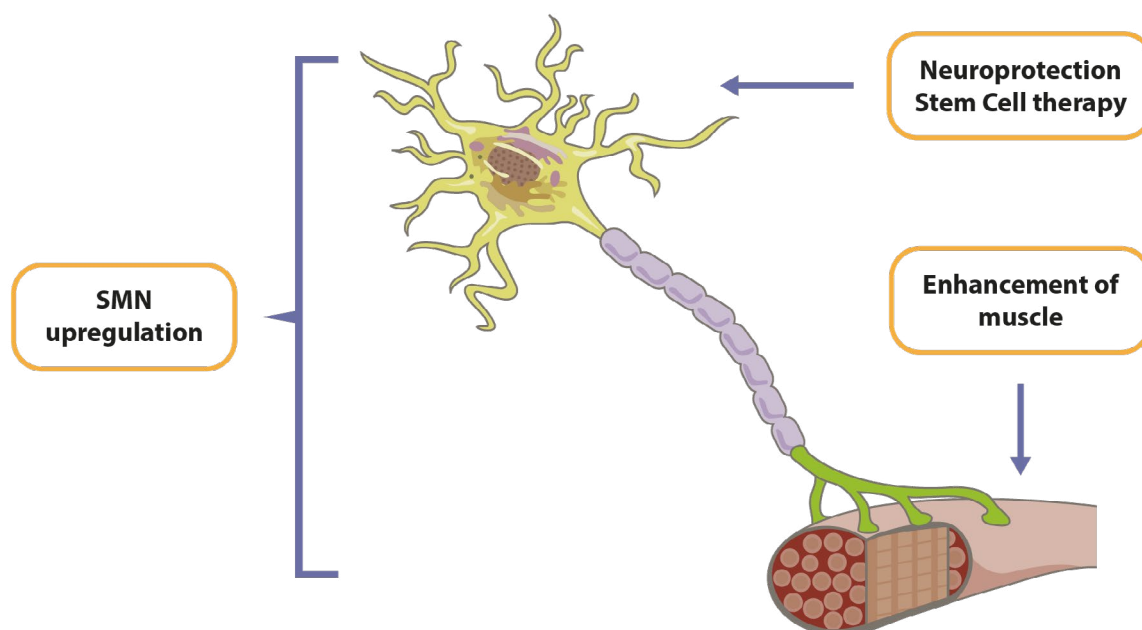


Figure 5. Treatment strategies for spinal muscular atrophy (SMA).

An overview of diverse strategies in therapeutic approaches are given in Table 2.

Table 2. Various approaches for SMA drug development

Therapeutic Approach	Examples
Upregulation of SMN protein	
<i>Increasing SMN2 Transcription</i>	HDAC inhibitors (VPA, PHA, SAHA, TSA) Other: prolactine, HU, quinazoline/RG3039, lncRNA oligos
<i>Correcting SMN2 splicing</i>	Antisense oligonucleotides (ASO) Small molecules (PTKSMA1, RG7800, LMI070)
<i>Stabilising SMN Transcript</i>	Celecoxib
<i>Increasing translation to SMN Transcript</i>	Indoprofene, Amynoglicosides
<i>Stabilising SMN protein</i>	BIP 135
<i>SMN Gene Therapy</i>	scAAV9SMN, AVXS-101
Neuroprotection	Olesoxime, Gabapentin, Riluzol
Enhancement of Muscle	Myostatin inhibitors, albuterol, SARMs, exercise, fast troponin activators (Tirasemtiv and CK-2127107, TWEAK inhibitors)
Stem Cell Therapy	Embryonic stem cells derived neural stem cells, human embryonic stem cells derived MN progenitors (hMNP)
Targeting modifiers	ROCK inhibitors (Fasudil)
Combined therapies	ASO or gene therapy in combination with muscle enhancers or neuroprotective

HDAC - histone deacetylase inhibitors, **VPA** - valproic acid, **PHA** - phenylbutyrate, **SAHA** - suberoylanilide hydroxamic acid, **TSA** - trichostatin, **SARM** - selective androgen receptor modulators, **TWEAK-TNF** - like weak inducer of apoptosis, **ROCK** - rho-associated protein kinase

Drug treatment (SMA Drug Pipeline)

Approved drug treatments

Currently there are two approved therapies for SMA in Europe:

- Antisense oligonucleotide - Spinraza™ (Nusinersen) that works by correcting the *SMN2* gene splicing. Spinraza™ was approved on December 23rd, 2016 by FDA (<https://www.sma-europe.eu/news/spinraza-nusinersen-approved-by-the-fda/>) and on June 1st 2017. by EMA (<https://www.ema.europa.eu/en/medicines/human/EPAR/spinraza>).

- Gene therapy - AVXS-101 which works by replacing the function of the missing or nonworking *SMN1* gene with a new, working copy. This gene therapy was approved on May 24th by FDA (<https://www.novartis.com/news/media-releases/avexis-receives-fda-approval-zolgensma-first-and-only-gene-therapy-pediatric-patients-spinal-muscular-atrophy-sma>) and on May 19th by EMA (<https://avexis.com/news-releases/news-release-details/avexis-receives-ec-approval-for-zolgensma.html>).

Survival of motor neuron 2 (*SMN2*) splicing modifier - Evrysdi (Risdiplam) - approved on August 7th by FDA.

Experimental drug treatments

Currently, as with all scientific research, it is difficult to predict which SMA drug programmes might be successful.

The SMA drug pipeline identifies possible treatment targets:

- SMN2 splicing modulation
- Gene replacement therapy
- Muscle targets
- Others

An updated version of the SMA Drug Pipeline (September 2020) is given in **Table 3**.

For further information on current treatment options see (Waldrop & Kolb, 2019).

For further information about Clinical Drug Development, please visit the following link:

<https://www.curesma.org/2019-researcher-meeting-summary4/>

About SMA Europe

SMA Europe campaigns to improve the quality of life of people who live with SMA, to bring effective therapies to patients in a timely and sustainable way and encourages optimal patient care.

We do this through different initiatives and activities:

Supporting research:

- SMA Europe funds research into the cause and treatment of SMA. Through our projects, we aim to promote and sustain scientific and medical research in SMA. SMA Europe runs a bi-annual call for research projects, which typically opens in February every other year. This Call aims to address different areas of SMA research.

We are supported by a Scientific Advisory Board (SAB), composed of neuroscientists and neurologists with particular expertise in spinal muscular atrophy research. The SAB identifies current and future priorities and through reviewing our grant applications, ensure that only the best science is funded.

Since 2008, SMA Europe has funded or committed to fund just over €5M to SMA research, spanning 31 projects.

- SMA Europe promotes research communication and collaboration through its scientific and clinical congresses on SMA

Advocating:

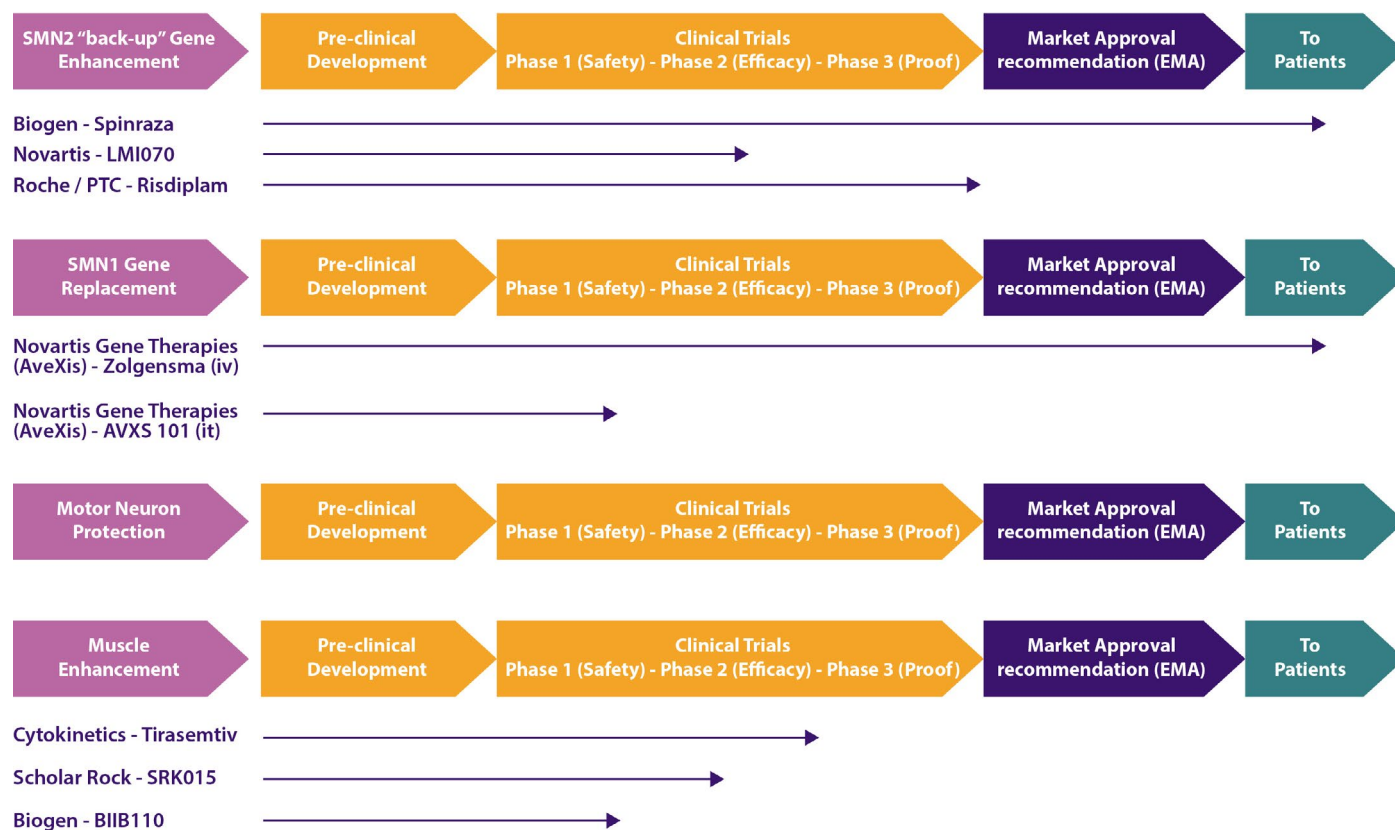
- We inform on patients' expectations
- We inform on the burden of living with the disease
- We inform on the potential economic and societal benefits of stabilising or improving health conditions of the SMA population
- Through our Patient Advisory Groups (PAGs), we provide important information that will inform the development of drugs which have the most meaningful effects to patients
- We advocate for new-born screening to be adopted by every country in Europe by 2025

For information please visit the SMA Europe web site <https://www.sma-europe.eu/>.

Table 3. SMA Drug Pipeline

SMA Drug Pipeline September 2020

By Treatment Target



ASO - antisense oligonucleotide; **GT** - gene therapy; **CNS** - central nervous system; **IV** - intravenous; **IT** - intrathecal; **SM** - small molecule

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NOTE: The quotes in orange bubbles represent patients' advocacy comments.

Glossary of medical terms

A

Ankylosis fixation and immobility of a joint.

Apoptosis (PCD) one of the mechanisms by which cell death occurs. Apoptosis is the mechanism responsible for the physiological deletion of cells and appears to be intrinsically programmed. It is characterized by distinctive morphologic changes in the nucleus and cytoplasm, chromatin cleavage at regularly spaced sites, and the endonucleolytic cleavage of genomic DNA at internucleosomal sites. This mode of cell death serves as a balance to mitosis in regulating the size of animal tissues and in mediating pathologic processes associated with different diseases.

Arthrogryposis persistent flexure or contracture of a joint.

Assay is an analysis done to determine the presence of a substance and the amount of that substance. Also, it can be performed to test the biological or pharmacological potency of a drug.

Aspiration is the inhalation of food into the lungs while eating

Astrocytes a class of large neuroglial (macroglial) cells in the central nervous system - the largest and most numerous neuroglial cells in the brain and spinal cord. Astrocytes (from "star" cells) are irregularly shaped with many long processes, including those with "end feet" which form the glial (limiting) membrane and directly and indirectly contribute to the blood-brain barrier. They regulate the extracellular ionic and chemical environment, and "reactive astrocytes" (along with microglia) respond to injury.

ATP adenosine-triphosphate, an adenine nucleotide containing three phosphate groups esterified to the sugar moiety. In addition to its crucial roles in metabolism adenosine triphosphate is a neurotransmitter.

Autonomic nervous system the enteric nervous system; parasympathetic nervous system; and sympathetic nervous system taken together. Generally speaking, the autonomic nervous system regulates the internal environment during both peaceful activity and physical or emotional stress.

Autophagy the segregation and degradation of cytoplasmic constituents by autophagosomes and their digestion by lysosomes. It plays an important role in biological metamorphosis and in the removal of bone by osteoclasts. Defective autophagy is associated with various diseases, including neurodegenerative diseases and cancer.

Autosomal dominant inheritance is an autosomal dominant disorder, the mutated gene is a dominant gene located on one of the non-sex chromosomes (autosomes). You need only one mutated gene to be affected by this type of disorder. A person with an autosomal dominant disorder - in this case, the father - has a 50% chance of having an affected child with one mutated gene (dominant gene) and a 50% chance of having an unaffected child with two normal genes (recessive genes).

Autosomal recessive mode of disease inheritance requires two copies of the mutated gene for the condition to develop. In SMA, the child who is affected by SMA inherits two copies of a mutated gene, one copy from each parent; while not typically affected by SMA, each parent carries one copy of the mutated SMA gene.

Axonal transport the directed transport of organelles and molecules along nerve cell axons. Transport can be anterograde (from the cell body) or retrograde (toward the cell body).

Axonogenesis the formation of new axons

B

Body mass index (BMI) is a measure of body fat based on height and weight that applies to adult men and women. The BMI is defined as the body mass divided by the square of the body height in meters

C

Carrier is a healthy individual who is not at risk of developing the disease but has a risk of passing the gene mutation to his or her offspring.

Chromosome in a prokaryotic cell or in the nucleus of a eukaryotic cell, is a structure consisting of or containing

DNA which carries the genetic information essential to the cell.

Crossing-over the reciprocal exchange of segments at corresponding positions along pairs of homologous chromosomes by symmetrical breakage and crosswise re-joining forming cross-over sites (holiday junctions) that are resolved during chromosome segregation. Crossing-over typically occurs during meiosis .

Cytokines proteins secreted by inflammatory leukocytes and some non-leukocytic cells, that act as intercellular mediators. They differ from classical hormones in that they are produced by a number of tissue or cell types rather than by specialized glands. They generally act locally.

Cytoskeleton the network of microfilaments, microtubules, and intermediary filaments which give shape, structure, and organization to the cytoplasm.

D

Denervation loss of nerve supply. Causes of denervation include disease, chemical toxicity, physical injury, or intentional surgical interruption of a nerve.

DNA a deoxyribonucleotide polymer that is the primary genetic material of all cells. Eukaryotic and prokaryotic organisms normally contain DNA in a double-stranded state, yet several important biological processes transiently involve single-stranded regions. DNA forms a double helix that is held together by hydrogen bonds between purines and pyrimidines (adenine to thymine and guanine to cytosine).

E

ECG electrocardiography, recording of the moment-to-moment electromotive forces of the heart as projected onto various sites on the body's surface, delineated as a scalar function of time. The recording is monitored by a tracing on slow moving chart paper or by observing it on a cardioscope, which is a cathode ray tube display.

Electromyography (EMG) is an electrodiagnostic technique for evaluating and recording the electrical activity produced by skeletal muscles. EMG is performed using an instrument called an electromyograph. An electromyograph detects the electric potential generated by muscle cells when these cells are electrically or neurologically activated.

Embryogenesis morphological and physiological development of embryos

Exon is a coding portion of DNA sequence

F

Fasciculations involuntary contraction of the muscle fibers innervated by a motor unit. Fasciculations may be visualized as a muscle twitch or dimpling under the skin, but usually do not generate sufficient force to move a limb. They may represent a benign condition or occur as a manifestation of motor neuron disease or peripheral nervous system diseases.

Fibrosis any pathological condition where fibrous connective tissue invades any organ, usually as a consequence of inflammation or other injury.

G

Gene conversion the asymmetrical segregation of genes during replication which leads to the production of non-reciprocal recombinant strands and the apparent conversion of one allele into another.

Gene expression the phenotypic manifestation of a gene or genes by the processes of genetic transcription and genetic translation.

Gene locus specific regions that are mapped within a genome. Genetic loci are usually identified with a shorthand notation that indicates the chromosome number and the position of a specific band along the P or Q arm of the chromosome where they are found.

Genotype all or part of the genetic constitution of an individual or group

Glia the non-neuronal cells of the nervous system. They not only provide physical support, but also respond to injury, regulate the ionic and chemical composition of the extracellular milieu, participate in the blood-brain barrier and blood-retinal barrier, form the myelin insulation of nervous pathways, guide neuronal migration during development, and exchange metabolites with neurons. Neuroglia have high-affinity transmitter uptake systems,

voltage-dependent and transmitter-gated ion channels, and can release transmitters, but their role in signalling (as in many other functions) is unclear.

Glucagon is pancreatic peptide and secreted by pancreatic alpha cells and plays an important role in regulation of blood glucose concentration, ketone metabolism, and several other biochemical and physiological processes.

H

Haplotypes the genetic constitution of individuals with respect to one member of a pair of allelic genes or sets of genes that are closely linked and tend to be inherited together such as those of the major histocompatibility complex.

HCP Health Care Professionals

Heterozygous are individuals, which have one faulty and one functioning copy of the specific gene

Homozygous deletion homozygous (biallelic) deletion refers to the loss of both alleles identified by allele-specific analysis in the clinical samples

H-reflex (or Hoffmann's reflex) is a refractory reaction of muscles after electrical stimulation of sensory fibers in their innervating nerves.

I

Incidence the rate of occurrence of new cases of a particular disease in a population being studied

Intron is a noncoding portion of DNA sequence

K

Ketonuria is a condition characterized by an abnormally elevated concentration of ketone bodies in the blood (acetonemia) or urine (acetonuria). It is a sign of diabetes complication, starvation, alcoholism or a mitochondrial metabolic disturbance.

L

Lower motor neurons (MNs) these cells extend from the spinal cord or brainstem to skeletal muscle. Lower motor neurons are responsible for all voluntary movement. Types of lower motor neurons include alpha motor neurons, beta motor neurons, and gamma motor neurons

M

Meiosis is a type of cell nucleus division, occurring during maturation of the germ cells. Two successive cell nucleus divisions following a single chromosome duplication (S phase) result in daughter cells with half the number of chromosomes as the parent cells.

Microdeletion loss of a tiny piece that may be too small to be seen readily through a microscope from a chromosome. Microdeletions can be detected via high-resolution chromosome banding, molecular chromosome analysis (with FISH), or DNA analysis.

Microglia the third type of glial cell, along with astrocytes and oligodendrocytes (which together form the macroglia). Microglia clearly are capable of phagocytosis and play an important role in a wide spectrum of neuropathologies. They have also been suggested to act in several other roles including in secretion (e.g., of cytokines and neural growth factors), in immunological processing (e.g., antigen presentation), and in central nervous system development and remodelling.

Motor neurons (MNs) neurons in ventral horn of spinal cord which activate muscle cells.

mRNA is an RNA sequences that serve as templates for protein synthesis. Eukaryotic mRNA is synthesized in the nucleus and must be exported to the cytoplasm for translation.

Muscle satellite cells are elongated, spindle-shaped, quiescent myoblasts lying in close contact with adult skeletal muscle. They are thought to play a role in muscle repair and regeneration.

Muscle spindles are skeletal muscle structures that function as the mechanoreceptors responsible for the stretch

or myotactic reflex (reflex, stretch). They are composed of a bundle of encapsulated skeletal muscle fibers, i.e., the intrafusal fibers (nuclear bag 1 fibers, nuclear bag 2 fibers, and nuclear chain fibers) innervated by sensory neurons.

Myosin is a diverse superfamily of proteins that function as translocating proteins. They share the common characteristics of being able to bind ACTINS and hydrolyze MgATP. Myosins generally consist of heavy chains which are involved in locomotion, and light chains which are involved in regulation

Myostatin is a growth differentiation factor that is a potent inhibitor of skeletal muscle growth. It may play a role in the regulation of myogenesis and in muscle maintenance during adulthood.

Myotubes are large, multinucleate single cells, either cylindrical or prismatic in shape, that form the basic unit of skeletal muscle. They consist of myofibrils enclosed within and attached to the sarcolemma.

N

Neurofilaments cytoplasmic filaments intermediate in diameter (about 10 nanometers) between the microfilaments and the microtubules. They may be composed of any of a number of different proteins and form a ring around the cell nucleus.

Neurotransmission the communication from a neuron to a target (neuron, muscle, or secretory cell) across a synapse. In chemical synaptic transmission, the presynaptic neuron releases a neurotransmitter that diffuses across the synaptic cleft and binds to specific synaptic receptors, activating them. The activated receptors modulate specific ion channels and/or second-messenger systems in the postsynaptic cell. In electrical synaptic transmission, electrical signals are communicated as an ionic current flow across electrical synapses.

NMJ (neuromuscular junction) the synapse between a neuron and a muscle.

Nuchal translucency is a prenatal ultrasonography measurement of the soft tissue behind the foetal neck.

Nucleotide is the monomeric units from which DNA or RNA polymers are constructed. They consist of a purine or pyrimidine base, a pentose sugar, and a phosphate group.

P

PCR test - Polymerase Chain Reaction, in vitro method for producing large amounts of specific DNA or RNA fragments of defined length and sequence from small amounts of short oligonucleotide flanking sequences (primers). The reaction is efficient, specific, and extremely sensitive.

Phenotype - the observable properties of an organism that are produced by the interaction of the genotype and the environment

Point mutation is a mutation caused by the substitution of one nucleotide for another. This results in the DNA molecule having a change in a single base pair.

Prevalence - the percentage of a population that is affected with a particular disease at a given time

Proprioception is a sensory function that transduce stimuli received by proprioceptive receptors in joints, tendons, muscles, and the inner ear into neural impulses to be transmitted to the central nervous system. Proprioception provides sense of stationary positions and movements of one's body parts and is important in maintaining kinesthesia and postural balance.

S

Sensitivity is a binary classification to assess test results. Sensitivity or recall rate is the proportion of true positives.

Specificity is the probability of correctly determining the absence of a condition.

Splicing (RNA) the ultimate exclusion of nonsense sequences or intervening sequences (introns) before the final RNA transcript is sent to the cytoplasm.

Synaptic vesicle (SV) membrane-bound compartments which contain transmitter molecules. Synaptic vesicles are concentrated at presynaptic terminals. They actively sequester transmitter molecules from the cytoplasm. In at least some synapses, transmitter release occurs by fusion of these vesicles with the presynaptic membrane, followed by exocytosis of their contents.

T

Telomeric region is a terminal section of a chromosome which has a specialized structure and which is involved in chromosomal replication and stability.

Trophic factors are helper molecules that allow a neuron to develop and maintain connections with its neighbours are called trophic factors. These small proteins work through their receptors on the surface of the nerve cells.

Transcription is the first step of DNA based gene expression (gene is a short part of DNA that encodes for a protein), in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzyme RNA polymerase. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

Translation refers to the process of creating proteins from an mRNA template. The sequence of nucleotides on the RNA is translated into the amino acid sequence of proteins and this reaction is carried out by ribosomes.

V

Ventral horn one of three central columns of the spinal cord. It is composed of grey matter.

