Briefing Document to the Clinical Trial Readiness in Spinal Muscular Atrophy (SMA) SMA Europe, TREAT-NMD and European Medicines Agency meeting

November 11, 2016, EMA, London, UK

The advent of clinical trials in spinal muscular atrophy has highlighted a number of challenges, such as the lack of natural history data, the lack of validated functional outcome measures and surrogate endpoints and biomarkers.

During the past 5 years, work has been ongoing to address these issues and substantial progress has been made. In light of this, SMA Europe and TREAT-NMD have organized a workshop with the European Medicines Agency (EMA) in an open forum setting (November 11, 2016, EMA, London). The workshop, which will serve to exchange knowledge and perspectives among the different parties involved, has the following objectives:

1) to present the EMA with data that have been collected by academia, industry and patient advocacy groups over the past few years related pre-clinical and clinical research studies and to discuss the relevance and meaningfulness of the data for the evaluation of the ongoing therapeutic approaches in the treatment of SMA.

2) to accelerate the elaboration of regulatory guidelines on medicinal products for the treatment of SMA.

A constructive dialogue on these objectives will help the neuromuscular community, especially patients and families, to plan for the future. **This document was created by SMA Europe and TREAT-NMD to serve as a tool facilitating the discussion during the event.**

Setting the scene

1.1 Definition of SMA

Spinal muscular atrophy (SMA) is one of the most common autosomal recessive inherited disorders in the human population. The term SMA is applied to a number of different conditions affecting predominantly the lower motor neurons, and multiple genes responsible for different SMA subtypes have been identified. However, for the scope of this document, we will concentrate on the most common form of SMA, an autosomal recessive disease linked to mutations (generally deletions) in the *SMN1* gene on chromosome 5q13.

1.2 SMA carrier frequency, incidence and prevalence

The prevalence of SMA carriers as determined by quantitative analysis of *SMN1* copies differs among ethnicities: Hutterites 1:8, Iranian population 1:16, Saudi Arabian and Egyptian population 1:20, Caucasian population 1:35, Asian population 1:50, Mexican population 1:105, and Malians 1:209 [1-6]. The *de facto* carrier frequency is even slightly higher due to various genetic constellations that are not identified by *SMN1* quantitative analysis such as *SMN1* point mutations (1.7% of carriers) and two *SMN1* copies per chromosome and null on the second chromosome 5 (2.4% of carriers) [1, 7]. Accordingly, the incidence of SMA has to vary between 1:256 in Hutterites to 1:175,000 in Malians. In Europe, the incidence is approximately 1:6000. All epidemiological studies on SMA prevalence have been carried out before the era of *SMN1* genetic testing, and therefore may include different non-5q13 SMA forms and have to be treated cautiously. However, based on the gene carrier frequency and on the current mean age of survival for individuals with SMA 1, 2 and 3, we can estimate that in the EU the cumulative prevalence should be between 21,000 and 25,000 affected individuals.

1.3 SMA classification

SMA is very heterogeneous with respect to age of onset, achieved motoric abilities, survival, mode of inheritance, affected muscles and progression of the disease [8].

As mentioned, in this document we specifically concentrate on the most frequent form, which is the autosomal recessive proximal spinal muscular atrophy (SMA) caused by mutations in *SMN1*, localized on chromosome 5q13 [9]. This accounts for about 80-90% of all forms of SMA.

The classification of SMA is traditionally based on clinical features including age at presenting

symptoms and maximal functional abilities achieved[10]. SMA is now known to represent a continuum in phenotypes of one genetically defined but clinically heterogeneous disease, ranging from very weak young infants who never achieve independent sitting (type I), to older infants who sit but never walk (type II), and to ambulant children (type III) and adults, (type IV)[11].

Type I SMA (acute form, Werdnig-Hoffmann disease, MIM #253300) is the most common and severe form accounting for more than 50% of all SMA cases, with severe generalized muscle weakness and hypotonia ("floppy infant"). The disease onset is within the first six months of life and these children are never able to sit. Survival is limited as type I infants without intensive supportive care usually die within the first 2 years[12] .

Type II SMA (intermediate form, MIM #253550) patients are able to sit but never able to walk unaided and usually present first symptoms after the first six months of life. While originally considered to be a condition associated with poor outcome already in childhood and adolescence[13], recent studies clearly indicate that with improved standard of care survival in adulthood is expected[14] [15-17].

Type III SMA (juvenile SMA, Kugelberg-Welander disease, MIM #253400) patients are able to sit and walk, and the lifespan is not reduced [13].

Type IV SMA (adult form, MIM #271150) patients are comparatively mildly affected with an age of onset later than 30 years; they have a normal life expectancy [13, 18]. This is a very rare SMA variant.

Type 0 has recently been reported with prenatal onset, contractures at birth and severe neonatal respiratory and motor impairment[19].

Table 1. *Classification of SMA based upon typical age of onset and maximal motor function achieved. Subtypes for types 1 and 3 are listed. The most common number of copies of the SMN2 gene and typical life expectancy for each type of SMA is highlighted.*

Type	Age at Symptom Onset		Incidence %	Prevalence %	Maximum Motor Function	SMN2 copy number	Life expectancy
0		Fetal	<1	0	Nil	**1**	Days -Weeks
1	< 6 Months	1A: B-2 Weeks 1B: <3 Months 1C: >3 Months	60	15	Never sits	1, **2**,3	< 2 years
2	6-18 Months		25	70	Never walks	2,**3**,4	20-40 years
3	1.5-10 Years	3A: <3 Years 3B: > 3 Years	15	15	Walks, regression	**3, 4**, 5	normal
4	>35 Years		<1	1	Slow decline	**4, 5**	normal

1.4 SMA diagnosis and genetic mechanism

The diagnosis of SMA is based on molecular genetic testing. SMA is caused in approximately 96% by homozygous absence of exons 7 and 8, or only of exon 7 of the *SMN1* gene [9, 20-24]. Among SMA patients with homozygous deletions of *SMN1*, about 90% reveal homozygous absence of both exons 7 and 8, while about 10% show only homozygous absence of exon 7 [24]. The genetic mechanism causing absence of *SMN1* is either deletion of *SMN1* or gene conversion of *SMN1* into *SMN2*. The region of conversion can encompass the complete *SMN* gene as well as only a part of it [9, 25, 26]. The majority of patients inherit the *SMN1* deletion from their parents. However, in 2% of SMA patients *de novo SMN1* deletion or gene conversion is observed [27]. In 3-4%, other mutations in *SMN1* can be found, most often with an *SMN1* deletion on the other allele [7, 20, 21, 24, 28-36]. These mutations can be more subtle, with more than 50 different mutations identified so far, many of them being missense mutations that disturb the proper SMN protein function [30, 37, 38]. The compound heterozygous constellation is found in approximately 3.4% of SMA patients[7].

Only few SMA patients worldwide, whose parents are consanguineous, have homozygous *SMN1* mutations [39, 40].

All SMA patients carry at least one *SMN2* copy gene. *SMN2* differs from *SMN1* by only 5 base pairs: one in each exon 7 and 8, one in intron 6 and two in intron 7 [41]. The genetic testing is based on the differences in the exons 7 and 8.

Based on this uniform mutation spectrum, genetic testing of *SMN1/SMN2* is straightforward and highly reliable. The gold standard of SMA genetic testing is quantitative analysis of *SMN1* and *SMN2* using either MLPA or quantitative genomic PCR [1, 42, 43]. Homozygous *SMN1* deletions can be identified also by PCR followed by restriction digest with Dra I, which cuts *SMN2* or Hinf I, which cuts *SMN1* for exon 7 and Dde I, which cuts *SMN2* for exon 8 [7, 44]. However, this method is unable to quantify the *SMN1* or *SMN2* gene products.

The number of copies of the *SMN2* gene is the major factor influencing the severity of the SMA phenotype [1, 20, 45, 46]. This usually varies between one and four, but can rarely reach up to eight copies. The underlying mechanism generating an increased number of copies of *SMN2* and a reduction or absence of *SMN1* is gene conversion [9, 25, 26]. The *SMN2* gene produces SMN transcripts, which are processed properly (FL-SMN2) for only 10%, while the other transcripts lack exon 7 (SMN2Δ7)[47]. Since each *SMN2* copy produces approximately 10% of FL-SMN2 transcripts, an increased number of *SMN2* leads to an increase in the absolute amount of FL-SMN2 transcripts, allowing production of more full-length SMN protein and thus influencing the severity of SMA [46, 48].

The majority of type I SMA patients carry two *SMN2* copies, type II SMA patients three *SMN2* copies, type IIIa SMA patients (age of onset before 3 years) three *SMN2* copies, type IIIb SMA patients (age of onset after 3 years) four *SMN2* copies, and type IV four to six *SMN2* copies (Table 1 and Figure 1) [1, 45]. Analysis of FL-SMN2 versus SMN2Δ7 transcripts of type I-III SMA patients showed a ratio of about 20:80 in type I, 30:70 in type II, and 40:60 in type III SMA patients [48]. On protein level, substantial differences are found especially when comparing type I and II SMA patients with type III patients and controls [48-50]. Individuals carrying five or six *SMN2* copies develop very mild SMA symptoms (type IV SMA)[45], whereas eight *SMN2* copies fully protect from developing SMA[51]. Similar phenotypic differences have been observed in transgenic SMA mice carrying two to eight copies of the human *SMN2* gene on a *Smn*-knockout background [52, 53].

Since *SMN2* copies strongly correlate with the severity of the disease and since for many therapies the inclusion criteria are based on *SMN2* copies, knowledge about the number of *SMN2* copies is very important.

Presence of *SMN1* but homozygous absence of *SMN2*, a genotype found in about 3-5% of control individuals, has no apparent phenotypic consequences [9]. The presence of at least one fully functional *SMN1* gene is sufficient to protect from SMA.
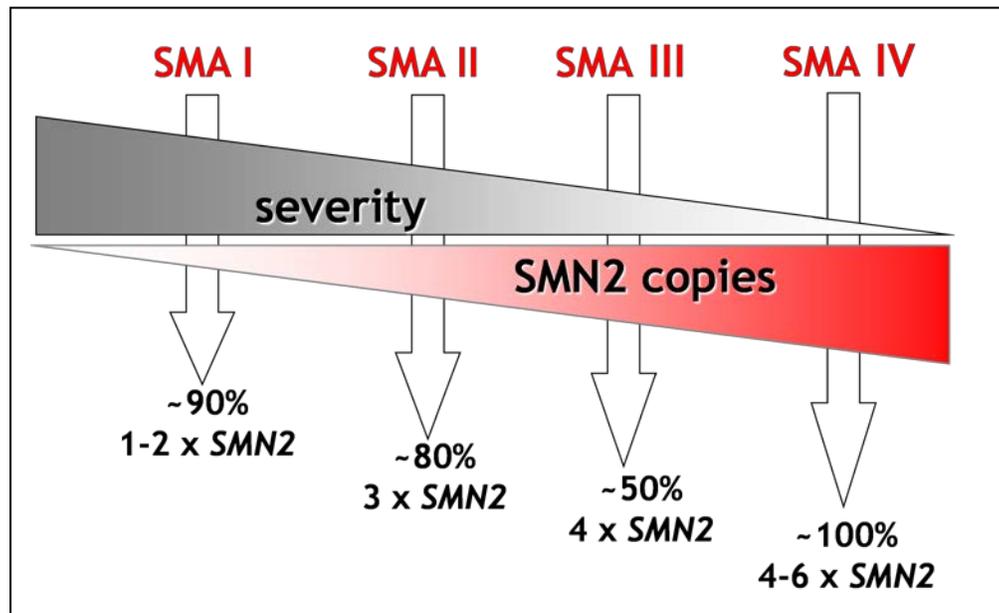

Figure 1. Correlation between SMA severity and SMN2 copy number

1.4.2 Alternative splicing of SMN2

SMN1 produces mainly full-length transcripts and protein, termed survival motor neuron (SMN). As mentioned, the *SMN2* copy gene produces instead mainly transcripts that lack exon 7 (Δ7SMN2) and only about 10% of full-length transcripts (FL-SMN2) [9, 48]. While the short version encodes a truncated and unstable protein, the FL-*SMN2* transcripts generate a protein that is identical to the one produced by *SMN1* [37]. The overall amount of SMN generated from *SMN2* copies in patients with less than 8 copies is insufficient to compensate for the *SMN1* loss. Nonetheless, *SMN2* is a major target for a number of current therapies (reviewed in [54]).

The recognition of *SMN* exon 7 and surrounding introns by the splice machinery is heavily regulated by a large number of exonic and intronic cis regulatory splice elements and trans-regulatory splicing factors (Figure 2) (reviewed in [55])

A C→T transition in *SMN2* exon 7 disturbs an exonic splicing enhancer (ESE)[56-58]. Together with an intronic splicing silencer in intron 7 (ISS-N1), the disruption of this ESE results in the removal of exon 7 during the splicing process in the majority of transcripts (Δ7SMN2). However, exon 7 includes another ESE that is responsible for the 10% of correctly spliced, full-length transcripts [59]. The ISS-N1 silencer in intron 7 is recognized by the splicing factor hnRNP-A1 [60]. If this motif is blocked by antisense oligonucleotides (SMN-ASOs), the binding of hnRNP-A1 to ISS-N1 is prevented, and exon 7 is better recognized by the splicing machinery, thus resulting in elevated levels of full-length transcripts *in vitro* and *in vivo* [61]. There are additional domains in intron 6 and intron 7 that can be blocked by ASOs and proved to increase exon 7 inclusion into *SMN2* mRNA [62, 63]. These observations have guided translational therapeutic strategies, discussed below.
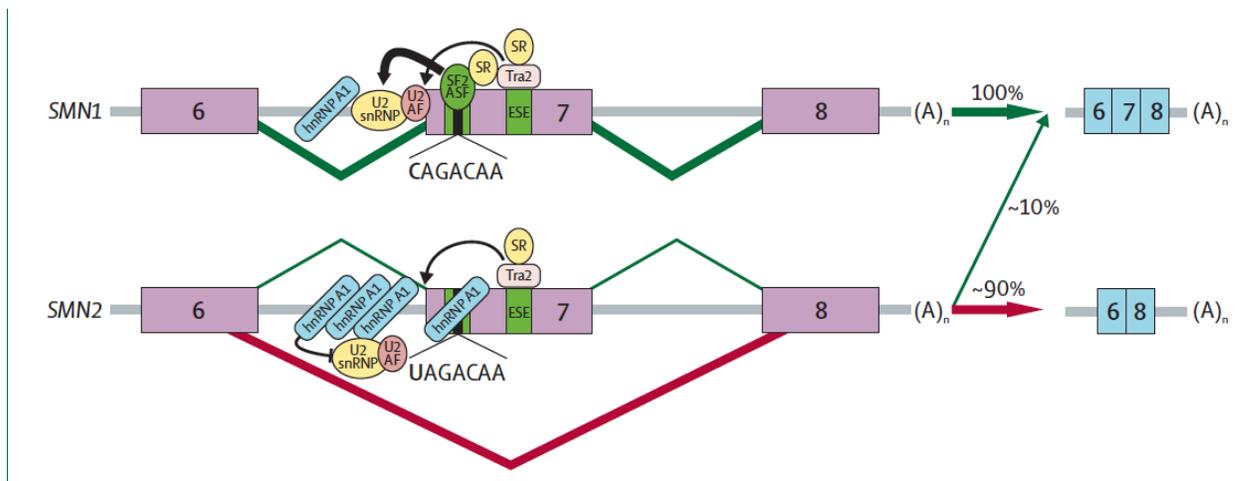

Figure 2. *Cis and trans regulation of SMN1 and SMN2 splicing and its impact on translation*

Schematic overview of splicing processes of the SMN exon 7 and its consequences on RNA- and protein level (from Lunn and Wang[64].)

1.5 Biomarkers in SMA

Biomarkers have been developed for SMA and have been used in different ways. These are summarized in table 2, with examples presented for the different categories of biomarkers[65]. This has been reviewed recently by Arnold et al[65].

Table 2: *Types of Biomarkers*

Type of Biomarker	Definition	Actual or Potential Examples in SMA	Validation Dependent Upon Effective Therapy?
Prognostic	Predicts a future clinical outcome	*SMN2* copy number	No
		→ Disease severity	
Disease Progression	Identifies the severity of disease impact	Compound muscle action potential amplitude	No
		→ Motor neuron loss	
		SMA-MAP panel	
Predictive	Predicts a future clinical response to therapy and helps stratify therapies	Reduced CMAP amplitude → Less response to SMN restoring therapies	Yes
Pharmacodynamic	Monitors or quantifies a therapeutic effect	Increased full-length SMN transcripts	Yes
		Increased SMN protein	
		→Effective induction of *SMN2* gene	

Compound Muscle Action Potential (CMAP), Motor Unit Number Estimation (MUNE), SMA-MAP panel of biomarkers developed by the SMA-Foundation which correlate with the Hammersmith motor function scale.

1.5.1 SMN2 copy number

While the *SMN2* copy number works well for cohorts of patients, it cannot be used as a definitive prognostic marker for an individual patient. Figure 1 shows the correlation of *SMN2* copy number for three clinically well classified populations. It can be noted that there is correlation, but there are also exceptions. For example, the occurrence of SMA type I cases with 3 copies of *SMN2* instead of the expected 2. In general, these cases clearly have a progression at the milder end of the clinical spectrum of typical type I cases, but nevertheless with an earlier onset and lower functional achievements than type II SMA[66-68]. It is also notable that there are cases with type II SMA that have two copies of *SMN2*. It should be emphasized that the number of *SMN2* copy number exceptions for type I and II SMA is rare in well characterized populations, 5-10% of type II

cases have 2 copies of *SMN2*. It is not unlikely that in some type I cases with additional copies of *SMN2,* the *SMN2* gene does not produce functional transcripts, e.g. due to a mutation[1].

1.5.2. Other modifiers of the SMN2 gene.

In addition to the number of copies of the *SMN2* gene, there is also a variant reported for *SMN2* that has been associated with a milder disease course. The variant SMNG859C has a frequency of 0.00348 in *SMN2* genes in the ExAC data base (http://exac.broadinstitute.org/). This variant has never been reported in a type I SMA, only in type II and III cases, suggesting a protective effect. This variant is more common in the Spanish population, where it accounts for approximately 50% of SMA II cases with a milder phenotype and 2 copies of *SMN2*. While the frequency may be lower in other populations, in a small sample in the USA, 3 of 7 milder patients were accounted for by this variant[69]. Notably, when using *SMN2* copy number as a prognostic marker, there are no exceptions to the phenotype in type I cases diagnosed before 6 months: all have 2 copies of *SMN2.* Thus a type I trial performed with patients presenting with muscle weakness before 6 months of age and 2 copies of *SMN2* will not include type II or III patients

While the clear majority of type II patients have 3 copies of *SMN2*, a considerable number (50%) of type III patients also have 3 copies of *SMN2*. When type III patients are stratified according to the severity of their phenotype, patients with 3 copies have an earlier onset and lower muscle function scores than those with 4 copies. Using age of onset and 3 copies of *SMN2,* a type II patient population can be defined very reliably for clinical trials.

There are very rare families where siblings have very different phenotypes but identical copy numbers of *SMN2* (usually 3 or 4)[20, 67, 70-72]. This indicates the presence of genetic modifiers acting in trans. Increased expression of PLS3 has been reported as a modifier in female patients[72]. Due to their rarity, these patients are not anticipated to have an impact on the overall natural history in the SMA population, and stratification for clinical trials is probably not needed.

1.5.3 Disease Progression SMA-MAP panel microRNA and IGF1

A panel of serum/plasma analytes correlating with SMA motor function scores and other outcome measures (muscle strength, electrophysiology, pulmonary function and quality of life) was developed using unbiased metabolomics and proteomic screening platforms. These data are publically available[73, 74] and can be analysed in multiplex ("SMA-MAP" test profile). In SMA mouse

models a subset of 10 of these markers can be used. Upon ASO treatment that increased the amount of SMN protein, 5 markers responded to treatment [75]. In SMA mouse models, IGF1 protein levels are reduced, but normalized upon ASO mediated increases in SMN protein[76]. The SMA-MAP panel has been evaluated in the NeuroNEXT trial natural history trial of type I SMA patients[65], with data pending analysis. Further validation of the SMA-MAP biomarker panel will be possible using longitudinal data that are currently obtained.

1.5.4. miRNAs

Lastly, miRNAs have been evaluated. miR-132 levels are increased in SMA patients and mouse models. Levels correlate with motor function scores in SMA patients[77], and normalized upon ASO treatment in SMA mice.

Response to treatment has not been tested for any of the markers in humans. Thus, these biomarkers remain exploratory endpoints at present.

1.5.5. *Electrophysiology as Prognostic and Disease Progression Biomarkers and potential predictive marker (Adapted from Arnold et al[75])*

Early descriptions of electromyography (EMG) findings in cohorts of SMA revealed a direct correlation between clinical severity and severity of electrophysiological motor unit loss[78, 79]. Critically, the compound motor action potential (CMAP) is a functional measure of the output from the motor neuron to the muscle or group of muscles being assessed. Most of the clinical SMA research has utilized CMAP recordings from ulnar innervated hand muscle (adductor digiti minimi). Unfortunately, bulbar and more proximal limb CMAP responses have not been closely investigated in SMA patients despite the proximal predominance of the disease. Nevertheless, ulnar CMAP from the abductor digiti minimi muscles has shown good potential as a prognostic biomarker in several different cohorts of SMA patients with good correlation with age, clinical severity and function [80-82].

Motor unit number estimation (MUNE) is a technique that allows assessment of the number of functional motor neurons/axons innervating a particular muscle or group of muscles [83]. In contrast to CMAP, which is commonly utilized in clinical diagnosis and care, MUNE is usually restricted to research purposes. In comparison to the CMAP, MUNE is more technically demanding. Several variations of MUNE technique have been used to estimate motor unit

numbers from the ulnar innervated abductor digiti minimi in SMA patient populations and these have all shown similar features of motor unit loss [80, 82, 84, 85]. In multicenter trials, it has proven difficult to use MUNE and to obtain reliable measures between centers. Thus, it is best used at a single site with the required expertise.

One aspect of SMA that is particularly curious is the inconstant progression of the disease. Overt motor deficits are often lacking prior to the rapid loss of motor function in patients with SMA, and following this rapid progression there may be periods of relative stability of function [80, 86-89]. Electrophysiological prognostic biomarkers including ulnar CMAP and MUNE have provided insight in that at least some patients appear to have a presymptomatic phase prior to rapid onset of motor unit degeneration [80, 87]. How the onset and rate of motor unit degeneration compare across severities of SMA remains uncertain.

1.5.6. *SMN protein and full length mRNA as a pharmacodynamic marker* (adapted from [65])

There are a large number of studies looking at the SMN protein or amount of full length SMN as a biomarker for SMA. SMN protein levels have been measured patients' lymphoblasts or fibroblasts[49, 50] and shown to correlate with the phenotype. Notably, it is critical to have the cells in the same growth state when doing these comparisons. Blood and white blood cells are more readily available. However, SMN protein levels in blood have not been shown to correlate well with clinical severity [90-93], thus precluding the use of SMN levels in blood as a stratification method. Very sensitive SMN protein measures in cerebrospinal fluid (CSF) have been used to study SMN levels before and after treatment in a small trial with nusinersen (ASO aiming to increase SMN protein levels)[94]. Using this method, SMN levels in blood or CSF (depending on the route of administration and biodistribution of the drug) could be used as a pharmacodynamic marker to confirm mechanism of action of SMN protein increasing therapies.

The amount of FL-SMN mRNA has also been measured in blood samples. While there is a clear difference in SMN transcript levels between type I SMA cases and controls[43, 90, 95-98], there seems to be extreme variability in SMN transcript levels in human white blood cells, with a lack of correlation to *SMN2* copy number in patients. Absolute quantification of SMN transcripts in the largest cohort studied to date showed that *SMN2* copy number strongly correlated with SMA severity, but was not a predictor of the amount of FL-SMN transcripts present in these cells. Transcript levels did not seem to be influenced by age or sex, but there was a high degree of inter-

individual variability, hindering the detection of very strong differences between SMA types. [43] Age, years from symptomatic onset or lean body mass in adult SMA patients did not correlate with FL-SMN transcripts levels; however, an association between FL-SMN levels and total or lower limb strength, using the Medical Research Council (UK) manual muscle testing scale, was observed in ambulant adult patients [96]. Overall, however, FL-SMN expression in blood does not correlate well with clinical severity of disease in SMA [43, 90, 93]. Consistent with this, there were no correlations between FL-SMN mRNA levels in the blood and age or motor function in a recent multicenter, natural history study of SMA infants less than 6 months of age using digital droplet PCR. However, FL-SMN mRNA levels were significantly lower than age-matched healthy controls[92]. While the level of FL-SMN is not useful for stratifying patients, measuring increases compared to baseline can be used in individual patients to confirm drug action for compounds aiming to increase exon 7 inclusion. [99].

1.5.7. *Considerations for therapeutic intervention*

While we are not sure of all aspects of response to therapy in man there are some key components of the basic biology that are important to consider. In SMA mouse models early introduction of SMN is clearly of greater benefit than late[100, 101]. In pigs a model of SMA has been created by knocking down pig SMN with a shRNA delivered by scAAV9[102]. The pig SMA model develops the classic electrophysiological defects found in SMA with decreased CMAP and MUNE and fibrillations due to denervation[102]. Introduction of therapy using scAAV9-humanSMN to restore SMN levels via CNS delivery had a remarkable impact. Early introduction prior to symptoms resulted in complete rescue whereas latter introduction in the symptomatic phase resulted in rescue of the motor neuron remaining[102].

In type I SMA analysis of pre-symptomatic individuals has shown that CMAP is relatively high before symptoms start but rapidly declines with onset[103, 104]. This suggests that also in humans, treatment before the onset of symptoms is expected to result in a larger therapeutic effect. Taken together a retained CMAP that is reasonably measurable in a type I case can represent that sufficient motor neurons are present to respond to an SMN inducing therapy and thus can be predictive of a response or the degree of response. This could also be true in milder SMA cases.

1.6 Experimental therapies for Spinal Muscular Atrophy.

A number of different approaches are under study in SMA, and, broadly speaking, these can be divided into experimental therapies focused on 1. increasing the level of SMN protein; 2. improving outcome irrespective of the primary SMN protein levels. Approaches with stem cells have also been experimented and will be briefly discussed.

1.6.1 Experimental therapies aiming at increasing SMN protein levels

A major focus of therapeutic developments in the last few years has been on increasing the full-length SMN protein by enhancing *SMN2* gene expression, increasing the inclusion of exon 7 in SMN2 transcripts, stabilizing the SMN protein, or replacing the *SMN1* gene with viral gene therapy. Ongoing clinical trials include a. antisense oligonucleotides, b. small-molecule drugs and c. gene therapy.

a) Antisense oligonucleotides (ASO). Splice switching ASO are synthetic RNA molecules that interfere with physiological splicing of exons. As all SMA patients carry at least one copy of *SMN2*, in which a single nucleotide change determines an exclusion of exon 7 in most of its transcripts, an attractive approach is to manipulate SMN2 splicing by ASOs so that exon 7 is retained in the mature transcript, and in this respect produce a mRNA and eventually a protein which is identical to the one produced by *SMN1*. These ASOs are highly effective at promoting inclusion of exon 7 in SMN2 transcripts and at increasing SMN protein levels both in vitro and in vivo, although they are not capable to cross the blood brain barrier. This requires repeated intrathecal administration in SMA patients in order to maintain adequate SMN protein level. One of such ASO, nursinersen, has been studied in the last few years in a comprehensive program of open label and randomised placebo controlled studies in type I and in patients with SMA type II and non-ambulant type III. Published data on a phase 1 study in 28 patients with type II and type III SMA aged 2–14 years demonstrated safety of 4 ascending doses and pharmacokinetics indicative of a prolonged CSF drug half-life of 4–6 months. In terms of exploratory efficacy, there was a significant increase in the functional Hammersmith Functional Motor Scale Expanded scale HFMSE in children receiving the highest ASO doses both at 3 months and at 14 months post doses. (Neurology® 2016;86:1–8). Data from a phase 2 study in infants with type I SMA presented at international meetings

continued to indicate safety and tolerability of nursinersen, with both respiratory and motor milestones demonstrating significant divergence from natural history of the condition. Specifically, not only the ventilation free survival of treated infants was significantly divergent from the natural history, but the majority of treated infants improved in their CHOP-INTEND functional scores (this is a remarkable finding as no child with SMA I ever demonstrates acquisition of novel milestones) and acquired independent sitting and in a few instances also standing. Very recently, the top line result of a randomised, placebo controlled study in type I SMA (Endear study) were announced by Biogen. Eighty children received the active compound while 41 received a sham injection. The study had a prespecified interim analysis after ~80 individuals had had the opportunity to be assessed at day 180 visit. The interim demonstrated a significant improvement in the proportion of nursinersen treated motor milestones responders vs sham procedure control, and in view of these encouraging results, the placebo controlled part of the study was interrupted and all patients are currently transitioning to an open label extension study as has been presented at the World Muscle Society Conference 2016. In addition, a study treating pre-symptomatic, genetically confirmed infants with nusinsersen is in progress. This open label study plans to recruit 25 infants and have both respiratory and functional endpoints assessment. So far, 20 infants have been dosed with the nursinersen, and the longest period of recruitment is 13 months. None of the treated children has reached the respiratory endpoint of the study, and a proportion has achieved sitting independently, standing and even walking. As this is a population of young infants, at the time of writing not all have reached the age at which acquisition of the sitting or walking milestone is expected. Nevertheless, the divergence of this group of children both from the respiratory perspective and the acquisition of new motor milestones and important function is certainly encouraging. At the time of writing, Ionis and Biogen have initiated an Extended Access Programme for infants with type SMA.

b) **Viral Gene therapy**. As a monogenic disease, SMA is a good target for vector-based gene replacement therapy to restore a normal form of the *SMN1* gene in patients. Viral-mediated *SMN* gene delivery has been remarkably successful in preclinical studies after intrathecal or intravenous viral vector administration. Indeed both systemic and intracerebroventricular injection of self-complementary adeno-associated viral vectors (scAAV) expressing SMN1 showed efficient transduction of motor neurons in both mice and non-human primates, as

well as nearly complete correction of the SMA phenotype in mice. In selecting a potential vector to deliver the *SMN1* gene, adeno-associated virus vectors (AAV) 9 appeared to be an excellent contender due its ability to cross the blood–brain barrier through the vascular system in mouse models. AveXis is currently conducting in US (Columbus University, Dr Jerry Mendell) the first gene therapy phase 1 clinical trial to assess the safety of multidose intravenous delivery of scAAV9-SMN in type I SMA infants. Two doses of AAV9 have been used so far in an ongoing phase 1 clinical trial. Children receiving the low dose of AAV9 remained stable and did not meet the respiratory endpoints of the trial. The results in the 12 children recruited into the higher dose cohorts indicated that all had improved their functional abilities, 2/3 of them had achieved the ability to sit unassisted and two patients achieved independent walking. These data are certainly encouraging also considering that not all children have currently achieved the age at which ambulation would be expected. Another evocative finding from this phase 1 study, which appears to echo the findings in the presymptomatic nursinersen trial, is that the children who were younger and with a shorter interval between diagnosis and initiation of therapy appear to have responded in a more robust way, a findings that is similar to the findings in the SMA mouse models. Further data from the nursinersen and the Avexis clinical trials will provide useful information on whether there is an optimal window for response to these interventions in SMA1 children

c) **Small molecules.** A number of low-molecular-weight drugs were in the past found to modestly increase levels of full-length SMN protein by activating the SMN2 promoter, increasing its expression, or altering the splicing pattern of SMN2 transcripts, so as to favour the inclusion of exon 7. The first generation of these drugs (for example histone deacetylase inhibitors) were tested in clinical trials, but showed little or no benefit. More recently, a number of orally bioavailable compounds were identified by high throughput screening to increase very substantially the level of SMN2 exon 7 inclusion in cells from patients from SMA and extend substantially the life expectancy of affected SMA mice. Roche is involved in the clinical development of 2 of these compounds (one, RG7800 was discontinued from the phase 1 clinical trial following some safety signals from chronic toxicology studies; while for the other one, RG7916 clinical trials recently started). Similarly, Novartis also identified a small molecule capable of affecting SMN2 gene splicing, LMI070, but also this phase 1 study

was put on hold due to some unexpected findings in the chronic preclinical toxicology studies.

1.6.2. Other therapeutic approaches

Neuroprotective compounds. Olesoxime is another small molecule that has showed neuroprotective properties in a number of in-vitro and in-vivo studies promoting neurite outgrowth and communication with the mitochondrial permeability transition pore.

This compound has been tested in a phase 2 randomized, multicenter, double blind, placebo-controlled trial completed in 2013. A total of 165 non-ambulant patients with SMA type II and III, aged 3 to 25 years, were studied for two years. The randomization ratio was 2:1 (108 in the Olesoxime group (10mg/kg), and 57 in the placebo group). Preliminary results suggested that Olesoxime maintains motor function and improves overall health status over the two-year treatment period, and a larger phase 3 study is being planned by Roche.

A separate approach is being developed by Cytokinetics in collaboration with Astellas. These companies are developing CK-107, a novel skeletal muscle troponin activator as a potential treatment for patients with SMA. The mechanism of action of this drug is the slowing of the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibers. CK-107 is currently in a phase 2 clinical trial, assessing its effect on multiple measures of muscle function in patients with Type II, Type III, or Type IV SMA, and one evaluating its effect on physical function and exercise tolerance in patients with COPD.

1.6.3. Stem cells

One of the goals of transplanted stem cells is to support endogenous motor neurons through the delivery of neuroprotective agents and ideally to also partially restore neuronal and non-neuronal cells.

Despite the positive results of neural stem cell transplantation in mice, its translational value in human is unclear, therefore, alternative protocols, which includes the use of embryonic stem cells or induced pluripotent stem cells for transplantation, have been tried in animal models. A protocol to test neuronal stem cells in SMA patients is currently on hold by the FDA. A controversial approach of allogenic mesenchimal cell transplantation, administered

intravenously and intrathecally, initiated by a private enterprise in Italy, was interrupted in 2014 by a panel of experts appointed by the Italian Ministry of Health, due to both lack of proven efficacy and serious concerns on the quality of the proposed drug as the mesenchymal cells given to patients were not grown under the approved EU strict set of standards.

2. Type I SMA

2.1 Classification

The first report of infantile onset SMA was by Oppenheim (1900), which he termed "amyotonia congenita", yet type I SMA has historically used the eponym "Werdnig-Hoffman disease", recognizing the two physicians who initially described the disease in older children. There has been an evolving process over the past 3 decades not only to classify and divide SMA into the these three most common types I, II and III based upon age of symptom onset and maximal motor function achieved, but also to identify subtypes within each form. SMA type I has been subdivided into types IA (symptom onset at birth to 2 weeks of age, early onset respiratory and feeding difficulty), IB (symptom onset 2 weeks to 3 months, poor head control) and IC (symptom onset generally 3 to 6 months of age, attains head control)[105]. Table 1 summarizes these features.

This classification has been useful for predicting survival and motor function, but only to a moderate degree. As mentioned, the number of copies of the "backup" *SMN2* gene is inversely related, in a general sense, to the severity of the phenotype and can serve as a prognostic biomarker. In general, type IA has a *SMN2* copy number of 1, IB has 2 copies and IC usually has 2 or 3 copies. Classic infantile SMA type I most commonly refers to type IB with 2 copies of the *SMN2* gene and this has the most homogeneous features and predictable course of survival and failure to gain motor skills[68]. Current clinical trials for symptomatic SMA type I have used *SMN2* copy number of 2 as an inclusion criterion, with the aim of enrolling a more homogeneous population with a predictable course.

2.2 Type I SMA: natural history and impact of standards of care on survival and function

2.2.1 Diagnosis

Diagnosis of SMA is usually prompted by referral for evaluation of the "floppy baby", typically between 1 and 4 months of age (range: birth to 8 months).[66, 68, 106] The diagnosis of type I SMA is made on clinical grounds, ultimately dependent upon the demonstration that independent sitting is not achieved after a period of observation. The delay between symptom onset and diagnosis averages 2 to 3 months.[68] While the presentation in a classical case is pretty unequivocal, the clinician can sometimes be challenged at the time of diagnosis in predicting accurately which infant will be type 1 based upon presenting features and laboratory studies.

This includes considering the age at symptom onset, the relative degree of hypotonia and weakness (especially head and neck control, and axial power), the extent of related feeding and breathing impairment, and the number of copies of the *SMN2* gene. Based upon these characteristics one can predict with over 90% accuracy that an infant will be type I, with less than 10% likelihood of achieving sitting, i.e. becoming a type II patient.

Pre-symptomatic infants with SMA can now be identified either by pre- or post-natal testing of infants at risk (parents having a prior affected child with SMA) or from newborn screening. Limited data on these infants have been published.[103, 104] Infants with an *SMN2* copy number of 2 or 3 are typically normal at birth and those with a copy number of 1 may be symptomatic at birth.

2.2.2 Clinical features

Infants with type I SMA typically present with hypotonia and have symmetric proximal > distal weakness in the lower > upper extremities.[107] Examination usually demonstrates tongue fasciculation and an inability to elicit muscle stretch reflexes. Muscle bulk may be reduced at the time of diagnosis. Sensation and sensorium are preserved. Weakness of bulbar muscles leads to fatigue in oral feeding, then progresses to dysphagia and risk of aspiration. Respiratory function is impaired eventually due to intercostal and accessory muscle weakness, with relative preservation of the diaphragm until later in disease process. This leads to the characteristic "bell-shaped" thorax, paradoxical ("see-saw") breathing pattern, and a feeble cough that impairs clearance of oral secretions. Respiratory failure can evolve in an indolent fashion, usually within 6 to 8 months of diagnosis, or can occur precipitously in the context of an acute upper or lower respiratory tract illness.

2.2.3. Survival

Epidemiologic studies of survival in type I SMA, prior to the advent of supportive care opportunities, have demonstrated that the median mortality for these infants was approximately 12 months (Brandt, 1950)[108] with 32% survival beyond 2 years (Zerres,1995)[86]. More recent natural history studies have focused upon a combined survival endpoint of age at death or a surrogate of survival free of permanent ventilation, generally accepted as intubation or tracheostomy with mechanical ventilation or > 16 hours/day non-invasive ventilation (NIV)

support for >14 consecutive days (16+/14+) in the absence of an acute reversible illness or following surgery. That is, the assumption is that the infant would have died without such support and a sufficient time period was allowed to ensure that the infant would not wean to <16 hours/day of NIV support. Table 3 summarizes published retrospective and prospective studies that address survival in type I SMA infants and notes the type of supportive care provided. Clearly, survival and even ventilation-free survival is highly dependent upon the nature and extent of supportive care, which may vary by country, institution and physician preference.[109, 110] Survival at age two years in a more recent German cohort study (2007), when analysed by *SMN2* copy number, was overall alive = 6%, with those with a copy number of 2 = 2% as compared to copy number of 3 = 67%.[66]

Table 3. *Survival data of patients with type I SMA. Selected examples are presented here. A more comprehensive summary is provided in the Appendix.*

Author, year published	Type of study	Age at death: MEDIAN (range, unless otherwise noted)
Oskoui 2007[111] **1980-1994** **1995-2006**	Retrospective Patient registry data (largely US)	 7.3 m (1.0-193.5 m) 10.0 m (2.5-112.0 m)
Cobben 2008 [106]	Retrospective, single site (Netherlands) Palliative care	6 m (95% CI: 5-7 m)
Rudnik-Schoneborn 2009[66] **All patients[b]** **2 copies *SMN2*** **3 copies *SMN2***	Retrospective German epidemiological data Strong nutritional support Variable ventilation support	 6.7 m (0.0-34.0 m) 6.5 m (0.5-30 m) 19.0 (10.1-55.1)
Ge 2012[112]	Retrospective, Chinese, epidemiological data, minimal support	7.0 m
Finkel 2014[68] **Overall** **2 copies *SMN2***	Prospective, multisite, US, combined endpoint[c] Largely proactive	 13.5 m (IQR: 8.1-22 m) 10.5 m (IQR: 8.1-13.6 m)

[b] Age of all patients at death/permanent ventilation.

[c] Age at death or requiring > 16 hours of BiPAP per day

2.2.4 Clinical Course

2.2.4.1 Motor function. Motor function in type I SMA has been studied using motor function scales, the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND)[92, 113] and the Test for Infant Motor Performance Items (TIMPSI)[92, 114] (section 2.2., below). Infants with SMA types IB and IC are normal at birth and present subacutely or precipitously with hypotonia and weakness. This is followed by a predictable overall decline in motor function, sometimes with a brief plateau phase.[68]

More recently, the development of motor milestones has been examined.[115] These infants, by definition, fail to gain independent sitting, and as a rule do not achieve motor skills beyond those which are present at the time of diagnosis. This observation is important to remember when considering the effect of enhanced standard of care and/or the therapeutic effect of a drug intervention.

2.2.4.2. Oral-motor dysfunction. If not already present at the time of diagnosis in type IB, oral-motor dysfunction typically evolves within the next few months (median age 8 months in one study, approximately 3 months prior to the median age of initiation of ventilation support)[68]. Failure to gain weight is often the subtle first sign of oral-motor dysfunction. Dysphagia can also lead to aspiration pneumonia, a potentially fatal condition in type I SMA infants. Supplemental feeding with nasogastric tube or gastrostomy tube placement is an option available to support infants with these limitations.[109, 116]

2.2.4.3. Respiratory insufficiency. If respiratory insufficiency is not already present at the time of diagnosis in type IB, it typically evolves after feeding compromise is present, but may be the heralding event in the face of an acute respiratory illness, leading to hospitalization and initiates the diagnostic effort. Supportive care options for these symptoms includes nasal and oral suctioning, chest percussion to loosen airway secretions, cough assist devices to increase lung expansion and to mobilize secretions, and ventilation support – non-invasive and invasive techniques.[117-121] Administering pulmonary function tests to type I SMA infants is challenging and limited to a research setting. [122, 123]

2.2.5 Impact of Standards of Care

Clinical care for infants and children with SMA has advanced over the past 2 decades and continues to evolve. Newer technologies, such as the cough assist devices, non-invasive ventilation support and gastrostomy tube placement, now offer home based pulmonary and nutritional management that previously was only available in hospital.

A summary of the published SMA type 1 natural history is presented in the appendix.

Figures 4 and 5 present data from a retrospective registry study[111] and compare the difference in Kaplan-Meier survival curves pre-NIV (1980-94) and post NIV (1995-2006), with improved survival beyond 2 years of age (left panel) but with increased dependence upon NIV support (right panel).[111]
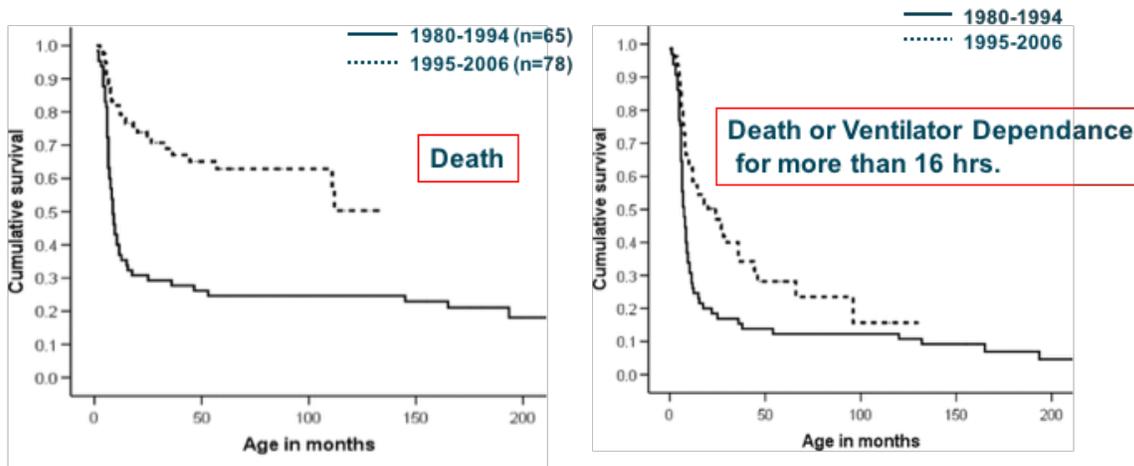
Figure 4 *Changing survival as a reflection of changing standard of care for patients with type I SMA. The 1980-94 cohort was prior to the availability of home-based non-invasive ventilation support and widespread application of gastrostomy feeding tubes. The 1995-2006 cohort reflects the availability of these interventions. Data is from a retrospective analysis of a US-based registry[111].*
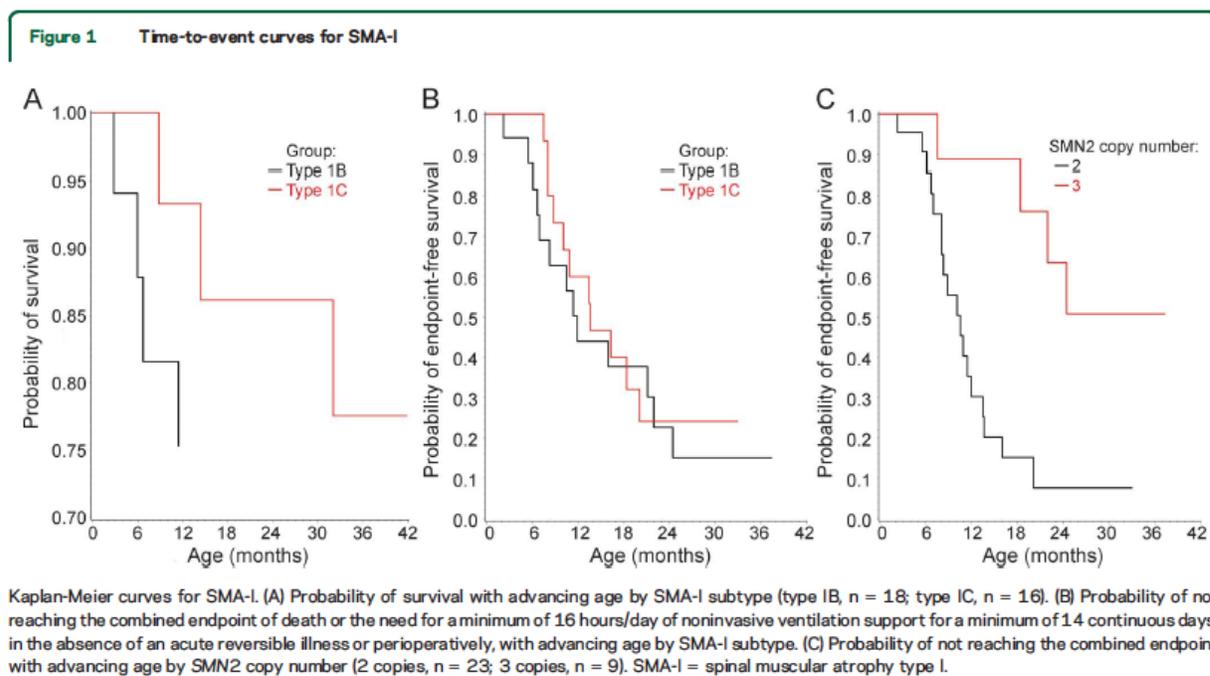

Kaplan-Meier curves for SMA-I. (A) Probability of survival with advancing age by SMA-I subtype (type IB, n = 18; type IC, n = 16). (B) Probability of not reaching the combined endpoint of death or the need for a minimum of 16 hours/day of noninvasive ventilation support for a minimum of 14 continuous days, in the absence of an acute reversible illness or perioperatively, with advancing age by SMA-I subtype. (C) Probability of not reaching the combined endpoint with advancing age by SMN2 copy number (2 copies, n = 23; 3 copies, n = 9). SMA-I = spinal muscular atrophy type I.

Figure 5. *Survival in type I SMA. Participants in this small prospective study demonstrated increased absolute survival for infants with type IC as compared with type IB (left panel) but no change when considering the combined endpoint of survival or 16+/14+ NIV support (middle panel) The right panel demonstrates a more linear decline for those participants with 2 copies of SMN2 as compared with those having 3 copies.[68]*

More recently, NIV is being started by some pulmonologists in a prophylactic way ("pro-active care"), and not waiting for signs of respiratory failure to occur ("reactive care"). The impact of this proactive approach is illustrated in Figure 6.[124] Those who received proactive care had better survival when compared to those who received reactive care. Parental choice may have factored into these observations.
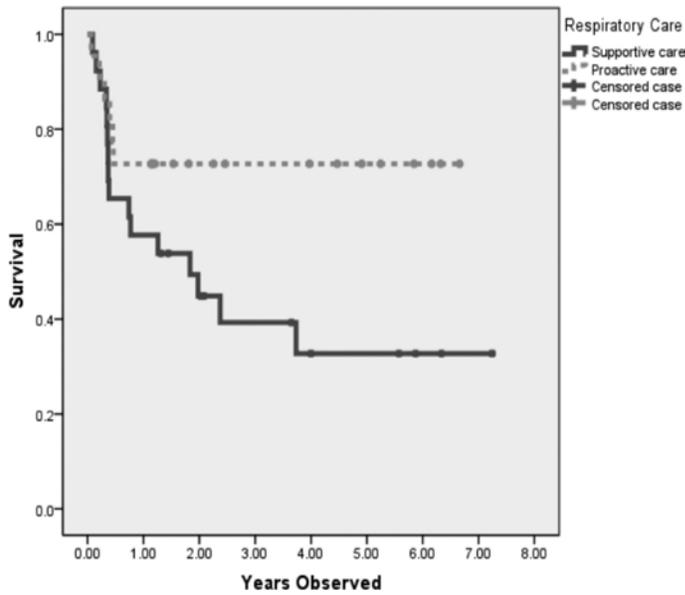

Figure 6. *Participants in this single site retrospective study were characterized by reactive ("supportive") care that was provided upon identification of the clinical need, versus proactive care that was initiated prior to any clinical identification of clinical need[124].*

2.2.5.1 Implications for clinical trials. An SMA workshop in 2005 suggested that a clinical trial for type I SMA infants could use survival or its surrogate of 16+/14+ ventilation support as the primary outcome measure.[105] Recognizing the impact of changing standards of care, however, presents a challenge for the use of survival as a primary outcome measure for type I SMA clinical trials, especially if multicentre and international where these standards may vary considerably.

Motor function scales for type I SMA, such as the CHOP INTEND, are also sensitive to changes in the infant's health. A decline may be noted during an acute illness and some rebound may occur if the infant recovers. The overall trend, however, is one of a steady decline from the baseline value, and improvement in the motor functional scales is very uncharacteristic of a SMA 1 child.

2.3 Type I SMA: Outcome Measures

2.3.1 Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND)

CHOP INTEND is a validated, 16-item, 64-point scale shown to be reliable in SMA Type I subjects[68, 113]. CHOP INTEND was derived in part from the Test of Infant Motor Performance (TIMP, see below) and was designed to measure motor function in weak infants with neuromuscular disease. It includes both active movements, spontaneous or goal-directed, and elicited reflexive movements, and assesses head, neck, trunk, and proximal and distal limb strength. CHOP INTEND does not include respiratory or feeding assessments, but it has been structured to move from easiest to hardest. The grading includes lower scores (gravity eliminated) and higher scores (antigravity movements). It should be noted, however, that it does not include sitting or weight bearing, so it measures motor function below the sitting milestone.

CHOP INTEND can be completed within a short period of time and does not require infants to be placed in positions that are poorly tolerated, like the prone position for more than a few seconds. Although subjects who require respiratory support score significantly lower, CHOP INTEND as well as other measures of infant motor performance, like TIMPSI, AIMS, and HINE (discussed below), do not measure or correlate with need for BiPAP.

The typical symptomatic SMA Type I infant has a CHOP INTEND score of approximately 20-22 points on the 0-64 point scale. In the studies from both the PNCR[68] and the NeuroNEXT SMA study[92], no infants with 2 copies of *SMN2* had a baseline value over 40 points. In the recently conducted NeuroNEXT SMA infant biomarker study, a total of 23 SMA infants and 14 control infants were assessed. The average CHOP INTEND score for SMA infants who had 2 copies of *SMN2* was 20.2 (SD = 7.4, n = 16, range = 10-33) and the maximum score in this subgroup was 33, a finding consistent with the results of the PNCR type I SMA natural history study. There was no correlation between CHOP INTEND scores and age in the SMA or control cohorts; the score for the entire SMA cohort, regardless of *SMN2* copy number, was 21.4 (range 10-52).

2.3.2 Motor milestones: Hammersmith Infant Neurological Examination- Part 2 (HINE-2)

The Hammersmith infant neurological examination (HINE) is a neurological examination for infants between 2 and 24 months of age and consists of 37 items divided into 3 sections. Section I: Neurological Examination, includes assessments of cranial nerve function, posture, movements, tone, reflexes, and reactions. Section II assesses developmental milestones: head

control, sitting, voluntary grasp, ability to kick, rolling, crawling, standing, and walking. Section III is a behavioural scale assessing the state of consciousness, emotional state, and social orientation. It should be noted that although items like pincer grasp have a developmental component, the HINE is a pure motor milestone test that was not developed specifically for SMA and has not been validated for SMA. It was utilized as an exploratory outcome measure in the Ionis CS3A open label infant SMA type I study of nusinersen, and because it was found to be informative, it evolved into a primary outcome measure in the randomized sham-controlled ENDEAR (CS3B) study. Each physical examination finding or milestone can be scored using a numerical scale, and therefore a global score can be created.

2.3.3 Test of Infant Motor Performance (TIMP), Test of Infant Motor Performance Screening Items (TIMPSI), and Alberta Infant Motor Scale (AIMS)

2.3.3.1 Test of infant motor performance (TIMP). The TIMP is a functional scale validated in premature infants under 4 months of age and includes both elicited and observed motor assessments. Because it is sensitive to age-related development and discriminates between those infants at low and high risk for motor problems, it predicts gross motor developmental delay in preterm infants. The TIMP has been shown to be a reliable test in type I SMA infants[125]. Infants with type I SMA exhibit profound weakness and significant motor delay, and tend to fatigue easily. A shorter version of the TIMP, therefore, which includes only the screening items, has been applied.

2.3.3.2 The Test of Infant Motor Performance Screening Items (TIMPSI). The TIMPSI is a 29-item, 99-point scale that has been shown to be valid and reproducible in infants with type I SMA. It tests rolling and crawling but does not test sitting. It includes many items administered in a prone position, which is not tolerated well by type I infants. It was utilized in the recent NeuroNEXT SMA infant biomarker study as a measure to screen the participating SMA and healthy infants' motor performance. Subjects who scored less than 41 on the TIMPSI were then evaluated using CHOP INTEND, and subjects who scored 41 or greater on the TIMPSI were evaluated using the Alberta Infant Motor Scale (AIMS, see below). The average TIMPSI score for the SMA cohort was 34.9 points, significantly lower than the average score for healthy controls (66.1, range = 50-96). SMA infants with 2 copies of *SMN2* had an average TIMPSI score of 27.2 (SD = 8.0, n = 16, range = 15-49). There was no correlation with age, and no SMA infant with 2

copies of *SMN2* had a TIMPSI score greater than 51. It should be noted, however, that the TIMPSI scale has not been utilized in any recent clinical trials, such as the Ionis or gene therapy Avexis ones, probably because (1) it is not well tolerated by type I infants, and (2) a number of items in CHOP INTEND have been derived from TIMPSI and, therefore, the 2 scales overlap. Both scales seem to show responsiveness to change.

2.3.3.3 Alberta Infant Motor Scale (AIMS). The AIMS is a 58-item observational scale that was developed to assess motor development in children from birth until independent walking[126, 127]. Like the TIMP, the AIMS scale also has many items in prone position, and it does not have fine motor and language domains. Although it was used as a motor scale and outcome measure in the study of Myozyme for treatment of Pompe disease, the AIMS has not been utilized in SMA clinical trials. In the NeuroNEXT infant biomarkers study, AIMS was employed to evaluate infants scoring 41 or higher on TIMPSI, following the mandatory 20-minute rest period. Only 3 SMA infants and 13 control infants were assessed using AIMS. No infants with 2 copies of *SMN2* received AIMS because they scored below 41 on the TIMPSI scale. The SMA infants evaluated by AIMS had 3 and 4 copies of *SMN2*. Despite the high *SMN2* copy number in these 3 infants, the AIMS score for the SMA cohort (8.7, SD = 3.5) was lower than for the control cohort (13.8, SD = 4.5). There was a positive correlation between AIMS score and age in the control cohort.

2.3.4 Bayley Scales of Infant Development

The Bayley-III includes assessment of motor function (gross and fine) as well as assessment of cognition and language (receptive and expressive) in infants and young children from 0-42 months. The Bayley-III language assessment is divided into expressive and receptive subtests; when these two subtests are combined, a composite score is determined. Similarly, the Bayley-III motor assessment includes scale scores for fine motor and gross motor development as well as a composite score. It has normative data, but in older/stronger children it takes a long time to administer. The main advantage of the Bayley scale is that it can also assess fine motor function, cognition and language; further, it can measure not only the acquisition of a milestone but also define when it was acquired. It has not been used extensively in ongoing clinical trials with the exception of the AveXis gene therapy type 1 trial.

2.3.5 Compound Motor Action Potential (CMAP) and Motor Unit Number Estimate (MUNE)

Compound motor action potential (CMAP) amplitude and motor unit number estimate (MUNE) were correlated with age, motor function, and *SMN2* copy number in a study by Swoboda and colleagues[104]. The study included 89 patients with types 1, 2, and 3 SMA; 29 type 1 patients were studied at baseline and 19 were followed longitudinally, including 3 pre-symptomatic patients. The symptomatic type 1 patients had reduced CMAP amplitudes that remained low over time. Four pre-symptomatic infants, 3 with type 1 and 1 with type 2 SMA, had normal CMAP amplitudes initially, but subsequently there was precipitous decline associated with the onset of symptoms.

Finkel et al.[68] identified similar baseline ulnar CMAP values in the PNCR cohort of type 1 patients (mean 0.3 mV) and described a single case of a pre-symptomatic type I neonate with rapid decline of CMAP that paralleled the decline in the CHOP INTEND motor function scale[103].

In the NeuroNEXT SMA infant biomarker study[92], ulnar CMAP measurements were obtained from the abductor digiti minimi (ADM) muscle by trained electromyographers using standardized methodology. It should be noted that this cohort of SMA patients included patients with 3 or 4 copies of *SMN2* as well as patients with an unknown number of *SMN2* copies. The average CMAP peak amplitude for the entire SMA cohort, 1.4 mV, was significantly lower than that of the control cohort, 5.5 mV. The average CMAP peak amplitude for SMA patients with 2 copies of *SMN2* was 0.5 mV (SD 1, n = 15). Although there was no similar positive correlation in the control infants, the CMAP amplitude values that were obtained in the entire SMA cohort had a positive correlation with motor functional ability as measured by TIMPSI and CHOP INTEND. Interestingly, however, in the subgroup of SMA infants with 2 copies of *SMN2*, there was no correlation with TIMPSI or CHOP INTEND.

Despite the protocol-dependent reliability issues, CMAP has been utilized as an electrophysiologic biomarker in the most recent type I SMA clinical trials.

2.6 Ethical considerations

Until investigational drugs have demonstrated clinically meaningful benefit and safety, and gain regulatory approval, it remains ethically appropriate for the clinician to counsel parents about the difficult choice of palliative versus proactive care for their infant. With the advent of new therapies showing promising results, parents are now more inclined to push forward with

proactive support, with the hope that such treatments will be available in a timely way and benefit their child. It remains a challenge for the clinician to counsel the parents about realistic expectations in this regard, when they are desperate for any treatment that might improve their child's pending demise. The common use of social media by parents of children with SMA helps them to connect to a community for advice, but can also generate unrealistic expectations or requests for supportive care that may not be warranted for their child. Supportive care is not universally available, leading some parents to feel that they are unable to provide the optimal support for their child. Hospital care may be limited as well. These inequities only heighten the disparity of health care resources among various countries. This may well limit the opportunity for some infants to enrol in clinical trials if standard of care resources are not available to them and inclusion/exclusion criteria cannot be met. Should standard of care guidelines be mandated for participation in a clinical trial? This is not ethically sound, as it removes parental autonomy, but would reduce the patient variability in a trial and allow for the effectiveness (or detriment) of an intervention to be assessed sooner and with fewer participants[128]. This topic warrants further discussion.

With the advent of effective therapeutics for type I SMA, other clinical trials will be challenged with a difficult quandary. New drugs would ideally be added to the approved drug, something that is problematic when both drugs share the same mechanism of action, e.g. *SMN2* splicing modifiers. Also, the phenotype for typeI SMA will be different due to the response to a gold standard drug. This will add uncertainty to the study design, selection of outcome measures, powering the study, etc. These issues will require considerable thought as the field evolves.

2.8 Patient perspective

2.8. 1 Diagnosis

Diagnosis can feel like a death sentence for parents, leaving them stunned, with little recourse, no hope and a total sense of despair, made worse by the fact that they have often never heard of SMA.

2.8.1.2 On-going care.

Babies will often receive care from a multidisciplinary healthcare team, which can sometimes feel like an overwhelming number of people. Parents may have contact with specialists in neuromuscular conditions, palliative   care, respiratory   medicine,

orthopaedics, physiotherapy, occupational therapy, speech and language therapy, dietetics and a hospital or community consultant paediatrician.

Standards of Care are available (and indeed are about to be relaunched) but type, level and quality of care can vary greatly, not only by country but by region.

Much of the babies' care takes place at home, with parents/carers taking responsibility for aspects of care such as:

Respiratory assistance, eg:

- Chest physiotherapy and positioning to maintain comfort

- Suction to remove secretions

- Medication to reduce secretions

- Pain relief to reduce any distress caused by breathlessness

- Non-invasive ventilation (NIV) with mechanical ventilation to increase comfort, in the management of acute infection or to correct night-time hypoventilation

- Invasive ventilation in some regions

Nutrition, eg management associated with:

- Nasogastric tube

- Nasojejunal tube

- Gastrostomy tube

Physiotherapy, eg:

- Passive exercises to enable movement and help circulation/prevent stiffening of the joints (contractures)

- Chest physiotherapy to help clear the chest when babies have difficulty coughing.

Ideally the goal of care is to enable children to enjoy a good quality of life at home with their family for as long as possible, with a minimum of hospital admissions. However, this puts considerable pressure on parents/carers in many different ways other than just time:

2.8.1.3 Emotional

The emotional toll of SMA is high and fraught with constant fear/worry about:

- The future: confronting the premature death of their child

- The next critical event which may necessitate quick decisions and possible hospitalization

- feeling helpless and unable to alter their child's eventual prognosis

2.8.1.4 Physical

Parents/carers live in a constant state of stress and exhaustion. They often suffer from lack of sleep as a result of having to provide 24hr care for their child, often having to tend to them several times each night.

2.8.1.5 Financial

One or both parents/carers often have to give up their jobs in order to care for their child. In addition, special equipment or adaptations to the home may be required, incurring significant costs.

2.8.1.6 Social

Parents/carers' social lives become extremely limited because of the time, practical and logistical requirements of caring for their child. Children inevitably tend to be excluded from normal opportunities for social interaction, such as nursery, school, playing with friends and generally exploring the world.

2.8.2 Parent attitudes to clinical trials

Type I parents/carers have differing opinions about what would be meaningful change and the risks they would tolerate, eg:

1. Some would only give a drug to their child if it could vastly improve their quality of life

2. Others would be willing to give any drug that could potentially extend the life of their child, regardless of toxicity and effect

3. Fewer respiratory events would lead to longer periods of time without non-invasive ventilation

Other concerns can include the stress and ethical issues involved if they end up being within the placebo group of a clinical trial.

3 Ambulant and non ambulant type II and III SMA

3.1 Classification

Type II SMA patients achieve the ability to sit unsupported and some of them are able to acquire standing position, but they do not acquire the ability to walk independently. Onset is generally between 7 and 18 months of age. There is a spectrum of severity ranging from weak children who are just able to sit unsupported and are more prone to respiratory signs and early scoliosis, to relatively stronger children who have much stronger trunk, limb and respiratory muscles.[16, 129] In these patients a subclassification may be achieved using functional scales that provide a measure of their level of ability.[130]

Patients with type III SMA [Kugelberg-Welander disease] all acquire independent walking but a number of them will develop proximal muscular weakness and need wheelchair assistance in childhood, whereas others continue to walk with minor muscular weakness. These can be further subdivided according to onset of clinical signs before (SMA type IIIa) and after age 3 years of age (SMA type IIIb).[131]

3.2   Type II and III SMA natural history/trajectories

The natural history of type II and III SMA has been reported in several studies that have helped to better define the progression of the disease. Both type II and III SMA are reported as progressive disorders but over the years there has been increasing attention to establish the variability of progression in these forms in relation to improvements in standards of care.

3.2.1 Survival

For patients with SMA types II and III, reports of survival probabilities have remained comparable across the literature since 1995 .[86, 117, 131, 132] For SMA type III, all reports of survival probabilities were 100 % up from the very early studies. For SMA type II survival probabilities were already very high in the earlier studies but this was reported to be 75 to 93 % at age 20 years.[86, 112, 117, 131-133] Improvements in standards of care, spinal management, better monitoring of respiratory function and a wider early use of non invasive ventilation and other respiratory tools, have resulted in a further improvement of the survival rate also in type II SMA.

3.2.2 Motor function and strength

Both type II and III SMA have been reported to be associated with significant evidence of loss of function over time. Dealing with a motor neuron disorder that causes muscle atrophy, it has been assumed that the progressive functional impairment should be mainly related to increasing muscle weakness. Several studies in the last two decades however have provided evidence that the progressive functional impairment is not always associated with parallel loss in muscle strength.[16] Longitudinal assessments of strength and motor function showed that SMA patients do not lose strength over time, while they lose motor function. The apparent discrepancy, that has been reported in several other subsequent studies[89, 134] is related to several factors, such as progressive scoliosis, changes in height and associated bone length or weight, contractures, serious inter-current illness and other factors that do not affect strength of the remaining muscles but will affect motor function. Some recent studies have shown that strength may also be progressively reduced but the reduction is very slow, can take years to be detected and does not follow the faster functional decline rate.[135, 136]

3.2.3 Natural history and prognostic factors predicting progression

Functional decline has been reported for both type II and III SMA and subsequent studies have shown that there is a variability in the rate of progression.

Recent studies that take into account the published standards of care have reported that over a 12-month follow-up period the overall motor function in these groups, as measured by different functional scales did not change significantly or only show minimal changes.[137, 138]

The Paediatric Neuromuscular Clinical Research (PNCR) Network for SMA, a consortium of clinical investigators at 3 clinical sites (Boston Children's Hospital, Columbia University, and Children's Hospital of Philadelphia), conducted a comprehensive, multi-centre, prospective natural history study including 65 participants with types II and III SMA, age 20 months to 45 years. Thirty-five patients had SMA type II and 30 had SMA type III.

Participants with types II and III SMA differed significantly in their baseline motor function values, but over a 12-month follow-up period, motor function as measured by Hammersmith Functional Motor Scale (HFMS), and Hammersmith Functional Motor Scale Expanded (HMFSE) and Gross Motor Functional Measure (GMFM, did not significantly change.[137]

The same network subsequently showed that following the same patients over 12 months of follow-up, the analysis showed some decline in motor function. The mean (SD) follow-up period was 25 (13) months, and the analysed data were derived from 79 SMA patients (41 type II SMA and 38 type III SMA). Regarding motor function, the mean change at 36 months was -1.71 for the HFMSE and -4.39 for the GMFM.[139]

A clear decline over a longer period was also observed in a French natural history study by Barois et al.)[140] where 168 enrolled patients with SMA were assessed over a 4-year period (1998-2002) using muscle strength and motor function. Statistical analysis performed 2 and 4 years after baseline showed significant worsening (about 20 %) during the follow-up period.

In another retrospective, multicenter study performed in patients aged 5.7 to 59 years using the Motor Function Measure (MFM), in 12 patients with type II SMA and 19 with type III SMA (mean ± SD follow-up, 25.8 ± 19mo), there was a moderate inverse relationship between age and the MFM total score. Patients with a longer follow-up showed a slow deterioration (-0.9 points/y for type 2 and -0.6 points/y for type 3).[141]

While there is consensus on the overall progression of both type II and III forms, the work of multiple researchers and national and international networks have allowed to establish that even within the same type of SMA, the progression is not linear and that, in some instances, patients may remain stable or even show some improvements.

Most of the previous studies are mostly concerned to establish whether type II and III SMA should be considered as progressive disorders or not, but until recently there has been no attempt to identify possible different trajectories of progression in subgroups of patients.

A recent international multicentric effort, collecting data from different existing large multicentric datasets, including one network based in the United States (PNCR) and 5 European centers (three in Italy, one in UK and one in Belgium) aimed to identify possible trajectories in a large cohort of 268 type II and III patients of age ranging between 2.5 and 55.5 years.[142] Sixty-eight were ambulant and 200 non ambulant at the time of the assessments (196 type II and 4 type III who lost ambulation).

The overall results confirmed that the mean 12 month changes in the whole cohort were very small but the range of individual changes was relatively wide. Although the majority of patients

fell within 2 points, there were a number of outliers including both patients increasing or decreasing more than two points. Having a larger cohort than those previously reported it was possible to identify different profiles of progression in ambulant and non ambulant patients, suggesting that age may play an important role in the progression of non ambulant patients.

For type II patients, children under 5 years of age were able to acquire milestones and many showed improved motor function in the absence of any therapeutic drug intervention. These results confirm previous observations in smaller cohorts.[137, 143] Non ambulant patients of age between 5 and 15 years have the greatest risk of decline in function, while those over 15 years of age are generally more stable.

For ambulant type III SMA, the trajectories were different, as possible improvements could also be noted also after the age of 5 years and the more sudden decline in scores occurred at prepubertal age.

This information, obtained in a large cohort, may be valuable at the time of selecting inclusion and stratification criteria for clinical trials or for the interpretation of the results of clinical trials.

For ambulant patients, the subdivision of type III SMA into IIIa and IIIb subtypes is very useful prognostically. Both type IIIa and type IIIb patients may lose ambulation. However, the probability of loss of ambulation is much higher in type IIIa.[86, 131, 144] By age 10 years approximately 55 to 75 % of type IIIa patients were ambulatory compared to age 20, where approximately 30 to 40 % were ambulatory. In contrast, among type IIIb patients approximately 50 to 70 % remained ambulatory until the age of 40 years.

3.2.4 Pulmonary function

Pulmonary function declines in both type II and III SMA patients over time; however, the decline is greater in type II patients.[134, 139, 140, 145, 146] Even if the available studies are not easily comparable because of different age of the patients described and of the duration of the studies, the rate of decline of respiratory function appears to have decreased over the years probably as a result of improved standards of care. In 1996 Souchon[134] reported that the decline in FVC in SMA type II and type III, first visit to last visit, was statistically significant, with declines of −17.5 % (p = 0.0001) and −13.6 % (p = 0.0012) respectively. Steffensen (2002)[146] described longitudinal changes in FVC in 12 SMA type II (n=10) and non-ambulant type III (n=2) patients, median age 25 years (range 11-57), who completed yearly assessments over 5 years. . The percent predicted

FVC declined from a median of 31% to 24% over these 5-years, an annual median decline of 1.4% and mean decline of 1.1%.

More recent studies by the PNCR showed that FVC values decreased by approximately 2% over 12 months, but the change was not statistically significant.[137, 138] When patients were followed beyond 12 months, as reported by PNCR in 2012,[139] pulmonary function showed decline; at both 24 and 36 months of follow-up, average FVC (percent predicted) decreased in a nonlinear fashion with a mean decline of approximately 3 %.[139] The decline of pulmonary function seems to be related to progression of the underlying disease.

3.3   Standards of care

An international, multidisciplinary SMA working group developed the initial standard of care guidelines for SMA.[147] This included a detailed review of the pertinent literature and used the Delphi technique to generate consensus (or lack thereof) based upon expert opinion. This guideline has become a practical roadmap for clinicians and emphasized the need for coordinated, individualized, multidisciplinary care from neurologists, pulmonologists, orthopaedic surgeons, gastroenterologists, dieticians, and physical, occupational and speech therapists, and with psychosocial support for the patient and the parents/caregivers. Palliative care begins at the time of diagnosis and emphasizes quality of life and comfort measures. This guideline has also been endorsed uniformly by pharmaceutical companies as the basis for specifying that there is an expectation for a certain degree of supportive care for participants in a clinical trial. This is often listed as an inclusion criterion. It has not been made a requirement, however, in most instances, and still allows for parental autonomy of choice during participation in a clinical trial). [148]

The standard of care guidelines are being updated following a workshop in February 2016. This included more patient input and allowed for comments from pharmaceutical representatives than was included in the initial 2007 effort. Working groups were convened to address nine topics: diagnosis, pulmonary, acute care, nutrition, orthopaedics, rehabilitation/therapy, medication, ethics and non-CNS organ system involvement. This work will be submitted for publication shortly.

3.4 Overview of outcome measures

3.4.1 *Motor function scales and motor milestones*

Functional outcome measures have been widely used over the years in SMA patients as clinical tools to record functional abilities and to follow changes related to disease progression. In the past, assessments of strength, either using qualitative methods such as the Medical Research Council (MRC) Scale for muscle strength or quantitative methods such as hand held myometry, (HHD) were typically included to assess children and adults with SMA, they were found to show the greatest variability particularly in the weakest individuals.[149] A measure of performance that relates to an individuals' function in everyday life carries more meaning and relevance than a measure that quantitates strength.

Over the past few years, while a number of therapeutic strategies were being developed, national and international clinical networks produced an impressive amount of work, validating existing functional scales and developing new ones.[130, 137, 139, 149-161] In 2014, as part of an ENMC workshop, an international group of researchers and industry representatives convened a meeting to update current knowledge on clinical trials and outcome measures for SMA.[162] Existing outcome measures were reviewed critically via published data and clinical experience to determine their suitability and readiness for use in clinical trials. Criteria were identified, including those deemed important by regulators and that were considered necessary for a scale to meet before inclusion in clinical trials. From the many outcome measures reported, they were prioritized based on the following criteria:

- Ease and time taken for their execution

- Suitability for SMA

- Reliability data were available

- Suitability for multicentric studies

- The outcome measure had been used in combination with other measures

- Longitudinal natural history data were available

- The outcome measure had already been successfully used in previous trials

- 'Clinically meaningful' for patients and their families.

Six functional outcome measures were selected as they fitted all criteria for conceptual framework, reliability, validity, disease-specific and normative data, and responsiveness to change, functional relevance, and clinical meaningfulness (Table 3)

Table 3: Outcome measures for clinical trials in SMA

Outcome measures for clinical trials in SMA: motor function scales, electrophysiological biomarkers and strength testing.

	HFMSE	MFM	6MWT	ULM	CHOP INTEND	TIMPSI	CMAP and MUNE	Myotools
Clinical utility: SMA subgroups	2 and weaker 3	2 and 3	3	2	1	1	✓	✓
Supports mechanisms of action	✓	✓	✓	✓	✓	✓	✓	✓
Conceptual framework for SMA	✓	✓	✓	✓	✓	✓	✓	✓
Reliability: Inter and intrarater	✓	✓	✓	✓	✓	✓	Protocol dependent	✓
Validation with other outcome measures	✓	✓	✓	✓	✓	✓	✓	✓
Normative ranges	✓	✓	✓	✓	✓	✓	✓	✓
Published natural history studies	✓	✓	✓	✓	✓	✓	✓	✓
Multicentre studies	✓	✓	✓	✓	✓	✗	Protocol dependent	✓
Responsiveness to change	✓	✓	✓	In progress	✓	✓	✓	✓
Clinical meaningfulness	✓	✓	✓	✓	✓	✓	✗	✓
MCID	✗	✗	✗	✗	✗	✗	✗	✗
Age-specific changes	✓	In progress	✓	In progress	✗	✗	✓	✓
Ambulant	✓	✓	✓	✗	✗	✗	✓	✓
Non-ambulant	✓	✓	✗	✓	✓	✓	✓	✓

HFMSE: Hammersmith Functional Motor Scale Expanded, MFM: Motor Function Measure, 6MWT: 6-minute walk test distance, ULM: upper limb module for SMA, CHOP INTEND: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders, TIMPSI: Test of Infant Motor Performance Screening Items, CMAP: compound motor action potential, MUNE: motor unit number estimate, MCID: minimal clinically important difference.

There was general consensus that functional rating scales capture motor performance in a more comprehensive way, appreciating that function is influenced by not only strength but by other factors such as contractures, fatigue, growth, and weight. Furthermore, functional rating scales are more suitable for reliable testing of younger patients below the age of 5 years, whereas myometry is reliable only above this age.

Motor performance in SMA may be assessed using a generic or disease specific rating scales. The Motor Function Measure (MFM) is a quantitative generic neuromuscular functional scale, targeting motor abilities in individuals with a wide spectrum of weakness distribution and severity.[160] Three clinically significant dimensions are included (standing position and transfers, axial and proximal motor function, and distal motor function) providing scores for each domain as well as a total score.

The MFM can be used for ambulatory and non-ambulatory children and adults aged 6 - 62 years, and for all levels of severity of the disease.[141, 163, 164] The MFM in its classic form (MFM32) is suitable for children older than 6 years and a modified version with 20 items (MFM20 version) has been validated for children under 6 years of age.[161]

Longitudinal data is available in a small sample of type II and III SMA patients demonstrating slow deterioration over follow-up greater than 6 months.[141] The scale has also been successfully used in recent clinical trials. Some disadvantages to the scale are related to the time it takes administer, potential gaps in items between the non-ambulant and ambulant phenotypes with a possible ceiling effect for stronger non-ambulant patients.[154]

The great majority of the available natural history studies have been using disease specific assessments.[130, 152, 165] It is important to note that disease specific assessments have the advantage that they are designed to target the functionally relevant problems common to SMA patients and are less likely to include items not appropriate to the disease phenotype.

The Hammersmith Function Motor Scale (HFMS), was specifically developed for type II SMA and the items included were ordered according to frequency distribution and the number of patients being able to achieve them.[130] The hierarchical organization of items permits characterization of patients across the spectrum of type II patients from those who are just able to sit to those who are able to stand with and without support.[130]

An expanded version (HFMSE) has been developed adding 13 relevant items to eliminate the ceiling effect of the original scale with ambulant type III SMA patients.[152] The expanded version (HFMSE) has been shown to have excellent reliability and is associated with other clinical and physiological measures of disease progression in SMA.[166]

The scale, in its original version for type II or in the expanded version, has been used in several national and international natural history studies both In US and Europe reporting data up to 1 year[138, 142] and, more recently, in a large series of type II and III patients systematically and prospectively followed up to 3 years.[139]

The extensive longitudinal experience with the HFMSE in large international populations of SMA patients permits a detailed understanding of the natural history of SMA and allows for targeted study design. The large number of patients available worldwide has allowed to identify different trajectories for subgroups of patients based on age and function and to highlight the possible role of a number of variables, such as development of contractures, scoliosis or growth over time, on the progression of the disease.

The Hammersmith scale, in its original version or in the subsequent adaptations, has also been successfully used in several clinical trials.[94, 167-171]

The disadvantage of the HFMSE is that it does not include items assessing upper limb activities that may be relevant in weaker type II patients. In order to address this point, the Upper Limb Module (ULM), an assessment of arm function has been specifically designed as an additional module.[150]

The ULM is intended to capture performance of activities of daily living not typically included in measures of gross motor function. This 9-item scale can be reliably performed in children and takes no more than 10 minutes to complete. The scale has been used in a multicentric setting and in clinical trials.

Increasing attention has been devoted in the last years to capture functional aspects that are specific to ambulant patients. The six minute walk test (6MWT) has been shown to be reliable and valid functional assessment in patients with SMA [172] and has the advantage that can also capture fatigue.[173, 174] Fatigue was demonstrated by a 17% decrease in gait velocity from the first minute to the last during the 6MWT.[151] This phenomenon was not observed in patients with other neuromuscular conditions and weakness, despite similar dysfunction, perhaps representative of a mechanistic defect of neurotransmission unique to SMA.[174] In addition to established validity and reliability, the longitudinal experience of the 6MWT in SMA has been reported.[153]

In addition to the 6MWT, the Timed Up and Go (TUG) test, quick, meaningful, and applied objective measure of balance, gait speed, and functional mobility, has been applied to ambulatory SMA patients.[175] TUG measures the time it takes a patient to stand up from an armchair, walk a distance of 3 meters, turn, walk back to the chair, and sit down.[176] Initial experience with TUG in SMA patients shows promise with initial convergent validity and test-retest reliability.[175]

3.4.1.1. Application of modern psychometrics on rating scales used in SMA

Rasch measurement methods are based on Rasch Measurement Theory[177] and allow us to examine the extent to which the rating scale data fit a mathematical model of a "perfect" scale, which would deliver reliable and valid measures.[178] This method is better able to identify strengths and weaknesses of rating scales [179] and at the same time deliver a similar assessment to that of traditional methods. In addition it can guide clinicians as to how to improve the psychometric properties of their scale based on the internal evidence and given a good fit of the rating scale data can generate a linearized version of the scale which allows change scores to mean the same across the breadth of the scale.[180] These methods are also supported by regulatory authorities such as the FDA.[181] .

The introduction of these methods to assess rating scales used to measure motor performance and patient reported outcomes in SMA has created an industrious review and in some case revision of several of the scales currently in use. This work is ongoing. Here we summarise the findings of a paper, which describes the results of Rasch analysis on nine commonly used scales in SMA - CHOP-INTEND, Egan Klassifikation Scale (EK2), GMFM, HFMS, HFMSE, MFM, MHFMS, North Star Ambulatory Assessment (NSAA) for SMA, TIMPSI [182] - and then describe scale development since that review.

All of the nine scales had issues with fit, which means, to varying degrees, items within scales were found to have reversed thresholds. This means response categories (for example item scores of 0, 1, 2 and 3) were not working as intended for individual items. All scales except the CHOP INTEND had issues with fit residuals and chi-squared tests. This suggests they contained items, which did not fit the intended construct of the scale, which may mean they are not measuring one domain i.e, not uni-dimensional. The TIMPSI, CHOP INTEND, HFMSE and MHFMS-Extend showed adequate targeting which indicates the items covered the range of motor performance seen in the patients. This was not true for all scales, and may relate to the intended population of the individual scales. Dependency was also an issue in most scales, which suggests that the scores of items may be influenced directly by the score on others. This may inflate reliability as assessed by Person separation Index (PSI), which for all scales was high (PSI > 0.83). All scales except for the TIMPSI were found to have differential item functioning (DIF) which indicates that age, type of SMA, assessment team, may alter how an individual scores on a scale. This review allowed the SMA community to gain a detailed understanding of the strengths and weakness of various scales. In particular this precipitated a review of the existing scales to improve their statistical robustness. A review of HFMSE and ULM applied a stepwise approach of revisions, starting from the original scales. This resulted in revised scales that while maintaining most of the features of the original scale, fit the conceptual framework for SMA capturing a wider spectrum of functional abilities across ambulatory and non-ambulatory individuals with SMA. Clinicians, patients with SMA and their caregivers were involved iteratively throughout the process to establish clinical meaningfulness and repeated Rasch analysis helped to improve the new scales robustness by highlighting possible issues that impact scale validity such as item fit, disordered thresholds or item redundancy.

3.4.1.2 PROM

The use of patient reported outcome measures (PROM) has been strongly suggested by regulatory authorities.

A recent paper reported the use of a PROM applying modern psychometric methods[115] and suggested that the Pediatric Evaluation of Disability Inventory Computerized Adaptive Test (PEDI-CAT) may be used to measure mobility skills in children and young adults with type III SMA, as well as daily activity skills in children and young adults with type II SMA. Analysis did reveal some issues including a floor effect and ceiling effect. In its present form this PROM may not be sufficiently sensitive to detect small functional changes over time in weaker individuals, or in stronger individuals for upper extremity function. However it may be a good tool for describing cross- sectional functional motor performance in children and young adults with SMA.

Other work involving modern psychometric methods has validated the ACTIVLIM for use in NMD's. This is a PROM, which assesses limitations in activities of everyday living. The analysis was based on a large sample (n=4146) which included a subset of 100 SMA patients. It showed a good reliability (R = 0.95), an appropriate targeting for 87% of the sample and an excellent invariance across age, gender, language and time.[183]

Other methods have been used to improve the statistical robustness of the existing scales. While the HFMSE, ULM, and 6MWT were developed and scored separately, an attempt to combine the scales into a composite measure was made.[184] Similar to the composite score of all three domains of the MFM, an unweighted average of the three individual percentage scores of the HFMSE, ULM, and 6MWT was nearly perfectly correlated with a composite score calculated from a principal component analysis.

3.5   Exploratory outcome measures (including MRI)

Seferian et al. [185] recently validated the use of highly sensitive key pinch and handgrip to quantify distal strength in 23 non-ambulant type II and III SMA patients. Interestingly, these measures detected a one year difference in the population of patients older than 14 years, yet this part of the cohort was rather limited (n=12). If confirmed in a larger cohort, this would help to define a framework of outcome according to patients' age. Indeed, functional scales like MFM32 or

HFMSE seem more sensitive in the 5-15 years population [141, 142], whilst strength measure would be more helpful in older patients. This also needs to be confirmed in larger cohort.

Regarding non-clinical outcome measure, the most reliable and standardized assessment in neurophysiology in SMA is the compound muscle action potential [139, 186, 187]. CMAP is well correlated with HFMSE [139] and MFM [186] and may accurately discriminate type II and III patients, but it does not change significantly over a one, and even on a three years period [139]. Motor Unit Number Estimation is also feasible in SMA population and correlated with HFMS [85, 187], yet the feasibility and standardization in a multicenter setting remains to be established.

Quantified Muscle resonance imaging (MRI) in SMA is in development. Sproule et al. [188] studied 14 patients with type I-III SMA. They demonstrated that muscle volume estimation is highly reliable (r=0.99), and correlated with MUNE, CMAP and HMFSE. Muscle volume estimation could discriminate between type II and III, but this did not reach statistical significance.

Durmus et al. [189] studied 25 patients with type IIIb SMA (onset of symptoms between 4-18 years, duration of symptoms between 1 and 30 years, median 10 years). They found a correlation between MRI score in gluteus (R=0.7) or triceps (r=0.54) and disease duration. Visual inspection of MRI in 4 patients re-evaluated after 8 years demonstrated progression of the disease.

3.6   Registries, and trial readiness

SMA is one of the most common inherited neuromuscular disorders. However, patient numbers are still limited. The incidence of SMA is estimated around 1 in 5000 to 1 in 10 000 [190]. This does not mean that this number of patients is also available for clinical trials.

The small numbers of patients and the lack of knowledge of where patient populations eligible for trials are located can delay the clinical trials process, resulting in individual trials taking several years to meet recruitment goals [191]. This has been recognised and a result the SMA patient registries initiative registries brought together as one of the TREAT-NMD's activities. TREAT-NMD [www.treat-nmd.eu] is a global neuromuscular network that aims to facilitate translational research in NMDs and was initially funded in 2007 as a 'network of excellence' under the European Union's Sixth Framework Programme. As part of TREAT-NMD's efforts an international network of patient registries for SMA, the Global SMA Patient Registry, was developed. The Global SMA Patient Registry, which comprises of 48 national SMA patient registries at various stages of development, as of September 2016 included the information on

about 6,500 genetically confirmed SMA patients. It was agreed that the primary focus of the Global SMA Patient Registry would be the facilitation of planning, feasibility studies and recruitment for clinical trials, and the data items they collect were harmonised to reflect this goal. The common dataset was agreed internationally and comprises a list of mandatory and highly encouraged items that all SMA registries affiliated with TREAT-NMD agree to collect. [109] Those items include patient demographics, molecular diagnosis (including number of *SMN2* copies where available), clinical diagnosis (type of SMA), motor function (best achieved and current), wheelchair use, gastric/nasal feeding information, history of scoliosis surgery; cardiac and respiratory function; clinical trial participation, and family history. The registries are an accurate source of information on patient numbers in various regions and countries and as a result allow the companies that conduct the clinical trials to assess trial feasibility and calculate the number of sites they might need to open to meet recruitment targets. By collecting contact information for those affected by SMA the registries allow potentially eligible patients to be informed about trials for which they may fulfil inclusion criteria.

Another resource that facilitates clinical trial readiness is the TREAT-NMD Care and Trial Sites Registry (CTSR). The CTSR is an online database of NMD-specialist clinical sites and medical centres [192]. The data collected includes information on numbers of patients seen by the centre, care settings, research and education as well as the infrastructure for clinical trials. As of September 2016, there were 340 centres for neuromuscular and/or neurodegenerative diseases in 51 countries, within those there is the information more than the centres in 42 countries that in total see over 6,500 SMA patients.

One of the issues for clinical trials is the wide phenotypic spectrum of the disease, making it more challenging to find a uniform patient group necessary for clinical trials. Furthermore, results found in one type of SMA cannot immediately be extrapolated to other types of SMA. We recently performed a study to estimate the worldwide prevalence of SMA combining different methods. We gathered information about their testing results from genetic testing laboratories and have done enquiries via the Global SMA Patient Registry, comprising national patient registries worldwide, and the Care and Trial Site Registry (CTSR), a database containing information of the facilities and patient cohorts at NMD-specialist clinical sites and medical

centres worldwide. The rates found in these registries were in general 2-5 times lower than results from genetic testing laboratories and in literature.

There could be several reasons for this. First of all, most literature studies about the SMA prevalence are scarce and often outdated, with many having been published before the disease causing gene was discovered or presenting the SMA prevalence only for a particular, small geographic region. Therefore, some of the patients with clinical phenotypes similar to SMA could have been misdiagnosed as having SMA, which could account for higher numbers found in literature.

The SMA patient registries collect information on the genetically confirmed patients and in some cases the genetic diagnosis is pending or in some countries or no genetic testing is available, which would limit the numbers of patients in the registries. In several countries the patient registry has only recently been set-up, therefore it is expected that the number of registered patients will increase with time. Moreover, most of the patient registries are mainly set-up with the purpose of clinical trials, so those patients who are not interested in participating in clinical trials may not wish to register, which would be additional factor for lower than expected number of patients. The CTSR contains information of specialised centres, so patients attending only non-specialised hospitals or centres may not be included in this database. However, even though the prevalence of SMA patients in the Global SMA Registry and CTSR are lower than what has been reported in literature, these sources provide a good estimate on the clinical trials ready population.

In summary, combining the information in the CTSR with the details on the number of potentially eligible patients from the SMA patient registries, can help companies planning a trial in SMA population with the establishment of the realistic recruitment targets and numbers of sites required for the trial.

3.7 Patient perspective to clinical benefit

3.7.1. Impact of type II / III SMA

With types II and III SMA it is important to consider the impact not only on the child or adult who has the disease but also their families and carers. As with type I, this impact manifests itself in many different aspects of their lives.

3.7.2. Practical/physical

People with type II / III SMA often need to access care from a wide range of specialists in neuromuscular conditions, respiratory medicine, orthopaedics, physiotherapy, occupational therapy, speech and language therapy, dietetics and a hospital or community consultant pediatrician. This can result in numerous appointments and take up a great deal of time. Again, as with type I, parents/carers take on a great deal of the on-going care at home, such as respiratory assistance, tube feeding and physiotherapy. This can be more than a full-time commitment, usually involving careful scheduling of events for the child, their caregivers and therapists, nursing help and night time help.

In a 2015 SMA Europe Survey (837 type II / III patients in 21 countries), important data around mobility, breathing and function were established:

- 71% were non-ambulant and 63% were unable to stand up

- 65% used a power wheelchair.

- 35% used ventilation assistance in some form.

- 78% were unable to dress themselves

- Over 70% were unable to transfer, go to the bathroom or toilet themselves

- 67% were unable to turn themselves in bed

The full results of this survey can be seen at the end of this document.

3.7.3 Emotional

Parents not only worry about whether their child will live or die but about his/her future and gradual loss of functionality and independence. There are also more immediate concerns such as the 'next event', which could be anything from a medical emergency to the loss of a function that marks the next stage in the deterioration of their child. Children with type III can find it particularly hard to adjust when it comes to losing the ability to walk.

For patients themselves, for those with type I or II, it is the only life they have ever known and so there is a certain level of acceptance, whereas for those with type III it can be difficult as they may well remember walking and then losing that function.

The main worry seems to be about further loss of function and, with it, independence.

3.7.4. Financial

As with SMA type I, parents/carers who have a child with type II/ III often have to give up/change their career or reduce working hours in order to care for their child. Financial support varies between countries but is still insufficient to cover all the additional expenditure required, such as special medical equipment, mobility aids, home adaptations etc. This situation continues to affect people with SMA type II/ III as they reach adulthood. Even though many of them achieve qualifications and successful careers, they are nevertheless confronted with all the financial pressures associated with having a severe disability of this kind.

The fact that peoples' minds are unaffected means that the importance of special equipment to enable them to participate fully in life is magnified. This manifests itself in many ways, e.g. in the home, at school and in work/social settings.

3.7.5. Social

There is also considerable impact on the social lives of those affected by type II/ III SMA and their families. Even though patients are well integrated in society, they are regularly limited by the activities in which they can become involved. Parents/carers have less time, cash and energy for social lives of their own.

Much of the feedback on the impact of SMA (type II and III in particular) is described in the results of two US studies, which are included at the end of this section.

3.8. Patient perspective on clinical trials and drug development

The prospect of drugs in the pipeline that could potentially provide significant improvements to SMA patients or, at the very least, slow down or halt deterioration, is creating high hopes within the SMA community. The potential for therapeutic benefit has highlighted the need to obtain reliable documented evidence of patient and caregivers input to support the meaningfulness of the measures used in natural history studies and in clinical trials[162, 184, 193, 194]. This approach has also been strongly encouraged by the FDA [195], which has also suggested that observer rated scales should be used to support the relevance of the observed functional changes[196].

Studies in the US[12] and Europe have consistently demonstrated that maintaining function and slowing down disease progression is the top priority for patients. Improvements are obviously hoped for but it is clear that even small changes such as dressing and going to the bathroom themselves would make a significant difference to patients' quality of life.

Changes measured in clinical trials need to be sufficiently sensitive to reflect an individual patient's starting point, whilst being broad enough to draw conclusions across a spectrum of different patients. Patients/carers are happy that current scales pick up larger differences, but there is a feeling that other more finely tuned measures might be necessary to pick up more granular changes. There is also a view that other factors such as fatigue and stamina would benefit from more emphasis as they have an important impact on quality of life as a whole. Another important characteristic of this patient/carer population in relation to clinical trials is their attitude to benefit/risk. They are prepared to accept a higher risk (side effects, drug not working etc.) because they know that if they do nothing, things will usually get worse anyway.

The SMA community wants safe and effective treatments and, for many patients and their families, each day without them brings new fears of losing essential functions and, in many cases, death. It is therefore vitally important for patients and carers to continue to be fully consulted and involved in the development of clinically meaningful outcome measures and how these are used in regulatory discussions and decisions.

There is a fear that the emphasis will be on treatments for young and even pre-symptomatic patients and that older patients with more advanced symptoms might be excluded from clinical trials and regulation processes.

The sense of urgency is enormous and everyone wants to have access to treatments that could benefit them, albeit in different ways.

Other surveys have tried to highlight the meaningfulness of the possible changes using the existing outcome measures. One of the challenges in SMA is that the disease is clinically very heterogeneous and, even when restricted to type II and III SMA the severity ranges from patients who can only sit to fully ambulant patients with minimal weakness. Another significant challenge is that, as reported by recent natural history studies, the progression of the disease in type II and III SMA can be variable, and that various factors, such as age or functional level, may be

[1] Qian et al. BMC Neurology (2015) 15:217

[2] http://www.curesma.org/news/voices-of-sma-fda-engagement.html

associated with different trajectories[142]. Because of this, it is not always easy to understand what to consider as a meaningful change in patients at different ages or functional levels. Moreover, it is not clear if similar quantitative improvements in the existing scales have the same meaning for patients who start at different extremes of the scale.

A recent paper describes a two part using focus groups including SMA patients and carers to explore their views on the clinical relevance of the individual activities included in the HFMSE. More specifically, the paper explores carers' and patients' view on the clinical meaningfulness of each individual HFMSE item, asking them to describe the relationship between the activity explored in the individual items and activities of daily living. Sixty-three individuals participated in the focus groups. This included 30 carers, 25 patients and 8 professionals who facilitated the discussion. The carers provided a comparison to activities of daily living for each of the HFMSE items. Each item of the scale was found to be clinically meaningful and could be related to activities of daily living (See appendix).

The paper also reports the results of a second survey where carers were asked to comment on the clinical relevance of possible changes of HFMSE scores over time. As functional data were available, the questions were tailored according to the patients' functional level on the HFMSE that were realistically close to their child's possible achievements rather than generic questions on activities, such as walking or running that would be highly desirable but, at least in a limited time frame, difficult or impossible to be achieved.

Of the 149 carers who agreed to complete the questionnaire, 72% would consider taking part into a clinical trial if the treatment was expected to slow down deterioration, 88% if it would stop deterioration and 97% if the treatment was expected to produce an improvement.

When asked if they would consider taking part into a trial if there was the possibility of achieving one, two or more than two activities, 75% would consider it even with just one activity achieved. Plotting the results according to their functional level, these were widely spread across functional levels and age.

This suggests that even if the activities that can be achieved are different, any improvement is considered to be meaningful, irrespective if the baseline score is very low, in the middle of the scale or very high. This is particularly important considering the ordinal nature of the scale that makes this comparison difficult.

Appendices

Comprehensive summary of the literature on survival in type I SMA.

Results of the questionnaire on clinical meaningfulness of the HFMSE.

Supplement

Synopses provided by the six pharmaceutical companies with drug in clinical trial that summarize their program.

References

1.   Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am J Hum Genet 2002;70:358-368.
2.   Hendrickson BC, Donohoe C, Akmaev VR, et al. Differences in SMN1 allele frequencies among ethnic groups within North America. J Med Genet 2009;46:641-644.
3.   Chong JX, Oktay AA, Dai Z, Swoboda KJ, Prior TW, Ober C. A common spinal muscular atrophy deletion mutation is present on a single founder haplotype in the US Hutterites. European journal of human genetics : EJHG 2011;19:1045-1051.
4.   Majumdar R, Rehana Z, Al Jumah M, Fetaini N. Spinal muscular atrophy carrier screening by multiplex polymerase chain reaction using dried blood spot on filter paper. Ann Hum Genet 2005;69:216-221.
5.   Sangare M, Hendrickson B, Sango HA, et al. Genetics of low spinal muscular atrophy carrier frequency in sub-Saharan Africa. Annals of neurology 2014;75:525-532.
6.   Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. European journal of human genetics : EJHG 2012;20:27-32.
7.   Wirth B, Herz M, Wetter A, et al. Quantitative analysis of survival motor neuron copies: identification of subtle SMN1 mutations in patients with spinal muscular atrophy, genotype-phenotype correlation, and implications for genetic counseling. Am J Hum Genet 1999;64:1340-1356.
8.   Wee CD, Kong L, Sumner CJ. The genetics of spinal muscular atrophies. Current opinion in neurology 2010;23:450-458.
9.   Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 1995;80:155-165.
10.   Pearn J. Classification of spinal muscular atrophies. Lancet (London, England) 1980;1:919-922.
11.   Russman BS. Spinal muscular atrophy: clinical classification and disease heterogeneity. Journal of child neurology 2007;22:946-951.
12.   Thomas NH, Dubowitz V. The natural history of type I (severe) spinal muscular atrophy. Neuromuscular disorders : NMD 1994;4:497-502.
13.   Munsat T, Davies K. Spinal muscular atrophy. 32nd ENMC International Workshop. Naarden, The Netherlands, 10-12 March 1995. Neuromuscular disorders : NMD 1996;6:125-127.
14.   Darras BT. Spinal muscular atrophies. Pediatric clinics of North America 2015;62:743-766.
15.   Zerres K, Wirth B, Rudnik-Schoneborn S. Spinal muscular atrophy--clinical and genetic correlations. Neuromuscular disorders : NMD 1997;7:202-207.
16.   Russman BS, Iannacone ST, Buncher CR, et al. Spinal muscular atrophy: new thoughts on the pathogenesis and classification schema. Journal of child neurology 1992;7:347-353.
17.   Hausmanowa-Petrusewicz I, Badurska-Modrzycka B, Ryniewicz B. On chaos in classification of childhood spinal muscular atrophy. Neuromuscular disorders : NMD 1992;2:429-430.
18.   Zerres K, Rudnik-Schoneborn S, Forkert R, Wirth B. Genetic basis of adult-onset spinal muscular atrophy. Lancet (London, England) 1995;346:1162.
19.   Dubowitz V. Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society 1999;3:49-51.
20.   McAndrew PE, Parsons DW, Simard LR, et al. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. Am J Hum Genet 1997;60:1411-1422.

21.   Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. Hum Mol Genet 1995;4:631-634.

22.   Wirth B, Rudnik-Schoneborn S, Hahnen E, Rohrig D, Zerres K. Prenatal prediction in families with autosomal recessive proximal spinal muscular atrophy (5q11.2-q13.3): molecular genetics and clinical experience in 109 cases. Prenatal diagnosis 1995;15:407-417.

23.   Velasco E, Valero C, Valero A, Moreno F, Hernandez-Chico C. Molecular analysis of the SMN and NAIP genes in Spanish spinal muscular atrophy (SMA) families and correlation between number of copies of cBCD541 and SMA phenotype. Hum Mol Genet 1996;5:257-263.

24.   Wirth B. An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). Hum Mutat 2000;15:228-237.

25.   Hahnen E, Schonling J, Rudnik-Schoneborn S, Zerres K, Wirth B. Hybrid survival motor neuron genes in patients with autosomal recessive spinal muscular atrophy: new insights into molecular mechanisms responsible for the disease. Am J Hum Genet 1996;59:1057-1065.

26.   van der Steege G, Grootscholten PM, Cobben JM, et al. Apparent gene conversions involving the SMN gene in the region of the spinal muscular atrophy locus on chromosome 5. Am J Hum Genet 1996;59:834-838.

27.   Wirth B, Schmidt T, Hahnen E, et al. De novo rearrangements found in 2% of index patients with spinal muscular atrophy: mutational mechanisms, parental origin, mutation rate, and implications for genetic counseling. Am J Hum Genet 1997;61:1102-1111.

28.   Bussaglia E, Clermont O, Tizzano E, et al. A frame-shift deletion in the survival motor neuron gene in Spanish spinal muscular atrophy patients. Nat Genet 1995;11:335-337.

29.   Hahnen E, Schonling J, Rudnik-Schoneborn S, Raschke H, Zerres K, Wirth B. Missense mutations in exon 6 of the survival motor neuron gene in patients with spinal muscular atrophy (SMA). Hum Mol Genet 1997;6:821-825.

30.   Sun Y, Grimmler M, Schwarzer V, Schoenen F, Fischer U, Wirth B. Molecular and functional analysis of intragenic SMN1 mutations in patients with spinal muscular atrophy. Hum Mutat 2005;25:64-71.

31.   Clermont O, Burlet P, Benit P, et al. Molecular analysis of SMA patients without homozygous SMN1 deletions using a new strategy for identification of SMN1 subtle mutations. Hum Mutat 2004;24:417-427.

32.   Ogino S, Wilson RB. Importance of standard nomenclature for SMN1 small intragenic ("subtle") mutations. Hum Mutat 2004;23:392-393.

33.   Ogino S, Wilson RB. Genetic testing and risk assessment for spinal muscular atrophy (SMA). Hum Genet 2002;111:477-500.

34.   Cusco I, Barcelo MJ, del Rio E, Baiget M, Tizzano EF. Detection of novel mutations in the SMN Tudor domain in type I SMA patients. Neurology 2004;63:146-149.

35.   Skordis LA, Dunckley MG, Burglen L, et al. Characterisation of novel point mutations in the survival motor neuron gene SMN, in three patients with SMA. Hum Genet 2001;108:356-357.

36.   Jedrzejowska M, Gos M, Zimowski JG, Kostera-Pruszczyk A, Ryniewicz B, Hausmanowa-Petrusewicz I. Novel point mutations in survival motor neuron 1 gene expand the spectrum of phenotypes observed in spinal muscular atrophy patients. Neuromuscular disorders : NMD 2014;24:617-623.

37.   Lorson CL, Strasswimmer J, Yao JM, et al. SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat Genet 1998;19:63-66.

38.   Buhler D, Raker V, Luhrmann R, Fischer U. Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. Hum Mol Genet 1999;8:2351-2357.

39.   Cusco I, Lopez E, Soler-Botija C, Jesus Barcelo M, Baiget M, Tizzano EF. A genetic and phenotypic analysis in Spanish spinal muscular atrophy patients with c.399_402del AGAG, the most frequently found subtle mutation in the SMN1 gene. Hum Mutat 2003;22:136-143.

40.   Kirwin SM, Vinette KM, Gonzalez IL, Abdulwahed HA, Al-Sannaa N, Funanage VL. A homozygous double mutation in SMN1: a complicated genetic diagnosis of SMA. Molecular genetics & genomic medicine 2013;1:113-117.

41.   Burglen L, Lefebvre S, Clermont O, et al. Structure and organization of the human survival motor neurone (SMN) gene. Genomics 1996;32:479-482.

42.   Arkblad E, Tulinius M, Kroksmark AK, Henricsson M, Darin N. A population-based study of genotypic and phenotypic variability in children with spinal muscular atrophy. Acta paediatrica (Oslo, Norway : 1992) 2009;98:865-872.

43.   Tiziano FD, Pinto AM, Fiori S, et al. SMN transcript levels in leukocytes of SMA patients determined by absolute real-time PCR. European journal of human genetics : EJHG 2010;18:52-58.

44.   van der Steege G, Grootscholten PM, van der Vlies P, et al. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. Lancet (London, England) 1995;345:985-986.

45.   Wirth B, Brichta L, Schrank B, et al. Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number. Hum Genet 2006;119:422-428.

46.   Burghes AH. When is a deletion not a deletion? When it is converted. Am J Hum Genet 1997;61:9-15.

47.   Lorson CL, Androphy EJ. An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. Hum Mol Genet 2000;9:259-265.

48.   Helmken C, Hofmann Y, Schoenen F, et al. Evidence for a modifying pathway in SMA discordant families: reduced SMN level decreases the amount of its interacting partners and Htra2-beta1. Hum Genet 2003;114:11-21.

49.   Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 1997;6:1205-1214.

50.   Lefebvre S, Burlet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 1997;16:265-269.

51.   Brahe C. Copies of the survival motor neuron gene in spinal muscular atrophy: the more, the better. Neuromuscular disorders : NMD 2000;10:274-275.

52.   Hsieh-Li HM, Chang JG, Jong YJ, et al. A mouse model for spinal muscular atrophy. Nat Genet 2000;24:66-70.

53.   Monani UR, Sendtner M, Coovert DD, et al. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(-/-) mice and results in a mouse with spinal muscular atrophy. Hum Mol Genet 2000;9:333-339.

54.   Kaczmarek A, Schneider S, Wirth B, Riessland M. Investigational therapies for the treatment of spinal muscular atrophy. Expert opinion on investigational drugs 2015;24:867-881.

55.   Wirth B, Garbes L, Riessland M. How genetic modifiers influence the phenotype of spinal muscular atrophy and suggest future therapeutic approaches. Current opinion in genetics & development 2013;23:330-338.

56.   Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc Natl Acad Sci U S A 1999;96:6307-6311.

57.   Cartegni L, Krainer AR. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. Nat Genet 2002;30:377-384.

58.   Kashima T, Manley JL. A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat Genet 2003;34:460-463.

59.   Hofmann Y, Lorson CL, Stamm S, Androphy EJ, Wirth B. Htra2-beta 1 stimulates an exonic splicing enhancer and can restore full-length SMN expression to survival motor neuron 2 (SMN2). Proc Natl Acad Sci U S A 2000;97:9618-9623.

60.   Singh NK, Singh NN, Androphy EJ, Singh RN. Splicing of a critical exon of human survival motor neuron is regulated by a unique silencer element located in the last intron. Mol Cell Biol 2006;26:1333-1346.

61.   Hua Y, Sahashi K, Rigo F, et al. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature 2011;478:123-126.

62.   Osman EY, Miller MR, Robbins KL, et al. Morpholino antisense oligonucleotides targeting intronic repressor Element1 improve phenotype in SMA mouse models. Hum Mol Genet 2014;23:4832-4845.

63.   Singh NN, Lawler MN, Ottesen EW, Upreti D, Kaczynski JR, Singh RN. An intronic structure enabled by a long-distance interaction serves as a novel target for splicing correction in spinal muscular atrophy. Nucleic Acids Res 2013;41:8144-8165.

64.   Lunn MR, Wang CH. Spinal muscular atrophy. Lancet (London, England) 2008;371:2120-2133.

65.   Kolb SJ, Coffey CS, Yankey JW, et al. Baseline results of the NeuroNEXT spinal muscular atrophy infant biomarker study. Annals of clinical and translational neurology 2016;3:132-145.

66.   Rudnik-Schoneborn S, Berg C, Zerres K, et al. Genotype-phenotype studies in infantile spinal muscular atrophy (SMA) type I in Germany: implications for clinical trials and genetic counselling. Clin Genet 2009;76:168-178.

67.   Cobben JM, van der Steege G, Grootscholten P, de Visser M, Scheffer H, Buys CH. Deletions of the survival motor neuron gene in unaffected siblings of patients with spinal muscular atrophy. Am J Hum Genet 1995;57:805-808.

68.   Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology 2014;83:810-817.

69.   Prior TW, Krainer AR, Hua Y, et al. A positive modifier of spinal muscular atrophy in the SMN2 gene. Am J Hum Genet 2009;85:408-413.

70.   Burghes AH, Ingraham SE, Kote-Jarai Z, et al. Linkage mapping of the spinal muscular atrophy gene. Hum Genet 1994;93:305-312.

71.   Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet 1995;4:1927-1933.

72.   Oprea GE, Krober S, McWhorter ML, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science 2008;320:524-527.

73.   Finkel RS, Crawford TO, Swoboda KJ, et al. Candidate proteins, metabolites and transcripts in the Biomarkers for Spinal Muscular Atrophy (BforSMA) clinical study. PloS one 2012;7:e35462.

74.   Kobayashi DT, Shi J, Stephen L, et al. SMA-MAP: a plasma protein panel for spinal muscular atrophy. PloS one 2013;8:e60113.

75.   Arnold DW, Duque S, Iyer C, et al. Normalization of Patient-Identified Plasma Biomarkers in SMNΔ7 Mice Following Postnatal SMN Restoration. PloS one submited.

76.   Hammond SM, Hazell G, Shabanpoor F, et al. Systemic peptide-mediated oligonucleotide therapy improves long-term survival in spinal muscular atrophy. Proc Natl Acad Sci U S A 2016;113:10962-10967.

77.   Catapano F, Zaharieva I, Scoto M, et al. Altered Levels of MicroRNA-9, -206, and -132 in Spinal Muscular Atrophy and Their Response to Antisense Oligonucleotide Therapy. Molecular therapy Nucleic acids 2016;5:e331.

78.   Buchthal F, Olsen PZ. Electromyography and Muscle Biopsy in Infantile Spinal Muscular Atrophy. Brain 1970;93:15-30.

79.   Hausmanowa-Petrusewicz I, Karwanska A. Electromyographic findings in different forms of infantile and juvenile proximal spinal muscular atrophy. Muscle & nerve 1986;9:37-46.

80.   Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: Relation to age, SMN2 copy number, and function. Annals of neurology 2005;57:704-712.

81.   Lewelt A, Krosschell KJ, Scott C, et al. Compound muscle action potential and motor function in children with spinal muscular atrophy. Muscle & nerve 2010;42:703-708.

82.   Kang PB, Gooch CL, McDermott MP, et al. The motor neuron response to SMN1 deficiency in spinal muscular atrophy. Muscle & nerve 2013.

83.   McComas AJ, Fawcett PR, Campbell MJ, Sica RE. Electrophysiological estimation of the number of motor units within a human muscle. Journal of neurology, neurosurgery, and psychiatry 1971;34:121-131.

84.   Galea V, Fehlings D, Kirsch S, McComas A. Depletion and sizes of motor units in spinal muscular atrophy. Muscle & nerve 2001;24:1168-1172.

85.   Bromberg MB, Swoboda KJ. Motor unit number estimation in infants and children with spinal muscular atrophy. Muscle & nerve 2002;25:445-447.

86.   Zerres K, Rudnik-Schoneborn S. Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications. Archives of neurology 1995;52:518-523.

87.   Finkel RS. Electrophysiological and motor function scale association in a pre-symptomatic infant with spinal muscular atrophy type I. Neuromuscular Disorders 2013;23:112-115.

88.   Russman BS, Melchreit R, Drennan JC. Spinal muscular atrophy: the natural course of disease. Muscle & nerve 1983;6:179-181.

89.   Iannaccone ST, Russman BS, Browne RH, Buncher CR, White M, Samaha FJ. Prospective analysis of strength in spinal muscular atrophy. DCN/Spinal Muscular Atrophy Group. Journal of child neurology 2000;15:97-101.

90.   Crawford TO, Paushkin SV, Kobayashi DT, et al. Evaluation of SMN protein, transcript, and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. PloS one 2012;7:e33572.

91.   Zaworski P, von Herrmann KM, Taylor S, et al. SMN Protein Can Be Reliably Measured in Whole Blood with an Electrochemiluminescence (ECL) Immunoassay: Implications for Clinical Trials. PloS one 2016;11:e0150640.

92.   Kolb SJ, Coffey CS, Yankey JW, et al. Baseline results of the NeuroNEXT spinal muscular atrophy infant biomarker study. Annals of clinical and translational neurology 2016;3:132-145.

93.   Czech C, Tang W, Bugawan T, et al. Biomarker for Spinal Muscular Atrophy: Expression of SMN in Peripheral Blood of SMA Patients and Healthy Controls. PloS one 2015;10:e0139950.

94.   Chiriboga CA, Swoboda KJ, Darras BT, et al. Results from a phase 1 study of nusinersen (ISIS-SMNRx) in children with spinal muscular atrophy. Neurology 2016;86:890-897.

95.   Simard LR, Belanger MC, Morissette S, Wride M, Prior TW, Swoboda KJ. Preclinical validation of a multiplex real-time assay to quantify SMN mRNA in patients with SMA. Neurology 2007;68:451-456.

96.   Tiziano FD, Lomastro R, Di Pietro L, et al. Clinical and molecular cross-sectional study of a cohort of adult type III spinal muscular atrophy patients: clues from a biomarker study. European journal of human genetics : EJHG 2013;21:630-636.

97.   Sumner CJ, Kolb SJ, Harmison GG, et al. SMN mRNA and protein levels in peripheral blood: biomarkers for SMA clinical trials. Neurology 2006;66:1067-1073.

98.   Vezain M, Saugier-Veber P, Melki J, et al. A sensitive assay for measuring SMN mRNA levels in peripheral blood and in muscle samples of patients affected with spinal muscular atrophy. European journal of human genetics : EJHG 2007;15:1054-1062.

99.   Naryshkin NA, Weetall M, Dakka A, et al. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science 2014;345:688-693.

100.   Lutz CM, Kariya S, Patruni S, et al. Postsymptomatic restoration of SMN rescues the disease phenotype in a mouse model of severe spinal muscular atrophy. J Clin Invest 2011;121:3029-3041.

101.   Le TT, McGovern VL, Alwine IE, et al. Temporal requirement for high SMN expression in SMA mice. Hum Mol Genet 2011;20:3578-3591.

102.   Duque SI, Arnold WD, Odermatt P, et al. A large animal model of spinal muscular atrophy and correction of phenotype. Annals of neurology 2015;77:399-414.

103.   Finkel RS. Electrophysiological and motor function scale association in a pre-symptomatic infant with spinal muscular atrophy type I. Neuromuscular disorders : NMD 2013;23:112-115.

104.   Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: relation to age, SMN2 copy number, and function. Annals of neurology 2005;57:704-712.

105.   Bertini E, Burghes A, Bushby K, et al. 134th ENMC International Workshop: Outcome Measures and Treatment of Spinal Muscular Atrophy, 11-13 February 2005, Naarden, The Netherlands. Neuromuscular disorders : NMD 2005;15:802-816.

106.   Cobben JM, Lemmink HH, Snoeck I, Barth PA, van der Lee JH, de Visser M. Survival in SMA type I: a prospective analysis of 34 consecutive cases. Neuromuscular disorders : NMD 2008;18:541-544.

107.   Wang CH, Lunn MR. Spinal muscular atrophy: advances in research and consensus on care of patients. Current treatment options in neurology 2008;10:420-428.

108.   Brandt S. Werdnig–Hoffmann's infantile progressive muscular atrophy. Opera ex domo biologiae hereditariae humanae universitatis hafniensis, vol. 22.
Copenhagen: Ejnar Munksgaard1950.

109.   Bladen CL, Thompson R, Jackson JM, et al. Mapping the differences in care for 5,000 spinal muscular atrophy patients, a survey of 24 national registries in North America, Australasia and Europe. Journal of neurology 2014;261:152-163.

110.   Gregoretti C, Ottonello G, Chiarini Testa MB, et al. Survival of patients with spinal muscular atrophy type 1. Pediatrics 2013;131:e1509-1514.

111.   Oskoui M, Levy G, Garland CJ, et al. The changing natural history of spinal muscular atrophy type 1. Neurology 2007;69:1931-1936.

112.   Ge X, Bai J, Lu Y, Qu Y, Song F. The natural history of infant spinal muscular atrophy in China: a study of 237 patients. Journal of child neurology 2012;27:471-477.

113.   Glanzman AM, Mazzone E, Main M, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. Neuromuscular disorders : NMD 2010;20:155-161.

114.   Krosschell KJ, Maczulski JA, Scott C, et al. Reliability and validity of the TIMPSI for infants with spinal muscular atrophy type I. Pediatric physical therapy : the official publication of the Section on Pediatrics of the American Physical Therapy Association 2013;25:140-148; discussion 149.

115.   De Sanctis R, Coratti G, Pasternak A, et al. Developmental milestones in type I spinal muscular atrophy. Neuromuscular disorders : NMD 2016;26:754-759.

116.   Durkin ET, Schroth MK, Helin M, Shaaban AF. Early laparoscopic fundoplication and gastrostomy in infants with spinal muscular atrophy type I. Journal of pediatric surgery 2008;43:2031-2037.

117.   Ioos C, Leclair-Richard D, Mrad S, Barois A, Estournet-Mathiaud B. Respiratory capacity course in patients with infantile spinal muscular atrophy. Chest 2004;126:831-837.

118.   Chatwin M, Bush A, Simonds AK. Outcome of goal-directed non-invasive ventilation and mechanical insufflation/exsufflation in spinal muscular atrophy type I. Archives of disease in childhood 2011;96:426-432.

119.   Hardart MK, Burns JP, Truog RD. Respiratory support in spinal muscular atrophy type I: a survey of physician practices and attitudes. Pediatrics 2002;110:e24.

120.   Han YJ, Park JD, Lee B, et al. Home mechanical ventilation in childhood-onset hereditary neuromuscular diseases: 13 years' experience at a single center in Korea. PloS one 2015;10:e0122346.

121.   Birnkrant DJ, Pope JF, Martin JE, Repucci AH, Eiben RM. Treatment of type I spinal muscular atrophy with noninvasive ventilation and gastrostomy feeding. Pediatric neurology 1998;18:407-410.

122.   Finkel RS, Weiner DJ, Mayer OH, McDonough JM, Panitch HB. Respiratory muscle function in infants with spinal muscular atrophy type I. Pediatric pulmonology 2014;49:1234-1242.

123.   Tangsrud SE, Carlsen KC, Lund-Petersen I, Carlsen KH. Lung function measurements in young children with spinal muscle atrophy; a cross sectional survey on the effect of position and bracing. Archives of disease in childhood 2001;84:521-524.

124.   Lemoine TJ, Swoboda KJ, Bratton SL, Holubkov R, Mundorff M, Srivastava R. Spinal muscular atrophy type 1: are proactive respiratory interventions associated with longer survival? Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies 2012;13:e161-165.

125.   Finkel RS, Hynan LS, Glanzman AM, et al. The test of infant motor performance: reliability in spinal muscular atrophy type I. Pediatric physical therapy : the official publication of the Section on Pediatrics of the American Physical Therapy Association 2008;20:242-246.

126.   Piper MC, Darrah J. Motor Assessment of the Developing Infant: Saunders, 1994.

127.   Blanchard Y, Neilan E, Busanich J, Garavuso L, Klimas D. Interrater reliability of early intervention providers scoring the alberta infant motor scale. Pediatric physical therapy : the official publication of the Section on Pediatrics of the American Physical Therapy Association 2004;16:13-18.

128.   Finkel RS, Bishop KM, Nelson RM. Spinal Muscular Atrophy Type I: Is It Ethical to Standardize Supportive Care Intervention in Clinical Trials? Journal of child neurology 2016.

129.   Dubowitz V. Chaos in the classification of SMA: a possible resolution. Neuromuscular disorders : NMD 1995;5:3-5.

130.   Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society 2003;7:155-159.

131.   Zerres K, Rudnik-Schoneborn S, Forrest E, Lusakowska A, Borkowska J, Hausmanowa-Petrusewicz I. A collaborative study on the natural history of childhood and juvenile onset proximal spinal muscular atrophy (type II and III SMA): 569 patients. Journal of the neurological sciences 1997;146:67-72.

132.   Farrar MA, Vucic S, Johnston HM, du Sart D, Kiernan MC. Pathophysiological insights derived by natural history and motor function of spinal muscular atrophy. The Journal of pediatrics 2013;162:155-159.

133.   Mannaa MM, Kalra M, Wong B, Cohen AP, Amin RS. Survival probabilities of patients with childhood spinal muscle atrophy. Journal of clinical neuromuscular disease 2009;10:85-89.

134.   Souchon F, Simard LR, Lebrun S, Rochette C, Lambert J, Vanasse M. Clinical and genetic study of chronic (types II and III) childhood onset spinal muscular atrophy. Neuromuscular disorders : NMD 1996;6:419-424.

135.   Deymeer F, Serdaroglu P, Parman Y, Poda M. Natural history of SMA IIIb: muscle strength decreases in a predictable sequence and magnitude. Neurology 2008;71:644-649.

136.   Werlauff U, Vissing J, Steffensen BF. Change in muscle strength over time in spinal muscular atrophy types II and III. A long-term follow-up study. Neuromuscular disorders : NMD 2012;22:1069-1074.

137.   Kaufmann P, McDermott MP, Darras BT, et al. Observational study of spinal muscular atrophy type 2 and 3: functional outcomes over 1 year. Archives of neurology 2011;68:779-786.

138.   Kaufmann P, McDermott MP, Darras BT, et al. Observational study of spinal muscular atrophy type 2 and 3: functional outcomes over 1 year. Arch Neurol 2011;68:779-786.

139.   Kaufmann P, McDermott MP, Darras BT, et al. Prospective cohort study of spinal muscular atrophy types 2 and 3. Neurology 2012;79:1889-1897.

140.   Barois A, Mayer M, Desguerre I, et al. [Spinal muscular atrophy. A 4-year prospective, multicenter, longitudinal study (168 cases)]. Bulletin de l'Academie nationale de medecine 2005;189:1181-1198; discussion 1198-1189.

141.   Vuillerot C, Payan C, Iwaz J, Ecochard R, Berard C, Group MFMSMAS. Responsiveness of the motor function measure in patients with spinal muscular atrophy. Archives of physical medicine and rehabilitation 2013;94:1555-1561.

142.   Mercuri E, Finkel R, Montes J, et al. Patterns of disease progression in type 2 and 3 SMA: Implications for clinical trials. Neuromuscular disorders : NMD 2016;26:126-131.

143.   Iannaccone ST, Browne RH, Samaha FJ, Buncher CR. Prospective study of spinal muscular atrophy before age 6 years. DCN/SMA Group. Pediatric neurology 1993;9:187-193.

144.   Russman BS, Buncher CR, White M, Samaha FJ, Iannaccone ST. Function changes in spinal muscular atrophy II and III. The DCN/SMA Group. Neurology 1996;47:973-976.

145.   Samaha FJ, Buncher CR, Russman BS, et al. Pulmonary function in spinal muscular atrophy. Journal of child neurology 1994;9:326-329.

146.   Steffensen BF, Lyager S, Werge B, Rahbek J, Mattsson E. Physical capacity in non-ambulatory people with Duchenne muscular dystrophy or spinal muscular atrophy: a longitudinal study. Developmental medicine and child neurology 2002;44:623-632.

147.   Wang CH, Finkel RS, Bertini ES, et al. Consensus statement for standard of care in spinal muscular atrophy. Journal of child neurology 2007;22:1027-1049.

148.   Mercuri E, Bertini E, Iannaccone ST. Childhood spinal muscular atrophy: controversies and challenges. The Lancet Neurology 2012;11:443-452.

149.   Iannaccone ST, American Spinal Muscular Atrophy Randomized Trials G. Outcome measures for pediatric spinal muscular atrophy. Archives of neurology 2002;59:1445-1450.

150.   Mazzone E, Bianco F, Martinelli D, et al. Assessing upper limb function in nonambulant SMA patients: development of a new module. Neuromuscular disorders : NMD 2011;21:406-412.

151.   Montes J, McDermott MP, Martens WB, et al. Six-Minute Walk Test demonstrates motor fatigue in spinal muscular atrophy. Neurology 2010;74:833-838.

152.   O'Hagen JM, Glanzman AM, McDermott MP, et al. An expanded version of the Hammersmith Functional Motor Scale for SMA II and III patients. Neuromuscular disorders : NMD 2007;17:693-697.

153.   Mazzone E, Bianco F, Main M, et al. Six minute walk test in type III spinal muscular atrophy: a 12month longitudinal study. Neuromuscular disorders : NMD 2013;23:624-628.

154.   Mazzone E, De Sanctis R, Fanelli L, et al. Hammersmith Functional Motor Scale and Motor Function Measure-20 in non ambulant SMA patients. Neuromuscular disorders : NMD 2014;24:347-352.

155.   Mercuri E, Mazzone E. Choosing the right clinical outcome measure: from the patient to the statistician and back. Neuromuscular disorders : NMD 2011;21:16-19.

156.   Mercuri E, Messina S, Battini R, et al. Reliability of the Hammersmith functional motor scale for spinal muscular atrophy in a multicentric study. Neuromuscular disorders : NMD 2006;16:93-98.

157.   Krosschell KJ, Maczulski JA, Crawford TO, Scott C, Swoboda KJ. A modified Hammersmith functional motor scale for use in multi-center research on spinal muscular atrophy. Neuromuscular disorders : NMD 2006;16:417-426.

158.   Krosschell KJ, Scott CB, Maczulski JA, et al. Reliability of the Modified Hammersmith Functional Motor Scale in young children with spinal muscular atrophy. Muscle & nerve 2011;44:246-251.

159.   Berard C, Fermanian J, Payan C. Outcome measure for SMA II and III patients. Neuromuscular disorders : NMD 2008;18:593-594; author reply 594-595.

160.   Berard C, Payan C, Hodgkinson I, Fermanian J, Group MFMCS. A motor function measure for neuromuscular diseases. Construction and validation study. Neuromuscular disorders : NMD 2005;15:463-470.

161.   de Lattre C, Payan C, Vuillerot C, et al. Motor Function Measure: Validation of a Short Form for Young Children With Neuromuscular Diseases. Archives of physical medicine and rehabilitation 2013.

162.   Finkel R, Bertini E, Muntoni F, Mercuri E, Group ESWS. 209th ENMC International Workshop: Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy 7-9 November 2014, Heemskerk, The Netherlands. Neuromuscular disorders : NMD 2015;25:593-602.

163.   Vuillerot C, Girardot F, Payan C, et al. Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. Developmental medicine and child neurology 2010;52:60-65.

164.   Vuillerot C, Payan C, Girardot F, et al. Responsiveness of the motor function measure in neuromuscular diseases. Archives of physical medicine and rehabilitation 2012;93:2251-2256 e2251.

165.   Mazzone ES, Mayhew A, Montes J, et al. Revised upper limb module for spinal muscular atrophy: Development of a new module. Muscle & nerve 2016.

166.   Glanzman AM, O'Hagen JM, McDermott MP, et al. Validation of the Expanded Hammersmith Functional Motor Scale in spinal muscular atrophy type II and III. Journal of child neurology 2011;26:1499-1507.

167.   Mercuri E, Bertini E, Messina S, et al. Pilot trial of phenylbutyrate in spinal muscular atrophy. Neuromuscular disorders : NMD 2004;14:130-135.

168.   Mercuri E, Bertini E, Messina S, et al. Randomized, double-blind, placebo-controlled trial of phenylbutyrate in spinal muscular atrophy. Neurology 2007;68:51-55.

169.   Pane M, Staccioli S, Messina S, et al. Daily salbutamol in young patients with SMA type II. Neuromuscular disorders : NMD 2008;18:536-540.

170.   Swoboda KJ, Scott CB, Crawford TO, et al. SMA CARNI-VAL trial part I: double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in spinal muscular atrophy. PLoS one 2010;5:e12140.

171.   Swoboda KJ, Scott CB, Reyna SP, et al. Phase II open label study of valproic acid in spinal muscular atrophy. PloS one 2009;4:e5268.

172.   Dunaway Young S, Montes J, Kramer SS, et al. Six-minute walk test is reliable and valid in spinal muscular atrophy. Muscle & nerve 2016;54:836-842.

173.   Montes J, Dunaway S, Montgomery MJ, et al. Fatigue leads to gait changes in spinal muscular atrophy. Muscle & nerve 2011;43:485-488.
174.   Montes J, Blumenschine M, Dunaway S, et al. Weakness and fatigue in diverse neuromuscular diseases. Journal of child neurology 2013;28:1277-1283.
175.   Dunaway S, Montes J, Garber CE, et al. Performance of the timed "up & go" test in spinal muscular atrophy. Muscle & nerve 2014;50:273-277.
176.   Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. Journal of the American Geriatrics Society 1991;39:142-148.
177.   Andrich D. Rating scales and Rasch measurement. Expert review of pharmacoeconomics & outcomes research 2011;11:571-585.
178.   Rasch G. Probabilistic models for some intelligence and attainment tests. Copenhagen: Danish Institute for Education Research. Chicago, IL: The University of Chicago Press, 1980.
179.   Hobart JC, Cano SJ, Zajicek JP, Thompson AJ. Rating scales as outcome measures for clinical trials in neurology: problems, solutions, and recommendations. The Lancet Neurology 2007;6:1094-1105.
180.   Hobart J, Cano S. Improving the evaluation of therapeutic interventions in multiple sclerosis: the role of new psychometric methods. Health technology assessment 2009;13:iii, ix-x, 1-177.
181.   Trials. ICCfSC. Meeting of the FDA SEALD Division and Representatives of the International Coordinating Committee for SMA Clinical Trials. 2010; New York.
182.   Cano SJ, Mayhew A, Glanzman AM, et al. Rasch analysis of clinical outcome measures in spinal muscular atrophy. Muscle & nerve 2014;49:422-430.
183.   Batcho CS, Van den Bergh PY, Van Damme P, et al. How robust is ACTIVLIM for the follow-up of activity limitations in patients with neuromuscular diseases? Neuromuscular disorders : NMD 2016;26:211-220.
184.   Montes J, Glanzman AM, Mazzone ES, et al. Spinal muscular atrophy functional composite score: A functional measure in spinal muscular atrophy. Muscle & nerve 2015;52:942-947.
185.   Seferian AM, Moraux A, Canal A, et al. Upper limb evaluation and one-year follow up of non-ambulant patients with spinal muscular atrophy: an observational multicenter trial. PloS one 2015;10:e0121799.
186.   Chabanon A PY, Daron A, Cances C, Vuillerot C, Fontaine S, Goemans N, De Waele L, Laugel V, Cuisset JM, Schara U, Gangfuss A, Gidaro T, Gargaun E, Marquet A, Villeret M, Phelep A, Annoussamy M, Servais L. Baseline data from a European prospective and longitudinal natural history study of patients with type 2 and 3 spinal muscular atrophy – NatHis-SMA. Neuromuscular disorders : NMD 2016;26.
187.   Gawel M, Kostera-Pruszczyk A, Lusakowska A, et al. Motor unit loss estimation by the multipoint incremental MUNE method in children with spinal muscular atrophy--a preliminary study. Neuromuscular disorders : NMD 2015;25:216-221.
188.   Sproule DM, Punyanitya M, Shen W, et al. Muscle volume estimation by magnetic resonance imaging in spinal muscular atrophy. Journal of child neurology 2011;26:309-317.
189.   Durmus H, Yilmaz R, Gulsen-Parman Y, et al. Muscle mri in spinal muscular atrophy 3: Selective and progressive involvement. Muscle & nerve 2016.
190.   Ogino S, Wilson RB, Gold B. New insights on the evolution of the SMN1 and SMN2 region: simulation and meta-analysis for allele and haplotype frequency calculations. European journal of human genetics : EJHG 2004;12:1015-1023.
191.   Sarkozy A, Bushby K, Beroud C, Lochmuller H. 157th ENMC International Workshop: patient registries for rare, inherited muscular disorders 25-27 January 2008 Naarden, The Netherlands. Neuromuscular disorders : NMD 2008;18:997-1001.
192.   Rodger S, Lochmuller H, Tassoni A, et al. The TREAT-NMD care and trial site registry: an online registry to facilitate clinical research for neuromuscular diseases. Orphanet J Rare Dis 2013;8:171.
193.   Mercuri E, Mayhew A, Muntoni F, et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. Neuromuscular disorders : NMD 2008;18:894-903.
194.   Kayadjanian N, Burghes A, Finkel RS, et al. SMA-EUROPE workshop report: Opportunities and challenges in developing clinical trials for spinal muscular atrophy in Europe. Orphanet J Rare Dis 2013;8:44.
195.   R. USDoHaHSFaDfDa. Guidance for Industry Use in Medical Product Development to Support Labeling Claims Guidance for Industry. . 2009.

196.	Qian Y, McGraw S, Henne J, Jarecki J, Hobby K, Yeh WS. Understanding the experiences and needs of individuals with Spinal Muscular Atrophy and their parents: a qualitative study. BMC neurology 2015;15:217.