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New Therapeutic Approaches to Spinal Muscular Atrophy

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Abstract

Bench to bedside progress has been widely anticipated for a growing number of neurodegenerative disorders. Of these, spinal muscular atrophy (SMA) is perhaps the best poised to capitalize on advances in targeted therapeutics development over the next few years. Several laboratories have achieved compelling success in SMA animal models using sophisticated methods for targeted delivery, repair, or increased expression of the survival motor neuron protein, SMN. The clinical community is actively collaborating to identify, develop, and validate outcome measures and biomarkers in parallel with laboratory efforts. Innovative trial design and synergistic approaches to maximize proactive care in conjunction with treatment with one or more of the promising pharmacologic and biologic therapies currently in the pipeline will maximize our chances to achieve meaningful outcomes for patients. This review highlights recent promising scientific and clinical advances bringing us ever closer to effective treatment(s) for our patients with SMA.

Keywords

Spinal muscular atrophy; SMA; SMN1; SMN2; Therapeutics; Clinical trials; Sensory; Motor; AAV9; Gene therapy; Humans; Animal models; Motor neuron; Spinal cord circuitry; Anterior horn cell; Natural history; Outcomes; Phenotype; Review; RNA splicing; Preclinical

Introduction

The term spinal muscular atrophy (SMA) encompasses a broad spectrum of disorders affecting the motor unit. However, by far the most common of the spinal muscular atrophies is proximal SMA due to a homozygous deletion and/or point mutations of the survival motor neuron gene *SMN1*. Proximal SMA is an autosomal-recessive disorder characterized by

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degeneration of motoneurons of the anterior horn of the spinal cord and brainstem, resulting in progressive muscular weakness, atrophy, respiratory insufficiency, and premature death in the majority of those affected.

SMA is one of the most common neuromuscular disorders, with a birth incidence of at least 1 in 10,000. Proximal SMA is classified into clinical subtypes based on age of disease onset and maximal motor function achieved (Table 1) [1]. A significant challenge in translating treatments successfully from bench to bedside is the early onset of symptoms in the majority of affected individuals. SMA type I, the severe infantile variant, accounts for at least 50% of cases. Those with SMA type II account for another 20% to 30%, with a mean onset of symptoms less than 1 year of age. At first glance, this apparent narrow window of opportunity for therapeutic intervention would seem an insurmountable barrier. However, the increasing potency and efficacy of new therapies have improved outcomes in severely affected animal models and provide great hope that significant progress toward truly effective and life-changing treatment interventions will be realized.

In this review, we explore recent insights gained regarding natural history, epidemiology, and pathophysiology of SMA; highlight particularly exciting and timely advances in both SMN-directed and non-SMN-directed therapeutic approaches; and briefly summarize recent clinical trial efforts, proactive care interventions, and rehabilitative strategies. We aim to complement other excellent recent reviews, including those by Pruss, Shababi, and Kolb, with the intent to extend or supplement their observations [2•, 3•, 4, 5].

Natural History, Epidemiology, and Genotype/Phenotype Correlations

Natural history studies in SMA are invaluable in increasing our understanding of the broad phenotypic spectrum and pattern of disease progression in SMA. A recent retrospective review of 237 cases from China emphasizes the worldwide burden of this disease, and reiterates key concepts regarding the natural history of disease progression [6]. The absence of aggressive respiratory interventions in this population provide valuable natural history data that parallels the important observations of Zerres et al. [7] from nearly two decades ago. Mean ages of onset for types I, II, and III were 3.1, 8.7, and 21.1 months, respectively. Survival probabilities for SMA type I children at 1, 2, and 5 years were 45%, 38%, and 29%. Type I infants with onset after 2 months of age had a significantly increased survival compared to those with earlier onset, reinforcing the prognostic value of age at onset of symptoms, which has been consistently demonstrated across and within subtypes as a significant predictor of phenotypic severity.

Signs and symptoms arising outside the motor unit in SMA animal models have been increasingly recognized. In the past two decades, peripheral sensory nerve involvement, congenital arthrogryposis, congenital bone fractures, and congenital heart defects have been reported in a relatively small proportion of severely affected infants with SMA, most often in those with apparent prenatal onset or early infantile phenotypes [8–11]. Although differences between patients and SMA animal models are to be expected, each new observation in SMA animal models deserves careful consideration of its potential relevance, if any, to human patients. Shababi et al. [12•] recently described developmental cardiac defects in a severely affected mouse model. They further characterized the evolution of pathologic responses including fibrosis and oxidative stress in a less severe model of disease, and concluded that cardiomyopathy is likely contributing to premature death in these animals [12•]. In parallel, Bevan et al. [13] demonstrated early heart failure in the SMN Δ 7 mouse model that was rescued with postnatal scAAV9-SMN delivery. Further study of both human patients and animal models will be required to better understand the

significance of these observations, particularly given the absence of recognized cardiomyopathy in autopsy studies of SMA type I infants and children.

In comparison to other forms of SMA, the breadth of phenotypes encompassed by 5q SMA is striking considering its common origin from a homozygous *SMN1* exon 7 deletion in the majority of those affected. In spite of its classification as a proximal SMA, neurophysiologic studies clearly illustrate profound distal denervation as an early manifestation of the severe infantile phenotype [14]. Wee et al. [15•] recently reviewed progress in defining the clinical and genetic features of at least 16 distinct forms of SMA inclusive of 5q proximal SMA. Each of these disorders presents a unique phenomenology with respect to age at onset, severity, and pattern and distribution of muscle weakness. Ultimately, continuing advances in the characterization of these different forms of SMA and their causative genes, which encode proteins ranging in function from transcriptional regulation to RNA processing to cytoskeletal dynamics, may help to identify shared pathways that contribute to the more severe motor neuron disease phenotype observed in infants with SMA type I.

For now, *SMN2* dosage remains the most potent modifier of phenotype, with an inverse relationship between *SMN2* dosage and disease severity. This observation has been instrumental in driving therapeutics development. Nevertheless, phenotype correlation with *SMN2* dosage remains insufficient for use in clinical practice for prognosis, a point worth emphasizing given the increasingly widespread application of this assay to newly diagnosed patients. Current molecular techniques used to determine *SMN2* dosage typically do not detect intact copies but rather target exon 7 only, providing potentially incomplete genotypic data. Although one must be cautious about using *SMN2* dosage for prognosis, the identification of two *SMN2* copies in a child with early infantile onset, particularly if respiratory muscle weakness is already apparent, is helpful to predict SMA type I.

Several studies in the past year sought to determine whether additional clinical criteria could improve the correlation between *SMN2* dosage and SMA severity within the standard classification of disease subtypes, defined largely by maximum achieved motor function (Table 1). Petit et al. [16] reported correlation between *SMN2* dosage and phenotype in a cohort of 103 patients with homozygous *SMN1* deletion. They characterized disease severity, age at death, loss of ambulation, and brainstem involvement (defined as the presence of facial weakness, chewing or swallowing difficulty, dysphonic voice, and/or tongue fasciculation with lingual atrophy). Among the three SMA types, *SMN2* dosage was significantly higher when these manifestations were absent. However, the presence of brainstem involvement did not correlate with *SMN2* dosage within a specific SMA cohort. For patients with SMA type I, there was a strong correlation between survival time and *SMN2* dosage, and for patients with SMA type III, loss of ambulation correlated with a significantly lower number of *SMN2* copies. Among patients able to walk, ambulation conservation, but not the age at which ambulation was lost, correlated with *SMN2* dosage [16].

In 2008, Oprea et al. [17] first proposed plastin 3 (PLS3) as a phenotypic modifier, when they discovered that *SMN1*-deleted female siblings appeared to be protected from disease progression in association with higher PLS3 expression. Stratigopoulos et al. [18] recently further explored the association of PLS3 expression with disease severity in a cohort of 88 patients with SMA types I, II, and III by measuring mRNA levels and examining the relationship to SMA type and *SMN2* copy number. Although younger patients demonstrated generally higher levels, PLS3 levels correlated with SMA type, *SMN2* copy number, and gross motor function only in post pubertal females. Further studies are clearly warranted to explore the relationship of PLS3 expression to age, gender, disease phenotype and progression.

Population-based carrier screening remains controversial, in large part due to the increasing complexity of options for genetic screening in the prenatal setting. However, improved technologies will continue to enhance opportunities for early diagnosis and counseling. Ben-Shachar et al. [19] reviewed the clinical implications of large-scale population carrier screening for SMA. Screening of 6000 individuals resulted in detection of 159 with a heterozygous deletion of SMN1 exon 7 in the absence of a family history of SMA. In 11%, two or more SMN1 exon 7 copies were in a cis configuration, confirming results from prior studies that a certain percentage of deletion carriers will be missed using multiplex ligation-dependent probe amplification or similar quantitative assays. Nevertheless, their observation of a carrier frequency of 1 in 40 supports population-based carrier screening for SMA [19].

Recent Advances in SMA Pathophysiology

SMN is critical for the biogenesis of small nuclear ribonucleoprotein particles (snRNPs) involved in spliceosomal assembly. Thus, multiple downstream targets for aberrant splicing could impact cellular function and contribute to disease pathogenesis; however, the critical downstream target(s) remain elusive. The relative selective vulnerability of motor neurons continues to provoke ongoing speculation, although it is increasingly apparent that, at least in infants with type I SMA, cells besides the lower motor neuron (LMN) are affected. The importance of non-LMN related symptoms as relevant therapeutic targets remains a point of controversy. Investigators over the past decade have performed a variety of complex experiments to determine when and where SMN expression is critical, and which tissues besides motor neurons are impacted by SMN deficiency, thus influencing the phenotype. Controversy about when and where the sentinel event occurs within the motor neuron, and whether it is the cell body, axon, or neuromuscular junction, remains as a topic of lively discussion. Ongoing speculation about contributions from skeletal muscle and its relevance as a therapeutic target also persist.

Arguably the most important new observation concerning pathophysiology in SMA relates to recent publications describing synaptic defects in the spinal and neuromuscular neuronal circuitry, with clear documentation of functional impairment of sensory-motor connectivity in the SMN Δ 7 mouse model. Ling et al. [20••] examined the neuromuscular circuitry as it pertains to hindlimb function, demonstrating that nearly all neuromuscular junctions remained fully innervated at the end stage of disease, and despite a reduced quantal content, were capable of eliciting muscle contraction. However, they observed an estimated 30% loss of synapses onto spinal motor neurons in the lateral columns of the lumbar spinal cord and a clear reduction in proprioceptive sensory neuron input, which they hypothesized contributed to the observed reduction in vesicular glutamate transporter 1-positive synapses. They further demonstrated that this loss of synaptic vesicles occurred progressively, indicating a primary defect in synaptic maintenance rather than a failure to form synapses. They presented a broader conceptualization of the pathogenesis in SMA that involved multilevel synaptic involvement. Mentis et al. [21••] extended these observations by demonstrating decreased proprioceptive reflexes that correlated with a decreased number and function of synapses on motor neuron cell bodies at a relatively early phase of disease pathogenesis. The authors hypothesize that deafferentation of motor neurons is an early event in SMA [20••, 21••].

Figure 1 represents an updated vision of the relevant circuitry involved in disease pathogenesis, indicating not only a motor neuronopathy, but also impaired synaptic feedback of proprioceptor neurons back onto α motor neurons within the spinal cord. In contrast to what is observed in the SMN Δ 7 mouse model, autopsy studies in SMA type I infants clearly show significant motor neuron loss within the spinal cord as well as severely denervated limb muscles. One might speculate that the recently observed cardiomyopathy affecting

these mice could conceivably be contributing to their demise before the full evolution to motor neuron cell death and severe peripheral denervation of limb musculature has occurred. However, the timing of these events as observed in the mouse model, and how they may relate to the process occurring in SMA patients, remains uncertain. An easily measurable electro-physiologic marker of the integrity of proprioceptive input onto motor neurons is the H-reflex. However, unless patients are identified and tested prior to symptom onset, demonstrating parallel pathogenic mechanisms in human patients is challenging, since H-reflexes are often already absent at the time of symptomatic presentation, at least in type I infants [22]. Should newborn screening permit early identification of infants at risk for disease progression, prospective monitoring of H-reflexes could provide compelling evidence to affirm the significance of these observations in the SMA animal model.

Ito et al. [23] performed detailed postmortem neuropathologic analyses on spinal cords obtained at autopsy from two fetuses with SMA, 5 SMA type I infants, a young teen with SMA type I, and an older teen with SMA type II, all with confirmed clinical and genetic diagnoses. Histopathologically, LMNs showed immature profiles characterized by fine Nissl bodies restricted to the periphery of small round somata with a few cell processes in the fetal period, and small-sized profiles in the postnatal period. Based on these observations, they hypothesized that LMNs undergo a reduction in size and number in the fetal period, ballooned neurons appear postnatally, and the remaining LMNs diminish with age. A relative preservation of surviving LMNs was reported in SMA type II compared with the SMA type I cases. One hypothesis is a developmental defect in maturation as well as a postnatal retrograde dying-back degeneration of LMNs in SMN-deficient SMA [23]. Their observations in SMA fetuses support previous observations by Parra et al. [24], who prospectively demonstrated normal fetal movements in the first trimester in those later determined via prenatal or postnatal diagnosis to be genetically affected.

Advances in Therapeutics Development from Preclinical Studies in Cell Culture and in SMA Animal Models

Several compelling advances in treatment have been demonstrated in preclinical studies in the SMA mouse model this past year, building further on advances made in the past few years. Table 2 provides a more comprehensive summary of the increasingly broad range of potential therapeutic strategies that have been identified [2•, 3•, 5, 17, 18, 25–28, 29•, 30–32, 33•, 34••, 35–54]. However, the most compelling examples, or those that represent novel therapeutic approaches, are highlighted below.

Dominguez et al. [25] injected SMN Δ 7 mice on postnatal day 1 with a self-complementary adeno-associated virus (scAAV9) vector containing *SMN1* along with a chimeric intron downstream of the phosphoglycerate kinase promoter to overexpress human SMN, successfully rescuing 100% of these mice, increasing life expectancy from a mean of 13 days to 199 days, with some animals surviving much longer. This intervention also completely rescued motor function and dramatically improved the weight loss phenotype. These results parallel recent observations in two additional laboratories, attesting to the reproducibility and robustness of a single, early delivery of SMN in abrogating disease severity [25–27].

Current SMA mouse models have been limited by their short lifespan, the significant delay in appearance of symptoms, or a subtle phenotype. Gladman et al. [28] recently generated a novel mouse model with a mouse minigene with the C>T mutation in exon 7 present that has been previously associated in humans with a later-onset type III phenotype. The C>T mutation appears to be sufficient to induce exon 7 skipping in the mouse minigene as in the human SMN2. The resulting mice exhibit exon 7 skipping and an adult-onset SMA

phenotype characterized by muscle weakness, decreased activity, and an alteration of the muscle fibers size. This will be yet another potentially valuable tool to assess promising therapeutics, particularly neuroprotective agents.

Bevan et al. [29•] recently reviewed the broader challenges facing systemic gene delivery. The early administration required to achieve such a robust improvement in phenotypic outcomes, however, should make us cautious about assumptions regarding its potential benefit as a therapeutic strategy in already symptomatic patients. Phase 1 studies in human subjects should probably be targeted to type I infants. Careful attention to clinical trial design, and to moderating expectations of parents regarding this extremely promising therapeutic strategy in already symptomatic type I infants, will be critical to the successful execution of clinical trials.

Antisense oligonucleotide (ASO)-based strategies are proving to be one of the most potent contenders in therapeutics development for SMA [30, 31]. Because the critical difference between *SMN1* and *SMN2* is a C-to-T transition in exon 7, and since all SMA patients, regardless of phenotypic severity have one or more *SMN2* copies, SMA is one of the most attractive disease targets for this evolving technology. ASOs are designed to base pair to specific nucleotide sequences, reducing potential off-target effects, particularly when compared with small molecule therapies such as histone deacetylase inhibitors. Much work has occurred over the past few years to identify the best target(s) for increasing full-length SMN (fSMN) by altering splicing of *SMN2*. ASO-10-27 is a specially modified ASO directed to an intronic splice silencer, and has been previously demonstrated in vitro to efficiently increase exon 7 inclusion in targeted transcripts [32]. Passini et al. [33•] recently demonstrated significant benefit of intraventricular delivery of ASO-10-27 to severely affected SMA mice, documenting improved muscle physiology, motor function, and survival. Previously, many in the field considered that central nervous system (CNS) delivery to motor neurons should be sufficient to fully rescue the phenotype across the disease spectrum. However, survival observed in the severe animal models was clearly not as robust as has been demonstrated with systemic adenoviral *SMN1* gene delivery. In another seminal publication, Hua et al. [34••] have now provided unequivocal evidence that peripheral SMN restoration is essential for long-term rescue in severe SMA animal models, further dramatically prolonging survival by using both CNS and systemic delivery. This has important therapeutic implications in humans, specifically for infants with SMA type I.

Biondi [35] recently published data indicating that in vivo NMDA receptor activation robustly extended the lifespan in two different relatively severe SMA mouse models. NMDA receptor activation appears to accelerate maturation of motor units postnatally, reduce apoptosis, and increase SMN expression via an impact on *SMN2* transcription. The pathway implicated in increased SMN expression appeared to involve reactivation of the CaMKII/AKT/CREB pathway. They propose NMDA receptor activation as a novel therapeutic neuroprotective strategy for preventing cell death of motor neurons in SMA.

Xu et al. [36] investigated the mechanism by hydroxyurea (HU) that increases fSMN transcripts in SMA patient-derived lymphocytes. HU had previously been shown to increase fSMN transcript in cultured lymphocytes from patients with SMA. Because nitric oxide (NO) is a major intracellular metabolite of HU, they decided to test whether NO donors themselves could enhance fSMN2 expression. Interestingly, they demonstrated that HU as well as other NO donors appears to enhance *SMN2* expression through the release of NO, and proposed that NO donors may themselves be considered as candidate therapeutics in SMA. However, the significance of these observations remains unclear in light of the absence of clearly demonstrated benefit in either SMA animal model studies or clinical trials to date.

Farooq et al. [37] demonstrated that the delivery of a novel therapy, prolactin, increases SMN expression in both mouse models of SMA and human cell lines via the STAT5 pathway. In a severe SMA animal model, subcutaneously delivered prolactin to upregulate SMN in brain and spinal cord resulted in a marked improvement in motor function, but only modest improvement in survival. Further studies are needed to understand the role of the STAT5 pathway as a therapeutic target in SMA.

Because patients present at various stages of disease progression and SMN restoration therapy in individuals with SMA will undoubtedly occur after symptom onset unless newborn screening becomes a reality, a key question in the field has been to better define when SMN upregulation or replacement is necessary to observe benefit. This issue is particularly important because the most dramatic rescue to date in SMA animal models has occurred with very early delivery, creating uncertainties about the viability of these therapies in already symptomatic patients. Lutz et al. [38] generated SMA model mice harboring an inducible *Smn* rescue allele. Inducing expression of SMN to an estimated 40% of wild-type levels following onset of symptoms at postnatal day 4 in an animal model of severe SMA resulted in a profound improvement in motor function and survival, providing additional confidence that delivery of SMN-directed therapeutics in already symptomatic patients is a viable therapeutic strategy, although clearly interpreting how these observations will relate to patients in clinical trials remains a challenge.

Jodelka et al. [39] identified an apparent feedback loop in SMN expression in which SMN protein abundance appears to affect the splicing of the SMN2 exon 7. Cells depleted of SMN, as well as patient-derived cells, demonstrated reduced SMN protein levels that correlated with decreases in cellular small nuclear RNA levels and SMN2 exon 7 splicing. Altering levels of individual snRNPs distinctly influenced SMN2 alternative splicing. These results indicate that a modest increase in SMN protein may cause a disproportionately larger increase in SMN expression. Understanding this mechanism may prove profoundly important for assessing the relative value of new therapies, which can capitalize best on this mechanism.

Stem cell technologies continue to prove fruitful for the investigation of motor neuron diseases, including SMA, and have potential treatment value via a variety of proposed mechanisms. Lunn et al. [5] comprehensively reviewed the different stem cell tools and strategies currently being utilized for mechanistic discovery and therapeutic development, including embryonic, adult, and induced pluripotent stem cells. They highlighted key issues that must be considered for the effective translation of stem cell therapies from bench to bedside, and reviewed preliminary data from the first-in-human phase 1 trial currently underway in patients with amyotrophic lateral sclerosis as a broader model for treatment intervention using such technologies in other motor neuron diseases, including SMA.

Recent Lessons from Clinical Trials and Studies in SMA

In interpreting results from clinical trials, it is important to carefully consider the strengths and limitations of each, including the potential power of any given trial to answer the specific questions posed by investigators. In particular, conclusions must be restricted to the scenario in which a given therapeutic intervention was applied to be rigorous, and to avoid being either overly optimistic, or pessimistic, about potential therapeutic value for a particular agent. Over the past few years, the synergistic collaboration of clinical investigators internationally has resulted in tremendous progress in our experience with a variety of outcome measures in diverse SMA populations that should prove instrumental in achieving success in the years ahead.

Three studies examining safety and/or efficacy of valproic acid (VPA) in various SMA populations have been reported in the past 2 years [40–42]. Swoboda et al. [40] reported the results of SMA CARNI-VAL Trial Part I, a double-blind, randomized, placebo-controlled trial of L-carnitine and VPA in nonambulatory SMA subjects 2 to 8 years of age. Sixty-one subjects were randomized 1:1 to placebo or treatment for the first 6 months; all received active treatment the subsequent 6 months. The primary outcome was a change in the MHFMS (Modified Hammer-smith Functional Motor Scale) score following 6 months of treatment. This study demonstrated no benefit from 6 months treatment. Weight gain, age, and treatment duration were clearly significant confounding variables. Post hoc analysis appeared to demonstrate potential benefit of treatment in the youngest children who received treatment over a 12-month period. Ideally, a phase 3 study focusing on an even younger cohort of children assessed over a full year would be desirable to further evaluate these observations. However, given that this medication is readily available for other uses, and other more potent agents may be on the horizon, such a study may prove challenging to realize.

Kissel et al. [41] reported the results of SMA CARNIVAL Trial Part II, a prospective, open-label study of L-carnitine and VPA in 33 ambulatory children ages 3 to 17 years, who were followed on treatment for a full year. The treatment regimen appeared safe and generally well tolerated, but weight gain was clearly a significant issue in a substantial proportion of participants. When considered in parallel with the apparent lack of improvement in motor function over a 12-month period of observation, enthusiasm for a phase 3 study, at least in this population, is low. However, this trial was extremely fruitful in demonstrating that the outcomes measures utilized, especially the MHFMS-Extend and the timed tests of motor function, were feasible, and were reliable and stable over a 12-month period, emphasizing their potential usefulness in ambulatory children with SMA across a wide age range.

Darbar et al. [42] reported their observations from an open-label trial of VPA in 22 patients with type II and III SMA, 2 to 18 years of age. Muscle strength and motor abilities were evaluated five times during a 1-year period. Motor function, as assessed using the HFMS (Hammer-smith Functional Motor Scale), showed modest improvement in the type II cohort; other measures were largely stable. The open-label nature of this study and the heterogeneity of the population preclude any conclusions regarding efficacy, but further expand our experience with a variety of outcome measures in these populations.

Chen et al. [43] reported the results of a randomized, double-blind, placebo-controlled trial of HU in which 28 SMA type II and 29 SMA type III subjects were randomized to drug or matching placebo in a 2:1 ratio. Dosing was titrated to 20 mg/kg/day, and followed frequently over a period of 18 months. The drug was generally well tolerated, and aside from neutropenia, adverse events did not differ between treatment and placebo groups. There was no clear difference in the primary outcome measures of strength (Manual Muscle Test), motor function (Gross Motor Function Measure), or fISMN transcript levels between cohorts. This very well executed trial, in which HU was administered in a fairly large, well-characterized cohort of patients who were compliant with dosing over a sufficient time frame to observe potential beneficial effect, provides Class I evidence that HU 20 mg/kg/day does not clearly alter strength, motor function, or fISMN mRNA levels in SMA.

Recent studies have suggested that SMN expression can be enhanced in vitro by salbutamol, a β_2 -adrenergic agonist. Tiziano et al. [44] evaluated the in vivo efficacy of salbutamol on increasing SMN levels in a pilot study of 12 type II and type III SMA patients who received treatment continuously over a period of 6 months. A significant and consistent increase in fISMN transcripts was proportional to *SMN2* copy number, suggesting that *SMN2* dosage might predict the molecular response to treatment. Their data support consideration of

salbutamol as a candidate for further assessment in a randomized, placebo-controlled phase 3 study.

New Tools and Outcome Measures for SMA Clinical Studies and Trials

Reliable outcome measures that reflect the underlying disease process and correlate with motor function in children with SMA are needed to better understand the natural history of disease progression and for use in demonstrating efficacy for new therapies. Currently, there is no single outcome measure that can assess the severity of the illness, provide prognostic information, document disease progression, and clearly respond to change. However, we highlight here progress in the continued identification and evaluation of promising outcome measures that are worthy of further investigation for use in the clinical care setting, the clinical trial setting, or both.

We described the performance of the ulnar maximum compound muscle action potential (CMAP) as an outcome measure for use in multicenter clinical trials in children with SMA ages 2 to 17 years, and evaluated the correlation between CMAP and gross motor function [55•]. CMAP proved reliable and easy to implement in the multicenter clinical trial setting, correlated well with motor function as measured using the MHFMS-Extend, and appears to have potential as a relevant surrogate for innervation and thus disease burden in patients with SMA. An evaluation of CMAP over time in individuals with SMA is ongoing and necessary to further understand this measure's utility in clinical and research settings.

Rutkove et al. [56] assessed the potential application of electrical impedance myography (EIM) in children with SMA. Preliminary results confirm that EIM can accurately distinguish patients with SMA type II from those with SMA type III. Because EIM requires no patient effort and is easy to apply, it has the potential to serve as a useful study outcome measure as well as an indicator of disease status in individuals with SMA. However, a significant limitation is the lack of change over time, at least as demonstrated in these initial studies. Additional studies are needed to further assess utility of EIM in SMA.

Sproule et al. [57] evaluated the potential use of MRI to assess muscle volume. Thigh muscle volume was assessed in 16 individuals with SMA types I, II, and III as young as 5 years of age. The documented reproducibility, tolerability, and strong correlation with clinical measures make MRI a candidate biomarker for clinical research. Additional longitudinal data are needed to confirm the reproducibility of their technique, as well as to document change over time in muscles of individuals with SMA. Pending further validation, MRI offers appealing potential as a biomarker and technique for assessing body composition in SMA in some settings.

Wu et al. [58] assessed the ability of quantitative ultrasound to evaluate muscles of 25 individuals with SMA types II and III, and compare between these two subtypes as well as with 21 normal subjects. Luminosity values for each muscle and the overlying subcutaneous fat were obtained, and a luminosity ratio was calculated by dividing the muscle by the subcutaneous fat luminosity. This ratio correlated well with strength, and discriminated between SMA type II and type III subtypes. Quantitative ultrasound is well tolerated, requires minimal time, and patient cooperation; therefore, it may be considered as a viable outcome measure in future SMA clinical trials.

Krosschell et al. [59•] assessed the reliability of the MHFMS in children less than 30 months of age with SMA, to complement existing published data on the performance of this outcome measure in older children. Excellent test-retest stability of MHFMS scores in young children with SMA type II suggests that the MHFMS can be used reliably in a younger SMA population for clinical trials and follow-up [58].

Montes et al. [60] performed a cross-sectional study using quantitative gait analysis during the 6-min walk test (6MWT), an increasingly popular measure of gross motor function and fatigue in ambulatory patients with SMA. Gait parameters of speed and dynamic balance were correlated with 6MWT distance in nine children and adults with SMA and nine controls. Gait analysis using the GAITRite system (CIR Systems, Havertown, PA) appeared to be a useful complement to the 6MWT and it offered a quantitative assessment of fatigue related changes in SMA.

New Information Relevant to Clinical Care in SMA

Obesity is a common problem in SMA and often directly contributes to a decline in function in older children and adults with ambulatory and nonambulatory forms of SMA. However, clinicians and even dietitians routinely underestimate the frequency and degree of obesity in their SMA patients. Plotting basic anthropometric measures (weight, height) on growth curves developed for use in healthy children often results in inaccurate and misleading information. Due to their almost universally substantially diminished lean body mass, children with SMA may have average fat mass even in the setting of significantly low weight for age. Dual-energy x-ray absorptiometry (DXA) is the gold standard for assessment of fat and lean body mass in clinical research. However, accessibility for its use clinically in assessing body composition may be limited at many centers. Sproule et al. [61] compared a painless and noninvasive method, bioelectrical impedance analysis, to the reference method, DXA, in 16 patients with SMA types II and III and proposed that bioelectrical impedance analysis may be of value as a screening tool for obesity in SMA.

Chatwin et al. [62] report their experience in SMA type I patients implementing a noninvasive respiratory care management protocol in the United Kingdom to control symptoms and facilitate care at home. They affirm previously published reports of the perceived benefit of using noninvasive positive pressure ventilation in facilitating the ability of parents to make informed care decisions in the outpatient setting [62].

SMA patients gradually lose muscle strength and function over time [63]. Rehabilitative interventions provide a complementary approach to proactive nutritional and respiratory management to help delay or prevent secondary impairments including muscle contractures, to slow the progression of the disease, and to maintain, or potentially improve, strength and motor function. Rehabilitative interventions in SMA include physical therapy, occupational therapy, speech therapy, use of orthotics and other adaptive equipment, bracing, strengthening and balance exercises, stretching or range of motion, aquatic therapy, physical activity, and community participation. Published data largely remain limited to case studies in these areas. Instructive recent case reports worth reviewing include those by Salem and Gropack [64] and Cha et al. [65]. However, interventional and longitudinal studies to assess the feasibility and functional impact of exercise in SMA patients, as well as other interventions, are critically needed; two such studies are currently in progress (NCT01233817 and NCT01166022).

Conclusions

The exponentially increasing progress in basic and clinical research provides unprecedented therapeutic opportunity for patients with SMA. The most difficult questions moving forward are how to choose and optimize the most promising leads within the rapidly expanding therapeutic portfolio. The close collaboration between basic scientists, industry, advocacy organizations, the National Institutes of Health, and clinicians has been a critical factor in the efficient progress to date. As we enter 2012, we have an unprecedented opportunity to identify one or more effective therapeutic interventions for SMA. Clinical trials represent

perhaps the most critical and defining aspect in the therapeutic development pipeline, and careful clinical trial design and broad consultation among clinicians and basic scientists working in the field will help to ensure success in effectively translating these exciting therapeutic opportunities to the clinic.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Zerres K, Wirth B, Rudnik-Schoneborn S. Spinal muscular atrophy-clinical and genetic correlations. *Neuromuscular Disorders*. 1997; 7:202–7. [PubMed: 9185186]
- 2•. Pruss RM, Giraudon-Paoli M, Morozova S, et al. Drug discovery and development for spinal muscular atrophy: lessons from screening approaches and future challenges for clinical development. *Future Med Chem*. 2010; 2:1429–40. In this excellent and comprehensive review, Pruss et al. review lessons learned and discuss potential pitfalls using screening approaches to identify pharmacologic therapies for SMA using cell-based assays, including examples from their experience with both SMN-and non-SMN-directed strategies. [PubMed: 21426138]
- 3•. Shababi M, Mattis VB, Lorson CL. Therapeutics that directly increase SMN expression to treat spinal muscular atrophy. *Drug News Perspect*. 2010; 23:475–82. This review nicely summarizes therapeutic approaches including nucleic acid-based and pharmacologic therapeutics that hold promise as potential treatment options in SMA. [PubMed: 21031163]
4. Kolb SJ, Kissel JT. Spinal muscular atrophy: a timely review. *Arch Neurol*. 2011; 68:979–84. [PubMed: 21482919]
5. Lunn JS, Sakowski SA, Federici T, et al. Stem cell technology for the study and treatment of motor neuron diseases. *Regen Med*. 2011; 6:201–13. [PubMed: 21391854]
6. Ge X, Bai J, Lu Y, et al. The Natural History of Infant Spinal Muscular Atrophy in China: A Study of 237 Patients. *J Child Neurol*. 2011
7. 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. *Arch Neurol*. 1995; 52:518–23. [PubMed: 7733848]
8. Rudnik-Schoneborn S, Goebel HH, Schlote W, et al. Classical infantile spinal muscular atrophy with SMN deficiency causes sensory neuropathy. *Neurology*. 2003; 60:983–7. [PubMed: 12654964]
9. Felderhoff-Mueser U, Grohmann K, Harder A, et al. Severe spinal muscular atrophy variant associated with congenital bone fractures. *J Child Neurol*. 2002; 17:718–21. [PubMed: 12503654]
10. Nadeau A, D’Anjou G, Debra G, et al. A newborn with Spinal Muscular Atrophy Type 0 presenting with a chilocopathological picture suggestive of myotubular myopathy. *J Child Neurol*. 2007; 22:1301–4.
11. Rudnik-Schoneborn S, Heller R, Berg C, et al. Congenital heart disease is a feature of severe infantile spinal muscular atrophy. *J Med Genet*. 2008; 45:635–8. [PubMed: 18662980]
- 12•. Shababi M, Habibi J, Yang HT, et al. Cardiac defects contribute to the pathology of spinal muscular atrophy models. *Hum Mol Genet*. 2010; 19:4059–71. The authors delineate cardiac defects in a severe model of SMA. Pathologic responses were also carefully delineated in a less severe model of disease. The authors provide evidence for altered cardiac development and function in severe mouse models of SMA. [PubMed: 20696672]
13. Bevan AK, Hutchinson KR, Foust KD, et al. Early heart failure in the SMNDelta7 model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery. *Hum Mol Genet*. 2010; 19:3895–905. [PubMed: 20639395]
14. Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: relation to age, SMN2 copy number, and function. *Ann Neurol*. 2005; 57:704–12. [PubMed: 15852397]

- 15•. Wee CD, Kong L, Sumner CJ. The genetics of spinal muscular atrophies. *Curr Opin Neurol*. 2010; 23:450–8. This is an excellent and thorough review of recent clinical, genetic, and therapeutic advances for the expanding spectrum of SMA, nicely delineating differences in clinical phenotypes and discussing pathways involved in pathogenesis. [PubMed: 20733483]
16. Petit F, Cuisset JM, Rouaix-Emery N, et al. Insights into genotype-phenotype correlations in spinal muscular atrophy: a retrospective study of 103 patients. *Muscle Nerve*. 2011; 43:26–30. [PubMed: 21171094]
17. Oprea GE, Kröber S, McWhorter ML, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. *Science*. 2008; 320:524–7. [PubMed: 18440926]
18. Stratigopoulos G, Lanzano P, Deng L, et al. Association of plastin 3 expression with disease severity in spinal muscular atrophy only in postpubertal females. *Arch Neurol*. 2010; 67:1252–6. [PubMed: 20937953]
19. Ben-Shachar S, Orr-Urtreger A, Bardugo E, et al. Large-scale population screening for spinal muscular atrophy: clinical implications. *Genet Med*. 2011; 13:110–4. [PubMed: 21233719]
- 20••. Ling KK, Lin MY, Zingg B, et al. Synaptic defects in the spinal and neuromuscular circuitry in a mouse model of spinal muscular atrophy. *PLoS One*. 2010; 5(11):e15457. The authors report their novel observations regarding a significant reduction in proprioceptive sensory neurons in the SMN Δ 7 animal model of SMA. This novel demonstration of broader involvement of the spinal circuitry may be an additional target for SMA therapeutics. [PubMed: 21085654]
- 21••. Mentis GZ, Blivis D, Liu W, et al. Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. *Neuron*. 2011; 69:453–67. This article demonstrates that motor neuron loss follows afferent synapse loss, and the subsequent motor neuron loss occurs in the same temporal and topographical order as the preceding decrease of proprioceptive reflexes to the specific muscles studied in an SMA mouse model. The authors provide the first evidence that deafferentation of motor neurons is an early event in SMA and occurs prior to loss of motor neurons in the efferent pathway. [PubMed: 21315257]
22. Renault F, Raimbault J, Praud JP, Laget P. Electromyographic study of 50 cases of Werdnig-Hoffmann disease. *Rev Electroencephalogr Neurophysiol Clin*. 1983; 13:301–5. [PubMed: 6672899]
23. Ito Y, Shibata N, Saito K, et al. New insights into the pathogenesis of spinal muscular atrophy. *Brain Dev*. 2011; 33:321–31. [PubMed: 20605078]
24. Parra J, Martinez-Hernandez R, Also-Rallo E, et al. Ultrasound evaluation of fetal movements in pregnancies at risk for severe spinal muscular atrophy. *Neuromuscul Disord*. 2011; 21:97–101. [PubMed: 21194946]
25. Dominguez E, Marais T, Chatauret N, et al. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice. *Hum Mol Genet*. 2011; 20:681–93. [PubMed: 21118896]
26. Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. *Nat Biotechnol*. 2010; 28:271–4. [PubMed: 20190738]
27. Valori CF, Ning K, Wyles M, et al. Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy. *Sci Transl Med*. 2010; 2:35ra42.
28. Gladman JT, Bebee TW, Edwards C, et al. A humanized Smn gene containing the SMN2 nucleotide alteration in exon 7 mimics SMN2 splicing and the SMA disease phenotype. *Hum Mol Genet*. 2010; 19:4239–52. [PubMed: 20705738]
- 29•. Bevan AK, Duque S, Foust KD, et al. Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. *Mol Ther*. 2011 This article summarizes the promising evidence for the future use of AAV9 for gene transfer to the CNS in a pediatric SMA population. The authors demonstrate efficient CNS and peripheral tissue targeting after intravenous administration to large animals at birth through 3 years of age. In addition, cerebrospinal fluid injection of AAV9 efficiently targeted motor neurons, suggesting that AAV9 has the potential to be a very powerful tool for gene delivery in individuals with SMA.
30. Burghes AH, McGovern VL. Antisense oligonucleotides and spinal muscular atrophy: skipping along. *Genes Dev*. 2010; 24:1574–9. [PubMed: 20679391]

31. Muntoni F, Wood MJ. Targeting RNA to treat neuromuscular disease. *Nat Rev Drug Discov.* 2011; 10:621–37. [PubMed: 21804598]
32. Hua Y, Sahashi K, Hung G, et al. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev.* 2010; 24:1634–44. [PubMed: 20624852]
33. Passini MA, Bu J, Richards AM, et al. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Sci Transl Med.* 2011; 3:72ra18. This report demonstrates compelling efficacy for CNS-directed ASO-10-27 therapy in mice with a severe form of SMA, including splice-mediated increases in SMN protein and in the number of motor neurons in the spinal cord, resulting in improvements in muscle physiology, motor function, and survival.
34. 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; 478:123–6. Subcutaneous injections of an ASO, ASO-10-27, to neonates rescued severe SMA mice more effectively than previously reported intracerebroventricular administration. Systemic ASO-10-27 treatment increased median lifespan by 25-fold and restored insulin-like growth factor 1 (IGF-1) to normal levels. These findings provide the evidence that reduced IGF-1 levels may be important in SMA pathogenesis, and compelling efficacy for the systemic administration of ASO-10-27 in a severe mouse model. [PubMed: 21979052]
35. Biondi O, Branchu J, Sanchez G, et al. In vivo NMDA receptor activation accelerates motor unit maturation, protects spinal motor neurons, and enhances SMN2 gene expression in severe spinal muscular atrophy mice. *J Neurosci.* 2010; 30:11288–99. [PubMed: 20739549]
36. Xu C, Chen X, Grzeschik SM, et al. Hydroxyurea enhances SMN2 gene expression through nitric oxide release. *Neurogenetics.* 2011; 12:19–24. [PubMed: 21174137]
37. Farooq F, Molina FA, Hadwen J, et al. Prolactin increases SMN expression and survival in a mouse model of severe spinal muscular atrophy via the STAT5 pathway. *J Clin Invest.* 2011; 121:3042–50. [PubMed: 21785216]
38. 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; 121:3029–41. [PubMed: 21785219]
39. Jodelka FM, Ebert AD, Duelli DM, Hastings ML. A feedback loop regulates splicing of the spinal muscular atrophy-modifying gene, SMN2. *Hum Mol Genet.* 2010; 19:4906–17. [PubMed: 20884664]
40. 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. [PubMed: 20808854]
41. Kissel JT, Scott CB, Reyna SP, et al. SMA CARNI-VAL TRIAL PART II: A prospective, single-armed trial of L-carnitine and valproic acid in ambulatory children with spinal muscular atrophy. *PLoS ONE.* 2011; 6:e21296. [PubMed: 21754985]
42. Darbar IA, Plaggett PG, Resende MB, et al. Evaluation of muscle strength and motor abilities in children with type II and III spinal muscle atrophy treated with valproic acid. *BMC Neurol.* 2011; 11:36. [PubMed: 21435220]
43. Chen TH, Chang JG, Yang YH, et al. Randomized, double-blind, placebo-controlled trial of hydroxyurea in spinal muscular atrophy. *Neurology.* 2010; 75:2190–7. [PubMed: 21172842]
44. Tiziano FD, Lomastro R, Pinto AM, et al. Salbutamol increases survival motor neuron (SMN) transcript levels in leucocytes of spinal muscular atrophy (SMA) patients: relevance for clinical trial design. *J Med Genet.* 2010; 47:856–8. [PubMed: 20837492]
45. Abbara C, Estournet B, Lacomblez L, et al. Riluzole pharmacokinetics in young patients with spinal muscular atrophy. *Br J Clin Pharmacol.* 2011; 71:403–10. [PubMed: 21284699]
46. Baughan T, Shababi M, Coady TH, et al. Stimulating full-length SMN2 expression by delivering bifunctional RNAs via a viral vector. *Mol Ther.* 2006; 14:54–62. [PubMed: 16580882]
47. Dupuis L, Echaniz-Laguna A. Skeletal muscle in motor neuron diseases: therapeutic target and delivery route for potential treatments. *Curr Drug Targets.* 2010; 11:1250–61. [PubMed: 20840067]

48. Garbes L, Riessland M, Holker I, et al. LBH589 induces up to 10-fold SMN protein levels by several independent mechanisms and is effective even in cells from SMA patients non-responsive to valproate. *Hum Mol Genet.* 2009; 18:3645–58. [PubMed: 19584083]
49. Hastings ML, Berniac J, Liu YH, et al. Tetracyclines that promote SMN2 exon 7 splicing as therapeutics for spinal muscular atrophy. *Sci Transl Med.* 2009; 1:5ra12.
50. Mattis VB, Ebert AD, Fosso MY, et al. Delivery of a read-through inducing compound, TC007, lessens the severity of a spinal muscular atrophy animal model. *Hum Mol Genet.* 2009; 18:3906–13. [PubMed: 19625298]
51. Riessland M, Ackermann B, Forster A, et al. SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy. *Hum Mol Genet.* 2010; 19:1492–506. [PubMed: 20097677]
52. Rose FF Jr, Mattis VB, Rindt H, Lorson CL. Delivery of recombinant follistatin lessens disease severity in a mouse model of spinal muscular atrophy. *Hum Mol Genet.* 2009; 18:997–1005. [PubMed: 19074460]
53. Tsai LK, Tsai MS, Ting CH, Li H. Multiple therapeutic effects of valproic acid in spinal muscular atrophy model mice. *J Mol Med (Berl).* 2008; 86:1243–54. [PubMed: 18649067]
54. Wyatt TJ, Keirstead HS. Stem cell-derived neurotrophic support for the neuromuscular junction in spinal muscular atrophy. *Expert Opin Biol Ther.* 2010; 10:1587–94. [PubMed: 20955113]
55. Lewelt A, Krosschell KJ, Scott C, et al. Compound muscle action potential and motor function in children with spinal muscular atrophy. *Muscle Nerve.* 2010; 42:703–8. This article demonstrates the viability of ulnar CMAP as a surrogate biomarker for denervation in SMA which correlates with gross motor function as measured using the MHFMS-EXTEND measure in children with SMA types II and III. [PubMed: 20737553]
56. Rutkove SB, Shefner JM, Gregas M, et al. Characterizing spinal muscular atrophy with electrical impedance myography. *Muscle Nerve.* 2010; 42:915–21. [PubMed: 21104866]
57. Sproule DM, Punyanitya M, Shen W, et al. Muscle volume estimation by magnetic resonance imaging in spinal muscular atrophy. *J Child Neurol.* 2011; 26:309–17. [PubMed: 20929908]
58. Wu JS, Darras BT, Rutkove SB. Assessing spinal muscular atrophy with quantitative ultrasound. *Neurology.* 2010; 75:526–31. [PubMed: 20697104]
59. 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; 44:246–51. This study demonstrates that the MHFMS, a measure of gross motor function requiring cooperation, can be reliably performed in SMA children less than 30 months of age. [PubMed: 21698647]
60. Montes J, Dunaway S, Montgomery MJ, et al. Fatigue leads to gait changes in spinal muscular atrophy. *Muscle Nerve.* 2011; 43:485–8. [PubMed: 21404286]
61. Sproule DM, Montes J, Dunaway SL, et al. Bioelectrical impedance analysis can be a useful screen for excess adiposity in spinal muscular atrophy. *J Child Neurol.* 2010; 25:1348–54. [PubMed: 20388937]
62. Chatwin M, Bush A, Simonds AK. Outcome of goal-directed non-invasive ventilation and mechanical insufflation/exsufflation in spinal muscular atrophy type I. *Arch Dis Child.* 2011; 96:426–32. [PubMed: 20573738]
63. Russman BS, Buncher CR, White M, et al. Function changes in spinal muscular atrophy II and III. The DCN/SMA Group. *Neurology.* 1996; 47:973–6. [PubMed: 8857729]
64. Salem Y, Gropack SJ. Aquatic therapy for a child with type III spinal muscular atrophy: a case report. *Phys Occup Ther Pediatr.* 2010; 30:313–24. [PubMed: 20868338]
65. Cha TH, Oh DW, Shim JH. Noninvasive treatment strategy for swallowing problems related to prolonged nonoral feeding in spinal muscular atrophy type II. *Dysphagia.* 2010; 25:261–4. [PubMed: 20087605]

Fig. 1.

Spinal and neuromuscular circuitry in SMA. Motor neuron degeneration dominates clinical phenotype(s) in SMA. SMN is critical for processes within the motor neuron cell body, axon, distal synapse, and neuromuscular junction. However, recent data from SMA animal models suggest broader involvement of key components within the spinal and neuromuscular circuitry; specifically, early functional impairment of proprioceptor feedback onto α motoneurons within the spinal cord. SMA—spinal muscular atrophy; SMN—survival motor neuron protein

Table 1

Phenotypic spectrum in proximal spinal muscular atrophy

SMA type and nomenclature	Age of onset	Natural lifespan	Clinical characteristics and maximum milestones achieved
0 SMA: arthrogryposis multiplex congenita type	Prenatal	< 6 months	Congenital hypotonia, weakness, respiratory failure, proximal joint contractures Unable to breathe unsupported
I Werdnig-Hoffman disease	Birth–6 months	~ 32% survival probability at 2 years [7]	Infantile onset of generalized hypotonia, weakness, impaired bulbar function, respiratory insufficiency. Unable to sit unsupported
II SMA, Dubowitz-type	6–12 months	~ 70% survival to adulthood [7]	Onset of proximal limb weakness in infancy, with progressive weakness, respiratory insufficiency, scoliosis, joint contractures in childhood. Achieve independent sitting
IIIa	> 1 year	Normal	Onset of proximal weakness in infant/toddler years, with progressive weakness, heel cord tightness and contractures; tremor and lumbar lordosis; increased risk for fracture and scoliosis. Achieve independent ambulation, but 50% lose this ability by 12 years of age [1]
IIIb Kugelberg-Welander Disease	> 3 years	Normal	Onset of proximal muscle weakness in childhood, with progressive weakness, heel cord tightness and contractures, tremor and lumbar lordosis; increased risk for fractures and scoliosis. Achieve independent ambulation
IV	Adulthood	Normal	Onset of proximal leg weakness in adulthood

SMA spinal muscular atrophy

Table 2

New therapeutic targets and strategies

Category	Target	Therapeutic agent	Preclinical/clinical investigation status
SMN upregulation	Histone deacetylase inhibition; activate SMN2 promoter	Valproic acid Sodium butyrate Phenylbutyrate	Several clinical trials completed and in progress; no clearly proven benefit to date
SMN upregulation	Histone deacetylase inhibition	Trichostatin A SAHA	Preclinical studies in mice; need less toxic compounds
SMN upregulation	Reduce methylation-dependent SMN2 silencing	LBH589 Romidepsin SAHA	Preclinical studies in SMA mice
SMN upregulation	Modulate SMN translation	TC007 ALB-X	Preclinical studies in SMA mice
SMN upregulation	Stat5 pathway	Prolactin	Preclinical studies in mice
Antisense oligonucleotides	SMN2 preRNA/splice regulators	Pro105 ASO-10–27	Preclinical studies mice and primates (both); phase 1 clinical trial planning in progress
DNA repair	SMN1 delivery	scAAV9-SMN	Preclinical studies in mice, primates
Neuroprotective strategies	Mitochondria; Excitotoxicity modification; NMDA receptor	Olesoxime Riluzole NMDA agonist(s)	Clinical trials completed or in progress for both compounds; preclinical studies in mice for all three strategies
C-5 substituted quinazolones	DcpS – impact mRNA metabolism	Quinazoline 495	Preclinical studies in mice; phase 1 safety trials completed
Tetracyclines	SMN2 splicing modulation	HU PTK-SMA1	Clinical trials completed (HU) Preclinical studies (PTK-SMA1)
Amino-glycosides	Stop codon read through – SMN2 Exon 8	TC007 PTC-X	Preclinical studies in mice
Disease modifier	Plastin 3 upregulation	None at present	Modification of phenotypic severity in human subjects
Stem cell therapy	Neurotrophic support	Human embryonic stem cell motoneuron progenitors	Preclinical studies in mice and rats; clinical trials under consideration
Bifunctional RNAs	Recruit splicing factors optimizing splicing	Under development	Preclinical studies in SMA mice
Myostatin antagonists	Skeletal muscle	Follistatin	Preclinical studies in SMA mice
β2-adrenergic agonists	Exon 7 inclusion	Albuterol Salbutamol	Ongoing clinical trials
Rho-kinase pathway	Neuromuscular junction	Y-27632	Preclinical studies in SMA mice

ASO antisense oligonucleotide; HU hydroxyurea; SAHA suberoylanilide hydroxamic acid; SMA spinal muscular atrophy; SMN survival motor neuron protein (Data from [2•, 3•, 5, 17, 18, 25–28, 29•, 30–32, 33•, 34••, 35–54].)