Perspectives on Clinical Trials in Spinal Muscular Atrophy

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Abstract

Spinal muscular atrophy is one of the most heterogeneous of the single-gene neuromuscular disorders. The broad spectrum of severity, with onset from the prenatal period to adulthood, presents unique challenges in the design and implementation of clinical trials. The clinical classification of subjects into severe (type 1), intermediate (type 2), and mild (type 3) subtypes has proved useful both in enhancing communication among clinicians internationally and in forging the collaborative development of outcome measures for clinical trials. Ideally, clinical trial design in spinal muscular atrophy must take into account the spinal muscular atrophy type, patient age, severity-of-affection status, nature of the therapeutic approach, timing of the proposed intervention relative to disease progression, and relative homogeneity of the cohort to be studied. Following is an overview of the challenges and opportunities, current and future therapeutic strategies, and progress to date in clinical trials in spinal muscular atrophy.

Keywords

spinal muscular atrophy; clinical trials; histone deacetylase inhibitors

Mutations involving the survival motor neuron (SNM) *SMN1* gene exon 7 are associated with disease phenotype in more than 95% of cases, and diagnostic and prenatal testing is readily available. The identification of a nearly identical gene, *SMN2*, which produces a fraction of the identical full-length SMN protein, plays a significant role in modifying phenotype. Homozygous deletion of *SMN1* in association with 2 or fewer *SMN2* copies is highly predictive of a severe infantile phenotype (type 1).[2] Spinal muscular atrophy subjects

with type 2, 3, or 4 (adult-onset) typically have 3 or more *SMN2* copies, and in general, an inverse relationship exists between disease severity and *SMN2* copy number.

Unfortunately, *SMN2* copy number alone has not proved reliably predictive of phenotype for all spinal muscular atrophy subjects. The recognition that this nearly identical gene might be exploited to produce increased amounts of the deficient or normal SMN protein has generated great optimism with regard to therapeutics development. Studies demonstrating rescue of the motor neuron disease pheno-type in a spinal muscular atrophy mouse model with an increased number of *SMN2* copies support such optimism.[3] These observations have stimulated efforts to identify strategies to upregulate overall *SMN2* expression, or to increase rates of exon 7 inclusion at the messenger ribonucleic acid (RNA) level to enhance production of the deficient and normal SMN protein. The ratio of deficient to normal SMN protein would vary depending on the specific strategy used. Successful efforts to identify therapies and move them efficiently to human clinical trials will require an international collaborative effort. Such efforts have begun and will be crucial in standardizing clinical trial design, choosing outcome measures, assessing potential surrogate markers, and prioritizing therapeutic strategies.[4]

Testing therapeutic strategies in animal models often plays an important role in clarifying pathophysiologic processes and demonstrating proof of concept for a given strategy; however, what appears to be straightforward in the laboratory often proves more complex in the clinic. The marked difference in phenotypic severity between type 1 and type 2 or 3 subjects with a 1.5-fold to 2.0-fold increase in *SMN2* copy number seems promising, but increasing expression of *SMN2* to the level required to rescue the spinal muscular atrophy mouse may prove untenable with a pharmaceutical agent alone. Furthermore, increased expression of *SMN* occurs from the embryonic period onward in this model.

Questions about the specific developmental window requiring upregulation of SMN to rescue motor neurons remain. It seems likely that prenatal upregulation of SMN expression may be necessary for motor neuron rescue in the most severe infantile cases. Preliminary data from electro-physiologic studies that include motor unit number estimation in infants and young children suggest that the most significant motor-neuron loss in most subjects occurs in the postnatal period (Figure 1).[5] Thus, the optimistic view that we might achieve significant amelioration of disease severity using such a strategy, assuming we can institute treatment early enough in the disease course, should not be discounted. Newborn screening would facilitate early diagnosis and enrollment in treatment trials. It would also facilitate a more anticipatory approach with regard to nutritional and respiratory interventions in infantile-onset cases that would almost certainly improve clinical outcomes.

Clinical Trial Design Considerations

Although potentially desirable from a patient recruitment standpoint, the widely variable phenotypic spectrum makes it difficult to include all spinal muscular atrophy subjects within a clinical trial. Disease progression with regard to motor-unit loss appears to be most rapid in the initial phases of the disease. It can be conceptually divided into 3 phases: a preclinical phase, a subacute phase, and a chronic phase (Figure 2).

During the preclinical phase, infants or children appear normal. Motor-unit loss is progressing but may not yet have reached a critical threshold, and reinnervation initially masks symptoms. This phase may be extremely short or even nonexistent in type 1 infants, but in type 2 and 3 infants and children, it may last many months or even years in the most mildly affected subjects.

The subacute phase is associated with motor-unit loss that has reached a critical threshold. Ongoing motor-unit loss during this period appears to be fairly rapid (associated with significant reduction of maximum compound muscle action potential amplitudes over a period of weeks to months) and can be exacerbated by stressors such as illness, nutritional compromise, or growth. Clinical symptoms evolve, ranging from frank weakness and progressive paralysis in the type 1 infant, to loss of ability to sit or roll in more severely affected type 2 subjects, to a more obvious slowing of acquisition of expected gross motor milestones in less severely affected type 2 and 3 infants and children.

After the subacute period, a chronic period ensues in which motor-unit loss appears to plateau. Ongoing reinnervation during this period may allow functional motor abilities to remain stable for prolonged periods of time, from months to years. Younger children may even reacquire gross motor skills that were previously lost, such as rolling, or slowly acquire some additional gross motor skills over time; however, denervation progresses with age.

The evolution from preclinical to subacute to chronic can be quite rapid in type 1, and most infants demonstrate severe denervation by 6 months of age. A slower evolution occurs in type 2 subjects, with progression within the subacute period occurring between 6 to 30 months before entry into the chronic phase. In type 3 subjects, this evolution appears to be even further extended over many months to years.

These observations have obvious implications for clinical-trial design. For instance, once patients enter the chronic phase of the disease, they are likely to demonstrate relatively stable functional motor abilities over a period of at least several months.[6] This allows documentation of a stable baseline and offers the opportunity to observe potential functional motor benefit if a therapy proves efficacious. On the other hand, if younger children who are still in the subacute phase of the disease are enrolled, they may well show functional decline over the course of the study.

Infants with spinal muscular atrophy type 1 present perhaps the greatest challenges in clinical trial design. Severe infantile spinal muscular atrophy accounts for most newly diagnosed cases, which are estimated to comprise 60% to 70% of all patients (Figure 3). These infants often experience profound weakness and respiratory insufficiency, making them quite fragile and thus more vulnerable to complications related to participating in a clinical trial, such as travel. In an open-label treatment trial with sodium phenylbutyrate, travel to and from the Utah site for research-related visits resulted in 1 flight diversion and 1 near-fatal incident due to respiratory insufficiency after several hours of travel in an infant car seat. Such infants must often depend on abdominal breathing because of severe intercostal weakness; thus, flat car beds are preferable, although they are not currently prescribed for most infants with spinal muscular atrophy type 1.

In addition, obtaining necessary laboratory tests for safety monitoring can result in episodes of respiratory decompensation because many of these infants have poor venous access. They are also quite vulnerable during assessments of motor function because they may be unable to tolerate certain positions owing to difficulties in management of oral secretions.

Aside from the obstacles of maintaining such fragile patients in clinical trials, the widely variable clinical care with regard to respiratory and nutritional interventions makes questionable observations of benefit based on survival as an outcome measure. Thus, more creative end points for these fragile infants will likely be needed, such as a requirement for 16 or more hours daily of noninvasive ventilatory support (eg, bilevel positive airway pressure). Standardizing recommended clinical-care interventions in such subjects will be vital in limiting variables that could overcome any apparent benefit from a pharmaceutical agent. Even with defined standard-of-care recommendations for this population of infants,

our inability to predict whether parents will choose such interventions may, unfortunately, limit their benefit to some extent.

Subjects with spinal muscular atrophy type 2 are able to sit at some point in their clinical course. Yet, the phenotypic variability exceeds that seen in type 1 infants, ranging from infants who sit transiently and demonstrate severe respiratory insufficiency to children who can sit, crawl, and even stand with support. Onset of scoliosis in early-to-middle childhood occurs in most children with spinal muscular atrophy type 2. Because progression of scoliosis is often rapid, it can be a confounding factor if it occurs during a clinical trial. Surgical intervention for scoliosis results in reduced mobility because of spinal rigidity, which may limit their ability to demonstrate a definite gross motor functional benefit from a specific therapeutic intervention.

Subjects with type 3 have an even wider spectrum with regard to age of onset. They are often subdivided into type 3a (onset < 3 years) and 3b (onset > 3 years) for purposes of epidemiologic studies. In general, they comprise a less fragile population than type 2 subjects with regard to respiratory or nutritional vulnerability. However, prior natural history studies suggest that loss of ambulatory capacity by 12 years of age occurs in about 50% of those with type 3a.[1] Loss of ambulatory status may also occur because of fracture or in association with contractures. Rapid progression of scoliosis associated with growth can confound assessment over a period of several months.

In addition, outcome measures appropriate for children with spinal muscular atrophy type 2 have a ceiling effect such that they are not adequate to assess change in motor function in type 3 subjects. Whether it is feasible to group subjects with type 2 and 3 together in a clinical trial depends largely on whether the chosen primary outcome measure can perform adequately across a large range of variability in scores. Such trial designs typically require a much larger number of targeted subjects than a trial aimed at a less heterogeneous population.

Denervation progresses rapidly in infants with the type 1 form of the disease, with a very short preclinical phase lasting only a few weeks in which neonates may appear completely normal. This is followed quickly by a subacute phase in which most infants fail to gain motor milestones as expected and may demonstrate onset of bulbar and respiratory weakness by 6 months of age (Figure 2). For this and other reasons, early identification and enrollment of such infants, facilitated by newborn screening, is perhaps the approach most likely to overcome such obstacles.

Acquisition of motor milestones in the first few months often occurs normally in type 2 and 3 subjects in the pre-clinical phase of the disease. The subacute phase is associated with the first clinical indication of weakness. Rather than a clear loss of function, these infants or children often show a slowing in the rate of acquisition of new motor milestones during this period, yet this period may be associated with a fairly rapid decline in motor units. The subacute phase is followed by a much slower yet progressive decline in functional abilities over time in the chronic phase of the disease (Figure 3).

The age of subjects and whether they are in a preclinical, subacute, or chronic phase of their illness may contribute to the way in which they respond to a specific therapeutic intervention. For instance, a relatively newly diagnosed 10-month-old child with type 2 spinal muscular atrophy is likely to be in the subacute phase of the illness and is predicted to have progressive motor-unit loss during the ensuing months. This progressive motor-unit loss may or may not be associated with a clear decline in motor function, dependent in part on how effective compensatory mechanisms are for reinnervation in that particular child. Thus, enrollment in a clinical trial along with other symptomatic children largely in the

chronic phase of their illness may confound expected results of a specific therapeutic intervention for the entire cohort, depending on which outcome measure is used to indicate efficacy. Further, infants and young children are often unable to cooperate with testing of strength or motor function or may demonstrate more variable results than older children. Yet this population has potentially the greatest to gain from a therapeutic intervention because more motor neurons at risk may possibly benefit from such intervention.

Because fatigue is a significant factor across all spinal muscular atrophy cohorts, the time of day of testing can have an important impact on reliability. For instance, testing an infant with type 1 spinal muscular atrophy in the morning (using a modified test of infant motor performance) can result in higher scores than those measured in the same infant later in the day, presumably due to fatigue. Thus, it is important to ensure that children are well rested and well nourished before testing, that the evaluation is done at a consistent time of day for each visit, and that testing be minimized to that which is necessary to avoid excessive fatigue. Such strategies will help ensure that any changes observed are more likely due to an effect of the therapy rather than a change in the testing paradigm. In the next sections, we review the currently proposed strategies for therapeutic intervention in spinal muscular atrophy as a starting point for discussion on prioritizing therapies for clinical trials.

Histone Deacetylase Inhibitors as Therapeutic Candidates

In 2000, Chang et al[2] demonstrated that sodium butyrate, a histone deacetylase inhibitor, increased full-length *SMN2* transcript levels and protein levels in cell lines derived from spinal muscular atrophy patients. Although the half-life of this compound made it an unlikely candidate for human trials, this observation stimulated interest in a wider spectrum of histone deacetylase inhibitors, including some already in clinical use. Control of the acetylation state of histones is one of several epigenetic mechanisms that regulate gene expression. When histones are acetylated within a region of chromatin, the resulting conformational change increases accessibility of DNA to the transcriptional machinery, enhancing transcriptional activity in that region.

The expression of *SMN2* is regulated by a nearly identical promoter to that of *SMN1*; thus, these promotors share common regulatory elements.[9,10] The SMN promoter is associated with the histone deacetylase 1 and 2 proteins that appear to modulate the histone acetylation state and thus play a role in regulating *SMN2* expression.[11] The *SMN2* promoter can be activated and full-length SMN messenger RNA and protein levels increased in spinal muscular atrophy patient−derived cells by several other histone deacetylase inhibitors, including phenylbutyrate, valproic acid, and suberoylanilide hydroxamic acid.[12-16]

Preliminary studies using a spinal muscular atrophy mouse model have demonstrated prolonged survival with sodium phenylbutyrate and other butyrate prodrugs.[17] It is of interest that this survival benefit occurs without a demonstrable increase in *SMN* protein expression, indicating that the observed effect may be due to other factors, such as the activation of antiapoptotic genes, as has been demonstrated with sodium phenylbutyrate in an amyotrophic lateral sclerosis mouse model.[18] Sodium phenylbutyrate and valproic acid have been in clinical use for decades for other indications; thus, safety and pharmacokinetic data are available, at least for other populations, and both are known to readily penetrate the central nervous system. It is not clear, however, that the dose range for these compounds for already established indications will be ideal for increasing *SMN* expression.

Sodium phenylbutyrate and valproic acid are under active study in ongoing and recently completed pilot clinical trials in Italy, the United States, and Germany (Table 1). Early pilot trials of phenylbutyrate in spinal muscular atrophy patients in Italy demonstrated that the drug was reasonably well tolerated and was associated with variable upregulation in SMN

messenger RNA expression in blood cells.[19] A recently completed placebo-controlled clinical trial of phenylbutyrate in spinal muscular atrophy type 2 subjects in Italy proved negative, although uncertainties regarding the chosen dosing regimen and the relatively short trial duration of 13 weeks make these results difficult to interpret.[20]

Hydroxyurea, another histone deacetylase inhibitor in clinical use for many years, has proved modestly effective in the treatment of sickle cell anemia and thalassemia by way of its demonstrated ability to upregulate fetal hemoglobin expression. It has also been reported to increase the amount of full-length SMN transcript, protein, and gems in spinal muscular atrophy–derived cell lines.[21] Placebo-controlled and open-label clinical trials are ongoing in the United States and Taiwan to further study this compound (Table 1). Newer generation histone deacetylase inhibitors that might act more potently at the SMN promoter are also being actively pursued, including suberoylanilide hydroxamic acid and trichostatin A.[15,22]

Alternative Strategies to Increase *SMN* Expression

Additional screening efforts to identify compounds to increase *SMN2* expression included a cell-based high-throughput screen of 550 000 compounds targeted for their ability to activate a 3.4-kb fragment of the *SMN2* promoter stably integrated in NSC34 cells (a motor neuron–derived cell line) and have yielded compounds that are currently undergoing further modification and testing.[23] Of interest is that these compounds do not appear to be histone deacetylase inhibitors.

Other strategies being pursued include altered splicing to enhance exon 7 inclusion from *SMN2*, neuroprotective strategies for SMN-deficient motor neurons, stabilization of the SMN protein already produced in reduced quantities, trophic or anabolic agents, and stem cell and gene-replacement therapies. Such strategies need not be mutually exclusive. It is likely that a combination of therapies will ultimately prove most fruitful. These strategies and their rationale are briefly reviewed in the following sections.

Enhancing SMN Exon 7 Inclusion

The molecular mechanisms that direct splicing of *SMN* transcripts have been investigated in detail and are reviewed elsewhere. A variety of exonic and intronic splice enhancer and silencer motifs appear to play an important role in SMN transcript splicing. Andreassi et al[24] demonstrated that the chemotherapy drug aclarubicin stimulates exon 7 inclusion and increased SMN protein level in skin fibroblasts derived from patients with spinal muscular atrophy. Unfortunately, the toxicity profile of this drug deemed it an unlikely candidate for long-term use in spinal muscular atrophy patients.

Another strategy to enhance exon 7 inclusion is the potential use of synthetic antisense oligonucleotides that bind to *SMN2*-derived transcripts and promote exon 7 inclusion during splicing.[25,26] Although such strategies have been successfully performed in vitro, it will be a much greater challenge to achieve efficient delivery of synthetic oligonucleotides to motor neurons in animal models and human subjects.

Strategies to Stabilize Survival Motor Neuron Protein

In a cell-based high-throughput screen of nearly 50 000 compounds, indoprofen increased *SMN2*-derived protein, but not *SMN1*-derived protein.[27] This drug appeared to act at the translational level, possibly by increasing the efficiency of translation of *SMN2*-derived transcripts through a cyclooxygenase-dependent mechanism.

Others have demonstrated that certain aminoglyco-sides can increase SMN protein levels and gem counts in patient-derived fibroblasts.[28] Aminoglycosides can alter translation by

promoting read-through of stop codons. This observation has led to a proposed treatment strategy for Duchenne muscular dystrophy patients with mutations in the dystrophin gene amenable to such read-through. In spinal muscular atrophy, the authors propose that these drugs enable read-through of the initial stop codon in exon 8 of *SMN2* transcripts, resulting in the production of an SMN protein with a slightly elongated C-terminus that increases the stability of SMN2 protein.

A third strategy is to interfere with SMN degradation, a process that occurs by way of the proteasome. Proteasome inhibitors have been identified that increase SMN protein levels in spinal muscular atrophy−derived cell lines, but toxicity remains a major limiting factor in the development of this particular strategy.[29]

The compounds identified to date exploiting the above strategies are poor candidates for human trials because of excessive toxicity or poor central nervous system penetration. Nonetheless, the search for related compounds targeting these strategies may provide important complementary therapeutics in the future.

Neuroprotective Strategies

Neuroprotective agents could be an extremely effective intervention to delay or limit motor neuron loss and disease progression in spinal muscular atrophy, particularly because early diagnosis through genetic screening is feasible. In addition, spinal muscular atrophy progresses more slowly than other motor neuron diseases (such as amyotrophic lateral sclerosis) once patients enter the chronic phase of their illness, making spinal muscular atrophy more amenable to intervention with neuroprotective agents. The most ideal time for implementation of such agents, however, would be before onset of symptoms, as has been demonstrated in spinal muscular atrophy animal models.

Even with ideal timing for a given intervention, proving that such agents are effective will be a significant challenge in the current environment because efficacy trials will likely require a larger number of subjects and a longer treatment duration compared with trials for agents expected to upregulate *SMN* expression. In the latter case, clear benefit in motor function or strength may be viable end points rather than a slowing of disease progression. It is evident from electrophysiologic studies that many spinal muscular atrophy patients maintain motor function for prolonged periods of time through collateral reinnervation.

Subjects with spinal muscular atrophy type 2 and 3 possess a subpopulation of large motor units that may have an increased vulnerability to oxidative stress. Additional stressors include increased metabolic demands related to growth during childhood, systemic hypoxia or acidosis associated with superimposed illness, and in adults, the expected attrition of motor units with age. Efforts to identify practical neuroprotective strategies in spinal muscular atrophy are likely to benefit from studies that have already been completed or are ongoing in other models of motor neuron disease, such as those for spinobulbar muscular atrophy or amyotrophic lateral sclerosis.

In amyotrophic lateral sclerosis clinical trials, riluzole has been found to be modestly effective in prolonging time to ventilation, presumably through a neuroprotective mechanism. Melki et al[30] have also reported modest benefit in survival in a spinal muscular atrophy mouse model. A small trial in infants with spinal muscular atrophy type 1 appeared to show good tolerability.[31] A second phase 1/2 trial of riluzole is ongoing in the United States in infants with spinal muscular atrophy type 1. A trial to assess efficacy in children and adults over a 2-year period has also been initiated in France.

Gabapentin has been proposed as a neuroprotective agent for clinical trials for both spinal muscular atrophy and amyotrophic lateral sclerosis as a result of encouraging data from preclinical studies in amyotrophic lateral sclerosis animal models of motor neuron disease. Two randomized, controlled clinical trials with gabapentin have been completed in spinal muscular atrophy patients. One study targeted adult subjects with spinal muscular atrophy type 2 and 3 and demonstrated no apparent efficacy.[32] The other trial targeted a very heterogeneous population of spinal muscular atrophy type 2 and 3 subjects ranging in age from 5 to 60 years and noted a possible modest gain in strength but no change in forced vital capacity (Table 1).[33]

No cell-based high-throughput screens to identify compounds that might be neuroprotective specifically in spinal muscular atrophy have been published to date, primarily because in vitro motor neuron models of spinal muscular atrophy have been lacking. Recent efforts to develop a model using embryonic stem cells differentiated into motor neurons may prove valuable. Complementary strategies to identify neuroprotective agents may also be possible with the development of invertebrate models of spinal muscular atrophy demonstrating a motor neuron phenotype.

Trophic and Anabolic Agents

Cardiotrophin-1, a neurotrophic factor, was evaluated in a spinal muscular atrophy mouse model in which deletion of SMN exon 7 was specific to motor neurons. Cardiotrophin-1 was introduced with intramuscular injection using an adenoviral vector and was associated with a modest benefit in survival and delayed onset of motor symptoms.[34] Several studies have documented the effect of β-adrenergic agonists on normal and diseased skeletal muscle. A single, small open-label trial of oral albuterol for 6 months in children with spinal muscular atrophy type 2 and 3 demonstrated modest effects on strength as measured by hand-held myometry and forced vital capacity.[35] This treatment, however, may have accelerated the progression of joint contractures. Placebo-controlled studies of this agent may be warranted and would help address whether tachyphylaxis is an issue during longer treatment intervals, as has been noted in other neuromuscular patient populations.[36]

Other potential proposed approaches in this category include strategies such as myostatin inhibition or follistatin upregulation. Myostatin inhibition in a rodent model of amyotrophic lateral sclerosis was associated with slowing of muscular atrophy.[37] Follistatin upregulation has been recently demonstrated to be associated with functional recovery of dystrophic muscles in mice treated with trichostatin A, a histone deacetylase inhibitor.

Stem Cell and Gene Therapy Strategies

A strategy that could theoretically lead to significant benefit in spinal muscular atrophy patients is replacement of *SMN1* using a gene therapy approach, assuming that such an intervention occurred before the loss of motor neurons. This strategy, like many of the others discussed, would not likely benefit patients who already have significant weakness. Technical difficulties with respect to efficient gene delivery to spinal cord motor neurons, as well as other current obstacles to gene therapy applications in human subjects, will require further studies before advancement to clinical trials in patients. Several studies support the pursuit of such techniques, however. In one study of spinal muscular atrophy mice, lentivirus gene delivery into muscle resulted in retrograde transfer of *SMN* to spinal cord motor neurons.[38] Another promising strategy involved intervention at the RNA level using antisense oligonucleotides, delivered with a viral vector, to increase incorporation of exon 7 in *SMN2* transcripts.[39]

Basic science research on the use of stem cells to help maintain or restore vulnerable motor neuron populations has been progressing rapidly.[40] In one study, differentiated embryonic stem cells transplanted into the spinal cord of rats with motor neuron injury successfully incorporated themselves within the ventral horn and reinnervated lower-extremity muscles.[41] The efficacy of this process must be augmented before stem cells can be considered an effective therapeutic strategy.

Exercise as a Therapeutic Modality

Families often ask clinicians whether exercise is helpful or even potentially harmful for their child. No human studies have addressed this issue in patients with spinal muscular atrophy; however, many of us encourage moderate exercise to the extent of the patient's ability as a means to limit the development of muscle and joint contractures, promote proper hip development, increase bone density, improve bowel motility, and provide a general sense of well-being.

A study of exercise in a spinal muscular atrophy mouse model suggested that exercise might actually increase *SMN* expression in the spinal cord. Mice with spinal muscular atrophy of intermediate severity were subjected to a training protocol in which they were either forced to run on a wheel or did not receive any training.[42] Trained mice showed a modest improvement in survival and a reduction in motor neuron loss compared with the controls. Similar observations have been noted with implementation of forced exercise in amyotrophic lateral sclerosis mouse models.[43]

Testing of Therapeutic Candidates in Animal Models

The spinal muscular atrophy mouse models developed to date have confirmed increased susceptibility of motor neurons to SMN deficiency and, more important, have demonstrated that motor neuron degeneration can be prevented by increasing *SMN2* dosage. Mice that are deleted for *SMN* and carry the human *SMN2* transgene are being actively used to test potential therapeutics. However, the fragility and limited duration of survival of these severely affected animals limit to some degree the mode and duration of therapeutic delivery. Efforts are ongoing to develop mice with a phenotype of more intermediate disease severity or an inducible phenotype that could be more easily used to test potential therapies.

Relying completely on such models to test therapies before consideration for human trials has some risks because efficacy, pharmacokinetics, and toxicities vary greatly between animals and humans. Clinicians may thus choose to pursue the judicious study of agents already available in the clinical realm despite the lack of preexisting animal model data. Pilot clinical trials using agents with a well-defined safety spectrum in human subjects, although not necessarily fully applicable to the spinal muscular atrophy population, has many advantages. Such a strategy allows for the proper establishment of clinical trial networks, refinement of outcome measures, family and patient education, and perhaps most importantly, optimization of protocol design for our often-fragile human subjects.

Other Nonpharmacologic Therapeutic Approaches

Although pharmacologic interventions and gene or stem cell therapies are compelling and generate a great deal of hope and optimism, proactive nutritional interventions, respiratory support, exercise, and rehabilitation can have an extremely powerful and often underestimated effect on quality of life. Used judiciously, they can prolong survival and well-being in the short-run until effective therapies are identified.

A Promising Future

Despite the obstacles discussed in this article, clinical trials in spinal muscular atrophy are moving forward at a rapid pace, and the early experiences have been positive. Participation in a clinical trial can facilitate optimal clinical care for patients while availability of clinical trials renews hope for families long used to hearing that we have little to offer. A collaborative initiative to develop standard-of-care guidelines for spinal muscular atrophy patients is one of the earliest fruits of such labors (reviewed by Ching et al in this issue). Some of the most difficult questions as we move forward involve the priori-tization of therapeutic candidates for clinical trials. This too will almost certainly require an ongoing international collaborative effort to choose outcome measures that allow us to examine data across studies. Such collaborations among researchers, clinicians, families, and patients will undoubtedly continue to foster progress, resulting in improved survival and well-being for our patients.

Acknowledgments

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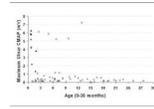
Figure 1.
Ulnar compound muscle action potential (CMAP) versus age in 46 infants with spinal muscular atrophy type 1. Control values from a single unaffected sibling (*SMN1* deletion carrier) are depicted by squares. Solid triangles indicate data from the first assessment in 8 subjects diagnosed with spinal muscular atrophy type 1 prenatally or early in the postnatal period due to a history of an affected sibling. Open triangles indicate data from symptomatic spinal muscular atrophy type 1 infants presenting at various ages. All data represent natural history data for subjects not yet enrolled in treatment protocols. The progressive decrease in compound muscle action potential values during the first 6 months of age represent the most active phase of progressive motor unit loss in these subjects, encompassing the preclinical and subacute phases of disease progression (see Figure 2). A less precipitous loss of compound muscle action potential amplitudes and motor units, of later onset, is observed in spinal muscular atrophy type 2 and 3 subjects.
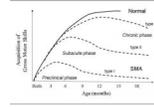
Figure 2.
Acquisition of gross motor milestones in controls versus infants with spinal muscular atrophy (SMA). Preclinical and subacute phases are characterized by more rapid loss of motor units. Loss of functional motor skills is modified by compensatory mechanisms for motor unit loss, including collateral reinnervation, central nervous system maturation and myelination, and peripheral nerve myelination.
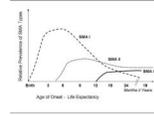
Figure 3.
Estimated spinal muscular atrophy (SMA) prevalence by age and type. Although an estimated 60% to 70% of children with a homozygous *SMN* deletion will manifest clinically with spinal muscular atrophy type 1, the prevalence of spinal muscular atrophy type 2 is higher because of significantly increased mortality in more severely affected infants.

Table 1

Completed and Ongoing Clinical Trials in Spinal Muscular Atrophy

Drug	Proposed Mechanism	Site	Study Type	Subject Cohort	Treatment Duration	Primary Outcome	Secondary Outcome	Result/Status
Albuterol	β-Adrenoreceptor agonist	UK	Open label pilot	13 type II/III children; age not given	6 months	Myometry	PFTs, lean body mass	Modest benefit in strength, lean mass
Gabapentin	Neuroprotection	US	Placebo-controlled, randomized	84 type II/III; age 21-?	12 months	Myometry	FVC, SMA-FRS, SIP	Negative
Gabapentin	Neuroprotection	Italy	Randomized to drug vs no treatment	120 type II/III; age 5-60	12 months	HH myometry	FVC, timed functional tests	Modest benefit in strength only
Riluzole	Neuroprotection	US	Placebo-controlled phase I/II	7 type I, 3 placebo; age 4-15 mo	9 months	Safety	Survival	Safe, possible increased survival
Riluzole	Neuroprotection	US	Open label phase I/II	44 type I; age 0-2	6 months	Safety, pK	TIMP, survival	Analysis pending–low enrollment
Phenylbutyrate	SMN2 upregulation	Italy	Open label pilot	10 type II; age 2.6-12.7	9 weeks; 7 days on/off dosing	Safety	HFMS	Variably increased mRNA, possible functional benefit
Phenylbutyrate	SMN2 upregulation	Italy	Placebo-controlled, randomized	94 type II; age 2.7-12.9	13 weeks; 7 days on/off dosing	HFMS, HH myometry		Negative
Phenylbutyrate	SMN2 upregulation	US	Open label pilot	20 type I/II; age 0-2	12-24 months	Safety	Biomarkers TIMP or M-HFMS	Final analysis pending
Acetyl-L-carnitine	Neuroprotection	Europe	Placebo-controlled, randomized	110 type I/II; age 4-57	9 months	HH myometry, FVC, timed function tests		Negative, publication pending
Creatine	Neuroprotection; energy metabolism optimization	US	Placebo-controlled, randomized	5 5 type II/III; age 2-18	6 months	GMFM	QMT, PFTs, QOL	Negative, In press
Hydroxyurea	SMN2 upregulation	US	Phase I/II placebo-controlled randomized	Types I/II/IIIa; age not yet given	6 months	Safety, biomarkers		Ongoing
Hydroxyurea	SMN2 upregulation	Taiwan	Open label pilot	33 type II/III; age 4-36	8 weeks; 3 dosage levels	Safety, SMN mRNA	FVC, HFMS, myometry	Publication pending
Valproic acid	SMN2 upregulation	US	Open label pilot	7 type III/IV; age 17-45	Variable, 1-15 months	HH myometry		Modest strength benefit
Valproic acid	SMN2 upregulation	US	Open label pilot phase I/II	42 type I/II/III; age 2-31	12 months	Safety	Biomarkers, PFTs, M-HFMS	Analysis pending
Valproic acid and carnitine (CARNI-VAL)	SMN2 upregulation	US	Randomized placebo-controlled intention to treat type II/ Open label type III	60 type II; age 2-8 30 type III; age 3-17	6-12 months	Safety	MHFMS, myometry, PFTs, lean mass (type II)	Ongoing

NOTE: UK, United Kingdom; PFTs = pulmonary function tests; US = United States; FVC = forced vital capacity; SMA = spinal muscular atrophy; SMA-FRS = SMA functional rating scale; SIP = mini-Sickness Impact Profile; HH = hand-held; pK = pharmacokinetics; TIMP = test of infant motor performance; HFMS = Hammersmith Functional Motor Scale for SMA; SMN2 = survival motor neuron protein; mRNA = messenger ribonucleic acid; M-HFMS = modified Hammersmith Functional Motor Scale for SMA; GMFM = Gross Motor Function Measure; QMT = quantitative muscle testing; QOL = Quality of Life parent questionnaire for the PDSQ neuromuscular module. Ages given in years unless otherwise indicated.