Therapeutic developments in spinal muscular atrophy

Douglas M. Sproule and Petra Kaufmann

Abstract: Spinal muscular atrophy (SMA), a potentially devastating disease marked by progressive weakness and muscle atrophy resulting from the dysfunction and loss of motor neurons of the spinal cord, has emerged in recent years as an attractive target for therapeutic intervention. Caused by a homozygous mutation to the Survival of Motor Neurons 1 (SMN1) gene on chromosome 5q, the severity of the clinical phenotype in SMA is modulated by the function of a related protein, Survival of Motor Neurons 2 (SMN2). SMN2 predominantly produces an unstable SMN transcript lacking exon 7; only about 10% of the transcription product produces a full-length, functional SMN protein. Several therapeutic strategies have targeted this gene with the goal of producing increased full-length SMN transcript, thereby modifying the underlying mechanism. Drugs that have increased SMN2 function, in vitro, are now explored for potential therapeutic benefit in this disease. Alternative approaches, including neuroprotective, muscle anabolic, gene and cell replacement strategies, also hold promise. The recent advances in preclinical research and the development of a wider range of animal models for SMA continue to provide cautious optimism that effective treatments for SMA will eventually emerge.

Keywords: spinal muscular atrophy, clinical trials, treatment, gene therapy

Introduction and background

Spinal muscular atrophy (SMA), a potentially devastating and clinically underrecognized neuromuscular disorder typically presenting early in life, has been recognized recently by the National Institute of Neurological Disorders and Stroke (NINDS) as ‘a very probably curable disease’, prompting excitement among clinicians and patients that a cure or treatment may be near at hand. Although the optimism has been tempered by the slow progress and limited success of recent clinical trials, the constellation of factors intrinsic to SMA continue to give hope that effective therapies will emerge in the near future. There are several excellent recent reviews that discuss clinical [Lunn and Wang, 2008; Oskoui and Kaufmann, 2008; Iannaccone, 2007; Prior, 2007; Russman, 2007; Wang et al. 2007], research [Kaufmann and Iannaccone, 2008; Darras and Kang, 2007; Swoboda et al. 2007], and molecular aspects [Kolb et al. 2007; Sumner, 2006] of this fascinating disease. In this review, the disease and important aspects of current (supportive) care are presented. Furthermore, the unusual genetic basis of this disease that drives current therapeutic strategies is discussed, as are the results of recent trials following these approaches. Lastly, alternative therapeutic strategies, including recent explorations into stem cell therapy for the treatment of SMA, are presented.

SMA is a leading genetic cause of death in infancy with an estimated incidence of 1/6000 to 1/10,000 live births [Merlini et al. 1992; Emery, 1991; Pearn, 1978, 1973], with over 95% of people with SMA harboring a homozygous deletion of the Survival of Motor Neurons 1 (SMN1) gene located on chromosome 5q13 [Melki et al. 1994; Gilliam et al. 1990]. The identification of this gene makes SMA readily diagnosable; the presence of a related, phenotype-altering gene (Survival of Motor Neurons 2, SMN2), offers an intriguing avenue to potential therapy [Wirth et al. 2006; Feldkotter et al. 2002; Lefebvre et al. 1997]. This disease affects motor neurons of the anterior horn of the spinal cord and lower brain stem, resulting in
their gradual dysfunction and ultimate loss, although the specific mechanisms driving the molecular pathophysiology of the disease remain incompletely understood. While distinct clinical phenotypes were described by 19th and 20th century neurologists [Kugelberg and Welander, 1956; Werdnig, 1891], clinicians and researchers have now recognized that SMA presents on a spectrum of severity rather than as discrete syndromes. The original classification schema remains useful in clinical practice, however, particularly with regards to descriptions of the extremes of the disease spectrum. Patients with SMA type 1 (Werdnig–Hoffmann) are the most severely affected and never achieve the ability to sit independently. They become symptomatic in infancy, often require a feeding tube to maintain adequate nutrition, and even with proactive respiratory management typically have a severely shortened life expectancy [Oskoui et al. 2007; Werdnig, 1891]. At the other end of the spectrum, patients with SMA type 3 (Kugelberg–Welander) can walk independently, at least for some period of their lives, but with varying degrees of disability [Kugelberg and Welander, 1956]. They can become symptomatic during or after childhood and typically have a normal life expectancy. Patients with SMA type 2 represent an intermediate phenotype. Such children develop the ability to sit independently but fail to achieve the ability to walk independently. With advances in medical care, individuals with SMA types 2 and 3 (SMA 2/3) often have a normal life expectancy, but remain severely physically disabled [Oskoui et al. 2007; Zerres et al. 1997; Zerres and Rudnik-Schoneborn, 1995].

**Advances in supportive care have altered the natural history of SMA**

Any review of therapy for SMA would be remiss without a discussion of the important and evolving role of multidisciplinary supportive care. SMA leads to predominantly proximal muscle atrophy and weakness, and the potential for medical complications such as scoliosis, joint contractures and ventilatory impairment [Wang et al. 2007]. This latter complication is primarily the result of respiratory muscle weakness, which prevents the normal expansion and clearance of the lungs leading to a restrictive defect. In recent years, advances in pulmonary care and the increasing application of noninvasive ventilatory support has dramatically improved the morbidity and mortality associated with pulmonary decline, particularly among children with severe (type 1 and 2) disease phenotypes [Oskoui et al. 2007; Bach et al. 2001, 2000]. Improvements in physical therapy management and advances in surgical approaches to scoliosis, including the use of vertical expandable prosthetic titanium rib and related ‘growing rods’ approaches [Hell et al. 2005], have allowed a more effective and timely management of secondary musculoskeletal complications. The optimization of nutritional management to avoid potential complications arising from both malnutrition [Messina et al. 2008] and obesity [Sproule et al. 2009] has also emerged as an area of increased attention in recent years [Wang et al. 2007].

In the context of such advances to supportive care, a notable improvement in the natural history of SMA has been observed over the last two decades despite an absence of efficacious therapy; this is particularly true for children with SMA type 1. Byers and Banker described 25 such subjects in 1961 with a mean age at death ($n = 23$) of 10 months (range 17 days to 52 months), and a mean age of 17 months in those who survived ($n = 2$), range 10–24 months [Byers and Banker, 1961]. A similar report by Zerres and Rudnik-Schoneborn published in 1995 found a survival probability of 32% at 2 years, of 18% at 4 years, of 8% at 10 years, and of 0% at 20 years among 197 children with SMA type 1 [Zerres and Rudnik-Schoneborn, 1995]. A recent analysis of 143 patients with SMA type 1, comparing those born from 1980 to 1994 ($n = 65$) with those born between 1995 and 2006 ($n = 78$) showed a 70% reduction in risk of death in the latter group, likely associated with improvements in clinical management, particularly with regards to non-invasive ventilatory management [Bach, 2007; Bach and Bianchi, 2003; Bach et al. 2000], and a trend toward more proactive care [Oskoui et al. 2007]. While recent efforts have been made to standardize clinical care with the publication of a consensus statement of care for patients with SMA [Wang et al. 2007], variability of clinical care between centers and the evolving natural history of the disease can both represent challenges for trial design, as we discuss below.

**Molecular genetics of SMA offers insights into the pathogenesis of the disease and routes to potential therapies**

Most people with SMA harbor a homozygous deletion of $SMN1$. For the purposes of this review, SMA will refer only to the vast majority of clinically manifesting subjects who harbor a
The most severe infantile forms of SMA, there is histological and electrophysiological evidence of reinnervation that can partially compensate for functional loss [Crawford, 2003; Crawford and Pardo, 1996], at least initially.

The development of a mouse model for the disease allows rapid and effective preclinical testing of promising drug compounds

Although the development of a murine model of SMA is invaluable for preclinical study, important caveats must be considered related to the extrapolation of data from mice to human populations. There are several existing transgenic mouse models for SMA that to some degree mimic the clinical phenotype; however, there is no direct animal model for SMA. As a consequence of the genetics of SMA (specifically the presence of SMN2 to partially rescue the phenotype), SMA is an exclusively human disease. There is no equivalent disease state among other species. In mice, homozygous mutation of the murine survival motor neuron gene (Smn) is an embryonic lethal; current mouse models are created by insertion of the human SMN2 gene, which partially rescues the phenotype in a manner analogous to that seen in the human disease [Monani et al. 2003]. Another model incorporates SMN2 cDNA lacking exon 7 into this SMA mouse (snn −/−; hSMN2 +/+; hSMN2 Δ7 +/+); this model extends mean survival from 5.2 [Monani et al. 2003] to 13.3 days [Le et al. 2005]. Models that produce alternative splicing and variable expression of Smn [DiDonato et al. 2001], as well as tissue-specific deletion of murine exon 7 [Cifuentes-Diaz et al. 2001; Frugier et al. 2000] have also been developed. Recently a model that incorporates a variable number of SMN2 sequences directly into the murine SMN1 locus was developed. This allows the potential generation of mice with 0, 1, 2, 3, 4, 5, 6 or 8 copies of SMN2, with a resultant phenotypic spectrum more closely reflective of the human disease [Lutz et al. 2009]. While these models prove extremely useful for preclinical research applications, in the absence of any known effective therapy for SMA in humans it is impossible to predict how results of treatment trials in a mouse model will translate to the human disease.

Despite this limitation, the development of multiple rodent models that effectively mimic a range of human clinical phenotypes offers invaluable opportunities to study prospective drug
treatments in a preclinical setting. This model has permitted rapid screening of several promising pharmaceutical and gene therapy treatment strategies for the disease, including several that have progressed to clinical trials in humans. While increasingly sophisticated mouse models will further amplify the effectiveness of such screening methods, ultimately the successful translation of preclinical success will depend on effective clinical trial designs applied to studies among human subjects.

Challenges to effective clinical trial design in SMA

The phenotypic heterogeneity of SMA presents a challenge to the development of effective trial design as no single design or set of clinical endpoints ‘fits’ all phenotypes. As noted above, the natural history of the disease is evolving, with lessened morbidity across the varied phenotypes of the disease and improved mortality, particularly among subjects with SMA type 1 [Oskoui et al. 2007]. While such advances are clearly welcome, the changing landscape of the disease renders comparisons of subjects with historical ‘controls’ misleading and inaccurate. For this reason, any observations based on such comparisons must be evaluated with caution.

Conducting clinical trials in severely affected infants with SMA is extremely difficult due to the medical fragility of these patients and the frequent development of intercurrent respiratory illnesses among these children [Iannaccone et al. 2007]. For this reason, most recent clinical trials have focused on patients who have at least achieved the motor milestone of independent sitting [Mercuri et al. 2007]. However, trial design among patients with milder phenotypes is also challenged by the slowly progressive nature of the disease among this subset of patients, particularly ambulatory subjects with SMA. In studies looking at acquisition and loss of motor milestones such as walking among patients with SMA type 3, a slow functional loss is only observed by observation over a period of several years [Zerres et al. 1997; Russman et al. 1996; Zerres and Rudnik-Schoneborn, 1995]. Given that most patients with SMA will initially present during childhood, the effect of normal development with its potential for gain in function must also be considered. Several candidate clinical outcome measures have been developed for use in this population, including measures of motor function such as the Gross Motor Function Measure (GMFM), Hammersmith Scale of Motor Function, pulmonary function, and quality of life [Iannaccone, 2002]. However, while patients and clinicians often note a very slow decline in function over time, longitudinal studies of subjects using available clinical measures such as the GMFM and forced vital capacity, and electrophysiological measures such as motor unit number estimation (MUNE), fail to detect significant declines in function over intervals of 1 year (unpublished data).

For these reasons, there is a strong interest in the development of biomarkers with sensitivity for early treatment effects. While several molecular (plasmin), electrophysiological (electrical impedance myography, MUNE) and muscle imaging (dual-energy X-ray absorptiometry, MRI) measures have been explored as potential candidates, none has been established as a suitable such marker of disease progression at this time. Owing to the aforementioned stability of the disease among milder disease phenotypes over the chronic phase of the disease, all trials to date have been designed to detect an improvement (rather than stability of disease) in the treatment group compared with the placebo group. Of course, treating patients in the initial stages of SMA is currently not feasible, because patients typically do not receive a diagnosis and present to an SMA clinical research center until they have developed clinical symptoms commensurate with having entered the chronic phase of the disease.

Current and future treatment approaches

Phase I and II trials carried out to date have targeted several mechanisms including (1) neuroprotection, (2) anabolic stimulation of muscle to increase muscle mass, and (3) increased production of SMN protein transcription.

Strategies to promote motor neuron survival

Motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and SMA are characterized by selective motor neuron cell loss leading to progressive denervation, weakness and atrophy of skeletal muscles. The clinical relevance of blocking these processes has been confirmed in animal studies showing that treatment with exogenous survival (neurotrophic) factors, or inhibition of endogenous cell death pathways leads to improved neurite outgrowth [Jablonska et al. 2006] and increased survival [Lesbordes et al. 2003]. Several agents thought to have neuroprotective properties have been explored for effectiveness
in SMA. While positive results from open-label studies have been reported, there have been no blinded, placebo-controlled studies demonstrating efficacy of any of these treatment approaches.

Riluzole. Riluzole, a neuroprotective agent with inhibitory effects on the presynaptic release of glutamate that provides a modest benefit in ALS, showed possible benefit in seven SMA type 1 patients compared with three placebo-treated patients, with a targeted follow-up period of 9 months [Russman et al. 2003], with three of the seven treated patients experiencing prolonged survival compared with none of the untreated patients. A subsequent open-label study had insufficient enrollment but pharmacokinetic studies in three subjects showed adequate blood levels after oral administration to infants [Swoboda et al. 2007].

Gabapentin. Gabapentin, a US Food and Drug Administration (FDA) approved agent with multiple molecular effects, has also been explored as a potential neuroprotective agent. Gabapentin could also have a protective action by reducing the pool of releasable glutamate in neurons, thereby diminishing the excitotoxicity potential of this amino acid, although its mechanism of action is not fully understood [Taylor, 1997]. Two placebo-controlled trials of gabapentin in SMA were negative [Merlini et al. 2003; Taylor, 1997]. The initial trial included 84 adult patients with SMA type 2 and 3 who were treated over 12 months and evaluated with myometry [Taylor, 1997]. A second trial included 120 patients with SMA type 2 and 3 aged 5–60 years who were also treated for 12 months and evaluated with myometry [Merlini et al. 2003].

Thyrotropin-releasing hormone. The use of thyrotropin-releasing hormone, thought to have possible beneficial neurotrophic effects on the anterior horn cell, has been reported in several individual case reports [Kato et al. 2009; Takeuchi et al. 1994]. A single controlled pilot study of six SMA patients treated for 5 weeks resulted in significant improvements in muscle strength; further effectively powered studies have not been performed or proposed [Tzeng et al. 2000].

Olesoxime [cholest-4-en-3-one]. A cholesterol-like molecule, olesoxime, was identified based on its survival-promoting activity in purified cultured rat motor neurons deprived of neurotrophic factors. The pro-survival activity of olesoxime is thought to be mediated through modulation of the opening of the mitochondrial permeability transition pore (mPTP), a critical step in cell apoptosis [Bordet et al. 2007]. Under stress conditions, including ATP depletion, the increase in reactive oxygen species and mitochondrial calcium overload results in irreversible pore opening, instead of the normal pore flicker. This causes apoptogenic molecules to be released from mitochondria, including cytochrome c, leading, ultimately, to programmed cell death. In addition to improving survival, olesoxime has been associated with increased neurite outgrowth similar to the outgrowth observed after treatment with trophic factors in several animal models of neurodegeneration [Bordet et al. 2007] and accelerates the spontaneous regenerative processes in a mouse model of nerve crush injury, with improved recovery of both the amplitude and latency of compound muscle action potentials and increased the number of hypermyelinated axons [Bordet et al. 2008]. This was associated with improved functional sciatic index measurements [Bordet et al. 2008].

It is currently being evaluated in Europe in a Phase IIa study to assess its efficacy in the treatment of peripheral diabetic neuropathic pain and in a Phase IIb (pediatric) pharmacokinetic study in children and adolescents with SMA.

Strategies to improve muscle mass

Albuterol. Albuterol and other beta agonists have attracted attention for use in neuromuscular diseases due to the low toxicity and wide availability of these medications, as well as perceived beneficial effects on muscle strength and mass in healthy volunteers [van Baak et al. 2000] and benefit in the mdx mouse model of Duchenne muscular dystrophy (DMD) [Harcourt et al. 2007]. Albuterol improved leg strength using manual muscle testing [Fowler et al. 2004] and increased lean body mass [Skura et al. 2008] in placebo-controlled studies of boys with DMD. Albuterol [Payan et al. 2009; van der Kooi et al. 2005; Kissel et al. 2001] has been tested in randomized, placebo-controlled, double-blind studies to treat facioscapulohumeral muscular dystrophy, although all such studies to date have failed to demonstrate improvement in the a priori primary outcome measure. Albuterol is thought to have an anabolic mechanism in muscle, but more recently has also been suggested as an upregulator of SMN2 function.
An open-label study of albuterol in 13 SMA type 2 and 3 patients over 6 months showed modest benefits in strength, pulmonary function and lean mass [Kinali et al. 2002]; a more recent open-label study of 23 children with SMA type 2 (aged 30 months to 6 years) demonstrated an improvement in Hammersmith Motor Function Scale score over a 12-month follow-up period [Pane et al. 2008]. Blinded, placebo-controlled studies of this intervention have not been performed in SMA.

**Myostatin inhibition.** Myostatin, a negative regulator of muscle satellite cells that acts as a gatekeeper to cell entry into the S-phase of mitosis [Rodino-Klapac et al. 2009], has attracted significant recent interest as a potential treatment target of neuromuscular diseases including DMD and other muscular dystrophies, and SMA. There is a ‘mighty mouse’ myostatin knockout model, as well as an example in nature, the heavily muscularized ‘Belgian Blue’ cow [Rodino-Klapac et al. 2009]. An otherwise healthy human infant with exceptional muscularization due to a homozygous mutation of myostatin was described [Schuelke et al. 2004]; this observation, in particular, has driven increased interest in the potential of this pathway to modify muscle bulk and composition. Myostatin deletion improves muscle architecture [Wagner et al. 2002] in the mdx mouse model of DMD, as well as improves extensor digitorum longus mass and contraction force [Bogdanovich et al. 2002], further prompting attention to the potential role of this pathway in affecting the severity of pathology in DMD and other neuromuscular diseases.

Myostatin is subject to posttranslational removal of a signal peptide followed by cleavage of the propeptide N-terminus and formation of a C-terminal dimer to form the active protein product. The myostatin dimer acts upon an activin IIb receptor complex, initiating a cascade of intracellular events that ultimately culminate in blockade of mitosis. Myostatin is inhibited by several molecules, including Gasp-1, FLRG and follistatin; follistatin, in particular, has garnered scrutiny as an attractive potential drug candidate [Rodino-Klapac et al. 2009]. This protein is expressed widely and has multiple effects including the inhibition of follicle-stimulating hormone secretion. While overexpression of this molecule can lead to infertility, follistatin is subject to posttranslational modification. The FS-315 isoform is thought to have minimal gonadal effects, thus providing a promising therapeutic target [Rodino-Klapac et al. 2009]. Overexpression of follistatin increased muscle weight in mdx mice by 327%, greater even than the effect seen with knockout of myostatin function, implying additional effects of follistatin on muscle beyond myostatin inhibition. Antagonism of the activin IIb receptor has also garnered attention as a potential therapeutic strategy. A recently completed phase I trial of one such antagonist (MYO-029) in adults with Becker muscular dystrophy, limb-girdle muscular dystrophy and facioscapulohumeral muscular dystrophy showed a trend towards increased muscle size using dual-energy X-ray absorptiometry [Wagner et al. 2008]. While the drug was well tolerated, the study probably did not have sufficient power to detect a small treatment effect.

Inhibition of myostatin function, either through follistatin administration or activin IIb receptor antagonism, has been explored in the SMA mouse model, with conflicting results. A study of follistatin administration to SMA mice increased muscle mass, led to some gross motor function improvement (specifically improvement in capacity to turn) and extended lifespan by 30% by preventing some early deaths [Rose et al. 2009]. SMN protein levels were unchanged, indicating that this therapeutic effect was SMN independent. A study by Sumner and colleagues, however, applying both follistatin inhibition and activin IIb receptor antagonism, failed to increase muscle mass or improve motor function or survival in the SMA mouse model [Sumner et al. 2009]. While further research is needed, it has been speculated that myostatin blockade is ineffective in the context of denervated muscle, raising serious questions about the relevance of this therapeutic strategy to SMA.

**Strategies to increase SMN protein transcript**

Therapeutic efforts in SMA to date have been dominated by drugs targeting an increase of SMN2 function. As noted above, SMN2 copy number is inversely correlated with phenotypic severity in SMA patients [Wirth et al. 2006; Harada et al. 2002; Lefebvre et al. 1997], and increasing the SMN2 copy number in transgenic mice produces a milder phenotype confirming the observations in the human [Le et al. 2005].
Therefore, drugs that would increase SMN2 function, such as exon-splicing modulators or histone deacetylase inhibitors, are expected to mitigate the deleterious effects of SMN1 deletion. Drugs increasing SMN2 function in vitro include compounds currently licensed by the FDA for use in other indications (such as valproate, hydroxyurea and the butyrates) as well as several others not licensed by the FDA.

Phenylbutyrate. Phenylbutyrate and sodium butyrate act as histone deacetylase inhibitors and preclinical studies have suggested a potential therapeutic role for these agents in SMA, as they appear to increase the expression of SMN2 full-length transcripts [Darras and Kang, 2007; Wirth et al. 2006; Kernochan et al. 2005]. Phenylbutyrate, in particular, has been shown to increase the SMN transcript expression in fibroblast cultures and leukocytes of patients with SMA [Brahe et al. 2005; Andreassi et al. 2004], ranging from 50% to 160% in SMA type 1 and from 80% to 400% in SMA types 2 and 3 cultures [Andreassi et al. 2004]. Histone deacetylase inhibitors also display a neuroprotective capacity against oxidative stress in vitro [Rouaux et al. 2007].

While phenylbutyrate showed initial promise, improving motor function in an open-label pilot study of 10 SMA type 2 patients treated for 9 weeks [Mercuri et al. 2004], a subsequent placebo-controlled trial of intermittent treatment over 13 weeks in 107 SMA 2 patients aged 2–13 years was negative [Mercuri et al. 2007]. This phase II, double-blind, randomized, placebo-controlled trial compared a 13-week course of oral phenylbutyrate 500 mg/kg/day divided into five doses using an intermittent schedule (7 days on treatment, 7 days off treatment) with placebo in a total of 107 patients with SMA type II. The study was designed to demonstrate functional motor benefit, with muscle strength and FVC as secondary measures [Mercuri et al. 2007].

Hydroxyurea. Another histone deacetylase inhibitor, hydroxyurea, has also been shown to increase SMN2 gene expression and production of SMN protein in cultured lymphocytes of SMA patients [Liang et al. 2008; Grzeschik et al. 2005]. An uncontrolled, open-label trial in two patients with SMA type 1, five patients with SMA type 2 and two patients with SMA type 3 showed improvement of muscle strength without significant side effects. A subsequent trial of 33 patients with SMA type 2 and 3 treated for 8 weeks with three different doses of hydroxyurea demonstrated an enhancement of SMN gene expression and a trend towards improvement in some clinical outcome measures [Liang et al. 2008]. Two different randomized, double-blind, placebo-controlled trials in children with SMA types 2 and 3 are ongoing.

Valproic acid. Similar to phenylbutyrate and hydroxyurea, valproic acid (VPA) is a histone deacetylase inhibitor that has been shown to increase full-length SMN levels in fibroblasts or lymphoblastoid cell lines from SMA patients [Hahnen et al. 2006; Brichta et al. 2003]. An initial open-label clinical study of VPA among SMA patients and SMN1 carriers resulted in increased SMN mRNA and SMN protein levels after treatment in 7 of 10 SMN1 mutation carriers and 7 of 20 SMA patients, but remained unchanged or decreased in the remaining 13 [Brichta et al. 2006]. In another open-label pilot study conducted in the United States, seven SMA type 3 patients aged 17–45 years treated with VPA for 8 months on average showed improvement in muscle strength and function [Weihl et al. 2006]. A larger open-label study among 42 subjects with SMA (2 type 1, 29 type 2 and 11 type 3) noted a clear decline in function among patients who experienced significant weight gain (a potential adverse effect of VPA), with improvement restricted almost entirely to participants under 5 years of age [Swoboda et al. 2006]. A placebo-controlled study of 60 SMA type 2 patients (2–8 years) has recently been completed, and preliminary reports suggest a negative result for the primary outcome measure, but positive trends in subgroup analysis for children under 3 years with improvements when adjusted for baseline weight [Jarecki, 2008; Swoboda et al. 2007]. A multicenter phase II trial of valproate plus carnitine in 90 patients with SMA type 2/3 has been completed but the results are not yet available [Swoboda et al. 2007]. Two additional studies, ‘Carni-Val Type 1’ and ‘VALIANT SMA’ are currently recruiting subjects to study the effect of levocarnitine and VPA in SMA type 1 patients (NCT00661453 at http://www.clinicaltrials.gov) and VPA in ambula-tory SMA type 3 patients, respectively (NCT00481013 at http://www.clinicaltrials.gov).

Antisense oligonucleotides. Antisense oligonucleotides (AONs), small synthetic RNAs, DNAs
or nucleotide analogs which hybridize to a specific target sequence thereby altering the function of the targeted gene, have garnered recent attention as potential treatment strategies for several neuromuscular diseases, including DMD, myotonic dystrophy and SMA. As discussed above, the vast majority of SMN2 transcripts skip exon 7, leading to protein instability and reduced function. While the specific effect of the C to T transition seen in SMN2 is unknown, it may induce exon splicing [Aartsma-Rus and van Ommen, 2009] or introduce an exon splice site [Aartsma-Rus and van Ommen, 2009]. An AON strategy to block exonic or intronic silencing sequences to enhance exon inclusion might lead to a more functional SMN2-derived protein. Using a systematic in vitro approach, Krainer and colleagues studied AONs in intron 6, exon 7 and intron 7 to identify the optimal AON for exon 7 inclusion [Hua et al. 2008, 2007]. AONs developed using this approach have been tested both in patient-derived fibroblasts and SMA (smn −/−; hSMN2 +/+ ) mice, leading to increased SMN protein levels in the liver and to a lesser extent in kidney and muscle, although not within the central nervous system [Hua et al. 2008, 2007]. An alternative strategy uses AONs with exon-splicing enhancer motives that will induce exon inclusion by acting as an enhancer to recruit the required splicing factors to facilitate exon inclusion. This approach has successfully increased SMN levels in patient fibroblasts in a dose-dependent manner [Skordis et al. 2003]. Since AONs are unable to cross the blood–brain barrier, direct intrathecal injection will likely be required [Dickson et al. 2008] for application of an AON strategy to treat SMA using presently available technology.

Gene therapy and stem cell therapy approaches

Several gene therapy and stem cell strategies are presently under investigation, although significant preclinical work and methodological advances remain ahead before these approaches can become clinically relevant. Lentivirus vectors expressing RNAs targeting exon 7 have been shown to enhance exon 7 inclusion in SMA patient-derived fibroblasts, accompanied by increased SMN levels [Marquis et al. 2009, 2007; Baughan et al. 2006]. A transgenic SMA mouse model (smn −/−; hSMN2 +/+ ) has also been developed that expresses antisense RNAs [Meyer et al. 2009]. Expression of antisense RNAs induced exon 7 inclusion, restored SMN production in motor neurons and partially rescued the clinical phenotype [Meyer et al. 2009]. Intramuscular injection of a lentivirus vector expressing human SMN into transgenic SMA mice has also been shown to restore SMN levels, reduce motor neuron cell death and increase life expectancy, compared with placebo-treated control mice [Azzouz et al. 2004], further proof-of-principle in support of this approach.

Stem cell therapies have likewise generated intense attention and promise as a cellular replacement strategy for a wide array of degenerative conditions, including SMA. The goal of transplantation is to provide a pool of cells that is able to support endogenous neurons through delivery of (absent) neuroprotective factors and provide a replacement population for lost motor neurons. Stem-cell-derived motor neurons have been shown to grow axons and successfully form neuromuscular junctions in vitro [Gao et al. 2007, 2005; Wichterle et al. 2002], and stem cell transplants have lead to axonal growth and some recovery in a rat model of paralysis [Deshpande et al. 2006; Harper et al. 2004]. Induced pluripotent stem cells have been derived from patients with SMA and used to generate motor neurons that show selective deficits compared with wild-type motor neurons in culture [Ebert et al. 2009]. Similarly, pluripotent stem cells have been generated from ALS patients and induced to generate motor neurons [Dimos et al. 2008]. More recently, spinal-cord-derived neural stem cells have been successfully transplanted into the mouse model of SMA, with modest amelioration of the clinical phenotype and generation of a viable population of motor neurons [Corti et al. 2008]. A subsequent study using pluripotent stem cells demonstrated similar successful stem cell engraftment and differentiation with improved survival and functional improvement in treated SMNΔ7 mice compared with controls, demonstrating the therapeutic potential of this approach [Corti et al. 2010].

While these reports suggest the intriguing possibility of stem cell therapy, several challenges must be addressed before the successful implementation of stem cell therapy can be fully realized. For this strategy to be viable, large numbers of stem cells need to be generated, to successfully populate the nervous system, properly differentiate into motor neurons and, critically, must successfully and correctly extend axons to and synapse upon muscle targets. Of course, even if all of these significant obstacles be overcome, the process must result in a
clinically meaningful improvement in function [Nayak et al. 2006]. While recent studies in animals have garnered much deserved attention and generated widespread enthusiasm among patient organizations, clinicians and researchers alike, it remains unclear when this therapeutic strategy will emerge as a practical approach to the treatment for SMA in the human population.

Conclusions
Despite the lack of positive results of trials to date and the significant methodological and scientific challenges to the development and assessment of potential therapies, SMA remains an attractive disease target for drug development. Although motor disability caused by the loss of motor neurons burdens persons with SMA, they maintain normal cognition and, in its milder forms, normal or near-normal life expectancy. Many people with SMA are able to maintain productive employment and make important societal contributions, despite their disease. There have been significant preclinical advances over the last decade, although clinical advances have not yet materialized in a similar fashion. Nevertheless, the increasing sophistication and organization of patient groups on both a national and international level has spurred investigator collaboration and the development of necessary clinical trial infrastructure for the disease. In the context of impressive recent advances in the laboratory, there is good reason for cautious optimism that an effective treatment will eventually be found for SMA.

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