

# Therapeutic developments in spinal muscular atrophy

Douglas M. Sproule and Petra Kaufmann

*Ther Adv Neurol Disord*

[2010] 0(0) 1–13

DOI: 10.1177/

1756285610369026

© The Author(s), 2010.

Reprints and permissions:

<http://www.sagepub.co.uk/journalsPermissions.nav>

**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

Correspondence to:

**Douglas M. Sproule, MD**

Division of Pediatric

Neurosciences,

Department of Neurology,

SMA Clinical Research

Center, Columbia

University Medical Center,

Harkness Pavilion,

HP-514, 180 Fort

Washington Avenue, New

York, NY 10032-3791, USA

[dsproule@](mailto:dsproule@)

[neuro.columbia.edu](mailto:neuro.columbia.edu)

**Petra Kaufmann, MD, MSc**

Division of Pediatric

Neurosciences,

Department of Neurology,

SMA Clinical Research

Center, Columbia

University Medical Center,

New York, NY, USA

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

homozygous mutation to the SMN1 protein. Therapeutic approaches to non-*SMN1*-related SMA are not discussed here. An inverted duplicate gene, *SMN2*, located on the same chromosome (5q13) can modify the phenotype [Lefebvre *et al.* 1997]. Owing to minor sequence differences compared with *SMN1*, exon 7 of *SMN2* is alternatively spliced resulting in an unstable SMN protein that can only partially compensate for the lack of SMN1 in SMA [Lorson and Androphy, 1998]. *SMN2* does produce approximately 10% full-length transcript, and thus *SMN2* function can partially rescue the phenotype [Lefebvre *et al.* 1997]. The clinical severity is inversely correlated with *SMN2* copy number but the clinical phenotype cannot always be predicted based on copy number alone: SMA type 1 patients typically have two copies of *SMN2*, SMA type 2 patients have two or three copies, and SMA type 3 patients typically have three or more copies [Harada *et al.* 2002]. More than four *SMN2* copies are associated with a mild SMA type 3 phenotype [Wirth *et al.* 2006], while persons with five or more copies may remain clinically asymptomatic. This observation has been replicated in mice by knocking out the murine *SMN* gene and introducing a human *SMN2* transgene [Monani *et al.* 2000]. The existence of the partially functional protein derived from *SMN2* offers a potential target for therapeutic intervention.

SMN is widely expressed in all tissues, and its function is incompletely understood. Also poorly understood is the motor-neuron-specific pathology of the disease. SMN is found diffusely in the cytoplasm, but in the nucleus it is concentrated in punctuate structures termed 'gems' [Liu and Dreyfuss, 1996]. SMN is thought to play an important role in mRNA splicing [Paushkin *et al.* 2002]. There is increasing evidence suggesting that the initial pathophysiology of the motor neuron may lie at the level of the neuromuscular junction and axon terminus, rather than the anterior horn cell body proper [Cifuentes-Diaz *et al.* 2004, 2002]. These observations suggest that there is a period of time between the development of dysfunction of neuromuscular transmission (with concomitant clinical weakness) and irreversible anterior horn cell loss, during which an effective treatment could theoretically lead to restoration of neuronal function. Regardless of the specific effect of the homozygous *SMN1* mutation, motor neurons are ultimately lost leading to muscle atrophy and weakness. In all but

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 (*smn*  $-/-$ ; *hSMN2*  $+/+$ ; *hSMN2*  $\Delta 7$   $+/+$ ); 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 [Jablonka *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 prosurvival 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 into 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 ambulatory 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 led 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 *SMN $\Delta$ 7* 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.

## Acknowledgments

The authors are supported by an SMA Foundation grant, a CTSA Award NIH 1 ULI RR024156 (PK), and a NINDS Neurological Sciences Academic Development Award (K12 NS01698) (DS). This manuscript is related to Dr Kaufmann's work while at Columbia University, and is not related to NIH/NINDS.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

## References

- Aartsma-Rus, A. and van Ommen, G.-J.B. (2009) Progress in therapeutic antisense applications for neuromuscular disorders. *Eur J Hum Genet*: <http://dx.doi.org/10.1038/ejhg.2009.160>
- Andreassi, C., Angelozzi, C., Tiziano, F.D., Vitali, T., De Vincenzi, E., Boninsegna, A. *et al.* (2004) Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy. *Eur J Hum Genet* 12: 59–65.
- Azzouz, M., Le, T., Ralph, G.S., Walmsley, L., Monani, U.R., Lee, D.C. *et al.* (2004) Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. *J Clin Invest* 114: 1726–1731.
- Bach, J.R. (2007) Medical considerations of long-term survival of Werdnig–Hoffmann disease. *Am J Phys Med Rehabil* 86: 349–355.
- Bach, J.R. and Bianchi, C. (2003) Prevention of pectus excavatum for children with spinal muscular atrophy type 1. *Am J Phys Med Rehabil* 82: 815–819.
- Bach, J.R., Ishikawa, Y. and Tatara, K. (2001) Pulmonary manifestations of neuromuscular disease. *Pediatr Pulmonol* 31: 89–90.
- Bach, J.R., Niranjana, V. and Weaver, B. (2000) Spinal muscular atrophy type 1: A noninvasive respiratory management approach. *Chest* 117: 1100–1105.
- Baughan, T., Shababi, M., Coady, T.H., Dickson, A.M., Tullis, G.E. and Lorson, C.L. (2006) Stimulating full-length SMN2 expression by delivering bifunctional RNAs via a viral vector. *Mol Ther* 14: 54–62.
- Bogdanovich, S., Krag, T.O., Barton, E.R., Morris, L.D., Whittemore, L.A., Ahima, R.S. *et al.* (2002) Functional improvement of dystrophic muscle by myostatin blockade. *Nature* 420: 418–421.
- Bordet, T., Buisson, B., Michaud, M., Abitbol, J.L., Marchand, F., Grist, J. *et al.* (2008) Specific antinociceptive activity of cholest-4-en-3-one, oxime (TRO19622) in experimental models of painful diabetic and chemotherapy-induced neuropathy. *J Pharmacol Exp Ther* 326: 623–632.
- Bordet, T., Buisson, B., Michaud, M., Drouot, C., Galea, P., Delaage, P. *et al.* (2007) Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis. *J Pharmacol Exp Ther* 322: 709–720.
- Brahe, C., Vitali, T., Tiziano, F.D., Angelozzi, C., Pinto, A.M., Borgo, F. *et al.* (2005) Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients. *Eur J Hum Genet* 13: 256–259.
- Brichta, L., Hofmann, Y., Hahnen, E., Siebzehrubl, F.A., Raschke, H., Blumcke, I. *et al.* (2003) Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. *Hum Mol Genet* 12: 2481–2489.
- Brichta, L., Holker, I., Haug, K., Klockgether, T. and Wirth, B. (2006) In vivo activation of SMN in spinal muscular atrophy carriers and patients treated with valproate. *Ann Neurol* 59: 970–975.
- Byers, R.K. and Banker, B.Q. (1961) Infantile muscular atrophy. *Arch Neurol* 5: 140–164.
- Cifuentes-Diaz, C., Bitoun, M., Goudou, D., Seddiqi, N., Romero, N., Rieger, F. *et al.* (2004) Neuromuscular expression of the BTB/POZ and zinc finger protein myoneurin. *Muscle Nerve* 29: 59–65.

- Cifuentes-Diaz, C., Frugier, T., Tiziano, F.D., Lacene, E., Roblot, N., Joshi, V. *et al.* (2001) Deletion of murine SMN exon 7 directed to skeletal muscle leads to severe muscular dystrophy. *J Cell Biol* 152: 1107–1114.
- Cifuentes-Diaz, C., Nicole, S., Velasco, M.E., Borra-Cebrian, C., Panozzo, C., Frugier, T. *et al.* (2002) Neurofilament accumulation at the motor endplate and lack of axonal sprouting in a spinal muscular atrophy mouse model. *Hum Mol Genet* 11: 1439–1447.
- Corti, S., Nizzardo, M., Nardini, M., Donadoni, C., Salani, S., Ronchi, D. *et al.* (2008) Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. *J Clin Invest* 118: 3316–3330.
- Corti, S., Nizzardo, M., Nardini, M., Donadoni, C., Salani, S., Ronchi, D. *et al.* (2010) Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice. *Brain* 133: 465–481.
- Crawford, T.O. (2003) Spinal muscular atrophies, In: Jones, R.H., De Vivo, D.C. and Darras, B.T. (eds). *Neuromuscular Disorders of Infancy, Childhood, and Adolescence: A Clinician's Approach*, Butterworth Heinemann: Philadelphia, PA, pp. 145–166.
- Crawford, T.O. and Pardo, C.A. (1996) The neurobiology of childhood spinal muscular atrophy. *Neurobiol Dis* 3: 97–110.
- Darras, B.T. and Kang, P.B. (2007) Clinical trials in spinal muscular atrophy. *Curr Opin Pediatr* 19: 675–679.
- Deshpande, D.M., Kim, Y.S., Martinez, T., Carmen, J., Dike, S., Shats, I. *et al.* (2006) Recovery from paralysis in adult rats using embryonic stem cells. *Ann Neurol* 60: 32–44.
- Dickson, A., Osman, E. and Lorson, C.L. (2008) A negatively acting bifunctional RNA increases survival motor neuron both in vitro and in vivo. *Hum Gene Ther* 19: 1307–1315.
- DiDonato, C.J., Lorson, C.L., De Repentigny, Y., Simard, L., Chartrand, C., Androphy, E.J. *et al.* (2001) Regulation of murine survival motor neuron (Smn) protein levels by modifying Smn exon 7 splicing. *Hum Mol Genet* 10: 2727–2736.
- Dimos, J.T., Rodolfa, K.T., Niakan, K.K., Weisenthal, L.M., Mitumoto, H., Chung, W. *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321: 1218–1221.
- Ebert, A.D., Yu, J., Rose, F.F.J., Mattis, V.B., Lorson, C.L., Thomson, J.A. *et al.* (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457: 277–280.
- Emery, A.E. (1991) Population frequencies of inherited neuromuscular diseases—a world survey. *Neuromuscul Disord* 1: 19–29.
- Feldkotter, M., Schwarzer, V., Wirth, R., Wienker, T.F. and Wirth, B. (2002) Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet* 70: 358–368.
- Fowler, E.G., Graves, M.C., Wetzel, G.T. and Spencer, M.J. (2004) Pilot trial of albuterol in Duchenne and Becker muscular dystrophy. *Neurology* 62: 1006–1008.
- Frugier, T., Tiziano, F.D., Cifuentes-Diaz, C., Miniou, P., Roblot, N., Dierich, A. *et al.* (2000) Nuclear targeting defect of SMN lacking the C-terminus in a mouse model of spinal muscular atrophy. *Hum Mol Genet* 9: 849–858.
- Gao, J., Coggeshall, R.E., Chung, J.M., Wang, J. and Wu, P. (2007) Functional motoneurons develop from human neural stem cell transplants in adult rats. *Neuroreport* 18: 565–569.
- Gao, J., Coggeshall, R.E., Tarasenko, Y.I. and Wu, P. (2005) Human neural stem cell-derived cholinergic neurons innervate muscle in motoneuron deficient adult rats. *Neuroscience* 131: 257–262.
- Gilliam, T.C., Brzustowicz, L.M., Castilla, L.H., Lehner, T., Penchaszadeh, G.K., Daniels, R.J. *et al.* (1990) Genetic homogeneity between acute and chronic forms of spinal muscular atrophy. *Nature* 345: 823–825.
- Grzeschik, S.M., Ganta, M., Prior, T.W., Heavlin, W.D. and Wang, C.H. (2005) Hydroxyurea enhances SMN2 gene expression in spinal muscular atrophy cells. *Ann Neurol* 58: 194–202.
- Hahnen, E., Eyupoglu, I.Y., Brichta, L., Haastert, K., Trankle, C., Siebzehnrubl, F.A. *et al.* (2006) In vitro and ex vivo evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy. *J Neurochem* 98: 193–202.
- Harada, Y., Sutomo, R., Sadewa, A.H., Akutsu, T., Takeshima, Y., Wada, H. *et al.* (2002) Correlation between SMN2 copy number and clinical phenotype of spinal muscular atrophy: three SMN2 copies fail to rescue some patients from the disease severity. *J Neurol* 249: 1211–1219.
- Harcourt, L.J., Ryall, J.G. and Lynch, G.S. (2007) Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul Disord* 17: 47–55.
- Harper, J.M., Krishnan, C., Darman, J.S., Deshpande, D.M., Peck, S., Shats, I. *et al.* (2004) Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. *Proc Natl Acad Sci U S A* 101: 7123–7128.
- Hell, A.K., Campbell, R.M. and Hefti, F. (2005) The vertical expandable prosthetic titanium rib implant for the treatment of thoracic insufficiency syndrome associated with congenital and neuromuscular scoliosis in young children. *J Pediatr Orthop B* 14: 287–293.
- Hua, Y., Vickers, T.A., Baker, B.F., Bennett, C.F. and Krainer, A.R. (2007) Enhancement of SMN2 exon 7

- inclusion by antisense oligonucleotides targeting the exon. *PLoS Biol* 5: e73.
- Hua, Y., Vickers, T.A., Okunola, H.L., Bennett, C.F. and Krainer, A.R. (2008) Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet* 82: 834–848.
- Iannaccone, S. (2007) Modern management of spinal muscular atrophy. *J Child Neurol* 22: 974–978.
- Iannaccone, S., Hynan, L.S. and Group, A. (2007) Challenges of enrollment for SMA type I clinical trials [abstract]. *Neuromuscul Disord* 17: 780.
- Iannaccone, S.T. (2002) Outcome measures for pediatric spinal muscular atrophy. *Arch Neurol* 59: 1445–1450.
- Jablonka, S., Karle, K., Sandner, B., Andreassi, C., von Au, K. and Sendtner, M. (2006) Distinct and overlapping alterations in motor and sensory neurons in a mouse model of spinal muscular atrophy. *Hum Mol Genet* 15: 511–518.
- Jarecki, J. (2008) Carni-Val Results. *Compass—A Publication dedicated to Research Updates, Families of SMA* Summer 2008: 2–3.
- Kato, Z., Okuda, M., Okumura, Y., Arai, T., Teramoto, T., Nishimura, M. *et al.* (2009) Oral administration of the thyrotropin-releasing hormone (TRH) analogue, taltireline hydrate, in spinal muscular atrophy. *J Child Neurol* 24: 1010–1012.
- Kaufmann, P. and Iannaccone, S.T. (2008) Clinical trials in spinal muscular atrophy. *Phys Med Rehabil Clin N Am* 19: 653–660.
- Kernochan, L.E., Russo, M.L., Woodling, N.S., Huynh, T.N., Avila, A.M., Fischbeck, K.H. *et al.* (2005) The role of histone acetylation in SMN gene expression. *Hum Mol Genet* 14: 1171–1182.
- Kinali, M., Mercuri, E., Main, M., De Biasia, F., Karatza, A., Higgins, R. *et al.* (2002) Pilot trial of albuterol in spinal muscular atrophy. *Neurology* 59: 609–610.
- Kissel, J.T., McDermott, M.P., Mendell, J.R., King, W.M., Pandya, S., Griggs, R.C. *et al.* (2001) Randomized, double-blind, placebo-controlled trial of albuterol in facioscapulohumeral dystrophy. *Neurology* 57: 1434–1440.
- Kolb, S.J., Battle, D.J. and Dreyfuss, G. (2007) Molecular functions of the SMN complex. *J Child Neurol* 22: 990–994.
- Kugelberg, E. and Welander, L. (1956) Heredofamilial juvenile muscular atrophy simulating muscular dystrophy. *AMA Arch Neurol Psychiatry* 75: 500–509.
- Le, T.T., Pham, L.T., Butchbach, M.E., Zhang, H.L., Monani, U.R., Coovert, D.D. *et al.* (2005) SMN $\Delta$ 7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum Mol Genet* 14: 845–857.
- Lefebvre, S., Burlet, P., Liu, Q., Bertrand, S., Clermont, O., Munnich, A. *et al.* (1997) Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet* 16: 265–269.
- Lesbordes, J.C., Cifuentes-Diaz, C., Miroglio, A., Joshi, V., Bordet, T., Kahn, A. *et al.* (2003) Therapeutic benefits of cardiostrophin-1 gene transfer in a mouse model of spinal muscular atrophy. *Hum Mol Genet* 12: 1233–1239.
- Liang, W.C., Yuo, C.Y., Chang, J.G., Chen, Y.C., Chang, Y.F., Wang, H.Y. *et al.* (2008) The effect of hydroxyurea in spinal muscular atrophy cells and patients. *J Neurol Sci* 268: 87–94.
- Liu, Q. and Dreyfuss, G. (1996) A novel nuclear structure containing the survival of motor neurons protein. *Embo J* 15: 3555–3565.
- Lorson, C.L. and Androphy, E.J. (1998) The domain encoded by exon 2 of the survival motor neuron protein mediates nucleic acid binding. *Hum Mol Genet* 7: 1269–1275.
- Lunn, M.R. and Wang, C.H. (2008) Spinal muscular atrophy. *Lancet* 371: 2120–2133.
- Lutz, C., Osborne, M., Winberg, M., Xue, Y., Rojas, J., Yasenchak, J. *et al.* (2009) Characterization of new mouse models of SMA. In: *Families of SMA Annual Meeting*, Cincinnati, OH, 18–20 June 2009, p.95.
- Marquis, J., Kampfer, S.S., Angehrn, L. and Schumperli, D. (2009) Doxycycline-controlled splicing modulation by regulated antisense U7 snRNA expression cassettes. *Gene Ther* 16: 70–77.
- Marquis, J., Meyer, K., Angehrn, L., Kampfer, S.S., Rothen-Rutishauser, B. and Schumperli, D. (2007) Spinal muscular atrophy: SMN2 Pre-mRNA splicing corrected by a U7 snRNA derivative carrying a splicing enhancer sequence. *Mol Ther* 15: 1479–1486.
- Melki, J., Lefebvre, S., Burglen, L., Burlet, P., Clermont, O., Millasseau, P. *et al.* (1994) De novo and inherited deletions of the 5q13 region in spinal muscular atrophies. *Science* 264: 1474–1477.
- Mercuri, E., Bertini, E., Messina, S., Pelliccioni, M., D'Amico, A., Colitto, F. *et al.* (2004) Pilot trial of phenylbutyrate in spinal muscular atrophy. *Neuromuscul Disord* 14: 130–135.
- Mercuri, E., Bertini, E., Messina, S., Solari, A., D'Amico, A., Angelozzi, C. *et al.* (2007) Randomized, double-blind, placebo-controlled trial of phenylbutyrate in spinal muscular atrophy. *Neurology* 68: 51–55.
- Merlini, L., Solari, A., Vita, G., Bertini, E., Minetti, C., Mongini, T. *et al.* (2003) Role of gabapentin in spinal muscular atrophy: results of a multicenter, randomized Italian study. *J Child Neurol* 18: 537–541.
- Merlini, L., Stagni, S.B., Marri, E. and Granata, C. (1992) Epidemiology of neuromuscular disorders in the under-20 population in Bologna Province, Italy. *Neuromuscul Disord* 2: 197–200.

- Messina, S., Pane, M., De Rose, P., Vasta, I., Sorletti, D., Aloysius, A. *et al.* (2008) Feeding problems and malnutrition in spinal muscular atrophy type II. *Neuromuscul Disord* 18: 389–393.
- Meyer, K., Marquis, J., Trub, J., Nlend, R., Verp, S., Ruepp, M.D. *et al.* (2009) Rescue of a severe mouse model for spinal muscular atrophy by U7 snRNA-mediated splicing modulation. *Hum Mol Genet* 18: 546–555.
- Monani, U.R., Pastore, M.T., Gavrilina, T.O., Jablonka, S., Le, T.T., Andreassi, C. *et al.* (2003) A transgene carrying an A2G missense mutation in the SMN gene modulates phenotypic severity in mice with severe (type I) spinal muscular atrophy. *J Cell Biol* 160: 41–52.
- Monani, U.R., Sendtner, M., Covert, D.D., Parsons, D.W., Andreassi, C., Le, T.T. *et al.* (2000) The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in *Smn(-/-)* mice and results in a mouse with spinal muscular atrophy. *Hum Mol Genet* 9: 333–339.
- Nayak, M.S., Kim, Y.S., Goldman, M., Keirstead, H.S. and Kerr, D.A. (2006) Cellular therapies in motor neuron diseases. *Biochim Biophys Acta* 1762: 1128–1138.
- Oskoui, M. and Kaufmann, P. (2008) Spinal muscular atrophy. *Neurotherapeutics* 5: 499–506.
- Oskoui, M., Levy, G., Garland, C.J., Gray, J.M., O'Hagen, J., De Vivo, D.C. *et al.* (2007) The changing natural history of spinal muscular atrophy type 1. *Neurology* 69: 1931–1936.
- Pane, M., Staccioli, S., Messina, S., D'Amico, A., Pelliccioni, M., Mazzone, E.S. *et al.* (2008) Daily salbutamol in young patients with SMA type II. *Neuromuscul Disord* 18: 536–540.
- Paushkin, S., Gubitz, A.K., Massenet, S. and Dreyfuss, G. (2002) The SMN complex, an assembly of ribonucleoproteins. *Curr Opin Cell Biol* 14: 305–312.
- Payan, C.A., Hogrel, J.Y., Hammouda, E.H., Lacomblez, L., Ollivier, G., Doppler, V. *et al.* (2009) Periodic salbutamol in facioscapulohumeral muscular dystrophy: a randomized controlled trial. *Arch Phys Med Rehabil* 90: 1094–1101.
- Pearn, J. (1973) The gene frequency of acute Werdnig–Hoffmann disease (SMA type 1). A total population survey in North-East England. *J Med Genet* 10: 260–265.
- Pearn, J. (1978) Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. *J Med Genet* 15: 409–413.
- Prior, T.W. (2007) Spinal muscular atrophy diagnostics. *J Child Neurol* 22: 952–956.
- Rodino-Klapac, L.R., Haidet, A.M., Kota, J., Handy, C., Kaspar, B.K. and Mendell, J.R. (2009) Inhibition of myostatin with emphasis on follistatin as a therapy for muscle disease. *Muscle Nerve* 39: 283–296.
- Rose, F.F.J., Mattis, V.B., Rindt, H. and Lorson, C.L. (2009) Delivery of recombinant follistatin lessens disease severity in a mouse model of spinal muscular atrophy. *Hum Mol Genet* 18: 997–1005.
- Rouaux, C., Panteleeva, I., René, F., Gonzalez de Aguilar, J.L., Echaniz-Laguna, A., Dupuis, L. *et al.* (2007) Sodium valproate exerts neuroprotective effects in vivo through CREB-binding protein-dependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. *J Neurosci* 27: 5535–5545.
- Russman, B.S. (2007) Spinal muscular atrophy: clinical classification and disease heterogeneity. *J Child Neurol* 22: 946–951.
- Russman, B.S., Buncher, C.R., White, M., Samaha, F.J. and Iannaccone, S.T. (1996) Function changes in spinal muscular atrophy II and III. The DCN/SMA Group. *Neurology* 47: 973–976.
- Russman, B.S., Iannaccone, S.T. and Samaha, F.J. (2003) A phase 1 trial of riluzole in spinal muscular atrophy. *Arch Neurol* 60: 1601–1603.
- Schuelke, M., Wagner, K.R., Stolz, L.E., Hübner, C., Riebel, T., Kömen, W. *et al.* (2004) Myostatin mutation associated with gross muscle hypertrophy in a child. *N Engl J Med* 350: 2682–2688.
- Skordis, L.A., Dunckley, M.G., Yue, B., Eperon, I.C. and Muntoni, F. (2003) Bifunctional antisense oligonucleotides provide a trans-acting splicing enhancer that stimulates SMN2 gene expression in patient fibroblasts. *Proc Natl Acad Sci U S A* 100: 4114–4119.
- Skura, C.L., Fowler, E.G., Wetzel, G.T., Graves, M. and Spencer, M.J. (2008) Albuterol increases lean body mass in ambulatory boys with Duchenne or Becker muscular dystrophy. *Neurology* 70: 137–143.
- Sproule, D.M., Montes, J., Montgomery, M., Battista, V., Koenigsberger, D., Shen, W. *et al.* (2009) Increased fat mass and high incidence of overweight despite low body mass index in patients with spinal muscular atrophy. *Neuromuscul Disord* 19: 391–396.
- Sumner, C.J. (2006) Therapeutics development for spinal muscular atrophy. *NeuroRx* 3: 235–245.
- Sumner, C.J., Wee, C.D., Warsing, L.C., Choe, D.W., Ng, A.S., Lutz, C. *et al.* (2009) Inhibition of myostatin does not ameliorate disease features of severe spinal muscular atrophy mice. *Hum Mol Genet* 18: 3145–3152.
- Swoboda, K.J., Kissel, J.T., Crawford, T.O., Bromberg, M.B., Acsadi, G., D'Anjou, G. *et al.* (2007) Perspectives on clinical trials in spinal muscular atrophy. *J Child Neurol* 22: 957–966.
- Swoboda, K.J., Scott, C., Wood, J., Reyna, S.P., Wride, M.C., Stokes, A. *et al.* (2006) Phase I/II Study of Valproic Acid: An open label assessment of safety, tolerability and dosing in SMA subjects greater than two years of age, *10th International Spinal Muscular Atrophy Research Group Meeting (FSMA)*, Montreal, Canada, <http://www.fsma.org/res2006news.shtml>

- Takeuchi, Y., Miyamoto, Y., Komatsu, H., Oomizono, Y., Nishimura, A., Okano, S. *et al.* (1994) Efficacy of thyrotropine-releasing hormone in the treatment of spinal muscular atrophy. *J Child Neurol* 9: 287–289.
- Taylor, C.P. (1997) Mechanisms of action of gabapentin. *Rev Neurol (Paris)* 153 (Suppl 1): S39–S45.
- Tzeng, A.C., Cheng, J., Fryczynski, H., Niranjani, V., Stitik, T., Sial, A. *et al.* (2000) A study of thyrotropin-releasing hormone for the treatment of spinal muscular atrophy: a preliminary report. *Am J Phys Med Rehabil* 79: 435–440.
- van Baak, M.A., Mayer, L.H., Kempinski, R.E. and Hartgens, F. (2000) Effect of salbutamol on muscle strength and endurance performance in nonasthmatic men. *Med Sci Sports Exerc* 32: 1300–1306.
- van der Kooij, E.L., Lindeman, E. and Riphagen, I. (2005) Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev* 1: CD003907.
- Wagner, K.R., Fleckenstein, J.L., Amato, A.A., Barohn, R.J., Bushby, K., Escolar, D.M. *et al.* (2008) A phase I/II trial of MYO-029 in adult subjects with muscular dystrophy. *Ann Neurol* 63: 561–571.
- Wagner, K.R., McPherron, A.C., Winik, N. and Lee, S.J. (2002) Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. *Ann Neurol* 52: 832–836.
- Wang, C.H., Finkel, R.S., Bertini, E.S., Schroth, M., Simonds, A., Wong, B. *et al.* (2007) Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol* 22: 1027–1049.
- Weihl, C.C., Connolly, A.M. and Pestronk, A. (2006) Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy. *Neurology* 67: 500–501.
- Werdnig, G. (1891) Zwei frühinfantile hereditäre Fälle von progressiver Muskelatrophie unter dem Bilde der Dystrophie, aber auf neurotischer Grundlage. *Arch Psychiatrie Nervenkrankheiten* 22: 437–480.
- Wichterle, H., Lieberam, I., Porter, J.A. and Jessell, T.M. (2002) Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110: 385–397.
- Wirth, B., Brichta, L., Schrank, B., Lochmuller, H., Blick, S., Baasner, A. *et al.* (2006) Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number. *Hum Genet* 119: 422–428.
- Zerres, K. and Rudnik-Schoneborn, S. (1995) Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications. *Arch Neurol* 52: 518–523.
- Zerres, K., Rudnik-Schoneborn, S., Forrest, E., Lusakowska, A., Borkowska, J. and Hausmanowa-Petrusewicz, I. (1997) A collaborative study on the natural history of childhood and juvenile onset proximal spinal muscular atrophy (type II and III SMA): 569 patients. *J Neurol Sci* 146: 67–72.

Visit SAGE journals online  
<http://tan.sagepub.com>

 SAGE JOURNALS  
 Online