Progress and Promise: The Current Status of Spinal Muscular Atrophy Therapeutics

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Abstract: Spinal muscular atrophy (SMA) is an inherited neuromuscular disorder that causes degeneration of α-motor neurons. Frequently, muscle weakness is very severe causing affected infants to die before reaching two years of age, but mild forms of the disease can be characterized by relatively static muscle weakness for many years. SMA is caused by recessive mutations of the \( \text{SMN1} \) gene, but all patients retain at least one copy of \( \text{SMN2} \), a similar gene capable of producing low levels of full-length SMN protein. No treatments currently exist for SMA patients, but the identification of therapeutic targets and the development of suitable animal models for preclinical testing have resulted in increased drug development efforts in the past ten years. Here, we review the current status of many of these programs, including those designed to activate \( \text{SMN2} \) gene expression, modulate splicing of \( \text{SMN2} \) preRNAs, stabilize SMN protein, replace \( \text{SMN1} \), provide neuroprotective support, and transplant neural cells.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disease that is the leading inherited cause of infant mortality with a prevalence of approximately 1 in 6,000-10,000 (Pearn, 1978; McAndrew et al., 1997). SMA was first described in the 1890s by physicians Guido Werdnig and Johan Hoffman when their autopsy studies highlighted the loss of α-motor neurons (MNs) from the ventral horn of spinal cord and skeletal muscle atrophy as the key pathological features of the disorder (Werdnig, 1891). Over the next century, SMA diagnosis continued to be made based principally on clinical examination and there was little hope for disease-modifying treatment. In 1995, the discovery of the disease-causing gene (Lefebvre et al., 1995) provided the ability to diagnose SMA with a simple molecular blood test. This has enhanced early diagnosis and resulted in established standards of care for patients (Wang et al., 2007). In addition, this discovery triggered a period of rapid advances in understanding the molecular and cellular basis of the disease. This has led to the identification of therapeutic targets and the development of SMA animal models, which can be used for preclinical therapeutics investigations. Consequently, therapeutics development in SMA now has strong academic, government, and industry involvement with companies such as Trophos, Repligen Corporation, CA Stem Cell, Inc., ISIS Pharmaceuticals, Inc., Genzyme Corporation, Paratek Pharmaceuticals, Inc., PTC Therapeutics, Novartis Pharmaceuticals, Inc., and Merck & Co., Inc. investing time and money in basic and clinical research. While clinical trials with repurposed drugs have been recently completed in SMA patients, Repligen’s recent phase 1 clinical trial approval from the FDA marks the first time a drug developed solely for the treatment of SMA has entered clinical trials, with more likely to come in the next few years. The promise of a treatment for this devastating neuromuscular disease has never been greater. In this review, we aim to highlight some of the therapies currently in development.

SMA Clinical Features

Patients with SMA exhibit severe, symmetrical proximal muscle weakness and atrophy, which affects the
legs more than the arms. In severe forms of SMA, the truncal muscles are also preferentially affected. SMA patients are typically classified into four groups based on age of disease onset and achievement of motor milestones. SMA type I or Werdnig-Hoffman disease is the most common and is characterized by disease symptoms before 6 months and no ability to sit independently. Without respiratory and nutritional support, these infants generally die before the age of 2 years. SMA type II patients begin showing symptoms between 6-18 months of life, and at some point achieve the ability to sit independently, but are never able to stand or walk. These patients generally live into their thirties, with age of death correlating with the required amount of respiratory support. SMA type III (Kugelberg-Welander) patients exhibit symptoms in childhood and while they eventually become wheelchair bound, are initially able to stand and walk independently. Patients with SMA type III have normal life expectancies. SMA type IV is the least common and is characterized by adolescent or adult-onset of disease and often retained ambulation. Although this classification system is widely used and accepted (Munsat and Davies, 1992), the phenotypic spectrum of SMA is continuous and symptoms are variable within SMA types. There are not adequate autopsy data available to know whether there are differences in the magnitude of MN loss that account for this vast range of clinical disease severity. However, electromyography (EMG) of severely affected patients shows extensive spontaneous fibrillations (indicating active denervation) associated with small voluntary motor units, while EMG of more mildly affected patients displays few fibrillations and large voluntary motor units indicating that collateral axonal sprouting has occurred (Crawford and Pardo, 1996). This suggests that in mild forms of SMA the distal terminal of the MN retains the ability to reinnervate neighboring, denervated myofibers, thereby maintaining muscle power and raises that possibility that therapeutic strategies to accentuate this process might be particularly effective for mild SMA patients. In all SMA patients, the highest rate of functional decline appears to occur soon after the onset of symptoms, after which muscle power remains relatively stable in many patients (Dubowitz, 1964; Crawford and Pardo, 1996; Iannaccone et al., 2000). Motor unit number estimation (MUNE) studies have also documented a precipitous loss of distal muscle motor units during disease progression (Swoboda et al., 2005). This has raised the possibility that the most effective therapy may require delivery prior to this decline.

Genetics of SMA

The SMA disease-causing gene was mapped in 1990 to a complicated region of chromosome 5q that contains an inverted duplication (Bruzustowicz et al., 1990; Melki et al., 1990). Lefebvre et al. (1995) later documented that mutations in one of the genes in this area, survival of motor neuron (SMN1), cause SMA. These mutations are usually homozygous deletions of exons 6-8; however, frameshift, missense, and nonsense SMN1 mutations have also been shown to cause SMA (Wirth, 2000). A duplicated and inverted centromeric copy of this gene, SMN2, exists in 90-95% of the normal population and in all patients with SMA. The only functional difference between the genes appears to be a C-T transition at position 6 in exon 7 in SMN2, which lies within an exonic enhancer sequence and leads to frequent exon 7 skipping during transcription of SMN2-derived pre-RNAs (Lorson et al., 1999). The resulting mRNA transcripts lacking exon 7 (SMNΔ7) encode a truncated protein product that is rapidly degraded (Lorson and Androphy, 2000; Burnett et al., 2009), but a small proportion of transcripts arising from the SMN2 gene are full-length and encode for the full length SMN protein (Figure 1). SMA is thus caused by reduced expression levels of SMN protein (Lefebvre et al., 1995; Coovert et al., 1997; Lefebvre et al., 1997). The ability of the SMN2 gene to correctly encode for functional SMN protein in the absence of SMN1 gene products plays a key role in modifying phenotypic outcome of SMA patients. There is an inverse relationship between SMN2 copy number and disease severity with most SMA type I patients having one or two SMN2 copies, most SMA type II patients having three SMN2 copies, and most SMA type III patients having 3 or 4 SMN2 copies (Parano et al., 1996; Feldkotter et al., 2002). Some individuals who lack SMN1 have been identified to retain 5 copies of the SMN2 gene, and do not display SMA symptoms, indicating that adequate SMN2 activity alone can prevent disease (Prior et al., 2004). This observation along with similar observations in mice (see below) has focused the majority of effort in SMA therapeutics development on identifying strategies to increase SMN levels.

SMN Protein Function

The evolutionarily conserved 38 kDa SMN protein is ubiquitously expressed, raising the question of why motor neurons are particularly susceptible to reduced SMN expression. SMN is present in the cytoplasm and the nucleus. When in the nucleus, it is often localized in “gems” which are closely related to and located near Cajal bodies, major sites of nuclear RNA transcription and processing (Liu and Dreyfuss, 1996). SMN forms a complex with Gemins 2-8 and unrip and this complex regulates the assembly of small nuclear ribonucleoprotein (snRNPs) (reviewed in Kolb et al., 2007;
Pellizzoni, 2007). snRNPs are RNA-protein complexes that are part of the spliceosome. Published work has documented reduced snRNP assembly in SMA patient-derived cell lines and SMA mouse tissues (Gabanella et al., 2005; Wan et al., 2005). Nonetheless, the mechanisms by which lowered snRNP assembly levels in all cells might cause selective motor neuron degeneration remain poorly defined. One recent hypothesis is that SMN deficiency results in preferential deficiency of U11 and U12 snRNPs (Gabanella et al., 2007; Zhang et al., 2008; Boullisfang et al., 2011), which are components of the minor spliceosome. The minor spliceosome is responsible for splicing of a minority of introns that are present in only a small proportion of genes, some of which are enriched in the nervous system such as voltage-gated ion channels and synaptic components. In this way, SMN deficiency could cause missplicing of neural-specific genes resulting in preferential motor neuron degeneration. Others have argued that any splicing changes that occur in SMA mice are only evident in late-stage disease and thus are not a primary event in SMA pathogenesis (Baumer et al., 2009).

Another hypothesis is that it is the disruption of an alternative, neuron-specific function of SMN that underlies the specific susceptibility of motor neurons to SMN protein deficiency. SMN is actively transported in discrete granules in the motor axon (Zhang et al., 2003) and is localized in growth cones of cultured neurons (Rossoll et al., 2003). Furthermore, SMN has been shown to co-localize with a variety of mRNA binding proteins including HuD (Akten et al., 2011; Fallini et al., 2011; Hubers et al., 2011) and hnRNP-R (Glinka et al., 2010) and with both actin mRNA (Rossoll et al., 2003) and candidate plasticity-related gene 15 (cpg15) mRNA (Akten et al., 2011) whose local accumulation is impaired in SMN-deficient neurons. These data have suggested that SMN plays a role in mRNA axonal transport and processing in axons and nerve terminals. It has been further postulated that these defects may lead to cytoskeletal disruptions of the nerve terminal. Interestingly, a gene encoding an actin modifying protein, Plastin 3, has been shown to be a protective modifier in human SMA (Oprea et al., 2008). Furthermore, inhibition of RhoA/ROCK signaling, which regulates actin dynamics, improves the outcome of SMA mice (Bowerman et al., 2009).

SMN likely has several molecular functions and the specific defect that causes motor neuron disease remains to be definitively determined. As a result, most high throughput, cell-based therapeutic screening

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**Figure 1.** SMA is caused by mutation of the *SMN1* gene and reduced expression of SMN protein. All patients retain one or more copies of the *SMN2* gene, but it is unable to fully compensate for loss of *SMN1*. *SMN2* contains a C to T transition (A) that leads to frequent exon 7 skipping during splicing of SMN2 pre-RNAs (B). The resulting truncated transcripts encode a truncated, unstable protein product (C) that is rapidly degraded. A minority of SMN2 transcripts contain exon 7 and encode a normal, full-length SMN protein (C).
assays to date have screened for SMN induction, rather than for an improvement in some aspect of SMN function. In the future, such functional screens (for snRNP assembly or SMN-dependent mRNA transport, for example) hold the promise of identifying novel SMA therapeutic candidates that improve SMN function independent of increasing SMN levels.

**Insights from Animal Models**

Fly, nematode, fish, and mouse models of SMA have provided key insights regarding the cellular pathogenesis of SMA and enabled *in vivo* preclinical studies of candidate therapeutics. The observation that complete knockout of SMN in any organism is embryonically lethal confirms that some SMN protein is required for survival of all cells (Schrank et al., 1997; Miguel-Aliaga et al., 1999; Chan et al., 2003; Burghes and Beattie, 2009). Embryonic lethality can be prevented by expression of some SMN protein, and SMA disease severity in mice correlates with transgenic *SMN2* copy number, recapitulating the relationship between SMN expression and disease severity seen in humans (Hsieh-Li et al., 2000; Monani et al., 2000). Whereas SMA mice expressing 2 copies of *SMN2* in the null background have a severe SMA phenotype living only one week, mice with 8 copies of *SMN2* are phenotypically normal (Monani et al., 2000). In recent studies, using conditional SMA mice in which restoration of SMN expression could be temporally controlled, it has been further demonstrated that increased SMN expression postnatally is capable of improving the disease phenotype, but the timing of this restoration is critical. Increased SMN expression at postnatal day 1 in SMA mice that would typically live 2 weeks results in a near complete rescue of many mice, but delaying SMN restoration until after the first postnatal week is ineffective (Le et al., 2011; Lutz et al., 2011). Detailed investigations of the pathology of SMA animal models may be providing some insights regarding the underlying basis of this “window of opportunity” for effective treatment. In severe SMA mice, studies have demonstrated a surprisingly modest degree of motor neuron death even at disease end-stage, although certain motor neuron pools are more severely affected than others (Mentis et al., 2011). There is also little evidence of profound distal axonal degeneration to suggest a dying back axonopathy causing muscle weakness. Rather early stages of disease are characterized by morphological and functional abnormalities of synapses (Figure 2), both the neuromuscular junctions (NMJs) (Chan et al., 2003; Kariya et al., 2008; Murray et al., 2008; Kong et al., 2009; Ling et al., 2010; Ruiz et al., 2010; Lee et al., 2011) and the synaptic inputs to motor neurons in the spinal cord (Ling et al., 2010; Mentis et al., 2011). These abnormalities are associated with simplified NMJ endplates and hypotrophic myofibers that fail to mature (Figure 2) (Kong et al., 2009; Lee et al., 2011). These early structural and functional abnormalities of synapses are likely followed by synapse loss, which in the case of the NMJ may directly result in denervated, weak muscle and in the case of central synapses may lead to further failed downstream activation of muscle. The temporal window for effective rescue in SMA mice may relate to the ability to restore connected, but dysfunctional synapses, which cannot be regained once lost. The finding of extensive synaptic abnormalities in SMA mice has also raised the question of where SMN must be delivered to restore synaptic function. Is SMN required just in motor neurons, or is it also required in other neurons or in muscle? Studies are ongoing to address these questions. Of note, therapeutic strategies

![Figure 2](image-url). SMA pathogenesis. Studies of SMA mouse models have uncovered multiple pathological and functional abnormalities of the motor system with particular abnormalities of both NMJ and MN synapses.
to improve muscle growth independent of SMN induction have shown only modest (Rose et al., 2009; Bosch-Marce et al., 2011) or no effect (Sumner et al., 2009) in severe SMA mice; however, such strategies need to be investigated in milder mouse models in which the capacity for collateral reinnervation may be more robust.

**Therapeutic Strategies**

Because SMN expression levels correlate with disease...
severity in humans and in mice and postnatal induction of SMN expression in mice is sufficient to prevent disease, a principal focus of therapeutics development in SMA has been to identify strategies to increase SMN protein levels either by activating SMN2 gene expression, increasing inclusion of exon 7 in SMN2-derived transcripts, stabilizing SMN protein, or by replacing the SMN1 gene (Figure 3). Other efforts have centered on neuroprotection and cell replacement. Some of the therapies currently in development for the treatment of SMA are discussed below (Figure 4).

**SMN2 Gene Expression Activation (Figure 3A)**

*Repurposed drugs: histone deacetylase inhibitors (HDACis), hydroxyurea, and prolactin*

HDACis activate gene expression by inhibiting histone deacetylases (HDACs), which deacetylate chromatin histones thereby promoting a tightly coiled, transcriptionally repressed region of chromatin. Ten years ago, Chang et al. (2001) were the first to show that the SMN2 gene could be activated by the HDACi sodium butyrate. Several HDACis including valproic acid (VPA), phenylbutyrate, trichostatin A (TSA), and suberoylanilide hydroxamic acid (SAHA) were subsequently shown to increase SMN2 expression in patient-derived cell lines and in animal models (Brichta et al., 2003; Sumner et al., 2003; Andreassi et al., 2004; Hahnen et al., 2006; Avila et al., 2007; Narver et al., 2008). This class of drug was also the first to show the ability to substantially improve the phenotype of SMA mice with TSA and SAHA increasing median survival by 40% and 30%, respectively, in different SMA mouse models (Narver et al., 2008; Riessland et al., 2010). As VPA and phenylbutyrate are in clinical use for other indications, they were both taken to clinical trials in SMA patients.
quickly despite their low potency as HDACis. Recent results from these studies in SMA type II and III patients have show little or no effect (Mercuri et al., 2007; Swoboda et al., 2010; Kissel et al., 2011). Studies with these compounds are ongoing in SMA type I infants as efficacy may be improved by early delivery. Efforts are also ongoing to identify other more potent, CNS penetrant, and perhaps HDAC enzyme-specific HDACis that may have efficacy in SMA. Identifying such drugs that do not have prohibitive toxicity for chronic use is a challenge for this class of compounds.

Hydroxyurea was shown to increase the ratio of full-length to truncated SMN mRNA in SMA patient-derived cell lines (Liang et al., 2008) perhaps via activation of nitric oxide (Xu et al., 2011). Hydroxyurea was also taken to clinical trials in SMA patients as it is FDA-approved for the treatment of sickle cell disease. Despite earlier work documenting modest improvements in manual muscle testing (MMT) scores in Taiwan (Liang et al., 2008), a placebo-controlled double-blind study was recently published that contradicted this finding, showing no improvements in the motor function or full-length SMN transcript levels of SMA type II and type III patients after hydroxyurea treatment (Chen et al., 2010).

Two groups have recently implicated Stat5 as a regulator of SMN expression (Ting et al., 2007; Farooq et al., 2011). Stat5 was first identified as a downstream target of three compounds able to promote SMN2 acivity, TSA, aclarubicin, and sodium vanadate, and Stat5 knockdown results in decreased SMN expression (Ting et al., 2007). More recently, prolactin, a known Stat5 activator, was shown to increase the median life span of severe SMA mice from 14 to 21 days (Farooq et al., 2011). Recombinant prolactin is FDA-approved for the treatment of prolactin deficiency in women and would be available for investigation in SMA clinical trials. It is unclear, however, whether the doses of recombinant prolactin typically used in clinical practice will be adequate to activate SMN2 in SMA patients.

Quinazoline derivatives

Quinazoline derivatives were originally found to increase SMN2 promoter activity in a screen of over 500,000 compounds in an NSC-34 cell-based screening assay (Jarecki et al., 2005). In a secondary screen, they increased SMN protein levels and gem number in SMA patient-derived fibroblasts (Jarecki et al., 2005). After structure activity relationship studies and lead optimization, a novel 2,4-diaminoquinazoline derivative was identified that showed good CNS penetration and a long half-life following oral dosing in mice (Thurmond et al., 2008). These compounds have been described to be potent inhibitors of DepS, a scavenger enzyme whose best-described role is in degradation of the 5′ cap during 3′ to 5′ mRNA decay (Wang and Kiledjian, 2001; Singh et al., 2008). Additionally, this enzyme has nuclear-cytoplasmic shuttling responsibilities and plays a role in first intron pre-mRNA splicing (Shen et al., 2008). A lead quinazoline compound was tested in vivo, and was shown to have modest behavioral and survival benefits in SMA mice (Butchbach et al., 2010). Our laboratory has confirmed a 29% increase in median survival and 15% increase in maximum body weight achieved in severe SMA mice using the current lead quinazoline derivative (Van Meerbeke et al., 2011). Phase I clinical trials with this compound have been initiated by the Repligen Corporation. The detailed mechanisms by which DepS inhibition leads to SMN2 promoter activation or provides survival and behavioral benefits in SMA mice remains under investigation.

Splicing Modulation (Figure 3B)

Repurposed drugs: salbutamol

Salbutamol (albuterol) is a short-acting β₂-adrenergic receptor agonist. It has been shown to increase full-length SMN transcript levels in SMA patient-derived cell lines (Angelozzi et al., 2008; Tiziano et al., 2010). Two pilot clinical studies in SMA type II and III patients suggested modest improvement of motor function over 6-12 month periods (Kinali et al., 2002; Pane et al., 2008). The drug was also well tolerated (Angelozzi et al., 2008; Pane et al., 2008). Larger randomized placebo-controlled trials are needed to further evaluate the efficacy of this drug in SMA.

Small molecules

In 2001, Andreassi et al. (2001) reported that aclarubicin increased SMN protein levels and gem numbers in SMA patient-derived fibroblasts by increasing exon 7 inclusion. Unfortunately, this compound has prohibitive toxicities for long-term use, as would likely be required for SMA patients. Based on their structural similarity to aclarubicin, tetracycline derivatives from Paratek Pharmaceutical’s chemical library were recently screened in a cell-free splicing assay and one derivative, PTK-SMA1, was found to increase the amount of exon 7 inclusion (Hastings et al., 2009). This assay utilized a minigene construct comprised of an SMN2-derived pre-mRNA including exon 6, exon 7, and the 5′ part of exon 8 (Hastings et al., 2009). PTK-SMA1 did not change the splicing patterns of other tested genes, suggesting a specific effect on SMN exon 7 splicing rather than a global effect. PTK-SMA1 also increased
exon 7 inclusion when delivered to a mild mouse model of SMA validating target engagement in vivo (Hastings et al., 2009).

PTC Therapeutics, a company focused on developing drugs that target post-transcriptional control of gene expression, has also recently identified compounds that increase exon 7 inclusion thereby increasing SMN protein expression. They have identified three different structural scaffolds that each increases SMN protein levels and extends median survival of severe SMA mice, in one case from 14 to 132 days. These compounds are orally bioavailable and can penetrate the blood-brain barrier, but the mechanism by which they alter SMN splicing has not yet been described (Naryshkin et al., 2011).

**Antisense oligonucleotides**

Another strategy that has been pursued to increase exon 7 inclusion is the use of antisense oligonucleotides (ASOs), modified nucleotides that bind specific mRNA sequences. This binding can mark a specific mRNA for degradation or in the case of SMA therapeutics, binding to specific cis-acting splicing regulatory motifs can promote exon 7 inclusion. Various sequences of SMN2 mRNAs have been targeted with the most efficacious molecules designed to target an intron splice silencer (ISS) sequence in the 5′ end of intron 7 (Singh et al., 2006; Hua et al., 2007; 2008). Different ASOs utilizing various chemistries (Williams et al., 2009; Hua et al., 2010; Passini et al., 2011) as well as a transsplicing molecule (Coady and Lorson, 2010) have been studied in cell and animal models. ISIS Pharmaceuticals has developed a 2′-O-2-methyoxyethyl-modified ASO (ASO-10-27 or ISIS SMNRx), which they have shown in collaboration with Adrian Krainer's laboratory and Genzyme is able to correct ear and tail necrosis in a mild mouse model of SMA and extend median survival in severe SMA mice from 16 to 26 days after ICV injection (Hua et al., 2010; Passini et al., 2011). Passini et al. (2011) also provided proof-of-principle evidence for the clinical relevance of this drug documenting putatively therapeutic ASO levels in the spinal cords of cynomolgus monkeys after intrathecal delivery. Furthermore, recent data show an even more striking benefit when ASO 10-27 is given systemically in severe SMA mice, with some mice surviving for more than 1 year (Hua et al., 2011). The enhanced benefit with systemic delivery suggests the requirement to restore SMN in other tissues aside from the CNS. Whether this is relevant to the human disease has yet to be determined. ISIS Pharmaceuticals plans to submit an IND (investigational new drug) in the coming months, with the goal of starting a phase 1 trial in SMA patients this winter using a single intrathecal ISIS SMNRx injection as a starting dose (personal communication, C. Frank Bennett, Ph.D.).

**Protein Modulation** (Figure 3C)

**Repurposed drugs: aminoglycosides and proteasome inhibition**

Aminoglycosides are known for their ability to induce translational readthrough of stop codons. In the case of SMN2 transcripts, it was postulated that readthrough of the initial stop codon would extend the length of the C-terminal of the truncated SMN protein thereby improving its stability. Aminoglycosides increased gem number and SMN protein levels in SMA patient-derived fibroblasts (Mattis et al., 2006) and geneticin (an aminoglycoside) improved motor behavior, but not survival in SMA mice with some evidence of toxicity (Heier and DiDonato, 2009). In contrast, a novel aminoglycoside, TC007, provided a ~30% increase in median survival when given to severe SMA mice by ICV injection (Mattis et al., 2009).

Recently, Kwon et al. (2011) showed that the FDA-approved ubiquitin proteasome inhibitor, Bortezomib, increased SMN protein levels in muscle and other peripheral tissues, but not in the CNS when delivered to severe SMA mice by intraperitoneal injection. When combined with TSA, which is a CNS penetrant drug, there was an improvement in survival of SMA mice that was better than that seen with TSA alone. These data provide proof of principle that inhibition of SMN protein degradation can reduce SMA disease severity. Nonetheless, both aminoglycosides and proteasome inhibitors will have to overcome challenges of drug toxicity before they can be legitimate treatments for SMA patients.

**SMA Project**

In 2003, the National Institute of Neurological Disorders and Strokes (NINDS) founded the SMA Project, a research initiative based at and directed by the NIH. This marked the first time that the NIH embarked on a mission to develop therapeutics in-house for a particular disease, with a goal of filing an IND in 5 years. SMA Project currently has two sets of compound series in development. The first, indoprofen analogs, are based on the observation that indoprofen increased SMN levels and nuclear gem counts in SMA patient-derived fibroblasts and provided a modest survival benefit to SMA mouse embryos (Lunn et al., 2004). The second set of compounds, approximately 200 benzimidazoles, was independently generated from
a high throughput screen and lead candidates are being generated from these hits (personal communication, Jill Heemskerk, Ph.D.).

**Gene Therapy** (Figure 3D)

Gene therapy provides the opportunity to restore a normal form of the *SMN1* gene to SMA patients; however, effective delivery to a difficult-to-access cell such as a motor neuron has been considered an almost impossible challenge until very recently. Several groups have accomplished this using self-complementary adeno-associated virus vectors (Foust et al., 2010; Passini et al., 2010; Valori et al., 2010; Dominguez et al., 2011). scAAV9 in particular was shown to have tropism for MNs in neonatal animals when injected intravascularly (Foust et al., 2009). Both *SMN1*-scAAV9 and scAAV8 were demonstrated to remarkably improve the SMA phenotype after early postnatal delivery in severe SMA mice. The first two papers outlining this work were published weeks apart and employed two different methods. Foust et al. (2010) injected SMN-scAAV9 intravenously (IV), while Passini et al. (2010) delivered SMN-scAAV8 by intracerebroventricular (ICV) injection. Both studies resulted in increased median survival of severe SMA pups compared to vehicle-treated counterparts, but interestingly delivery of the gene intravenously provided a greater survival benefit when compared to ICV injections, with 6 of 7 severe SMA mice living past 250 days compared to a median survival of 157 days in the ICV-injected mice (Foust et al., 2010; Passini et al., 2010). Other studies have also shown dramatic extensions of median lifespan including ALS and SMA is Olesoxime (TRO19622), a cholesterol-like compound being developed by Trophos. This drug was identified in a cell-based screen for compounds that protected rat primary motor neurons from degeneration induced by trophic factor withdrawal (Bordet et al., 2007). Although the drug mechanisms are not entirely understood, the drug has been shown to bind TSPO and VDAC, proteins located on the outer mitochondrial membrane, suggesting a role of mitochondrial signaling in the mechanism of drug action (Bordet et al., 2007). Recruitment for an ongoing clinical trial of Olesoxime in SMA patients in Europe was recently completed (personal communication, Rebecca Pruss, Ph.D.).

**Cell Replacement**

Another treatment strategy that is being actively investigated in SMA is cell replacement. Embryonic stem (ES) cells can be differentiated into neural stem cells and then functional MNs under stringent growth and differentiation conditions usually involving retinoic acid, sonic hedgehog, and neurotrophic factors (Wichterle et al., 2002; Shin et al., 2005). One group reported the *in vivo* benefit of neural stem cell injections in severe SMA mice using intrathecal injections after differentiating neural stem cells from mouse spinal cord neurospheres (Corti et al., 2006; Corti et al., 2008). SMA mice treated with these neural stem cells displayed increased survival and motor behavioral benefits, as well as increased MN number and size in the spinal cord when compared to SMA littermates (Corti et al., 2008).

Riluzole is an FDA-approved drug for the treatment of amyotrophic lateral sclerosis (ALS) and there is interest to evaluate it for efficacy in SMA. A phase I clinical trial showed Riluzole to be safe in SMA type I patients (Russman et al., 2003) and a recent study indicated that this compound has similar pharmacokinetic properties in SMA patients to those seen in ALS patients (Abbara et al., 2011).

The beta-lactam antibiotic ceftriaxone was demonstrated to increase glutamate reuptake and improve the phenotype of ALS mice (Rothstein et al., 2005). Consequently, a clinical trial of ceftriaxone is ongoing in ALS patients. This drug was evaluated in severe SMA mice and shown to modestly extend survival (Nizzardo et al., 2011).
This group has also demonstrated phenotypic benefits in animal models of ALS, SMA, and spinal cord injury, further validating the potential of this therapeutic (Wyatt et al., 2011).

**Ongoing Screens**

There are many ongoing efforts to identify other novel drugs for SMA. These include high throughput screening campaigns by Novartis and by Merck in collaboration with Gideon Dreyfuss. In the latter effort, native SMN protein-inducing compounds have been identified during a high throughput screen of over one million small molecules in SMA patient-derived cells. Hits are currently being optimized and their mechanisms of action are being characterized (personal communication, Gideon Dreyfuss, Ph.D.). Two additional recent studies highlight the utility of novel screening assays. Lee Rubin’s group at the Harvard Stem Cell Institute used a new image-based method that identifies increases in gem number, gem intensity, and SMN levels independent of cellular location (Makhortova et al., 2011). In addition, by screening compounds at varying concentrations and those with known targets and pathways instead of diverse chemical libraries, the investigators were able to provide mechanistic evidence that implicates GSK-3 kinase as an SMN regulator (Makhortova et al., 2011). Elliot Androphy’s group created a new luciferase-based screening assay that simultaneously allows detection of hits that increase SMN2 promoter activity, increase exon 7 inclusion, and/or stabilize the SMN protein (Xiao et al., 2011). In a primary screening of over 200,000 compounds, over 6,000 compounds were initially identified (Xiao et al., 2011). After elimination of many compounds postulated to interfere with the assay and those that also nonspecifically increased activity of an SMN1-luciferase construct, 21 compounds were tested in a secondary assay (Xiao et al., 2011). From these compounds, one lead series underwent structure and relationship studies that led to the identification of 2 compounds that showed good oral bioavailability and CNS penetration in mice (Xiao et al., 2011). These compounds are aryl piperidines whose mechanism of action has yet to be described (Xiao et al., 2011).

**Challenges of Clinical Trials**

The challenges of SMA clinical trials are unique and specific to this disease. The most reliable outcome measures in a disease with such variable clinical severity have yet to be determined and the need to identify informative biomarkers is high. Clinical trials in infants with type I SMA have proven to be particularly difficult because of the fragility of this patient population (Russman et al., 2003). Conversely, SMA type II and type III patients often have stable muscle strength for months and years making the detection of meaningful changes difficult. The mouse studies have suggested that the timing of drug delivery may be critical for efficacy, but when this window of opportunity occurs or if it occurs in human patients is unknown. Given that current data suggests that early intervention could substantially affect treatment success, there has been interest in having SMA added to the panel of diseases for population-wide newborn screening (Prior, 2010).

**Conclusion**

The knowledge of SMA pathogenesis and the development of clinical candidates have increased considerably since the discovery of the disease-causing gene. Drug development in SMA has been characterized by robust collaborative efforts between academic, government, pharmaceutical, and non-profit organizations. As the promise of a treatment for this devastating disease continues to grow, we are hopeful that progression over the next 15 years will be even more rapid than the last.

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**Disclosure**

The authors have no conflicts of interest.

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