HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2017 February 01.

Published in final edited form as:

Ann N Y Acad Sci. 2016 February ; 1366(1): 5–19. doi:10.1111/nyas.12813.

Developing therapies for spinal muscular atrophy

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Abstract

Spinal muscular atrophy is an autosomal-recessive pediatric neurodegenerative disease characterized by loss of spinal motor neurons. It is caused by mutation in the survival of motor neuron 1 gene (*SMN1*) leading to loss of function of the full-length SMN protein. SMN has a number of functions in neurons, including RNA-splicing and snRNP-biogenesis in the nucleus, and RNA-trafficking in neurites. The expression level of full-length SMN protein from the *SMN2* locus modifies disease severity. Increasing full-length SMN protein by a small amount can lead to significant improvements in the neurological phenotype. Currently available interventions for spinal muscular atrophy my patients are physical therapy, orthopedic, nutritional, and pulmonary interventions; these are palliative or supportive measures and do not address the etiology of the disease. In the last decade there has been a push for developing therapeutics to improve motor phenotypes and increase lifespan of spinal muscular atrophy patients. These therapies are aimed primarily at restoration of full-length SMN protein levels; but other neuroprotective treatments have been investigated as well. Here, we discuss recent advances in basic and clinical studies towards finding safe and effective treatments of spinal muscular atrophy using gene therapy, antisense oligonucleotides, and other small molecule modulators of SMN expression.

Keywords

spinal muscular atrophy; antisense oligonucleotides; gene therapy; clinical trials

Introduction

Spinal muscular atrophy (SMA) is a severe neurodegenerative disease that results in degeneration and cell death of lower motor neurons in the spinal cord and is the leading genetic cause of infant mortality. It is an autosomal-recessive disorder with an incidence of 1:11,000 live births.[1] SMA is caused by homozygous deletions or mutations involving the survival of motor neuron 1 gene (*SMN1*) that encodes the SMN protein.[2] There are five clinical types of SMA defined by disease severity and age of onset, although the various types represent a continuum of severity with indistinct borders.[3] Type I SMA, Werdnig-Hoffmann disease, is a severe infantile form diagnosed prior to six months of age with mortality occurring before two years of age. Type I patients are severely hypotonic, have denervation of muscles, and are never able sit up unaided. Type II SMA, Dubowitz disease,

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diagnosed between 6–18 months of age is less severe; patients can progress to sitting unsupported at some point, but they never gain the ability to walk unassisted and have a shortened lifespan. Type III SMA, Kugelberg–Welander disease, is diagnosed after 18 months of age; patients are able to stand and walk unaided, and have an approximately normal lifespan. Type 0 and type IV SMA are rare variants. Type 0, also referred to as type IA, is the most severe form of SMA; it is characterized by prenatal onset as determined by reduced fetal movement and arthrogryposis at birth, and mortality before 6 months of age.[4] Type IV SMA is similar to type III, but with onset in adulthood and milder symptoms.

Humans possess an additional gene, *SMN2,* which is almost identical to *SMN1* but produces only 10% of full-length SMN protein.[5] *SMN2* has a C-T substitution in exon 7 that leads to exclusion of exon 7 and formation of a truncated, unstable protein (SMNΔ7) that is rapidly degraded.[5–7] In SMA patients, *SMN2* is the primary modifier of the disease phenotype; the number of copies of *SMN2* a patient carries is inversely correlated with disease severity. SMA type I patients typically carry only 1–2 copies of *SMN2*, while SMA type II and III patients carry between two and four copies of *SMN2*.[8]

To date there is no U.S. Food and Drug Association (FDA)-approved treatment for SMA, and only palliative therapies are available to patients. In recent years, several promising treatments have entered advanced clinical trials with promising results (Table 1). The therapeutic aim in the majority of these trials is to increase the amount of functional full-length SMN protein expressed, either by use of antisense oligonucleotide (ASO)–mediated exon 7 inclusion at the *SMN2* locus or by exogenous expression of *SMN1* by viral-mediated gene therapy vectors. Additionally, a number of other therapies that modulate SMN levels or function are being investigated. Here we describe current progress in cell culture, mouse models, and clinical trials towards an effective SMA treatment.

Multifaceted functions of SMN

SMN protein is ubiquitously expressed very early in development and plays diverse roles in RNA processing and trafficking as summarized in Figure 1. In the cell body, SMN protein forms the SMN complex with eight other proteins: Gemin2–8 and unr-interacting protein (UNRIP) (reviewed in Ref. 9). This complex is involved in biogenesis of small nuclear ribonucleoproteins (snRNPs) via recruitment and binding of the seven Sm protein core to snRNA.[10] SMN complex binding of Sm proteins confers specificity and efficiency of the Sm proteins to accurately bind specific snRNAs.[11] SMN bound snRNPs localize to Cajal bodies where they are incorporated into the spliceosomal machinery. The SMN complex is also integral to U7 snRNP function and histone mRNA processing via interaction with a different subset of Sm proteins.[12, 13] Furthermore, there is evidence that SMN expression levels are regulated in part by components of the spliceosome. UA1 protein, a component of the U1 snRNP complex, regulates SMN expression by binding to the 3′-UTR of SMN mRNA to inhibit polyadenylation and decrease SMN protein levels.[14]

An additional role for SMN was first reported by Bassell and colleagues, who described the presence and trafficking of SMN protein, in association with Gemin2 or Gemin3, in motor neuron axons and dendrites.[15] In neuronal processes, SMN regulates RNA via direct

interaction with several RNA binding proteins including FMRP,[16] KSRP,[17] HuD,[18–20] and Imp1.[21] Loss of SMN in neurons affects trafficking of SMN binding partners and target mRNAs.[22] This leads to changes in axon and dendrite outgrowth and morphology, as well as aberrant growth cone dynamics and disrupted local translation of a number of axonal RNAs.[18, 23, 24] In motor neuron axons, SMN-containing granules interact with the coatomer complex of coat protein I (COPI) vesicle family member α-COP, linking SMN function to the Golgi network.[25] Disruption of this interaction by either knockdown of α-COP or inhibition of Golgi-mediated granule secretion decreases SMN in the cellular processes resulting in aberrant neurite outgrowth and morphology.[26, 27] Further, *in vitro* evidence suggests SMN may associate with polyribosomes in the cytoplasm and act as a translational repressor in RNA granules.[28] The relative contribution of each distinct role for SMA throughout the nucleus, cell body, and processes of motor neurons to the SMA phenotype is not yet known. However, understanding SMN protein functions and mechanisms of action in a subcellular context could shed light on additional pathways for therapeutic intervention and biomarkers.

Interestingly, aberrant RNA splicing and metabolism is common to other motor neuron diseases including amyotrophic lateral sclerosis (ALS). New evidence of a direct interaction between the SMN complex and FUS, an RNA binding protein mutated in familial ALS, is a functional link between the two disorders. Loss of nuclear FUS due to ALS-associated mutations leads to decreased levels of nuclear gems and cytoplasmic localization of SMN.[29] As a result, snRNPs are unable to reenter the nucleus, thus leading to aberrant spliceosomal function.[30] In ALS neurons, localization of SMN within cytoplasmic FUS granules leads to axonal SMN reduction, which can be rescued by SMN overexpression.[31] Depletion of another ALS-associated protein, TDP-43, can increase SMN alternative splicing and decrease its expression,[32] and may contribute to U12 minor spliceosome function.[33] Overexpression of SMN in a mutant SOD1 mouse model of ALS alleviated motor deficits and inhibited neuron death, providing further evidence for SMN as a modifier of ALS phenotypes.[34] Interactions between ALS and SMA disease pathways are indicative of common molecular mechanisms in motor neuron loss and may inform preclinical studies for other degenerative motor neuron diseases.

Models for development of SMA therapeutics

Mouse models

Complete loss of SMN expression is lethal very early in gestation,[35] and therefore *Smn*$^{-/-}$ mice are not a viable model to study SMA disease progression and pathology. While humans with SMA have at least one copy of the disease-modifying *SMN2*, rodents do not carry the *SMN2* gene. As a result, mouse models of SMA often include human *SMN2* transgenes or modifications to the mouse *Smn* to mimic *SMN2* exon 7 splicing. To date, a number of mouse models of SMA have been described that cover the spectrum of disease phenotypes from severe infantile onset to milder type III (reviewed in[36]). Of these models, two have emerged as the most widely used for studies of SMA therapeutics. The first, referred to as the Taiwanese model (*Smn*$^{hung-/-}$;*SMN2Hung*$^{tg/-}$) expresses a transgene with two copies of *SMN2* and can be bred with *Smn* heterozygotes to obtain a range of phenotypes.[37] The

second model, SMNΔ7 (*Smn*$^{-/-}$;*SMN2*$^{tg/tg}$;*SMNΔ7*$^{tg/tg}$), is homozygous for human *SMN2* and *SMNΔ7* and exhibits a severe motor phenotype, significant motor neuron loss, and mean lifespan of 15 days.[38] These models allow for investigation of potential therapeutics with outcome measures ranging from increased lifespan and gross motor function improvement to neuromuscular junction (NMJ) morphology, motor neuron survival, and subcellular mechanistic readouts.

Invertebrate models

Invertebrate models of SMA are integral to understanding aberrant development, axon outgrowth, and splicing. Both flies and worms have *SMN* homologs that are roughly 30% similarity to the human *SMN1* gene. *C. elegans* has been used in RNAi-based screens for *smn* modifier genes.[39] *Drosophila* are genetically tractable, have a short lifecycle, and are a good system for investigating motor behaviors. The generation of an allelic series of *Drosophila* mutants with SMA patient–specific point mutations allowed in-depth investigation of point-mutation effects on SMN binding, function, and phenotype.[40] RNAi-mediated muscle-, neuron-, or motor neuron–specific SMN loss in the fly also has elucidated cell type–specific effects on neuromuscular junction morphology and electrophysiology.[41]

In zebrafish, the SMA axonal phenotype is recapitulated by administration of an antisense morpholino that knocks down Smn1 mRNA expression.[42] Studies investigating compounds that modulate axon growth in zebrafish have used this morpholino model and found that kinesins,[43] Nrxn2,[44] and molecules involved in ubiquitin homeostasis[45] modify the *Smn1* axon phenotype. Zebrafish genetic models of SMA also have severe motor axon defects and can be used for rescue experiments to look at temporal effects of *Smn1* loss on motor axons. [46, 47]

Large animal models

Large animal and primate models of SMA have been used extensively for bioavailability and safety studies, particularly for antisense oligonucleotide (ASO) and viral vector–mediated gene therapy development. However, they lack the *SMN2* gene and therefore cannot be used for efficacy studies of SMN2 modulators. Pigs lack the intron splicing silencer in exon 7 and have modified splicing of *SMN1*, so they are not a good model for splice modulation therapies.[48] However, Burghes and colleagues have developed a porcine model using intrathecal delivery of scAAV9-mediated SMN mRNA knockdown that recapitulates the SMA phenotype of motor neuron loss and axon degeneration, as determined by assessment of motor unit function with electromyography (EMG), compound motor action potential (CMAP), and motor unit number estimation (MUNE).[49] Further, the phenotype is rescued by administration of human *SMN1* via scAAV9-SMN, providing evidence that this is a very useful pre-clinical model for gene therapy approaches.

In vitro human SMA stem-cell model

Induced pluripotent stem cells (iPSCs) are increasingly used to model CNS diseases including Alzheimer's disease, Parkinson's disease, and ALS (reviewed in Ref. 50). iPSCs derived from SMA patient fibroblasts can be differentiated into neural lineage cells and have low SMN expression and decreased nuclear gems.[51] iPSCs, and the motor neurons derived

from them, are an *in vitro* model of SMN loss that can be used to study cellular phenotypes and potential treatment compounds.[51, 52] Wild-type mouse[53] and human[54] motor neuron progenitors injected into the cerebrospinal fluid (CSF) of SMA mouse models differentiate into neurons and provide trophic support for endogenous motor neurons to increase survival. Gene editing is used to correct mutations in iPSCs to directly compare the effect of a specific mutation in otherwise genetically identical cell lines. Correction of the mutation in SMA iPSC lines increases SMN expression and decreases motor neuron cell death *in vitro,* and injection in SMNΔ7 mice increases lifespan by forty percent.[55]

Antisense oligonucleotides

ASO technology and therapeutic approach

ASOs are single-strand, DNA-like molecules 15–35 nucleotides long that can modulate target gene expression by hybridization to the pre-mRNA. Targets can be regulated by several different mechanisms including restoration of the correct open reading frame, decreasing gene expression, alternative splicing, or, in the case of SMA, exon inclusion. ASOs are being developed as therapeutics for a number of rare genetic diseases caused by aberrant mRNA splicing, including Duchenne muscular dystrophy, fibrodysplasia ossificans progressiva, Leber congenitial amaurosis, and tauopathies.[56] While a growing number of ASOs are being tested in clinical trials, to date only two have been FDA-approved, both reduce target gene expression: fomivirsen sodium for cytomegalovirus retinitis in immune-compromised AIDS patients and mipomersen sodium for familial hypercholesterolemia.[57]

The pharmaceutical properties of ASOs can be altered by the chemical structure of the nucleotide backbone or sugar ring. Each modification of the backbone confers slightly different stability, toxicity, and function to the ASO. In addition, bifunctional ASOs have added high-affinity splice enhancer sequences that promote binding of SR proteins and increase splicing.[58–60] One ASO modification extensively used in the clinical application to SMA for *SMN2* alternative splicing is 2′-OME, which provides enhanced target RNA binding affinity and resistance to endonuclease cleavage conferring stability to the ASO.[61, 62]

SMN is an attractive candidate for ASO therapy as patients have a functional copy of *SMN2* that is an ideal target for modulation. As compared to SMN1, a single C-T substitution in exon7 of *SMN2* weakens a binding site for the splicing activator SRSF1 leading to exon skipping and a 90% decrease in full-length SMN protein. Early studies of ASOs in SMA patient fibroblasts proved that they could be used to promote the inclusion of SMN2 exon 7 (Ref. 63) and identified the intronic splicing silencer N1 (ISS-N1) site in *SMN2* exon 7 as a particularly potent target for ASO binding. Adjacent to the exon 7 5′-splice site, ISS-N1 contains a binding site for the A1-dependent ISS hnRNP-A1, which decreases exon 7 inclusion. ASOs targeted to the ISS-N1 sequence competitively bind the mRNA and prevent repressor binding by hnRNP to increase exon 7–containing SMN2 expression, in both *in vitro* and *in vivo* in mouse models of SMA.[64–66]

Development of ASO for clinical trials in SMA

Preclinical studies of the specific oligonucleotide ISIS 396442 have shown that either peripheral[67] or intraventricular (ICV) injection of this ASO into either mouse model (SMNΔ7 or Taiwanese) results in stable *in vivo* rescue of full-length SMN in the spinal cord.[68, 69] *In utero* ICV injection at E15.5 and postnatal injection partially rescued ear and tail necrosis in a milder SMA type III mouse model.[68] Intrathecal infusion of the ASO into cynomolgus monkeys was sufficient for ASO distribution at therapeutic levels throughout the spinal cord.[69] In comparison to the other chemically-modified ASOs, ISIS 396443 not only is more potent, but also yields more efficient *SMN2* splicing correction throughout the CNS, both in the spinal cord and brain. Furthermore, administration of a single intrathecal bolus injection leads to better distribution and accumulation than infusion, which has important clinical significance for dosing.[70] These studies laid the foundation for clinical trials of ASO 396643 (also known as ISIS-SMNRx). In a Phase I clinical trial, ISIS-SMNRx was administered by intrathecal injection into type II/III SMA patients and seemed well tolerated (NCT01494701). In a Phase II trial with type II/III SMA patients, multiple injections were well tolerated, and patients showed improvements in the Expanded Hammersmith Functional Motor Scale (HFMSE) scores at all doses (NCT01703988). Two double blind sham-controlled Phase III clinical trials are currently enrolling infants with type I (NCT02193074) and children with type II (NCT02292537) for intrathecal administration of ISIS-SMNRx, and will run through 2017 (Table 1).

Other ASOs with different chemistries and binding sites are also being developed and tested in clinical trials. Short 8-mer ASOs were developed to target a GC-rich site adjacent to the ISS-NI.[66, 71] The 8-mer 3UP8i has fewer off-target effects than the longer ASOs, significantly increases inclusion of SMN2 exon 7, and ameliorates neuromuscular junction (NMJ) pathology and increases survival in a mild SMA mouse model.[72] ICV injection of an E1 intronic-repressor-binding ASO, $E1^{MO}$-ASO, in the SMNΔ7 mouse model was sufficient to induce SMN expression in the spinal cord and brain, leading to increased weight, longer lifespan, and improved motor behaviors.[73] A screen for orally-available, small-molecule SMN splicing modifiers in SMA patient fibroblasts led to the discovery of three small molecules: SMN-C1, SMN-C2, and SMN-C3,[74] which are associated with a dose-dependent increase in SMN protein following oral treatment in the SMNC/C and SMNΔ7 mouse models. Orally-available molecules are now being tested in a Phase I clinical trial for safety and tolerability of RO6885247 (NCT02240355). Due to the invasive nature of other therapeutic strategies that rely on intravenous and intrathecal injections, an effective orally-available molecule could be beneficial for patient safety and dosing.

Gene therapy

Viral vectors for exogenous expression of gene targets

One main approach to mitigating the effects of SMN loss is exogenous expression of full-length SMN protein by introducing genetic material directly into motor neurons. The gene of interest, *SMN1* in the case of SMA, is packaged into a carrier vector that is usually an attenuated or modified viral vector. For CNS targeting, lentiviral (LV) or adeno-associated viral (AAV) vectors are most common as they are able to transduce non-dividing cells like

neurons (reviewed in Refs 75 and 76, respectively). Additionally, non-viral techniques use direct injection of genetic material or rely on cationic lipids or polymers to facilitate entry into the cells.[77] A number of different therapeutic strategies have been investigated for development of *in vivo* gene therapy vectors for expression of full-length SMN.

The earliest promising SMA gene therapy efforts used intramuscular administration of LV vectors that were retrogradely transferred to the spinal cord and spinal motor neurons to demonstrate phenotypic improvement in SMNΔ7 mice.[78] However, more recently AAV gene therapy vectors have been developed for the treatment of a number of monogenic CNS disorders. Because of their long-term gene expression, efficient transduction of non-dividing target cells without integrating into the host genome, and limited immunogenic potential, AAV are advantageous.[79] Specifically, the AAV9 serotype has emerged as a frontrunner in CNS gene therapy trials because it can cross the BBB,[80] and thus it can be administered intravenously.

Gene therapy development in SMA

SMA model mouse studies from several different labs have shown that systemic administration of scAAV9-SMN not only leads to widespread motor neuron transduction and SMN expression,[81] but significantly rescues the SMNΔ7 phenotype and increases life span.[82–84] Direct administration of scAAV9-SMN to the CNS via ICV or intrathecal injection also rescues the SMNΔ7 model and significantly increases lifespan.[85, 86] Injection of scAAV9-SMN into the CSF is more invasive and carries additional risks, but requires a significantly lower dose of viral vector to achieve high levels of motor neuron transduction in both pig and primate models.[87, 88] In addition to AAV9, several studies have investigated other viral vectors. Administration of AAV8-SMN directly into the CNS by intraventricular injection also improved NMJ morphology and muscle function of SMNΔ7 mice and increased life span.[85] New lentiviral vectors that are integration-deficient are being developed, which are safer and more efficient in *in vitro* assays; however, they require extensive testing.[89]

Begun in 2014, the first Phase I clinical trial for gene therapy in SMA (NCT02122952) will evaluate the safety and efficacy of escalating doses of scAAV9.CB.SMN, by intravenous infusion, of three cohorts of SMA type I infants. The study is currently recruiting participants and is expected to finish data collection in mid-2017. A number of other AAV-SMN vectors are in pre-clinical development and, hopefully, will be advanced for clinical trials in the coming years.

Modulatory therapeutic strategies

While directly increasing full-length SMN expression has a significant impact on ameliorating the SMA phenotype, many therapeutic approaches do not directly target SMN expression (Fig. 2). Instead, these therapies are aimed at modulating molecules and cellular pathways involved, such as neuroprotective mechanisms, cell survival, gene expression, and axon outgrowth. A number of drugs have been investigated (reviewed in Ref. 90); the following provides an overview of the current modulatory therapies in SMA trials.

Olesoxime

Olesoxime (TRO19622) was originally identified in a small molecule screen for potential modifiers of motor neuron degeneration in ALS.[91] It is a cholesterol-like molecule that acts on mitochondria to inhibit cell death, rescue neurite outgrowth,[91] and prevent astrogliosis.[92] Additionally, olesoxime treatment has been shown to accelerate myelination in response to injury.[93] Olesoxime entered a two-year Phase II/III clinical trial in SMA type II/III patients that ended in October 2013 (NCT01302600). Two year oral dosing prevented loss of motor function on the Motor Function Measure Scale (MFMS) and disease-related adverse events, compared to the placebo control group.[94] If approved by the FDA, olesoxime will be the first neuroprotective treatment for SMA.

Quinazolines

A small-molecule screen for SMN2 transcriptional activators first identified the quinazoline family of compounds that increase SMN mRNA and protein as well as nuclear Cajal bodies in patient fibroblasts.[95] The compound was manipulated to improve half-life, blood-brain barrier permeability, and SMN2 promoter activation for development in clinical trials.[96] Oral treatment of neonatal SMNΔ7 mice with quinazoline compounds increased *Smn* promoter activity, ameliorated the neuromuscular phenotype, and increased lifespan by up to 30 percent.[97] Quinazoline increases SMN levels by binding and inactivating DcpS, a decapping enzyme, to stabilize the mRNA.[98] One quinazoline compound, RG3039, had good CNS distribution, was a potent inhibitor of DcpS, modestly increased Smn levels, and improved survival and motor function in multiple severe mouse models.[99, 100] Additionally, RG3039 was shown to have a long half-life and increased not only the number of cells with nuclear gems, but also the number of gems per cell.[100] RG3039 was well tolerated in a Phase 1 clinical trial for safety in healthy controls and clinical studies are ongoing.

Histone deacetylase inhibitors

Histone deacetylase (HDAC) inhibitors are a class of compounds that are epigenetic regulators of gene expression. *In vitro* studies of HDAC inhibitor treatment with valproic acid (VPA) or phenylbuterate (PB) in SMA patient-derived fibroblasts show a significant increase in full-length SMN levels.[101–103] HDAC inhibitors traditionally increase transcriptional activation via hyperacetylation of a gene's promoter. However, conflicting studies of *SMN* promoter acetylation state have shown that the mechanism could be more complex. RNAi studies of individual HDAC enzymes *in vitro* have shown that different enzymes affect *SMN* promoter activity differently.[104] VPA treatment has also been shown to modulate SMN pre-RNA splicing by increasing expression of splicing factors SF2/ASF and decreasing hnRNPA1.[105] Interestingly, SMN was recently reported to have a chromatin-binding ability, and may interact with methylated histone H3K79.[106] Whether this function has implications for disease pathogenesis is not yet clear.

In a number of Phase I and Phase II clinical trials, VPA and PB have been tested for safety and efficacy in SMA type II/III patients. Increase in blood levels of full-length SMN, and patient motor benefits were moderate and inconsistent between studies (reviewed in Ref. [107]). Reports of weight gain and loss of carnitine led to combined therapy of VPA with L-carnitine, but in multiple human trials there was no improvement with the combined

treatment.[108–110] Interestingly, heterogeneity in a cohort of 16 SMA patients correlated with increased expression of fatty acid translocase CD36, which repressed the response to VPA treatment in patient-derived IPS cells.[111] In the future, CD36 may be a marker to identify patient populations that might receive the most benefit from VPA treatment.

Trichostatin A (TSA), LBH589 and suberoylanilide hydroxamic acid (SAHA) are potent inhibitors of a broader spectrum of HDACs[112] and have been shown to significantly increase full-length SMN expression.[112–115] Furthermore, TSA or SAHA treatment has been demonstrated to ameliorate motor deficits and increase lifespan in both the SMNΔ7[114, 116] and the Taiwanese mouse models.[117] Interestingly in SMN2b model mice, which lack the *SMN2* transgene, TSA treatment is able to rescue the phenotype without directly modulating SMN expression.[118] Additionally, two polyphenol compounds, curcumin and resveratrol, have HDAC inhibitor and neurotrophic properties that increase *SMN2* gene expression[119–121] and interact with SMN to affect neurite outgrowth.[122] While the exact mechanism of action of these compounds is unclear, they are potential candidates for further exploration in mouse models and SMA clinical studies.

Additional pathways for drug targets and clinical intervention

Several studies have implicated the PTEN/mTOR/Akt pathway in SMA. Loss of PTEN expression by shRNA injection in SMNΔ7 mouse muscle rescues NMJ morphology, while systemic administration of an AAV9 PTEN knockdown construct increases life span and rescues motor function in the same mouse model.[123] In the CNS, insulin-like growth factor 1 (IGF-1) plays an important role in normal brain development, axon outgrowth, and cell survival,[124] making it an interesting target for SMA therapy. Previous studies using recombinant IGF-1 treatment (IPLEX)[125] or AAV-IGF-1 administration to the CNS[126] rescued motor neuron cell death but did not lead to significant increases in motor function or life span. Increases in mouse life span were only reported when IGF-1 was administered in combination with an ASO specific to SMN.[127] Further, transgenic expression of mouse IGF-1 under a skeletal muscle–specific promoter in the SMNΔ7 mouse background led to an increase in life span and myofiber size, but did not ameliorate motor behavior, indicating muscle-specific rescue is not comprehensive.[128] Interestingly, systemic delivery by intravenous injection of AAV1 expressing human IGF-1 at postnatal day 0 led to hepatic induction and increased serum levels of IGF-1; this reduced motor neuron death and increased motor function and lifespan into a severe mouse model of SMA.[129] These results highlight not only the efficacy of IGF-1 in alleviating motor neuron pathology, but also the need for systemic IGF-1 expression for potential therapeutic value.

Our lab recently demonstrated that microRNA miR-183 is increased in SMA models and directly leads to altered expression of mTOR pathway components, as well as aberrant axonal local translation.[23] Repressed mTOR activity and protein synthesis in SMN-deficient cells suggest that increasing full-length SMN transcript alone may not be sufficient because protein synthesis machinery may still be suppressed in SMA. If so, it may be necessary to “prime” the protein synthesis machinery by activating mTOR within critical therapeutic windows. These findings may have implications for therapeutic trial design in the future.

Other molecules that have shown promise in *in vitro* studies have been unsuccessful in clinical trials, including lithium[130] and somatotropin.[131] Such results highlight the differences between rodent and human cellular models, and the importance of clinical trials for confirming efficacy in human patients.

Therapeutic challenges

Therapeutic targeting: CNS or systemic?

Although SMN is ubiquitously expressed, spinal motor neurons are most susceptible to loss of full-length SMN protein; the specificity of this motor neuron degeneration remains an important, unanswered question. Notably, disruption of astrocytes,[132] Schwann cells,[133] and microglial activation in the spinal cord during motor neuron loss have also been reported.[134] Ideally, therapeutic strategies would also target these other cell types.

A number of studies have described additional SMA pathology outside the CNS in SMA animal models and have highlighted the need for systemic or combination therapies in clinical trials. Targeted loss of SMN only in motor neurons recapitulates the SMA neuromuscular phenotype, however, the mice have lower mortality, and even improvement in some phenotypes with age.[135] Conversely, motor neuron specific rescue of *SMN* expression in a severe SMA mouse model attenuates the motor phenotype and NMJ innervation, but does not improve lifespan due to disrupted autonomic innervation of the heart.[136] Further, inducible *SMN* expression under the *Emx1* promoter that expresses in cortical neurons also failed to extend lifespan.[137] In fact, a number of cardiac abnormalities due to altered autonomic innervation have been reported in SMA mouse models, including heart failure and arrhythmia.[138–140] In humans, several small cohort studies have noted arrhythmia and abnormal vascular perfusion in SMA infants, but further investigation is required to understand how this might affect SMA patient outcomes and treatment response.[141, 142]

Studies on ASO- and gene therapy vector–dosing to the CNS, versus systemic administration, in SMA mouse models have shown varying amounts of efficacy for each strategy. CNS targeting of SMA by ASO therapy is accomplished by intrathecal injection into the spinal cord. The scAAV9 vectors used in gene therapy cross the BBB and can be administered either intrathecally or systemically and still reach critical cell populations. Intrathecal injections are invasive and costly, and if repeated doses are necessary, treatments must be timed for maximum efficacy. Longer lasting, less invasive therapies that still have extensive motor neuron targeting would be ideal when designing new therapeutics and clinical trials for SMA.

Critical windows for SMA treatment

Due to the irreversible cell death and degeneration of the spinal motor neurons, determining an appropriate treatment window for SMA is crucial for the ultimate success of these therapies on SMA patients. To that end, studies on the exact timing of motor neuron loss and therapy efficacy are being completed in mouse and primate models.[76] In an inducible hypomorphic mouse model, low *SMN* expression and early embryonic lethality can be rescued by induction of full-length SMN early in development (E7.5) but not later

(E13.5).[143] In an effort to diagnose SMA patients early for early intervention, a non-invasive blood-based postnatal screen for homozygous loss of *SMN1* is also being developed (NCT02123186).

Clinically relevant outcome measures

Current clinical trial designs for SMA therapeutics rely on a number of different outcome measures to determine success. Among the most commonly used are compound muscle activated potential (CMAP), motor unit number estimation (MUNE), and, more recently, electrical impedance myography (EIM); but because of the age range of the patients and the heterogeneity of the rate of decline, these measures can be difficult to interpret. The discovery of new biomarkers for SMA is critical for identifying factors that could be measured as additional benchmarks of positive outcomes in these trials. To this end, several groups have looked for biomarkers in SMA patient samples[144, 145] and in mouse models.[146] SMN transcript levels do not correlate well with protein levels or disease severity, possibly due to variation in patient age, expression, and even sample storage and handling.[147–149] A new two year longitudinal natural history study (NCT01736553) is in progress and will measure both SMN mRNA and protein in addition to total plasma protein levels with secondary motor function outcomes to address these issues and identify biomarkers.

Conclusions

In the two decades since the discovery of the SMN gene many important discoveries have contributed to our basic understanding of SMA pathogenesis and the development of treatment paradigms (Fig. 3). Due to the well-characterized discrete genetic basis of the disease, SMA is a good candidate for *SMN2* splice-site modulating ASOs, as well as exogenous *SMN1* replacement by gene therapy vectors. *In vitro* and *in vivo* animal studies have provided strong evidence that the SMA neuromuscular phenotype can be significantly ameliorated by expression of full-length SMN protein or by treatment with neuroprotective compounds. There are still many questions as to the relative importance and timing of SMN expression in motor neurons and its subcellular functions in cell bodies versus axons and dendrites that are important for designing novel therapeutics. However, recent advances in pre-clinical drug development and clinical trials have shown great promise towards viable therapies for SMA patients.

Acknowledgments

We thank Basil Darras and members of the Sahin lab for critically reviewing the manuscript. Due to limited space, we have not quoted all literature in the field, and we apologize to those whose articles are not referenced. Research in the Sahin lab has been supported by the NIH (P30 HD018655), Slaney Family Fund, Whitehall Foundation, SMA foundation, CureSMA, Boston Children's Hospital Translational Research Program, Novartis, Roche, and Shire.

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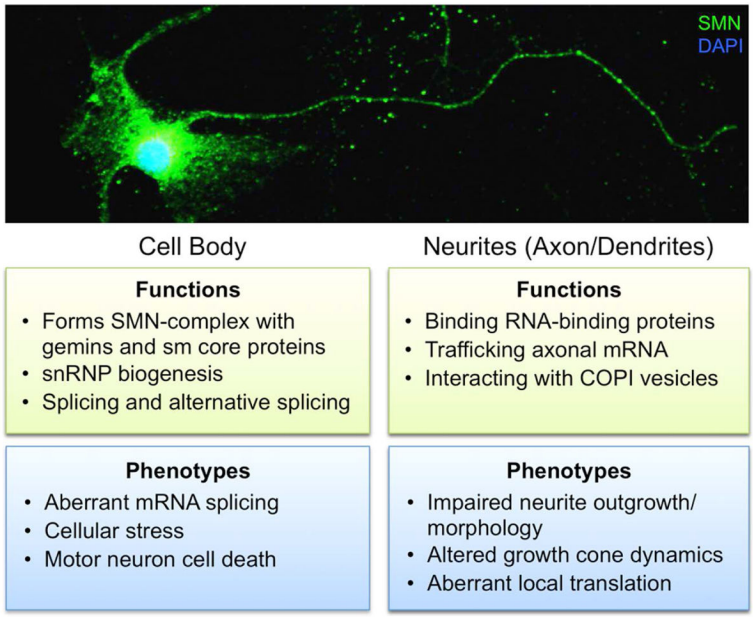

Figure 1.

Subcellular localization of SMN function in motor neurons. The top image shows immunofluorescent staining of SMN protein throughout the cell body and processes of a primary rodent spinal motor neuron in culture. SMN has a number of different functions throughout the motor neuron cell body axon/neurites. Loss of SMN leads to perturbations in these cellular functions and therefore specific SMA phenotypes are associated with these subcellular functions.

Name	Mechanism of action	SMN Expression
Antisense Oligonucleotide	↑ Exon 7 inclusion in SMN2	↑ Full-length SMN from SMN2
Gene Therapy	AAV delivery for exogenous SMN	↑ SMN expression
Olesoxime	⊣ Cell death	N/A
Quinazoline	⊣ DcpS ↓ RNA turnover	↑ Full-length SMN from SMN2
HDAC Inhibitors	↑ Promotor acetylation ↑ SF2/ASF ↓ hnRNPA1	↑ Full-length SMN expression
IGF-1	↓ Motor neuron cell death	N/A
mTOR	↑ Protein Synthesis	N/A
Stem Cell Replacement	↑ Wildtype motor neuron number	↑ Full-length SMN expression

Figure 2.

Mechanisms of action of SMA therapeutics. There are a number of different therapeutic strategies. SMA therapies not only directly increase the full-length SMN expression, but also indirectly modulate pathways that effect SMN expression, and act as neuroprotective compounds to inhibit motor neuron cell death and provide trophic support for surviving cells.

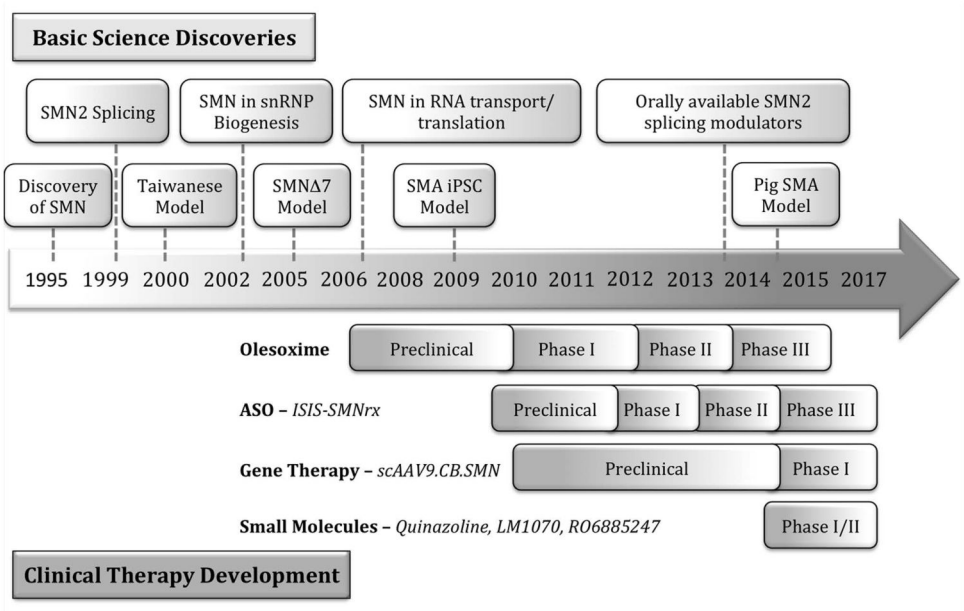

Figure 3.

Important milestones in SMA basic science and clinical development. Timeline of important basic science discoveries in SMA: SMN basic biology and SMA modeling from the discovery of SMN to today (top), emerging and ongoing pre-clinical development and clinical trials of SMA therapeutics (bottom).

Table 1

Current Open Clinical Trials for SMA Therapies. Open clinical trials enrolling patients as published on clinicaltrials.gov as of January 5th 2015.

Treatment	SMA patients	Identifier/phase	Study design	Primary outcome measures
ISIS-SMN-Rx	Type I infants	NCT02193074 Phase III	Randomized, double-blind, sham- controlled safety and efficacy study Intrathecal administration	Time to death or permanent ventilation
ISIS-SMN-Rx	Type II/III	NCT02292537 Phase III	Randomized, double-blind, sham- controlled safety and efficacy study Intrathecal administration	Change from baseline HFMS
scAAV9.CB.SMN	Type I infants	NCT02122952 Phase I	Non-random, open label safety and efficacy study IV delivery escalating viral dose in three cohorts	Grade three of higher treatment toxicity, mortality
LMI070	Type I infants	NCT02268552 Phase II	Open-label multi-part safety and efficacy study Oral administration of escalating doses in three cohorts	Change from baseline in physical exam, blood chemistry, urinalysis, vital signs, and cardiac function
RO6885247	Type I/II/III	NCT02240355 Phase I	Randomized, double-blind, placebo- controlled, safety and efficacy study Oral administration	Incidence of adverse evens (AEs)
Pyridostigmine bromide	Type III adults	NCT02227823 Phase II	Non-random, open-label Oral delivery of anti-cholinesterase for patients with abnormal NMJs	Change in baseline distance in 6 minute walk test
4-AP (dalfampridine)	Type III	NCT01645787 PhaseII/III	Randomized, double blind, placebo- controlled Oral administration	Change in six-minute walk test Kinematic gait evaluation
Biomarker identification	Type I infants	NCT01736553	Prospective observational study of SMA infants vs. healthy controls	SMN mRNA, SMN protein plasma proteins, CMAP, EIM, motor function