Current and emerging treatment options for spinal muscular atrophy

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Abstract: Spinal muscular atrophy is one of the most common inherited neuromuscular conditions; our understanding of the genetic pathology and translational research coming from this insight has made significant progress over the past decade. This short review provides the background of the disease along with the bench to bedside progress of some promising treatment options to develop better understanding of the present state of the disease.
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Introduction

Spinal muscle atrophy (SMA) is an autosomal recessive neurodegenerative disease and a leading global genetic cause of infant death.[1] It is characterized by the loss of α motor neurons from the anterior horn of the spinal cord resulting in muscle weakness, trunk paralysis, and muscle atrophy.[2] With an estimated prevalence of 1 in 6,000–11,000 live births and a carrier frequency of 1 in 40 to 1 in 60 among different ethnic groups and geographical location,[3–7] SMA is the most common monogenic disease fatal to infants and one of the most common forms of neuromuscular disorder in childhood.

SMA is classified into three major groups based on the age of onset and severity of the disease;[8–11] Type I SMA (Werdnig–Hoffmann disease) is the most prevalent and severe form of the disease with postnatal onset within the first six months. These patients are never able to sit and usually succumb within 2–5 years of age due to respiratory failure.[11–13] Patients with the intermediary form or type II SMA develop muscle weakness within 6–18 months of age. Although these patients can sit, due to progressive muscle weakness, they can never stand or walk. Type III SMA (Kugelberg–Welander disease) has an onset between 18 months and 30 years of age with patients able to walk on their own (with some assistance).[11,12,14,15] The diagnosis of SMA is typically established through physical examination, patient history, electromyography followed by confirmatory genetic testing.[3] Muscle biopsy may also be done in some cases[16] although less frequently since the advent of genetic testing.

The lack of functional survival motor neuron (SMN) protein due to deletion or mutation in the *SMN1* gene is the cause of SMA.[17] SMN is an evolutionary conserved RNA-associated protein required for cellular viability; complete loss of functional full-length SMN protein is therefore embryonically lethal.[18–20] Humans due to an evolutionary recent duplication event at chromosome 5q[17,21] possess a nearly identical SMN paralog, *SMN2* (Figure 1) and thus uniquely among all species can survive loss of *SMN1*.

Although, *SMN2* has only few translationally insignificant nucleotide differences compared with *SMN1*, the C to T transition at position 6 of exon 7 results in the

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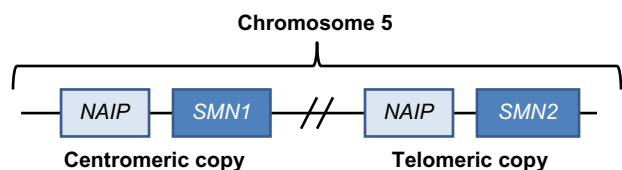Tel +971 2 509 9942
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Chromosome 5

Figure 1 Human SMN locus on chromosome 5q showing inverted duplication of *SMN1* and *NAIP* genes.
Abbreviations: NAIP, neuronal apoptosis inhibitory protein; SMN, survival motor neuron.

production of an alternative splice variant. Consequently, *SMN2* produces only ~10% of the full-length functional SMN protein produced by *SMN1*; the remaining mRNA lacks exon 7 and is translated into a truncated, unstable non-functional protein called SMNΔ7 which, failing to oligomerize, is quickly degraded (Figure 2).[22,23]

Greater than 95% of all SMA patients have homozygous deletions of *SMN1* gene.[17] All SMA patients have at least one copy of *SMN2*, which produces low levels of functional SMN protein and acts as a disease modifier. There is an inverse correlation between *SMN2* gene copy number and disease severity, ie, an increase in the *SMN2* gene copy number decreases the SMA severity.[24,25] Typically, SMA type I patients have one or two copies of *SMN2* gene compared with two to three copies in type II, and three to four copies in types III and IV. Individuals with more than four copies of the *SMN2* gene are completely asymptomatic notwithstanding the deletion of *SMN1*.

A central function of 294 amino acid long SMN protein is the assembly of small nuclear ribonucleic proteins (snRNPs) which are essential for splicing.[26–35] With mutations in the ubiquitously expressed SMN shown to cause SMA, we are left with a question posed in this disease gene cloning era; how do mutations in a gene which is expressed everywhere

(ie, SMN) mainly impact neurons (despite several recent studies suggesting a role for SMN in other tissue types as well[36–44]). Although the precise pathogenic molecular mechanism of SMA is not known, it is believed that the lack of motor neuron SMN protein may lead to a synaptopathy resulting in apoptotic death of motor neurons.[20,45]

Presently, only multidisciplinary supportive care including, critically, respiratory support is available for most children with SMA.[10] Although these interventions have improved both life expectancy and quality of the life, an effective therapy for SMA is eagerly awaited by patients, their families, researchers, clinicians, and support care staff alike. Several approaches are currently being pursued as the following section outlines.

Therapeutic strategies for SMA
Gene therapy

Gene therapy is one of the most promising therapeutic advances for SMA. In the past 6 years, several groups have used self-complementary adeno-associated virus serotypes 8 and 9 carrying human *SMN1* cDNA to treat mouse models of SMA. The most encouraging results (amelioration of disease phenotype and dramatic extension in the life span) were observed with pre-symptomatic treatment of SMA mice.[46–49]

These promising results helped scientists at Nationwide Children's Hospital to receive a fast track designation from the US Food and Drug Administration (FDA) for ChariSMA™ (gene therapy product) to be tested as a SMA therapeutic. At the time of writing, a phase I clinical trial at Nationwide Children's Hospital in collaboration with AveXis Inc. and The Sophia's Cure Foundation is underway to evaluate safety
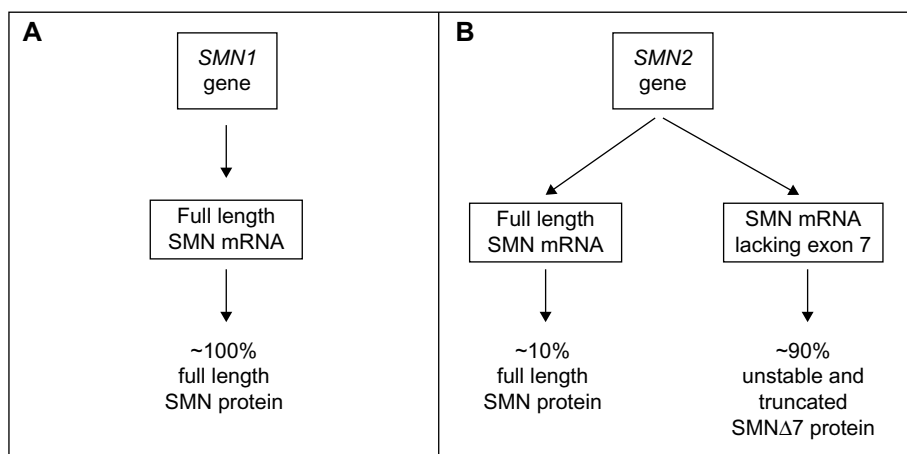

Figure 2 Schematic diagram showing *SMN1* (**A**) and *SMN2* (**B**) genes along with their corresponding mRNA and protein products.
Abbreviation: SMN, survival motor neuron.

and efficacy of gene transfer (systemic AAV9-delivered *SMN1* gene) in SMA type I patients. The major challenges to bring this treatment into clinics are clinical safety, the cost of virus and the possibility of an immune response neutralizing AAV.[50]

SMN2-dependent therapies

Given the role of *SMN2* as a modifier gene for SMA, the inverse correlation between the *SMN2* gene copy number and disease severity, the gene itself has become a drug target. Strategies includes a) inducing the expression of *SMN2*, b) modulating splicing of *SMN2*-derived transcript, and c) stabilizing the full-length *SMN2*-derived mRNA and/or protein (Figure 3).[51,52]

Activation of SMN2 promoter

Histone deacetylases (HDACs) through chromatin condensation are known to repress transcription of genes such as *SMN2*. Several HDAC inhibitors including sodium butyrate, valproic acid (VPA), and phenyl butyrate have been assessed for *SMN2* induction in cellular and animal models of the disease as well as in clinical trials as potential therapeutic for SMA.[53–57] Although they showed promise in cell culture and SMA mouse models, no significant clinical improvement has been observed in SMA patients with HDAC inhibitors.[2,58,59]

The STAT5 pathway has been implicated in the activation of *SMN2* promoter.[60–62] The human lactation hormone prolactin (PRL) and human growth hormone (HGH) have been shown to activate the STAT5 pathway which results in an increase in both *SMN2* gene transcription and full-length SMN protein both in vitro and in vivo, resulting in attenuation of the SMA mouse model severity. PRL treatment resulted in significant survival and attenuation of disease phenotype in the SMA mouse model, a possible reflection of the significant SMN induction observed with PRL treatment in vivo.[61] Although, PRL has been proven safe and was successfully tested in humans for the treatment of lactation-deficient mothers,[63] the absence of clinical grade PRL is delaying its further assessment as a potential SMA therapeutic in the patient population. A Phase II trial in SMA type II/III patients showed no improvement in muscle function or strength after 3 months of treatment with HGH.[64] However, it should be noted that only a single low dose of HGH was used and peripheral white blood cell SMN levels were not assessed in the patient population. Before crossing HGH off the list of SMA therapeutics, it may be beneficial to do a HGH dose escalation study especially in younger SMA patient population assessing muscle strength and monitoring changes in lymphocyte SMN levels.

The deCODE project initiated by Families of SMA (now cureSMA) identified C5-substituted quinazoline activity in increasing *SMN2* promoter activity and thus SMN protein in cell-based assays. The DcpS inhibitor RG3039, a derivative of quinazolines, has since been tested and reported to mildly improve both survival and motor function in two mouse models of SMA through an increase in full-length
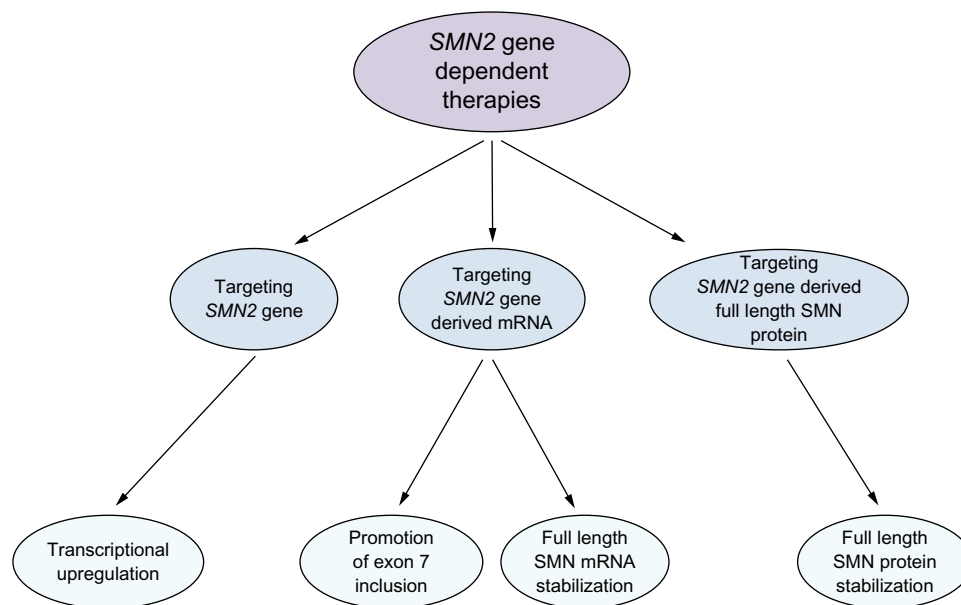

Figure 3 Current *SMN2* gene-derived therapeutic approaches to SMA.
Abbreviations: SMN, survival motor neuron; SMA, spinal muscle atrophy.

SMN levels.[65,66] The compound was initially out-licensed to Repligen Corporation (Waltham, MA, USA) which in 2012 out-licensed to Pfizer Pharmaceuticals (New York, NY, USA); however, the program has now been halted.

Correction of splicing

The significant majority of transcripts arising from *SMN2* gene lack exon 7; agents that suppress exon 7 skipping have thus become the goal of a number of laboratories. Several small compounds including HDAC inhibitors such as VPA, TSA, and sodium butyrate have been assessed for their capacity to increase full-length SMN transcript by altering the splicing process in vitro.[53–57,67] However, regardless of any putative effect observed in vitro, no beneficial effect has carried over to clinical trials.

Recently, RO6885247 from PTC Therapeutics (South Plainfield, NJ, USA) and Hoffmann-LaRoche (Nutley, NJ, USA) has been shown to profoundly affect the splicing of *SMN2* gene to include more exon 7 showing a dramatic positive impact on SMA model mice.[68] A Phase I clinical trial is currently underway to assess the safety profile along with tolerability of this compound.

Antisense oligos (ASOs) are increasingly being used for therapeutic/experimental purposes to treat a number of diseases including SMA, duchenne muscular dystrophy, and myotonic dystrophy.[41,69–72] A bifunctional ASO complementary to *SMN2* exon 7 pre-mRNA sequences has been designed and tested to promote the inclusion of exon 7 and at the same time inhibit binding of negative splicing factors which ultimately results into increased production of full-length SMN mRNA.[41,71]

ASOs are not blood brain barrier (BBB) penetrant, which initially posed a hurdle in their use as a SMA therapeutics.[73] Several reports have shown that intrathecal injections of ASOs results into improvement in survival and disease phenotype in SMA mice.[71] However, in 2011, Hua et al reported an increase in SMN levels along with attenuation of SMA phenotype in SMA mice with systemic delivery of ASO.[41] ISIS pharmaceutical has successfully completed early phase trials to show safety, tolerability, and pharmacokinetics of intrathecal ISIS-SMN Rx (ASOs) in SMA patients. A Phase III has been initiated to test the safety and efficacy of intrathecal administration of ISIS-SMN Rx in patients with infantile-onset and later onset of SMA. This represents a significant breakthrough in the field of SMA as this is the first compound which has reached Phase III clinical trial. The results from these trials are eagerly awaited by the SMA community.

Full-length SMN transcript and protein stabilization

In addition to the upregulation of *SMN2* transcription and the modulation of splicing, the stabilization of either mRNA or protein is an often overlooked but nonetheless viable potential therapeutic approach. In this regard, SMN mRNA has a specific AU-rich element region in its 3′-untranslated region which marks the mRNA for degradation.[74,75] In silico mining of gene expression data sets was used to identify the p38 pathway as a means of enhancing *SMN2* levels. In the initial study, it was shown that treatment of neuronal cells with the p38 activator anisomycin results in the translocation of protein HuR to the cytoplasm where it binds the 3′-untranslated region of SMN mRNA and stabilizes the transcript inhibiting its degradation which in turn increases the SMN protein level.[74] Since then several p38-activating compounds have been identified and reported to increase the SMN protein level in vitro as well as in vivo.[76,77]

Celecoxib, a safe, well-tolerated prescription medication used widely for arthritis and in some pediatric rheumatologic diseases, was identified as a BBB penetrant, p38-activating compound.[78,79] Treatment with low dose of celecoxib increased SMN protein levels in both human and mouse nerve cell cultures, as well as in patient fibroblasts. In a severe SMA mouse model, mice treated with celecoxib showed an increase in the SMN protein in central nervous system (CNS) tissues. In addition, SMA mice also showed an improved motor function and a statistically significant 40% extension of survival as compared to mice treated with placebo.[76] A Phase I/II clinical trial is planned for this FDA approved agent in 2015; it may be with the hope that celecoxib may serve as an adjunctive therapy for SMA, particularly given the low safe doses are required for SMN induction.

Aminoglycoside antibiotics, such as tobramycin and amikacin which have been shown to mask premature stop codon mutations in some genes, have been used to increase SMN protein levels in patient fibroblasts. However, there efficacy and safety has yet to be tested successfully in animal models of the disease.[80–82] An alternative potential therapeutic approach involves targeting the ubiquitin–proteasome pathway which targets many proteins including SMN for degradation. The FDA-approved proteasome inhibitor bortezomib has been shown to increase SMN levels both in vitro and in vivo.[83] However, the major obstacle to the use of bortezomib as a therapeutic is its inability to cross the BBB.

Finally, through the experience gained from treatments in animal models of the disease, it is the general consensus that, as with most disorders, early timing of the treatment is

Table I Current therapeutic pipeline for SMA

Therapeutic approach	Name of the product	Current Status	Sponsors
Gene therapy	ChariSMA™	Phase I clinical trial	• Jerry R Mendell (Nationwide Children's Hospital) • AveXis Inc. • The Sophia's Cure Foundation
Promoter activation	Prolactin	Pre-clinical stage	• Children's Hospital of Eastern Ontario
	RG3039	Pre-clinical stage	• Pfizer Pharmaceuticals
Inclusion of exon 7 product	RO6885247	Phase I clinical trial	• PTC Therapeutics • Hoffmann-LaRoche
	ISIS-SMN Rx (ASO)	Phase III clinical trial	• ISIS Pharmaceuticals
mRNA stabilization product	Celecoxib	Phase I/II clinical trial	• Children's Hospital of Eastern Ontario • University of Calgary

Abbreviations: ASO. Antisense oligos; SMA, spinal muscle atrophy.

critical for maximum benefit; optimally, this would involve pre-symptomatic identification of infants with SMA. Newborn screening is therefore an important step in the most effective use of novel therapies of SMA allowing intervention before the clinical course is set.[51,84,85] Hopefully through early intervention along with the promising therapeutic candidates described in Table 1, there will be an effective therapy and possibly a cure for SMA in the near future.

Disclosure

The authors report no conflicts of interest in this work.

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