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Spinal Muscular Atrophy: The Development and Implementation of Potential Treatments Running Head: Spinal Muscular Atrophy

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Abstract

In neurodegenerative disorders effective treatments are urgently needed, along with methods to detect that the treatment worked. In this review we discuss the rapid progress in the understanding of recessive proximal spinal muscular atrophy and how this is leading to exciting potential treatments of the disease. Spinal muscular atrophy is a caused by loss of the Survival Motor Neuron 1 (*SMN1*) gene and reduced levels of SMN protein. The critical downstream targets of SMN deficiency that result in motor neuron loss are not known. However, increasing SMN levels has a marked impact in mouse models, and these therapeutics are rapidly moving towards clinical trials. Promising preclinical therapies, the varying degree of impact on the mouse models, and potential measures of treatment effect are reviewed. One key issue discussed is the variable outcome of increasing SMN at different stages of disease progression.

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) describes a group of lower motor neuron disorders with genotypic and phenotypic diversity that can be inherited as dominant, recessive or X-linked traits. The focus of this review will be on the most common form of SMA, 5q proximal recessive SMA caused by loss or mutation of the Survival Motor Neuron 1 gene (*SMN1*) and retention of the *SMN2* gene[1]. SMA has a frequency of 1/11,000 new births[2-4] and carrier frequencies that range from 1/47-1/72 depending on racial group[4]. SMA represents the most common genetic cause of infant death[5]. Many other types of SMA are related to mutated genes that are expressed in not just the nervous system but in a wide range of tissues. This is also the case with SMN expression, and the reason for selective motor neuron or motor circuit involvement in 5q SMA is not known[1, 6]. Proximal 5q SMA can be classified clinically into five subtypes based on severity and onset[7]. Type 0 is the most severe subtype and is characterized by weakness at birth. Type 1 is the most common subtype and is associated with onset prior to 6 months of age and the lack of ability to sit independently. Without ventilatory support, death usually occurs prior to age 2 in type 1 SMA. Onset of type 2 occurs between 6-18 months and the ability to sit upright is achieved while ambulation is not. Type 3 has onset after 18 month of age and ambulation is at least temporarily achieved. The mildest subtype is type 4 characterized by mild proximal weakness with adult onset[8, 9].

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Genetics of 5q SMA and phenotype modification in man

The loss or mutation of *SMN1* and the retention of *SMN2* causes SMA[1, 6]. *SMN1* and *SMN2* differ by a single nucleotide in exon7 that does not alter an amino acid but does alter a splice modulator[10-12]. The majority of the transcript from *SMN2* lacks exon7 thus the resulting SMN protein does not oligomerize efficiently and is degraded[13-16]. The copy number of both *SMN1* and *SMN2* vary in the population, which is particularly relevant to the severity of this disease[4, 17]. Additional copies of the *SMN2* gene can modify the SMA phenotype with an inverse correlation of phenotypic severity and copy number[17, 18]. Spinal muscular atrophy has been modeled in mice by placing a human *SMN2* transgene on the background of a homozygous loss of function mouse *Smn* allele[19-21]. The introduction of two copies of *SMN2* into a *Smn* knockout mouse results in a severe SMA like phenotype and death at 5 days. The presence of eight copies of *SMN2* on this background results in mice that are essentially normal. The addition of a transgene expressing SMNΔ7 (SMN lacking the exon7 sequence) along with two copies of SMN2 extends lifespan of the mouse to ∼14 days. In addition to alterations in the *SMN2* copy number, variants in *SMN2* gene have been identified that result in increased full-length SMN production. One such variant is 859G>C in exon7 of *SMN2* that increases full-length transcript by about 20% and is found in patients with mild SMA[22-24]. Interestingly, this variant occurs in two copies in milder type 3b patients, one copy in type 2 patients, and does not occur at all in severe type 1 patients[22-24]. This leads to the prediction that a 20% increase in full-length SMN mRNA in 2 copy *SMN2* patients will likely result in type 3b SMA and most likely a 25% increase in full-length SMN mRNA in those same patients would result in no SMA phenotype[22, 24].

In addition to variants within the *SMN2* gene there are also modifiers of SMA that lie outside the SMN locus. This is clear from haploidentical siblings with the same copy number of *SMN2* that have different SMA severities[17, 25-27]. While families with type 2 and 3 SMA siblings are most common, a similar phenomenon also occurs with type 1 and type 2 SMA siblings[28, 29]. Plastin 3 mRNA has been reported to be markedly elevated in some milder siblings and is suggested to be a modifier of SMA[30]. However, high Plastin 3 mRNA levels are also found in female siblings with the more severe SMA phenotype[31]. One possibility is the Plastin 3 modifier is female dependent and incompletely penetrant. An alternative theory is that Plastin 3 is not a critical modifier of SMA phenotype. The role of Plastin 3 in SMA remains uncertain as no DNA changes in the Plastin 3 gene itself, nor any activator of Plastin 3 expression that segregates with the mild sibling, have been reported. The regulators of splicing in the *SMN1* and *SMN2* genes that alter incorporation of exon7 have been studied extensively. Numerous sites have been found that bind either a negative or a positive regulator of splicing[32]. Within some of these regulators exists a series of variants in the single nucleotide polymorphism databases. These variants could alter the activity of the splicing regulator. While to date it has not been reported, at least one possibility to explain the alteration of SMN expression in haploidentical discordant siblings could be a mutation in one of the numerous regulators of *SMN2* splicing.

SMN Function

SMN has a clear canonical function in the assembly of Sm proteins onto snRNAs[33]. Thus it is not surprising that complete loss of SMN is lethal both to an organism and to a cell, since the assembly of snRNAs is essential in splicing[6, 19, 34, 35]. It remains unclear whether disruption of SMN's essential splicing function, an additional axonal SMN function, an unknown function, or some combination thereof is critical for the SMA phenotype. We have previously discussed the potential mechanisms of SMA in a review[6]. Understanding the mechanism of SMA is of critical importance for therapeutic development of clinically

applicable targets directly downstream of SMN. The investigation of downstream genes will provide valuable targets that can be altered to improve SMA disease phenotype.

Assays of the ability of SMN to perform assembly of Sm proteins onto snRNA show a very tight correlation to SMA phenotypic severity in cells and extracts from SMA mouse spinal cord[36, 37]. Furthermore, there is a correlation with ability to perform snRNP assembly and the ability of a transgene to correct SMA[6, 38]. The predicted outcome of reduced snRNP assembly is an alteration in gene splicing due to reduced snRNP levels[6, 39]. As the snRNPs most affected by SMN reduction are those involved in splicing minor introns, genes containing minor introns are predicted to be the primary target of reduced snRNP assembly[37-39].

Splicing has been examined in tissues where SMN is reduced and, provided samples are assayed early in the SMA disease progression, there are minimal splicing changes[40]. Thus it appears that SMN deficiency does not produce a plethora of splicing changes[40]. We do not consider small (2-5%) changes likely to have any major consequence on the cell. Using laser capture microdissection Ruggi et al have shown that the amount of full-length SMN from *SMN2* is lower in motor neurons in normal mice than in other neuronal cell types, providing a partial explanation of why motor neurons are selectively affected[41]. To date there is no comprehensive data on the splice changes that occur specifically in SMA motor neurons. It is likely that there are only a few critical downstream targets altered upon SMN deficiency as it appears that not all, or even most genes, are significantly affected by reduced SMN.

One change with SMN reduction that has been reported in both Drosophila and mouse is the splicing of the minor intron in the stasimon gene[39, 42]. The stasimon gene shows an approximately 30% reduction of a spliced isoform in motor neurons and 40% in proprioceptive neurons of the SMA mouse[39]. In Drosophila with reduced SMN the total larvae shows a similar level of splice alteration (30%). The exact level of alteration in either the Drosophila proprioceptive neurons or motor neurons is not clear[39]. Expression of stasimon in the SMN deficient fly does correct some of the larval NMJ defects but not all. In addition, it is not clear whether the exon deficient isoform shows any rescue ability as opposed to the full-length isoform[39]. While this data clearly shows that a U11/U12 intron is affected in SMA mice, the crucial nature of the target in SMA needs to be confirmed by additional experiments. For instance, does knockdown of stasimon *in vivo* in mouse neurons produce an SMA like phenotype or does replacement of stasimon in the SMA mouse have any effect?

In Drosophila the mutant SMN alleles are non-functional, and the larvae are reliant on maternal SMN[43]. SMN deficient Drosophila show decreased movement, defective motor rhythm and abnormal neurotransmitter release at the neuromuscular junction in larva[42]. These phenotypes can be corrected by expression of SMN in cholinergic neurons, but not by expression of SMN in glutamatergic or GABAergic neurons[42]. In Drosophila the motor neuron is glutamatergic whereas the proprioceptive neuron is cholinergic. Previous studies in the SMA mouse have suggested the importance of the proprioceptive neurons in effecting the output of the motor neuron[44]. In addition, correction of SMN in just motor neurons or just muscle of SMA mice does not have a major impact on survival yet correction in all neurons does[45-47]. Interestingly, the expression of SMN in motor neurons can correct the neurotransmitter release properties at the NMJ and restore the synaptic stripping on the motor neuron[46, 47]. Importantly, removal of SMN from the motor neuron in the presence of two copies of *SMN2* does result in a clear motor neuron phenotype although the mice do survive longer than Δ7SMA mice[48]. There is profound reduction in muscle bulk and changes in developmental markers of muscle in SMA[49]. This has led to the suggestion that high SMN levels above those from two copies of *SMN2* are required in muscle tissue. While

this is possible, it is difficult to separate the indirect atrophic effects of denervation from direct effects of SMN deficiency on muscle atrophy and development. High expression of SMN in just muscle does not correct the SMA phenotype in mice[45]. Whether complete SMA treatment will require expression of high SMN levels in multiple tissues including muscle remains to be determined.

The mouse and human have considerably more introns than lower organisms. Therefore it remains very important to obtain a complete catalogue of splice alterations in neurons. In this regard, it is essential to have RNA-seq data on motor neurons along with suppression and knockdown studies using scAAV9 delivery in SMA mice. This will allow the definition of critical downstream targets. Induced pluripotent stem cells have been developed from SMA patients and neurons/motor neurons do show a mild phenotype[50] These cells are being used in screens to identify drug compounds. Again RNA-seq data from these cells would be useful along with the identification of factors that suppress phenotypes in these cells. This will afford the opportunity to compare the changes occurring *in vivo* in animal models of SMA with those found *in vitro* in human cells.

SMN has been reported to interact with a large number of proteins. Whether all these interactions really contribute to a function in the cell remains debatable[6]. It is important to note that biochemical studies using SMN missense mutations *in vitro* in culture need to be interpreted with care due to the ever present full-length wild-type SMN in the cell. *In vivo* in the mouse, mild SMN missense mutants interact with wild-type SMN to form functional complexes (complementation) whereas SMN missense mutants on their own are nonfunctional[38].

It is important to consider functions other than snRNP assembly that could be involved in the development of SMA. SMN is found in low amounts in the axon and reduction of SMN leads to reduced β -actin mRNA transport and axon defects[51-53]. This has led to the suggestion that SMN has a unique function and interacts with some different proteins in the axon. The question that arises include: What is this axonal complex and can it be assayed biochemically? Certainly it is possible that the Lsm proteins[54] or others could be assembled onto mRNA for transport down the axon. If this is the case this assembly reaction can be measured and correlated to reduced Sm assembly in SMA[6]. SMN has been reported to interact with the golgi adaptor protein Alpha Cop[55, 56] as well as HuD[57, 58]. These proteins are present in some RNA granules in the axons yet it is difficult to reconcile the significance of these SMN complexes when relatively few particles in the axon show complete overlap. Furthermore, how are these various complexes maintained in equilibrium in the cytoplasm where different SMN partners are competing with each other for the same spot on SMN? Finally, overexpression of these binding partners should act in dominant negative manner to compete out the other SMN functions if these multiple complexes do in fact occur in the same cell. If transport of mRNA is a critically affected function in SMA then it becomes important to determine what will suppress the phenotype. Our preference is that a clear strong suppression of the SMA phenotype be obtained in the mouse. For example, overexpression of HuD has been reported to suppress axonal defects in cultured cells but this finding has not been tested *in vivo* by scAAV9 delivery into the SMA mouse[57]. If strong suppression can be shown then this is both a new target for therapeutics and evidence for the importance of that particular mechanism in SMA.

Current therapies and what has been tested in SMA

The clinical management of SMA is designed to address the secondary effects of muscle weakness, and the standards of care for SMA have been described elsewhere[59]. Outside of supportive care, there are currently no effective therapeutic interventions available for SMA.

A number of drug compounds have been tested in SMA clinical trials, but to date none have proven clearly effective. These studies include both presumed neuroprotective agents and those expected to induce SMN. Studies with gabapentin and riluzole for neuroprotective effects showed no benefit[60, 61]. Several small molecule compounds, some of which are available clinically for other non-SMA related FDA-approved indications, have been shown to promote inclusion of exon7 in *SMN2* transcripts by alteration of splicing and or induction of SMN expression levels. However in all cases these compounds were found by induction of SMN in patient fibroblast lines. Given this is a dividing cell and not a motor neuron the possibility exists that these compounds do not induce SMN *in vivo* in the required cell types. Indeed we have found this to be the case for a number of molecules when tested in mice (unpublished observation). Of the compounds reported to induce SMN in cultured cells, phenylbutyrate, hydroxyurea, and valproic acid have been taken to clinical trial without evidence of clinical benefit[62-67]. Salbutamol increased full-length SMN protein production in fibroblasts from SMA patients[68], but clinical trials showed only a modest effect and blinded, placebo controlled studies have not been performed[69]. There are multiple factors that could contribute to the failure of these clinical trials. First and foremost would be the lack of clear data that the compounds induce SMN in the required cell types *in vivo*. Second is the inappropriate timing of treatment delivery (i.e. in late symptomatic patients). It is increasingly becoming clear that at least in SMA model mice there is a therapeutic window when increased SMN protein is needed for motor neuron survival and an improvement in phenotype[70, 71]. Most SMA patients enrolled in these trials have possibly been outside this therapeutic window where increasing SMN levels would be predicted to have an effect. One key aspect that is not fully understood is the requirement for increased SMN in the different types of SMA, and whether increasing SMN later in the course of disease in type 2 and 3 patients will allow the remaining motor neurons to function better or not. The timing of motor neurons loss in SMA type 2 and 3 and whether there is a specific window of development which overlaps type 1 is not known. To get complete answers to these questions will require human clinical trials with the strong SMN restoring agents that have recently been developed. While we cannot be certain that early induction of SMN is required for correction of SMA in humans, understanding the biology of SMA and the consideration of this possibility is important in clinical trial design and interpretation. Although there have been problems with the initial drug compounds evaluated, there are now SMN inducers in the pipeline that clearly have a major impact on the SMA mouse models *in vivo*.

Therapeutic Pipeline for SMA in 2013

Currently the main targets for therapeutics in SMA are increasing SMN from *SMN2* or restoration of SMN levels using gene therapy. Other therapeutic possibilities such as stem cells that can differentiate into motor neurons, neuroprotective strategies and the use of targets downstream of SMN deficiency (once defined) are significantly behind the progress of SMN restoration. The effects of stem cell therapies, to date, are related to trophic support of the motor neurons rather than functional motor neuron replacement[72-74]. The requirement of implantation of stem cells along the full length of the spinal cord and establishment of synaptic connections remain significant challenges, and currently this is an experimental concept requiring much further development. The required targets of neuroprotective therapies remain unknown, and to date, in SMA and other neurological disorders, impressive results are lacking.

Therapies targeting SMN protein restoration levels are the best supported by preclinical work and hold the most promise for an effective treatment (table 1 and 2). When SMN is restored early in SMA mouse models, a clear rescue of SMA phenotype and increase of survival occurs[71, 75, 76]. Approaches to increase SMN include gene therapy for *SMN* replacement, antisense oligonucleotides (ASO) to modify *SMN2* splicing, small molecule

therapies targeting modification of *SMN2* splicing, extending the stability of SMN protein, and activating the *SMN2* promoter (Table 1 and Table 2). Earlier reports of gene therapy in the SMA mouse demonstrated transduction of *SMN* to the motor neurons in the lumbar spinal cord after delivery to multiple muscles and retrograde delivery of the rabies G pseudotyped virus to the motor neurons. However this transduction is not as efficient as subsequent studies, and the lentivirus studies produced a minimal impact on survival in the SMA mouse[77].

In 2010/2011 dramatic and successful rescue of the SMA mice was reported by four groups using gene therapies to replace SMN with an adeno-associated virus-based vector[76, 78-80]. The AAV used was serotype 9 and self-complementary or scAAV9, this virus has the ability to cross the blood brain barrier and results in rapid expression. Various routes of delivery of scAAV9 SMN including intravenous, intracerebroventricular, and combined routes have been investigated[79, 81, 82]. The combined findings of preclinical work support that sufficient viral titer and transduction within the central nervous system will be critical in future clinical trials. The delivery of scAAV9 has been explored in larger animals including both primates and the pig. In large animals, scAAV9, when introduced into the vasculature, crosses the blood brain barrier and results in efficient transduction of motor neurons in various regions of the spinal cord[83-85]. Preclinical toxicology studies in both primates and mice indicate good safety of scAAV9-SMN, and in the near future an IND will be filed on scAAV9-SMN for an initial clinical trial in type 1 SMA using vascular delivery (Brian Kaspar and Jerry Mendell, personal communication). In addition to vascular delivery, intrathecal delivery has been investigated in large animals; again this results in efficient transduction of motor neurons and allows for a reduced viral dose to be used[85-87]. Studies are underway to fully optimize this route of delivery and to obtain the required toxicology studies to move this treatment to clinical trials. Gene therapy is well placed for the treatment of SMA with clear preclinical efficacy and a good toxicology profile. Autoimmunity against restored SMN, as seen in other gene therapy trials, is not predicted to occur in SMA due to the presence of endogenous SMN levels. scAAV9-SMN offers the potential one-time dosing without the requirement of repeated treatment. The main disadvantage currently is the production of the large amount of virus required for treatment.

Antisense oligonucleotides (ASOs) are powerful tools for therapeutic and investigative applications. Utilizing complementary base pair recognition to bind mRNA, ASOs can be used for gene suppression (blocking translation of RNA to protein) or modification of RNA processing and therefore exon content. ASO therapy for SMA can be designed to modify *SMN2* by correcting pre-mRNA splicing (increased incorporation of SMN exon7), either by promotion of binding of splicing factors (bifunctional ASO's) or blocking hnRNPA1 binding at splice suppressor sites. We have recently extensively reviewed the use of ASOs in SMA in particular in preclinical studies[88]. Here we will briefly indicate the most salient features. Bifunctional ASO's are thus named due to the presence of both a domain complementary to a specific RNA and a secondary domain to facilitate splicing factors such as SR proteins. These ASOs have been used predominantly in cells in culture to induce incorporation of *SMN2* exon7 *in vitro* and not tested in mice extensively *in vivo*. Morpholino and 2′-*O*-methoxyethyl (MOE) chemistries in particular have been used to block the negative regulators of the ISS-N1 sequence. Both chemistries result in remarkable rescue of the SMA mouse. The morpholino gives a rescue of over 100 days in SMA model mice with cerebrospinal fluid delivery[89-91]. In contrast MOE gives reduced efficacy with a single cerebrospinal fluid delivery but an enhanced efficacy when delivered at multiple time points and at high doses peripherally with a survival benefit of well over 100 days[92]. There appears a clear difference here; however it has to be remembered that the blood brain barrier in mice is relatively open at the stage of development when this ASO is delivered. Therefore it is difficult to predict exact distribution with peripheral delivery. It is our view, for numerous

reasons, that motor neurons and neurons are the critical target, but which is the best chemistry to use in clinical trials for the treatment of SMA will require testing of both chemistries with rigorous preclinical data in both mice and primates. In essence the ability of ASOs to increase full-length SMN protein has been demonstrated *in vitro* and *in vivo*, and preclinical studies successfully rescue mouse models of SMA when delivered early[90-96]. Recently two early phase trials have been initiated by ISIS Pharmaceuticals to investigate the safety and pharmacokinetics of intrathecal delivery of MOE ASO in patients with infantile-onset SMA and in older children with milder disease. The results of these studies are eagerly anticipated. Initial results indicate that for the MOE chemistry that they are safe[97]. The ASOs have clearly shown efficacy in animal models now the question remains how this translates into human studies. What ASO chemistry works the best with intrathecal delivery, when it needs to be given, as well as the repeat dosing requirement will all become important questions. The advantage of an ASO is the relative simple manufacture, the lack of toxicity, the clear efficacy and the specificity to target which should give minimal toxicity. The disadvantage is the lack of clear knowledge on the optimal chemistry and the difficulty of repeat dosing in a simple manner.

Table 2 lists small molecule drugs that have been developed, the associated proposed mechanism of effect, and the impact on survival in mouse models of SMA. Several histone deacetylase (HDAC) inhibitors have been investigated in SMA mouse models *in vivo* with variable effects on survival, but a major problem is that currently all pan HDAC inhibitors have shown Ames positive tests and indicate a major issue for a pediatric indication such as SMA. However a number of other small molecule drugs that increase SMN production from *SMN* or alter splicing of *SMN2* to increase incorporation of exon7 have been identified with high throughput screening. Quinazolines are shown to increase *SMN*2 promoter activity, and derivatives have been shown to increase SMA mouse survival to a greater or lesser degree depending on the severity of the model used.[98-100] The drug is currently moving to phase one clinical trials. However drug compounds of a second generation have now been reported these compounds have been developed by PTC and Roche using HTS screens. They identified molecules that alter the splicing of *SMN2* such that more exon7 is incorporated and more full length SMN is produced. These molecules have a remarkable impact on the SMA model mice increasing live span to at least 150 days when drug is removed. Thus clearly small molecules conventional drugs to alter *SMN2* splicing and hence amount of SMN produced by a gene can be developed, and this offers exciting prospects for the development of conventional drugs for treatment of SMA. Potential advantages of a drug compound include straight forward manufacture, easy delivery with a reasonable expectation that the compound will be distributed to where it is required. Possible disadvantages include the potential for toxicity of the compound, in particular, with requirement of sustained use.

Future Parallel Measurements of Treatment Response in Humans and Mouse

Candidate outcome measures include muscle strength testing, motor function testing, muscle mass imaging, functional scales, quality of life questionnaires, survival, time to ventilator dependence, electrophysiology, and others[101]. Clinical functional scales, vital for measuring treatment effect in clinical trials, are variably hampered by the wide range of disease severity, and variably onset and progression, and age dependent factors, an issue highlighted by a report of a Rasch analysis of SMA motor scales[102]. Until there is an effective treatment for SMA, it remains uncertain which outcomes will be sensitive to treatment effect. Therefore, sensitive and reliable biomarkers with predictive, prognostic, and pharmacodynamics functionality are needed for effective translation of promising

therapeutics, and it is ideal if markers can be similarly applied in animal models and humans to obtain parallel measurements. Proof of concept and correlation of treatment effect in target tissues using animal models can provide powerful validation of a particular biomarker's potential. Ideally measures should be tested in preclinical models using randomized, double blind, placebo intervention study design to predict findings in early clinical trials. Without accurate biomarkers and surrogate endpoints the risk is that effective treatments will be deemed ineffective due to incorrect timing or delivery or incorrect patient selection. Molecular, electrophysiological and imaging tools have been investigated as potential biomarkers and surrogate endpoints. Currently there are biomarker panels for SMA that correlate with severity of weakness and function[103, 104], but whether these markers are related to the biology of the disease and will have predictive or surrogate endpoint ability remain to be determined. SMN transcripts and protein levels can be reliably measured in the peripheral blood, but these levels do not correlate with function[105]. Imaging modalities including ultrasound, dual-energy X-ray absorptiometry, and magnetic resonance have been investigated but currently have technical limitations that limit utility of the techniques. Due to the inaccessibility of the motor system and target tissues to endpoint analysis in humans, electrophysiological markers are particularly promising tools of motor unit assessment *in vivo*.

Compound muscle action potential (CMAP) is an electrophysiological measure of the total output of the motor units supplying a particular muscle. Failure of any portion of the motor unit (the motor neuron, axon, synapses, or innervated muscle fibers) may result in reduced CMAP size. CMAP is a simple technique, a distinct advantage, but the indirect nature of CMAP response does not take into account the process of collateral reinnervation. Therefore, the CMAP response may be partially or fully recovered with less severe motor neuron loss. Recording repetitive CMAP responses with trains of nerve stimulation can quantify failure at the synapse as suggested to occur in animal models and patients[106-108]. Motor unit number estimation (MUNE) is a modification of CMAP that allows an estimation of the functional motor units supplying the muscle being tested. The technique of MUNE compensates for the process of reinnervation and gives a more direct estimation of the number of motor units and the average size of individual motor unit potentials within the CMAP response. Despite this more direct assessment, the technique of MUNE requires more evaluator skill can be prone to bias, and these factors can potentially limit MUNE's applicability to multicenter clinical trials.

Clinically, CMAP and MUNE correlate with disease severity, functional status, SMN2 copy number, and age[109-111]. CMAP and MUNE have not been fully investigated in preclinical models of SMA. CMAP and MUNE can be used in mouse models to determine the precise timing of motor unit loss, and the availability of preclinical treatments with robust effect can be used to determine if CMAP and MUNE are valid surrogate endpoints of motor unit rescue. It is expected CMAP and MUNE will have predictive biomarker ability (i.e. if CMAP and MUNE are severely reduced; a robust response with SMN restoration would be less likely) and surrogate endpoint potential (measurement of a treatment effect). In SMNΔ7 mice CMAP and MUNE are reduced at onset of SMA phenotype and fully restored with early SMN restoration (unpublished observation). It is predicted that complete rescue would lead to normal CMAP and MUNE results. Whereas delayed and incomplete rescue would lead to partially preserved MUNE and the CMAP would to an extent be normalized depending on the capacity for remaining motor units for reinnervation. It remains to be determined whether SMN restoration improves the function of the motor neurons that would have otherwise survived without intervention. Thus, another possible outcome with late or delayed treatment could include no change in MUNE (no rescue of motor neurons) but increased CMAP due to enlargement of the territories of the surviving motor neurons (increased divergence or output) (figure 1). Another particularly promising technique often

grouped with electrophysiology is electrical impedance myography. EIM determines impedance characteristics of muscle tissue but does not assess physiology of muscle or the motor unit and has shown significant promise as a longitudinal measure in SMA, in particular due to ease of application and non-invasive nature of the technique[112].

Unmet Needs and New Directions for Research

The downstream targets of SMN remain a central and important unanswered question. The effect of SMN deficiency on splicing changes remains the most likely pathway affected but the downstream targets remain to be identified. Therefore it is critical to define all the splicing changes that occur in motor neurons when SMN is deficient, and because of non-autonomous function of motor neurons, this determination should occur with motor neuron *in situ* in the spinal cord. Once candidate genes are identified it is important to confirm whether identified targets can suppress the SMA phenotype. To date no such large impact genes have been found. Importantly, the expression profile of identified targets would indicate fundamental biology of the disease which can influence the development of biomarkers, understanding of the timing of the disease, and design of future therapies.

Why SMN deficiency results in motor neuron dysfunction remains uncertain. Furthermore, the specificity of the effects of SMN deficiency on motor the system has been questioned. The extra-motor phenotypic features in mouse models have prompted a closer assessment of the phenotype in human SMA. Distal extremity necrosis in mild mouse models lacking an overt phenotype of weakness and aged rescued severe mouse models have prompted the consideration of a vascular role of SMN.[21, 76, 92, 96] Additionally, cardiac defects, possibly related to autonomic involvement, are described and have been corrected with SMN targeting therapies in mouse models.[46, 85, 113] Other features of disordered autonomic function has been reported in aged rescued animals including priapism, bowel obstruction, and bladder distention.[96] In mice, where *Smn* is specifically reduced and there is no dependence on the human SMN promoter, extra-motor features are lacking[8]. This suggests that these features may be phenomena of the human SMN promoter in the mouse rather than a true reflection of disease state. Rarely features outside the motor system have been reported, typically in patients with more severe disease, and features of autonomic involvement are incompletely defined in patients with SMA and need additional investigation. It remains an important consideration that partial restoration of SMN levels in human trials could unmask other tissues that are susceptible to low levels of SMN.

Preclinical treatments are positioned to have dramatic effects in early clinical trials provided treatments sufficiently restore SMN at the correct time and in the required target tissues. SMA natural history data, albeit limited, suggest that motor function and electrophysiological measures such as CMAP and MUNE are preserved prior to symptoms onset, even in infants with severe disease (type 1)[109, 114]. It is expected that treatment prior to onset of clinical and electrophysiological features of motor dysfunction will be required for optimal effects. It will be ideal to design early trials for treatment either prior to overt symptoms or as early as possible after symptom onset. The majority of clinical and electrophysiological natural history are derived from patients at later time points in the course of the disease. Additional work is required to fully define the natural history of SMA at the onset of disease, particularly in mild cases, and the determination is required regarding how long after symptom onset SMN restoration will have significant effect. Therefore we do not have a clear picture of the events that occur at the start of the disease and the timing of these events, particularly in different severities of SMA. Natural history work is ongoing using motor function measures and molecular and electrophysiological biomarkers in early symptomatic infants with SMA to further define these outcome markers in patients (ClinicalTrials.org ID: NCT01736553). This work will provide the foundation for early

trials investigating SMN restoring therapies. Despite these hurdles the positive development of strong therapeutics with clear targets brings the hope that SMA can be treated or prevented if the therapeutic is provided at the correct time.

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References

1. Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995 Jan 13; 80(1):155–65. [PubMed: 7813012]
2. Pearn J. Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. J Med Genet. 1978 Dec; 15(6):409–13. [PubMed: 745211]
3. Prior TW, Snyder PJ, Rink BD, et al. Newborn and carrier screening for spinal muscular atrophy. Am J Med Genet A. 2010 Jul; 152A(7):1608–16. [PubMed: 20578137]
4. Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. Eur J Hum Genet. 2012 Jan; 20(1):27–32. [PubMed: 21811307]
5. Roberts DF, Chavez J, Court SD. The genetic component in child mortality. Archives of disease in childhood. 1970 Feb; 45(239):33–8. [PubMed: 4245389]
6. Burghes AH, Beattie CE. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nature reviews Neuroscience. 2009 Aug; 10(8):597–609.
7. Zerres K, Rudnik-Schoneborn S. Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications. Archives of neurology. 1995 May; 52(5):518–23. [PubMed: 7733848]
8. Bowerman M, Murray LM, Beauvais A, Pinheiro B, Kothary R. A critical smn threshold in mice dictates onset of an intermediate spinal muscular atrophy phenotype associated with a distinct neuromuscular junction pathology. Neuromuscular Disorders. 2012 Mar; 22(3):263–76. [PubMed: 22071333]
9. Piepers S, van den Berg LH, Brugman F, et al. A natural history study of late onset spinal muscular atrophy types 3b and 4. J Neurol. 2008 Sep; 255(9):1400–4. [PubMed: 18575920]
10. Monani UR, Lorson CL, Parsons DW, et al. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum Mol Genet. 1999 Jul; 8(7):1177–83. [PubMed: 10369862]
11. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc Natl Acad Sci U S A. 1999 May 25; 96(11):6307–11. [PubMed: 10339583]
12. Cartegni L, Krainer AR. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. Nat Genet. 2002 Apr; 30(4):377–84. [PubMed: 11925564]
13. Gennarelli M, Lucarelli M, Capon F, et al. Survival motor neuron gene transcript analysis in muscles from spinal muscular atrophy patients. Biochem Biophys Res Commun. 1995 Aug 4; 213(1):342–8. [PubMed: 7639755]
14. Lorson CL, Strasswimmer J, Yao JM, et al. SMN oligomerization defect correlates with spinal muscular atrophy severity. Nat Genet. 1998 May; 19(1):63–6. [PubMed: 9590291]

15. Lorson CL, Androphy EJ. An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. Hum Mol Genet. 2000 Jan 22; 9(2):259–65. [PubMed: 10607836]
16. Burnett BG, Munoz E, Tandon A, Kwon DY, Sumner CJ, Fischbeck KH. Regulation of SMN protein stability. Mol Cell Biol. 2009 Mar; 29(5):1107–15. [PubMed: 19103745]
17. McAndrew PE, Parsons DW, Simard LR, et al. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. Am J Hum Genet. 1997 Jun; 60(6):1411–22. [PubMed: 9199562]
18. Burghes AH. When is a deletion not a deletion? When it is converted. Am J Hum Genet. 1997 Jul; 61(1):9–15. [PubMed: 9245977]
19. Schrank B, Gotz R, Gunnersen JM, et al. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci U S A. 1997 Sep 2; 94(18):9920–5. [PubMed: 9275227]
20. Monani UR, Sendtner M, Coovert DD, et al. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(-/-) mice and results in a mouse with spinal muscular atrophy. Hum Mol Genet. 2000 Feb; Dec; 9(3):333–9. [PubMed: 10655541]
21. Hsieh-Li HM, Chang JG, Jong YJ, et al. A mouse model for spinal muscular atrophy. Nat Genet. 2000 Jan; 24(1):66–70. [PubMed: 10615130]
22. Prior TW, Krainer AR, Hua Y, et al. A positive modifier of spinal muscular atrophy in the SMN2 gene. Am J Hum Genet. 2009 Sep; 85(3):408–13. [PubMed: 19716110]
23. Vezain M, Saugier-Veber P, Goina E, et al. A rare SMN2 variant in a previously unrecognized composite splicing regulatory element induces exon 7 inclusion and reduces the clinical severity of spinal muscular atrophy. Hum Mutat. 2010 Jan; 31(1):E1110–25. [PubMed: 19953646]
24. Bernal S, Alias L, Barcelo MJ, et al. The c.859G>C variant in the SMN2 gene is associated with types II and III SMA and originates from a common ancestor. J Med Genet. 2010 Sep; 47(9):640–2. [PubMed: 20577007]
25. Burghes AH, Ingraham SE, Kote-Jarai Z, et al. Linkage mapping of the spinal muscular atrophy gene. Human genetics. 1994 Mar; 93(3):305–12. [PubMed: 8125483]
26. Cobben JM, van der Steege G, Grootscholten P, de Visser M, Scheffer H, Buys CH. Deletions of the survival motor neuron gene in unaffected siblings of patients with spinal muscular atrophy. Am J Hum Genet. 1995 Oct; 57(4):805–8. [PubMed: 7573039]
27. Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet. 1995 Oct; 4(10):1927–33. [PubMed: 8595417]
28. DiDonato CJ, Morgan K, Carpten JD, et al. Association between Ag1-CA alleles and severity of autosomal recessive proximal spinal muscular atrophy. Am J Hum Genet. 1994 Dec; 55(6):1218–29. [PubMed: 7977383]
29. Wirth B, el-Agwany A, Baasner A, et al. Mapping of the spinal muscular atrophy (SMA) gene to a 750-kb interval flanked by two new microsatellites. Eur J Hum Genet. 1995; 3(1):56–60. [PubMed: 7767657]
30. Oprea GE, Krober S, McWhorter ML, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science. 2008 Apr 25; 320(5875):524–7. [PubMed: 18440926]
31. Bernal S, Also-Rallo E, Martinez-Hernandez R, et al. Plastin 3 expression in discordant spinal muscular atrophy (SMA) siblings. Neuromuscular disorders : NMD. 2011 Jun; 21(6):413–9. [PubMed: 21546251]
32. Bebee TW, Gladman JT, Chandler DS. Splicing regulation of the survival motor neuron genes and implications for treatment of spinal muscular atrophy. Front Biosci. 2011; 15:1191–204.
33. Pellizzoni L. Chaperoning ribonucleoprotein biogenesis in health and disease. EMBO Rep. 2007 Apr; 8(4):340–5. [PubMed: 17401408]
34. Cifuentes-Diaz C, Frugier T, Tiziano FD, et al. Deletion of murine SMN exon 7 directed to skeletal muscle leads to severe muscular dystrophy. J Cell Biol. 2001 Mar 5; 152(5):1107–14. [PubMed: 11238465]
35. Vitte JM, Davoult B, Roblot N, et al. Deletion of murine Smn exon 7 directed to liver leads to severe defect of liver development associated with iron overload. Am J Pathol. 2004 Nov; 165(5):1731–41. [PubMed: 15509541]

36. Wan L, Battle DJ, Yong J, et al. The survival of motor neurons protein determines the capacity for snRNP assembly: biochemical deficiency in spinal muscular atrophy. Mol Cell Biol. 2005 Jul; 25(13):5543–51. [PubMed: 15964810]
37. Gabanella F, Butchbach ME, Saieva L, Carissimi C, Burghes AH, Pellizzoni L. Ribonucleoprotein Assembly Defects Correlate with Spinal Muscular Atrophy Severity and Preferentially Affect a Subset of Spliceosomal snRNPs. PLoS ONE. 2007; 2(9):e921. [PubMed: 17895963]
38. Workman E, Saieva L, Carrel TL, et al. A SMN missense mutation complements SMN2 restoring snRNPs and rescuing SMA mice. Hum Mol Genet. 2009 Jun 15; 18(12):2215–29. [PubMed: 19329542]
39. Lotti F, Imlach WL, Saieva L, et al. An SMN-dependent U12 splicing event essential for motor circuit function. Cell. 2012 Oct 12; 151(2):440–54. [PubMed: 23063131]
40. Baumer D, Lee S, Nicholson G, et al. Alternative splicing events are a late feature of pathology in a mouse model of spinal muscular atrophy. PLoS Genet. 2009 Dec.5(12):e1000773. [PubMed: 20019802]
41. Ruggiu M, McGovern VL, Lotti F, et al. A role for SMN exon 7 splicing in the selective vulnerability of motor neurons in spinal muscular atrophy. Mol Cell Biol. 2012 Jan; 32(1):126–38. [PubMed: 22037760]
42. Imlach WL, Beck ES, Choi BJ, Lotti F, Pellizzoni L, McCabe BD. SMN is required for sensory-motor circuit function in Drosophila. Cell. 2012 Oct 12; 151(2):427–39. [PubMed: 23063130]
43. Chan YB, Miguel-Aliaga I, Franks C, et al. Neuromuscular defects in a Drosophila survival motor neuron gene mutant. Hum Mol Genet. 2003 Jun 15; 12(12):1367–76. [PubMed: 12783845]
44. Mentis GZ, Blivis D, Liu W, et al. Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. Neuron. Feb 10; 69(3):453–67. [PubMed: 21315257]
45. Gavrilina TO, McGovern VL, Workman E, et al. Neuronal SMN expression corrects spinal muscular atrophy in severe SMA mice while muscle-specific SMN expression has no phenotypic effect. Hum Mol Genet. 2008 Apr; 1517(8):1063–75. [PubMed: 18178576]
46. Gogliotti RG, Quinlan KA, Barlow CB, Heier CR, Heckman CJ, Didonato CJ. Motor neuron rescue in spinal muscular atrophy mice demonstrates that sensory-motor defects are a consequence, not a cause, of motor neuron dysfunction. J Neurosci. 2012 Mar 14; 32(11):3818–29. [PubMed: 22423102]
47. Martinez TL, Kong L, Wang X, et al. Survival motor neuron protein in motor neurons determines synaptic integrity in spinal muscular atrophy. J Neurosci. 2012 Jun 20; 32(25):8703–15. [PubMed: 22723710]
48. Park GH, Maeno-Hikichi Y, Awano T, Landmesser LT, Monani UR. Reduced survival of motor neuron (SMN) protein in motor neuronal progenitors functions cell autonomously to cause spinal muscular atrophy in model mice expressing the human centromeric (SMN2) gene. J Neurosci. Sep 8; 30(36):12005–19. [PubMed: 20826664]
49. Bowerman M, Murray LM, Boyer JG, Anderson CL, Kothary R. Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy. BMC medicine. 2012; 10:24. [PubMed: 22397316]
50. Ebert AD, Yu J, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature. 2009 Jan 15; 457(7227):277–80. [PubMed: 19098894]
51. Rossoll W, Jablonka S, Andreassi C, et al. Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol. 2003 Nov 24; 163(4):801–12. [PubMed: 14623865]
52. McWhorter ML, Monani UR, Burghes AH, Beattie CE. Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding. J Cell Biol. 2003 Sep 1; 162(5):919–32. [PubMed: 12952942]
53. Rossoll W, Bassell GJ. Spinal muscular atrophy and a model for survival of motor neuron protein function in axonal ribonucleoprotein complexes. Results Probl Cell Differ. 2009; 48:289–326. [PubMed: 19343312]
54. di Penta A, Mercaldo V, Florenzano F, et al. Dendritic LSm1/CBP80-mRNPs mark the early steps of transport commitment and translational control. J Cell Biol. 2009 Feb 9; 184(3):423–35. [PubMed: 19188494]

55. Todd AG, Lin H, Ebert AD, Liu Y, Androphy EJ. COPI transport complexes bind to specific RNAs in neuronal cells. Hum Mol Genet. 2013 Feb 15; 22(4):729–36. [PubMed: 23175440]
56. Peter CJ, Evans M, Thayanithy V, et al. The COPI vesicle complex binds and moves with survival motor neuron within axons. Hum Mol Genet. 2011 May 1; 20(9):1701–11. [PubMed: 21300694]
57. Hubers L, Valderrama-Carvajal H, Laframboise J, Timbers J, Sanchez G, Cote J. HuD interacts with survival motor neuron protein and can rescue spinal muscular atrophy-like neuronal defects. Hum Mol Genet. 2011 Feb 1; 20(3):553–79. [PubMed: 21088113]
58. Fallini C, Zhang H, Su Y, et al. The survival of motor neuron (SMN) protein interacts with the mRNA-binding protein HuD and regulates localization of poly(A) mRNA in primary motor neuron axons. J Neurosci. 2011 Mar 9; 31(10):3914–25. [PubMed: 21389246]
59. Wang CH, Finkel RS, Bertini ES, et al. Consensus statement for standard of care in spinal muscular atrophy. J Child Neurol. 2007 Aug; 22(8):1027–49. [PubMed: 17761659]
60. Russman BS, Iannaccone ST, Samaha FJ. A phase 1 trial of riluzole in spinal muscular atrophy. Arch Neurol. 2003 Nov; 60(11):1601–3. [PubMed: 14623733]
61. Merlini L, Solari A, Vita G, et al. Role of gabapentin in spinal muscular atrophy: results of a multicenter, randomized Italian study. J Child Neurol. 2003 Aug; 18(8):537–41. [PubMed: 13677579]
62. Mercuri E, Bertini E, Messina S, et al. Randomized, double-blind, placebo-controlled trial of phenylbutyrate in spinal muscular atrophy. Neurology. 2007 Jan 2; 68(1):51–5. [PubMed: 17082463]
63. Liang WC, Yuo CY, Chang JG, et al. The effect of hydroxyurea in spinal muscular atrophy cells and patients. Journal of the neurological sciences. 2008 May 15; 268(1-2):87–94. [PubMed: 18166199]
64. Swoboda KJ, Scott CB, Reyna SP, et al. Phase II open label study of valproic acid in spinal muscular atrophy. PLoS One. 2009; 4(5):e5268. [PubMed: 19440247]
65. Swoboda KJ, Scott CB, Crawford TO, et al. SMA CARNI-VAL trial part I: double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in spinal muscular atrophy. PLoS One. 2010; 5(8):e12140. [PubMed: 20808854]
66. Kissel JT, Scott CB, Reyna SP, et al. SMA CARNIVAL TRIAL PART II: a prospective, single-armed trial of L-carnitine and valproic acid in ambulatory children with spinal muscular atrophy. PLoS One. 2011; 6(7):e21296. [PubMed: 21754985]
67. Kissel JT, Elsheikh B, King WM, et al. SMA VALIANT Trial: A prospective, double-blind, placebo controlled trial of valproic acid in ambulatory adults with spinal muscular atrophy. Muscle & nerve. 2013 May 16.
68. Angelozzi C, Borgo F, Tiziano FD, Martella A, Neri G, Brahe C. Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells. J Med Genet 2007. 2007 Oct 11; 45(1): 29–31. 2008 Jan.
69. Pane M, Staccioli S, Messina S, et al. Daily salbutamol in young patients with SMA type II. Neuromuscular disorders NMD. 2008 Jul; 18(7):536–40. [PubMed: 18579379]
70. Le TT, McGovern VL, Alwine IE, et al. Temporal requirement for high SMN expression in SMA mice. Human Molecular Genetics. 2011 Sep 15; 20(18):3578–91. 2011. [PubMed: 21672919]
71. Lutz CM, Kariya S, Patruni S, et al. Postsymptomatic restoration of SMN rescues the disease phenotype in a mouse model of severe spinal muscular atrophy. The Journal of Clinical Investigation. 2011; 121(8):3029–41. [PubMed: 21785219]
72. Corti S, Nizzardo M, Nardini M, et al. Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. The Journal of Clinical Investigation. 2008; 118(10):3316–30. [PubMed: 18769634]
73. Corti S, Nizzardo M, Nardini M, et al. Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice. Brain. 2010 Feb 1; 133(2):465–81. 2010. [PubMed: 20032086]
74. Corti S, Nizzardo M, Simone C, et al. Genetic correction of human induced pluripotent stem cells from patients with spinal muscular atrophy. Sci Transl Med. 2012 Dec 19.4(165):165ra2.
75. Le TT, McGovern VL, Alwine IE, et al. Temporal requirement for high SMN expression in SMA mice. Hum Mol Genet. 2011 Sep 15; 20(18):3578–91. [PubMed: 21672919]

76. Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotech. 2010; 28(3):271–4.
77. Azzouz M, Le T, Ralph GS, et al. Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. The Journal of Clinical Investigation. 2004; 114(12):1726–31. [PubMed: 15599397]
78. Valori CF, Ning K, Wyles M, et al. Systemic Delivery of scAAV9 Expressing SMN Prolongs Survival in a Model of Spinal Muscular Atrophy. Science Translational Medicine. 2010 Jun 9.2(35):35ra42. 2010.
79. Passini MA, Bu J, Roskelley EM, et al. CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. The Journal of Clinical Investigation. 2010; 120(4):1253–64. [PubMed: 20234094]
80. Dominguez E, Marais T, Chatauret N, et al. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice. Human Molecular Genetics. 2011 Feb 15; 20(4):681–93. 2011. [PubMed: 21118896]
81. Glascock JJ, Shababi M, Wetz MJ, Krogman MM, Lorson CL. Direct central nervous system delivery provides enhanced protection following vector mediated gene replacement in a severe model of spinal muscular atrophy. Biochem Biophys Res Commune. 2012 Jan 6; 417(1):376–81.
82. Benkhelifa-Ziyyat S, Besse A, Roda M, et al. Intramuscular scAAV9-SMN injection mediates widespread gene delivery to the spinal cord and decreases disease severity in SMA mice. Mol Ther. 2013 Feb; 21(2):282–90. [PubMed: 23295949]
83. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nature biotechnology. 2009 Jan; 27(1):59–65.
84. Duque S, Joussemet B, Riviere C, et al. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. Mol Ther. 2009 Jul; 17(7):1187–96. [PubMed: 19367261]
85. Bevan AK, Duque S, Foust KD, et al. Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. Mol Ther. 2011 Nov; 19(11):1971–80. [PubMed: 21811247]
86. Federici T, Taub JS, Baum GR, et al. Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs. Gene Ther. 2012 Aug; 19(8):852–9. [PubMed: 21918551]
87. Gray SJ, Nagabhushan Kalburgi S, McCown TJ, Jude Samulski R. Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates. Gene Ther. 2013 Apr; 20(4):450–9. [PubMed: 23303281]
88. Porensky PN, Burghes AH. Antisense oligonucleotides for the treatment of spinal muscular atrophy. Hum Gene Ther. 2013 May; 24(5):489–98. [PubMed: 23544870]
89. Porensky PN, Mitrpant C, McGovern VL, et al. A single administration of morpholino antisense oligomer rescues spinal muscular atrophy in the mouse. Human Molecular Genetics. 2011 Dec 20. 2011.
90. Zhou H, Janghra N, Mitrpant C, et al. A novel morpholino oligomer targeting ISS-N1 improves rescue of severe spinal muscular atrophy transgenic mice. Hum Gene Ther. 2013 Mar; 24(3):331–42. [PubMed: 23339722]
91. Mitrpant C, Porensky P, Zhou H, et al. Improved Antisense Oligonucleotide Design to Suppress Aberrant SMN2 Gene Transcript Processing: Towards a Treatment for Spinal Muscular Atrophy. PLoS One. 2013; 8(4):e62114. [PubMed: 23630626]
92. Hua Y, Sahashi K, Rigo F, et al. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature. 2011; 478(7367):123–6. [PubMed: 21979052]
93. Baughan TD, Dickson A, Osman EY, Lorson CL. Delivery of bifunctional RNAs that target an intronic repressor and increase SMN levels in an animal model of spinal muscular atrophy. Hum Mol Genet. 2009 May 1; 18(9):1600–11. [PubMed: 19228773]
94. Williams JH, Schray RC, Patterson CA, Ayitey SO, Tallent MK, Lutz GJ. Oligonucleotide-mediated survival of motor neuron protein expression in CNS improves phenotype in a mouse

model of spinal muscular atrophy. The Journal of neuroscience the official journal of the Society for Neuroscience. 2009 Jun 17; 29(24):7633–8. [PubMed: 19535574]

95. Passini MA, Bu J, Richards AM, et al. Antisense Oligonucleotides Delivered to the Mouse CNS Ameliorate Symptoms of Severe Spinal Muscular Atrophy. Science Translational Medicine. 2011 Mar 2.3(72):72ra18. 2011.
96. Porensky PN, Mitrpant C, McGovern VL, et al. A single administration of morpholino antisense oligomer rescues spinal muscular atrophy in mouse. Hum Mol Genet. 2012 Apr 1; 21(7):1625–38. [PubMed: 22186025]
97. Claudia Chiriboga KS, Basil Darras, Susan Iannaccone, Jacqueline Montes, Heather Allen, Rebecca Parad, Shanda Johnson, Darryl De Vivo, Daniel Norris, Katie Alexander, Frank Bennett, Kathie Bishop. Results of an Open-Label Escalating Dose Study To Assess the Safety Tolerability Does Range Finding of a Single Intrathecal Dose of ISIS-SMNRx in Patients with Spinal Muscular Atrophy. AAN Meeting Abstracts. 2013; 80(S36.002)
98. Butchbach MER, Singh J, Þorsteinsdóttir M, et al. Effects of 2,4-diaminoquinazoline derivatives on SMN expression and phenotype in a mouse model for spinal muscular atrophy. Human Molecular Genetics. 2010 Feb 1; 19(3):454–67. 2010. [PubMed: 19897588]
99. Gogliotti RG, Cardona H, Singh J, et al. The DcpS inhibitor RG3039 improves survival, function and motor unit pathologies in two SMA mouse models. Human Molecular Genetics. 2013 Jun 4. 2013.
100. Van Meerbeke JP, Gibbs RM, Plasterer HL, et al. The DcpS inhibitor RG3039 improves motor function in SMA mice. Human Molecular Genetics. 2013 May 31. 2013.
101. Montes J, Gordon AM, Pandya S, De Vivo DC, Kaufmann P. Clinical Outcome Measures in Spinal Muscular Atrophy. Journal of child neurology. 2009; 24(8):968–78. [PubMed: 19509409]
102. Cano SJ, Mayhew A, Glanzman AM, et al. Rasch analysis of clinical outcome measures in spinal muscular atrophy. Muscle & nerve. 2013:n/a–n/a.
103. Crawford TO, Paushkin SV, Kobayashi DT, et al. Evaluation of SMN protein, transcript, and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. PLoS One. 2012; 7(4):e33572. [PubMed: 22558076]
104. Finkel RS, Crawford TO, Swoboda KJ, et al. Candidate proteins, metabolites and transcripts in the Biomarkers for Spinal Muscular Atrophy (BforSMA) clinical study. PLoS One. 2012; 7(4):e35462. [PubMed: 22558154]
105. Sumner CJ, Kolb SJ, Harmison GG, et al. SMN mRNA and protein levels in peripheral blood: biomarkers for SMA clinical trials. Neurology. 2006 Apr 11; 66(7):1067–73. [PubMed: 16481599]
106. Kong L, Wang X, Choe DW, et al. Impaired Synaptic Vesicle Release and Immaturity of Neuromuscular Junctions in Spinal Muscular Atrophy Mice. The Journal of Neuroscience. 2009 Jan 21; 29(3):842–51. 2009. [PubMed: 19158308]
107. Ling KKY, Lin MY, Zingg B, Feng Z, Ko CP. Synaptic Defects in the Spinal and Neuromuscular Circuitry in a Mouse Model of Spinal Muscular Atrophy. PLoS One. 2010; 5(11):e15457. [PubMed: 21085654]
108. Wadman RI, Vrancken AF, van den Berg LH, van der Pol WL. Dysfunction of the neuromuscular junction in spinal muscular atrophy types 2 and 3. Neurology. 2012; 79(20):2050–5. 2012 Nov 13. [PubMed: 23115209]
109. Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: Relation to age, SMN2 copy number, and function. Annals of Neurology. 2005; 57(5):704–12. [PubMed: 15852397]
110. Lewelt A, Krosschell KJ, Scott C, et al. Compound muscle action potential and motor function in children with spinal muscular atrophy. Muscle & nerve. 2010; 42(5):703–8. [PubMed: 20737553]
111. Finkel RS. Electrophysiological and motor function scale association in a pre-symptomatic infant with spinal muscular atrophy type I. Neuromuscular Disorders. 2013; 23(2):112–5. [PubMed: 23146148]
112. Rutkove SB, Gregas MC, Darras BT. Electrical impedance myography in spinal muscular atrophy: a longitudinal study. Muscle & nerve. 2012 May; 45(5):642–7. [PubMed: 22499089]

113. Shababi M, Habibi J, Ma L, Glascock JJ, Sowers JR, Lorson CL. Partial restoration of cardiovascular defects in a rescued severe model of spinal muscular atrophy. J Mol Cell Cardiol. 2012 May; 52(5):1074–82. [PubMed: 22285962]

114. Finkel RS. Electrophysiological and motor function scale association in a pre-symptomatic infant with spinal muscular atrophy type I. Neuromuscular disorders NMD. 2013 Feb; 23(2):112–5. [PubMed: 23146148]

115. Benkhelifa-Ziyyat S, Besse A, Roda M, et al. Intramuscular scAAV9-SMN Injection Mediates Widespread Gene Delivery to the Spinal Cord and Decreases Disease Severity in SMA Mice. Mol Ther. 2013; 21(2):282–90. [PubMed: 23295949]

116. Coady TH, Lorson CL. Trans-splicing-mediated improvement in a severe mouse model of spinal muscular atrophy. The Journal of neuroscience : the official. journal of the Society for Neuroscience. 2010 Jan 6; 30(1):126–30.

117. Shababi M, Glascock J, Lorson CL. Combination of SMN trans-splicing and a neurotrophic factor increases the life span and body mass in a severe model of spinal muscular atrophy. Hum Gene Ther. 2011 Feb; 22(2):135–44. [PubMed: 20804424]

118. Riessland M, Ackermann B, Forster A, et al. SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy. Hum Mol Genet. 2010 Apr 15; 19(8):1492–506. [PubMed: 20097677]

119. Avila AM, Burnett BG, Taye AA, et al. Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. J Clin Invest. 2007 Mar; 117(3):659–71. [PubMed: 17318264]

120. Narver HL, Kong L, Burnett BG, et al. Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. Ann Neurol. 2008 Oct; 64(4):465–70. [PubMed: 18661558]

121. Farooq F, Abadia-Molina F, Mackenzie D, et al. Celecoxib increases SMN and survival in a severe spinal muscular atrophy mouse model via p38 pathway activation. Hum Mol Genet. 2013 May 10.

122. Kwon DY, Motley WW, Fischbeck KH, Burnett BG. Increasing expression and decreasing degradation of SMN ameliorate the spinal muscular atrophy phenotype in mice. Hum Mol Genet. 2011 Sep 15; 20(18):3667–77. [PubMed: 21693563]

123. Mattis VB, Ebert AD, Fosso MY, Chang CW, Lorson CL. Delivery of a read-through inducing compound, TC007, lessens the severity of a spinal muscular atrophy animal model. Human Molecular Genetics. 2009 Oct 15; 18(20):3906–13. 2009. [PubMed: 19625298]

124. Bowerman M, Beauvais A, Anderson CL, Kothary R. Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. Hum Mol Genet. 2010 Apr 15; 19(8):1468–78. [PubMed: 20097679]

125. Cherry JJ, Osman EY, Evans MC, et al. Enhancement of SMN protein levels in a mouse model of spinal muscular atrophy using novel drug-like compounds. EMBO molecular medicine. 2013 Jul; 5(7):1103–18.

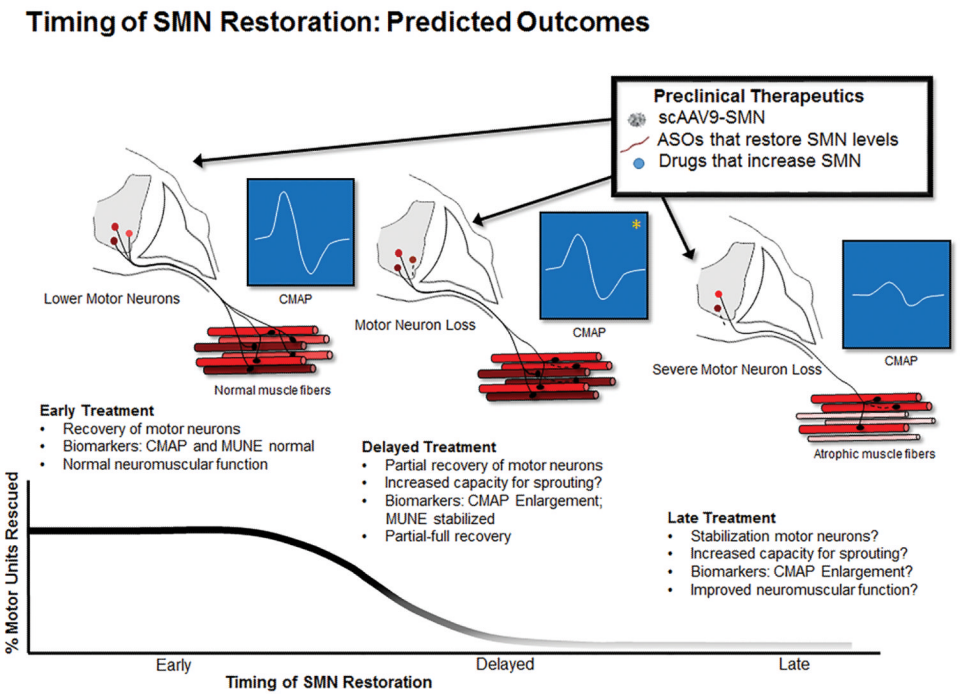

Figure 1.

Timing of SMN Restoration and Predicted Outcomes: SMA is caused by reduced levels of SMN protein. Therapies that provide early restoration of SMN are anticipated to fully rescue motor neurons and the motor unit. When SMN restoration is delayed it is anticipated that rescue will be reduced in a time-dependent fashion. CMAP: compound muscle action potential; MUNE: motor unit number estimation. *Following delayed treatment, CMAP size may be fully corrected if there is sufficient collateral reinnervation from the remaining motor neurons.

Table 1

Preclinical Gene Therapy, ASO, and Stem Cell Strategies that Extend Survival in SMA Mouse Models

Strategy	Dose and Delivery	Mouse	Survival Ratio (days) (Treated/Untreated)	Authors
ASO to alter *SMN2* splicing				
ASO(-10–27)	8 μg ICV on P1	005025	1.6 (25/16)	Passini et al. 2011
ASO(-10−27)	20 μg ICV on P2 2× 50 μg/g SC P1.4 20μg ICV +2× 50μg/g SC 2 × 160 μg/g SC P1+P4	005058	1.7 (17/10) 10.8 (108/10) 17.3 (173/10) 24.8 (248/10)	Hua et al. 2011
PMO(-10-29)	81μg ICV on P1	005025	7.5 (112/15)	Porensky et al. 2012
PMO25(-10-34)	40μg/g ICV on P1	005058	8.5 (85.5/9.5)	Zhou et al. 2013
PMO(−10−34)	6 mM ICV on P1	005025	8.4 (126/15)	Mitrpant et al. 2013
Gene therapy to restore SMN				
scAAV9-*SMN*	5×10^{11} vg IV on P1	005025	16 (>250/15.5)	Foust et al. 2010
	1×10^{11} vg IV on P1	005025	5 (69.1/13.9)	Valori et al. 2010
	4.5×10^{10} vg IV on P1	005025	14.5 (199/13.7)	Dominguez et al. 2011
	2×10^{10} vg IV on P2 2×10^{10} vg ICV on P2+3	005025	2.5 (~33/~13) 9.6(~125/~13)	Glascock et al. 2012
	2×10^{11} vg on P2	005024	2.4 (17/7)	Glascock et al. 2012
	7×10^{10} vg IM on P1 (with IV spread)	005025	13.6 (163/12)	Benkhelifa-Ziyyat et al. 2013
scAAV8-*SMN*	1.7×10^{10} vg ICV + Lumbar cord P1	005025	10.5 (157/15)	Passini et al. 2010
SMN Trans-Splicing Vector	1.14×10^{12} plasmid copies ICV on P1	005024	Alone 1.8 (7/4)	Coady et al 2010
SMN Trans-splicing/IGF-1 Dual Vector	10 μg plasmid DNA ICV on P2	005024	1.5 (6/4) Trans-Splicing 1.8 (7/4) IGF-1 alone 2 (8/4) Combined	Shababi et al. 2011
Stem Cell				
Neural Stem Cells (NSCs)	ICV on P1	005025	1.4 (18.2/13)	Corti et al. 2008
Embryonic stem cell-derived NSCs	ICV on P1	005025	1.6 (21.0/12.8)	Corti et al. 2010
Genetically corrected-Induced pluripotent stem Cell-derived MNs	MNs on P1 into cervical and lumbar spinal cord	005025	1.4 (19/14) SMA skinfibroblasts 1.5(21/14) Non-SMAskin fibroblasts 1.5 (21/14) Corrected SMA skin fibroblasts	Corti et al. 2012

Mouse models (Jackson Lab Catalog number if available): 005024 (*SMN2;Smn*-/-); 005025 (*SMN2;Smn*-/-); 005058 ((*SMN2)2Hung Smn1*^{tm1Hung}/J). The 5024 and 5025 mouse lines contain targeted deletion in the mouse Smn gene and are null for mouse SMN. Both lines also contain the human *SMN2* transgene derived from line 89 which contains a single copy of SMN2 thus when homozygous as in these lines the mice contain two copies of *SMN2*. The 5025 line also contains two copies of a second transgene, *SMNΔ7*. The *SMN2* in these two lines is expressed in all tissues tested to date. The line 5058

was developed by Hsieh-Le et al and contains a mouse Smn allele that disrupts exon7, and therefore has the potential to produce a truncated mouse Smn lacking exon7. The 5058 line also has the human SMN2 transgene from line 2 and has two copies human *SMN2* transgene per chromosome, thus, four copies in the homozygous state. In the homozygous state line 5058 has a normal life span but shows necrosis of limbs. In general 5058 is used with the *SMN2* gene in a heterozygote state so that there is 2 copies of SMN2 and the deletion in a homozygous state for a SMA mouse. The mouse line *Smn 2B*/- has disruption of the mouse Smn gene. The advantage of this line is that it does not show necrosis indicating that the necrosis apparent in the aforementioned models likely result from of the uneven expression of SMN due to the human promoter in a mouse background. This line also seems very sensitive to treatments when compared to 5025. The disadvantage of *Smn 2B*/- is that it does not contain the human SMN2 gene which is a desired therapeutic target in humans. The predictive power of these mouse lines is not currently known as there have been no successful treatments in humans. ASO: antisense oligonucleotide; PMO: phosphorodiamidate morpholino oligonucleotides; vg: viral genomes; ICV: intracerebroventricular; P: postnatal day with first day of life starting at P1; MN: motor neurons
*Days

Table 2

Preclinical small molecule drugs that can successfully extend survival in mouse models of SMA

Strategy	Delivery/Timing	Mouse	Survival (days) (treated/untreated)	Authors
DcpS Inhibitor				
RG3039	IP on P4	*Smn 2B/-*	6 (112/18)	Gogliotti et al. 2013
		005058	1.4 (10/13.8)	
RG3039	IP on P1	005025	1.3 (23/18)	Van Meerbeke et al 2013
		$ChAT^{Cre}Smn^{Res}$	1.7 (41.5/25)	
2,4-diaminoquinazoline	Oral on P4	005025	1.3 (17/14)	Butchbach et al. 2010
Histone Deacetylase Inhibitor				
Suberoylanilide Hydroxamic Acid	Oral on E15	005024	Rescue of embryonic lethality	Riessland et al. 2010
	Oral on P1	005058	1.3 (12.9/9.9)	
Trichostatin A	IP on P5	005025	1.2 (19/16)	Avila et al. 2007
Trichostatin A + Nutrition	IP on P1 + nutrition P8	005025	1.7 (38/14)	Narver et al. 2008
P38 and HuR Protein Activator				
Celecoxib	IP on P1-P6	005025	1.4 (18/13)	Farooq et al. 2013
Proteasome Inhibitor				
Bortezomib	IP on P5	005025	No effect alone 1.4 (20/14) with TSA	Kwon et al. 2011
Read-through Inducing Compound				
TC007	ICV P3,5,7	005025	1.3(16/12.6)	Mattis et al. 2009
Rho-kinase Inhibitor				
(Y-27632)	Oral on P3	*Smn 2B/-*	∼14-33 wks/∼4 wks	Bowerman et al. 2010
	E14 +P3	005024	No effect	
Fasudil	Oral P3	*Smn 2B/-*	9.8 (300/30.5)	Bowerman et al. 2012
STAT5 Activator				
Prolactin	IP on P1	005025	1.6 (21/14)	Farooq et al. 2011
Mechanism Undefined				
PTC compounds	IP on P3	005025	11 (150/14)	Naryshkin et al. 2012
LDN-76070	IP on P2	005025	1.4 (17/11.5)	Cherry et al. 2013

Mouse models (Jackson Lab Catalog number, if available): 005024 (*SMN2;Smn-/-*); 005025 (*SMN2;Smn-/-*; SMNΔ7+/+); 005058 ((*SMN2)2Hung Smn1*^{tm1Hung}/J); Smn 2B- (Bowerman et al. 2012). $ChAT^{Cre}Smn^{Res}$:5025 line with SMN restored in motor neurons but not other cell types See table 1 legend for mouse model genetics and characteristics. IP: intraperitoneal; P: postnatal day with first day of life starting at P1; E: gestational day; TSA: trichostatin A