Published in final edited form as:

Future Neurol. 2018 August ; 13(3): 161–172. doi:10.2217/fnl-2018-0008.

Motor neuron biology and disease: A current perspective on infantile-onset spinal muscular atrophy

Narendra N. Jha, PhD^{1,3}, Jeong-Ki Kim, PhD^{1,3}, Umrao R. Monani, PhD^{1,2,3,*}

¹Department of Pathology & Cell Biology, 630 W. 168th St., Columbia University Medical Center, New York, NY 10032

²Department of Neurology, 630 W. 168th St., Columbia University Medical Center, New York, NY 10032

³Center for Motor Neuron Biology & Disease, 630 W. 168th St., Columbia University Medical Center, New York, NY 10032

Abstract

Infantile-onset spinal muscular atrophy (SMA) is a prototypical disease in which to investigate selective neurodegenerative phenotypes. Caused by low levels of the ubiquitously expressed Survival Motor Neuron (SMN) protein, the disease mainly targets the spinal motor neurons. This selective phenotype remains largely unexplained, but has not hindered the development of SMN repletion as a means to a treatment. Here we chronicle recent advances in the area of SMA biology. We provide a brief background to the disease, highlight major advances that have shaped our current understanding of SMA, trace efforts to treat the condition, discuss the outcome of two promising new therapies and conclude by considering contemporary as well as new challenges stemming from recent successes within the field.

Keywords

Spinal muscular atrophy; survival motor neuron; therapies; motor neuron; neurodegeneration

Clinical phenotype and genetic principles of SMA

Proximal spinal muscular atrophy (SMA) is an infantile-onset and frequently fatal autosomal recessive neuromuscular disorder. While formally considered a rare disease, SMA is nevertheless the most common inherited cause of infant mortality. Estimates of carriers within the human population range from 1 in every 37 to 125 individuals; Caucasians are reportedly three times more likely to carry a disease allele than are individuals of Hispanic descent [1, 2]. This carrier frequency translates into a disease incidence of approximately one affected infant among every 5500 – 62,500 newborns. SMA severity varies quite considerably. Patients are typically classified as severe (type I), intermediate (type II) or

*Corresponding author: Umrao R. Monani, Mailing address: Umrao R. Monani, P&S, Room 5-422, 630 W. 168th St., New York, NY 10032, Tel: (212)-342-5132, Fax: (212)-305-5438, um2105@cumc.columbia.edu.

Conflict of interest: The authors have declared that no conflict of interest exists

mildly affected (type III) [3]. This classification (Table 1) is based on age at onset and severity of the disease. Severely affected patients, who constitute the majority (60%) of cases, manifest with weakness in the first 6 months of life and fail to ever walk independently. Those with the intermediate form of the disease account for ~27% of all patients, become symptomatic between 6 and 18 months of age, acquire the ability to sit independently, but never become ambulant. Mildly affected patients (~12% of all cases) begin exhibiting a disease phenotype after 18 months of age and can frequently walk unassisted. However, weakness in later life often results in the need for a walking aid or wheelchair. In addition to these three SMA types, patients sometimes exhibit a profoundly severe (type 0) or extremely mild (type IV) form of the disease [4, 5]. However, these patients constitute less than 1% of affected individuals.

The classical SMA phenotype is characterized by weakness of the proximal rather than distal muscles. Moreover, the disease affects the lower limbs more than it does the upper ones, the intercostals more than the diaphragm and axial muscles rather than appendicular muscle groups. This weakness stems from profound atrophy of affected muscles which in turn is commonly attributed to early, postnatal loss of the spinal motor neurons (Fig. 1) and consequent denervation. Medial motor neurons are particularly vulnerable to death or dysfunction. Precisely what determines the vulnerability of certain motor pools and thus specific muscle groups to disease remains an enduring mystery, although recent studies are beginning to cast light on this question [6, 7]. Reports of defects in other cells of the central nervous system (CNS) and in peripheral organs have gradually emerged and gained attention [8–14]. However, there is general agreement that the neuromuscular system bears the brunt of the disease.

The genetic cause of SMA was identified more or less a century after the disease was first described by Werdnig and Hoffman [15–18], and found to arise from homozygous mutations in the Survival of Motor Neuron 1 (*SMN1*) gene [19]. This discovery, which was made by scientists in France, also led to the identification of an *SMN1* paralog, *SMN2*, which is invariably present in SMA patients. *SMN2* is only present in humans and is thought to have arisen from an evolutionarily recent duplication on chromosome 5q [20]. However, it is rendered less than optimally functional by a C→T transition 6bp inside exon 7 of the gene [21, 22]. The transition alters the efficiency with which the exon is included in *SMN* transcripts, most likely by the creation of an exonic splicing silencer (ESS) in the *SMN2* gene [23]. Accordingly, *SMN2* produces mostly transcripts lacking exon 7. Although these *SMN* 7 transcripts are no less stable than their full-length (FL) counterparts, they are translated into a protein that is practically non-functional and rapidly degraded [24]. *SMN2* therefore produces relatively little (~10%) functional *SMN* protein compared to *SMN1* and is unable, on its own, to prevent disease (Fig. 2). Notwithstanding its inability, in most instances, to compensate for *SMN1* loss, multiple *SMN2* copies – arising from frequent rearrangements/duplications in the 5q SMA locus – are generally associated with higher levels of the *SMN* protein [25, 26]. Accordingly, greater *SMN2* copies in patients commonly result in milder phenotypes; fewer copies of the gene correlate with more severe forms of SMA [27, 28]. Still, recent reports suggest that not all *SMN2* copies are functionally equivalent. Indeed, subtle mutations within the gene can dramatically affect its ability to express the intact transcript and thus functional protein [29, 30]. These collective findings

largely influenced the initial design of therapies for SMA, targeting *SMN2* expression as a means to a treatment – also see subsequent sections.

The biology of the SMN protein

The 294aa SMN protein is ubiquitously expressed and resides in both the nucleus as well as cytoplasm [31]. In the nucleus, it partitions to bright, sub-nuclear foci that frequently co-localize with Cajal bodies [31, 32] and were therefore christened Gems (Gemini of Cajal bodies). In the period since these observations were first made, SMN has been assigned a plethora of functions. Perhaps the most widely-accepted is its dual role in orchestrating the biogenesis of spliceosomal small nuclear ribonucleoprotein (snRNP) particles and in pre-mRNA splicing [33–35]. SMN does not perform these functions alone, but rather as a multimeric complex that includes seven Gemin proteins (Gemin 2–8) and a factor termed UNRIP [36, 37]. The complex ensures the non-promiscuous assembly of a heptameric ring of Sm proteins (B/B', D1, D2, D3, E, F and G) onto one of nine (U1, U2, U4, U5, U6, U11, U12, U4atac and U6atac) uridine-rich small nuclear RNAs (snRNAs) in the cytoplasm [38]. The assembled snRNPs are then imported into the nucleus [39, 40] where they mediate the removal of introns from nascent pre-mRNAs. Considering how fundamental these processes are to cellular function, it is hardly surprising that ablation of SMN or many of the Gemin is incompatible with life [41–46]. Nor is the diminished capacity of the cell to assemble spliceosomal snRNPs following SMN knockdown unexpected [47, 48]. In contrast, explaining the decidedly neuromuscular SMA phenotype based on disruptions of the canonical splicing function of the SMN protein has proved challenging. This is despite the identification of splicing defects in genes such as *Cacna1b*, *Agrn* and *Tmem41b* deemed important to neuromuscular function [49–51] which, in any case, have modest effects when corrected, and frequently cited correlations between snRNP assembly activity and SMA severity [52, 53]; such correlations cease to exist in more severe mouse models of SMA, notwithstanding unequivocal phenotypic differences between them [53]. Proponents of the canonical function of SMN in SMA pathogenesis have furthermore argued that it is the intrinsic inability of spinal motor neurons to efficiently splice and therefore express SMN from *SMN2* that underlies the selective vulnerability of these cells in the disease [54]. Such inability is thought to be especially profound in SMA motor neurons owing to reduced baseline levels of functional SMN protein in these cells. However, this assertion has been contested in a study by Jodelka *et al* in which the authors showed that such inefficient splicing is not a selective characteristic of spinal motor neurons, but rather a common feature of all cells within the spinal cord [55]. Moreover, if spinal motor neurons are truly unable to efficiently express SMN protein from *SMN2*, it is unclear why certain pools such as those controlling the extraocular muscles are relatively spared [56]. This question could be extended to cortical motor neurons which are also largely intact in classical SMA [57]. Accordingly, it is not unreasonable to conclude that other SMN functions contribute to the health and survival of motor neurons.

While one or more major mis-spliced transcripts linking the canonical, housekeeping function of SMN to the neuromuscular SMA phenotype may yet emerge, the protein has in the interim been assigned a number of non-canonical functions, including roles in transcription, translation and ubiquitin and mitochondrial homeostasis [58–61]. Many of

these functions have been inferred from the SMN interactome in motor neurons or neuronal cell lines, and continue to be investigated in these cells based on the selective vulnerability of α -motor neurons to low protein. Further rationale for these types of investigations derived from observations of particularly early morphological defects in SMN-depleted axons [62–64] and the neuromuscular junctions (NMJs) of SMA model mice [65–67]. Among the most widely reported functions stemming from these studies are those that involve the chaperone activity of the SMN protein in motor axons and the NMJ. In this regard, SMN was found to constitute a number of Gemin2/3-containing ribonucleoproteins (RNPs) that are trafficked along axons and into nerve terminals, implying functions in axon outgrowth and synaptogenesis. Interestingly, these complexes do not contain the spliceosomal Sm proteins [68]. Arguably, the most fascinating interactions that have emerged from these studies are those between SMN and the hnRNP-R, HuD, KSRP and ZBP-1 proteins [69–72]. These RNA-binding proteins traffic a number of neuronal transcripts down axons and into growth cones. HuD, in particular, binds RNAs for GAP43, tau and acetylcholine esterase [72]. Proteins corresponding to these transcripts have been shown to be important for axon generation and maintenance [73–76]. In SMN-depleted motor neurons, HuD levels are greatly reduced [77]. Moreover, HuD knockdown causes axonal defects in zebrafish that phenocopy effects seen under low SMN concentrations [77]. Over-expressing HuD can rescue axonal defects in SMA fish. These findings, in addition to reports of defects in local axonal translation [76] in SMN-depleted cells, make a compelling case for a non-canonical but disease-relevant function for the protein in motor neurons.

Prompted by the continued interest in identifying novel, phenotypically relevant SMN functions, recent reports have focused on factors that have very specific effects – at the NMJ – on neurotransmission. These studies arose from observations of discordant siblings (sibs) – patients with identical SMA haplotypes but distinct disease phenotypes [78]. Exploiting such families as a means of studying SMA biology resulted in the identification of a number of proteins (Plastin3 – PLS3, Coronin1 C – CORO1C, Neurocalcin Delta – NCALD) that are either differentially expressed in the affected sibs or interact in some manner with the differentially expressed proteins [79–81]. In every instance, the proteins that were identified were found to regulate endocytosis following neurotransmitter release at the NMJ. In SMA, neurotransmission is compromised, perhaps due to defective synaptic endocytosis [82, 83]. Modulating the expression of the proteins identified in the discordant sibs is thought to rescue this defect. Importantly, the physiological relevance of the modifier proteins was validated in mouse models, albeit mutants fortified with SMN-enhancing reagents [80, 81]. Still, precisely what links SMN to the modifiers remains to be determined. One plausible connection is the actin cytoskeleton. Reports of perturbed actin dynamics in SMN-depleted cells support this contention [84, 85] as do studies purporting to correct cytoskeletal defects stemming from low SMN [86, 87]. The putative functions of SMN at the NMJ are intuitively appealing and continue to be subject to intense investigation. Indeed, one approach that involved the examination of differentially vulnerable motor neuron pools in SMA has already uncovered several factors, including α -synuclein, that support the role of the SMN protein in motor axons and nerve terminals [6]. Follow-up studies to investigate these factors and the manner in which they relate to SMN are bound to add to our understanding of SMA.

Given the especially detrimental effect of low SMN on spinal motor neurons, it is perhaps not surprising that the search for non-canonical functions of the protein has involved neuronal cells/tissue. Yet it is clear, mainly from studies in model organisms, that low protein in non-CNS tissue is also responsible for disease [88, 89 and references therein]. Cardiac, hepatic, lymphatic, bone and reproductive tissue have all been reported, at least in model mice, to be affected to some extent by low SMN. However, the effects of the protein on skeletal muscle may be the most relevant in the context of the neuromuscular SMA phenotype. There continues to be lack of consensus about the need for wild-type SMN levels in this tissue [90–92]. Although such lack of agreement is perplexing, the current data might merely mean that the role of muscle in SMA is nuanced and potential disease phenotypes arising from low protein in this tissue more subtle than expected. Investigating potentially novel and disease-relevant SMN functions in muscle is therefore more relevant than ever.

Therapies for SMA

Just a few years ago, the only available treatments for SMA were palliative at best. Today, there is a burgeoning pipeline of SMA drugs that have either shown promise in pre-clinical models or in human patients (Table 2). The rapid advance in the development of these therapies is owed to three main factors. First, SMA is a monogenic and autosomal recessive disorder. The underlying cause – SMN paucity – of the disease was identified more than two decades ago and quickly recognized as a loss in function. Restoring the protein therefore became an intuitively appealing approach and was rapidly adopted as a means to a treatment. Equally serendipitous was the existence of the *SMN2* gene and its invariable presence in patients affected with the disease. A clear understanding of the mechanisms that disrupt *SMN2* splicing and thus function while preserving the integrity of the *SMN1* gene provided insight into how the paralog might be exploited as a therapeutic target. A final reason underlying the therapeutic successes witnessed so far stems from the early development of humanized model mice [93, 94] that provided the first direct evidence of the feasibility of targeting *SMN2* to treat SMA and then served as invaluable pre-clinical tools with which to test and optimize therapeutic strategies. Paradoxically, the SMN-dependent therapeutic approaches have evolved without the need to fully understand *how* paucity of protein causes SMA. Still, the developments in SMA treatments over the last two years are to be celebrated.

As summarized in Table 2, the therapeutic approaches to treating SMA are broadly divided into two categories – those that seek to rescue the disease phenotype by directly restoring SMN and those that propose to do so without altering levels of the protein. Both approaches merit consideration here. Not surprisingly, it is the SMN-repletion approaches that have witnessed the most rapid development. Two such strategies are worth detailing here. The first, exemplified by the antisense drug, Spinraza, rectifies the aberrant splicing of the *SMN2* gene. The drug, a splice-switching oligonucleotide, blocks a critical intronic element, ISS-N1, immediately downstream of exon 7 that normally binds the splicing repressor, hnRNPA1 and serves to exclude the exon from the mature transcript [95]. Accordingly, following treatment, *SMN2* begins expressing greater levels of the FL-SMN transcript and thus greater levels of functional SMN protein. Based on initial data in mice [96], followed by promising results in a cohort of SMA patients [97, 98], Spinraza was approved in December, 2016 by the FDA for use as a treatment for the disorder [99]. Small molecules

that similarly attempt to modulate *SMN2* splicing are also in the pipeline as treatments for SMA. It is expected that those in late-stage clinical trials will be granted regulatory approval in the U.S. before the end of the decade and eventually become universally available.

A complementary approach to restoring the SMN protein involved viral vector technology and the discovery that certain adeno-associated virus serotypes such as AAV9 are not only potent transducers of a wide variety of cells in the intact organism, but also traverse the blood-brain barrier and efficiently deliver their payload to CNS cells [100, 101]. Accordingly, a self-complementary AAV9 vector bearing the SMN transcript (scAAV9-SMN) was initially used to demonstrate clinical efficacy in SMA model mice and then found to be equally efficacious in a larger (pig) model of the disease [102, 103]. These studies served as the springboard to testing the safety and then efficacy of scAAV9-SMN in human patients. The clinical trials, led by Avexis, Inc. have demonstrated remarkable promise, albeit in a somewhat limited number of 15 patients treated with the therapeutic vector [104]. Indeed, whereas the vast majority (~92%) of the patients in a natural history cohort had succumbed to disease by 20 months of age, none of the 15 patients administered the therapeutic vector had either died or were in need of permanent-assisted ventilation. Accordingly, it is expected that gene replacement as a therapy for SMA will receive regulatory approval by the end of the year.

Chief among the myriad SMA therapeutics currently being tested without claiming to raise SMN levels are Olesoxime and CK-2127107. Olesoxime, a neuroprotective agent thought to counter mitochondrial stress [105] is reported to provide modest benefit to typeII/III patients between the ages of 6 and 15, and is currently in phase 2 clinical trials [106]. CK-2127107, a proprietary small molecule being developed by Cytokinetics Inc., is a fast skeletal muscle troponin activator and is intended to improve muscle function in patients with SMA by sensitizing myofibers to Ca^{2+} release. It is reported to amplify skeletal muscle response to nerve activation [106] and was tested [108] but deemed not to alter disease in patients with another common motor neuron disorder, amyotrophic lateral sclerosis (ALS). Together with Olesoxime, CK-2127107 adds to the arsenal of drugs being developed to treat SMA, although neither is expected to have more than a modest therapeutic effect.

Notwithstanding the important therapeutic milestones exemplified by the approval of Spinraza and future promise of gene replacement for SMA, it is abundantly clear that an outright cure for the disease is yet to be realized. The currently available therapies are undeniably salutary in effect but remain mere treatments. Moreover, the outcomes of the SMN-repletion strategies have focused attention on key issues of concern. Of greatest import is the need for timely intervention. Early treatment with the splice-switching oligonucleotide or scAAV9-SMN clearly resulted in the most pronounced effect. This was predicted in pre-clinical studies [109] and emphasizes the need to institute routine newborn screens for SMA. Of equal importance is to ensure systemic SMN repletion. The somewhat superior outcome in the gene replacement trial compared to the results of the treatment with Spinraza could well be explained by the manner in which the therapeutics were delivered [97]. Spinraza is routinely delivered intrathecally, restricting SMN repletion to CNS tissue; scAAV9-SMN was introduced intravenously and thus expected to transduce *all* tissues. If peripheral organs contribute to the overall SMA phenotype, ensuring that they are also treated will be of

paramount importance. In this regard, orally available drugs capable of crossing the blood-brain barrier will constitute a significant advance for the treatment of SMA. Besides effecting repletion systemically, such drugs will also preclude the invasive manner in which scAAV9-SMN and Spinraza are administered. A third concern has to do with how patients will respond in the long term to Spinraza or gene replacement. Pre-clinical studies have indicated that while an early intervention endows the SMA organism with the greatest benefit, waning protein levels following an acute period of treatment will not be without consequences [110]. Both muscle and motor nerves require a sustained minimum amount of protein to prevent the onset of pathology. Conversely, chronic exposure to small molecules or oligonucleotide enhancers of SMN protein, are bound to trigger toxicity and/or precipitate off-target effects. A fourth consideration stemming from the temporal requirements for the SMN protein concerns older patients who have lived with the disease for a protracted period of time and have therefore, in all likelihood, already lost motor units. Already it is clear that such patients will derive only modest benefit from SMN repletion [98, 111]. This emphasizes the need for a continued investment in basic studies that might spawn alternative or combinatorial therapies. A final concern deriving from current and future SMA therapies involves the financial burden to patients, as well as society at large, of implementing the treatments. Spinraza is reported to cost \$750,000 for the first year of treatment alone [112]. Treatment with scAAV9-SMN is expected to be dearer still. Such exorbitant costs, albeit to admittedly bespoke treatments, will surely limit affordability and may merit governmental regulation in the interest of making drug available to all who require it. Until such time, competition in the form of alternatives may be the best way of controlling costs. In this regard, regulatory approval of SMA drugs in late stage clinical trials is eagerly anticipated.

Concluding remarks

To the community of scientists who engage in the study and treatment of rare disorders, the SMA story might be construed to have concluded successfully. Yet, to those of us who have witnessed the field evolve over the last quarter century, there is clearly much to be done. Indeed, despite the successes, our understanding of how SMN maintains the health and viability of the spinal motor neurons and why certain populations of these cells succumb when deprived of the protein remains narrow and quite limited. It might be argued that addressing such basic questions is clinically irrelevant so long as one restores SMN function in a timely manner. On the contrary, such an assertion is not only naïve, but also terribly short-sighted. It will be years before a proper and reliable pronouncement can be made on the true long-term efficacy of currently available treatment options. Moreover, it is already evident that SMN repletion may not be equally effective in all patients. We therefore advocate for a continued, robust investment in basic studies, cautioning fellow scientists, governmental institutions and family advocacy groups against the sort of complacency that often follows an initial success. Instead, it is important to build on the sorts of studies that led to the realization of SMN repletion as a viable treatment for SMA. Among the most important questions to be addressed in this endeavor are the following: What are the precise biochemical and genetic pathways that link low SMN to the neuromuscular SMA phenotype? How might one compensate for the functional loss of motor units in SMA, particularly as it relates to individuals who have missed the clinical window of opportunity

during which SMN repletion is effective? Is early SMN repletion truly sufficient to permanently ward off disease? What are the long-term requirements for the protein within and outside the CNS? Might SMN function in distinct capacities in different tissues? We believe that a careful and systematic consideration of these questions will not only impact the field of SMA but is also likely to inform our broader understanding of motor neuron biology and disease.

Future perspectives

The degree of progress that has been achieved in the field of SMA may be measure in a number of ways. First, there is a great deal more awareness about the disease. Social media has undoubtedly helped, but one cannot overlook the efforts of an increasingly vocal and dedicated group of family foundations that have aggressively fund-raised and advocated for patients. Second, notwithstanding questions of the link between low SMN and selective motor neuron loss in SMA, our understanding of the biology of the disease has rapidly advanced. Finally, despite the caveats associated with the current SMN repletion therapies, patients and families have, for the first time, a realistic means of mitigating disease. Over the course of the next decade and as newborn screening and early treatment for SMA becomes routine practice in the developed world, we believe that the high rate of infant mortality associated with the disease will drop dramatically. The long-term outcome of current treatments is uncertain, but we predict that early benefit and the decline in early mortality will eventually unmask a more chronic form of disease. This may, in future, be attenuated once a systemic form of SMN repletion is approved as a means of a treatment, but is unlikely to result in complete restoration of health. The prospect of such a future has already galvanized long-time SMA investigators to double down and focus on resolving how low SMN triggers neurodegeneration, recognizing that such investigations will likely produce novel and more effective therapies. We would not be surprised if these efforts have implications well beyond the field of SMA, proving instructive to the treatment of other motor neuron disorders and even, perhaps, neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Executive summary

Clinical phenotype and genetic principles of SMA

- SMA is a clinically heterogeneous disorder manifesting as muscle weakness that usually develops in infancy in severe, intermediate or mild forms.
- The pathological hallmarks of SMA include spinal motor neuron loss and attendant skeletal muscle atrophy; proximal muscles are preferentially affected in the disease.
- SMA is a result of mutations in *SMN1*. However, patients invariably harbor *SMN2*, an almost identical but functionally deficient paralog.
- A greater number of *SMN2* copies results in greater amounts of SMN protein and a milder SMA phenotype.

The biology of the SMN protein

- The most widely-cited functions of the SMN protein are in the biogenesis of snRNP particles and pre-mRNA splicing.
- A number of less well-recognized functions have been deduced from the many proteins that SMN reportedly binds.
- Although the precise function of SMN in SMA is still not clear, there is substantial evidence of a role for the protein in motor axons and at the NMJ.

Therapies for SMA

- Rapid advances in the treatment of SMA have been made in the last decade, largely focusing on SMN repletion as a therapeutic strategy.
- Spinraza, a splice-switching oligonucleotide that raises SMN levels by altering the splicing of the *SMN2* gene, showed considerable promise for the treatment of SMA and was recently approved for use by the FDA and other regulatory agencies.
- SMN gene replacement using AAV9 technology has also been shown to be efficacious in clinical trials and is expected to be approved for the treatment of SMA before the end of 2018.
- A number of therapeutic drugs for SMA that do not rely on raising SMN levels are currently in late stage trials for the treatment of the disease.
- Despite progress in the therapeutic arena for the treatment of SMA, it is clear that currently available treatments are far from outright cures for the disease.

Concluding remarks

- Although SMN repletion has now been translated into a clinically meaningful treatment for SMA, much remains to be understood about the basic biology of the disease.
- Additional research to elucidate molecular mechanisms underlying human SMA is critical to refining and improving current therapies.

Acknowledgements

We apologize to those of our colleagues whose original work could not be cited in this article owing to space restrictions. Work in the authors' laboratory is supported by AFM-France, Cure SMA and grants from the National Institutes of Health (R01 NS057482, R21 NS0999921 and R56 NS104218).

References

* Of interest

** Of considerable interest

1. 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* 20(1), 27–32 (2012). [PubMed: 21811307]
2. Hendrickson BC, Donohoe C, Akmaev VR. et al. Differences in SMN1 allele frequencies among ethnic groups within North America. *J. Med Genet* 46(9), 641–644 (2009). [PubMed: 19625283]

3. Wang CH, Lunn MR. Spinal muscular atrophy: advances in research and consensus on care of patients. *Curr. Treat. Options Neurol* 10(6), 420–428 (2008). [PubMed: 18990310]
4. Grotto S, Cuisset JM, Marret S. et al. Type 0 Spinal Muscular Atrophy: Further Delineation of Prenatal and Postnatal Features in 16 Patients. *J. Neuromuscul. Dis* 3(4), 487–495 (2016). [PubMed: 27911332]
5. Clermont O, Burlet P, Lefebvre S. et al. SMN gene deletions in adult-onset spinal muscular atrophy. *Lancet* 346 (8991–8992) 1712–1713 (1995).
6. Kline RA, Kaifer KA, Osman EY. et al. Comparison of independent screens on differentially vulnerable motor neurons reveals alpha-synuclein as a common modifier in motor neuron diseases. *PLoS Genet.* 13(3), e1006680 (2017). [PubMed: 28362802]
7. Boyd PJ, Tu WY, Shorrock HK. et al. Bioenergetic status modulates motor neuron vulnerability and pathogenesis in a zebrafish model of spinal muscular atrophy. *PLoS Genet.* 13(4), e1006744 (2017). [PubMed: 28426667]
8. Boyer JG, Murray LM, Scott K, De Repentigny Y, Renaud JM, Kothary. Early onset muscle weakness and disruption of muscle proteins in mouse models of spinal muscular atrophy. *Skelet. Muscle* 3(1), 24 (2013). [PubMed: 24119341]
9. Bowerman M, Swoboda KJ, Michalski JP. et al. Glucose metabolism and pancreatic defects in spinal muscular atrophy. *Ann. Neurol* 72(2), 256–268 (2012). [PubMed: 22926856]
10. Bowerman M, Michalski JP, Beauvais A, Murray LM, DeRepentigny Y, Kothary R. Defects in pancreatic development and glucose metabolism in SMN-depleted mice independent of canonical spinal muscular atrophy neuromuscular pathology. *Hum. Mol. Genet* 23(13), 3432–3444 (2014). [PubMed: 24497575]
11. Deguise MO, Kothary R. New insights into SMA pathogenesis: immune dysfunction and neuroinflammation. *Ann. Clin. Transl. Neurol* 4(7), 522–530 (2017). [PubMed: 28695153]
12. Somers E, Stencel Z, Wishart TM, Gillingwater TH, Parson SH. Density, calibre and ramification of muscle capillaries are altered in a mouse model of severe spinal muscular atrophy. *Neuromuscul. Disord* 22(5), 435–442 (2012). [PubMed: 22153987]
13. Hunter G, Aghamaleky Sarvestany A, Roche SL, Symes RC, Gillingwater TH. SMN-dependent intrinsic defects in Schwann cells in mouse models of spinal muscular atrophy. *Hum. Mol. Genet* 23(9), 2235–2250 (2014). [PubMed: 24301677]
14. Rindt H, Feng Z, Mazzasette C, Glascock JJ. et al. Astrocytes influence the severity of spinal muscular atrophy. *Hum. Mol. Genet* 24(14), 4094–4102 (2015). [PubMed: 25911676]
15. Werdnig G Zwei frühinfantile hereditäre Fälle von progressiver Muskelatrophie unter dem Bilde der Dystrophie, aber auf neurotischer Grundlage. *Arch. Psychiat. Nervenkr* 22, 437–480 (1891).
16. Hoffmann J Ueber chronische spinale Muskelatrophie im Kindesalter, auf familiärer Basis. *Deutsch Z Nervenheilk* 3, 427–470 (1893).
17. Hoffmann J Weiterer Beitrag zur Lehre von der hereditären progressiven spinalen Muskelatrophie im Kindesalter nebst Bemerkungen über den fortschreitenden Muskelschwund im Allgemeinen. *Deutsch Z Nervenheilk* 10, 292–320 (1897).
18. Hoffmann J Dritter Beitrag zur Lehre von der hereditären progressiven spinalen Muskelatrophie im Kindesalter. *Deutsch Z Nervenheilk* 18, 217–224 (1900).
19. Lefebvre S, Burglen L, Reboullet S. et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80(1), 155–165 (1995). [PubMed: 7813012] ** Described the identification of the SMA genes.
20. Rochette CF, Gilbert N, Simard LR. SMN gene duplication and the emergence of the SMN2 gene occurred in distinct hominids: SMN2 is unique to *Homo sapiens*. *Hum. Genet* 108(3), 255–266 (2001). [PubMed: 11354640]
21. 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* 8(7), 1177–1183 (1999). [PubMed: 10369862]
22. Lorson CL, Hahnen E, Androphy EJ, and Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc. Natl. Acad. Sci. USA* 96(11), 6307–6311 (1999). [PubMed: 10339583]

23. Kashima T, Manley JL. A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat. Genet* 34(4), 460–463 (2003). [PubMed: 12833158]
24. Cho S, Dreyfuss G. A degron created by SMN2 exon 7 skipping is a principal contributor to spinal muscular atrophy severity. *Genes Dev.* 24(5), 438–442 (2010). [PubMed: 20194437]
25. Coovert DD, Le TT, McAndrew PE. et al. The survival motor neuron protein in spinal muscular atrophy. *Hum. Mol. Genet* 6(8), 1205–1214 (1997). [PubMed: 9259265]
26. Lefebvre S, Burlet P, Liu Q. et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat. Genet* 16(3), 265–269 (1997). [PubMed: 9207792]
27. 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* 60(6), 1411–22 (1997). [PubMed: 9199562]
28. Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am. J. Hum. Genet* 70(2), 358–368 (2002). [PubMed: 11791208]
29. Prior TW, Krainer AR, Hua Y. et al. A positive modifier of spinal muscular atrophy in the SMN2 gene. *Am. J. Hum. Genet* 85(3), 408–413 (2009). [PubMed: 19716110]
30. Wu X, Wang SH, Sun J, Krainer AR, Hua Y, Prior TW. A-44G transition in SMN2 intron 6 protects patients with spinal muscular atrophy. *Hum. Mol. Genet* 26(14), 2768–2780 (2017). [PubMed: 28460014]
31. Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. *EMBO J.* 15(14), 3555–3565 (1996). [PubMed: 8670859]
32. Young PJ, Le TT, thi Man N, Burghes AH, Morris GE. The relationship between SMN, the spinal muscular atrophy protein, and nuclear coiled bodies in differentiated tissues and cultured cells. *Exp. Cell Res* 256(2), 365–374 (2000). [PubMed: 10772809]
33. Liu Q, Fischer U, Wang F, Dreyfuss G. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell* 90(6), 1013–1021 (1997). [PubMed: 9323129]
34. Fischer U, Liu Q, Dreyfuss G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* 90(6), 1023–1029 (1997). [PubMed: 9323130] * Along with ref. 35, this article describes one of the most widely-cited functions for the SMN protein.
35. Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G. A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell* 95(5), 615–624 (1998). [PubMed: 9845364] * Along with ref. 34, this article describes one of the most widely-cited functions for the SMN protein
36. Chari A, Paknia E, Fischer U. The role of RNP biogenesis in spinal muscular atrophy. *Curr. Opin. Cell Biol* 21(3), 387–393 (2009). [PubMed: 19286363]
37. Grimm M, Otter S, Peter C, Müller F, Chari A, Fischer U. Unrip, a factor implicated in cap-independent translation, associates with the cytosolic SMN complex and influences its intracellular localization. *Hum. Mol. Genet* 14(20), 3099–3111 (2005). [PubMed: 16159890]
38. Chari A, Golas MM, Klingenhäger M. et al. An assembly chaperone collaborates with the SMN complex to generate spliceosomal SnRNPs. *Cell* 2008 135(3), 497–509 (2008). [PubMed: 18984161]
39. Narayanan U, Ospina JK, Frey MR, Hebert MD, Matera AG. SMN, the spinal muscular atrophy protein, forms a pre-import snRNP complex with snurportin1 and importin beta. *Hum. Mol. Genet* 11(15), 1785–1795 (2002). [PubMed: 12095920]
40. Narayanan U, Achsel T, Lührmann R, Matera AG. Coupled in vitro import of U snRNPs and SMN, the spinal muscular atrophy protein. *Mol. Cell* 16(2), 223–234 (2004). [PubMed: 15494309]
41. Schrank B, Götz R, Gunnensen 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* 94(18), 9920–9925 (1997). [PubMed: 9275227]
42. Jablonka S, Holtmann B, Meister G. et al. Gene targeting of Gemin2 in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death. *Proc. Natl. Acad. Sci. U S A* 99(15), 10126–10131 (2002). [PubMed: 12091709]

43. Mouillet JF, Yan X, Ou Q. et al. DEAD-box protein-103 (DP103, Ddx20) is essential for early embryonic development and modulates ovarian morphology and function. *Endocrinology* 149(5), 2168–2175 (2008). [PubMed: 18258677]
44. Shpargel KB, Praveen K, Rajendra TK, Matera AG. Gemin3 is an essential gene required for larval motor function and pupation in *Drosophila*. *Mol. Biol. Cell* 2009 20(1), 90–101 (2009). [PubMed: 18923150]
45. Meier ID, Walker MP, Matera AG. Gemin4 is an essential gene in mice, and its overexpression in human cells causes relocalization of the SMN complex to the nucleoplasm. *Biol. Open* 7(2). pii: bio032409 (2018).
46. Gates J, Lam G, Ortiz JA, Losson R, Thummel CS. rigor mortis encodes a novel nuclear receptor interacting protein required for ecdysone signaling during *Drosophila* larval development. *Development* 131(1), 25–36 (2004). [PubMed: 14645129]
47. 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* 25(13), 5543–5551 (2005). [PubMed: 15964810]
48. Zhang Z, Lotti F, Dittmar K. et al. SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. *Cell* 133(4), 585–600 (2008). [PubMed: 18485868]
49. Kim JK, Caine C, Awano T, Herbst R, Monani UR. Motor neuronal repletion of the NMJ organizer, Agrin, modulates the severity of the spinal muscular atrophy disease phenotype in model mice. *Hum. Mol. Genet* 26(13), 2377–2385 (2017). [PubMed: 28379354]
50. Lotti F, Imlach WL, Saieva L. et al. An SMN-dependent U12 splicing event essential for motor circuit function. *Cell* 151(2), 440–454 (2012). [PubMed: 23063131]
51. Zhang Z, Pinto AM, Wan L. et al. Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. *Proc. Natl. Acad. Sci. USA* 110(48), 19348–19353 (2013). [PubMed: 24191055]
52. Workman E, Saieva L, Carrel TL. et al. A SMN missense mutation complements SMN2 restoring snRNPs and rescuing SMA mice. *Hum. Mol. Genet* 18(12), 2215–2229 (2009). [PubMed: 19329542]
53. 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* 2(9), e921 (2007). [PubMed: 17895963]
54. 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* 32(1), 126–38 (2012). [PubMed: 22037760]
55. Jodelka FM, Ebert AD, Duelli DM, Hastings ML. A feedback loop regulates splicing of the spinal muscular atrophy-modifying gene, SMN2. *Hum. Mol. Genet* 19(24), 4906–4917 (2010). [PubMed: 20884664]
56. Comley LH, Nijssen J, Frost-Nylen J, Hedlund E. Cross-disease comparison of amyotrophic lateral sclerosis and spinal muscular atrophy reveals conservation of selective vulnerability but differential neuromuscular junction pathology. *J. Comp. Neurol* 524(7), 1424–1442 (2016). [PubMed: 26502195]
57. Steiman GS, Rorke LB, Brown MJ. Infantile neuronal degeneration masquerading as Werdnig-Hoffmann disease. *Ann. Neurol* 8(3), 317–324 (1980). [PubMed: 7436375]
58. Wishart TM, Mutsaers CA, Riessland M. et al. Dysregulation of ubiquitin homeostasis and β -catenin signaling promote spinal muscular atrophy. *J. Clin. Invest* 124(4), 1821–1834 (2014). [PubMed: 24590288]
59. Ripolone M, Ronchi D, Violano R. et al. Impaired Muscle Mitochondrial Biogenesis and Myogenesis in Spinal Muscular Atrophy. *JAMA Neurol.* 72(6), 666–675 (2015). [PubMed: 25844556]
60. Sanchez G, Dury AY, Murray LM. et al. A novel function for the survival motoneuron protein as a translational regulator. *Hum. Mol. Genet* 22(4), 668–684 (2013). [PubMed: 23136128]

61. Strasswimmer J, Lorson CL, Breiding DE. et al. Identification of survival motor neuron as a transcriptional activator-binding protein. *Hum. Mol. Genet* 8(7), 1219–1226 (1999). [PubMed: 10369867]
62. Rossoll W, Kröning AK, Ohndorf UM, Steegborn C, Jablonka S, Sendtner M. Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons? *Hum. Mol. Genet* 11(1), 93–105 (2002). [PubMed: 11773003]
63. 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* 162(5), 919–931 (2003). [PubMed: 12952942]
64. Kariya S, Mauricio R, Dai Y, Monani UR. The neuroprotective factor Wld(s) fails to mitigate distal axonal and neuromuscular junction (NMJ) defects in mouse models of spinal muscular atrophy. *Neurosci. Lett* 449(3), 246–251 (2009). [PubMed: 19010394]
65. Murray LM, Comley LH, Thomson D, Parkinson N, Talbot K, Gillingwater TH. Selective vulnerability of motor neurons and dissociation of pre- and post-synaptic pathology at the neuromuscular junction in mouse models of spinal muscular atrophy. *Hum. Mol. Genet* 17(7), 949–962 (2008). [PubMed: 18065780]
66. Ling KK, 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* 5(11), e15457 (2010). [PubMed: 21085654]
67. Kariya S, Park GH, Maeno-Hikichi Y. et al. Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. *Hum. Mol. Genet* 17(16), 2552–2569 (2008). [PubMed: 18492800]
68. Zhang H, Xing L, Rossoll W, Wichterle H, Singer RH, Bassell GJ. Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. *J. Neurosci* 26(33), 8622–8632 (2006). [PubMed: 16914688] * The first report alluding to an axon-specific function of the SMN protein.
69. Rossoll W, Kröning AK, Ohndorf UM, Steegborn C, Jablonka S, Sendtner M. Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons? *Hum. Mol. Genet* 11(1), 93–105 (2002). [PubMed: 11773003]
70. Akten B, Kye MJ, Hao le T. et al. Interaction of survival of motor neuron (SMN) and HuD proteins with mRNA cpg15 rescues motor neuron axonal deficits. *Proc. Natl. Acad. Sci. USA* 108(25), 10337–10342 (2011). [PubMed: 21652774]
71. Tadesse H, Deschênes-Furry J, Boisvenue S, Côté J. KH-type splicing regulatory protein interacts with survival motor neuron protein and is misregulated in spinal muscular atrophy. *Hum. Mol. Genet* 17(4), 506–524 (2008). [PubMed: 17998247]
72. Hubers L, Valderrama-Carvajal H, Laframboise J, Timbers J, Sanchez G, Côté J. HuD interacts with survival motor neuron protein and can rescue spinal muscular atrophy-like neuronal defects. *Hum. Mol. Genet* 20(3), 553–579 (2011). [PubMed: 21088113]
73. Mobarak CD, Anderson KD, Morin M. et al. The RNA-binding protein HuD is required for GAP-43 mRNA stability, GAP-43 gene expression, and PKC-dependent neurite outgrowth in PC12 cells. *Mol. Biol. Cell* 11(9), 3191–3203 (2000). [PubMed: 10982410]
74. Aranda-Abreu GE, Behar L, Chung S, Furneaux H, Ginzburg I. Embryonic lethal abnormal vision-like RNA-binding proteins regulate neurite outgrowth and tau expression in PC12 cells. *J. Neurosci* 19(16), 6907–6917 (1999). [PubMed: 10436048]
75. Deschênes-Furry J, Mousavi K, Bolognani F. et al. The RNA-binding protein HuD binds acetylcholinesterase mRNA in neurons and regulates its expression after axotomy. *J. Neurosci* 27(3), 665–675 (2007). [PubMed: 17234598]
76. Fallini C, Donlin-Asp PG, Rouanet JP, Bassell GJ, Rossoll W. Dynamics of survival of motor neuron (SMN) protein interaction with the mRNA-binding protein IMP1 facilitates its trafficking into motor neuron axons. *J. Neurosci* 36(13), 3811–3820 (2016). [PubMed: 27030765]

77. Hao le T, Duy PQ, An M. et al. HuD and the Survival Motor Neuron Protein Interact in Motoneurons and Are Essential for Motoneuron Development, Function, and mRNA Regulation. *J. Neurosci* 37(48), 11559–11571 (2017). [PubMed: 29061699]
78. Wirth B, Tessarolo D, Hahnen E. et al. Different entities of proximal spinal muscular atrophy within one family. *Hum. Genet* 100(5–6), 676–680 (1997). [PubMed: 9341891]
79. Oprea GE, Kröber S, McWhorter ML. et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. *Science* 320(5875), 524–527 (2008). [PubMed: 18440926]
80. Hosseinibarkooie S, Peters M, Torres-Benito L. et al. The Power of Human Protective Modifiers: PLS3 and CORO1C Unravel Impaired Endocytosis in Spinal Muscular Atrophy and Rescue SMA Phenotype. *Am. J. Hum. Genet* 99(3), 647–665 (2016). [PubMed: 27499521]
81. Riessland M, Kaczmarek A, Schneider S. et al. Neurocalcin Delta Suppression Protects against Spinal Muscular Atrophy in Humans and across Species by Restoring Impaired Endocytosis. *Am. J. Hum. Genet* 100(2), 297–315 (2017). [PubMed: 28132687]
82. Torres-Benito L, Neher MF, Cano R, Ruiz R, Tabares L. SMN requirement for synaptic vesicle, active zone and microtubule postnatal organization in motor nerve terminals. *PLoS One* 6(10), e26164 (2011). [PubMed: 22022549]
83. Tejero R, Lopez-Manzaneda M, Arumugam S, Tabares L. Synaptotagmin-2, and -1, linked to neurotransmission impairment and vulnerability in Spinal Muscular Atrophy. *Hum. Mol. Genet* 25(21), 4703–4716 (2016). [PubMed: 28173138]
84. Bowerman M, Anderson CL, Beauvais A, Boyl PP, Witke W, Kothary R. SMN, profilin Ila and plastin 3: a link between the deregulation of actin dynamics and SMA pathogenesis. *Mol. Cell. Neurosci* 42(1), 66–74 (2009). [PubMed: 19497369]
85. Hensel N, Claus P. The Actin Cytoskeleton in SMA and ALS: How Does It Contribute to Motoneuron Degeneration? *Neuroscientist* 24(1), 54–72 (2018). [PubMed: 28459188]
86. Bowerman M, Beauvais A, Anderson CL, Kothary R. Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. *Hum. Mol. Genet* 19(8), 1468–1478 (2010). [PubMed: 20097679]
87. 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 Med* 10:24 (2012). [PubMed: 22397316]
88. Hamilton G, Gillingwater TH. Spinal muscular atrophy: going beyond the motor neuron. *Trends Mol. Med* 19(1), 40–50 (2013). [PubMed: 23228902]
89. Nash LA, Burns JK, Chardon JW, Kothary R, Parks RJ. Spinal Muscular Atrophy: More than a Disease of Motor Neurons? *Curr. Mol. Med* 16(9), 779–792 (2016). [PubMed: 27894243]
90. 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* 17(8), 1063–1075 (2008). [PubMed: 18178576]
91. Iyer CC, McGovern VL, Murray JD. et al. Low levels of Survival Motor Neuron protein are sufficient for normal muscle function in the SMN^{-/-} mouse model of SMA. *Hum. Mol. Genet* 24(21), 6160–6173 (2015). [PubMed: 26276812]
92. Martinez TL, Kong L, Wang X. et al. Survival Motor Neuron Protein in Motor Neurons Determines Synaptic Integrity in Spinal Muscular Atrophy. *J. Neurosci* 32(25), 8703–8715 (2012). [PubMed: 22723710]
93. Monani UR, Sendtner M, Covert 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* 9(3), 333–339 (2000). [PubMed: 10655541]
94. Hsieh-Li HM, Chang JG, Jong YJ, Wu MH, Wang NM, Tsai CH, Li H. (2000) A mouse model for spinal muscular atrophy. *Nat. Genet* 24(1), 66–70 (2007).
95. Singh NK, Singh NN, Androphy EJ, Singh RN. Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. *Mol. Cell. Biol* 26(4), 1333–1346 (2006). [PubMed: 16449646] ** The original report that served as the basis for the SMA drug, Spinraza.

96. Hua Y, Sahashi K, Hung G. et al. Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am. J. Hum. Genet* 82(4), 834–848 (2008). [PubMed: 18371932]
97. Kim JK, Monani UR. Augmenting the SMN Protein to Treat Infantile Spinal Muscular Atrophy. *Neuron* 97(5), 1001–1003 (2018). [PubMed: 29518354]
98. Finkel RS, Mercuri E, Darras BT. et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N. Engl. J. Med* 377(18), 1723–1732 (2017). [PubMed: 29091570] ** First description of a treatment for human SMA based on modulating SMN2 expression.
99. <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm>
100. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat. Biotechnol* 27(1), 59–65 (2009). [PubMed: 19098898]
101. Bucher T, Colle MA, Wakeling E. et al. scAAV9 intracisternal delivery results in efficient gene transfer to the central nervous system of a feline model of motor neuron disease. *Hum. Gene Ther* 24(7), 670–82 (2013). [PubMed: 23799774]
102. 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* 28(3), 271–274 (2010). * The original pre-clinical study upon which gene replacement for SMA was based.
103. Duque SI, Arnold WD, Odermatt P. et al. A large animal model of spinal muscular atrophy and correction of phenotype. *Ann. Neurol* 77(3), 399–414 (2015). [PubMed: 25516063]
104. Mendell JR, Al-Zaidy S, Shell R. et al. Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N. Engl. J. Med* 377(18), 1713–1722 (2017). [PubMed: 29091557] ** First description of a treatment for human SMA based on SMN gene replacement.
105. Bordet T, Berna P, Abitbol JL, Pruss RM. Olesoxime (TRO19622): A Novel Mitochondrial-Targeted Neuroprotective Compound. *Pharmaceuticals (Basel)*. 3(2), 345–368 (2010). [PubMed: 27713255]
106. Bertini E, Dessaud E, Mercuri E. et al. Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular atrophy: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*. 16(7), 513–522 (2017). [PubMed: 28460889]
107. Andrews JA, Miller TM, Vijayakumar V. et al. CK-2127107 amplifies skeletal muscle response to nerve activation in humans. *Muscle Nerve* Nov 18. doi: 10.1002/mus.26017. (2017).
108. Andrews JA, Cudkowicz ME, Hardiman O. et al. VITALITY-ALS, a phase III trial of tirasemtiv, a selective fast skeletal muscle troponin activator, as a potential treatment for patients with amyotrophic lateral sclerosis: study design and baseline characteristics. *Amyotroph. Lateral Scler. Frontotemporal Degener* 19(3–4), 259–266 (2018). [PubMed: 29402141]
109. Lutz CM, Kariya S, Patruni S. et al. Post-symptomatic restoration of SMN rescues the disease phenotype in a mouse model of severe spinal muscular atrophy. *J. Clin. Invest* 121(8), 3029–3041 (2011). [PubMed: 21785219]
110. Kariya S, Obis T, Garone C. et al. Requirement of enhanced Survival Motoneuron protein imposed during neuromuscular junction maturation. *J. Clin. Invest* 124(2), 785–800 (2014). [PubMed: 24463453]
111. Mercuri E, Darras BT, Chiriboga CA. et al. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. *N. Engl. J. Med* 378(7), 625–635 (2018). [PubMed: 29443664]
112. van der Ploeg AT. The dilemma of two innovative therapies for spinal muscular atrophy. *N. Engl. J. Med* 377(18), 1786–1787 (2017) [PubMed: 29091554]

Figure 1 –.
Motor neuron loss in SMA. Lumbar spinal motor neurons in a severely affected PND10 “ 7” SMA mouse and its control littermate were stained with an antibody to choline acetyltransferase. Roughly 25% of the motor neurons in the mutant are lost. Scale bar – 50µM.

Figure 2 –.

The genetic basis of SMA. Depicted are the two SMN genes, the single base difference between them and their major splice products. The proclivity of *SMN2* to exclude exon 7 from transcripts results in little FL-SMN transcript and therefore inadequate functional SMN protein. This triggers loss of the spinal motor neurons and the SMA phenotype. Note: ESS – exon splice silencer.

Table 1 –

The major types of proximal spinal muscular atrophy

Type of SMA	Age at onset	Signature features	Time of Death	% of total SMA
I	< 6 months	Proximal muscle weakness; patients unable to sit without support	< 2 years	60
II	6–18 months	Proximal muscle weakness; patients able to sit unaided but cannot stand or walk independently	> 2 years	27
III	> 18 months	Proximal muscle weakness in juvenile age; patients able to walk unaided	Normal life span	12
IV	Adulthood	Proximal muscle weakness in adulthood; patients may require walking aid	Normal life span	1

Table 2 –

Summary of notable therapies that are either approved or in late stage development for the treatment of spinal muscular atrophy

Treatment Strategy	Therapeutic Drug and Source	Current Status
SMN Repletion	Spinraza (Biogen; antisense oligo <i>SMN2</i> splice enhancer)	FDA, EMA approved
	RG-7916 (Roche; small molecule <i>SMN2</i> splice enhancer)	Phase III clinical trials
	AVXS-101 (Avexis; virally delivered <i>SMN1</i> replacement)	Phase III clinical trials
	LM-10170 (Novartis; small molecule <i>SMN2</i> splice enhancer)	Phase II clinical trials
Non-SMN approach	Olesoxime (Roche; neuroprotection)	Phase II clinical trials
	CK-2127107 (Cytokinetics Inc., muscle enhancer)	Phase II clinical trials
	SRK-015 (Scholar Rock Inc., muscle enhancer)	Pre-clinical phase