

# DEVELOPMENT AND TRANSLATION OF THERAPIES FOR SPINAL MUSCULAR ATROPHY

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## ABSTRACT

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterised by widespread loss of lower motor neurons from the spinal cord, leading to progressive weakness and muscle atrophy. SMA is largely caused by homozygous loss of the *survival motor neuron (SMN) 1* gene, resulting in reduced levels of full-length SMN protein. Although no approved treatment is currently available for SMA, several clinical trials investigating different approaches to increase SMN levels are showing promising early results. Trials investigating the use of therapies targeting muscle strength and neuroprotective pathways are also in progress, generating the possibility of delivering combination therapies utilising both SMN-dependent and SMN-independent targets. Due to an increased understanding of the cellular and molecular consequences of SMN depletion, a second wave of therapies targeted at pathways downstream of SMN are currently undergoing preclinical development. As these therapies move forward towards the clinic, new treatment options are likely to become available, raising the potential to generate an effective 'cure' for SMA.

**Keywords:** Spinal muscular atrophy (SMA), motor neuron, therapy, treatment, clinical trials.

## INTRODUCTION

Spinal muscular atrophy (SMA) is a hereditary motor neuron disease characterised by loss of motor neurons from the anterior grey horn of the spinal cord.<sup>1</sup> With an incidence of 1 in ~10,000 live births and a carrier frequency of 1 in ~35-50, this autosomal recessive disease is the most common genetic cause of infant mortality.<sup>2,3</sup> In 95% of cases, SMA is caused by homozygous deletion of the *survival motor neuron (SMN) 1* gene.<sup>4</sup> In humans, there are two SMN genes, a telomeric *SMN1* copy and an almost identical, centromeric *SMN2* copy.<sup>4</sup> The *SMN2* gene has a C to T substitution that results in the exclusion of exon 7 and the production of a truncated protein product, which is rapidly degraded.<sup>4,5</sup> However, exclusion of exon 7 is incomplete, resulting in approximately 10-15% of the protein produced from *SMN2* being full-length SMN.<sup>6</sup> As complete loss of SMN protein is embryonically lethal,<sup>7</sup> SMA patients are dependent on SMN protein produced by the *SMN2*

gene,<sup>8</sup> with variability in protein levels occurring as a result of differences in *SMN2* copy number; individuals with a higher *SMN2* copy number have a less-severe disease phenotype.<sup>9</sup> Thus, *SMN2* copy number represents the primary determinant of disease severity in SMA.<sup>1</sup>

The degeneration of lower motor neurons that occurs in SMA leads to a progressive decline in motor development, manifesting as muscle atrophy and weakness, primarily affecting proximal muscle groups.<sup>9</sup> The profile of disease progression can vary substantially between patients with some phases of plateau in the decline of motor development.<sup>10</sup> Based on the age of onset, motor function achieved, and typical age of death, SMA can be classified into as many as five distinct clinical subgroups with varying severity.<sup>11</sup> In all subtypes, molecular genetic analysis is now the gold standard for diagnosis.<sup>12</sup> Type 0 onset occurs *in utero* with life not extending beyond the first few weeks after birth.<sup>1</sup> Type 1 SMA, otherwise known as Werdnig-Hoffmann's disease, is the

most common type of SMA.<sup>11</sup> Disease onset occurs by 6 months and patients are unable to sit without support and cannot control head movement.<sup>2</sup> Poor bulbar function and weak intercostal muscles lead to difficulties in feeding and breathing, resulting in death within the first 2 years of life in the absence of palliative care.<sup>12</sup> Patients with Type 2 SMA (Dubowitz's syndrome) have an intermediate phenotype, being able to maintain a sitting position unaided, but without the ability to walk independently.<sup>12</sup> Onset occurs between 7 months and 18 months of age and death frequently occurs during adolescence due to respiratory problems.<sup>2</sup> Type 3 SMA (Kugelberg-Welander's disease) shows marked symptom heterogeneity; some patients are able to walk independently and have some muscular weakness, while others begin to walk but require wheelchair assistance in childhood.<sup>2</sup> Type 4 SMA disease onset typically occurs in the second or third decade of life, with patients suffering from muscular weakness without respiratory problems.<sup>12</sup> For both SMA Type 3 and Type 4, life expectancy is often comparable to that of the general population.<sup>11</sup> Several rare forms of SMA also exist, caused by mutations in genes other than *SMN1*. These include X-linked SMA, SMA with respiratory distress, and spinal and bulbar muscular atrophy (Kennedy's disease).<sup>12</sup>

Following the identification of the disease-causing gene, it has become possible to model SMA in various animal systems.<sup>13,14</sup> Studies in these animal models have led to an increased understanding of the molecular pathogenesis of SMA and have indicated that SMN replacement therapies may be a viable therapeutic strategy to treat SMA.<sup>1,2,12</sup>

## THE SURVIVAL MOTOR NEURON PROTEIN

In order to successfully target the SMN protein as a therapeutic approach for SMA, it is necessary to understand the functions of the SMN protein and the downstream effects of SMN reduction. SMN protein is a ubiquitously expressed, multifunctional protein that forms a macromolecular complex, essential for the splicing of pre-messenger RNAs (mRNAs).<sup>15-17</sup> SMN associates with Gemins 2-8 and Unrip to form a complex that enables Sm core proteins and uridine-rich small nuclear ribonucleic acids to form small nuclear ribonucleoproteins (snRNPs).<sup>15,16</sup> During pre-mRNA splicing, snRNPs are essential for the excision of

introns from mRNA precursors in the nucleus.<sup>17</sup> Reduced SMN levels lead to a tissue-specific decrease in snRNP assembly that correlates with phenotypic severity in mouse models of SMA.<sup>18</sup> Moreover, widespread splicing defects have been found in SMA tissues with a wide diversity of genes being affected,<sup>19</sup> including genes encoding splicing regulators and proteins required for motor circuit function.<sup>20,21</sup> However, it has also been suggested that splicing defects may represent a late feature of SMA indicating that alternative splicing events could simply represent a consequence of disease progression in SMA, rather than the primary cause.<sup>22</sup>

Aside from its housekeeping function in snRNP assembly, SMN has been shown to have additional, non-canonical roles that may contribute to disease pathogenesis in SMA. During axonogenesis and axonal sprouting there is a progressive shift of SMN towards an axonal localisation in the human spinal cord, suggesting an axonal function of SMN.<sup>23</sup> Indeed, SMN localises to axonal transport granules that deliver mRNAs to the synapse, where local translation can occur.<sup>24</sup> SMN is also involved in axonal elongation, with loss of SMN leading to defects in axon outgrowth.<sup>14,25,26</sup> Through interaction with mRNA binding proteins, SMN is involved in the localisation of beta-actin and *beta-actin* mRNA to growth cones of developing motor neurons, which leads to axonal elongation and growth cone size regulation.<sup>26</sup> Interestingly, while SMN deficient motor neurons have reduced growth cone size,<sup>25,26</sup> mice that lack beta-actin in motor neurons do not,<sup>27</sup> indicating that other pathways contribute to the defective axonal elongation phenotype in SMA. Indeed, inappropriate activation of the Rho A/Rho-kinase (ROCK) pathway has been shown to lead to defects in neuritogenesis in SMA.<sup>28,29</sup> Moreover, it has been demonstrated that insulin-like growth factor 1 (IGF-1) is essential for enhancing axonal outgrowth of motor neurons. Intriguingly, circulating IGF-1 levels are also reduced as a consequence of the SMN reduction in SMA.<sup>30,31</sup>

Several other cellular and molecular pathways are also dysregulated in SMA due to reduced SMN levels. For example, ubiquitin homeostasis is altered in SMA whereby SMN depletion in mouse models of SMA leads to downregulation of ubiquitin-like modifier-activating enzyme 1 (UBA1) and accumulation of its downstream targets.<sup>32,33</sup> UBA1 activates ubiquitin as the first step of the ubiquitin conjugation process to mark proteins for

degradation by the proteasome.<sup>34</sup> The proteasome has also been implicated in SMA pathogenesis as SMN degradation is mediated by the ubiquitin-proteasome system (UPS)<sup>35</sup> and inhibiting proteasome function has been shown to increase SMN levels.<sup>36</sup> Furthermore, the deubiquitinase *Usp9x* associates with and stabilises the SMN complex through interaction with SMN, which it deubiquitinates.<sup>5</sup> *Usp9x* does not, however, deubiquitinate and stabilise the truncated SMN protein produced by *SMN2*, which is therefore rapidly degraded.<sup>33</sup> The identification of these novel cellular and molecular functions of SMN opens up the possibility of developing SMN-independent therapies for the treatment of SMA.

## SPINAL MUSCULAR ATROPHY AS A MULTISYSTEM DISORDER

Regardless of whether therapies being developed are SMN-dependent or SMN-independent, one key element of any successful therapy is the ability to deliver it to cells, tissues, and organs affected by the disease. In the case of SMA, the main pathological target is undoubtedly alpha motor neurons;<sup>37</sup> ~25–30% of motor neuron cell bodies are lost from the spinal cord of late symptomatic SMA mice.<sup>7</sup> However, neuromuscular pathology is apparent before the overt loss of motor neuron cell bodies occurs in SMA.<sup>14,38</sup> Thus, the neuromuscular system appears to develop relatively normally, but early on in the disease structural and functional defects are seen in both nerve and muscle.<sup>39,40</sup> These include early pathological changes at the neuromuscular junction (NMJ), including nerve terminal loss, synaptic accumulation of neurofilament proteins, and defective maturation of acetylcholine receptor clusters.<sup>39–41</sup> Alongside these early changes in distal extremities of motor neurons, intrinsic defects have been reported in skeletal muscle, including smaller myotubes as well as reduced proliferation and fusion defects of myoblasts.<sup>42,43</sup> These pathological changes in muscle occur independently of neuron degeneration and correlate with SMN reduction in model systems.<sup>38,42–44</sup>

Aside from lower motor neurons and skeletal muscle, low SMN levels also affect other cell types throughout the nervous system in SMA. For example, a defective myelination phenotype has been observed, resulting from intrinsic defects in Schwann cells in mouse models of SMA;<sup>45–47</sup> altered function of astrocytes has also been implicated in

SMA pathogenesis.<sup>48,49</sup> Furthermore, pathological changes have been reported in the thalamus, cerebral cortex, brainstem, and dorsal root ganglia in severe cases of SMA.<sup>9</sup> Alongside pathological changes in the nervous system and skeletal muscle, defects in peripheral tissues and organs including the heart, pancreas, blood vessels, and intestine have also been reported.<sup>8,9,50,51</sup> One working hypothesis to explain the presence of extra-neuronal pathology in SMA is the 'threshold hypothesis' where differential thresholds for low SMN levels exist in different cell types, with motor neurons being most vulnerable in SMA due to their exceptional sensitivity to low levels of SMN.<sup>8,13</sup>

Although pathological extra-neuronal organ system phenotypes may often manifest at the subclinical level in SMA patients, they are becoming of increasing importance as therapies prolonging a patient's survival run the risk of unmasking disorders of other organ systems. In addition, several recent studies have demonstrated that restoring SMN in motor neurons or skeletal muscle alone is insufficient to correct disease pathology in SMA mice, and that peripheral SMN restoration is likely to be essential for long-term rescue of SMA.<sup>30,52</sup> Taken together, these findings suggest that any successful therapy for SMA will need to target not only motor neurons and skeletal muscle, but also more widespread, systemic pathology.

## DEVELOPMENT OF NOVEL THERAPIES FOR SPINAL MUSCULAR ATROPHY

At present, no curative or disease-modifying therapies are available for patients with SMA. Palliative care options can assist with management of symptoms and prevention of complications. There are, however, several clinical trials aimed at modifying the disease that are currently underway in SMA patient cohorts. Given the central role that SMN plays in the disease, it is not surprising to find that the majority of clinical trials are aimed at increasing SMN protein levels. The current clinical trials can be split into four main groups, three based around a variety of approaches to increase SMN levels and one group of complementary therapies mainly comprising neuroprotective factors or muscle strength-enhancing compounds (Table 1).

Gene therapy to replace the faulty *SMN1* gene is one of the main technological approaches entering clinical trials, based on very promising preclinical data from animal models.<sup>80–83</sup>

**Table 1: Current overview of clinical trials for spinal muscular atrophy as of June 2016.**<sup>53-79</sup>

Trial number	Study	Status	Type	Sponsor
<b>Gene therapy</b>				
NCT02122952 <sup>55</sup>	Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1	Ongoing - not recruiting	Phase I	Jerry R. Mendell
<b>Molecular therapy with ASO</b>				
NCT02386553 <sup>56</sup>	A Study of Multiple Doses of ISIS SMNRx (ISIS 396443) Delivered to Infants With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy	Recruiting	Phase II	Biogen
NCT02462759 <sup>57</sup>	A Study to Assess the Safety and Tolerability of ISIS 396443 (ISIS SMNRx) in Participants With Spinal Muscular Atrophy (SMA)	Ongoing - not recruiting	Phase II	Biogen
NCT02594124 <sup>58</sup>	An Open-label Study (SHINE) for Patients With Spinal Muscular Atrophy (SMA) Who Participated in Studies With IONIS-SMNRx	Recruiting via invitation	Phase III	Ionis Pharmaceuticals, Inc.
NCT02292537 <sup>59</sup>	A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Patients With Later-onset Spinal Muscular Atrophy	Ongoing - not recruiting	Phase III	Ionis Pharmaceuticals, Inc.
NCT02193074 <sup>60</sup>	A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Infants With Spinal Muscular Atrophy	Ongoing - not recruiting	Phase III	Ionis Pharmaceuticals, Inc.
NCT02052791 <sup>61</sup>	An Open-label Safety and Tolerability Study of Ionis SMNRx in Patients With Spinal Muscular Atrophy Who Previously Participated in IONIS SMNRx-C52 or IONIS SMNRx-CS10	Ongoing - not recruiting	Phase I	Ionis Pharmaceuticals, Inc.
NCT01839656 <sup>62</sup>	A Study to Assess the Safety and Pharmacokinetics of IONIS SMNRx in Infants With Spinal Muscular Atrophy	Ongoing - not recruiting	Phase II	Ionis Pharmaceuticals, Inc.
NCT01780246 <sup>63</sup>	An Open-label Safety and Tolerability Study of ISIS SMNRx in Patients With Spinal Muscular Atrophy Who Previously Participated in ISIS 396443-C51	Completed	Phase I	Ionis Pharmaceuticals, Inc.
NCT01703988 <sup>64</sup>	An Open-label Safety, Tolerability and Dose-range Finding Study of Multiple Doses of ISIS SMNRx in Patients With Spinal Muscular Atrophy (SMNRx - C52)	Completed	Phase I/II	Ionis Pharmaceuticals, Inc.
NCT01494701 <sup>65</sup>	An Open-label Safety, Tolerability and Dose-range Finding Study of ISIS SMNRx in Patients With Spinal Muscular Atrophy	Completed	Phase I	Ionis Pharmaceuticals, Inc.
<b>Small molecules enhancing SMN</b>				
NCT02268552 <sup>66</sup>	An Open-label Study of LM1070 in Type 1 Spinal Muscular Atrophy (SMA)	Ongoing - not recruiting	Phase I/II	Novartis Pharmaceuticals
NCT02240355 <sup>67</sup>	A Study of RO6885247 in Adult and Pediatric Patients With Spinal Muscular Atrophy	Suspended	Phase I	Hoffman-La Roche
NCT01671384 <sup>68</sup>	Valproate and Levocarnitine in Children With Spinal Muscular Atrophy	Recruiting	Phase III	All India Institute of Medical Sciences, New Delhi
NCT00661453 <sup>69</sup>	CARNIVAL Type I: Valproic Acid and Carnitine in Infants With Spinal Muscular Atrophy (SMA) Type 1	Completed	Phase I/II	University of Utah
NCT00481013 <sup>70</sup>	Valproic Acid in Ambulant Adults With Spinal Muscular Atrophy (VALIANTSMA)	Completed	Phase II	University of Utah
NCT02633709 <sup>71</sup>	A Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of RO7034067 (RG7916) Given by Mouth in Healthy Volunteers	Recruiting	Phase I	Hoffman-La Roche



**Table 1 continued.**

Trial number	Study	Status	Type	Sponsor
<b>Complementary/other</b>				
NCT02644668 <sup>72</sup>	A Study of CK-2127107 in Patients With Spinal Muscular Atrophy	Recruiting	Phase II	Cytokinetics
NCT02628743 <sup>73</sup>	A Study to Evaluate Long Term Safety, Tolerability, and Effectiveness of Olesoxime in Patients With Spinal Muscular Atrophy	Recruiting	Phase II	Hoffmann-La Roche
NCT01302600 <sup>74</sup>	Safety and Efficacy of Olesoxime (TRO19622) in 3-25 Years SMA Patients	Completed	Phase II	Trophos
NCT02227823 <sup>75</sup>	Safety and Efficacy Study of Pyridostigmine on Patients With Spinal Muscular Atrophy Type 3 (EMOTAS)	Recruiting	Phase II	Centre Hospitalier Régional de la Citadelle
NCT01645787 <sup>76</sup>	Short and Long Term Treatment With 4-AP in Ambulatory SMA Patients	Completed	Phase II/III	Columbia University
NCT00533221 <sup>77</sup>	Pilot Study of Growth Hormone to Treat SMA Type II and III	Completed	Phase II	University Hospital Freiburg
NCT00528268 <sup>78</sup>	Study to Evaluate Sodium Phenylbutyrate in Pre-symptomatic Infants With Spinal Muscular Atrophy (STOPSMA)	Completed	Phase I/II	NICHD
NCT00324454 <sup>79</sup>	Levetiracetum for Cramps, Spasticity and Neuroprotection in Motor Neuron Disease	Completed	Phase II	Duke University

**SMA: Spinal muscular atrophy; SMN: survival motor neuron; ASO: antisense oligonucleotides.**

Several studies demonstrated that adeno-associated virus mediated SMN gene replacement resulted in widespread expression of SMN in the spinal cord and significantly increased survival of SMA model mice.<sup>80-83</sup> In the ongoing Phase I gene therapy clinical trial, SMN within a self-complementary adeno-associated virus serotype 9 (scAAV9) vector is being delivered intravenously to Type 1 SMA patients (Table 1). As the use of gene therapy approaches are still not commonplace in the clinical setting, this initial clinical trial is evaluating both the safety and efficacy of the treatment in the small group of infants enrolled in the study (Table 1).

A second therapeutic approach to increase SMN levels is to direct antisense oligonucleotides (ASOs) against sequences that inhibit the inclusion of *SMN2* exon 7. The binding of ASOs to the regulatory motif prevents the binding of repressor factors. This promotes the inclusion of exon 7, thereby increasing the amount of full-length SMN produced by *SMN2*.<sup>84,85</sup> The use of such ASOs in mouse models of SMA has shown promising results with survival prolonged by >100 days in some cases.<sup>86</sup> ASOs to treat SMA are currently in Phase II and Phase III of clinical trials (Table 1). Interestingly, trials using ASOs to treat other diseases, such as muscular dystrophy, are well advanced and have

been instrumental in informing the study design and methodology used for ASOs trials in SMA.<sup>87</sup> The third SMN-targeted therapeutic approach entering clinical trials for SMA is based around small molecules that modify *SMN2* splicing and increase SMN levels, including the use of histone deacetylase inhibitors such as valproic acid.<sup>88</sup> Studies taking this more traditional pharmacological approach are currently at different stages of the clinical trial process, again after promising results from screens in rodent and cell models of SMA (Table 1).<sup>89</sup>

Regardless of the technological approach used to increase SMN levels, the ‘therapeutic time-window’ within which therapy must be delivered in order to have a maximal effect needs to be considered. Several studies have indicated that SMN is required at the early stages of development and that sufficient SMN levels are essential for NMJ maturation in the early post-natal period.<sup>88-92</sup> It has also been demonstrated that late stage SMN gene replacement using scAAV9 failed to ameliorate NMJ defects, while pre-symptomatic delivery of scAAV9-SMN resulted in near complete rescue of the SMA phenotype.<sup>93</sup> Together, these studies indicate that treatment using SMN-enhancing therapies will need to occur before overt symptoms are apparent for a full restoration of the SMA phenotype.

While the first-generation of SMN-dependent therapies progress through the clinical trial process there is a wave of second-generation SMN-independent therapies currently in preclinical and clinical development. The most clinically advanced of these are centred around administering neuroprotective factors, or enhancing muscle strength (Table 1). Olesoxime is one potential neuroprotective factor currently in Phase II clinical trials for Type 2 or 3 SMA patients. This treatment acts by binding to components of the mitochondrial permeability pore, through which it can exert neuroprotective effects.<sup>94</sup> Several drugs in trials to improve muscle strength have previously been approved for other disorders involving weakness of the neuromuscular system, while others are more recent developments, including CK-2127107 (Table 1). This is a skeletal muscle troponin activator currently in trials to treat neuromuscular dysfunction, muscular weakness, and muscle fatigue.<sup>95</sup> IGF-1 has also been used to try and improve muscular symptoms in SMA patients. Intravenous administration of AAV1-IGF-1 increased tissue levels of SMN, ameliorated muscle atrophy, and increased survival in SMA model mice.<sup>31</sup> However, in SMA patient trials, IGF-1 failed to reduce muscle atrophy.<sup>96</sup> It has recently been found that the IGF-1 receptor is increased in the spinal cord of SMA mice.<sup>97</sup> Hence, the beneficial effects of increasing IGF-1 might be restricted by the overexpression of IGF-1 receptor.<sup>97</sup> Indeed, the same group showed that reducing IGF-1 receptor expression protects motor neurons and improves motor behaviour in a mouse model of SMA.<sup>97</sup>

Following on from recent developments in our understanding of the cellular and molecular pathways dysregulated downstream of SMN reduction in SMA (as discussed earlier), therapeutic strategies that target these pathways are also being developed. For example, members of the ROCK pathway have become attractive therapeutic targets due to their known potential to modulate axon outgrowth and growth cone motility. Although targeting downstream effectors of ROCK did not rescue the SMA phenotype,<sup>28</sup> inhibiting ROCK led to positive outcomes in mouse models of SMA.<sup>98,99</sup> Both Fasudil and Y-27632 inhibit ROCK and lead to an increase in the survival of SMA mice and improved NMJ maturation and muscle fibre size.<sup>98,99</sup> Although toxicity was associated with Fasudil at high doses and motor neuron cell death was not reduced, the increase in survival points

towards the importance of targeting muscle in SMA treatment strategies.<sup>98,99</sup>

The UPS has also recently been highlighted as a potentially attractive therapeutic target for SMA. Initial indications came from studies showing a role for the UPS in SMN degradation and stabilisation. Indeed, it has been demonstrated that inhibiting the chymotrypsin-like activity of the 26S proteasome using bortezomib increases SMN levels in peripheral tissues of SMA mice.<sup>36</sup> This resulted in improved motor function and increased survival. When SMA mice were treated with a combination therapy of bortezomib and trichostatin A, a histone deacetylase inhibitor that increases SMN protein levels, the improvements observed across all aspects of the SMA phenotype were greater than when mice were treated with only one treatment.<sup>36</sup> This study therefore provides proof-of-principal that combination therapies increasing SMN gene transcription and reducing SMN protein degradation may represent a viable therapeutic approach for SMA. However, due to toxicity issues associated with using available proteasome inhibitors such as bortezomib, targeting E3 ligases responsible for the specific ubiquitination of SMN may be a more suitable therapeutic approach to stabilise SMN.<sup>34</sup> E3 ligases are the enzymes responsible for conferring the specificity of the UPS, therefore targeting them would be predicted to lead to a reduction in the non-specific effects associated with targeting the proteasome.<sup>34</sup> Indeed, mind bomb 1 was recently identified as an E3 ligase responsible for the ubiquitination of SMN.<sup>100</sup> Interestingly, loss of the *Caenorhabditis elegans* orthologue of mind bomb 1 ameliorated the neuromuscular defects seen due to *SMN1* loss of function.<sup>100</sup>

Alongside evidence suggesting that targeting SMN protein stability via the UPS may be an attractive therapeutic approach for SMA, recent experiments have demonstrated that UBA1, a key E1 ubiquitin-activating enzyme required for UPS function, is a major downstream target of SMN whose levels are robustly depleted in SMA.<sup>32,33</sup> This SMN-induced reduction of UBA1 levels leads to disruption of UBA1-dependent targets, such as an accumulation of beta-catenin.<sup>33</sup> Experiments in SMA animal models targeting these UBA1-dependent pathways have demonstrated improvements in neuromuscular phenotype, suggesting that these pathways are amenable to therapeutic intervention.<sup>33</sup>

## FUTURE DEVELOPMENT AND CONCLUDING REMARKS

At present, there are no curative therapies for patients with SMA; however, several clinical trials aimed at modifying the disease are underway in patient cohorts. The focus of many of these clinical trials is augmenting full-length SMN protein levels. However, there is a second wave of SMN-independent therapies in preclinical and clinical development that target cellular and molecular pathways dysregulated downstream of SMN reduction in SMA.

Several major issues and questions will need answering before we are able to design and deliver fully effective therapies. For example, debate is still ongoing regarding whether restoration of SMN levels will be more important in the central nervous system and/or in peripheral tissues to successfully treat SMA.<sup>30,52,101</sup> As previously mentioned, several studies in rodent models of the disease have shown that SMN restoration in extra-neural tissues and organ systems will likely be necessary for amelioration of the systemic SMA phenotype.<sup>30,52</sup> Were this situation to be recapitulated in human SMA patients, it could have significant consequences for the success of current clinical trials, many of which aim to increase SMN levels using delivery methods targeting the central nervous system (Table 1).

One other major issue that remains to be fully resolved concerns the presence of a therapeutic

time-window after which therapy delivery can only have a minimal effect. Several studies have indicated that for maximal benefit, SMN-replacement therapies will need to be delivered before the onset of overt symptoms.<sup>91,93</sup> It will therefore be essential to understand how the recent mouse work relates to the temporal development of SMA pathogenesis in human patients.

It is possible that combination therapies will have the greatest potential to ameliorate the SMA disease phenotype. Combining SMN enhancement therapies with muscle strength-enhancing drugs or neuroprotective factors may help to preserve and strengthen the connections between neurons and muscles. This approach may result in effective treatment of SMA symptoms beyond the therapeutic time-window in which SMN-dependent therapies alone will be effective. An alternative combinatorial therapeutic avenue is the possibility of combining therapies to enhance SMN gene transcription with treatments to reduce SMN degradation and stabilise the protein (see previous discussion). This approach has the benefit of a possible dose reduction of the SMN-enhancing agent, which may help to reduce toxicity and increase efficacy.<sup>36</sup> While the future looks bright for the progress of emerging treatments for SMA, it is necessary to continue investing in research covering a range of therapeutic approaches in order to get the best chance of success for developing an effective cure for SMA.

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