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Biographies

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Key points

- The approval of nusinersen represents an important milestone for spinal muscular atrophy (SMA) research and treatment
- Promising results from clinical trials indicate that several additional treatment options, such as gene therapy, could be available for patients with SMA in the near future
- Preclinical research has highlighted the powerful potential of combinatorial targeting of both survival motor neuron protein (SMN)-dependent and SMN-independent pathways ('SMN-plus' therapy) to deliver maximal therapeutic benefits
- The rapid emergence of many new therapeutic options for SMA raises major issues concerning the coordination of future clinical trials in this small population of patients
- The development of therapies for SMA has the potential to offer important insights and tools that are applicable to patients with other neuromuscular and neurodegenerative conditions

Abstract

Spinal muscular atrophy (SMA) is a devastating motor neuron disease that predominantly affects children and represents the most common cause of hereditary infant mortality. The condition results from deleterious variants in *SMN1*, which lead to depletion of the survival motor neuron (SMN) protein. Now, 20 years after the discovery of this genetic defect, a major milestone in SMA and motor neuron disease research has been reached with the approval of the first disease-modifying therapy for SMA by US and European authorities — the antisense oligonucleotide nusinersen. At the same time, promising data from early stage clinical trials of *SMN1* gene therapy have indicated that additional therapeutic options are likely to emerge for patients with SMA in the near future. However, the approval of nusinersen has generated a number of immediate and substantial medical, ethical and financial implications that have the potential to resonate beyond the specific treatment of SMA. Here, we provide an overview of the rapidly-evolving therapeutic landscape for SMA, highlighting current achievements and future opportunities. We also discuss how these developments are providing important lessons for the emerging second generation of combinatorial ('SMN-plus') therapies that are likely to be required to generate robust treatments that are effective across a patient's lifespan.

Competing interests statement

T.H.G. and K.T. serve as Chair of the Scientific & Clinical Advisory Board, and Trustee of the SMA Trust respectively.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive, progressive neurodegenerative disease characterised primarily by the degeneration of alpha motor neurons in the ventral grey horn of spinal cord. In >95% of patients, SMA is caused by deleterious variants in *SMN1*, the gene encoding the survival motor neuron protein (SMN)[1]. The prevalence of SMA is one in ~10,000 live births, which makes it the most common causes of infant genetic mortality[2]. Historically, as with most hereditary neurodegenerative conditions, therapies for SMA essentially have been supportive, and have exerted no effect on the underlying disease process. However, several decades of intensive research efforts have culminated — spectacularly — in the global approval of nusinersen, marketed as Spinraza (Biogen), the first disease-modifying therapy for SMA. This major milestone is one to be celebrated, but also raises important questions and challenges for basic and clinical researchers. In this Review, we discuss the ethical, clinical and financial implications that have arisen with the approval of nusinersen, and address how these are likely to affect therapy development for SMA and related conditions.

Clinical and genetic background of SMA

Clinically, SMA is divided into different subtypes on the basis of severity and age of onset[3]. SMA type 0 is the most severe form of the disease, with patients requiring respiratory support from or soon after birth and leads to death within weeks, even with intensive respiratory support. The most common form of the disease (SMA type I, also known as Werdnig–Hoffmann disease) is also severe, and is characterised by very early disease onset before 6 months of age. These children are never able to sit unsupported and typically die within the first 2 years of life. SMA type II is of intermediate severity: onset is usually between 6 and 18 months of age and affected children can sit unsupported. Although life expectancy is reduced in patients with SMA type II, largely owing to respiratory complications, the majority of these individuals live into adulthood, albeit with substantial disability. The onset of SMA type III (also known as Kugelberg–Welander disease) most often occurs around the age of 3 years; patients achieve the ability to walk and have a normal life expectancy, albeit with considerable neuromuscular weakness and clinical heterogeneity. Finally, SMA type IV is the mildest form of SMA with onset in adulthood, normal life expectancy and modest disability compared to other types of SMA.

The diverse clinical phenotypes of SMA all result from the same genetic defect: homozygous deletion or point mutation of *SMN1*, which lead to loss of SMN protein expression[1]. Humans are unique in having a second copy of this gene, *SMN2,* which has an almost identical coding sequence (FIG. 1a). However, a one nucleotide difference at the start of exon 7 leads to alternative splicing of most *SMN2* transcripts[4,5]. Normally, splicing of *SMN1* leads to full-length mRNA that is translated into full-length SMN protein (294 amino acids, 32 kDa) (FIG. 1b). By contrast, alternative splicing causes removal of exon 7 in *SMN2,* resulting in a shortened mRNA that encodes a truncated and unstable form of the SMN protein (SMNΔ7; 282 amino acids, 30.5 kDa) that is quickly degraded[6]. However, a minority of *SMN2* pre-mRNA transcripts retain exon 7 after splicing, which results in the production of variable levels of full-length SMN protein[7], influenced by developmental stage and tissue type[8-10]. Importantly, the presence of *SMN2* provides a backup, effectively, for the loss of *SMN1* in patients. The recognition that differences between *SMN1* and *SMN2*-derived protein originate from alternative splicing led to intensive investigation of the underlying mechanism and ultimately resulted in the development of nusinersen (FIG. 1c, reviewed in detail elsewhere[11,12]).

SMN1 and *SMN2* are located in an unstable genomic region; consequently, the number of copies of *SMN2* varies between individuals[13]. All patients carry at least one copy of this gene and the complete absence of any form of *SMN* is embryonic lethal[14]. Most phenotypic variability in patients with SMA, therefore, is explained by variation in copy number of *SMN2*. Interestingly, however, the number of *SMN2* copies does not always correlate directly with the observed clinical phenotype[15]. A complete understanding of this relationship is complicated by the fact that levels of SMN protein in peripherally available tissues, such as blood and fibroblasts, are variable and do not always correlate with *SMN2* copy number and mRNA levels[16,17]. In addition, genetic modifiers, such as *PLS3*, *CORO1C* and *NCALD*, also influence disease severity and symptoms[18-20], further illustrating the high genetic complexity that can be associated even with a largely monogenetic disorder such as SMA.

Cellular roles of SMN

The cellular functions of SMN have been extensively studied with a view to gain insight into how loss of protein function leads to pathogenesis of SMA[21]. SMN interacts with many other proteins and has been implicated in numerous cellular processes, including ribonucleoprotein (RNP) formation, ubiquitin homeostasis, cytoskeleton dynamics, and

endocytosis (FIG. 2). Depletion of SMN results in defects in all of these pathways, and tissue-specific disruption of subsets of these cellular pathways might help to explain differential vulnerability between cell types in SMA. For example, within the CNS, motor neurons are the primary pathogenic target[22], but a variety of other cell types — including astrocytes, Schwann cells and sensory neurons — have also been shown to both be affected by and influence SMA pathogenesis[23-26].

RNP formation. The cellular role of SMN that has been studied most extensively is its canonical role in the formation of small nuclear RNPs (snRNPs)[27]. snRNPs consist of seven Sm core proteins and one snRNA, and form part of the spliceosome in the nucleus. SMN facilitates the formation, transport and maturation of snRNPs as part of a multiprotein complex that consists of SMN, several Gemin proteins (Gemin2–8) and serine-threonine kinase receptor-associated protein (also known as Unrip)[28-30]. Research has now shown that SMN plays a general part in RNP assembly[31,32]. This role includes the assembly, regulation and function of small nucleolar RNPs (which are required for post-transcriptional modification of non-coding RNAs, including ribosomal RNAs)[33], signal recognition particles (which are required for the transport of newly synthesised proteins)[34] and telomerase[35], which extend beyond the canonical cellular role of SMN in snRNP biogenesis.

NMJ maturation and stability. In contrast to defects in splicing, which seem to exert their influence largely during the late stages of SMA pathogenesis[36,37], the neuromuscular junction (NMJ) is an early pathogenic target in SMA[38]. A number of NMJ defects have been extensively characterised in SMA, including cytoskeletal abnormalities (such as the accumulation of neurofilaments) and denervation of endplates, which indicate a neuronal dying-back pathology[39-41]. These defects are observed before the onset of pronounced clinical symptoms and are preceded by functional synaptic defects[42,43]. Importantly, these defects have been identified not only in animal models of SMA, but also in patients with SMA, in whom neurofilament accumulation, denervation and electrophysiological defects have been detected[44,45].

The molecular mechanisms linking depletion of SMN to pathology at the NMJ in individuals with SMA are incompletely understood. A further complication is that, at least in mice, degenerative changes largely occur during the developmental timeframe normally associated with postnatal maturation of the NMJ. Consequently, experiments exploring these pathogenic changes have highlighted important temporal requirements for SMN expression at the NMJ during development: for example, whereas depletion of SMN in

neonatal mice leads to pathogenic changes at the NMJ, SMN depletion in adult mice was not associated with SMA phenotypes[46]. Thus, SMN expression is carefully regulated in motor neurons throughout development, which has clear ramifications for NMJ pathology and also has profound implications regarding the optimal timing of delivery for SMN-therapies.

Ubiquitin homeostasis and mitochondrial dysfunction. The occurrence of early pathogenic changes at the NMJ in SMA highlights the importance of synaptic stability for the maintenance of motor neuron homeostasis. Synaptic stability is maintained by several molecular pathways, including the control of energy metabolism by mitochondria and the regulation of ubiquitin homeostasis[47]. Both of these pathways are disrupted in SMA. Depletion of ubiquitin-like modifier activating enzyme 1 (UBA1), the primary E1 ubiquitin-activating enzyme, has been reported across a range of animal models of SMA and in patients with the disease[48-50]. Moreover, downstream targets of UBA1, including β-catenin (CTNNB1), were found to accumulate as a result of UBA1 depletion[50]. Therapeutic targeting of UBA1 or its downstream targets (such as CTNNB1) has shown considerable promise in animal models of SMA[50,51], which suggests that this pathway represents an additional therapeutic target for SMA.

Studies in animal models of SMA and in tissue from patients with SMA have also pointed towards impaired mitochondrial function as a key consequence of SMN depletion in SMA[52,53]. For example, depletion of SMN in cell lines leads to reduced levels of ATP and defects in mitochondrial trafficking[54-56]. Furthermore, changes in bioenergetic pathways regulated by PGK1 have been shown to modify motor neuron vulnerability by retrograde labelling of motor neuron pools controlling muscles with varying degrees of denervation at the NMJ in models of SMA[57]. Interestingly, these pathways could be modulated both by genetic overexpression or knockdown of PGK1 and by the PGK1-activating small molecule terazosin, indicating that they are also therapeutically targetable[57].

Cytoskeletal dynamics and endocytosis. Evidence from cellular and animal models of SMA, as well as from patients with SMA, indicates that changes in the cytoskeleton and endocytosis pathways are affected downstream of SMN depletion and play an important part in SMA[58-60]. Cytoskeletal dynamics have a crucial role in neuronal development and morphology and are of particular importance in processes involving neuronal differentiation, including maturation of the NMJ. Therapeutic modulation of these pathways in models of SMA increases survival by correcting NMJ defects and improving muscle function[61,62]. The

importance of cytoskeletal and actin dynamics in SMA is further illustrated by findings in patients with SMA. In individuals from families discordant for SMA, upregulation of the actin-binding protein plastin 3 (PLS3) protected those carrying a homozygous *SMN1* deletion from developing SMA[19]. Functionally, PLS3 upregulation in SMA models corrected numerous SMA phenotypes, including NMJ morphology[18,63].

In addition to the regulation of NMJ morphology, cytoskeletal dynamics also have a central role in the regulation of endocytosis. Endocytotic processes are essential to synaptic function — including signal transduction at the NMJ — and have been shown to be defective in SMA. In a zebrafish model of SMA, interaction between PLS3 and coronin 1C (CORO1C) increases the amount of F-actin, which rescues endocytosis and axonal phenotypes[18]. The relevance of this pathway is further illustrated by the identification of neurocalcin delta (NCALD) as a genetic modifier of SMA[20]. NCALD also binds to actin and clathrin, a calcium-sensitive regulator of endocytosis. Targeting NCALD in models of SMA restores defects in endocytosis and improves NMJ function[20].

Defective local protein translation might link molecular pathways in SMA. Taken together, the cellular pathways discussed here indicate a broad role for SMN in motor neuron biology and SMA pathogenesis that extends far beyond its canonical role in snRNP biogenesis. However, many fundamental questions remain regarding SMN biology, with obvious implications for the development of appropriately-targeted and effective therapies (BOX. 1). Several of the identified functions of SMN — such as in ubiquitin and energy homeostasis, cytoskeleton dynamics, endocytosis, and NMJ stability — might be more closely inter-related than they initially seem. For example, carefully-controlled local protein translation is a unifying factor of all of these processes[64,65]. SMN has been shown to regulate transport and translation of specific mRNAs, including β-actin, cpg15, CARM1 and gap43[66-73]. The proteins encoded by these RNAs are important for neuronal development, and their decreased expression results in morphological changes in cellular models of SMA, suggesting an involvement with defective NMJ development. SMN has also been shown to play an important part in the regulation of global protein translation *in vivo* — most likely via its influence on ribosome biogenesis[74]. Thus, correction of defective protein translation will probably be a crucial requirement for SMA therapies if they are to fully address the underlying biological mechanisms regulating disease pathogenesis.

Advances in SMA therapy development

In contrast to other neurodegenerative diseases (for example, amyotrophic lateral sclerosis; ALS), the combination of a well-established, largely monogenetic cause for SMA and the availability of accurate and robust animal models of the disease (BOX 2) has put therapy development at the centre of SMA research. In addition to the approval of nusinersen, several other therapies are in advanced stages of preclinical and clinical development (TABLE 1), which suggests that more advanced and effective therapies will continue to emerge over the coming years.

SMN-targeted therapies. Since the discovery that SMA is caused by a homozygous deletion of *SMN1*, efforts to treat SMA have largely focussed on developing the ability to restore SMN expression. Currently, several different approaches to increase SMN levels have progressed through to the late stages of clinical development. First, several approaches are being developed that target splicing of *SMN2* to increase the levels of the full-length mRNA and protein. Several antisense oligonucleotides (ASOs) have been developed that are directed against sequences in *SMN2* that either enhance or inhibit *SMN2* exon 7 splicing[12] (FIG. 1c), which have proved to be highly effective in preclinical studies[75,76]. Nusinersen is at the forefront of these ASO-based therapies, and has now been approved in numerous countries around the world (including the USA, Europe, Canada and Japan) for the treatment of all types of SMA. Although around half of patients with type I SMA show substantial improvements in motor function following treatment, nusinersen can have a more modest effect in other patients[77-79]. This variation is probably due — at least in part — to delays that exist between diagnosis and delivery of treatment, as patients who were started on treatment early performed substantially better than those who started treatment late in the disease course[79]. The NURTURE trial, which is currently underway, addresses this issue further by including only children with presymptomatic SMA. Moreover, other trials are underway that aim to further assess the efficacy of nusinersen in older patients with SMA type II and III. These are important studies for which the outcomes will be essential to generate a more complete picture of the efficacy of nusinersen across patients with SMA.

One particular disadvantage of most current ASO approaches is the need for repeated intrathecal injections, which limits distribution of the ASO to the periphery, is expensive and can pose an additional burden to patients and clinicians as it is an invasive treatment that – during the initial loading phase – needs to be regularly repeated. Next-generation ASOs might overcome these issues by improving efficacy and enabling

alternative, less invasive delivery routes[80]. Moreover, clinical studies are currently underway in patients with SMA to assess the efficacy of orally-administered small molecule therapies that enhance the inclusion of exon 7 in the splicing of *SMN2*. Preclinical studies indicate that several of these compounds can be highly effective in rescuing SMA phenotypes[81]. Other studies have attempted to increase SMN protein levels either by inhibiting histone deacytelases, increasing STAT5 activity or inhibiting SMN ubiquitination[82-86], but although these approaches seemed to hold promise in early preclinical experiments, clinical studies so far have been disappointing[87-89].

In addition, efforts are being made to restore SMN expression via gene replacement therapy. A self-complementary adeno-associated virus serotype 9 is currently under clinical development as AVXS-101 that aims to re-introduce SMN systemically following a one-off, intravenous injection. Preclinical studies have shown that this approach can lead to extensive amelioration of SMA phenotypes in different animal models by widespread restoration of SMN expression, including in the spinal cord[90-93]. An initial clinical safety and efficacy study in patients with type I SMA indicates encouraging progress[94]. In particular, no major adverse events were reported, suggesting that gene therapy treatment was safe and well-tolerated in young children. All patients who received a high, therapeutic dose in the study survived beyond time-points normally expected, and reached motor milestones not normally associated with a diagnosis of type I SMA[94]. However, this study was based on a very small, carefully-selected group of patients. Consequently, more-extensive multicentre trials are required to establish the potential efficacy of this gene therapy approach in a larger and more diverse cohort of patients with SMA. Moreover, despite the promise of this approach, viral gene therapy is not a commonly used procedure in clinical practice. Safe and effective delivery of gene therapy to all patients with SMA, therefore, is likely to require substantial developments in infrastructure to make it widely available.

Neuroprotective and muscle-enhancing therapies. In addition to SMN-targeted therapies, several SMN-independent therapies are moving towards clinical development. These include neuroprotective therapies, of which olesoxime is at the most advanced stage of clinical trials. Olesoxime is an oral drug that is thought to exert its neuroprotective effects by preventing increased permeability of mitochondria under stress conditions[95]. Initial results from a phase II clinical trial in non-ambulant patients with SMA type II and III suggest that olesoxime is safe and leads to a modest functional improvement[95], but further studies are required to reach a definitive conclusion on the efficacy of olesoxime for the treatment of patients with SMA. Moreover, drugs that have demonstrated efficiency in supporting muscle

function in several preclinical studies, including CK-2127107 and SRK-015, are now being tested with a view to improve muscular symptoms in patients with SMA. These drugs target different aspects of muscle function, but both aim to reduce muscle fatigue and weakness[96]. In addition to these novel compounds, drugs that have been approved for use in other diseases, including pyridostigmine (approved for myasthenia gravis) and 4-AP (approved for multiple sclerosis), are being clinically investigated in SMA for their abilities to reduce muscle fatigability and increase muscle endurance. With nusinersen expected to prolong survival in a growing number of patients with SMA, such muscle-enhancing and neuroprotective treatments — in combination with recommendations concerning exercise and physiotherapy — could provide additional support for patients by limiting issues related to muscle fatigability and weakness. In addition, the SMN-independent nature of these therapeutic developments suggests that their ability to provide general support and protection to the neuromuscular system might also make them promising candidates for the treatment of other neuromuscular diseases.

Therapeutic targeting of SMA modifiers and combinatorial therapy approaches. In addition to the SMN-targeted and neuroprotective therapies that are in advanced stages of clinical development, the discovery of other therapeutically targetable cellular pathways and modifiers of SMA pathology (such as actin dynamics and ubiquitin homeostasis[50,51,62]) provides hope that additional therapies can be developed to further supplement SMN-dependent treatments. Importantly, these discoveries provide opportunities to develop next-generation 'SMN-plus' combinatorial therapies, in which multiple molecular targets and body tissues can be targeted simultaneously to generate a robust amelioration of disease symptoms across the lifespan. Indeed, a number of studies within the past few years have illustrated the preclinical promise of such combinatorial approaches for SMA. For example, a partial rescue of disease phenotypes in a mouse model of SMA has been achieved using SMN-targeted ASOs at suboptimal doses, thereby mimicking the situation in patients with SMA for whom SMN-targeted therapies do not provide a complete rescue of symptoms. Genetic modifiers of SMA have been therapeutically targeted in the context of this suboptimal SMN-targeted ASO model. Increased expression of *PLS3* resulted in a marked improvement in survival in these mice, in parallel with restoration of defects in actin dynamics and endocytosis[18,63]. Similarly, down-regulation of NCALD (a genetic modifier that has a negative effect on severity of SMA) in a combinatorial approach with suboptimal SMN-ASO treatment improved a number of neuromuscular phenotypes in a mouse model of SMA[20]. Taken together, these studies provide an important proof-of-principle that

combinatorial ‘SMN-plus’ approaches can provide a promising avenue for the development of future, next-generation therapies for SMA.

Clinical, financial and ethical implications

The approval of nusinersen for the treatment of SMA, obtained from authorities in both the USA and Europe, was greeted with unanimous relief and praise from the research and patient communities[97,98]. This success has fundamentally transformed the clinical landscape into one where a realistic hope of disease-modifying therapies now exists for patients. Nusinersen has acted as a trailblazer for ongoing and future preclinical studies and clinical trials by showing that treatment of a devastating neurological condition is possible via therapeutic development based on sound scientific rationale — in this case, targeting the splicing of *SMN2*[12]. The approval of nusinersen has had a profound effect not only on the field of SMA research, but also across the wider discipline of neuromuscular disease research, in which it will stimulate attempts to develop splice-modulating oligonucleotide therapies[99]. The proof-of-principle that splice modulating oligonucleotide therapies can be both safe and effective across a fairly diverse range of patients brings this cutting-edge technology firmly into the armoury of the neurology and neuromuscular disease clinic.

Perhaps expectedly, these notable clinical successes have also raised a range of challenging issues that now need to be considered[100]. With regards to nusinersen, the most pressing of these is that of access to therapy for patients. As discussed, repeated intrathecal injections are currently needed to deliver nusinersen[101], which is both expensive and clinically challenging, thereby providing a potential barrier to access for some patients. For example, the clinical infrastructure required to deliver such therapies across large geographical areas and to vulnerable (for example, very young) individuals presents logistical issues on a scale that have not previously been encountered for a patient population with a neuromuscular disease such as SMA. The majority of animal-based and patient-based data indicate that delivery of SMN-targeted therapies is required as early as possible in the disease time-course to generate maximal therapeutic benefits[79,94,102]; consequently, there is considerable urgency to develop an appropriate infrastructure that can deliver nusinersen — and, potentially, also future therapies — to an expectant patient population quickly after diagnosis.

For this arrangement to become a reality, newborn or maternal screening programs for SMA will probably be required. This type of program is technically feasible through the

use of approaches such as real-time PCR and short-amplicon melt profiling[103,104] for newborn screening, and non-invasive prenatal testing using fetal DNA from maternal blood — as is now routinely used for testing for aneuploidy and rhesus factors during pregnancy. Excitingly, several American states have succeeded in introducing assessment for SMA in newborn screening programs. Any successes that can be attributed to these early adopters of screening programs are likely to be key in persuading other geographical regions to follow suit. Emerging clinical data seem to support findings by preclinical studies that highlight the importance of starting treatment for SMA as early as possible[78,79,94], and the adoption of screening programs offers one realistic means of improving the effectiveness of therapies for SMA in the near future. Nevertheless, these programs will have to overcome substantial logistical and practical hurdles, as well as considerable ethical issues, in order to be widely implemented. One example of an ethical dilemma that surrounds newborn screening programs stems from the current uncertainty as to how patients with mild forms of SMA will respond to available therapies, together with our limited knowledge regarding the long-term safety and efficacy of newly emerging therapies in patients with SMA, which results in an unknown benefit-to-risk ratio of the treatments in these individuals. Interestingly, however, research suggests that many parents would be willing to pay to test their newborn children for SMA[105] despite the controversy surrounding such tests. This view is likely to be further reinforced by the emergence of genuine therapeutic options for the condition.

Balancing expectations of patients with SMA and their families. Once issues of access have been overcome, clinicians will need to balance the expectations of patient and carers regarding the therapeutic benefits that nusinersen — or other emerging therapies — might provide. Currently available clinical efficacy data for nusinersen clearly indicates that ASO therapies have variable effects in different patients[77-79]. Thus, even in the patients who respond best to therapy, neuromuscular performance might still lag behind comparable neuromuscular development achieved by unaffected children. Future studies will be required to explain these effects, as well as to detail any sustained benefits that can be achieved in the long-term. Likewise, long-term monitoring of patient populations with SMA who have received SMN-targeted treatments will provide answers to the question of whether sustained amelioration of neuromuscular symptoms can lead to the unmasking of sub-clinical systemic symptoms (for example in heart, vascular system and other non-neuronal tissues and organs[106-112]).

The careful clinical counselling and discussions that are required to appropriately manage expectations of potential benefits and risks of treatment will be facilitated by results

from a recent investigation, which examined how patients with SMA and their families define 'meaningful change' in association with treatment[113]. This study indicated that small functional changes are often already perceived as meaningful, as long as they are maintained, and that patients and their families find outcome measures in therapy studies related to quality of life of particular importance[113]. However, these discussions also need to consider the possibility that the potential benefits of treatment often can be unwittingly exaggerated by a range of sources, including social media[114]. Likewise, given the broad spectrum of clinical phenotypes that exist between patients with severe (type 0–I) or milder (type III–IV) SMA, and the focus on patient groups with type I SMA in many clinical trials, another important consideration is how effective — or otherwise — treatments such as nusinersen are likely to be for type III and IV patients who have been living with milder forms of the condition for an extended period of time, or for patients with very severe SMA with an onset at or immediately after birth. Clinical trials in patients with mild SMA are more difficult to undertake than those in patients with type I SMA, particularly with regards to the identification of reliable and robust outcome measures, and will naturally take longer to complete. Moreover, the effects of SMN-targeted therapies on very severe, type 0 cases of SMA are currently unclear, as treatment in these individuals would need to be delivered *in utero*. Until such data are available, establishment of clear clinical expectations of therapeutic benefits of the first generation of SMN-targeted therapeutics for all patients with SMA might be difficult.

Financial burden of SMN-targeted therapies. Even if all of the complications and issues that have been discussed here can be dealt with successfully, thereby providing widespread access to SMN-targeted therapies for all patients with SMA, the financial implications of such treatments still represent one of the largest barriers to overcome. As with the arrival of any new clinical treatment — especially with those for rare diseases that are based on cutting-edge technology — the controversial issue of pricing has been at the forefront of public discussions concerning nusinersen[115]. Following approval of nusinersen, Biogen announced its pricing structure in the United States: US$750,000 for six injections during the first year of treatment and $375,000 per year for each subsequent year of treatment. Such pricing inevitably leads to a range of complex issues that affect patient groups across the globe and across healthcare systems — whether treatment provision is through insurance-based schemes in the United States or through National Institute for Health and Care Excellence approval for delivery via the National Health Service in the United Kingdom, for example — and is likely to lead to geographical inequalities in availability and access.

Orphan drug prices can vary widely across European countries[116]. Moreover, crucial differences have emerged between countries worldwide with regards to the development of legislation and regulations concerning access to orphan drugs. As a result, countries such as China and India currently lack legislative protocols provided by e.g. the American Food and Drug Administration (FDA) and European Medicines Agency (EMA), required to ensure access to emerging therapies for conditions such as SMA[117]. As the prevailing trend is for continued increases in the price of speciality drugs[118], these issues are unlikely to be simply transient and, consequently, they will only dissipate once additional treatments enter the marketplace to compete with nusinersen. It is crucial, therefore, that all stakeholders — from patients and advocacy groups through to scientific researchers, industry, and regulatory agencies — continue to work together to ensure that the exciting and promising therapies emerging from research pipelines are successfully translated into accessible and effective treatments for all patients. Importantly, these efforts need to be placed in the context of the well-known psychosocial costs of SMA[119], as prolonged survival in SMA will likely increase the need for specialised care and thereby further increase the already substantial psychosocial and financial burden that SMA patients and their families experience[120-122].

Future clinical trials in the post-nusinersen landscape: SMA and beyond. Finally, consideration should be given to how clinical trials of treatments for SMA will be conducted in the post-nusinersen landscape. Given that nusinersen now represents an approved treatment for SMA, with gene therapy approaches likely to follow closely behind, recruitment of patients into new clinical trials for SMA therapies are likely to become ever more difficult. Researchers engaged in the development and testing of new therapies will be faced with the challenge of recruiting patients onto new clinical trials with the potential risk of a patient being assigned to a placebo group or receiving a dose or therapy that remains ineffective, in the knowledge that a proven therapy known to have the capacity to influence disease severity and progression already exists.

The research findings detailed in this Review also strongly suggest that SMN restoration alone — whether via an ASO, gene therapy and/or small molecule based approach — does not represent a therapy that is sufficient to ameliorate all symptoms in every patient with SMA. Whether this deficit is due to the presence of a critical time-window for therapy delivery, or to the existence of different pathological mechanism underlying the slower, more progressive decline observed in patients with mild forms of the disease, additional non-SMN targeted therapies will probably be required to deliver effective therapy that is capable of covering the whole lifespan. Thus, the next generation of therapies for

SMA are expected to be of a primarily combinatorial 'SMN-plus' nature. Although this idea is scientifically and clinically exciting, development and robust assessment of the efficacy of such treatment approaches will be challenging when the majority of patients who are available to participate in trials are already receiving an SMN-targeted therapy (such as nusinersen or one of the emerging gene therapy or small molecule-based approaches), which have varying effectiveness on a case-by-case basis. Indeed, recruitment of drug naïve patients into new clinical trials might be nearly impossible in the near future. This added level of complexity will need to be addressed by further strengthening the collaborations between stakeholders to develop clinical trial capability and methodologies that are capable of assessing combinatorial therapies in an unbiased and reliable manner.

Despite these challenges, the lessons being learnt from past and current clinical trials involving patients with SMA are serving to influence and inform therapy development and testing strategies that have relevance to a wide range of other neurological and neuromuscular conditions. For example, both the development of robust outcome measures and standards of care for SMA[123-126] have been important for the generation of an effective clinical trial infrastructure. For example, outcomes measures such as the modified Hammersmith scale, CMAP and 6-minute walk test were tested and optimised during past trials and are now routinely used in current trials where and when appropriate. Moreover, the current challenges with regards to newborn screening and with respect to management of multiple clinical trials for different therapies in the post-nusinersen landscape are likely to be watched closely by investigators from other fields who are wrestling with similar issues for other clinical conditions.

The possibility also exists that at least some of the therapies and treatments currently being developed for SMA have the potential to act as effective therapies for other, related conditions. For example, an awareness is a growing regarding the molecular overlap between SMA caused by defects in *SMN1* and other, rarer *SMN1*-independent forms of SMA (such as X-linked SMA caused by mutations in *UBE1*[49-51,127]). The molecular mechanisms governing disease pathogenesis in SMA overlap substantially with those in ALS[128-132]. Thus, the considerable research efforts that are beginning to yield clinical promise for patients with SMA might also result in longer-term therapeutic benefits for a larger, more diverse group of diseases and patients.

Conclusion

The broad approval of nusinersen for the treatment of SMA has generated considerable optimism within research and patient communities regarding the future therapeutic landscape. However, a number of major challenges remain that demand the development of a more detailed understanding of the molecular mechanisms underlying SMA in order to deliver the next-generation of therapies that will be required to supplement current and future SMN-targeted therapies. Moreover, the approval of nusinersen raises a number of ethical, logistical and financial issues. How these issues are addressed over the coming years will be highly instructive for future therapy development, not only for SMA but also for other neurological conditions. The ongoing dialogue between basic scientists, clinicians, patients with SMA and SMA advocacy groups — which is something that the SMA field has always excelled at — will be of vital importance for the delivery of continuing successes in SMA therapy development.

Box 1 | Fundamental outstanding questions

Since the initial discovery that SMA is caused by homozygous deletion of *SMN1*, much progress has been made in our understanding of the pathogenesis of the disease and the cellular mechanisms that are affected by SMN depletion. However, various fundamental questions remain unanswered.

Motor neuron specificity. Although SMA is a multi-systemic disorder that affects tissues other than motor neurons, motor neurons are undoubtedly the primary pathological target. Why motor neurons are more sensitive to SMN depletion than other tissues and cell types remains to be discovered.

Temporal and tissue requirements for SMN expression. How absolute levels of SMN protein vary over time and between tissues under normal conditions remains to be fully determined. Similarly, whether all tissues show equal amounts of SMN depletion in individuals with SMA remains unknown.

Identification of modifiers and other determinants of SMA subtypes. Although the main genetic cause of SMA is well-defined and the SMA subtype correlates well with the number of *SMN2* copies in general, substantial clinical variability is observed between patients of the same genotype. A better understanding of other modifiers of SMA (including genetic modifiers) will enable more accurate prognosis. Moreover, therapeutic targeting of endogenous SMA modifiers represents a promising approach for the development of future treatments.

Mechanistic links between non-canonical SMN pathways. Despite known effects of SMN depletion on non-canonical pathways in SMA (such as ubiquitin homeostasis, mitochondrial dysfunction, and actin dynamics), the molecular mechanisms linking these pathways — individually or collectively — to disease pathogenesis need further exploration.

Box 2 | Disease model systems for SMA.

The SMA field has benefited greatly from the availability of a number of reliable mouse models of the disease, generated relatively soon after the discovery of the genetic cause of the disease. Several mouse models, each with their own specific characteristics, are now routinely used to study SMA disease mechanisms and to undertake preclinical testing of possible new therapies. Indeed, all therapies that are currently in clinical development were first shown to be efficient in mouse models of SMA. In addition, non-mammalian small animal models, such as *Drosophila* and zebrafish, are facilitating high-throughput screening of therapeutic compounds. However, the advent of SMA therapies brings a need for further development and refinement of disease models. For example, pharmacological mouse models that are based on a partial rescue of the disease phenotype using nusinersen-like ASOs are likely to be valuable for testing the efficacy of combinatorial therapeutic approaches. Other types of disease models, such as larger mammalian models (such as pigs) and patient-based cells lines (such as iPSC-derived motor neurons), will add further to the researcher's toolbox. Central to achieving further progress in advancing our understanding of the role of SMN in SMA pathogenesis will be the ability to take advantage of this wide range of model systems by combining data from the full range of animal models (from mice and zebrafish through to pigs) and patient samples (such as iPSC-derived motor neurons). Use of these tools will enable researchers to fully establish the key molecular mechanisms underlying SMA and aid the development of future, next-generation therapies for patients with SMA.

Figure Legends

Figure 1 | Genetics of SMA. a | Overview of the human chromosome 5q13 locus, which contains a telomeric copy (*SMN1*) and a centromeric (*SMN2*) copy of the two human *SMN* genes. This genomic locus is highly variable with ~200kb duplications commonly observed that are associated with the copy number variability for *SMN1* and *SMN2*. b | Splicing and protein production from *SMN1* and *SMN2* mRNA. Splicing of *SMN1* pre-mRNA produces full-length *SMN1* mRNA that is translated into full-length, functional SMN protein. Full-length SMN protein contains multiple functional domains that mediate its interaction, function and localisation, including a tudor domain (which regulates the interaction of SMN with RGG-domain containing proteins, including Sm proteins), a proline-rich region (which regulates SMN–profilin interaction) and a YG-box domain (which regulates SMN self-oligomerization). By contrast, splicing of *SMN2* pre-mRNA produces full-length mRNA in only a minority (~10%) of cases. Most splicing of *SMN2* pre-mRNA produces *SMN2* mRNA that lacks exon 7 (*SMN2Δ7*), which results in a truncated protein that is rapidly degraded. Importantly, the exact tissue and time-dependent regulation of SMN protein expression, including *SMN2*-derived full-length SMN protein, remains to be determined. Note that exon 8 is non-coding and therefore not represented as part of the full-length protein; however, after alternative splicing of *SMNΔ7*, 4 amino acids of exon 8 are included before translation is terminated. c | In *SMN1*, the CAGACAA-site at the 5'-end of exon 7 represents an exonic splice enhancer (ESE) sequence. This sequence is associated with binding of splicing factors that normally promote its inclusion, and thereby the formation of full-length *SMN1* protein. The C to T transition within this sequence that differentiates *SMN2* from *SMN1* leads to formation of an exonic splice suppressor (ESS), which, through binding of hnRNPA1, promotes the exclusion of exon 7 and thereby the predominant production of *SMN2Δ7* mRNA and unstable SMNΔ7 protein. The intronic splice silencer, ISS-N1, just upstream of exon 7, contains two hnRNPA1 binding sites and has a much stronger effect on exon 7 exclusion than does the C to T transition in the exonic sequence. Nusinersen and other ASOs target ISS-N1, thereby preventing the binding of hnRNPA1 and promoting exon 7 inclusion and the production of full-length SMN protein. Next-generation ASOs and small molecules targeting *SMN2* splicing also include compounds that target other splicing elements, such as element 1 and 2.

Figure 2 I Cellular pathways and therapeutic targets in SMA. First-generation SMA therapies, including nusinersen, are predominantly aimed at increasing SMN protein levels. These therapies therefore mainly target the nucleus and cell body of motor neurons (and other cells). Next-generation therapies for SMA, aimed at providing neuroprotection and / or targeting modifiers of SMA, largely target other regions of the motor neuron (e.g. the axon and neuromuscular junction).

Figure 1

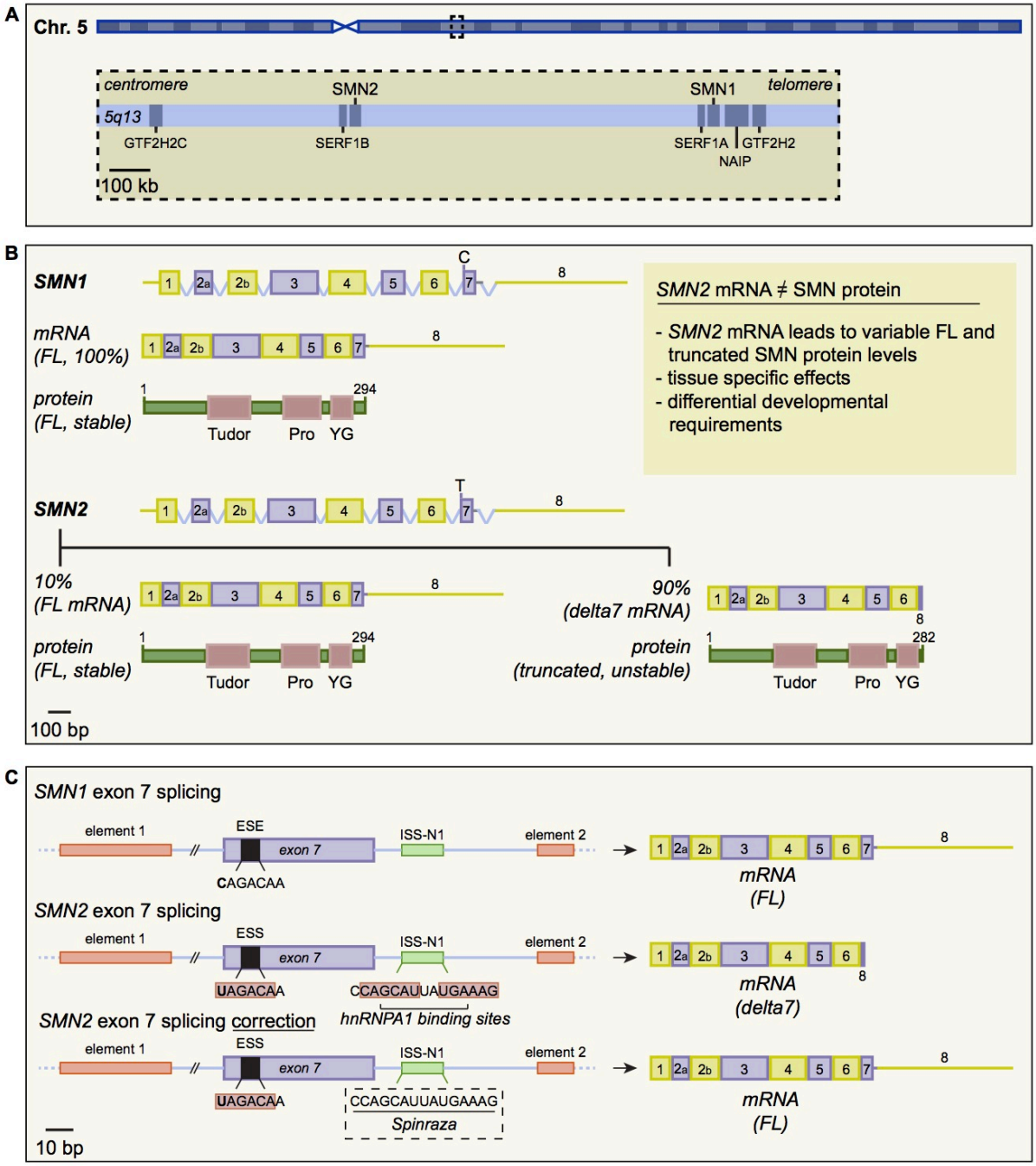
A
Chr. 5
centromere
SMN2
SMN1
telomere
5q13
GTF2H2C
SERF1B
SERF1A
GTF2H2
NAIP
100 kb
B
SMN1
C
8
mRNA
(FL, 100%)
protein
(FL, stable)
294
Tudor
Pro
YG
SMN2 mRNA ≠ SMN protein
- SMN2 mRNA leads to variable FL and truncated SMN protein levels
- tissue specific effects
- differential developmental requirements
SMN2
T
10%
(FL mRNA)
90%
(delta7 mRNA)
protein
(truncated, unstable)
282
100 bp
C
SMN1 exon 7 splicing
element 1
ESE
exon 7
ISS-N1
element 2
CAGACAA
mRNA
(FL)
SMN2 exon 7 splicing
ESS
UAGACAA
CCAGCAUUAUGAAAG
hnRNPA1 binding sites
mRNA
(delta7)
SMN2 exon 7 splicing correction
CCAGCAUUAUGAAAG
Spinraza
10 bp

Figure 2

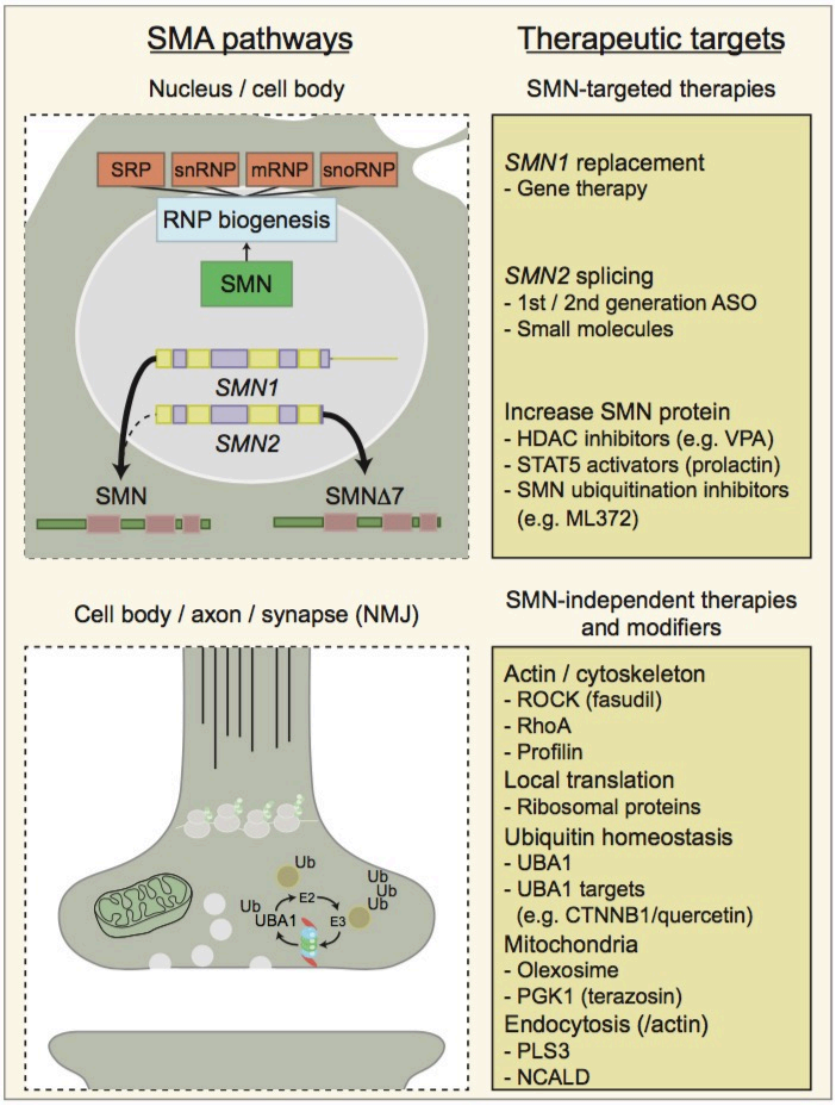
SMA pathways
Therapeutic targets
Nucleus / cell body
SMN-targeted therapies
SRP
snRNP
mRNP
snoRNP
RNP biogenesis
SMN
SMN1
SMN2
SMN
SMNΔ7
SMN1 replacement
- Gene therapy
SMN2 splicing
- 1st / 2nd generation ASO
- Small molecules
Increase SMN protein
- HDAC inhibitors (e.g. VPA)
- STAT5 activators (prolactin)
- SMN ubiquitination inhibitors
(e.g. ML372)
Cell body / axon / synapse (NMJ)
SMN-independent therapies
and modifiers
Ub
Ub
Ub
Ub
Ub
E2
UBA1
E3
Actin / cytoskeleton
- ROCK (fasudil)
- RhoA
- Profilin
Local translation
- Ribosomal proteins
Ubiquitin homeostasis
- UBA1
- UBA1 targets
(e.g. CTNNB1/quercetin)
Mitochondria
- Olexosime
- PGK1 (terazosin)
Endocytosis (/actin)
- PLS3
- NCALD

Table 1. Concise overview of current therapeutic developments for SMA.

Therapeutic approach	**Therapy**	**Target**	**Stage of development**
SMN targeted therapies	Nusinersen (ASO)	*SMN2* splicing	Approved
	Other experimental ASOs	*SMN2* splicing	Clinical trial (phase I/II/III), preclinical
	Small molecules: RG7910, LMI070	*SMN2* splicing	Clinical trial (phase I/II)
	Gene therapy: AVXS-101	*SMN1* replacement	Clinical trial (phase I)
Neuroprotection	Olesoxime	Mitochondria	Clinical trial (phase II / III)
Muscle enhancement	CK2127107	Fast troponin (activator)	Clinical trial (phase I/II)
	SRK-015	Myostatin (inhibitor)	Clinical trial (phase I)
	Pyridostigmine, 4- aminopyridine	Fatigability and endurance	Clinical trial (phase II/III)
	Exercise and/or physiotherapy	Overall muscle strength	NA
Modifiers of SMA	Upregulation of UBA1: possible gene therapy or small molecule therapy	Ubiquitin homeostasis	Preclinical
	Upregulation of PLS3: possible gene therapy or small molecule therapy	Actin dynamics	Preclinical
	Downregulation of NCALD: possible gene therapy or small molecule therapy	Endocytosis	Preclinical
	Quercetin-mediated inhibition of CTNNB1	Motor neuron stability	Preclinical
	Fasudil-mediated inhibition of ROCK	Actin dynamics	Preclinical

ASO, antisense oligonucleotide; CTNNB1, β-catenin 1; NCALD, neurocalcin-delta; PLS3, plastin-3; NA, not applicable; SMA, spinal muscular atrophy; SMN, survival motor neuron protein; UBA1, ubiquitin-like modifier-activating enzyme 1

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