Author/s:
Farrar, MA;Park, SB;Vucic, S;Carey, KA;Turner, BJ;Gillingwater, TH;Swoboda, KJ;Kiernan, MC

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Emerging Therapies and Challenges in Spinal Muscular Atrophy

Michelle A. Farrar, PhD,[1] Susanna B. Park, PhD,[2] Steve Vucic, PhD,[3] Kate A. Carey, PhD,[1] Bradley J. Turner, PhD,[4] Thomas H. Gillingwater, PhD,[5] Kathryn J. Swoboda, MD,[6] and Matthew C. Kiernan, DSc[2]

Spinal muscular atrophy (SMA) is a hereditary neurodegenerative disease with severity ranging from progressive infantile paralysis and premature death (type I) to limited motor neuron loss and normal life expectancy (type IV). Without disease-modifying therapies, the impact is profound for patients and their families. Improved understanding of the molecular basis of SMA, disease pathogenesis, natural history, and recognition of the impact of standardized care on outcomes has yielded progress toward the development of novel therapeutic strategies and are summarized. Therapeutic strategies in the pipeline are appraised, ranging from *SMN1* gene replacement to modulation of *SMN2* encoded transcripts, to neuroprotection, to an expanding repertoire of peripheral targets, including muscle. With the advent of preliminary trial data, it can be reasonably anticipated that the SMA treatment landscape will transform significantly. Advancement in presymptomatic diagnosis and screening programs will be critical, with pilot newborn screening studies underway to facilitate preclinical diagnosis. The development of disease-modifying therapies will necessitate monitoring programs to determine the long-term impact, careful evaluation of combined treatments, and further acceleration of improvements in supportive care. In advance of upcoming clinical trial results, we consider the challenges and controversies related to the implementation of novel therapies for all patients and set the scene as the field prepares to enter an era of novel therapies.

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Spinal muscular atrophy (SMA) is characterized by muscle weakness and severe physical disability attributed to motor neuron degeneration in the spinal cord and brainstem. It continues to represent the leading genetic cause of infant death attributed to respiratory insufficiency, with a pan-ethnic incidence of approximately 1 in 11,000 live births and a carrier frequency of 1 in 40 to 67.[1] The most common form of SMA is caused by mutations in the survival motor neuron 1 (*SMN1*) gene, resulting in SMN protein deficiency.[2] The almost identical survival motor neuron 2 (*SMN2*) gene produces a small amount of functional SMN protein, and SMN2 copy number is recognized as a major modulator of the SMA phenotype.[3] There have been significant advances in the understanding of the underlying pathogenic process in SMA. Concomitantly, there has been progress in defining disease progression and natural history, with a concerted effort in developing outcome measures and clinical trial readiness. Consequently, novel genetic therapies, aimed at modulating SMN protein expression, have resulted in significant clinical improvement in SMA patients for the first time, thereby providing much needed hope for the treatment of this devastating disease. As such, the present review will focus on recent advances made toward developing novel therapeutic strategies and future challenges as the field enters into a new treatment era.

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Address correspondence to Dr Michelle Farrar, School of Women's and Children's Health and Sydney Children's Hospital, High Street Randwick, NSW 2031, Australia. E-mail: m.farrar@unsw.edu.au

From the [1]Discipline of Paediatrics, School of Women's and Children's Health, UNSW Medicine, The University of New South Wales, Sydney, Australia; [2]Brain & Mind Centre and Sydney Medical School, University of Sydney, Sydney, Australia; [3]Department of Neurology, Westmead Hospital and Western Clinical School, University of Sydney, Sydney, Australia; [4]Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australia; [5]Euan MacDonald Centre for Motor Neurone Disease Research, University of Edinburg, Edinburg, United Kingdom; and [6]Center for Human Genetics Research, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

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TABLE 1. Classification and Subtypes of Spinal Muscular Atrophy

Type	Age of Onset	Maximal Motor Milestone	Motor Ability and Additional Features	Prognosis[c]
SMA 0	Before birth	None	Severe hypotonia; unable to sit or roll[a]	Respiratory insufficiency at birth; death within weeks
SMA I	2 weeks (Ia) 3 months (Ib) 6 months (Ic)	None	Severe hypotonia; unable to sit or roll[b]	Death/ventilation by 2 years
SMA II	6 to 18 months	Sitting	Proximal weakness; unable to walk independently	Survival into adulthood
SMA III	<3 years (IIIa) >3 years (IIIb) >12 years (IIIc)	Walking	May lose ability to walk	Normal life span
SMA IV	>30 years or 10 to 30 years	Normal	Mild motor Impairment	Normal life span

[a]Need for respiratory support at birth; contractures at birth, reduced fetal movements.
[b]Ia joint contractures present at birth; Ic may achieve head control.
[c]Prognosis varies with phenotype and supportive care interventions.

Progress in Understanding the Natural History of SMA

SMA has a broad range of age of onset, severity, rate of progression, and variability between and within subtypes (Table 1).[4,5] In type I SMA, earlier age of onset is associated with worse prognosis and mortality[6]; the median age to death or ventilation (>16 hours per day) is 13.5 months and 10.5 months for patients with 2 copies of *SMN2*.[5] Patients with SMA type II have a better prognosis than those with type I disease, with 93% surviving to 25 years.[6] After age 15, a relative stability in function develops with subsequent gradual decline over time.[7] Age of onset is also a predictor of functional ability, with patients classified as SMA IIIa having a 73% probability of walking 10 years after diagnosis, whereas SMA type IIIb patients have a 97% probability of walking 10 years after diagnosis.[8]

A better understanding of the natural history of SMA has been crucial to the development of relevant outcome measures and implementation of clinical trials. Understanding variability in the rate of clinical progression in SMA related to age, SMA type, and ambulation status will assist in the development of appropriate motor function scales that are able to monitor subtle, but clinically meaningful changes (see Finkel et al[9]). The clinical heterogeneity in motor function is a challenge and connected with the range of possibilities for change in short time frames. The pattern of age-related changes in motor function in SMA types II and III is nonlinear, and there are different patterns of progression between ambulant and nonambulant patients.[7,10] In nonambulant patients, variable improvement in motor function occurs up to 4 to 5 years of age, before functional ability (eg, in upper limbs) declines between 5 and 15 years. After age 15, a relative stability in function develops with subsequent gradual decline over time.[7] Decline in motor and respiratory function within a 12-month period was often minor, although progression was variable between individuals.[7,9] This slow rate of progression, particularly in milder phenotypes, poses a major challenge to clinical trials in SMA because most trials need to be completed within 1 to 2 years. Rather than SMA phenotype, ambulant status may be more relevant to the trajectory of disease progression and consequently in trial design and outcome measure development. For instance, different outcome measures are required to monitor clinically important differences among ambulant and nonambulant cohorts, yet it will also be important to better connect scales that measure different functional levels to be able to more accurately demonstrate improvements.[10]

In addition to motor function scales, neurophysiological studies have provided insights into clinical progression, timing of motor neuron loss, and compensation in SMA; however, these studies have predominantly focused on later stages of disease. Electrophysiological outcomes compound muscle action potential (CMAP) amplitude and motor unit number estimation (MUNE) correlate with age, SMA type, functional status, and

TABLE 2. Current Management of Spinal Muscular Atrophy

	Assessments	Interventions
Respiratory	Cough effectiveness; respiratory muscle function tests; overnight oximetry; forced vital capacity (>6 years)	Referral to respiratory specialist Routine immunizations Annual influenza vaccination
	Overnight polysomnography if disordered breathing suspected	Airway clearance techniques and cough assistance—chest physiotherapy, postural drainage, mechanical or manual cough assistance Noninvasive ventilation (nocturnal and/or daytime if indicated)[a]
	Acute respiratory infections	Antibiotics intensified airway clearance, increased ventilation support[a]
Gastrointestinal and nutritional	Feeding and swallowing assessment Assess caloric intake	Nutritional supplementation, modifying food consistency, optimizing oral intake, positioning and seating alterations Nasogastric, nasojejunal, or percutaneous gastronomy—as soon as reduced oral intake is recognized
	Assess for signs of reflux or aspiration	Nissen fundoplication (if indicated)
	Assess for constipation	Hydration, regular oral aperients
Orthopedic and rehabilitation	Posture, mobility, function Contractures	Equipment to assist with mobility, self-care, and function
	Scoliosis	Physiotherapy, standing frames, orthoses
	Hip subluxation/dislocation	Spinal surgery[b]
Psychological	Assess for depression/anxiety	Counseling, pharmacotherapy

The management of SMA incorporates a multidisciplinary and supportive approach, including neurologists (adult and pediatric), respiratory physicians, geneticists, gastroenterologists, palliative care physicians, rehabilitation specialists, orthopedic surgeons, and allied health.
[a]The appropriate level of interventional support to prolong life, particularly in SMA type1, is controversial and the consensus statement[16] recognizes the importance of discussions with the family to explore and define potential quality-of-life and palliative care issues. The philosophy and introduction of proactive respiratory support in patients with SMA type 1 varies considerably and practice varies internationally.
[b]There is no consensus on management of scoliosis or hip subluxation/dislocation in nonambulant patients.

SMN2 copy number.[11] Gradual decline in motor and respiratory function may, in part, be related to physical growth in SMA types II and III, with the provision that CMAP amplitude remains stable.[9] In addition, the CMAP amplitude may also remain constant despite reduction in MUNE values, a finding explained by the presence of motor unit loss with compensatory collateral sprouting and supported by axonal excitability studies.[12] Transcranial magnetic stimulation techniques assessing central motor networks also suggest adaptive changes.[13] Neurophysiological studies are rare in presymptomatic infants, but suggest that motor function is initially relatively preserved.[11,14] In SMA type I, this loss of motor function is followed by early and precipitous reductions in CMAP and MUNE responses. The onset, time course, and extent of motor neuron loss has not been established in SMA types II and III, yet is important in determining whether there is a specific therapeutic window. Preclinical studies of SMN restoring therapies, such as gene therapy and antisense oligonucleotides to correct SMN2 messenger RNA (mRNA) splicing, provide support for the utilization of electrophysiological biomarkers for treatment stratification, determining response and defining therapeutic windows.[15]

Clinical Care

In parallel with preclinical advances, continued improvements in multidisciplinary care and technological advances have altered the natural history of SMA since the Consensus Statement for standards of care in SMA was published in 2007 (Table 2; Appendix 1)[16]; however,

even in areas of general consensus, marked variability in the implementation of the standards of care, particularly in the use of ventilation, nutritional support, and scoliosis surgery, have been observed.[17,18] For patients participating in clinical trials, it is crucial to standardize the management of modifiable factors, particularly nutrition and respiratory support, given that differences in care practices may impact outcome. However, at the most severe end of the SMA phenotypic spectrum, in the setting of uncertain therapeutic efficacy, this remains a challenge. Advances in drug development are likely to impact the standards of care for SMA, particularly given that successful disease modification will inevitably alter natural history and necessitate new standards of supportive care and interventions.

Respiratory Management

Respiratory complications are the major cause of morbidity and mortality in SMA. The onset of peripheral hypoventilation may be asymptomatic and initially occur during sleep, but with deterioration daytime respiratory dysfunction develops. Objective measures of respiratory function are not routinely performed in children younger than 4 to 6 years because of complexity of the required maneuvers; potential alternative measures of respiratory function, for example, sniff nasal inspiratory pressure and forced oscillation techniques, have been proposed in this population because they are noninvasive and require less patient cooperation.[19] Attempts to identify nighttime hypoventilation using pulmonary testing in SMA patients have been largely unsuccessful,[20] highlighting the continued benefit of overnight polysomnography. Assessment frequency needs to be individualized, based on current functional status and rate of disease progression, and should be supplemented with other clinical observations, such as assessment for paradoxical breathing and chest wall growth, among others.

The major respiratory complications faced by SMA patients include impaired cough—resulting in reduced clearance of lower airway secretions—hypoventilation, chest wall and lung underdevelopment, and recurrent infections that exacerbate muscle weakness. Although contentious, proactive management with noninvasive ventilation, even before the onset of paradoxical respirations, has led to improved survival, prevention, and improvement in chest wall deformity as well as improved quality of life.[21,22] In addition, optimizing airway clearance is important for acute and chronic management of SMA patients with secretion mobilization techniques, such as assisted coughing,[23–25] physiotherapy, and postural drainage.[26] The decision to progress to invasive ventilation with tracheostomy remains an ethical dilemma, and considerable variability exists between countries with no consensus in guidelines.[17,18] The goal of interventions should always be to improve quality of life with the provision of support and assistance to parents in making difficult decisions consonant with their values and beliefs.

Nutritional Support

Malnutrition is prevalent in SMA, with bulbar dysfunction and deterioration of nutritional status preceding and exacerbating respiratory failure with disease progression.[27,28] Appropriate nutritional management of SMA patients is critical for improving quality of life and optimizing survival,[29] although no clear consensus exists on the timing of initiation of nutritional support.

Orthopedic Considerations

Scoliosis is a common complication of SMA, present in 60% to 95% of patients, secondary to progressive muscle weakness. In SMA types I and II, scoliosis occurs earlier and a more-severe, progressive curvature is evident compared to SMA type III.[30] Progression of scoliosis may exacerbate respiratory dysfunction, gastrointestinal reflux, and increase postural discomfort.[30] Management of scoliosis includes nonsurgical options, such as physical therapy, bracing, and seating modification, and depending on the strategy used, may slow, but not necessarily prevent, curve progression. Additionally, surgical approaches are utilized in progressive scoliosis, most frequently in type II and III SMA. Posterior spinal fusion is typically implemented after skeletal maturity during adolescence in SMA, with iliac fixation used to assist correction of pelvic obliquity. Innovative surgical techniques utilizing growing rods (eg, vertical prosthetic titanium rib or magnetic rods) enable spinal growth while avoiding repeated invasive surgeries; however, medium- to longer-term implications remain unclear. Although surgery does not reverse the respiratory reserve lost because of scoliosis, it leads to improved life quality[31] and can slow deterioration of respiratory function.[32] Finally, with an intrathecally administered therapy showing promise in phase 3 clinical trials, construction of bony windows may be considered to facilitate drug administration.

Genetic and Environmental Insights Into Pathogenesis

Whereas mutations in *SMN1* characterize SMA, disease severity is also linked to a number of genetic modifiers. These modifiers are of relevance in enabling patient stratification in clinical trials, better prediction of an individual's prognosis, and establishing newborn screening. Patients have variable copy numbers of the *SMN2* gene, a related gene that differs from *SMN1* by only five

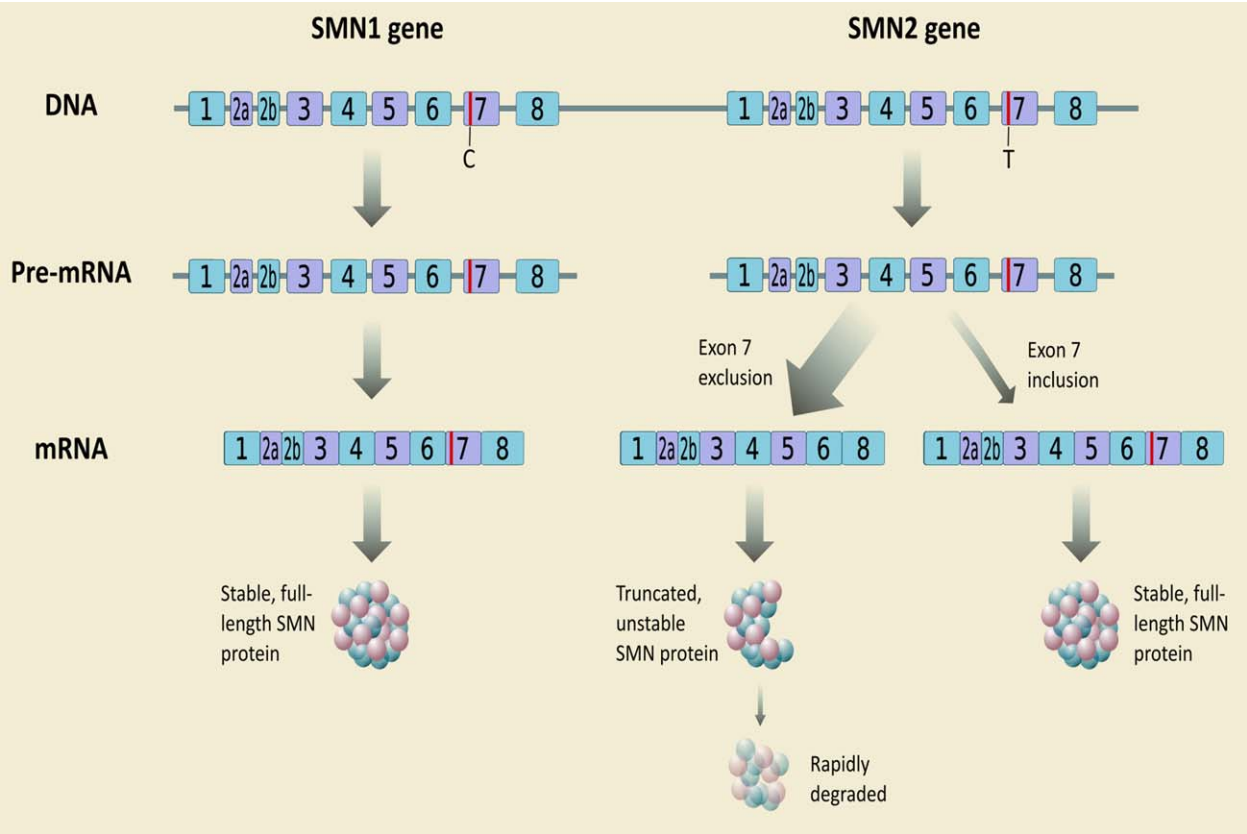

FIGURE 1: Genetics of Spinal Muscular Atrophy. In humans, the SMN protein is encoded by the *SMN1* and *SMN2* genes. The C to T substitution in exon 7 of *SMN2* is translationally silent, but alters splicing such that the majority of SMN2 transcripts lack exon 7 and the truncated protein is unstable. Normally, *SMN1* produces abundant SMN protein. In SMA, homozygous mutation of *SMN1* results in only a small amount of functional SMN protein contributed by the varying copy numbers of SMN2. mRNA = messenger RNA; SMN = survival motor neuron [Color figure can be viewed at wileyonlinelibrary.com].

nucleotides, altering splicing and leading to transcription of a nonfunctional SMN protein lacking exon 7 in the majority of transcripts (Fig 1).[33] SMN2 copy number is the main determinant of phenotype, although not solely sufficient to predict severity.[34] Sequence variations within the *SMN2* gene and upregulation of modifier proteins, such as plastin 3, may also positively modify phenotype.[35–37] In addition, nutritional deficiency, oxidative stress, and hypoxia, partly attributed to gastrointestinal dysfunction, may cause widespread splicing alterations, including *SMN2*, and accelerate SMA progression.[38–40]

How Low Levels of SMN Cause SMA

Recent insights into the role of SMN within motor neurons have furthered our understanding of the implications of SMN deficiency.[41] The best characterized role of the SMN complex is in the assembly of Sm proteins (a distinctive family of RNA associated small proteins) onto small nuclear RNAs (snRNAs), forming small nuclear ribonucleoproteins (snRNPs), which are essential components of pre-mRNA splicing machinery in cells.[42] SMN deficiency and therefore reduced snRNP assembly capacity are proposed to cause aberrant splicing or transport of RNPs to the detriment of motor neurons.[43] A recent study showed transcriptional dysregulation in motor neurons isolated from very young presymptomatic SMA mice that preferentially affected a small subset of genes involved in synaptogenesis and maintenance of neuromuscular junctions (NMJs).[43] Furthermore, some of these dysregulated motor neuron relevant genes showed underlying splicing changes, strengthening a potential link between aberrant splicing and motor neuron vulnerability.[43]

A second view of SMA pathogenesis contends that SMN has axonal function independent of splicing that may be disrupted in SMA. Consequently, SMN deficiency may impair targeting and local translation of axonal mRNAs essential for motor neuron development and maintenance.[44,45] Furthermore, SMN regulates several other fundamental cellular processes in the neuronal cytoplasm that are critical for maintaining axonal and synaptic health, including endocytic pathways, local translation, mitochondrial transport, and targeting to axons and ubiquitin homeostasis.[46–51]

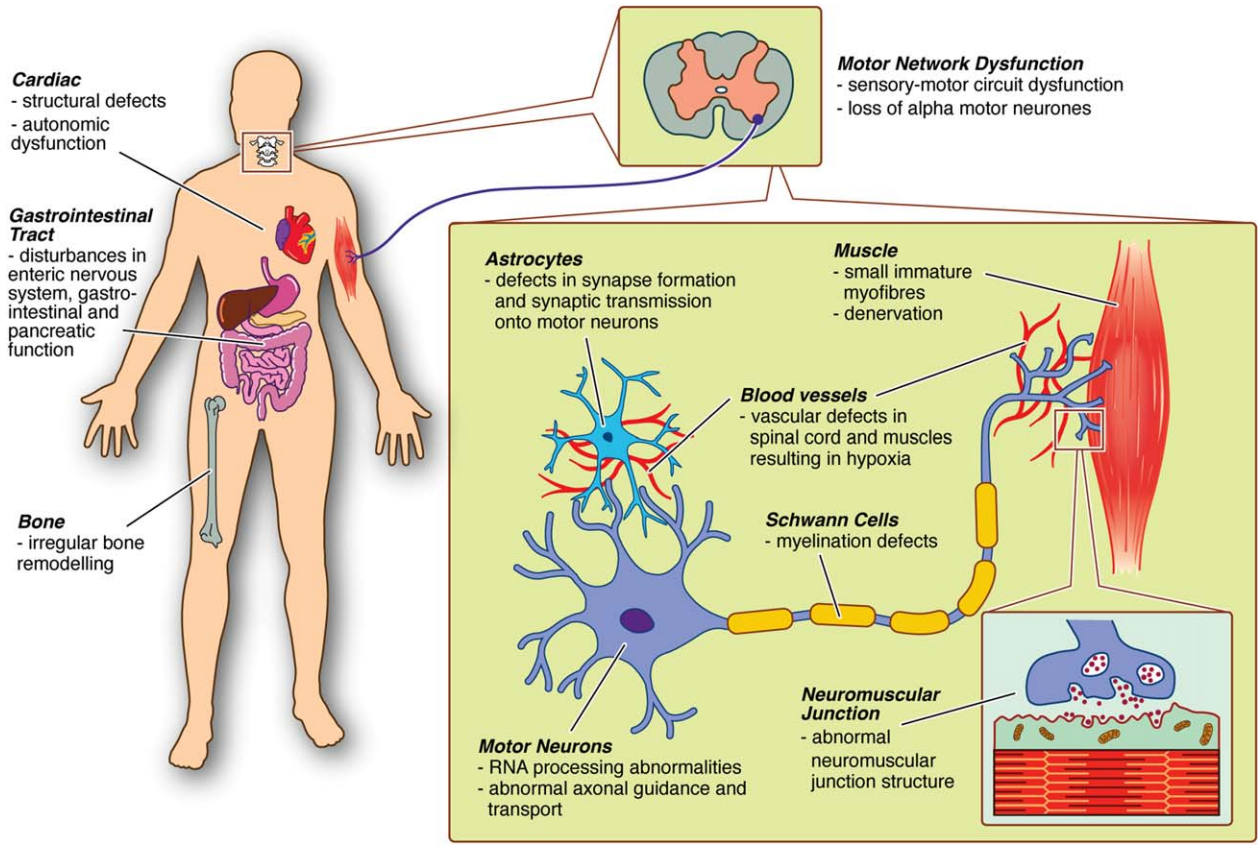

FIGURE 2: Pathophysiological findings in SMA. Multiple functional abnormalities in motor networks have been identified in SMA mice and humans, including defects in astrocytes, Schwann cells, motor neurons, and skeletal muscle. Disease-associated phenotypes have also been reported across a range of other organs in SMA mice (in some cases supported by data from human patients), including cardiac structural and functional abnormalities, gastrointestinal tract dysfunction, and irregular bone remodeling. One potential unifying factor may be a deficiency in the development of vasculature in SMA, with the resulting hypoxia likely impacting a range of cell types. SMA = spinal muscular atrophy. [Color figure can be viewed at wileyonlinelibrary.com]

Animal Models of SMA

Current understanding of SMA pathogenesis has been generated largely as a result of the availability of mouse models of SMA. These have been largely generated by targeting the endogenous mouse *Smn* gene, while using transgenic strategies to add variable copy numbers of the human *SMN2* transgene.[52] These animal models tend to phenocopy severe forms of the human disease, resulting in the majority of animal-based work focusing on early postnatal phenotypes. Recently, alternative strategies have been used to generate mice modeling less-severe forms of SMA, allowing investigation of disease pathogenesis and preclinical drug testing more relevant to type II and III SMA.[53,54] Additional animal models of SMA are also beginning to play an important role in SMA research, including *Drosophila* and zebrafish,[55,56] and recent developments suggesting that large animal models (eg, pigs) may be forthcoming.[57]

Although alpha motor neurons in the spinal cord remain the primary pathological target in SMA,[58] there is now accumulating evidence suggesting that other cells, tissues, and organs contribute to disease symptoms (Fig 2).[59,60] For example, there is now experimental evidence suggesting a non-cell-autonomous contribution to motor neuron degeneration from astrocytes and Schwann cells.[61,62] Likewise, low levels of SMN in skeletal muscle have been implicated in SMA pathogenesis with significant disruption of the molecular composition of skeletal muscle evident in presymptomatic severe SMA mice in the absence of detectable changes in lower motor neurons.[63] One potential unifying factor may be a deficiency in the development of vasculature in SMA; the resulting hypoxia would likely impact motor neurons as well as skeletal muscle and possibly contribute to the gastrointestinal defects (gastroesophageal reflux, constipation, and delayed gastric emptying) commonly observed in SMA patients.[64]Although the mechanisms mediating the effects of vascular depletion have not been fully elucidated, hypoxia has been identified as a modifier of *SMN2* splicing, potentially explaining some of the splicing alterations observed in SMA.[38,39,65]

Disease-associated phenotypes have been reported across a range of other organs in SMA mice (in some

cases supported by data from human patients). These include functional and structural cardiac defects,[66] abnormal development of the gastrointestinal tract, liver, and spleen,[64,67,68] and irregular bone remodeling and skeletal pathology.[69] These findings suggest that successful treatment of SMA may require systemic targeting of a range of affected tissues. However, how these findings will translate to humans is uncertain, for example, heart defects are rare in humans.

Defining the Therapeutic Window in Animal Models

In severe SMA mice, induction of SMN expression at gestation or in the early postnatal period substantially improves survival, whereas later induction is less effective.[70,71] Mice are resistant to SMN depletion after early postnatal stages, suggesting that there is a period of sensitivity to low SMN levels and that high SMN levels are required during this early postnatal stage.[70,72] Notably, in mouse models, the time period when SMN function is required coincides with the neonatal period of NMJ establishment, development, and maturation, suggesting that the mechanistic underpinnings of the therapeutic window are based on the pathways driving normal NMJ maturation. These observations imply that early correction of SMN levels in SMA types II and III is likely to be necessary and sufficient to protect the neuromuscular system, and lifelong expression of SMN may not be required.

Whereas most preclinical studies investigating SMN restoration in animals were limited to evaluating presymptomatic administration of gene therapy with viral vectors or antisense oligonucleotides to correct SMN2 splicing and enhance SMN expression, which, in most cases, robustly improves the health of SMA mice, some studies have tested the impact of pre- and postsymptomatic SMN restoration. Systemic delivery of these approaches largely rescued SMA mice's motor function, neuromuscular physiology, and life span when delivered within the first 3 postnatal days (P0–P3), but were less effective beyond P5 and gene therapy was not effective at P10, confirming the presence of a narrow therapeutic window.[73,74] However, SMN restoration using intravenous injection of self-complementary adeno-associated virus (scAAV9)-SMN vectors given at symptom onset had a marked effect with amelioration of severe proximal weakness and electrophysiological indices, in a porcine model of SMA[57] which may represent a more-relevant model for predicting efficacy in humans. Even so, presymptomatic delivery prevented the development of symptoms, suggesting that therapeutic windows are still critical in this model. Whereas it is accepted that animal models cannot recapitulate human SMA precisely, the translation of concepts of motor neuron degeneration to humans suggests that presymptomatic or early-symptomatic restoration of SMN (during NMJ maturation) will likely produce the best response to therapy. An unresolved issue remains as to whether commencing therapy in older patients will be effective. The time course and extent of motor neuron loss in type III or IV has never properly been mapped, largely attributed to a paucity of robust animal models of less-severe forms of SMA. Encouragingly, recent results from models of milder SMA phenotypes suggest that some therapeutic efficacy may be possible even at late disease stages.[75] Although the therapeutic window for SMA types III and IV has not been defined, the normal early motor development may suggest that it is linked to age of presentation and broader than types I and II. Preliminary clinical trial data are emerging and indicating that with SMN repletion motor neurons may not be irreversibly doomed. However, animal models recapitulating severe SMA show rapid postnatal motor neuron attrition and reduced efficacy with delayed treatments, such that the optimal success may ultimately arise from presymptomatic provision of therapy.[73,76]

Therapeutic Developments

The pipeline of therapies for SMA encompasses four different strategies, including *SMN1* gene replacement, modulation of *SMN2* encoded full-length protein levels, neuroprotection, and targeted improvements of muscle strength and function (Fig 3). Translational research continues to progress and clinical trials have recently reporting positive preliminary results related to safety and efficacy of the newest approaches (Table 3). This follows a number of negative clinical trials of repurposed drugs, including valproic acid and acetyl-L-carnitine, phenylbutyrate hydroxyurea, riluzole, and somatotropin,[77–79] despite promising preclinical data. Importantly, these negative studies have informed clinical trial design, validated the reliability and feasibility of specific outcome measures, and highlighted the importance of patient stratification.

In a mouse model of severe SMA, postnatal intravenous gene therapy using a viral vector rescued motor function and neurophysiology and extended survival from 2 weeks to beyond 250 days.[70] A phase 1/2a clinical trial for AVXS-101(a self-complementary AAV9 carrying the SMN gene under the control of a hybrid cytomegalovirus enhancer/chicken-β-actin promoter) in SMA type I infants has completed enrollment and initial observations in safety, survival, and motor function have been promising with all patients event free (death or

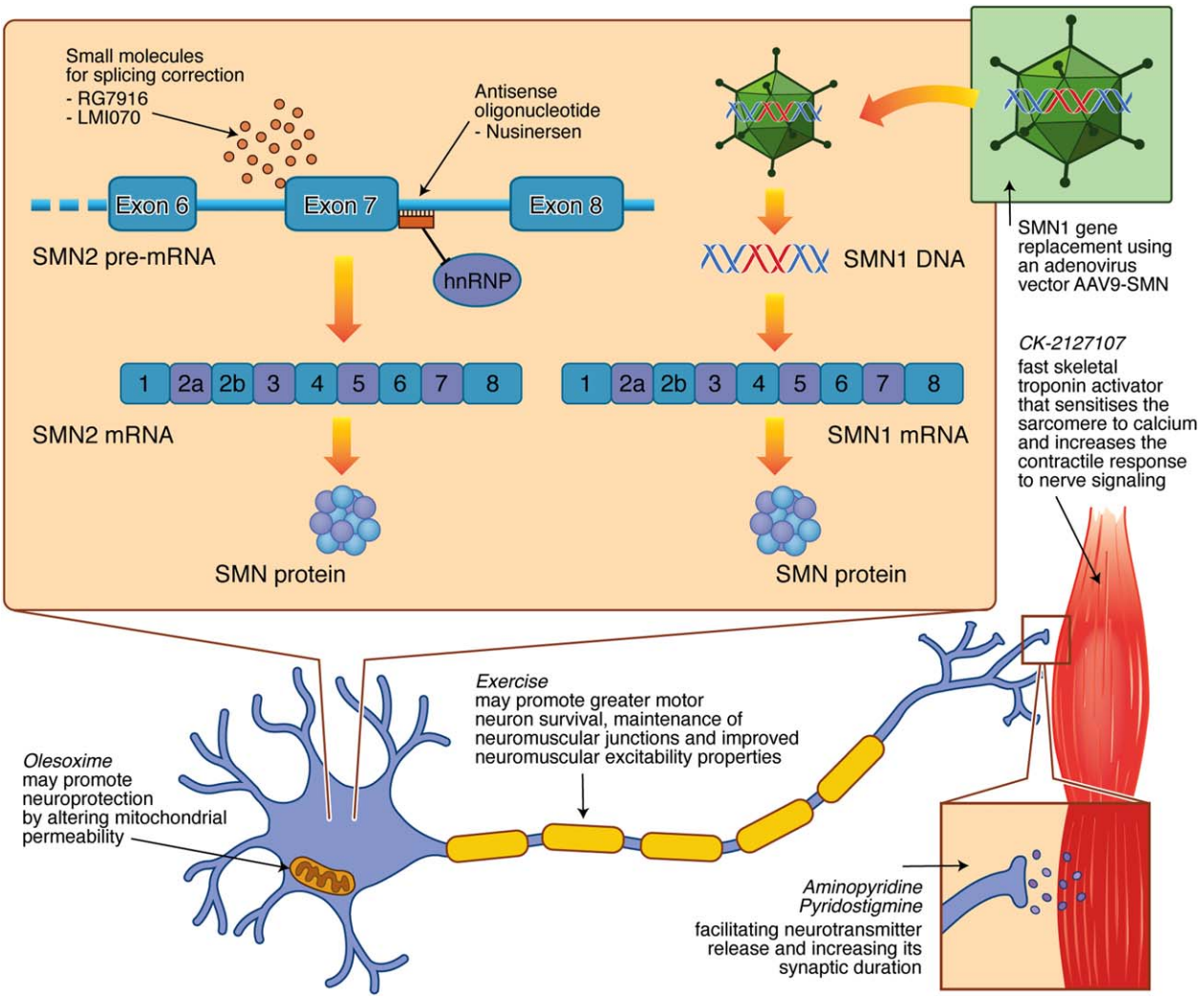

FIGURE 3: Therapeutic targets for SMA being investigated in clinical trials. SMN1 gene replacement therapy utilizes a self-complementary adeno-associated viral vector (AAV9-SMN) that crosses the blood–brain barrier following intravenous administration. Compounds that increase the production of fully functional SMN protein by modifying the splicing of SMN2 include the orally available small molecules, RG7916 and LMI070, and the intrathecally administered antisense oligonucleotide, nusinersen, which acts by displacing heterogenous nuclear ribonucleoprotein (hnRP) proteins from the intronic splicing silencer site on the SMN2 pre-mRNA. The neuroprotective effects of olesoxime, through altered mitochondrial permeability, and exercise, through greater motor neuron survival, maintenance of neuromuscular junctions, and improved neuromuscular excitability properties, are being investigated. Additional strategies focused on improving neuromuscular function and physical performance include CK-2127107, a fast skeletal troponin activator that sensitizes the sarcomere to calcium and increases the contractile response to nerve signaling, and 4-aminopyridine and pyridostigmine that may facilitate neurotransmitter release and increase its synaptic duration. mRNA = messenger RNA; SMN = survival motor neuron. [Color figure can be viewed at wileyonlinelibrary.com]

continuous noninvasive ventilation greater than 16 hours per day) and stabilization of pulmonary outcomes reported.[80] Modest improvements in motor function were observed in patients receiving a low dose of the study drug and greater improvements were shown in patients receiving the proposed therapeutic dose; 2 patients achieved normal motor function 4.9 and 10.3 months following treatment as measured by the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), a marked change from the natural history of SMA type I.[81]

The most advanced compound known to increase production of fully functional SMN protein is nusinersen (IONIS-SMN_{RX}), an antisense oligonucleotide administered intrathecally that modifies the splicing of *SMN2*. A phase 1 open label study of nusinersen in 28 SMA II and III patients aged 2 to 14 years reported that the drug was well tolerated with no safety concerns identified.[82] Transient back pain and postlumbar puncture headache were of a similar frequency to previous reports in infants and children undergoing lumbar puncture. Favorable time- and dose-dependent increases in muscle

TABLE 3. New Therapeutic Approaches in Spinal Muscular Atrophy: Current Clinical Trials

Approach	Study Description	Preliminary Results
SMN1 gene replacement		
AVXS-101[a,b]	Phase 1/2a gene transfer of SMN1 in SMA type I infants Two cohorts treated with a single dose of AVXS-101 delivered intravenously	Safety, survival, and motor function have been promising with all patients event free (death or continuous noninvasive ventilation greater than 16 hours per day) and stabilization of pulmonary outcomes.[80]
Modulation of SMN2 full-length protein		
Nusinersen (IONIS–SMN_{RX})[a]	10 phase 1 to phase 3 studies Nusinersen administered by intrathecal injection in SMA type I infants (0–6 months) and later-onset type II/III participants (age range, 2–14 years)	Favorable safety, tolerability, and encouraging clinical efficacy[83,84]; intrathecal administration tolerated, no drug-related adverse events[82] Phase 3 ENDEAR study in SMA type I and phase 3 CHERISH study in childen aged 2 to 12 years with SMA type II; primary endpoint met in each study at interim analysis with statistically significant improvement in motor milestones
RG7916 (RO7034067)	Phase 2 in adult and paediatric patients with type II and III SMA with oral delivery	
LMI070	Phase 1/2 study in infants with type I SMA (1–7 months) of oral LMI070	
Neuroprotection: promote survival of motor neurons		
Olesoxime (TRO19622)	Phase 2 studies in 3- to 25-year-olds with type II or nonambulant type III SMA	A greater percentage of patients were stable or improved compared with placebo; however, the primary endpoint was not met ($p = 0.07$).[89]
Exercise	Pilot study of a physiotherapeutic approach tailored to type II and III SMA patients aged 5 to 10 years	
Exercise	Muscle-strengthening program using hand weights and resistance bands in combination with a home-based cycle ergometry in type III patients aged 8 to 50 years	
Enhancing Nerve or Muscle Function		
CK-2127107	A phase 2 oral compound in SMA type II to IV (aged 12 years+).	
Pyridostigmine	Phase 2 study in SMA type III (aged 6 years+)	
4-aminopyridine	Phase 2/3 study assessing changes in walking ability and endurance in 18- to 50-year-olds with SMA type III	

[a]AVXS-101 and Nusinersen have been granted US Food and Drug (FDA) and European Medicines Agency orphan drug status and FDA fast-track approval.
[b]AVXS-101 has been granted FDA Breakthrough Therapy Designation. Following a FDA Type B meeting on September 30, 2016, a single-arm pivotal trial has been announced.

function were reported in patients 9 to 14 months post-dosing.[83] Observations from a phase 2 open-label study of nusinersen in 20 infants with SMA type I show increases in motor function, ranging from stable independent sitting to walking (the latter patients having 3 copies of SMN2, not the standard type I SMA copy number of 2), with no evidence of a therapeutic plateau in motor skills yet.[84] Interim analyses of phase 3 clinical trials evaluating nusinersen, including infants with SMA type I (ENDEAR) and children with SMA type II (CHERISH), have reached primary endpoints with improvement in motor milestones and favorable safety profiles. Regulatory filings have recently been submitted and an expanded access program initiated for SMA type I. The effect of presymptomatic administration of nusinersen is currently being evaluated and will provide pivotal insights into therapeutic windows. Further advances include the development of peptide-mediated oligonucleotides to enable systemic therapy and overcome difficulties with central nervous system delivery by repeated intrathecal injections, with recent efficacy demonstrated in rodent models.[85]

Orally bioavailable small molecules are being developed for selective *SMN2* splicing correction, and several (RG 7916 and LM1070) are entering early phase clinical trials. Administration of these compounds to mice with severe SMA increased SMN protein levels, motor function, and survival (from 18 to beyond 150 days).[76,86] Further clinical trials are also needed to define the efficacy of salbutamol, a β agonist that promotes exon 7 inclusion in SMN2 transcripts, following encouraging early results from several pilot studies in SMA patients.[87] Increasing the expression of *SMN2* with small molecules, such as quinazoline-derived compounds, moderately increased SMN mRNA and protein levels as well as survival in severe SMA mice.[88] However, plans to progress beyond phase 1 clinical trials have been terminated, reflecting challenges in translating disease-modifying benefits from mouse to human.

Olesoxime has entered clinical trials in SMA patients following demonstration of its neuroprotective properties motor neurons in cell culture and SMA mice. Phase 2 trials have been completed and though the primary endpoint was not statistically significant, a greater percentage of patients were stable or improved compared to placebo, suggesting that olesoxime may slow decline in motor function over 2 years in already symptomatic patients with SMA types II and III.[89] However, further data are needed to determine whether this is a clinically meaningful effect.

Additional strategies focused on improving neuromuscular function and physical performances in SMA patients are also being assessed in clinical trials. Among these is CK-2127107, which slows calcium release from fast skeletal muscle troponin and sensitizes the sarcomere to calcium thus increasing contractile response to nerve signalling; studies have demonstrated its efficacy in mouse models of motor neuron disease and it appears safe in healthy human volunteers.[90] Additionally, exercise-induced neuroprotection has recently been demonstrated in SMA-like mice with greater motor neuron survival, maintenance of neuromuscular junctions, and improved neuromuscular excitability properties, accompanied by positive metabolic and behavioral changes.[91] The benefits and risks of different types of exercise are being evaluated in SMA patients, and initial studies have demonstrated that resistance training is feasible, safe, and well tolerated and aerobic training increases oxidative capacity.[92,93] Further outcomes will be important in planning patient therapy and rehabilitation.

A number of potential SMN independent therapeutic targets have been identified in preclinical studies. These include the compounds, Fasudil and Y-27632, that regulate actin cytoskeleton integrity through Rho-associated protein kinase inhibition,[94,95] the antioxidant flavonoid, quercetin, that suppresses beta-catenin signaling,[96] BAY 55-9837 that indirectly stabilizes *SMN* mRNA,[97] and compounds that activate the mammalian target of rapamycin pathway.[98] In addition, RNA sequencing of motor neurons may identify novel downstream targets of splicing alterations. Stabilization of endogenous SMN protein provides a further therapeutic strategy, with STL-182 showing promising preclinical efficacy.[99]

Conclusions and Future Directions

There have been tremendous advances in therapeutic development in SMA, with treatment options rapidly evolving and preliminary results of clinical trials in patients producing new hope. In parallel, there has been substantial progress in understanding clinical disease progression and natural history to accelerate the implementation of clinical trials. Rodent models suggest that requirements for normal SMN levels are paramount during development of the motor unit, with SMN restoring therapies most effective early. The translation of these concepts to humans is needed to determine whether therapy in later stages of disease is beneficial. Critical for timely access to novel disease-modifying treatments is the rapid recognition of clinical manifestations and diagnosis, with presymptomatic diagnosis to guard against disease onset and progression the ultimate aim. Population-based newborn screening pilot programs are determining the feasibility and reliability of presymptomatic diagnosis,

and effective molecular methods have been validated on dried blood spots, including real-time polymerase chain reaction and high-resolution melting analysis.[100–103]

Improvements in multidisciplinary clinical care, together with advances in technology, have changed the natural history for patients with SMA. With new therapeutics emerging, it is likely that profound shifts in management approaches will transpire in severe SMA. These will also necessitate additional validation of nonmotor standardized and reliable outcome measures, particularly respiratory assessments, given that these are functionally meaningful and contribute substantially to morbidity. Secondary complications, such as scoliosis and contractures, may further limit the value of existing motor outcome measures. Furthermore, individual motor function scales are relevant to specific levels of SMA severity, and it will be important to better connect scales that measure different functional levels to be able to more accurately demonstrate improvements. Whereas motor function scales are a major focus and most relevant to SMA, strength testing, electrophysiological assessments, and metabolomic and proteomic outcomes measures are also being integrated into natural history studies and clinical trials in SMA.[104]

With the tantalizing prospect of novel therapies moving closer to clinical reality, more questions arise, compelling the formation of collaborative and linked future monitoring programs to determine the impact of these therapies. Longer-term monitoring programs should also include assessments of cognition, growth, autonomic function, and adverse events and enable a comparison and evaluation of combined treatments. These will serve to understand how novel therapies may affect phenotype over the longer term and the duration of effect—will they reduce progression, as well as stabilize or improve function? How will they affect requirements for permanent ventilation or age of death? What is the potential variability of responsiveness of different motor neuron subpopulations attributed to drug distribution or inherent differences in reversibility? Furthermore, the possibility that reinnervation may stress remaining motor neurons, resulting in a post-polio-like condition with late deterioration, must be considered. It is likely that combined therapies increasing SMN levels while also enhancing and preserving neuromuscular function and preventing additional systemic pathology will provide the best approach. In the setting of a first-in-class approved therapy, continued progress in developing second-generation and combination therapies will require innovative approaches in trial design. In addition, new challenges are arising with emerging therapies, including difficulties with access to treatment associated with the complexities, costs, and expertise required with intrathecal administration. Further efforts to ascertain optimal routes of drug delivery and distribution and defining the therapeutic window will be essential.

As the field looks toward a new treatment era, it is necessary to focus on timely access to novel, disease-modifying therapy and endeavoring to develop therapies for patients with SMA of all ages and severities.

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Author Contributions

The authors contributed equally to the literature search and the writing and formatting of the review, and to critically reviewing the manuscript.

Potential Conflicts of Interest

Dr Farrar has received an honoraria from Biogen. Professor Gillingwater is named on a patent application filed by the University of Edinburgh covering the use of beta-catenin inhibitors for the treatment of SMA (DD/P206869GB, filed October 7, 2013).

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