Spinal muscular atrophy: antisense oligonucleotide therapy opens the door to an integrated therapeutic landscape

Matthew JA Wood[1], Kevin Talbot[2], Melissa Bowerman[1*]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, UK

[2]Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, UK

*To whom correspondence should be addressed: melissa.bowerman@dpag.ox.ac.uk

ABSTRACT

Spinal muscular atrophy (SMA) is a devastating neuromuscular disorder characterized by loss of spinal cord motor neurons, muscle atrophy and infantile death or severe disability. It is caused by severe reduction of the ubiquitously expressed survival motor neuron (SMN) protein, due to loss of the *SMN1* gene. This would be completely incompatible with survival without the presence of a quasi-identical duplicated gene, *SMN2*, specific to humans. *SMN2* harbours a silent point mutation which favours the production of transcripts lacking exon 7 and a rapidly degraded non-functional SMNΔ7 protein, but from which functional full length (FL) SMN protein is produced at very low levels (~10%). Since the seminal discovery of the SMA-causing gene in 1995, research has focused on the development of various SMN replacement strategies culminating, in December 2016, in the approval of the first precise molecularly targeted therapy for SMA (nusinersen), and a pivotal proof of principle that therapeutic antisense oligonucleotide (ASO) treatment can effectively target the central nervous system (CNS) to treat neurological and neuromuscular disease. Nusinersen is a steric block ASO that binds the *SMN2* mRNA and promotes exon 7 inclusion and thus increases FL SMN expression. Here, we consider the implications of this therapeutic landmark for SMA therapeutics and consider how future developments will need to address the challenges of delivering ASO therapies to the CNS, with appropriate efficiency and activity, and how SMN-based therapy should be used in combination with complementary strategies to provide an integrated approach to treat CNS and peripheral pathologies in SMA.

INTRODUCTION

Spinal muscular atrophy (SMA) is one of the commonest genetic causes of death in infancy, with an incidence of 1:6000-10,000 births (1, 2). Autosomal recessive inheritance of inactivating mutations, in most cases deletion, of the survival motor neuron 1 (*SMN1*) gene (3), which produces the ubiquitously expressed SMN protein, leads to death of alpha motor neurons in the ventral horn of the spinal cord, denervation and muscle atrophy (2, 4). However, there is now significant evidence supporting additional functionally significant pathology in heart (5–7), liver (8), spleen (9, 10), gastrointestinal tract (11), pancreas (12), brain (13), vasculature (14), Schwann cells (15), lung (16) and bone (17). Complete loss of the SMN protein is embryonic lethal (18) but in humans, an evolutionarily recent duplication has given rise to a second gene, *SMN2*, identical to *SMN1* apart from 5 nucleotides (3, 19–22). Four of these single-nucleotide changes are inconsequential to the function of *SMN2*, in contrast to a critical C to T substitution at position 6 of exon 7, which causes aberrant splicing of the SMN transcript (20, 23). Whether inducing loss of an exon splicing enhancer (ESE) or the gain of an exon splicing silencer (ESS) (24, 25), this nucleotide change leads to skipping of exon 7 in ~90% of the produced mRNA transcripts, and to a non-functional and rapidly degraded SMNΔ7 protein (3). The ~10% of full-length (FL) SMN protein produced by *SMN2* is fully functional and sufficient to allow survival in the absence of FL SMN protein from the *SMN1* gene (Fig. 1A). Given that the number of copies of *SMN2* varies between individuals, *SMN2* is a critical determinant of disease severity whereby the number of copies defines the amount of FL SMN generated and establishes a dose-dependent relationship with the severity of SMA pathology (3, 26). This complexity at the genomic level thus results in a clinically heterogeneous disease classified by age of onset and disease severity. Type 0 is detected *in utero* and is the most severe form of SMA (27, 28) while Type IV has an adult onset and is the mildest form of the disease (29). Types I, II and III account for the majority of cases and have a childhood onset, with Type I patients typically dying before the age of 2, Type II patients being unable to walk and living until adulthood and Type III patients reaching a normal life expectancy albeit with variable ambulatory deficiencies (30, 31).

Since the discovery of the SMA-causing gene in 1995, intensive effort has been invested in the development of gene augmentation approaches such as delivering FL SMN via an adeno-associated virus (AAV) or promoting production of FL SMN from *SMN2* via histone deacetylase inhibitors (HDACi) or small molecules, all of which have been actively evaluated in clinical trials (Table 1). These strategies show promise and have been expertly

reviewed by others (32, 33). In December 2016, however, the therapeutic landscape for SMA drastically changed following the US Food and Drug Administration (FDA) approval of nusinersen (Spinraza™), also known as ISIS-SMN_{Rx} or ISIS 396443. Nusinersen is an antisense oligonucleotide (ASO) designed to promote *SMN2* exon 7 inclusion (Fig. 1B), which was developed by Ionis Pharmaceuticals and taken into clinical trial in partnership with Biogen (34, 35). On April 21st 2017, the European Medicines Agency (EMA) announced that the Committee for Medicinal Products for Human Use (CHMP) recommended approval of nusinersen for SMA patients in the European Union (EU). Nusinersen has thus quickly become the new standard of care benchmark against which new treatments for SMA will be compared and the data from published and forthcoming clinical trials will rapidly establish the successes and limitations of this approach, guiding future therapeutic endeavours. Here, we discuss what can be learnt from the nusinersen clinical trial data (34, 35) as we move forward in the development of next generation treatment strategies, with a particular emphasis on integrating a range of different therapeutic approaches that will benefit patients with all Types of SMA.

NUSINERSEN: FROM MOLECULAR TARGET TO CLINICAL TRIALS

The road to the first FDA-approved ASO therapy for SMA was paved by numerous research groups and years of detailed pre-clinical work, which has been previously expertly described (36–38). This concerted effort led to the identification of an intron splicing silencer N1 (ISS-N1) sequence in intron 7 of the *SMN2* gene, which favours skipping of exon 7 and thus production of SMNΔ7 (39, 40). Experiments further demonstrated that antagonizing the inhibitory ISS-N1 sequence using ASOs, small nucleotide sequences designed to bind a specific pre-mRNA sequence and modify its pre-mRNA splicing (38), promotes exon 7 inclusion and the production of FL SMN (Fig. 1B) as well as significantly extending survival in a severe SMA mouse model (39, 41). The transition from pre-clinical to clinical studies was then rapidly led by Ionis Pharmaceuticals and Biogen and to date, results from phase 1 and 2 studies have been published (34, 35).

The purpose of the phase 1 trial was primarily to determine the safety, tolerability and pharmacokinetics of a single intrathecal dose of nusinersen (34). A total of 28 Type II and III SMA patients were divided in 4 groups that received a single dose of the drug (1, 3, 6 or 9 mg). Nusinersen was administered directly to the cerebrospinal fluid (CSF) intrathecally, as its chemistry (2'-O-(2-methoxyethyl) (2'-MOE)) does not allow it to cross the blood brain barrier (BBB) and penetrate the central nervous system (CNS) when administered

systemically (e.g. intravenous, subcutaneous) (42). Nusinersen was deemed safe and well tolerated as no serious adverse events (AEs) were reported and all participants remained in the study. The presence of nusinersen could be detected by a modified ELISA method, in both plasma (> 24 hrs) and CSF (7 days), following the single dose in all groups and in the 9 mg group and the drug could even be measured in the CSF 29 days post-dose. To evaluate activity of the drug, SMN protein levels were assessed in CSF 9-14 months after the single-dose and showed that SMN levels more than doubled in the 6 and 9 mg groups. Given that small incremental increases in SMN expression would be predicted to lead to drastic improvements in neuromuscular function and lifespan (26, 43), the impact of nusinersen on SMN levels was considered to be a strong predictor of a potential positive effect on functional outcomes for SMA patients if the drug could be given earlier. Clinical assessment of participants revealed improvement in participants from the 9 mg group when compared to their baseline evaluations. Combined, the phase 2 study defined nusinersen as being safe and well-tolerated by Type II and III SMA patients as well as showing long-lasting functional activity in a dose-dependent manner (Table 2). Although unblinded and not placebo-controlled this study was important in laying the foundations for ongoing phase II and III nusinersen clinical trials in SMA patients.

A further open-label phase 2 study aimed at evaluating the safety and tolerability, pharmacokinetics and functional activity of multiple doses of intrathecal nusinersen in Type I SMA patients (35). In this case, it was performed with a historical control group, using data compiled from a published natural history case series (PNCR) (44). Twenty participants were divided into 2 groups, the first receiving an escalating dose of 6 to 12 mg while the second followed the same dosing schedule with a 12 mg dose from the outset. Here, all participants reported mild and moderate AEs while 80% reported serious AEs (77 in total), which were deemed to be a consequence of the natural history of the disease and not of the drug itself. Importantly, the drug could be detected in the CSF up to 168 days after dosing. For most participants, motor function assessment scores increased during the dosing regimen while the PNCR data shows a slow decline during disease progression. Survival was also significantly improved such that, at time of publication, most subjects were surviving without need of ventilation, in contrast with data from the PNCR study where median survival is approximately 10 months. Of note, one participant in the 6-12 mg group and two participants in the 12 mg group died during the trial. This provided an unprecedented opportunity to validate nusinersen target engagement with analysis of SMN protein and mRNA demonstrating the presence of the drug in neuronal cells as well as an increase in *FL*

SMN2 throughout the CNS compared to similar tissues obtained from non-SMA and untreated SMA infants. Thus, despite the limitations of the open-label trial design and the use of historical rather than in trial control subjects, this phase II nusinersen clinical trial demonstrates incontestable activity and functional benefits of the drug (Table 3). However, the death of 3 out of 20 participants (15%), and evidence of incomplete restoration of motor function, both features of subjects with established disease, highlights the need for further trials to specifically address the timing of nusinersen treatment for maximal benefit. Indeed, this is currently being investigated in a phase 2 study aimed at treating pre-symptomatic patients (NTC02386553) alongside a phase 3 phase 3 randomized, double-blind and sham-controlled study in later-onset SMA patients (> 6 months) NCT02292537).

BEYOND NUSINERSEN: CONSIDERATIONS AS WE MOVE FORWARD WITH ASO THERAPIES

The recent success of nusinersen is a strong proof-of-principle that ASOs can be used to target the CNS for SMA therapy and opens the door for applications in other neurodegenerative and neuromuscular diseases. However, while the results obtained in the nusinersen clinical trials are impressive and much needed within the community, they do point to several issues that need to be carefully considered as we proceed in the evaluation of second generation ASO strategies for SMA. Indeed, regardless of the approach utilized, improving efficiency and delivery of the ISS-N1 ASO is essential to reduce required dose as well as drug- and procedure-related AEs reported by the nusinersen clinical trials.

As mentioned previously, the ASO designed by Ionis Pharmaceuticals is in a 2'-MOE chemistry that requires that it be delivered directly to the CNS due to its inability to cross the BBB. This is carried out via a sensitive, invasive and technically difficult lumbar puncture (LP) that leads to severe post-LP headaches in some patients for up to one week. It is therefore critical that future development of ASOs incorporates novel methodologies to improve their capability to reach the motor neurons in spinal cord and brain, either via different chemistries or the use of vehicles to aid in their trans-BBB and intracellular delivery. WAVE Life Sciences™ is for example evaluating the efficiency of stereochemically optimised ASOs, whereby they eliminate the stereoisomer heterogeneity and variable activity that occurs in typical ASO preparation, thus rendering a purer and potentially more powerful drug. While they are currently in the pre-clinical discovery phase for *SMN2* ASOs, results from these experiments will be awaited with interest. Another approach to improve activity and delivery of ASOs is to link

them to a cell-penetrating peptide (CPP) (45), which not only promotes easier access to cellular compartments such as the nucleus, but also allows for a less invasive intravenous systemic administration due to the increased stability they confer to ASOs (46). We have recently combined the *SMN2* ISS-N1 ASO in a neutral phosphorodiamidate morpholino (PMO) chemistry to a CPP termed peptide nucleic acids/PMO internalization peptide 6a (Pip6a) and demonstrated that treatment of neonatal SMA pups via a facial vein injection results in a dramatic rescue in survival accompanied by improvement in neuromuscular phenotype (47). Importantly, we show that Pip6a-PMO is able to reach and upregulate FL SMN expression to therapeutic levels in spinal cord, brain and skeletal muscle. Sarepta Therapeutics™ is also investing in the development of CPPs to deliver ASOs and have recently reported significant benefits in heart and skeletal muscle of a canine model of Duchenne muscular dystrophy following the intravenous injection of a B peptide-PMO (48). Other strategies to modulate ASO formulation and delivery such as microparticles, nanoparticles, or injectable implants could hold extreme promise (49), although, to the best of our knowledge, are not yet initiated for SMA therapy.

In addition, ASOs targeted to alternate regulatory regions of *SMN2* are also currently being investigated. Indeed, a long non-coding RNA (lncRNA), termed *SMN-AS1*, has recently been identified that originates from the *SMN2* antisense strand and promotes inhibition of *SMN2* transcription, particularly in neuronal cells (50). Inactivating the *SMN-AS1* sequence with a targeted ASO resulted in increased SMN expression and crucially, combination of the *SMN-AS1* ASO with the *ISS-N1* ASO had an additive effect on SMN levels, significantly increasing the lifespan of severe SMA mice (50). RaNA Therapeutics™ have also published promising data showing that RN-005, a mixmer ASO composed of locked nucleic acid (LNA)-modified nucleotides interspersed with 2'-O-methyl nucleotides that binds to *SMN-AS1* lncRNA, increases FL SMN expression in patient fibroblasts and induced pluripotent cell (iPSC)-derived motor neurons (51). They also demonstrate the additive activity of *SMN-AS1* inactivation and the *ISS-N1* ASO on FL SMN levels in neuronal cells (51). Up-to-date information reveals that their product is currently still in the pre-clinical discovery phase.

The strategies discussed in this section were focused on those not yet implemented in clinical trials. As discussed above, several SMN1- and *SMN2*-targeting compounds are currently being evaluated in patients (Table 1). As these promising drugs eventually obtain regulatory approval for patient use, how their delivery route, activity and efficiency compare to those of nusinersen, will significantly impact and change, yet again, the

SMA therapeutic landscape. In light of the pre-clinical observations that combinatorial approaches can have additive effects, the evaluation of the therapeutic potential of a "cocktail" of treatments composed of *SMN2* transcription promoting molecules (e.g. RO7034067), SMN1 augmentation technologies (e.g. AAV9-SMN1), nusinersen and second generation ASO strategies will be of utmost importance to exploit the optimal benefits from each strategy (Fig. 2). Finally, the push from several advocacy groups for pre-natal screening of SMA, highlighted by the recent announcement that the U.S. Department of Health and Human Services will be reviewing an application for the inclusion of SMA on their Recommended Uniform Screening Panel (RUSP), also points to imminent changes to the therapeutic landscape. Early diagnosis may require adapted doses and delivery methods of SMN-dependent therapies and result in a greater population of mildly affected SMA patients that will require long-term care management and may develop non-CNS symptoms over time.

AN INTEGRATED THERAPEUTIC APPROACH FOR SMA

When discussing combinatorial therapies, it is important that we also consider the use of SMN-specific therapies in combination with SMN-independent strategies. This has recently been reviewed in the context of promising therapeutic targets that are still in pre-clinical stages (*Bowerman et al*., *Disease Models and Mechanisms*). However, the leading non-SMN compound is from Cytokinetics™, a fast skeletal troponin activator (CK-2127107) designed with the intent of improving muscle function and performance. In a rat model of heart failure that displays skeletal muscle atrophy, oral administration of CK-2127107 improved exercise tolerance and performance (52). CK-2127107 has already been evaluated in a phase 1 clinical trial (and is being assessed in an ongoing phase 2 study (NCT02644668)). While this muscle-targeting molecule may lead to significant improvements in SMA patients, further research is still needed to better understand how SMN-independent and non-CNS approaches in particular can be incorporated in the evolving SMA therapeutic landscape.

Data from animal models and patients indicates the involvement of peripheral organs including muscle, liver, heart and pancreas in the most severely affected SMA patients (53). The consequences of individuals treated with SMN-inducing treatments surviving past the natural history of the disease and reaching puberty and adulthood are currently unknown (54). One interesting commonality between the peripheral organs pathologically affected in SMA is that they all contribute to the maintenance of whole-body metabolic homeostasis and health (Fig. 3). For example, skeletal muscle, liver, pancreas and heart play central roles in the

modulation and utilization of glucose metabolism and dysfunction of one or more of these tissues can lead to whole-body perturbations as demonstrated in diabetes and cardiovascular diseases (55–57). Furthermore, the accurate regulation of glucose metabolism is critical for the function and activity of peripheral and CNS tissues (58, 59). Importantly, we and others have demonstrated that both SMA animal models and patients display pathologies and clinical symptoms indicative of disturbances in glucose metabolism (12, 60–63). Interestingly, many therapeutic strategies that demonstrate a positive effect on SMA pathogenesis such as systemic administration of the HDACi trichostatin A (TSA) (64), inactivation of phosphatase and tensin homolog (PTEN) (65), muscle treatment with insulin growth factor 1 (IGF-1) (66), exercise (67) and systemic dosing of the natural compound loganin (68), all influence glucose metabolism (69–73), even though they were not originally evaluated for that purpose. Irregularities in fatty acid (74–76), lipid (77, 78) and amino acid (Walter *et al*., *in preparation*) metabolism also occur in SMA patients and pre-clinical models and could similarly be modulated by interventions that have beneficial repercussions on metabolic homeostasis and consequently, disease progression and pathology. The observed influence of diet composition on lifespan and neuromuscular phenotype in SMA mice (62, 79, 80) emphasizes the importance of considering a systemic approach as we enter this new era for SMA therapeutic development. Understanding pathological pathways in peripheral tissues, and identifying relevant treatment strategies to restore them can have an unintended but beneficial ripple effect on whole-body metabolic homeostasis. The recent regulatory approval of nusinersen, a SMN- and CNS-directed therapy, has rapidly changed the SMA therapeutic landscape, whereby peripheral and metabolic components of the disease that are not fully targeted by this and other gene therapies that are in the pipeline, will gain more attention from researchers, clinicians and patients as critical therapeutic targets.

CONCLUSION

In the present review, we have focused on the clinical data from the nusinersen trials that led to the recent and first ever approval of an ASO gene therapy for SMA. Importantly, we have discussed how nusinersen is not the end of the road for therapeutic development and the caveats of nusinersen point to a need for second generation ASO-related approaches alongside alternative SMN upregulation strategies. Furthermore, there is a requirement for a better understanding of how therapeutically targeting peripheral metabolic defects that are most likely a consequence of intrinsic perturbations in multiple key tissues such as skeletal muscle, pancreas, liver and heart, can lead to whole-body benefits. Awaited results from the nusinersen phase 3 study in later-

onset SMA patients (NCT02292537) and the phase 2 trial aimed at pre-symptomatic patients (NTC02386553) will most likely provide additional insight into the optimal therapeutic window for SMN-dependent treatments and support for the requirement of a integrated approach to SMA therapy. However, the new SMA therapeutic landscape will have to be broader than nusinersen alone and integrate various SMN-dependent and -independent strategies as well as CNS- and systemically-directed approaches whether they be in the form of pharmacological compounds, dietary interventions or exercise paradigms. Importantly, the integrated treatment of SMA will be part of a life-long strategy for management of disease symptoms and be tailored to each individual patient (Fig. 4).

ACKNOWLEDGEMENTS

M.B. is an SMA Trust Career Development Fellow. Work in the Wood and Talbot laboratories on SMA is funded by the SMA Trust.

REFERENCES

1. Pearn,J. (1978) Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. *J. Med. Genet.*, **15**, 409–413.

2. Crawford,T.O. and Pardo,C.A. (1996) The neurobiology of childhood spinal muscular atrophy. *Neurobiol. Dis.*, **3**, 97–110.

3. Lefebvre,S., Bürglen,L., Reboullet,S., Clermont,O., Burlet,P., Viollet,L., Benichou,B., Cruaud,C., Millasseau,P. and Zeviani,M. (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, **80**, 155–165.

4. Murray,L.M., Comley,L.H., Thomson,D., Parkinson,N., Talbot,K. and Gillingwater,T.H. (2008) Selective vulnerability of motor neurons and dissociation of pre- and post-synaptic pathology at the neuromuscular junction in mouse models of spinal muscular atrophy. *Hum. Mol. Genet.*, **17**, 949–962.

5. Shababi,M., Habibi,J., Yang,H.T., Vale,S.M., Sewell,W.A. and Lorson,C.L. (2010) Cardiac defects contribute to the pathology of spinal muscular atrophy models. *Hum. Mol. Genet.*, **19**, 4059–4071.

6. Heier,C.R., Satta,R., Lutz,C. and DiDonato,C.J. (2010) Arrhythmia and cardiac defects are a feature of spinal muscular atrophy model mice. *Hum. Mol. Genet.*, **19**, 3906–3918.

7. Rudnik-Schöneborn,S., Heller,R., Berg,C., Betzler,C., Grimm,T., Eggermann,T., Eggermann,K., Wirth,R., Wirth,B. and Zerres,K. (2008) Congenital heart disease is a feature of severe infantile spinal muscular atrophy. *J. Med. Genet.*, **45**, 635–638.

8. Szunyogova,E., Zhou,H., Maxwell,G.K., Powis,R.A., Francesco,M., Gillingwater,T.H. and Parson,S.H. (2016) Survival Motor Neuron (SMN) protein is required for normal mouse liver development. *Sci. Rep.*, **6**, 34635.

9. Deguise,M.-O., De Repentigny,Y., McFall,E., Auclair,N., Sad,S. and Kothary,R. (2017) Immune dysregulation may contribute to disease pathogenesis in spinal muscular atrophy mice. *Hum. Mol. Genet.*, 10.1093/hmg/ddw434.

10. Thomson,A.K., Somers,E., Powis,R.A., Shorrock,H.K., Murphy,K., Swoboda,K.J., Gillingwater,T.H. and Parson,S.H. (2017) Survival of motor neurone protein is required for normal postnatal development of the spleen. *J. Anat.*, **230**, 337–346.

11. Gombash,S.E., Cowley,C.J., Fitzgerald,J.A., Iyer,C.C., Fried,D., McGovern,V.L., Williams,K.C., Burghes,A.H.M., Christofi,F.L., Gulbransen,B.D., *et al.* (2015) SMN deficiency disrupts gastrointestinal and enteric nervous system function in mice. *Hum. Mol. Genet.*, **24**, 3847–3860.

12. Bowerman,M., Swoboda,K.J., Michalski,J.-P., Wang,G.-S., Reeks,C., Beauvais,A., Murphy,K., Woulfe,J., Screaton,R.A., Scott,F.W., *et al.* (2012) Glucose metabolism and pancreatic defects in spinal muscular atrophy. *Ann. Neurol.*, **72**, 256–268.

13. Wishart,T.M., Huang,J.P.-W., Murray,L.M., Lamont,D.J., Mutsaers,C.A., Ross,J., Geldsetzer,P., Ansorge,O., Talbot,K., Parson,S.H., *et al.* (2010) SMN deficiency disrupts brain development in a mouse model of severe spinal muscular atrophy. *Hum. Mol. Genet.*, **19**, 4216–4228.

14. Somers,E., Stencel,Z., Wishart,T.M., Gillingwater,T.H. and Parson,S.H. (2012) Density, calibre and ramification of muscle capillaries are altered in a mouse model of severe spinal muscular atrophy. *Neuromuscul. Disord. NMD*, **22**, 435–442.

15. Hunter,G., Aghamaleky Sarvestany,A., Roche,S.L., Symes,R.C. and Gillingwater,T.H. (2014) SMN-dependent intrinsic defects in Schwann cells in mouse models of spinal muscular atrophy. *Hum. Mol. Genet.*, **23**, 2235–2250.

16. Schreml,J., Riessland,M., Paterno,M., Garbes,L., Roßbach,K., Ackermann,B., Krämer,J., Somers,E., Parson,S.H., Heller,R., *et al.* (2013) Severe SMA mice show organ impairment that cannot be rescued by therapy with the HDACi JNJ-26481585. *Eur. J. Hum. Genet. EJHG*, **21**, 643–652.

17. Vaidla,E., Talvik,I., Kulla,A., Sibul,H., Maasalu,K., Metsvaht,T., Piirsoo,A. and Talvik,T. (2007) Neonatal spinal muscular atrophy type 1 with bone fractures and heart defect. *J. Child Neurol.*, **22**, 67–70.

18. Schrank,B., Götz,R., Gunnersen,J.M., Ure,J.M., Toyka,K.V., Smith,A.G. and Sendtner,M. (1997) Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. *Proc. Natl. Acad. Sci. U. S. A.*, **94**, 9920–9925.

19. Rochette,C.F., Gilbert,N. and Simard,L.R. (2001) SMN gene duplication and the emergence of the SMN2 gene occurred in distinct hominids: SMN2 is unique to Homo sapiens. *Hum. Genet.*, **108**, 255–266.

20. Monani,U.R., Lorson,C.L., Parsons,D.W., Prior,T.W., Androphy,E.J., Burghes,A.H. and McPherson,J.D. (1999) A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum. Mol. Genet.*, **8**, 1177–1183.

21. Monani,U.R., McPherson,J.D. and Burghes,A.H. (1999) Promoter analysis of the human centromeric and telomeric survival motor neuron genes (SMNC and SMNT). *Biochim. Biophys. Acta*, **1445**, 330–336.

22. Boda,B., Mas,C., Giudicelli,C., Nepote,V., Guimiot,F., Levacher,B., Zvara,A., Santha,M., LeGall,I. and Simonneau,M. (2004) Survival motor neuron SMN1 and SMN2 gene promoters: identical sequences and differential expression in neurons and non-neuronal cells. *Eur. J. Hum. Genet. EJHG*, **12**, 729–737.

23. Lorson,C.L., Hahnen,E., Androphy,E.J. and Wirth,B. (1999) A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **96**, 6307–6311.

24. Cartegni,L. and Krainer,A.R. (2002) Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nat. Genet.*, **30**, 377–384.

25. Kashima,T. and Manley,J.L. (2003) A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat. Genet.*, **34**, 460–463.

26. Lefebvre,S., Burlet,P., Liu,Q., Bertrandy,S., Clermont,O., Munnich,A., Dreyfuss,G. and Melki,J. (1997) Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat. Genet.*, **16**, 265–269.

27. MacLeod,M.J., Taylor,J.E., Lunt,P.W., Mathew,C.G. and Robb,S.A. (1999) Prenatal onset spinal muscular atrophy. *Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc.*, **3**, 65–72.

28. Dubowitz,V. (1999) Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. *Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc.*, **3**, 49–51.

29. Pearn,J.H., Hudgson,P. and Walton,J.N. (1978) A clinical and genetic study of spinal muscular atrophy of adult onset: the autosomal recessive form as a discrete disease entity. *Brain J. Neurol.*, **101**, 591–606.

30. Pearn,J. (1980) Classification of spinal muscular atrophies. *Lancet Lond. Engl.*, **1**, 919–922.

31. Munsat,T.L. and Davies,K.E. (1992) International SMA consortium meeting. (26-28 June 1992, Bonn, Germany). *Neuromuscul. Disord. NMD*, **2**, 423–428.

32. Mohseni,J., Zabidi-Hussin,Z. a. M.H. and Sasongko,T.H. (2013) Histone deacetylase inhibitors as potential treatment for spinal muscular atrophy. *Genet. Mol. Biol.*, **36**, 299–307.

33. Faravelli,I., Nizzardo,M., Comi,G.P. and Corti,S. (2015) Spinal muscular atrophy--recent therapeutic advances for an old challenge. *Nat. Rev. Neurol.*, **11**, 351–359.

34. Chiriboga,C.A., Swoboda,K.J., Darras,B.T., Iannaccone,S.T., Montes,J., De Vivo,D.C., Norris,D.A., Bennett,C.F. and Bishop,K.M. (2016) Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. *Neurology*, **86**, 890–897.

35. Finkel,R.S., Chiriboga,C.A., Vajsar,J., Day,J.W., Montes,J., De Vivo,D.C., Yamashita,M., Rigo,F., Hung,G., Schneider,E., *et al.* (2016) Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet Lond. Engl.*, 10.1016/S0140-6736(16)31408-8.

36. Singh,N.N., Lee,B.M., DiDonato,C.J. and Singh,R.N. (2015) Mechanistic principles of antisense targets for the treatment of spinal muscular atrophy. *Future Med. Chem.*, **7**, 1793–1808.

37. Porensky,P.N. and Burghes,A.H.M. (2013) Antisense oligonucleotides for the treatment of spinal muscular atrophy. *Hum. Gene Ther.*, **24**, 489–498.

38. Douglas,A.G.L. and Wood,M.J.A. (2013) Splicing therapy for neuromuscular disease. *Mol. Cell. Neurosci.*, **56**, 169–185.

39. Hua,Y., Vickers,T.A., Okunola,H.L., Bennett,C.F. and Krainer,A.R. (2008) Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am. J. Hum. Genet.*, **82**, 834–848.

40. Singh,N.K., Singh,N.N., Androphy,E.J. and Singh,R.N. (2006) Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. *Mol. Cell. Biol.*, **26**, 1333–1346.

41. Porensky,P.N., Mitrpant,C., McGovern,V.L., Bevan,A.K., Foust,K.D., Kaspar,B.K., Wilton,S.D. and Burghes,A.H.M. (2012) A single administration of morpholino antisense oligomer rescues spinal muscular atrophy in mouse. *Hum. Mol. Genet.*, **21**, 1625–1638.

42. Geary,R.S., Yu,R.Z. and Levin,A.A. (2001) Pharmacokinetics of phosphorothioate antisense oligodeoxynucleotides. *Curr. Opin. Investig. Drugs Lond. Engl. 2000*, **2**, 562–573.

43. Bowerman,M., Murray,L.M., Beauvais,A., Pinheiro,B. and Kothary,R. (2012) A critical smn threshold in mice dictates onset of an intermediate spinal muscular atrophy phenotype associated with a distinct neuromuscular junction pathology. *Neuromuscul. Disord. NMD*, **22**, 263–276.

44. Finkel,R.S., McDermott,M.P., Kaufmann,P., Darras,B.T., Chung,W.K., Sproule,D.M., Kang,P.B., Foley,A.R., Yang,M.L., Martens,W.B., *et al.* (2014) Observational study of spinal muscular atrophy type I and implications for clinical trials. *Neurology*, **83**, 810–817.

45. Mitchell,D.J., Kim,D.T., Steinman,L., Fathman,C.G. and Rothbard,J.B. (2000) Polyarginine enters cells more efficiently than other polycationic homopolymers. *J. Pept. Res. Off. J. Am. Pept. Soc.*, **56**, 318–325.

46. Kurrikoff,K., Gestin,M. and Langel,Ü. (2016) Recent in vivo advances in cell-penetrating peptide-assisted drug delivery. *Expert Opin. Drug Deliv.*, **13**, 373–387.

47. Hammond,S.M., Hazell,G., Shabanpoor,F., Saleh,A.F., Bowerman,M., Sleigh,J.N., Meijboom,K.E., Zhou,H., Muntoni,F., Talbot,K., *et al.* (2016) Systemic peptide-mediated oligonucleotide therapy improves long-term survival in spinal muscular atrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **113**, 10962–10967.

48. Echigoya,Y., Nakamura,A., Nagata,T., Urasawa,N., Lim,K.R.Q., Trieu,N., Panesar,D., Kuraoka,M., Moulton,H.M., Saito,T., *et al.* (2017) Effects of systemic multiexon skipping with peptide-conjugated morpholinos in the heart of a dog model of Duchenne muscular dystrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **114**, 4213–4218.

49. Mitragotri,S., Burke,P.A. and Langer,R. (2014) Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat. Rev. Drug Discov.*, **13**, 655–672.

50. d'Ydewalle,C., Ramos,D.M., Pyles,N.J., Ng,S.-Y., Gorz,M., Pilato,C.M., Ling,K., Kong,L., Ward,A.J., Rubin,L.L., *et al.* (2017) The Antisense Transcript SMN-AS1 Regulates SMN Expression and Is a Novel Therapeutic Target for Spinal Muscular Atrophy. *Neuron*, **93**, 66–79.

51. Woo,C.J., Maier,V.K., Davey,R., Brennan,J., Li,G., Brothers,J., Schwartz,B., Gordo,S., Kasper,A., Okamoto,T.R., *et al.* (2017) Gene activation of SMN by selective disruption of lncRNA-mediated recruitment of PRC2 for the treatment of spinal muscular atrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **114**, E1509–E1518.

52. Hwee,D.T., Kennedy,A.R., Hartman,J.J., Ryans,J., Durham,N., Malik,F.I. and Jasper,J.R. (2015) The small-molecule fast skeletal troponin activator, CK-2127107, improves exercise tolerance in a rat model of heart failure. *J. Pharmacol. Exp. Ther.*, **353**, 159–168.

53. Hamilton,G. and Gillingwater,T.H. (2013) Spinal muscular atrophy: going beyond the motor neuron. *Trends Mol. Med.*, **19**, 40–50.

54. McGraw,S., Qian,Y., Henne,J., Jarecki,J., Hobby,K. and Yeh,W.-S. (2017) A qualitative study of perceptions of meaningful change in spinal muscular atrophy. *BMC Neurol.*, **17**, 68.

55. Rask-Madsen,C. and Kahn,C.R. (2012) Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.*, **32**, 2052–2059.

56. Ashrafian,H., Frenneaux,M.P. and Opie,L.H. (2007) Metabolic mechanisms in heart failure. *Circulation*, **116**, 434–448.

57. Ali,S. and Drucker,D.J. (2009) Benefits and limitations of reducing glucagon action for the treatment of type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab.*, **296**, E415-421.

58. Roh,E., Song,D.K. and Kim,M.-S. (2016) Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. *Exp. Mol. Med.*, **48**, e216.

59. Argilés,J.M., Campos,N., Lopez-Pedrosa,J.M., Rueda,R. and Rodriguez-Mañas,L. (2016) Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease. *J. Am. Med. Dir. Assoc.*, **17**, 789–796.

60. Bruce,A.K., Jacobsen,E., Dossing,H. and Kondrup,J. (1995) Hypoglycaemia in spinal muscular atrophy. *Lancet Lond. Engl.*, **346**, 609–610.

61. Davis,R.H., Miller,E.A., Zhang,R.Z. and Swoboda,K.J. (2015) Responses to Fasting and Glucose Loading in a Cohort of Well Children with Spinal Muscular Atrophy Type II. *J. Pediatr.*, **167**, 1362–1368.e1.

62. Butchbach,M.E.R., Rose,F.F., Rhoades,S., Marston,J., McCrone,J.T., Sinnott,R. and Lorson,C.L. (2010) Effect of diet on the survival and phenotype of a mouse model for spinal muscular atrophy. *Biochem. Biophys. Res. Commun.*, **391**, 835–840.

63. Bowerman,M., Michalski,J.-P., Beauvais,A., Murray,L.M., DeRepentigny,Y. and Kothary,R. (2014) Defects in pancreatic development and glucose metabolism in SMN-depleted mice independent of canonical spinal muscular atrophy neuromuscular pathology. *Hum. Mol. Genet.*, **23**, 3432–3444.

64. Avila,A.M., Burnett,B.G., Taye,A.A., Gabanella,F., Knight,M.A., Hartenstein,P., Cizman,Z., Di Prospero,N.A., Pellizzoni,L., Fischbeck,K.H., *et al.* (2007) Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. *J. Clin. Invest.*, **117**, 659–671.

65. Little,D., Valori,C.F., Mutsaers,C.A., Bennett,E.J., Wyles,M., Sharrack,B., Shaw,P.J., Gillingwater,T.H., Azzouz,M. and Ning,K. (2015) PTEN depletion decreases disease severity and modestly prolongs survival in a mouse model of spinal muscular atrophy. *Mol. Ther. J. Am. Soc. Gene Ther.*, **23**, 270–277.

66. Bosch-Marcé,M., Wee,C.D., Martinez,T.L., Lipkes,C.E., Choe,D.W., Kong,L., Van Meerbeke,J.P., Musarò,A. and Sumner,C.J. (2011) Increased IGF-1 in muscle modulates the phenotype of severe SMA mice. *Hum. Mol. Genet.*, **20**, 1844–1853.

67. Grondard,C., Biondi,O., Armand,A.-S., Lécolle,S., Della Gaspera,B., Pariset,C., Li,H., Gallien,C.-L., Vidal,P.-P., Chanoine,C., *et al.* (2005) Regular exercise prolongs survival in a type 2 spinal muscular atrophy model mouse. *J. Neurosci. Off. J. Soc. Neurosci.*, **25**, 7615–7622.

68. Tseng,Y.-T., Chen,C.-S., Jong,Y.-J., Chang,F.-R. and Lo,Y.-C. (2016) Loganin possesses neuroprotective properties, restores SMN protein and activates protein synthesis positive regulator Akt/mTOR in experimental models of spinal muscular atrophy. *Pharmacol. Res.*, **111**, 58–75.

69. Aguirre,G.A., De Ita,J.R., de la Garza,R.G. and Castilla-Cortazar,I. (2016) Insulin-like growth factor-1 deficiency and metabolic syndrome. *J. Transl. Med.*, **14**, 3.

70. Sun,C. and Zhou,J. (2008) Trichostatin A improves insulin stimulated glucose utilization and insulin signaling transduction through the repression of HDAC2. *Biochem. Pharmacol.*, **76**, 120–127.

71. Hodakoski,C., Hopkins,B.D., Barrows,D., Mense,S.M., Keniry,M., Anderson,K.E., Kern,P.A., Hawkins,P.T., Stephens,L.R. and Parsons,R. (2014) Regulation of PTEN inhibition by the pleckstrin homology domain of P-REX2 during insulin signaling and glucose homeostasis. *Proc. Natl. Acad. Sci. U. S. A.*, **111**, 155–160.

72. Röhling,M., Herder,C., Stemper,T. and Müssig,K. (2016) Influence of Acute and Chronic Exercise on Glucose Uptake. *J. Diabetes Res.*, **2016**, 2868652.

73. Liu,K., Xu,H., Lv,G., Liu,B., Lee,M.K.K., Lu,C., Lv,X. and Wu,Y. (2015) Loganin attenuates diabetic nephropathy in C57BL/6J mice with diabetes induced by streptozotocin and fed with diets containing high level of advanced glycation end products. *Life Sci.*, **123**, 78–85.

74. Zolkipli,Z., Sherlock,M., Biggar,W.D., Taylor,G., Hutchison,J.S., Peliowski,A., Alman,B.A., Ling,S.C. and Tein,I. (2012) Abnormal fatty acid metabolism in spinal muscular atrophy may predispose to perioperative risks. *Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc.*, **16**, 549–553.

75. Crawford,T.O., Sladky,J.T., Hurko,O., Besner-Johnston,A. and Kelley,R.I. (1999) Abnormal fatty acid metabolism in childhood spinal muscular atrophy. *Ann. Neurol.*, **45**, 337–343.

76. Tein,I., Sloane,A.E., Donner,E.J., Lehotay,D.C., Millington,D.S. and Kelley,R.I. (1995) Fatty acid oxidation abnormalities in childhood-onset spinal muscular atrophy: primary or secondary defect(s)? *Pediatr. Neurol.*, **12**, 21–30.

77. Kölbel,H., Hauffa,B.P., Wudy,S.A., Bouikidis,A., Della Marina,A. and Schara,U. (2017) Hyperleptinemia in children with autosomal recessive spinal muscular atrophy type I-III. *PloS One*, **12**, e0173144.

78. Sproule,D.M., Montes,J., Montgomery,M., Battista,V., Koenigsberger,D., Shen,W., Punyanitya,M., De Vivo,D.C. and Kaufmann,P. (2009) Increased fat mass and high incidence of overweight despite low body mass index in patients with spinal muscular atrophy. *Neuromuscul. Disord. NMD*, **19**, 391–396.

79. Narver,H.L., Kong,L., Burnett,B.G., Choe,D.W., Bosch-Marcé,M., Taye,A.A., Eckhaus,M.A. and Sumner,C.J. (2008) Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. *Ann. Neurol.*, **64**, 465–470.

80. Butchbach,M.E.R., Singh,J., Gurney,M.E. and Burghes,A.H.M. (2014) The effect of diet on the protective action of D156844 observed in spinal muscular atrophy mice. *Exp. Neurol.*, **256**, 1–6.

LEGENDS TO FIGURES

Figure 1. A) The *SMN1* gene is correctly spliced while the *SMN2* gene contains a C to T substitution in exon 7 which leads to aberrant exclusion of exon 7. **B)** An ASO targeting the inhibitory intron splicing silencer N1 (ISS-N1) promotes *SMN2* exon 7 inclusion.

Figure 2. The optimal SMN-dependent treatment strategy for SMA would be a combination of *SMN2* enhancing small molecules, *SMN2* targeting antisense oligonucleotides (ASO)s, and *SMN1* enhancing adeno-associated viruses (AAVs), resulting in an additive increase in functional SMN expression.

Figure 3. The heart, liver, skeletal muscle and pancreas are pathologically affected peripheral and metabolic tissues in SMA that maintain inter- and intra-organ metabolic homeostasis via the regulation, of glucose, amino acid, lipid and fatty acid metabolism.

Figure 4. In the new and evolving SMA therapeutic landscape, an integrated treatment paradigm should consider both CNS and peripheral targets, pharmacological, physiological and dietary interventions as well as the specific symptoms and needs that arise at different developmental time-points.

TABLES

Table 1. Current non-ASO *SMN1* and *SMN2* targeting therapies evaluated in clinical trials for Spinal Muscular Atrophy*

General Strategy	Name of Pharmacological Compound	ClinicalTrials.gov Identifier
AAV9-*SMN1*	AVXS-101	NCT02122952
HDAC Inhibitors	Valproic Acid	NCT00227266, NCT00481013 NCT00661453 NCT00374075 NCT01033331
	Valproate	NCT01671384
	Sodium Phenylbutyrate	NCT00528268 NCT00439218 NCT00439569
Small Molecules	LMI070	NCT02268552
	RO7034067	NCT03032172 NCT02913482 NCT02908685 NCT02633709
	Hydroxyurea	NCT00485511 NCT00568698 NCT00568802
	Celecoxib	NCT02876094

* as of May 2017

Table 2. Summary of results from phase 1 nusinersen clinical trial

Evaluated Parameters	Outcome Measures	Key Conclusions
Safety	Physical/neurological examinations; Vital signs; Clinical laboratory tests; Electrocardiograms; CSF laboratory tests	No significant changes
Tolerability	Adverse events	Only mild and moderate AEs were reported
Pharmacokinetics	Nusinersen in plasma; Nusinersen and SMN in CSF	Dose-dependent increase in drug half-life and SMN levels
Efficacy	HFMSE; PedsQL	Dose-dependent improvement over baseline scores

Table 3. Summary of results from phase 2 nusinersen clinical trial

Evaluated Parameters	Outcome Measures	Key Conclusions
Safety	Physical/neurological examinations; Vital signs; Clinical laboratory tests; CSF Laboratory Tests; ECGs	No significant changes
Tolerability	Adverse events	3 deaths and 77 serious adverse events
Pharmacokinetics	Nusinersen in plasma; Nusinersen in CSF; SMN in CNS;	Drug and increased SMN levels in spinal cord and brain tissues
Efficacy	HINE-2; CHOP-INTEND; CMAPs; Survival; Permanent Ventilation	Overall improvement compared to baseline values or to natural history case series

FIGURES

Figure 1.

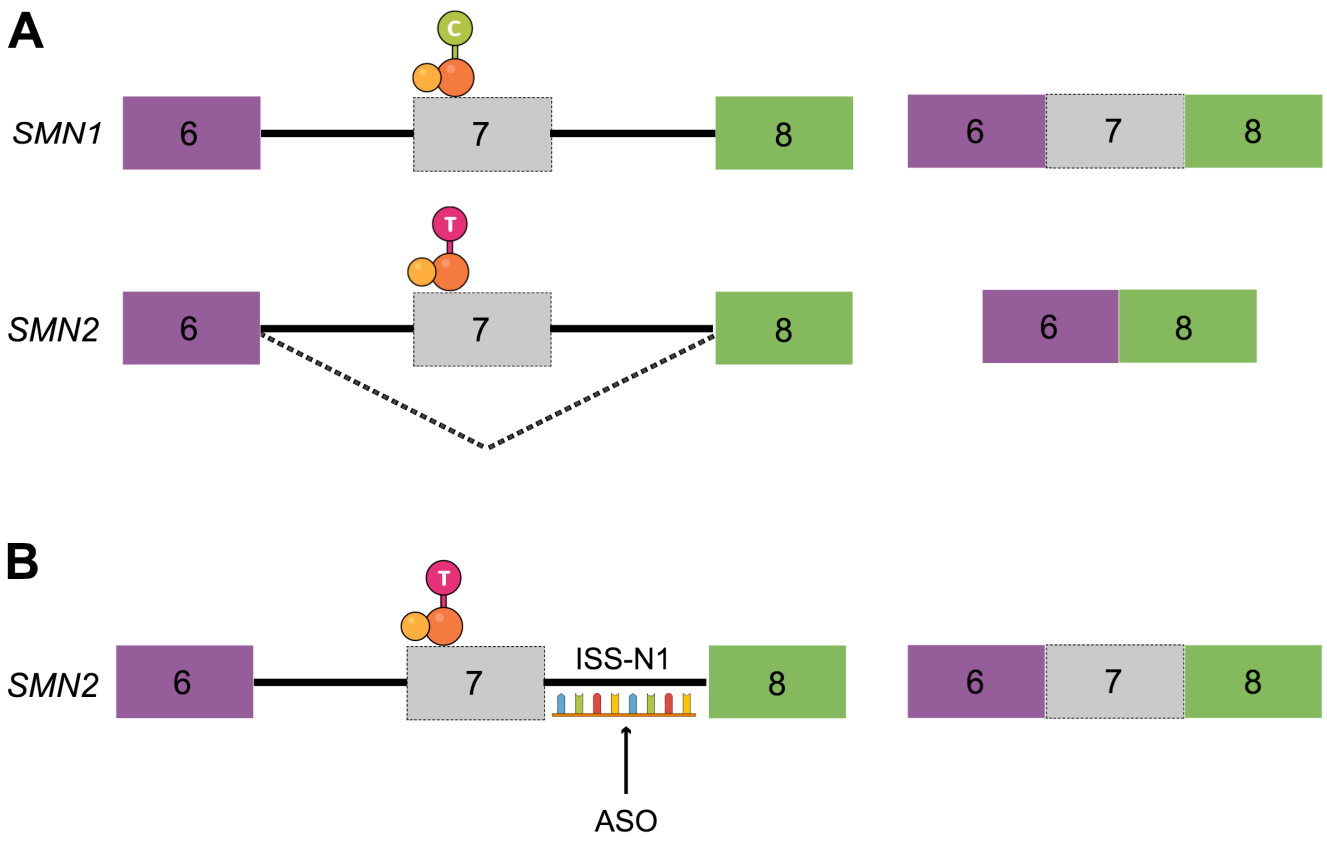


Figure 2.

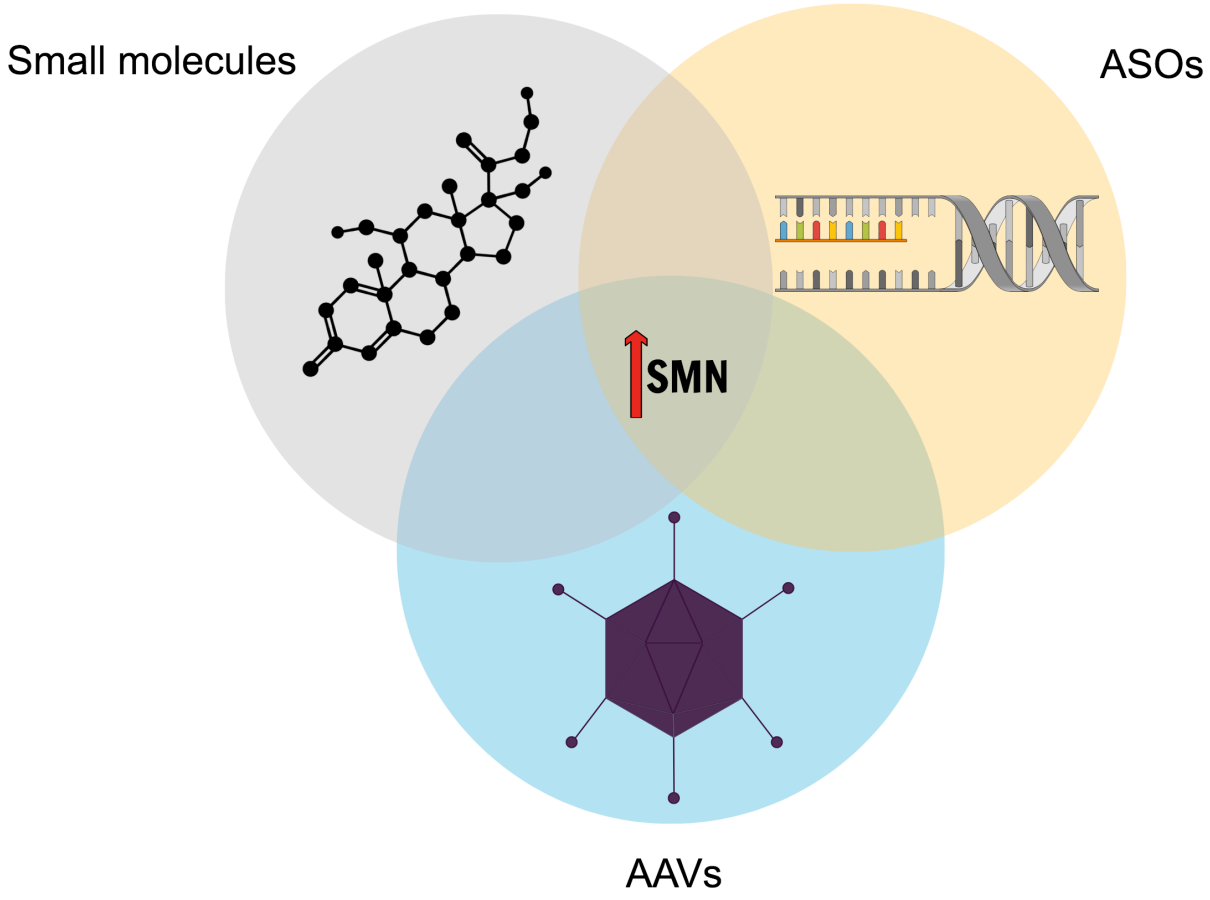


Figure 3.

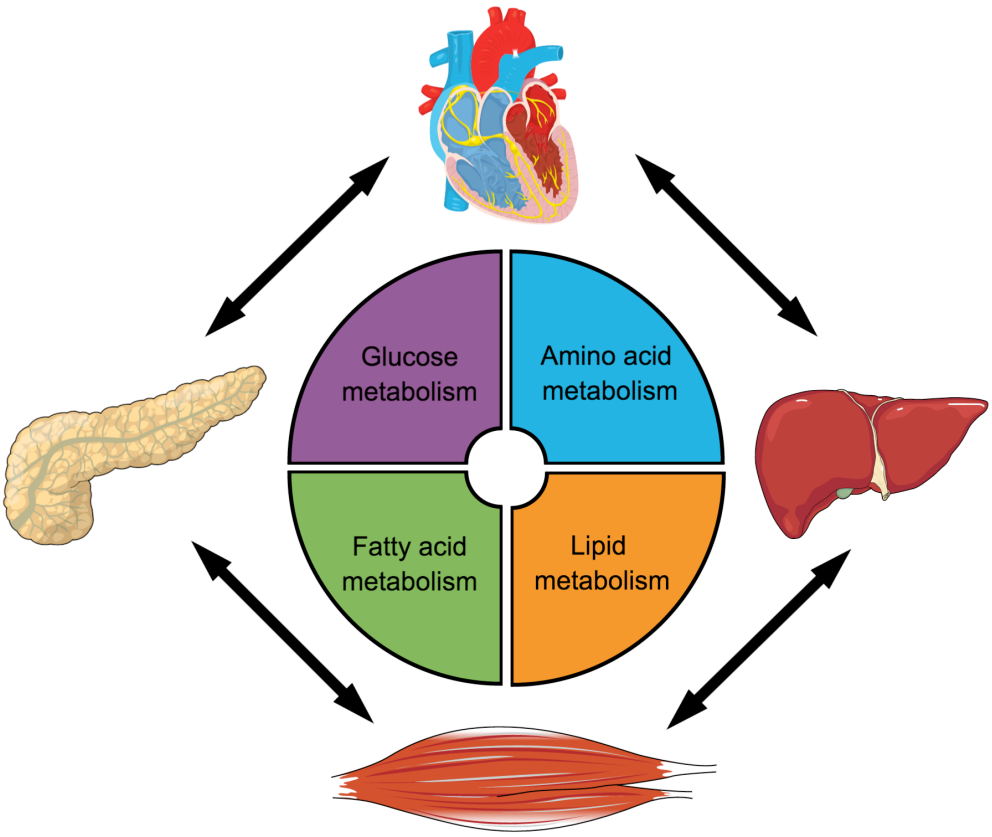
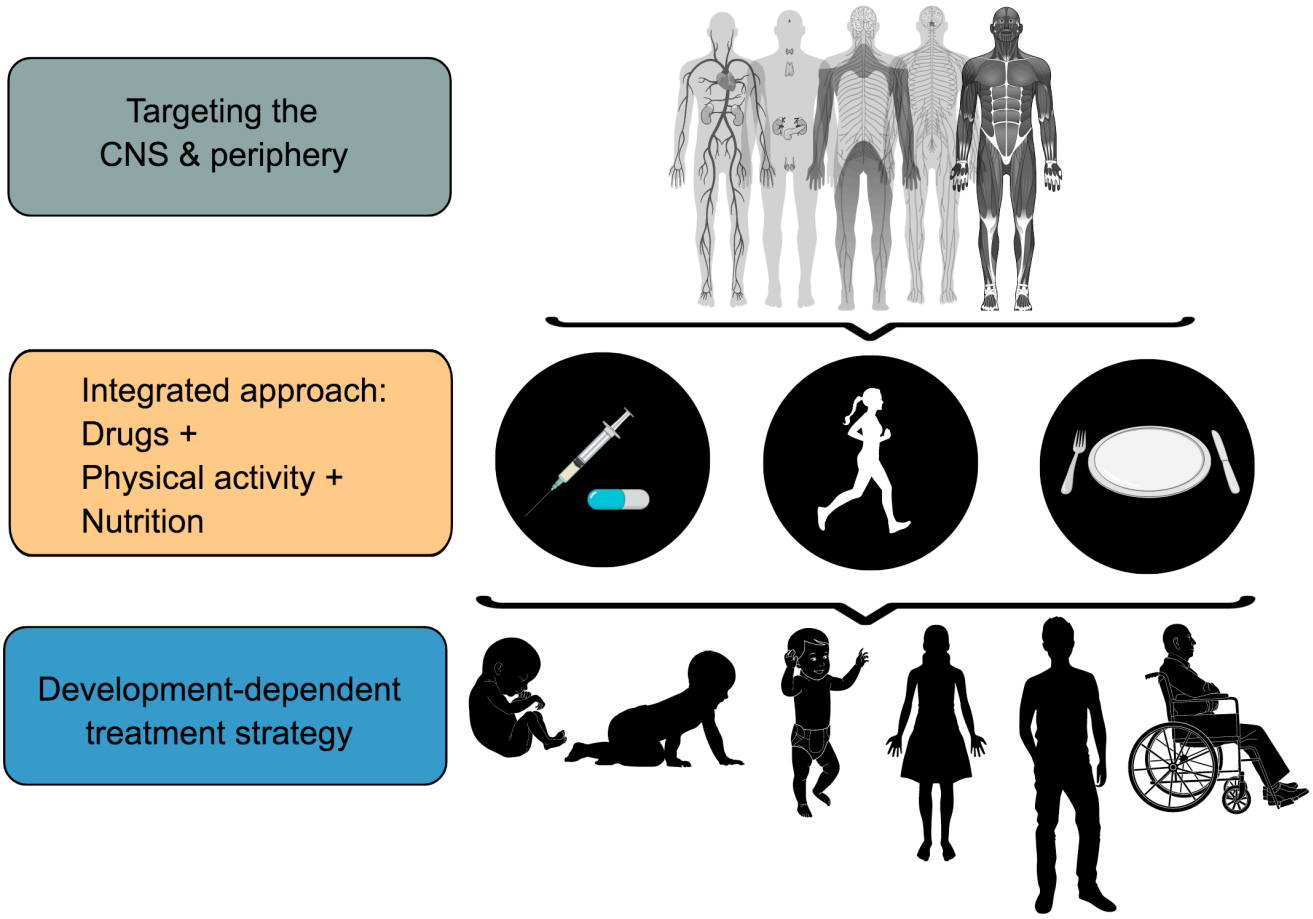

Glucose metabolism
Amino acid metabolism
Fatty acid metabolism
Lipid metabolism

Figure 4.


Targeting the
CNS & periphery
Integrated approach:
Drugs +
Physical activity +
Nutrition
Development-dependent
treatment strategy

ABBREVIATIONS

2'-MOE: 2'-O-(2-methoxyethyl)

AE: Adverse Event

ASO: Antisense Oligonucleotide

BBB: Blood Brain Barrier

CHMP: Committee for Medicinal Products for Human Use

CNS: Central Nervous System

CPP: Cell-penetrating peptide

CSF: Cerebrospinal fluid

EMA: European Medicines Agency

FDA: Food and Drug Administration

FL: Full Length

HDACi: Histone Deacytelase Inhibitor

IGF-1: Insulin Growth Factor 1 (IGF-1)

iPSC: induced pluripotent stem cell

ISS-N1: Intron Splicing Silencer N1

LNA: locked nucleic acid

lncRNA: long non-coding RNA

LP: Lumbar Puncture

mRNA: messenger RNA

NMJ: Neuromuscular Junction

Pip6a: peptide nucleic acids/PMO internalization peptide 6a

PMO: phosphorodiamidate morpholino

PNCR: Published Natural History Case Series

PTEN: Phosphatase and Tensin Homolog

RUSP: Recommended Uniform Screening Panel

SMA: Spinal Muscular Atrophy

SMN: Survival Motor Neuron

TSA: Trichostatin A