Therapeutic approaches for spinal muscular atrophy (SMA)

Authors: Scoto M[1], Finkel RS[2], Mercuri E[3], Muntoni F[1*]

[1] Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health, London, UK

[2] Division of Pediatric Neurology, Nemours Children's Hospital, University of Central Florida, College of Medicine Orlando, USA

[3] Pediatric Neurology, Catholic University and Centro Nemo, Policlinico Gemelli, Rome, Italy

*Corresponding author

Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder characterized by progressive muscle wasting and loss of muscle function due to severe motor neuron dysfunction, secondary to mutations in the survival motor neuron 1 (*SMN1*) gene. A second neighboring centromeric gene, *SMN2*, is intact in all patients but contains a C-to-T variation in exon 7 that affects a splice enhancer and determines exclusion of exon 7 in the majority of its transcript, leading to an unstable protein that cannot substitute for mutant SMN1.

Following successful studies on disease models and intensive studies on SMN functions in the past decade, SMN upregulation targeting *SMN2*, has been suggested as a possible

therapeutic approach. Recently we have witnessed an historical turning point with the first disease-modifying treatment receiving Food and Drug Administration (FDA) approval and now being available to patients also outside the clinical trial. This innovative treatment is an antisense oligonucleotide (ASOs) which, administered intrathecally, is able to increase exon 7 inclusion in the majority of the *SMN2* mRNA, and increase the production of fully functional SMN protein. Alternative advanced therapies, such as viral vector mediated gene therapy and orally available small molecules are also showing promising results in early clinical trial phases.

Article

Spinal muscular atrophy (SMA) is a monogenic autosomal recessive disorder having an incidence of ~1 in 10000 live births. [1, 2] Since the disease-causing genetic defect responsible for SMA was identified in 1995, there has accrued significant understanding of SMA pathogenesis, genetic, biologic and cellular mechanisms leading to crucial recent breakthroughs in its treatment. Historically the treatment for SMA was divided between optimisation of clinical management on one end and experimental therapies on the other, and a recent Cochrane review on treatment for SMA reached the conclusion that no drug treatment for SMA has been proven to have significant efficacy. [3, 4]

Recently the treatment's scenario has dramatically changed: the 23rd of December 2016 an oligonucleotide drug, called Spinraza, has received FDA approval for the treatment of SMA in the US. The FDA approval has been echoed by the European Medicine Agency (EMA) on 21 April 2017, when the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion, recommending the granting of a marketing authorization for

the medicinal product Spinraza, intended for the treatment of 5q spinal muscular atrophy (SMA).

Spinraza is the first of a relatively rich list of experimental therapy compounds under evaluation to arrive to the goalpost of FDA/EMA approval. There are indeed a number of alternative approaches that are attractive therapeutic strategies, developed to either increase SMN protein level (orally bioavailable small-molecule drugs that modulate the splicing of SMN2; *SMN1* gene replacement using viral vector) or act as neuroprotective drugs to improve motor neuron survival.

In this article we will review the most recent and promising therapeutic approaches for spinal muscular atrophy. (Figure 1)

Approved and experimental therapies aiming at increasing SMN protein levels

With greater understanding of the molecular basis of SMA in the past 2 decades, a major focus of therapeutic developments has been on increasing the full-length SMN protein by: increasing the inclusion of exon 7 in *SMN2* transcripts; enhancing *SMN2* gene expression; stabilizing the SMN protein, or replacing the *SMN1* gene.

Splice switching antisense oligonucleotides *(*ASOs) are synthetic RNA molecules that can interfere with physiological splicing of exons. They can either be designed to exclude an exon from the pre-mRNA (as in the case of the exon skipping strategy utilised in Duchenne muscular dystrophy) or induce the inclusion of an exon that would otherwise be removed (as it is the case for *SMN2*). Indeed all SMA patients carry at least one copy of *SMN2*, in which a single nucleotide change at a splice enhancer site excludes exon 7 in approximately 90% of its transcripts and results in the translation of a non-functional protein. The manipulation of

this splicing, inducing an increase in exon 7 retention in *SMN2* pre-mRNA, is therefore an attractive therapeutic approach, both because it is applicable to all patients with SMA, and because the resulting mRNA, and eventually protein product, is identical to the one produced by *SMN1*. These ASOs are highly effective at promoting inclusion of exon 7 in *SMN2* transcripts and at increasing SMN protein levels both in vitro and in vivo, although they are not capable of crossing the blood-brain barrier, so they require repeated intrathecal administration. [5, 6]

Early open label clinical trials of the ASO Spinraza (also known as Isis 396443, SMN_{Rx} and nusinersen), demonstrated a good safety profile and encouraging efficacy data both in type I and type II SMA individuals. [7] (table 1 shows a list of clinical trials using the ASO Spinraza).

Field Code Changed

A subsequent large randomised double blind controlled clinical trial (ENDEAR) in which infants under 7 months of age with type I SMA received either Spinraza or sham procedure (control arm) was interrupted early following the positive interim efficacy analysis, allowing to all participants to be rolled over into an open label study (called SHINE). The positive results from this study prompted the submission of the new drug application with the FDA.

While the drug is currently licensed in US for patients with SMA, and at the time the application for EMA approval has been submitted, the pharmaceutical sponsor, Biogen, has offered to the trial sites in several European countries, the possibility to enrol more patients with type I SMA via an Expanded Access Program (EAP). (For more information visit www.biogen.com) The interim results from the randomized control study in type II patients and an open label study of Spinraza in pre-symptomatic infants have also been very favourable.

Small molecules. A number of low-molecular-weight drugs that can increase levels of full-length SMN protein by different mechanisms, from activating the *SMN2* promoter to increasing its expression, or forcing read-through of the SMN2 product, are being studied. [8, 9]

Field Code Changed

Histone deacetylase inhibitor compounds can increase SMN2 mRNA levels and had shown promising results in mouse models and cell lines derived from SMA patients but, when tested in clinical trials, they invariably showed little or no benefit. These have included clinical trials with sodium phenylbutyrate, valproic acid and hydroxyuria. [10-13] (NCT00485511; NCT00568698; NCT00528268; NCT00439218; NCT00439569; NCT00227266)

Other small-molecule drugs such as aminoglycosides promote ribosomal reading through the stop codon of SMNΔ7 transcripts, enabling the translation of a protein variant with increased stability when compared to the native product of the *SMN2* gene lacking exon 7. Subcutaneous administrations of a read-through inducing compound (TC007), while not extending survival, did result in increased gross motor function in treated SMA transgenic mice [14]

Field Code Changed

A different class of more potent drugs capable of altering the splicing pattern of *SMN2* transcripts to favour the inclusion of exon 7 has been more recently developed. These drugs have very substantial efficacy in improving outcome in the SMA transgenic mice and are currently in early clinical trials.

One of these molecules was identified by PTC Therapeutics using a high throughput drug screening platform. This demonstrated unequivocal and robust efficacy in preclinical SMA transgenic mice studies. [15] Roche then chemically optimized this compound and brought it into the clinic as an orally bioavailable drug. A phase 1 multicentre randomized, double blind, placebo-controlled study was initiated in 2015 to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of RG7800 following 12 weeks of treatment in

adult and pediatric patients with SMA (MOONFISH study; NCT02240355). After recruiting the first cohort of patients, the sponsor placed the trial on clinical hold due to unexpected eye safety findings observed in the parallel chronic preclinical toxicology study of RG7800. This clinical trial was eventually terminated. More recently Roche has initiated two phase I/II studies to investigate the safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of a similar compound, RG7916, in infants with type 1~~ SMA~~ (FIREFISH; NCT02913482) and in Type 2 and 3 Spinal Muscular Atrophy (SUNFISH; NCT02908685). Both studies are currently ongoing and recruiting patients.

Novartis is pursuing a similar strategy with a small molecule also capable of increasing exon 7 retention in the SMN2 transcript and capable of substantially increase life expectancy in SMA transgenic mice [16]; an open-label phase I/II study of oral LMI070 in infants with Type 1 spinal muscular atrophy was initiated in April 2015 in four European countries (NCT02268552). In middle 2016 the pharmaceutical sponsor has decided to pause the enrollment study as parallel chronic preclinical toxicology studies, using daily dosing for a year compared to weekly dosing in the human study, showed unexpected injuries to the peripheral nerves and spinal cord, testes, and blood vessels in the kidney. Since the announcement, all patients enrolled in the trial were closely monitored and the study is currently ongoing but not recruiting participants.

Viral Gene therapy. As a monogenic disease, SMA is a good target for vector-based gene replacement therapy to restore a normal form of the *SMN1* gene in patients. Viral-mediated *SMN* gene delivery has been remarkably successful in preclinical studies. Both systemic and intra-cerebro-ventricular injection of self-complementary adeno-associated viral vectors (scAAV) expressing SMN showed efficient transduction of motor neurons in both mice and

non-human primates, as well as nearly complete correction of the SMA phenotype in mice. (17-19)

In selecting a potential vector to deliver the *SMN1* gene, the adeno-associated virus vectors (AAV) ~~8 and~~ 9 appeared to be an excellent contenders due to ~~their~~ its ability to cross the blood–brain barrier after systemic (intravenous) delivery in mouse models (20, 21)

AveXis is currently conducting a single site study in the US (Nationwide Children's Hospital, Columbus, Ohio, Dr Jerry Mendell), the first gene therapy phase I clinical trial to assess the safety of intravenous delivery of scAAV9-SMN in type 1 SMA infants. (NCT02122952) This open-label, dose-escalation clinical trial of AVXS-101 injected intravenously through a peripheral limb vein is currently active but not recruiting. A total of 15 infants have been enrolled in this study; participants were allocated in 2 cohorts receiving 6.7e13 vg/kg of AVXS-101 (n=3) and 2.0e14 vg/kg of AVXS-101 (n=12) delivered as a single intravenous administration.

The primary analysis for efficacy will be assessed when all patients reach 13.6 months of age with an estimate study completion in December 2017.

Encouraging preliminary data were presented at several international conferences in 2016, and AveXis is planning a larger multicentre Phase III open-label single-dose, by intravenous infusion, gene replacement therapy clinical trial for patients with SMA type 1 both in US and EU.

Other therapeutic approaches:

Neuroprotective compounds. Olesoxime is another small molecule that has shown neuroprotective properties in a number of in-vitro and in-vivo studies promoting neurite outgrowth and communication with the mitochondrial permeability transition pore. In-vitro neuronal cell death studies demonstrated a dose-dependent increase in cell survival with the

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

use of olesoxime in trophic factor deprivation assays. Furthermore, in SOD1G93A transgenic mouse models of ALS, treatment with olezoxime resulted in the prevention of weight loss, a delay in severe muscle function decline, and a 10% increase in lifespan compared to vehicle-treated controls. [22]

This drug has been tested in a phase II randomized, multicentre, double blind, placebo-controlled trial completed in 2013. A total of 165 non-ambulant patients with SMA type II and III, aged 3 to 25 years, were recruited in 23 sites in different European countries (France, Germany, Italy, UK, Poland, Netherlands, Belgium) and followed in the study for approximately two years. The randomization ratio was 2:1, with 108 to the olesoxime group (10mg/kg), and 57 to the placebo group. Preliminary results suggested that olesoxime maintains motor function and improves overall health status over the two-year treatment period.

An open-label study sponsored by Hoffmann-La Roche enrolling patients who participated in the phase II study to evaluate long term safety, tolerability, and effectiveness of olesoxime (OLEOS; NCT02628743) in patients with Spinal Muscular Atrophy is currently ongoing. The estimated study completion date is December 2020.

Skeletal muscle troponin activation. This type of therapeutic approach using another small-molecule is intended to slow the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibers, which may improve muscle function and physical performance in people with SMA. In collaboration with Astellas, Cytokinetics has developed CK-2127107 (CK-107), a novel skeletal muscle troponin activator which in preclinical models of spinal muscular atrophy, has demonstrated increases in submaximal skeletal muscle force in response to neuronal input and delays in the onset and reductions in the degree of muscle fatigue. [23]

A Phase 2, Double-Blind, Randomized, Placebo-Controlled, Study of CK-2127107 in Two Ascending Dose Cohorts of ambulant and non-ambulant Patients With SMA type II, III and IV is currently recruiting patients in the US and Canada. (NCT02644668)

Albuterol. Albuterol is a beta-adrenergic agonist that is recognized to have a positive anabolic effect in healthy individuals. This property has been evaluated in a pilot study on SMA type II and III patients that showed a significant improvement of myometry, FVC and DEXA scores at 6 months evaluation. [24] A following open label pilot study using oral salbutamol, which is a form of albuterol, showed an improvement of the functional scores at the Hammersmith Functional Motor Scale (HFMS) after 6 and 12 months of treatment. [25] In-vitro studies have also shown that salbutamol can unexpectedly increase the ratio of full length to truncated SMN mRNA, SMN protein and gem numbers by promoting the exon 7 inclusion and this effect was found to be directly proportional to the *SMN2* gene copy number. [26, 27]

Stem cells. One of the goals of transplanted stem cells is to support endogenous motor neurons through the delivery of neuroprotective agents and, ideally, to also partially restore neuronal and non-neuronal cells. [28-30] Neural stem cells obtained from the spinal cord administered intrathecally to SMA mice showed appropriate migration into the parenchyma and the capability to generate a small proportion of motor neurons. These treated mice exhibited improved motor unit and neuromuscular function and showed a 38% increase in life expectancy. [31]

Despite the positive results of neural stem cell transplantation in mice, its translational value in human is unclear. Alternative protocols, which include the use of embryonic stem cells or induced pluripotent stem cells for transplantation, have been tried in animal models. These cells have the ability to differentiate in vitro and in vivo into neural stem cells and motor

neurons. [32-34] Immune-suppression therapy may be necessary for this strategy to be successful.

The findings of improved SMA phenotype in mice following the intrathecal transplantation of embryonic stem cell-derived neural stem cells included proper migration to target tissue in the spinal cord, neuroprotective function, and a 58% increase in lifespan. [35]

A protocol to test neuronal stem cells in SMA patients is currently on hold by the FDA, however there are no imminent clinical trials expected in humans. [36]

Similarly, a controversial approach of allogenic mesenchymal cell transplantation, administered intravenously and intrathecally, initiated by a private enterprise in Italy, was interrupted in 2014 by a panel of experts appointed by the Italian Ministry of Health due to both lack of proven efficacy and serious concerns on the quality of the proposed drug as the mesenchymal cells given to patients were not grown under the approved EU strict set of quality control standards.

While the SMA research field is rapidly expanding with all the above therapeutic opportunities, and the outcome of the recently concluded phase 3 trials of Spinraza are extremely encouraging, nevertheless, there are still several questions that remain unsolved. A question is whether there is a defect of motor neurons development, a progressive loss of motor neurons or both. The timing for optimal intervention for all these approaches is not clear in the human, and in particular at which point there is irreversible pathology that precludes any meaningful therapeutic response in the various subtypes of SMA. Indeed, while a precise relationship between timing of the therapeutic intervention and response has been identified in several studies in the SMA mouse model, the equivalent information in the human is currently not available. Nor is it clear if clinical responses to these therapies will be sustained over time, especially in the growing child. In addition, animal models and limited but instructive patients case-reports have provided evidence that SMA pathology is not

Field Code Changed

Field Code Changed

restricted to motor neurons, but rather is a composite of pathology involving also skeletal muscle, neuromuscular junctions, interneurons and sensory-motor neurotransmission. [37-42] Systemic organ dysfunction or structural changes have been described in the most severe end of the SMA spectrum. It remains uncertain whether treatments that target motor neurons and not systemic tissues will lead to the development of multi-organ system dysfunction over time. Questions like "when, how, and which cell types should be targeted?" remain still critical to design innovative therapeutic strategies, and in particular the potential for a therapeutic advantage when targeting both the peripheral tissues and the CNS versus targeting exclusively the CNS needs to be demonstrated. Considering the therapeutic tools under development, it is likely that the answer to these questions will come from the studies in patients in the years to come.

Acknowledgements.

FM is supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London. The MRC Centre for Neuromuscular Diseases Biobank and the support of the MDUK and of the SMA Trust to the activities of the Dubowitz Neuromuscular Centre is also gratefully acknowledged

Conflict of interests.

FM is involved as principal investigator in the following clinical trials: nusinersen (SHINE, sponsored by Ionis and Biogen); olesoxime (OLEOS, sponsored by Roche). He has participated in scientific advisory board activities for Roche; Biogen and Avexis, and is also a

member of the Pfizer rare disease scientific advisory board. MS is involved as sub-investigator in SHINE clinical trial and is principal investigator in OLEOS clinical trial.

RF is involved as principal investigator in the following SMA clinical trials: nusinersen (CS3A, ENDEAR, CHERISH, NURTURE and SHINE, sponsored by Ionis and Biogen) and CK-2127107 (CY 5021 study, sponsored by Cytokinetics and Astellas). He has participated in scientific advisory board activities for Ionis, Biogen, Roche, Novartis and AveXis; has served on the DSMB for the Roche RG7800 and AveXis AVXS-101 phase 1 study; and has served as an advisor to CureSMA (US), the SMA Foundation (US), SMA REACH (UK) and SMA Europe.

EM is involved as principal investigator in the following clinical trials: nusinersen (SHINE, sponsored by Ionis and Biogen); olesoxime (OLEOS, sponsored by Roche). He has participated in scientific advisory board activities for Ionis, Roche; Biogen and Avexis,

References

1. Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlfs EM, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. European journal of human genetics : EJHG. 2012;20(1):27-32.
2. MacDonald WK, Hamilton D, Kuhle S. SMA carrier testing: a meta-analysis of differences in test performance by ethnic group. Prenatal diagnosis. 2014;34(12):1219-26.
3. Wadman RI, Bosboom WM, van der Pol WL, van den Berg LH, Wokke JH, Iannaccone ST, et al. Drug treatment for spinal muscular atrophy type I. The Cochrane database of systematic reviews. 2012(4):Cd006281.
4. Wadman RI, Bosboom WM, van der Pol WL, van den Berg LH, Wokke JH, Iannaccone ST, et al. Drug treatment for spinal muscular atrophy types II and III. The Cochrane database of systematic reviews. 2012(4):Cd006282.
5. Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett CF, et al. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature. 2011;478(7367):123-6.
6. Porensky PN, Mitrpant C, McGovern VL, Bevan AK, Foust KD, Kaspar BK, et al. A single administration of morpholino antisense oligomer rescues spinal muscular atrophy in mouse. Human molecular genetics. 2012;21(7):1625-38.

7. Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet. 2016;388(10063):3017-26.
8. Tsai LK, Tsai MS, Ting CH, Li H. Multiple therapeutic effects of valproic acid in spinal muscular atrophy model mice. Journal of molecular medicine (Berlin, Germany). 2008;86(11):1243-54.
9. Hastings ML, Berniac J, Liu YH, Abato P, Jodelka FM, Barthel L, et al. Tetracyclines that promote SMN2 exon 7 splicing as therapeutics for spinal muscular atrophy. Science translational medicine. 2009;1(5):5ra12.
10. Avila AM, Burnett BG, Taye AA, Gabanella F, Knight MA, Hartenstein P, et al. Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. The Journal of clinical investigation. 2007;117(3):659-71.
11. Narver HL, Kong L, Burnett BG, Choe DW, Bosch-Marce M, Taye AA, et al. Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. Annals of neurology. 2008;64(4):465-70.
12. Garbes L, Riessland M, Holker I, Heller R, Hauke J, Trankle C, et al. LBH589 induces up to 10-fold SMN protein levels by several independent mechanisms and is effective even in cells from SMA patients non-responsive to valproate. Human molecular genetics. 2009;18(19):3645-58.
13. Riessland M, Ackermann B, Forster A, Jakubik M, Hauke J, Garbes L, et al. SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy. Human molecular genetics. 2010;19(8):1492-506.
14. Mattis VB, Ebert AD, Fosso MY, Chang CW, Lorson CL. Delivery of a read-through inducing compound, TC007, lessens the severity of a spinal muscular atrophy animal model. Human molecular genetics. 2009;18(20):3906-13.
15. Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, et al. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science (New York, NY). 2014;345(6197):688-93.
16. Palacino J, Swalley SE, Song C, Cheung AK, Shu L, Zhang X, et al. SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. Nature chemical biology. 2015;11(7):511-7.
17. Foust KD, Wang X, McGovern VL, Braun L, Bevan AK, Haidet AM, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nature biotechnology. 2010;28(3):271-4.
18. Passini MA, Bu J, Roskelley EM, Richards AM, Sardi SP, O'Riordan CR, et al. CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. The Journal of clinical investigation. 2010;120(4):1253-64.
19. Valori CF, Ning K, Wyles M, Mead RJ, Grierson AJ, Shaw PJ, et al. Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy. Science translational medicine. 2010;2(35):35ra42.
20. Duque SI, Arnold WD, Odermatt P, Li X, Porensky PN, Schmelzer L, et al. A large animal model of spinal muscular atrophy and correction of phenotype. Annals of neurology. 2015;77(3):399-414.
21. Duque S, Joussemet B, Riviere C, Marais T, Dubreil L, Douar AM, et al. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. Molecular therapy : the journal of the American Society of Gene Therapy. 2009;17(7):1187-96.
22. Bordet T, Buisson B, Michaud M, Drouot C, Galea P, Delaage P, et al. Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis. The Journal of pharmacology and experimental therapeutics. 2007;322(2):709-20.
23. Calder AN, Androphy EJ, Hodgetts KJ. Small Molecules in Development for the Treatment of Spinal Muscular Atrophy. Journal of medicinal chemistry. 2016;59(22):10067-83.
24. Kinali M, Mercuri E, Main M, De Biasia F, Karatza A, Higgins R, et al. Pilot trial of albuterol in spinal muscular atrophy. Neurology. 2002;59(4):609-10.
25. Pane M, Staccioli S, Messina S, D'Amico A, Pelliccioni M, Mazzone ES, et al. Daily salbutamol in young patients with SMA type II. Neuromuscular disorders : NMD. 2008;18(7):536-40.
26. Angelozzi C, Borgo F, Tiziano FD, Martella A, Neri G, Brahe C. Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells. Journal of medical genetics. 2008;45(1):29-31.
27. Tiziano FD, Lomastro R, Pinto AM, Messina S, D'Amico A, Fiori S, et al. Salbutamol increases survival motor neuron (SMN) transcript levels in leucocytes of spinal muscular atrophy (SMA) patients: relevance for clinical trial design. Journal of medical genetics. 2010;47(12):856-8.
28. Hedlund E, Hefferan MP, Marsala M, Isacson O. Cell therapy and stem cells in animal models of motor neuron disorders. The European journal of neuroscience. 2007;26(7):1721-37.

29. Koliatsos VE, Xu L, Yan J. Human stem cell grafts as therapies for motor neuron disease. Expert opinion on biological therapy. 2008;8(2):137-41.
30. Suzuki M, Svendsen CN. Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. Trends in neurosciences. 2008;31(4):192-8.
31. Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Ronchi D, et al. Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. The Journal of clinical investigation. 2008;118(10):3316-30.
32. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. Cell. 2002;110(3):385-97.
33. Li XJ, Du ZW, Zarnowska ED, Pankratz M, Hansen LO, Pearce RA, et al. Specification of motoneurons from human embryonic stem cells. Nature biotechnology. 2005;23(2):215-21.
34. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science (New York, NY). 2008;321(5893):1218-21.
35. Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Ronchi D, et al. Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice. Brain : a journal of neurology. 2010;133(Pt 2):465-81.
36. Taylor JL, Lee FK, Yazdanpanah GK, Staropoli JF, Liu M, Carulli JP, et al. Newborn blood spot screening test using multiplexed real-time PCR to simultaneously screen for spinal muscular atrophy and severe combined immunodeficiency. Clinical chemistry. 2015;61(2):412-9.
37. Cifuentes-Diaz C, Faille L, Goudou D, Schachner M, Rieger F, Angaut-Petit D. Abnormal reinnervation of skeletal muscle in a tenascin-C-deficient mouse. Journal of neuroscience research. 2002;67(1):93-9.
38. Kariya S, Mauricio R, Dai Y, Monani UR. The neuroprotective factor Wld(s) fails to mitigate distal axonal and neuromuscular junction (NMJ) defects in mouse models of spinal muscular atrophy. Neuroscience letters. 2009;449(3):246-51.
39. Kariya S, Park GH, Maeno-Hikichi Y, Leykekhman O, Lutz C, Arkovitz MS, et al. Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. Human molecular genetics. 2008;17(16):2552-69.
40. Mentis GZ, Blivis D, Liu W, Drobac E, Crowder ME, Kong L, et al. Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. Neuron. 2011;69(3):453-67.
41. Szunyogova E, Zhou H, Maxwell GK, Powis RA, Francesco M, Gillingwater TH, et al. Survival Motor Neuron (SMN) protein is required for normal mouse liver development. Scientific reports. 2016;6:34635.
42. Khairallah MT, Astroski J, Custer SK, Androphy EJ, Franklin CL, Lorson CL. SMN deficiency negatively impacts red pulp macrophages and spleen development in mouse models of Spinal Muscular Atrophy. Human molecular genetics. 2017.