

Spinal muscular atrophy disease: a literature review for therapeutic strategies

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Abstract

Currently, there is no cure for the treatment of spinal muscular atrophy (SMA). Based on the available clinical and molecular findings, different therapeutic strategies were tested *in vitro* and *in vivo* and clinical trials are ongoing. The main therapeutic direction is focused on the enhancement of *SMN* expression by increasing the full-length (fl) *SMN2* transcript levels, preventing the *SMN* exon 7 from skipping or from protein stabilizing. In addition, the action of neurotrophic, neuroprotective or anabolic agents is tested and stem cell and gene therapy approaches are in a promising development.

• **Keywords:** neuromuscular disorder, SMN gene, therapeutically drugs, mutations •

Introduction

Spinal muscular atrophy is the second most common lethal autosomal recessive disorder in Caucasians, after cystic fibrosis [1,2]. The disease is characterized by the progressive degeneration of the alpha motoneurons, which leads to muscle atrophy, paralysis and even death [3]. Several clinical types were described for SMA, according to the age onset and the progression of the symptoms. The most recent classification [4] includes four types: type I (Werdnig-Hoffman), type II (intermediate), type III (Kugelberg – Welander) and type IV (adult form). Type I is the most severe form, with an onset before the age of 6 months and a life expectancy of around 2 years. Types II and III are known as chronic forms and are less severe, with an onset between 6-18 months (type II) and respectively after 18 months (for type III). In many cases, Type IV mimics the symptoms of type III, but the onset is typically around 30 years old. A normal life expectancy is characteristic for this adult form. Some research studies also reported the existence of type 0 or embryonic form, which is characterized by reduced movement of the fetus between 30-36 weeks of the pregnancy and a very short life expectancy [5].

The SMA determining gene, termed survival motor neuron (*SMN1*) was mapped on 5q11.2-13.3 region [6]. The homozygous deletion of *SMN1* exon 7 is the most common mutation found in SMA patients, but there are

also many cases of compound heterozygous subjects, for whom deletions and different point mutations have been detected. The *SMN1* paralog and *SMN2*, differ with a single nucleotide in exon 7 (C-T), that alters the splicing process. A few models were proposed in order to explain this mechanism. The first hypothesis sustains the existence of an Exonic Splicing Enhancer (ESE) in *SMN1* gene, known as a binding site for splicing regulatory factors like arginine/serine rich (SR) protein. In *SMN2* gene, the T nucleotide in position 6 of exon 7 disrupts this ESE and, in an implicit way, the binding of the SR protein [7,8,9,10]. Another model proposed the existence of an Exonic Splicing Silencer only in *SMN2* gene, which is a binding site for the hnRNP A1 repressor protein [11]. An extended analysis of these two models confirm the first one and sustain the role of hnRNP A1 protein in exon 7 skipping, but for both genes, not only for the *SMN2*. A new hypothesis emerged from these observations sustains that the hnRNP A1 protein acts in a general manner to block splicing of exon 7, in the absence of SF2/ASF dependent ESE [12]. No matter which of these models is the real one, the result of the splicing factors action was obvious: only 20% of the *SMN2* gene transcripts contain a message for the fl-SMN protein, while the remaining 80% are responsible for a truncated form. In SMA patients, the fl-SMN protein is synthesized in a small quantity, while the truncated protein is degraded and unable to sustain the motor neuron survival.

These molecular mechanisms indicated that *SMN2* gene could be a good target for SMA disease therapy, meaning the increase of fl-SMN protein level. Different compounds are tested in order to accomplish this aim. However, alternative strategies using neuroprotective agents, stem cells transplantation or gene therapy are ongoing.

Enhancement of SMN expression

The ability of *SMN2* gene to encode for the fl-SMN protein has been hardly exploited, by several mechanisms:

a. Increasing the fl-SMN transcript level by activating *SMN2* gene promoter

Some researchers reported that the promoters of the *SMN* genes have similar positive regulatory elements [13]. This information was extremely useful in finding the compounds able to increase the fl-SMN2 transcript level and, in an implicit way, the normal fl-SMN protein quantity. It is well known that histone acetyltransferases (HATs) enhance the transcription through the chromatin relaxation, while the histone deacetylases (HDACs) induce the transcriptional repression by chromatin condensation [14]. In this context, HDAC inhibitors became good candidates in finding a way to increase the fl-SMN level. For the first time, these kinds of compounds were used in 2001: a revolutionary increase of fl-SMN2 transcript level could be noticed following the sodium butyrate treatment of SMA patients, derived cell lines and SMA knockout transgenic mice [15]. Additional researches indicated that the half-life of sodium butyrate does not recommend its use in clinical trials. Anyway, these studies concerning the effect of sodium butyrate on *SMN* gene expression were the beginning for new researches in testing the action of HDAC inhibitors compounds on *SMN* expression.

A sodium butyrate derivate (sodium 4 phenylbutyrate - PB) has been tested on fibroblast cells derived from SMA patients [16] and then in clinical trials [17,18]. The emerging conclusion was that PB improves motor neuron function in SMA type II patients, by enhancing the fl-SMN transcript and protein levels. Although patients with urea cycle disorders [19] tolerate well this compound, its clinical use in SMA is still reserved, because of its short half-life of approximately 1 hour.

Another HDAC inhibitory compound with a promising effect in SMA treatment is hidroxyurea. In cultured lymphocytes from SMA patients, hidroxyurea is able to increase the ratio of full-length to truncated SMN messenger RNA, the SMN protein level and intranuclear gems number (the structures containing SMN protein) [20]. Based on this data, clinical trials were opened in United States [21] and Taiwan [22]. Preliminary results showed an increase of fl-SMN mRNA level at only 8 weeks of treatment.

The most studied HDAC inhibitor in SMA treatment is valproic acid (VPA) also known as 2 - propylpentanoic acid. This drug was already used in the treatment of epilepsy and with therapeutically indication in bipolar disorders. Taking into account that VPA is a fatty acid and a HDAC inhibitor, just like sodium butyrate, it was hypothesized that it may increase SMN2 mRNA level. If sodium butyrate has a short half-life of only six minutes in human serum, the VPA has the advantage of 8 to 10 hours half-life. The administration of VPA in therapeutic doses (0.5–1000µM), on fibroblast lines in SMA patients, for a 16-hour period, has determined the increase of fl-SMN2 mRNA level from 2 to 4 fold. As it was expected, a stronger response was obtained when VPA low doses (0.5-5µM) were administrated on fibroblast lines in SMA patients with more than two *SMN2* copies. By testing the action of this drug on rat organotypic hippocampal slice cultures (often used for Central Nervous System disorders studies), it was proved that VPA also increases SMN level in the neuronal tissue [23,24]. It is presupposed that VPA action is mediated by binding AP1 and SP1 transcription factors through the human and rat *SMN* promoter [23]. The VPA effects on neurogenesis and astrocyte proliferation, as well as on increased levels of Bcl-2 and Bcl-x anti-apoptotic factors, were also discussed in researches involving type III like - SMA mouse models [25]. These *in vitro* and *in vivo* studies have encouraged the opening of VPA clinical trials. Consequently to the pilot trial which showed an increased SMN protein level in healthy carriers as well in SMA patients [26], four clinical trials with VPA and carnitine (to avoid the possible toxicity of the drug) have been started [27].

HDAC inhibitors from the second generation like Trichostatin A, SAHA, M344 benzamide, MS-275, m-Carboxycinnamic acid, bis-Hydroxamide were included in several recent studies [28,29]. Of these compounds, SAHA seems to be the most promising drug for therapy, as it increases SMN levels at low concentrations, has a favorable profile cytotoxicity and it is well tolerated [29].

b. Preventing the *SMN2* exon 7 from skipping

Researches regarding the molecular mechanism of SMA physiopathology revealed that the production of the SMN truncated protein is the result of an alternative splicing of exon 7 in the majority of patients. Preventing this exon from skipping is one of the most common ways to increase the full-length of SMN protein level.

The first identified compound that helps in preventing splicing of the endogenous *SMN2* exon 7 is sodium vanadate, an inhibitor of protein tyrosine phosphatases, alkaline phosphatases and ATPases. Among several signaling pathways modulators on splicing, only treatment with sodium vanadate (50-100µM) for 10 to 24 hours showed an important increase in fl-SMN2 mRNA. The treatment with 50µM sodium vanadate caused the highest amount of the fl- transcript, while a concentration over 100µM resulted in the cell's death. The

long treatment period is explained by the time needed for cellular processes like transcription or phosphorylation. In this context, it is hypothesized that the phosphorylation of some cellular factors may be involved in SMN2 splicing process and that the most probable proteins influenced by sodium vanadate treatment are SR, snRNP and hnRNP [30].

The HDAC inhibitors have effects not only on chromatin relaxation, but also on *SMN* exon 7 retaining, as it could be noticed in the case of sodium butyrate treatment on lymphoid cell lines. According to this treatment, the SR protein induction was detected [15]. This dual action exerted on SMN protein level was also observed in the case of VPA and Trichostatin A.

Aclarubicin, a drug from anthracycline antibiotic class is frequently used in chemotherapy against solid tumors and leukemia. However, aclarubicin seems to significantly increase the gems number and the SMN level in a SMA I patient derived fibroblasts, by altering the splicing process. The high toxicity profile has embedded the use of this drug in treating SMA patients [31].

Trying to avoid the discovery of the potential therapeutic drugs that cannot cross the blood brain barrier, a recent research [32] has focused on the action of some polyphenol botanical compounds with known ability in passing this difficulty. Curcumin, resveratrol and epigallocatechin galate were previously reported to have some modifying actions in neurodegenerative diseases [33]. An increase in fl-SMN transcripts and protein levels was noticed after the administration of these compounds in cultures of SMA derived fibroblast cells. Additionally, it seems that curcumin up-regulate some genes encoding for SR proteins, essentially in splicing process.

Among natural or synthesized compounds, physical exercises are reported to be involved in the SMN2 splicing regulation, probably by modifying the expression pattern of pre-mRNA splicing factors in motoneurons [34]. Another study based on type 2 SMA mouse model proved that physical exercises have benefic effects in motor neuron protection, acceleration of muscle maturation and lifespan gain through the NMDA-receptor activity modulation [35]. A combined therapy of drugs and regular physical exercises is thought to improve clinical signs in SMA patients.

c. Stabilizing SMN Protein

In order to identify the compounds able to stabilize the SMN protein, by acting at the translational level, a high throughput screening of approximately 47000 compounds with potential effect on SMN protein was performed. Only indoprofen increases the SMN protein level in the treated fibroblast cells with about 13 percent, reported to untreated cells [36]. Moreover, as a result of indoprofen treatment, the number of gems was enhanced *in vitro* and a reduced embryonic lethality was noticed in SMA mutant mice.

SMN stabilizing protein compounds are also aminoglycosides. This class of antibiotics have *in vitro* capacities to suppress premature stop mutations in some genes (e.g. CFTR [37] and dystrophin genes [38]) and to affect the translation termination process, by altering the conformation of ribosomal decoding site [39]. Based on these findings, a research group reported an important increase of SMN protein level of fibroblast treatment with tobramycin and amikacin [40]. Other six aminoglycosides which were found to increase SMN protein level in fibroblast cells [41] may be used for therapeutic purposes.

There are also studies indicating that proteasome inhibitors may have a benefic effect on SMN protein stability [42]. It is already known that the ubiquitin/proteasome pathway has an important role in the proteolysis of intracellular proteins. By using pulse-chase analysis, it was confirmed that SMN protein is ubiquitinated and degraded by the ubiquitin/proteasome system [43].

Neurotrophic, neuroprotective and anabolic agents' administration

Neurotrophic factors have been referred to be good candidates for therapeutic strategies of several motor neurons diseases, based on the ability to slow down motor neuron death and axon degeneration [44]. Cardiotrophin-1 is a cytokine from interleukin-6 family, with known benefic effects on survival of motor neurons during embryonic period [45]. The cardiotrophin-1 delivery to SMA mutant mice revealed that the protective role for proximal and distal motoneurons is also present in postnatal period [46].

Neuroprotective agents were proposed for clinical trials in SMA, following the *in vitro* and *in vivo* studies. Because a hypothesis regarding SMA pathogenesis includes glutamate cytotoxicity, factors with anti-glutamate action have been tested in order to improve the motor skills and life expectancy. Gabapentin is an excitatory amino acid neurotransmitter with neuroprotective properties, proposed to have therapeutic effects for Amyotrophic Lateral Sclerosis (ALS). Two clinical trials with gabapentin were developed for patients with SMA types II and III, for a one-year period. If the first gabapentin study (developed on 84 SMA patients) has not revealed any potential therapeutic effects [47], the second one (applied on 120 patients) revealed a small improvement of muscle strength [48].

Another anti-glutamate factor is riluzole, a compound with neuroprotective effects tested in a SMA mutant mouse model [49]. The first clinical trial of riluzole in SMA patients was started in 2003 [50] and enrolled 10 SMA type I patients. The authors of this study concluded that it is safe to use riluzole in infants, but a more statistical powerful study is needed to ascertain the benefactor effects of this drug. At this moment, two

riluzole trials are ongoing on SMA type I patients in USA and in France [51].

Anabolic agents such as albuterol (also known as salbutamol) were reported to have significant results in clinical state improvement. Myometry, forced vital capacity and lean body mass were increased after 6-months of oral administration [52]. Starting from these clinical findings, a few years later, another research group studied these effects of the drug and reported an increase of SMN mRNA and protein level in fibroblast cells derived from SMA patients [53]. It is thought that the salbutamol is acting on SMN protein through the influence of the *SMN2* exon 7 splicing event.

It was recently reported that Follistatin [54] is an anabolic agent with an ability to increase muscle mass, to improve motor neurons' function and to prolong life survival, in SMA mutant mice. Taking into account that the SMN protein level was not increased by follistatin administration, it was suggested that this glycoprotein exerts its beneficial effects by a SMN independent mechanism. It is thought that follistatin is acting, at least in part, through a myostatin (a TGF- β family member) pathway, a negative regulator of muscle mass. The over expression of follistatin in myostatin null mice, results also in an enhancement of muscle mass, suggesting the existence of additional pathways or acting mechanisms [55].

A combined therapy of neurotrophic, neuroprotective or anabolic agents may lead to the improvement of SMA phenotype and, in an implicit manner, to the lifespan duration.

Stem cell transplantation and gene therapy

Stem cell transplantation and gene therapy represent a promising future in the treatment of motor neuron diseases, including SMA. It is known that neuronal stem cell transplantation can lead to an improvement of motor neuron disease phenotype, through different mechanisms, such as replacement of non-neuronal cells [56], delivery of neuroprotective factors [57] or reduction of toxic compounds [58]. For the first time, stem cells transplantation was used in a SMA model as putative therapeutic pathway in 2008 [59]. The motor neuron function improvement and survival of SMA mice led to the conclusion that neural stem cells transplantation has positive effects on SMA disease phenotype.

Lesbordes was reported to have conducted the first gene therapy study on SMA in 2003, [46]. He used the intra-muscular injection with an adenoviral vector expressing cardiotrophin-1, whose effects were discussed previously in this paper. The method had been tested few years earlier [60], when cardiotrophin-1 appeared to be a protective factor in progressive motor neuropathy model mice. In 2004, a new gene therapy study was reported: a lentivector developed from infectious equine anemia virus was used in gene transfer in SMA mice, consequently in *in vitro* testing. The SMN lentivector was injected in two days

of age SMA mice at the level of the voluntary muscles. Following this therapy, the SMA mice life expectancy was increased with 3 to 5 days and a motor neuron death reduction could be recorded [61].

Remarkable results for SMA potential therapy were obtained by using RNA strategies. The enhancing of exon 7 incorporation in SMN message was found to be a successful strategy in the increase of SMN protein level, but drugs such as sodium vanadate, sodium butyrate and aclarubicin were not recommended because of their short half-life or toxicity. A bifunctional antisense oligonucleotides strategy was developed in order to increase *SMN2* gene expression [62]. The antisense oligonucleotides (about 20 nucleotides length) are complementary to *SMN2* exon 7 sequence but also contain non-complementary tails, able to bind splicing factors and to stimulate the natural splicing reaction. Applying this strategy, additional to the action on *SMN2* exon 7 splicing, a partial restoration of gems number in SMA fibroblast cells could be observed. *In vivo*, delivery of bifunctional RNAs was possible by using plasmid-based and rAAV recombinant adeno-associated virus vectors [63]. The advantage of rAAV vectors is the high tropism for myocytes and neuronal cells and the retrograde transport to neurons after the intramuscular injection. An improvement of the antisense oligonucleotides strategy was the use of modified U7snRNA, as vehicle for SMN antisense, RNA complementary to the 3' splice site of SMN exon 8 [64]. This way, the researchers were able to identify anti SMN U7snRNAs that can alter the splicing process and stimulate the *SMN2* exon 7 retaining. Compared to the others antisense oligonucleotides strategies that use chemical modified RNAs, the SMN U7snRNAs provide the advantage of the continuous delivery in the cell and the increased half-life of the RNAs.

Another RNA strategy for preventing *SMN2* exon7 from skipping is known as trans – splicing system. Trans-splicing is a natural but rare process that has been recently induced in therapeutic assays for cystic fibrosis [65] or Alzheimer disease [66]. This strategy is based on the competition for splices sites between endogenous RNA (mutant) and the therapeutic RNA (able to provide the correct RNA sequence through a trans splicing process). Initially, plasmids and rAAVs were used for delivery of trans splicing RNAs, in order to restore small nuclear ribonucleoprotein (snRNP) assembly *in vitro* [67]. As this method had no results *in vivo*, a new system was developed: a single vector that co-expresses the trans splicing RNA. This way, antisense RNA is able to increase the snRNP assembly and SMN level protein *in vitro* as well as *in vivo* [68]. Although the regulatory networks for this constructs are not yet clarified, the results are promising and there is a hope of improving therapeutic strategies for SMA, life expectancy and quality of life for patients.

Conclusions

A large spectrum of compounds that act through various mechanisms were tested in order to identify a therapeutically drug in treating spinal muscular atrophy disease. However, no compound is currently available in SMA therapy, as toxicity and half-life duration are some of

the limiting factors. A special care is needed when results obtained in animal models are extrapolated in clinical trials on humans, taking into account the genetics and physiopathological differences between species. The stem cell and gene therapy give promising results, but the confirmation for a drug and its way to delivery, are still being waited for.

References

1. Pearn J. (1978). Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. *Journal of Medical Genetics*, 5, 409-413.
2. Melki J., Lefebvre S., Burglen L., Burlet P., Clermont O., Millasseau P., Reboullet S., Benichou B., Zeviani M., LePaslier D., et al. (1994). De novo and inherited deletions of 5q13 region in spinal muscular atrophies. *Science*, 264, 1474-1477.
3. Crawford T.O, Pardo C.A. (1996). The neurobiology of childhood spinal muscular atrophy. *Neurobiology of Disease*, 3, 97-110.
4. Kaplan J.C. (2009). Gene table of monogenic neuromuscular disorders (nuclear genome only) Vol 19. No 1 January 2009. *Neuromuscular Disorders*, 19, 77-98.
5. Dubowitz V. (1999). Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. *European Journal of Paediatric Neurology*, 3, 49-51.
6. Lefebvre S., Burglen L., Reboullet S., Clermont O., Burlet P., Viollet L., Benichou B., Cruaud C., Millasseau P., Zeviani M. et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, 80, 155-165.
7. Lorson C.L., Hahnen E., Androphy E.J., Wirth B. (1999). A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proceeding of the National Academy of Sciences of the United States of America*, 96, 6307-6311.
8. Lorson C.L., Androphy E.J. (2000). An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. *Human Molecular Genetics*, 9, 259-265.
9. Hofmann Y., Lorson C.L., Stamm S., Androphy E.J., Wirth B. (2000). Htra2-beta 1 stimulates an exonic splicing enhancer and can restore full-length SMN expression to survival motor neuron 2 (SMN2). *Proceedings of the National Academy of Sciences of the United States of America*, 97, 9618-9623.
10. Cartegni L., Krainer A.R. (2002). Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nature Genetics*, 30, 377-384.
11. Kashima T, Manley J.L. (2003). A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nature Genetics*, 34, 460-463.
12. Cartegni L., Hastings M.L., Calarco J.A., De Stanchina E., Krainer A.R. (2006). Determinants of exon 7 splicing in the spinal muscular atrophy genes, SMN1 and SMN2. *American Journal of Human Genetics*, 78, 63-77.
13. Echaniz-Laguna A., Miniou P., Bartholdi D., Melki J. (1999). The promoters of the survival motor neuron gene (SMN) and its copy (SMNc) share common regulatory elements. *American Journal of Human Genetics*, 64, 1365-1370.
14. Kernochan L.E., Russo M.L., Woodling N.S., Huynh T.N., Avila A.M., Fischbeck K.H., Sumner C.J. (2005). The role of histone acetylation in SMN gene expression. *Human Molecular Genetics*, 14, 1171-1182.
15. Chang J.G., Hsieh-Li H.M., Jong Y.J., Wang N.M., Tsai C.H., Li H. (2001). Treatment of spinal muscular atrophy by sodium butyrate. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 9808 - 9813.
16. Andreassi C., Angelozzi C., Tiziano F.D., Vitali T., De Vincenzi E., Boninsegna A., Villanova M., Bertini E., Pini A., Neri G., Brahe C. (2004). Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy. *European Journal of Human Genetics*, 12, 59-65.
17. Mercuri E., Bertini E., Messina S., Pelliccioni M., D'Amico A., Colitto F., Mirabella M., Tiziano F.D., Vitali T., Angelozzi C., Kinali M., Main M., Brahe C. (2004). Pilot trial of phenylbutyrate in spinal muscular atrophy. *Neuromuscular Disorders*, 14, 130 - 135.
18. Brahe C., Vitali T., Tiziano F.F., Angelozzi C., Pinto A.M., Borgo F., Moscato U., Bertini E., Mercuri E., Neri G. (2005). Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients. *European Journal of Human Genetics*, 13, 256-259.
19. Maestri N.E., Brusilow S.W., Clissold D.B., Bassett S.S. (1996). Long-term treatment of girls with ornithine transcarbamylase deficiency. *The New England Journal of Medicine*, 335, 855 - 859.
20. Grzeschik S.M., Ganta M., Prior T.W., Heavlin W.D., Wang C.H. (2005). Hydroxyurea enhances SMN2 gene expression in spinal muscular atrophy cells. *Annals of Neurology*, 58, 194 - 202.
21. www.clinicaltrials.gov, identification number NCT00568698, accessed on August 2009.
22. www.clinicaltrials.gov, identification number NCT00485511, accessed on August 2009.
23. Brichita L., Hofmann Y., Hahnen E., Siebzehrubel F.A., Raschke H., Blumcke I., Eyupoglu I.Y., Wirth B. (2003). Valproic acid increases the SMN2 protein level: a well-known drug as potential therapy for spinal muscular atrophy. *Human Molecular Genetics*, 12, 2481-2489.
24. Sumner C.J., Huynh T.N., Markowitz J.A., Perhac J.S., Hill B., Coovert D.D., Schussler K., Chen X., Jarecki J., Burghes A.H., Taylor J.P., Fischbeck K.H. (2003).

- Valproic acid increases SMN levels in spinal muscular atrophy patient cells. *Annals of Neurology*, 54, 647-654.
25. Tsai L.K., Tsai M.S., Ting C.H., Li H. (2008). Multiple therapeutic effects of valproic acid in spinal muscular atrophy model mice. *Journal of Molecular Medicine*, 86, 1243-1254.
 26. Brichta L., Holker I., Haug K., Klockgether T., Wirth B. (2006). In vivo activation of SMN in spinal muscular atrophy carriers and patients treated with valproate. *Annals of Neurology*, 59, 970-975.
 27. www.clinicaltrials.gov, identification numbers NCT00227266, NCT00374075, NCT00481013, NCT00661453, accessed on August 2009.
 28. 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., Sumner C.J. (2007). Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. *The Journal of Clinical Investigation*, 117, 659-671.
 29. Hahnen E., Eyupoglu I.Y., Brichta L., Haastert K., Trankle C., Siebzehrubl F.A., Riessland M., Holker I., Claus P., Romstock J., Buslei R., Wirth B., Blumcke I. (2006). In vitro and ex vivo evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy. *Journal of Neurochemistry*, 98, 193-202.
 30. Zhang M.L., Lorson C.L., Androphy E.J., Zhou J. (2001). An in vivo reporter system for measuring increased inclusion of exon 7 in SMN2 mRNA: potential therapy of SMA. *Gene Therapy*, 8, 1532-1538.
 31. Andreassi C., Jarecki J., Zhou J., Coover D.D., Umrao R.M., Chen X., Whitney M., Pollok B., Zhang M., Androphy E., Burghes A.H. (2001). Aclarubicin treatment restores SMN levels to cells derived from type I spinal muscular atrophy patients. *Human Molecular Genetics*, 10, 2841-2849.
 32. Sakla M.S., Lorson C.L. (2008). Induction of full-length survival motor neuron by polyphenol botanical compounds. *Human Genetics*, 122, 635-643.
 33. Ramassamy C. (2006). Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. *European Journal of Pharmacology*, 545, 51-64.
 34. Grondard C., Biondi O., Armand A.S., Lecolle S., Della Gaspera B.D., Pariset C., Li H., Gallien C.L., Vidal P.P., Chanoine C., Charbonnier F. (2005). Regular exercise prolongs survival in a type 2 spinal muscular atrophy model mouse. *The Journal of Neuroscience*, 25, 7615-7622.
 35. Biondi O., Grondard C., Lecolle S., Deforges S., Pariset C., Lopes P., Cifuentes - Diaz C., Li H., della Gaspera B., Chanoine C., Charbonnier F. (2008). Exercise-induced activation of NMDA receptor promotes motor unit development and survival in a type 2 spinal muscular atrophy model mouse. *The Journal of Neuroscience*, 28, 953-962.
 36. Lunn M.R., Root D.E., Martino A.M., Flaherty S.P., Kelley B.P., Coover D.D., Burghes A.H., Man N., Morris G. E., Zhou J., Androphy E.J., Sumner C.J., Stockwell B.R. (2004). Indoprofen upregulates the survival motor neuron protein through a cyclooxygenase-independent mechanism. *Chemistry & Biology*, 11, 1489-1493.
 37. Howard M., Frizzell R.A., Bedwell D.M. (1996). Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. *Nature Medicine*, 2, 467-469.
 38. Barton-Davis E.R., Cordier L., Shoturma D. I., Leland S.E., Sweeney H.L. (1999). Aminoglycoside antibiotics restore dystrophin function to skeletal muscle of mdx mice. *The Journal of Clinical Investigations*, 104, 375-381.
 39. Manuvakhova M., Keeling K., Bedwell D.M. (2000). Aminoglycoside antibiotics mediate context-dependent suppression of termination codons in a mammalian translation system. *RNA*, 6, 1044-1055.
 40. Wolstencroft E.C., Mattis V., Bajer A.A., Young P.J., Lorson C.L. (2005). A non-sequence-specific requirement for SMN protein activity: the role of aminoglycosides in inducing elevated SMN protein levels. *Human Molecular Genetics*, 14, 1199-1210.
 41. Mattis V.B., Rai R., Wang J., Chang C.W., Coody T., Lorson C.L. (2006). Novel aminoglycosides increase SMN levels in spinal muscular atrophy fibroblasts. *Human Genetics*, 120, 589-601.
 42. Chang H.C., Hung W.C., Chuang Y.J., Jong Y.J. (2004). Degradation of survival motor neuron (SMN) protein is mediated via ubiquitin/proteasome pathway. *Neurochemistry International*, 45, 1107-1112.
 43. Burnett B.G., Munoz E., Tandon A., Kwon D.Y., Sumner C.J., Fischbeck H. (2009). Regulation of SMN protein stability. *Molecular and Cellular Biology*, 29, 1107-1115.
 44. Thoenen H., Hughes R.A., Sendtner M. (1993). Trophic support of motoneurons: physiological, pathophysiological, and therapeutic implications. *Experimental Neurology*, 124, 47-55.
 45. Oppenheim R.W., Wiese S., Prevette D., Armanini M., Wang S., Houenou L.J., Holtmann B., Gotz R., Pennica D., Sendtner M. (2001). Cardiotrophin-1, a muscle-derived cytokine, is required for survival of subpopulations of developing motoneurons. *The Journal of Neuroscience*, 21, 1283-1291.
 46. Lesbordes J.C., Cifuentes-Diaz C., Miroglio A., Joshi V., Bordet T., Kahn A., Melki J. (2003). Therapeutic benefits of cardiotrophin-1 gene transfer in a mouse model of spinal muscular atrophy. *Human Molecular Genetics*, 12, 1233-1239.
 47. Miller R.G., Moore D.H., Dronskey V., Bradley W., Barohn R., Bryan W., Prior T.W., Gelinas D.F., Iannaccone S., Kissel J., Leshner R., Mendell J., Mendoza M., Russman B., Samaha F., Smith S; SMA Study Group. (2001). A placebo-controlled trial of gabapentin in spinal muscular atrophy. *Journal of the Neurological Science*, 191, 127-131.
 48. Merlini L., Solari A., Vita G., Bertini E., Minetti C., Mongini T., Mazzoni E., Angelini C., Morandi L. (2003). Role of gabapentin in spinal muscular atrophy: results of a multicenter, randomized Italian study. *Journal of Child Neurology*, 18, 537-541.

49. Haddad H., Cifuentes-Diaz C., Miroglio A., Roblot N., Joshi V., Melki J. (2003). Riluzole attenuates spinal muscular atrophy disease progression in a mouse model. *Muscle Nerve*, 28, 432-437.
50. Russman B.S., Iannaccone S.T., Samaha F.J. (2003). A phase 1 trial of riluzole in spinal muscular atrophy. *Archives of Neurology*, 60, 1601-1603.
51. Swoboda K.J., Kissel J.T., Crawford T.O., Bromberg M.B., Acsadi G., D'Anjou G., Krossschell K.J., Reyna S.P., Schroth M.K., Scott C.B., Simard L.R. (2007). Perspectives on clinical trials in spinal muscular atrophy. *Journal of Child Neurology*, 22, 957 – 966.
52. Kinali M., Mercuri E., Main M., De Biasia F., Karatza A., Higgins R., Banks L.M., Manzur A.Y., Muntoni F. (2002). Pilot trial of albuterol in spinal muscular atrophy. *Neurology*, 59, 609 - 610.
53. Angelozzi C., Borgo F., Tiziano F.D., Martella A., Neri G., Brahe C. (2008). Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells. *Journal of Medical Genetics*, 45, 29-31.
54. Rose Jr.F., Mattis V.B., Rindt H., Lorson C.L. (2009). Delivery of recombinant follistatin lessens disease severity in a mouse model of spinal muscular atrophy. *Human Molecular Genetics*, 18, 997-1005.
55. Lee S.J. (2007). Quadrupling muscle mass in mice by targeting TGF-beta signaling pathways. *PLoS ONE*, 2, e789.
56. Lee J.P., Jeyakumar M., Gonzalez R., Takahashi H., Lee P.J., Baek R.C., Clark D., Rose H., Fu G., Clarke J., McKercher S., Meerloo J., Muller F.J., Park K.I., Butters T.D., Dwek R.A., Schwartz P., Tong G., Wenger D., Lipton S.A., Seyfried T.N., Platt F.M., Snyder E.Y. (2007). Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nature Medicine*, 13, 439 – 447.
57. Flax J.D., Aurora S., Yang C., Simonin C., Wills A.M., Billinghamurst L.L., Jendoubi M., Sidman R.L., Wolfe J.H., Kim S.U., Snyder E.Y. (1998). Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. *Nature Biotechnology*, 16, 1033-1039.
58. Park K.I., Teng Y.D., Snyder E.Y. (2002). The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nature Biotechnology*, 20, 1111-1117.
59. Corti S., Nizzardo M., Nardini M., Donadoni C., Salani S., Ronchi D., Saladino F., Bordoni A., Fortunato F., Del Bo R., Papadimitriou D., Locatelli F., Menozzi G., Strazzer S., Bresolin N., Comi G. P. (2008). Neural stem cells transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. *The Journal of Clinical Investigation*, 118, 3316-3330.
60. Bordet T., Schmalbruck H., Pettmann B., Hagege A., Castelnau-Ptakhine L., Kahn A., Haase G. (1999). Adenoviral cardiotrophin-1 gene transfer protects pmn mice from progressive motor neuronopathy. *The Journal of Clinical Investigation*, 104, 1077-1085.
61. Azzouz M., Le T., Ralph G.S., Walmsley L., Monani U.R., Lee D.C.P., Wilkes F., Mitrophanous K.A., Kingsman S.M., Burghes A.H.M., Mazarakis N.D. (2004). Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. *The Journal of Clinical Investigation*, 14, 1726-1731.
62. Skordis L.A., Dunckley M.G., Yue B., Eperon I.C., Muntoni F. (2003). Bifunctional antisense oligonucleotides provide a trans-acting splicing enhancer that stimulates SMN2 gene expression in patient fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 4114-4119.
63. Baughan T., Shababi M., Coady T., Dickson A., Tullis G., Lorson C. (2006). Stimulating full-length SMN2 expression by delivering bifunctional RNAs via a viral vector. *Molecular Therapy*, 14, 54-62.
64. Madocsai C., Lim S.R., Geib T., Lam B.J., Hertel K.J. (2005). Correction of SMN2 Pre-mRNA Splicing by Antisense U7 Small Nuclear RNAs. *Molecular Therapy*, 12, 1013-1022.
65. Liu X., Jiang Q., Mansfield S.G., Puttaraju M., Zhang Y., Zhou W., Cohn J.A., Garcia-Blanco M.A., Mitchell L.G., Engelhardt J.F. (2002). Partial correction of endogenous DeltaF508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing. *Nature Biotechnology*, 20, 47-52.
66. Rodriguez-Martin T., Garcia-Blanco M.A., Mansfield S.G., Grover A.C., Hutton M., Yu Q., Zhou J., Anderton B.H., Gallo J.M. et al. (2005). Reprogramming of tau alternative splicing by spliceosome-mediated RNA trans-splicing: implications for tautopathies. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 15659-15664.
67. Coady T.H., Shababi M., Tullis G., Lorson C. (2007). Restoration of SMN function: delivery of a trans-splicing RNA re-directs SMN2 pre-mRNA splicing. *Molecular Therapy*, 15, 1471-1478.
68. Coady T.H., Baughan T.D., Shababi M., Passini M.A., Lorson C. (2008). Development of a single vector system that enhances trans-splicing of SMN2 transcripts. *Plos One* (www.plosone.org) 3, e3468.