Review Article • DOI: 10.2478/v10134-010-0045-4 • Translational Neuroscience • 1(4) • 2010 • 308-321

VERSITA

Translational Neuroscience

Heidi R. Fuller[1,2],
Marija Barišić[3],
Đurđica Šešo-Šimić[4],
Tea Špeljko[5],
Glenn E. Morris[1,2],
Goran Šimić[5,*]

[1]*Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, United Kingdom*
[2]*Institute of Science and Technology in Medicine, Keele University, United Kingdom*
[3]*Department of Ophthalmology, University Hospital Center "Zagreb", Zagreb, Croatia*
[4]*Department of Paediatrics, Zagreb-East Medical Center, Zagreb, Croatia*
[5]*Department of Neuroscience, Croatian Institute for Brain Research, University of Zagreb Medical School, Zagreb, Croatia*

Received 17 December 2010
accepted 17 December 2010

TREATMENT STRATEGIES FOR SPINAL MUSCULAR ATROPHY

Abstract
Progress in understanding the genetic basis and pathophysiology of spinal muscular atrophy (SMA), along with continuous efforts in finding a way to increase survival motor neuron (SMN) protein levels have resulted in several strategies that have been proposed as potential directions for efficient drug development. Here we provide an overview on the current status of the following approaches: 1) activation of *SMN2* gene and increasing full length *SMN2* transcript level, 2) modulating *SMN2* splicing, 3) stabilizing SMN mRNA and SMN protein, 4) development of neurotrophic, neuroprotective and anabolic compounds and 5) stem cell and gene therapy. The new preclinical advances warrant a cautious optimism for emergence of an effective treatment in the very near future.

Keywords
Clinical trials • Spinal muscular atrophy • *SMN1* gene • *SMN2* gene • SMN protein • Treatment • Therapy

INTRODUCTION

Spinal muscular atrophy (SMA) is the second most common autosomal recessive disorder and one of the leading known genetic causes of infant mortality. It is a neuromuscular disorder of childhood and young adults with an incidence of at least 1/10000 live births [1]. SMA is characterized by a progressive denervation of skeletal muscles, presumably due to the loss of anterior horn cells, and consequently symmetrical hypotonia, muscle weakness, atrophy and paralysis of varying severity [2-6]. SMA is clinically classified into four types according to the age at onset and disease severity. In SMA type I, or Werdnig-Hoffmann disease, onset is before 9 months of age and affected infants never sit unaided. Death occurs within the first two years, most commonly due to respiratory failure. SMA type II is defined by onset of symptoms between 3 and 15 months, with less severe symptoms than seen in SMA type I patients. Affected children are able to sit unaided, but they are wheelchair-bound and most survive to the 2nd or 3rd decade [7]. In SMA type III, or Kugelberg-Welander disease, onset is from 4-15 years, children walk unaided, and due to the slow progression of the muscle weakness, they live into adulthood [8]. SMA type IV is a rare, mild form of the disease, with onset after 30 years of age and normal life expectancy [9]. In most developed countries, the diagnosis is based on clinical features and DNA testing (genotyping) in nearly all cases. Sometimes, the diagnosis is also confirmed by muscle biopsy, electromyography and MRI of the spine.

The underlying genetic defect is on the chromosome 5q13 (*SMN* gene). Humans posses two copies of this gene, centromeric (*SMN1*) and telomeric (*SMN2*), which differ in only 8 single nucleotide changes, two of which are in exons 7 and 8 [10,11]. The *SMN1* gene produces majority of full length SMN (FL-SMN) proteins in healthy individuals, because *SMN2* gene C to T transition at codon 280 in exon 7 causes skipping of this exon during alternative splicing of the *SMN2* gene. Consequently, about 80-90% of *SMN2* transcripts lack exon 7 and produce dysfunctional protein. In homozygous absence of *SMN1* gene, the 10% of full length SMN protein produced by the *SMN2* gene is not sufficient to compensate for the absence of *SMN1* gene and prevent the disease [12-14]. However, a higher number of *SMN2* copies will generate more than 10% of FL-SMN protein and this will lead to less severe SMA types [15-19].

THERAPEUTIC STRATEGIES FOR SMA

A better understanding of pathogenesis and huge preclinical progress are leading to new ideas for SMA therapeutic strategies. The current main therapeutic strategies are: 1) activation of the *SMN2* gene and increasing full length *SMN2* transcript level, 2) modulation of *SMN2* splicing, 3) stabilization of SMN mRNA and SMN protein, 4) the use of neurotrophic, neuroprotective and anabolic compounds [7, 20], 5) stem cell and gene therapy. Supportive care to prevent and manage the secondary effects of muscle atrophy is still the basis for treatment of SMA, so the progress in multidisciplinary clinical care should also be developed and nourished [21-23].

* *E-mail: gsimic@hiim.hr*

1. Compounds that activate *SMN2* gene and increase full length *SMN2* transcript level

Both SMN genes are regulated by a promoter that is almost identical [14,24] in sequence and activity. Besides cAMP binding protein, Sp family of proteins and interferon regulatory factor [25,26], SMN promoter activity is also modulated by histone deacetylase (HDAC) 1 and 2 proteins. Once the core histone NH2-terminus is acetylated, relaxation of corresponding chromatin region enables it to be more transcriptionally active [7,27,28]. The balance between histone acetyltransferase and histone deacetylase activity determines level of transcription and is an important epigenetic mechanism that regulates gene expression.

The HDAC inhibitor **sodium butyrate** was one of the first drugs shown to influence SMN expression. Treatment of SMA lymphoid cell lines with sodium butyrate increased full-lenght *SMN2* transcript and protein levels, while treatment of SMA-like mice resulted in an increased expression of SMN protein in motor neurons of the spinal cord and significant improvement of SMA clinical symptoms [29]. Unfortunately, studies indicate that the short half-life of sodium butyrate in human serum does not recommend its use in clinical trials [30].

A derivative of sodium butyrate - sodium-4-phenyl-butyrate, also known as **phenylbutyrate (PB)**, was also shown to increase SMN transcript expression, SMN protein and gem counts in fibroblast cells derived from SMA patients [31,32]. It has a well-known pharmacokinetic and safety profile and good penetration into the central nervous system, and so appeared to be a promising candidate for the treatment of SMA. In a small uncontrolled trial an improvement of motor function in 10 SMA type II patients was found after nine weeks of treatment with oral PB, with no apparent major side effects [33]. However, a placebo-controlled trial of intermittent treatment over 13 weeks suggested that there was no improvement in functional scores under the conditions used [34,35]. A phase I/II study initiated by the US National Institute of Neurological Disorders and Stroke to identify the maximum tolerated dose in SMA I patients and to evaluate for biological activity was terminated due to extremely slow recruitment, but an open label phase I/II study to evaluate safety, tolerability and potential efficacy in presymptomatic SMA type I and type II genotyped infants is still ongoing in US [36].

Another HDAC inhibitory compound with a promising effect in SMA treatment is **hydroxyurea (HU)**. This compound has been previously shown to activate the fetal globin gene and is now used to treat patients with sickle cell disease and thalassemias.

In vitro, HU increased the amount of full-length SMN transcript and production of SMN protein in patient-derived lymphoblastoid cell lines [37,38]. A larger randomized uncontrolled trial from the same investigators included 33 patients with SMA type II and III who were treated for 8 weeks with three different doses of HU. The study demonstrated an improvement in muscle power and clinical improvement in SMA type II and III patients, without side effects, but its efficacy remains to be established in larger randomized controlled trials [39]. We are currently awaiting the final results of a randomized, double-blind, placebo-controlled trial in children with SMA type I that has completed recruitment in the US, and for the two different phase II/III, placebo-controlled randomized safety/efficacy trials, with SMA type II and III patients that should be soon completed in US and Taiwan [36].

Valproic acid (VPA) or 2 - propylpentanoic acid is the most studied HDAC inhibitor and neurotrophic factor in SMA treatment. It is widely used in the treatment of epilepsy and bipolar disorders [40]. Unlike other HDAC inhibitors, VPA has the advantage of a long half-life of up to 8-10 hours. A few *in vitro* studies proposed VPA as a promising drug for SMA treatment due to its effect on increasing FL-*SMN2* mRNA and protein levels in skin fibroblasts from type I SMA patients [41,42]. As expected, a stronger response was obtained when low doses of VPA (0.5-5μM) were administrated on fibroblast lines in SMA patients with more than two *SMN2* copies [41].

In vitro and also *in vivo* studies [43] have encouraged the opening of VPA clinical trials. In some patients VPA improved the clinical condition together with an increased SMN protein level and improvement in muscle strength and function; however, in some patients it had no effect or even caused worsening of symptoms [44-46]. The most recent data obtained showed that treatment with VPA for 3 months resulted in an increase in SMN protein levels (27-289%) in SMA type I and II patients as measured by ELISA in PBMC (peripheral blood mononuclear cells) [47]. VPA treatment has also been shown to improve gross motor function in patients less than 5 years of age (42 test subjects), but carnitine depletion was frequent, and decline in motor function occurred in several subjects in association with weight gain [48]. Bone mineral density increased, FL-SMN levels were unchanged and SMNΔ7 levels decreased.

A few clinical trials with VPA and carnitine (to avoid the possible toxicity of the drug) have been also recently started [36,43,48]. A phase II non-randomized, safety/efficacy open label study, with 42 patients provided good evidence that VPA can be used safely in SMA subjects over 2 years of age, with close monitoring of carnitine status. The data also appeared to demonstrate that there was an improvement in gross motor function in younger non-ambulatory type II children [49]. However, data from the CARNI-VAL Trial (a double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in SMA) showed that no statistically significant improvement was detected during six months treatment with VPA and L-carnitine in non-ambulatory SMA type II and III sitters 2-8 years of age. Weight gain, age and treatment duration were significant confounding factors that would need to be considered in the design of future trials [50]. Another CARNI-VAL phase I/II trial for SMA type I patients is currently underway and is expected to be completed by December 2010 [36].

Recently, a new mechanism of action of HDAC inhibitors was described. Even though *SMN2* copy number has been identified as the major SMA disease modifier, it is clear that patients with identical *SMN1* mutations and *SMN2* copy numbers have differences in disease progression [16,17,51,52]. Recent evidence suggests that differences in DNA methylation can affect *SMN2* expression [52] and also that *SMN2* sequence variations, for example c.859G>C substitution, can also affect

the disease severity [53]. Histone deacetylase (HDAC) inhibitors including **vorinostat** and **romidepsin** have been identified as able to bypass *SMN2* gene silencing by DNA methylation, while others, such as valproic acid and phenylbutyrate, are not, due to HDAC isoenzyme specificities [52]. The finding that DNA methylation is functionally important in SMA disease progression and pharmacological *SMN2* gene activation might have implications for future SMA therapy regimens.

The second generation HDAC inhibitors such as **Trichostatin A, suberoylanilide hydroxamic acid (SAHA), M344 benzamide, MS-275, m-Carboxycinnamic acid, bis-Hydroxamide** were used in several recent studies [54-57]. Out of these compounds, SAHA seems to be the most promising drug for therapy, as it increases SMN levels at low concentrations, has a favorable cytotoxicity profile and is well tolerated [28,55]. Moreover, it has just been shown that oral administration of SAHA rescued embryonic lethality of US-SMA mice (carrying one copy of *SMN2* per allele) [57], and increased survival (by 30%) of Taiwanese SMA mice (2 *SMN2* copies per allele). The treatment slightly improved weight progression and significantly ameliorated motor impairment. This study once again confirmed the notion that SAHA augments the *SMN2* RNA and SMN protein levels in both spinal cord and muscle. In addition, SAHA increased motor neuron number at PND10, arborization complexity and neuromuscular junction size, as well as the size of muscle fibers (most likely due to the protective activity of SAHA on α-motor neurons and elevated level of SMN protein in muscle).

LBH589 (**Panobinostat**), a hydroxamic acid-derived HDACi is already widely used in cancer clinical trials and is a promising candidate for treatment of SMA. Treatment of SMA fibroblasts with LBH589 significantly increased SMN protein levels, gem number and transcription of Gemin2 and Gemin3 (highest values between 6-and8-fold) [58]. The increased SMN production was shown to be due to splicing correction of *SMN2* transcription (maybe through hTRA2-ß1 splicing factor up-regulation), and also by reducing its ubiquitinylation and stabilizing it. The mechanism by which LBH589 reduces SMN ubiquitinylation is also not understood, but one possibility is that enhanced incorporation of SMN into the SMN complex reduces its accessibility to the ubiquitin proteasome system (UPS). Alternatively, LBH589 might cause differential expression of the proteins involved in SMN ubiquitinylation. LBH589 was also shown to increase *SMN2* expression in human neural stem cell cultures, in the spinal cord of SMA mice and in fibroblasts derived from VPA-non-responding as well as negative-responding patients [58].

Molecular docking studies have shown that **(E)-resveratrol**, a polyphenolic compound, exhibits the highest binding capacity toward HDAC8 enzyme (the first human HDAC for which 3D and X-ray structure was described), compared to TSA, SAHA and VPA [59]. Whilst (E)-resveratrol inhibited HDAC activity in a HeLa nuclear extract, only one SMA type I fibroblast cell line showed 1.3-fold increase in FL-*SMN2* mRNA levels, a decrease in *SMNΔ7* mRNA, as well as temporal increase in SMN protein level.

A new direction for the development of drugs that are capable of increasing the expression of SMN protein levels could be the use of compounds that change cellular **pH microenvironment** since they can modulate pre-mRNA alternative splicing *in vivo*. In SMA cells, 5-(N-ethyl-N-isopropyl)-amiloride, a Na^+/H^+ exchanger inhibitor, significantly increased *SMN2* exon 7 inclusion and SMN protein production [60].

2. Compounds that modulate *SMN2* splicing

In the majority of SMA patients, production of truncated SMN protein is the result of alternative splicing of exon 7 in *SMN2* gene transcripts. Preventing this exon from skipping is currently one of the most attractive approaches to increase the amount of full-length of SMN protein [61,62]. The phosphatase inhibitor **sodium vanadate**, was the first compound identified that can stimulate exon 7 inclusion into transcripts derived from the endogenous *SMN2* gene [63].

Aclarubicin, a drug from anthracylcine antibiotic class and frequently used as a chemotherapeutic, was shown to significantly increase the full-length SMN protein level in SMA type I patient derived fibroblasts by stimulating exon 7 inclusion in *SMN2* gene transcripts. Unfortunately, the side effects and known toxicity profile of this drug prohibits its use in treating young SMA patients [64]. Some polyphenolic botanical compounds have been shown to provide benefit in varied genetic diseases [65]. In transient reporter assays, **curcumin** was shown to increase exon 7 inclusion of *SMN2*. Full-length SMN mRNA and protein was increased in SMA patient fibroblasts, as well as the formation of SMN-containing nuclear gems [66]. These compounds appear to up-regulate some genes encoding serine/arginine-rich (SR) proteins, which are essential in splicing processes [67], so this may explain the alternative splicing effects on *SMN2* exon 7. Importantly, there is evidence that several polyphenolic compounds can pass the blood brain barrier, which is one of the challenging factors for developing drugs to target motor neurons [65].

Two studies based on mouse models reported that **physical exercise** can affect *SMN2* splicing regulation, either by modifying the expression pattern of pre-mRNA splicing factors in motoneurons [68] or through the NMDA–receptor activity modulation [69]. Interestingly, *in-vivo* NMDA receptor activation in type II SMA mice has been shown to exhibit neuroprotective effects including acceleration of motor unit maturation, protection of spinal motor neurons and enhancment of *SMN2* gene expression. In addition, life span was strongly extended in two different mouse models of severe SMA. The benefits of NMDA appeared to be dose-dependent as high doses favored apoptotic pathways and decreased SMN expression. Analysis of signaling cascades revealed that NMDA receptor activation appears to reactivate the CamKII/AKT/CREB (cAMP response element-binding protein) [70]. Since SMN contains a cAMP-response element (CRE-II) that can positively regulate the expression of the SMN gene [71], it is possible that NMDA receptor activation enhances SMN gene expression via this pathway.

A non-pharmacological strategy to enhance exon 7 inclusion is the use of **synthetic antisense oligonucleotides** that bind to *SMN2*-derived transcripts and promote exon 7 inclusion

during splicing. This strategy works well *in vitro*, but delivery of these oligonucleotides to motor neurons remains to be an unresolved problem and the main challenge of this approach [61,72].

An antisense oligonucleotide, **2'OMe ASO**, has been identified to enhance SMN exon 7 inclusion and to completely reverse the *SMN2* splicing pattern towards full-length SMN in SMA patient fibroblasts [73]. More recently, bilateral intracerebroventricular (ICV) injections of 2'OMe ASO in SMA mice at PND1, 3, 5, 7 and 10 were shown to increase spinal cord and brain SMN protein expression (as much as 50% of the level of healthy littermates) at PND12 and was accompanied by significant improvement in bodyweight and righting response, although motor function correction was only partial [74].

Another antisense oligonucleotide called **18mer 2'-O-(2-methoxyethyl) (MOE) ASO** (ASO 10-27) was shown to increase SMN protein levels after ICV administration in type III SMA homozygous mice [75]. It also increased FL-*SMN2* mRNA and protein levels in type III SMA heterozygous mice. Embryonic ICV administration resulted in an increase in exon 7 inclusion at PND7, which decreased, but was still significantly higher than control, at PND30. In addition, ICV administration of MOE ASO at E15 was shown to rescue, or delay, ear and tail necrosis in the mild SMA mouse model, as a result of reinnervation of the trail muscle. The authors suggested that MOE ASO may be more suitable than 2'-OMe ASO for SMA therapy since it appears safer and much more effective because it does not induce inflammation in the mouse CNS.

A class of **bifunctional RNAs** (with an antisense component) have been described that are designed to elevate SMN through either the recruitment of splicing factors or inhibition of negatively regulating sequences. Baughan *et al.* [76] constructed bifunctional RNAs that target an inhibitory sequence of SMN exon 7, called E1. Transfection of SMA type I patient fibroblasts with the bifunctional RNAs increased SMN protein levels in cytoplasm and nucleus, as well as gem numbers. The same was observed after adding a 2'-O-methyl group for stabilization of the RNA. ICV injection at PND2 in SMA mice increased SMN protein levels in brain and spinal cord. The bifunctional RNA Tra2-E1 was also shown to increase weight-gain and lifespan of a more severe SMA mouse model ($SMN2^{+/+}Smn^{-/-}$). Delivery of a trans-splicing system that involves the co-expression of a *SMN2* trans-splicing RNA and an antisense RNA that blocks a downstream splice site in *SMN2* pre-mRNA was recently shown to improve severity in a severe mouse model of SMA [77]. After ICV injections of vectors expressing tsRNA and anti-senseRNA (plasmid pMU3) in severe SMA (SMN-/-*SMN2*+/+) mice, trans-splicing was detectable in the CNS and at distal sites including the kidney and liver. It was determined that pMU3 increased levels of small ribonucleoproteins (snRNP) assembly activity in the major (U1) and minor (U11) splicing pathways, and SMN protein levels were increased in lumbar spinal cords of severe SMA mice. The treatment increased lifespan by approximately 70%, but not bodyweight.

Synthetic small-molecule compounds offer an alternative to antisense oligonucleotide therapies. In particular, a **tetracycline derivative, PTK-SMA1** has been shown to increase FL-*SMN2* transcript levels in HeLa cell nuclear extracts [78]. Unlike other compounds that increase SMN via *SMN2* promoter, PTK-SMA1 acts by directly stimulating splicing of exon 7. Further analysis specifically determined that PTK-SMA1 stimulates splicing from exon 6 to exon 7 and inhibits exon 7 skipping. PTK-SMA1 increased transcript and protein levels in SMA patient fibroblasts, as well as the number of SMN-containing gems. A 6-day course of treatment with PTK-SMA1 increased transcript and protein levels in SMA type I-like and type III-like mice.

3. Compounds that stabilize SMN mRNA and SMN protein

Aminoglycosides are well-known antibiotics that alter translation by reading-through initial stop codons. Wolstencroft et al. [79] showed that the SMN exon 7 sequence is not specifically required, rather that it functions as a "tail" that facilitates proper localization. They proposed that activity of SMNΔ7 protein could be restored by suppression recognition of the stop codon to induce a longer C-terminus. Furthermore, they demonstrated that treatment of SMA patient fibroblasts with aminoglycosides tobramycin and amikacin resulted in an increase in SMN-positive gems and increase in SMN protein. Mattis and collaborators proposed six aminoglycosides which were found to increase SMN protein level in fibroblast cells that may be used for therapeutic purposes [80], and later, showed that a novel aminoglycoside, TC007, increased gem numbers in iPS-SMA cells (induced pluripotent stem cells from a type I primary patient fibroblasts) [81]. ICV injection at PND5 of SMA mice elevated SMN protein levels in brain and spinal cord. The observed increase was attributed to read-through activity, since the splicing ratio and the total amount of *SMN2* mRNA was unaffected. TC007 treatment increased survival of ventral horn cells at end-stage of the disease (PND14), improved motor function and survival, and importantly, appeared to be non toxic for unaffected animals. When TC007 was delivered directly to the central nervous system (CNS) of an intermediate SMA model ($SMN^{-/-}$; $SMN2^{+/+}$; SMNΔ7) it induced SMN expression in both the brain and spinal cord, increased lifespan by about 30% and increased ventral horn cell number (consistent with its ability to increase SMN levels in induced pluripotent stem cell-derived human SMA motor neuron cultures) [81]. However, when TC007 was delivered by subcutaneous administration, it increased gross motor function and muscle integrity in SMA mice at mid-stage of disease, but did not extend the life-span significantly (16%), nor significantly increase the level of SMN protein [82].

The aminoglycoside **geneticin (G418)** was identified as an agent capable of suppressing termination at the target D7-SMN stop codon by inducing read-through. Intraperitoneal injection of G418 in SMNΔ7 mice at PND5-10 resulted in an increase in SMN protein level, measured in the brain, kidney and spinal cord, but not muscle (at PND13 SMN level was still high in kidney, but not significantly in spinal cord and brain). SMN protein level was also increased when SMA patient fibroblasts were treated with G418 (and to a greater extent than with amikacin, tobramycin, VPA and forskolin). G418 improved motor function of SMA mice but bodyweight and lifespan of affected animals was not improved. In addition, toxicity

after 9 day administration in unaffected mice was noted [83].

Ultrahigh-throughput screening recently identified **substituted quinazolines** as potent *SMN2* inducers [84]. Oral administration of several piperidine 2,4-diaminoquinazoline derivatives to neonatal mice induced SMN promoter activity in the central nervous system. Oral administration at PND04 of one particular derivative, D156844, increased SMN protein expression (nonstatistically significant 1.7-fold increase) in the spinal cord of neonatal SMNΔ7 mice and improved their phenotype and lifespan (21% longer than control).

Acting on a translational level, **indoprofen** is able to increase SMN protein level in the treated fibroblast cells by 13%, and to reduce embryonic lethality in SMA mutant mice. Unfortunately, both indoprofen and aminoglycosides have poor blood–brain barrier penetration [85].

There is an increasing interest in improving the stability of SMN and SMN complexes as an approach to therapy, in addition to increasing SMN synthesis. Activation of PKA through cyclic AMP appears to inhibit SMN degradation by facilitating incorporation of SMN into complexes [86]. There are also studies indicating that the SMN protein is degraded by the **ubiquitin–proteasome** system and that drugs that inhibit this pathway increase SMN protein levels in patient-derived fibroblasts [86,87]. SMN interacts, directly or indirectly, stably or transiently, with a large number of other proteins, some of which contribute to SMN stability and may also be potential targets for SMA therapy. Further understanding of SMN protein stability and dynamics may lead to other SMA therapeutic targets.

4. Neuroprotective, neurotrophic and anabolic compounds

4.1 Neuroprotective compounds

One of the therapeutic strategies in SMA is to protect SMN-deficient motor neurons from degeneration [88]. There is still no *in vitro* motoneuron model of SMA, therefore there have not yet been cell-based high-throughput screens to identify compounds that might be neuroprotective. Hopefully, efforts in developing motor neurons differentiated from embryonic stem cells will soon help to find a satisfactory solution.

Riluzole is a compound with neuroprotective effect, based on its anti-glutamatergic activity. Glutamate is released after presynaptic depolarization and insufficient glutamate elimination leads to increased levels of free radicals and motor neuron degeneration [89, 90].

Riluzole's neuroprotective efficacy was tested in an SMA mouse mode, where it attenuated the disease progression [91]. In a randomized placebo-controlled study with only 10 SMA type I patients [92], authors concluded that it was not possible to prove that riluzole is an efficacious treatment in SMA type I. Despite this, and even though treatment groups were not comparable, it was very intriguing that three out of seven patients treated with riluzole were still alive at ages two, three and five years and used BiPAP ventilation support only at night. A subsequent open-label study of 44 SMA I patients had insufficient enrollment, and randomized placebo controlled trial (RCT) of riluzole is ongoing on 150 SMA type II and III patients in France [49]. Treatment of type I SMA patients with a drug such as riluzole, that may prolong survival but does not appear to improve motor function is ethically debatable [93-95].

Creatine increases muscle mass and strength through its role as an energy shuttle between mitochondria and working musculature, and it could also have neuroprotective effects [96-98]. In a double-blind randomized placebo-controlled trial in 55 patients with SMA type II and III, unfortunately there was no evidence for a therapeutic effect of oral creatine after six months of treatment [99].

Gabapentin, an excitatory amino acid neurotransmitter, is another compound with neuroprotective effects due to its anti-glutamate action [90-101]. From one large randomized unblinded and uncontrolled trial with gabapentin in 120 patients with SMA type II and III it was not possible to draw conclusions regarding the efficacy of gabapentin [90]. In the randomized controlled trial in adult patients of 21 years or older with SMA type II and III there was no evidence for significant efficacy of twelve months treatment with oral gabapentin [102].

L-carnitine is an essential cofactor for the beta-oxidation of long-chain fatty acids and it inhibits mitochondrial injury and apoptosis both *in vitro* and *in vivo*. A decrease of carnitine was found in muscles of patients with SMA type I. L-carnitine treatment restored the level of free carnitine to normal in an animal model [103]. **Acetyl-L-carnitine**, the non-acetylated derivative of L-carnitine, has neuroprotective and neurotrophic activity in motoneuron cultures [104]. Although the CARNI-VAL trial (a randomized, placebo-controlled trial of L-carnitine and valproic acid in SMA) proved no benefit from six months treatment, we are waiting for the results of another randomized, placebo-controlled trial for SMA type I [36].

4.2 Neurotrophic compounds

Cardiotrophin-1 is a cytokine from interleukin-6 family, with known beneficial effects on survival of motor neurons during the embryonic period [105]. A preclinical study in SMA mice showed that cardiotrophin-1 delivery to SMA mutant mice resulted in protection of proximal and distal motoneurons, which was present even in postnatal period [106]. In this study, cardiotrophin-1 was delivered by intramuscular injection with an adenoviral vector expressing cardiotrophin-1 and it was able to improve survival and delay the motor defect in SMAΔ7 mice in neurons even at low doses. It provided good evidence that neurotrophic factors deserve further study as potential drugs in SMA [20].

Thyrotropin releasing hormone (TRH) has been shown to have a neurotrophic effect on spinal motor neurons of SMA patients [107]. A small double-blind randomized placebo-controlled trial in patients with SMA type II and III compared intravenous thyrotropin releasing hormone therapy with placebo, over a 29 day period [107,108]. A significant improvement in muscle strength was only reported in one patient and so a larger crossover comparison study is required in order to draw any conclusions regarding the efficacy of TRH treatment. Kato *et al.* [110] reported that oral administration of **taltirelin hydrate**, a TRH analogue, increased muscle strength of an 18-year-old male SMA type III patient, without any clinical adverse effects encountered by intravenous injection of TRH.

Salbutamol acts as a beta2-adrenoceptor agonist on human skeletal muscle [111-113] and has been shown to increase *SMN2* full length mRNA and the SMN protein levels in fibroblast cells derived from SMA patients [114]. In an open label pilot trial with thirteen SMA type II and III patients, there was a significant increase in muscle strength measured by myometry and forced vital capacity (FVC) after six months of salbutamol treatment. The therapy was well tolerated, but in several patients an increase in contractures was seen that may have been due to a stronger effect of salbutamol on agonist muscles [115]. In another open trial on 23 SMA type II patients, the functional scores were significantly higher after 6 and 12 months of treatment with salbutamol and there were no major side effects [116]. Tiziano *et al.* [117] reported that oral administration of salbutamol (albuterol) for 6 months, induced a significant and persistent increase in *SMN2*-FL transcript levels in leukocytes of 12 type II/III SMA patients (mean increase of 91.8%). The increase positively correlated with the *SMN2* copy number.

The open and uncontrolled study on safety and tolerability of recombinant **ciliary neurotrophic factor (CNTF)** included 10 patients with SMA type I. Six patients died, no change in muscle function and strength was noted and a difference between disease course and side effects could not be made [118].

The anabolic agent, **follistatin**, is a natural antagonist of myostatin, a member of TGF-ß super-family and a negative regulator of skeletal muscle mass [119]. Intraperitoneal follistatin injections in SMA mice appeared to increase muscle mass (between 20 and 25%), significantly improved motor function and extended the lifespan by approximately 30% [120]. Follistatin did not increase SMN or plastin-3 protein levels in spinal cord and triceps muscle, suggesting that its mechanism may be independent of SMN. However, Sumner *et al.* [121] reported that transgenic overexpression of follistatin in SMA mice did not increase muscle mass significantly, and did not affect motor function and survival of the animals.

It is possible that a combined therapy of neurotrophic, neuroprotective and anabolic agents may lead to the improvement of SMA phenotype and, in an implicit manner, to the lifespan duration [122].

4.3 Stem cell therapy for SMA

An important new strategy for SMA treatment is cell replacement. Embryonic stem cells (ESCs) are omnipotent cells that can be directed to differentiate into motor neurons [7]. Some interesting and promising data show that these stem cell-derived motor neurons can grow axons and form neuromuscular junctions in preclinical experiments [123,124]. Transplantation of differentiated ESCs into rat spinal cord after induced motor neuron injury also shows promising results: cells survived, but also produced axons that were able to grow into the ventral root [125,126]. The motor neuron function improvement and survival of SMA mice led to the conclusion that neural stem cell transplantation has positive effects on SMA disease phenotype [127]. The improvement in motor neuron function can arise through different mechanisms, such as replacement of non-neuronal cells, delivery of neuroprotective factors or reduction of toxic compounds [128-130].

Stem cell therapy could have promising results in the future, but at present, a major obstacle is the generation of a large amount of pure and differentiated human motor neuron populations from stem cells [131]. Once this is possible, other challenges will be to ensure that they are able to populate the nervous system and survive, grow axons, create synapses, and in the end, result in a significant functional improvement [21].

Corti *et al.* [132] generated cultures of motor neuron cells (double positive for HB9 and ChAT) from embryonic stem cells. These cells migrated extensively to spinal cords of SMA mice (after intrathecal transplantation at PND1) and produced NeuN and ß-III-tubulin, as determined by confocal immunohistochemical analysis. A fraction differentiated into motoneurons (ChAT and SMI32 expression and morphology) and there was no tumor formation. The cells exhibited neuroprotective characteristics on endogenous motor neurons – there were 6-7% of donor cells at PND13, but the reduction of motor neurons was significantly attenuated. It was determined that ESC-NSC produced GDNF, BDNF, TGF-α and NT3.

4.4 Gene therapy for SMA

Gene replacement and manipulation is another new treatment approach that could lead to a cure for SMA. Similarly to stem cell therapy, gene therapy development is also limited due to technical difficulties, such as efficient gene delivery to target tissues and problems related to random insertion of the therapeutic gene into the host DNA [7,20].

One of the first gene therapy studies on SMA was conducted on mice, with the intramuscular injection of adenoviral vector expressing cardiotrophin-1, whose delivery to SMA mutant mice resulted in protection of proximal and distal motoneurons, present even in postnatal period [106]. Another vector, used for *SMN1* gene transfer, was lentivector, developed from infectious equine anemia virus. It was injected into the voluntary muscles of mice and resulted in retrograde transport of *SMN1* gene, expression of SMN in spinal motor neurons, reduction of motor neuron death and consequently led to a modest increase in life expectancy of SMA mice by approximately 3–5 days [133].

RNA strategies have produced some promising results for potential SMA therapy. *SMN1* and *2* genes are almost identical in sequence, apart from a substitution of C to T at position +6 of exon 7, which is likely the predominant functional difference between the two genes. This change alters RNA splicing, which results in the removal of exon 7 from the mature mRNA, and as a result, only 10% full-length transcripts are produced from the *SMN2* gene. Considering these facts, a new strategy involving single base mutation gene repair, has been developed. **Single-stranded oligonucleotides (ODN)** that are specific for the *SMN2* sequence have been used to direct the exchange of T to C in skin fibroblasts from an SMA type I patient. Genotype analysis of these cells showed direct genetic conversion of the *SMN2* genotype to *SMN1* and an increase in production of full-length SMN mRNA was detected [13].

A single nucleotide difference in *SMN2* exon 7, which disrupts an exonic splicing enhancer,

can also be modified by **bifunctional antisense oligoribonucleotides**. These are complementary to exon 7, but also contain non-complementary tails, and are able to bind splicing factors and to stimulate the natural splicing reaction. These tailed oligoribonucleotides were shown to increase *SMN2* exon 7 splicing *in vitro* and rescued the incorporation of *SMN2* exon 7 in SMA patient fibroblasts, as well as partial restoration of SMN-containing nuclear gems [72].

Plasmid-based and recombinant adeno-associated virus (rAAV) vectors enable delivery of **bifunctional RNAs** [134]. The advantage of rAAV vectors is the high tropism for muscles and neurons, the retrograde transport to neurons *in vivo* after the intramuscular injection and increased half-life of RNA [135]. An improved antisense oligonucleotide strategy was the use of **modified U7snRNA** as a vehicle for the antisense RNA complementary to the 3′ splice site of SMN exon 8. Transduction of HeLa cells with *SMN2* minigenes and anti-SMN U7snRNA expressing adenovirus type 5-derived vectors was recently shown to increase exon 7 inclusion by as much as 33 to 60% [137]. Infection of SMA patient fibroblast cell lines also resulted in an increase of FL-*SMN2* transcripts.

Meyer *et al.* [138] used a U7 snRNA construct, U7-ESE-B, which targeted the 3′ part of exon 7 and carried an ESE sequence that attracted stimulatory splicing factors for a lentiviral (LV)-mediated transgenesis in SMA mice. This approach resulted in prolonged survival of those animals (123 days for SMA/U7, 6.5 for SMA, ~20-fold increase in survival time). Weight-gain seen in transgenic pups implied that SMA symptoms had been suppressed, although it depended on the number of copies of the U7-ESE-B transgene and results were variable. The inclusion of exon 7 in *SMN2* transcripts of SMA/U7 mice increased to as much as 44% and a moderate increase in SMN protein expression was seen in total spinal cord and also within the motoneurons. The researchers presume, by a recent observation, that a higher expression could be achieved in human patients.

Valori *et al.* [139] constructed a self-complementary adeno-associated viral vector (scAAV9) with a codon-optimized *SMN1* cDNA that rescued a SMNΔ7 mouse model (80% of treated animals). The expression of *SMN1* increased in several tissues examined and the phenotype of transfected (intravenous delivery at PND1) animals improved. In another study, scAAV9-SMN delivery to SMNΔ7 mice was shown to help correct the early heart failure seen in this model [140]. Bradycardia was abolished and a decrease in the severity of heart defect symptoms was noted.

Intracerebroventricular and lumbar injection of AAV8-hSMN at PND00 in SMA mice elevated SMN protein levels in lumbar, thoracic and cervical segments of the spinal cord, measured at PND16 [141]. Myofibers (in quadriceps, gastrocnemius, intercostal muscle) were by 2-fold larger than the ones from untreated animals. The treatment improved NMJ structure and motor function, and a substantial increase in survival (up to 66 days) was observed. Treatment with scAAV-hSMN (human SMN-expressing self-complementary AAV vector) resulted in an improvement in median survival of 157 days. A significant decrease in the number of collapsed structures in NMJs from quadriceps and intercostal muscles was seen with scAAV-hSMN compared with AAV8-hSMN. However, there was an increase in the number of aberrant NMJs at 216-269 days.

Foust *et al.* [142] injected intravenously (IV) scAAV9-SMN in SMA mice at PDN01 and this was shown to increase SMN protein levels in brain, spinal cord and muscle, and improve motor function, bodyweight and lifespan. In addition, treatment with scAAV9-SMN appeared to fully correct the reduction in synaptic current. The results suggested that there is a developmental period in which scAAV9 therapy was of maximum benefit, since treatment at postnatal day 5 resulted in partial correction, whereas treatment at postnatal day 10 had little effect. They also reported that IV injection in a male cynomolgus macaque at PND01 of scAAV9-GFP successfully transfected dorsal root ganglia and motor neurons, demonstrating that it has the potential to cross the blood-brain barrier in primates.

An alternative RNA strategy for preventing *SMN2* exon 7 from skipping is to use a t**rans–splicing system**. It has been recently introduced in therapeutic assays for cystic fibrosis [143] and Alzheimer's disease [144]. This strategy is based on the competition for splicing sites between endogenous RNA (mutant) and the therapeutic RNA (able to provide the correct RNA sequence through a trans-splicing process). *In vivo* delivery of an optimized trans-splicing vector in SMA mice appears to increase snRNP assembly, which is an important SMN-dependent activity. A single delivery of the vector to severe SMA mice neonates resulted in reduced phenotype severity and extended survival by about 70% [145].

In addition to RNA strategies that target SMN, manipulation of other proteins involved in motor neuron survival may be a useful approach. **Phosphatase and tensin homolog (PTEN)** is a negative regulator of the rapamycin (mTOR) pathway and is enriched in axon terminals of purified motor neurons. Knock-down of PTEN in motor neurons resulted in an increase in growth cone size, promotion of axonal elongation and increased cell survival [146]. In addition, PTEN downregulation in *SMN*$^{-/-}$ *SMN2* motor neurons restored ß-actin protein levels in the growth cones. Delivery of AAV6 expressing siPTEN into the hind limb muscle of PND1 SMA mice resulted in >35% increase in survival of lumbar spinal cord motoneurons at PND10.

5. Other approaches

The base of treatment in SMA is still s**upportive care**, which requires multidisciplinary medical care [21]. Due to intensive supportive care, such as ventilation, the use of mechanical insufflation-exsufflation device and tube feeding, the survival of patients with SMA type I has significantly increased in recent years [22]. For future therapeutic trials it is very important that the supportive care in different treatment facilities is the same, because variability in clinical care among centers can affect the clinical course of the disease.

To address variability in current practice, The International Standard of Care Committee for Spinal Muscular Atrophy was formed in 2005. Their efforts resulted in development of consensus statement for standard of care in SMA. They achieved consensus on 5 care areas: diagnostic/new interventions, pulmonary, gastrointestinal/nutrition, orthopedics/rehabilitation, and palliative care.

Consensus was achieved on several topics related to common medical problems in spinal muscular atrophy, diagnostic strategies, and recommendations for assessment and monitoring, and therapeutic interventions in each care area. The 5 care areas are divided in 3 functional levels of the patients: non sitter, sitter, and walker. It is the authors' intention that this document is used as a guideline, not as a practice standard for their care. A practice standard for SMA is urgently needed to help with the multidisciplinary care of these patients [124].

Since **forced exercise** was found to be of benefit in ALS mouse models [147,148] it was recently studied in SMA mice. Type II SMA mice developed by Hsieh-Li *et al.* [149] were used in the interesting study of Grondard *et al.* [68]. The mice were subjected to a training protocol of forced run on a wheel or no training. Trained mice showed improved survival of approximately 57% as well as reduced motor neuron loss. Interestingly, in trained animals there was also evidence of increased full-length SMN transcript and protein in spinal cord tissues. The authors speculate that exercise may activate genes that promote exon 7 inclusion in *SMN2*-derived transcripts [68]. Another study based on a type 2 SMA mouse model proved that physical exercise has beneficial effects in motor neuron protection, acceleration of muscle maturation and lifespan gain through the NMDA–receptor activity modulation [69]. A combined therapy of drugs and regular physical exercises is therefore thought to improve clinical signs in SMA patients.

Butchbach *et al.* [150] determined that the diet of dams influenced lifespan and motor function of pups. PicoLab20 opposed to Harlan-Tekland 22/5 (with fat content 9% opposed 5.2%) had increased lifespan for 21%, and improved righting reflex at PND07. Pups from PicoLab20-fed dams (WT and SMNΔ7) had higher blood glucose levels, and lower ß-hydroxybutyrate levels. Plastin-3 and SMN levels were similar for both diets.

CONCLUSION

There has been dramatic preclinical progress in the last two decades with the discovery of the gene and accordingly gene therapy that provides one of several promising targets for treatment [21]. However, we can conclude that there is still no satisfactory drug for SMA treatment. Drug treatment in future trials should be given for an extended period of time and patient follow up should be sufficiently long (preferably at least one year for both treatment and follow up) [109].

Outcome measures should be unique in all trials in order to be able to compare different studies and their therapy efficacy [21]. The aggravating circumstance regarding the treatment of this relatively rare disease is the wide variability of severity which makes it difficult to collect a homogenous study population or to use the same outcome measures for all patients. Recent international consensus has emphasized the importance of designing and performing separate trials of patients with type I SMA and those with type II and III and even to stratify within these groups to obtain as homogeneous study population as possible [151].

Successful clinical trials for SMA require reliable, feasible, economical, sensitive to change, and clinically meaningful outcome measures [35,49,152]. Motor function measures, pulmonary measures, quantitative muscle strength testing and quality of life measures have been studied as outcomes in SMA and were found to be reliable [153-156]. Innovative study design could help overcome some of the challenges in conducting trials in treating this relatively rare disease [21].

Discovering efficient **biomarkers** that reflect the pathogenesis, severity and clinical improvement of SMA would have potential advantages of decreasing the sample size required in early phase II trials, as well as accessing therapeutic efficacy because they are more easily measured than different clinical outcomes [157,158]. Future treatment trials in SMA are complicated by some ethical concerns. It is ethically debatable whether it is desirable to treat patients with SMA type I with a drug that has an effect on survival, but does not appear to improve motor function and has no effect on achieving motor milestones such as rolling, sitting, standing or walking. Recently, arguments for and arguments against were described [93-95].

Altogether, there has been a huge progress in new approaches and development of a wider range of animal models for SMA, which provides cautious optimism that effective treatment for SMA will eventually emerge [159].

ACKNOWLEDGEMENT

This article resulted from fruitful discussions during the 7th UK SMA Researchers' conference that was held at Lake Vyrnwy, North Wales in October 2010. The authors would like to thank the SMA Trust and the Jennifer Trust for SMA for their sponsorship and help with organising the meeting.

References

[1] Botta A., Tacconelli A., Bagni I., Giardina E., Bonifazi E., Pietropolli A., *et al.*, Transmission ratio distortion in the spinal muscular atrophy locus: data from 314 prenatal tests, Neurology, 2005, 65, 1631-1635

[2] Bromberg M. B., Swoboda K. J., Motor unit number estimation in infants and children with spinal muscular atrophy, Muscle Nerve, 2002, 25, 445-447

[3] Crawford T. O., Pardo C. A., The neurobiology of childhood spinal muscular atrophy, Neurobiol Dis, 1996, 3, 97-110

[4] Dubowitz, Disorders of the lower motor neuron, the spinal muscular atrophy, 1995, in: Dubowitz (ed), Muscle disorders in childhood, Saunders, London, 325-369

[5] Greenfield J.C., Stern R.O., The anatomical identity of the Werdnig-Hoffmann and Oppenheim forms of infantile muscular atrophy, Brain, 1927, 50, 652-686

[6] Pearn J., Classification of spinal muscular atrophies, Lancet, 1980, 1, 919-922

[7] Simic G., Pathogenesis of proximal autosomal recessive spinal muscular atrophy, Acta Neuropathol, 2008, 116, 223-234
[8] Zerres K., Rudnik-Schoneborn S., Natural history in proximal spinal muscular atrophy. Clinical analysis of 445 patients and suggestions for a modification of existing classifications, Arch Neurol, 1995, 52, 518-523
[9] Wharton S., Ince P.G., Pathology of motor neuron disorders, 2003. In: Shaw P.J., Strong M.J. (eds) Motor neuron disorders. Blue books of practical neurology, book 28. Butterworth-Heineman, Elsevier Science, Philadelphia, pp 17-49
[10] Burglen L., Lefebvre S., Clermont O., Burlet P., Viollet L., Cruaud C., *et al.*, Structure and organization of the human survival motor neurone (SMN) gene, Genomics, 1996, 32, 479-482
[11] Parsons D. W., McAndrew P. E., Iannaccone S. T., Mendell J. R., Burghes A. H., Prior T. W., Intragenic telSMN mutations: frequency, distribution, evidence of a founder effect, and modification of the spinal muscular atrophy phenotype by cenSMN copy number, Am J Hum Genet, 1998, 63, 1712-1723
[12] Brahe C., Bertini E., Spinal muscular atrophies: recent insights and impact on molecular diagnosis, J Mol Med, 1996, 74, 555-562
[13] DiMatteo D., Callahan S., Kmiec E. B., Genetic conversion of an *SMN2* gene to SMN1: a novel approach to the treatment of spinal muscular atrophy, Exp Cell Res, 2008, 314, 878-886
[14] Monani U. R., Lorson C. L., Parsons D. W., Prior T. W., Androphy E. J., Burghes A. H., *et al.*, A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene *SMN2*, Hum Mol Genet, 1999, 8, 1177-1183
[15] Brahe C., Copies of the survival motor neuron gene in spinal muscular atrophy: the more, the better, Neuromuscul Disord, 2000, 10, 274-275
[16] Feldkotter M., Schwarzer V., Wirth R., Wienker T. F., Wirth B., Quantitative analyses of SMN1 and *SMN2* based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy, Am J Hum Genet, 2002, 70, 358-368
[17] Mailman M. D., Heinz J. W., Papp A. C., Snyder P. J., Sedra M. S., Wirth B., *et al.*, Molecular analysis of spinal muscular atrophy and modification of the phenotype by *SMN2*, Genet Med, 2002, 4, 20-26
[18] McAndrew P. E., Parsons D. W., Simard L. R., Rochette C., Ray P. N., Mendell J. R., *et al.*, Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number, Am J Hum Genet, 1997, 60, 1411-1422
[19] Wirth B., Brichta L., Schrank B., Lochmuller H., Blick S., Baasner A., *et al.*, Mildly affected patients with spinal muscular atrophy are partially protected by an increased *SMN2* copy number, Hum Genet, 2006, 119, 422-428
[20] Sumner C. J., Therapeutics development for spinal muscular atrophy, NeuroRx, 2006, 3, 235-245
[21] Oskoui M., Kaufmann P., Spinal muscular atrophy, Neurotherapeutics, 2008, 5, 499-506
[22] Oskoui M., Levy G., Garland C. J., Gray J. M., O'Hagen J., De Vivo D. C., *et al.*, The changing natural history of spinal muscular atrophy type 1, Neurology, 2007, 69, 1931-1936
[23] Wang C. H., Finkel R. S., Bertini E. S., Schroth M., Simonds A., Wong B., *et al.*, Consensus statement for standard of care in spinal muscular atrophy, J Child Neurol, 2007, 22, 1027-1049
[24] Echaniz-Laguna A., Miniou P., Bartholdi D., Melki J., The promoters of the survival motor neuron gene (SMN) and its copy (SMNc) share common regulatory elements, Am J Hum Genet, 1999, 64, 1365-1370
[25] Baron-Delage S., Abadie A., Echaniz-Laguna A., Melki J., Beretta L., Interferons and IRF-1 induce expression of the survival motor neuron (SMN) genes, Mol Med, 2000, 6, 957-968
[26] Rouget R., Vigneault F., Codio C., Rochette C., Paradis I., Drouin R., *et al.*, Characterization of the survival motor neuron (SMN) promoter provides evidence for complex combinatorial regulation in undifferentiated and differentiated P19 cells, Biochem J, 2005, 385, 433-443
[27] Kernochan L. E., Russo M. L., Woodling N. S., Huynh T. N., Avila A. M., Fischbeck K. H., *et al.*, The role of histone acetylation in SMN gene expression, Hum Mol Genet, 2005, 14, 1171-1182
[28] Lunke S., El-Osta A., The emerging role of epigenetic modifications and chromatin remodeling in spinal muscular atrophy, J Neurochem, 2009, 109, 1557-1569
[29] Chang J. G., Hsieh-Li H. M., Jong Y. J., Wang N. M., Tsai C. H., Li H., Treatment of spinal muscular atrophy by sodium butyrate, Proc Natl Acad Sci U S A, 2001, 98, 9808-9813
[30] Miller A. A., Kurschel E., Osieka R., Schmidt C. G., Clinical pharmacology of sodium butyrate in patients with acute leukemia, Eur J Cancer Clin Oncol, 1987, 23, 1283-1287
[31] Andreassi C., Angelozzi C., Tiziano F. D., Vitali T., De Vincenzi E., Boninsegna A., *et al.*, Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy, Eur J Hum Genet, 2004, 12, 59-65
[32] Brahe C., Vitali T., Tiziano F. D., Angelozzi C., Pinto A. M., Borgo F., *et al.*, Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients, Eur J Hum Genet, 2005, 13, 256-259
[33] Mercuri E., Bertini E., Messina S., Pelliccioni M., D'Amico A., Colitto F., *et al.*, Pilot trial of phenylbutyrate in spinal muscular atrophy, Neuromuscul Disord, 2004, 14, 130-135
[34] Mercuri E., Bertini E., Messina S., Solari A., D'Amico A., Angelozzi C., *et al.*, Randomized, double-blind, placebo-controlled trial of phenylbutyrate in spinal muscular atrophy, Neurology, 2007, 68, 51-55
[35] Kaufmann P., Muntoni F., Issues in SMA clinical trial design. The International Coordinating Committee (ICC) for SMA Subcommittee on SMA Clinical Trial Design, Neuromuscul Disord, 2007, 17, 499-505
[36] www.clinicaltrials.gov
[37] Grzeschik S. M., Ganta M., Prior T. W., Heavlin W. D., Wang C. H., Hydroxyurea enhances *SMN2* gene expression in spinal muscular atrophy cells, Ann Neurol, 2005, 58, 194-202
[38] Liang W. C., Yuo C. Y., Chang J. G., Chen Y. C., Chang Y. F., Wang H. Y., *et al.*, The effect of hydroxyurea in spinal muscular atrophy cells and patients, J Neurol Sci, 2008, 268, 87-94

[39] Chang J.G., Tsai F.J., Wang W.Y., Jong Y.J., Treatment of spinal muscular atrophy by hydroxyurea, American Journal of Human Genetics, 2002, 7, issue 4:2402 suppl.

[40] Terbach N., Williams R. S., Structure-function studies for the panacea, valproic acid, Biochem Soc Trans, 2009, 37, 1126-1132

[41] Brichta L., Hofmann Y., Hahnen E., Siebzehnrubl F. A., Raschke H., Blumcke I., *et al.*, Valproic acid increases the *SMN2* protein level: a well-known drug as a potential therapy for spinal muscular atrophy, Hum Mol Genet, 2003, 12, 2481-2489

[42] Sumner C. J., Huynh T. N., Markowitz J. A., Perhac J. S., Hill B., Coovert D. D., *et al.*, Valproic acid increases SMN levels in spinal muscular atrophy patient cells, Ann Neurol, 2003, 54, 647-654

[43] Brichta L., Holker I., Haug K., Klockgether T., Wirth B., In vivo activation of SMN in spinal muscular atrophy carriers and patients treated with valproate, Ann Neurol, 2006, 59, 970-975

[44] Weihl C. C., Connolly A. M., Pestronk A., Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy, Neurology, 2006, 67, 500-501

[45] Wirth B., Brichta L., Hahnen E., Spinal muscular atrophy and therapeutic prospects, Prog Mol Subcell Biol, 2006, 44, 109-132

[46] Wirth B., Brichta L., Hahnen E., Spinal muscular atrophy: from gene to therapy, Semin Pediatr Neurol, 2006, 13, 121-131

[47] Piepers S., Cobben J. M., Sodaar P., Jansen M. D., Wadman R. I., Meester-Delver A., *et al.*, Quantification of SMN protein in leucocytes from spinal muscular atrophy patients: effects of treatment with valproic acid, J Neurol Neurosurg Psychiatry, 2010

[48] Swoboda K. J., Kissel J. T., Crawford T. O., Bromberg M. B., Acsadi G., D'Anjou G., *et al.*, Perspectives on clinical trials in spinal muscular atrophy, J Child Neurol, 2007, 22, 957-966

[49] Swoboda K. J., Scott C. B., Reyna S. P., Prior T. W., LaSalle B., Sorenson S. L., *et al.*, Phase II open label study of valproic acid in spinal muscular atrophy, PLoS One, 2009, 4, e5268

[50] Swoboda K. J., Scott C. B., Crawford T. O., Simard L. R., Reyna S. P., Krosschell K. J., *et al.*, SMA CARNI-VAL trial part I: double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in spinal muscular atrophy, PLoS One, 2010, 5, e12140

[51] Cusco I., Barcelo M. J., Rojas-Garcia R., Illa I., Gamez J., Cervera C., *et al.*, *SMN2* copy number predicts acute or chronic spinal muscular atrophy but does not account for intrafamilial variability in siblings, J Neurol, 2006, 253, 21-25

[52] Hauke J., Riessland M., Lunke S., Eyupoglu I. Y., Blumcke I., El-Osta A., *et al.*, Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylase inhibition, Hum Mol Genet, 2009, 18, 304-317

[53] Prior T. W., Krainer A. R., Hua Y., Swoboda K. J., Snyder P. C., Bridgeman S. J., *et al.*, A positive modifier of spinal muscular atrophy in the *SMN2* gene, Am J Hum Genet, 2009, 85, 408-413

[54] Avila A. M., Burnett B. G., Taye A. A., Gabanella F., Knight M. A., Hartenstein P., *et al.*, Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy, J Clin Invest, 2007, 117, 659-671

[55] Hahnen E., Eyupoglu I. Y., Brichta L., Haastert K., Trankle C., Siebzehnrubl F. A., *et al.*, In vitro and ex vivo evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy, J Neurochem, 2006, 98, 193-202

[56] Riessland M., Brichta L., Hahnen E., Wirth B., The benzamide M344, a novel histone deacetylase inhibitor, significantly increases *SMN2* RNA/protein levels in spinal muscular atrophy cells, Hum Genet, 2006, 120, 101-110

[57] 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, Hum Mol Genet, 2010, 19, 1492-1506

[58] 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, Hum Mol Genet, 2009, 18, 3645-3658

[59] Dayangac-Erden D., Bora G., Ayhan P., Kocaefe C., Dalkara S., Yelekci K., *et al.*, Histone deacetylase inhibition activity and molecular docking of (e)-resveratrol: its therapeutic potential in spinal muscular atrophy, Chem Biol Drug Des, 2009, 73, 355-364

[60] Yuo C. Y., Lin H. H., Chang Y. S., Yang W. K., Chang J. G., 5-(N-ethyl-N-isopropyl)-amiloride enhances *SMN2* exon 7 inclusion and protein expression in spinal muscular atrophy cells, Ann Neurol, 2008, 63, 26-34

[61] Cartegni L., Krainer A. R., Correction of disease-associated exon skipping by synthetic exon-specific activators, Nat Struct Biol, 2003, 10, 120-125

[62] Lorson C. L., Androphy E. J., An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN, Hum Mol Genet, 2000, 9, 259-265

[63] Zhang M. L., Lorson C. L., Androphy E. J., Zhou J., An in vivo reporter system for measuring increased inclusion of exon 7 in *SMN2* mRNA: potential therapy of SMA, Gene Ther, 2001, 8, 1532-1538

[64] Andreassi C., Jarecki J., Zhou J., Coovert D. D., Monani U. R., Chen X., *et al.*, Aclarubicin treatment restores SMN levels to cells derived from type I spinal muscular atrophy patients, Hum Mol Genet, 2001, 10, 2841-2849

[65] Ramassamy C., Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets, Eur J Pharmacol, 2006, 545, 51-64

[66] Sakla M. S., Lorson C. L., Induction of full-length survival motor neuron by polyphenol botanical compounds, Hum Genet, 2008, 122, 635-643

[67] Skommer J., Wlodkowic D., Pelkonen J., Gene-expression profiling during curcumin-induced apoptosis reveals downregulation of CXCR4, Exp Hematol, 2007, 35, 84-95

[68] Grondard C., Biondi O., Armand A. S., Lecolle S., Della Gaspera B., Pariset C., *et al.*, Regular exercise prolongs survival in a type 2 spinal muscular atrophy model mouse, J Neurosci, 2005, 25, 7615-7622

[69] Biondi O., Grondard C., Lecolle S., Deforges S., Pariset C., Lopes P., *et al.*, Exercise-induced activation of NMDA receptor promotes motor unit development and survival in a type 2 spinal muscular atrophy model mouse, J Neurosci, 2008, 28, 953-962

[70] Biondi O., Branchu J., Sanchez G., Lancelin C., Deforges S., Lopes P., *et al.*, In vivo NMDA receptor activation accelerates motor unit maturation, protects spinal motor neurons, and enhances *SMN2* gene expression in severe spinal muscular atrophy mice, J Neurosci, 2010, 30, 11288-11299

[71] Majumder S., Varadharaj S., Ghoshal K., Monani U., Burghes A. H., Jacob S. T., Identification of a novel cyclic AMP-response element (CRE-II) and the role of CREB-1 in the cAMP-induced expression of the survival motor neuron (SMN) gene, J Biol Chem, 2004, 279, 14803-14811

[72] Skordis L. A., Dunckley M. G., Yue B., Eperon I. C., Muntoni F., Bifunctional antisense oligonucleotides provide a trans-acting splicing enhancer that stimulates *SMN2* gene expression in patient fibroblasts, Proc Natl Acad Sci U S A, 2003, 100, 4114-4119

[73] Singh N. K., Singh N. N., Androphy E. J., Singh R. N., 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, 2006, 26, 1333-1346

[74] Williams J. H., Schray R. C., Patterson C. A., Ayitey S. O., Tallent M. K., Lutz G. J., Oligonucleotide-mediated survival of motor neuron protein expression in CNS improves phenotype in a mouse model of spinal muscular atrophy, J Neurosci, 2009, 29, 7633-7638

[75] Hua Y., Sahashi K., Hung G., Rigo F., Passini M. A., Bennett C. F., *et al.*, Antisense correction of *SMN2* splicing in the CNS rescues necrosis in a type III SMA mouse model, Genes Dev, 2010, 24, 1634-1644

[76] Baughan T. D., Dickson A., Osman E. Y., Lorson C. L., Delivery of bifunctional RNAs that target an intronic repressor and increase SMN levels in an animal model of spinal muscular atrophy, Hum Mol Genet, 2009, 18, 1600-1611

[77] Coady T. H., Lorson C. L., Trans-splicing-mediated improvement in a severe mouse model of spinal muscular atrophy, J Neurosci, 2010, 30, 126-130

[78] Hastings M. L., Berniac J., Liu Y. H., Abato P., Jodelka F. M., Barthel L., *et al.*, Tetracyclines that promote *SMN2* exon 7 splicing as therapeutics for spinal muscular atrophy, Sci Transl Med, 2009, 1, 5ra12

[79] Wolstencroft E. C., Mattis V., Bajer A. A., Young P. J., Lorson C. L., A non-sequence-specific requirement for SMN protein activity: the role of aminoglycosides in inducing elevated SMN protein levels, Hum Mol Genet, 2005, 14, 1199-1210

[80] Mattis V. B., Rai R., Wang J., Chang C. W., Coady T., Lorson C. L., Novel aminoglycosides increase SMN levels in spinal muscular atrophy fibroblasts, Hum Genet, 2006, 120, 589-601

[81] Mattis V. B., Ebert A. D., Fosso M. Y., Chang C. W., Lorson C. L., Delivery of a read-through inducing compound, TC007, lessens the severity of a spinal muscular atrophy animal model, Hum Mol Genet, 2009, 18, 3906-3913

[82] Mattis V. B., Ebert A. D., Fosso M. Y., Chang C. W., Lorson C. L., Delivery of a read-through inducing compound, TC007, lessens the severity of a spinal muscular atrophy animal model, Hum Mol Genet, 2009, 18, 3906-3913

[83] Heier C. R., DiDonato C. J., Translational readthrough by the aminoglycoside geneticin (G418) modulates SMN stability in vitro and improves motor function in SMA mice in vivo, Hum Mol Genet, 2009, 18, 1310-1322

[84] Butchbach M. E., Singh J., Thorsteinsdottir M., Saieva L., Slominski E., Thurmond J., *et al.*, Effects of 2,4-diaminoquinazoline derivatives on SMN expression and phenotype in a mouse model for spinal muscular atrophy, Hum Mol Genet, 2010, 19, 454-467

[85] Lunn M. R., Root D. E., Martino A. M., Flaherty S. P., Kelley B. P., Coovert D. D., *et al.*, Indoprofen upregulates the survival motor neuron protein through a cyclooxygenase-independent mechanism, Chem Biol, 2004, 11, 1489-1493

[86] Burnett B. G., Munoz E., Tandon A., Kwon D. Y., Sumner C. J., Fischbeck K. H., Regulation of SMN protein stability, Mol Cell Biol, 2009, 29, 1107-1115

[87] Chang H. C., Hung W. C., Chuang Y. J., Jong Y. J., Degradation of survival motor neuron (SMN) protein is mediated via the ubiquitin/proteasome pathway, Neurochem Int, 2004, 45, 1107-1112

[88] Thoenen H., Hughes R. A., Sendtner M., Trophic support of motoneurons: physiological, pathophysiological, and therapeutic implications, Exp Neurol, 1993, 124, 47-55

[89] Bryson H. M., Fulton B., Benfield P., Riluzole. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in amyotrophic lateral sclerosis, Drugs, 1996, 52, 549-563

[90] Merlini L., Solari A., Vita G., Bertini E., Minetti C., Mongini T., *et al.*, Role of gabapentin in spinal muscular atrophy: results of a multicenter, randomized Italian study, J Child Neurol, 2003, 18, 537-541

[91] Haddad H., Cifuentes-Diaz C., Miroglio A., Roblot N., Joshi V., Melki J., Riluzole attenuates spinal muscular atrophy disease progression in a mouse model, Muscle Nerve, 2003, 28, 432-437

[92] Russman B. S., Iannaccone S. T., Samaha F. J., A phase 1 trial of riluzole in spinal muscular atrophy, Arch Neurol, 2003, 60, 1601-1603

[93] Bach J. R., The use of mechanical ventilation is appropriate in children with genetically proven spinal muscular atrophy type 1: the motion for, Paediatr Respir Rev, 2008, 9, 45-50; quiz 50; discussion 55-46

[94] Bosboom W. M., Vrancken A. F., van den Berg L. H., Wokke J. H., Iannaccone S. T., Drug treatment for spinal muscular atrophy type I, Cochrane Database Syst Rev, 2009, CD006281

[95] Ryan M. M., The use of invasive ventilation is appropriate in children with genetically proven spinal muscular atrophy type 1: the motion against, Paediatr Respir Rev, 2008, 9, 51-54; discussion 55-56

[96] Bessman S. P., Geiger P. J., Transport of energy in muscle: the phosphorylcreatine shuttle, Science, 1981, 211, 448-452

[97] Ellis A. C., Rosenfeld J., The role of creatine in the management of amyotrophic lateral sclerosis and other neurodegenerative disorders, CNS Drugs, 2004, 18, 967-980

[98] Tarnopolsky M., Martin J., Creatine monohydrate increases strength in patients with neuromuscular disease, Neurology, 1999, 52, 854-857

[99] Wong B., Hynan L.S., Iannaccone S.T., AmSMART Group, A randomized, placebo-controlled trial of creatine in children with spinal muscular atrophy, J Clin Neuromuscul Dis, 2007, 8, 101-110

[100] Greensmith L., Vrbova G., Possible strategies for treatment of SMA patients: a neurobiologist's view, Neuromuscul Disord, 1995, 5, 359-369

[101] Taylor J. E., Thomas N. H., Lewis C. M., Abbs S. J., Rodrigues N. R., Davies K. E., *et al.*, Correlation of SMNt and SMNc gene copy number with age of onset and survival in spinal muscular atrophy, Eur J Hum Genet, 1998, 6, 467-474

[102] Miller R. G., Moore D. H., Dronsky V., Bradley W., Barohn R., Bryan W., *et al.*, A placebo-controlled trial of gabapentin in spinal muscular atrophy, J Neurol Sci, 2001, 191, 127-131

[103] Bresolin N., Freddo L., Tegazzin V., Bet L., Armani M., Angelini C., Carnitine and acyltransferase in experimental neurogenic atrophies: changes with treatment, J Neurol, 1984, 231, 170-175

[104] Bigini P., Larini S., Pasquali C., Muzio V., Mennini T., Acetyl-L-carnitine shows neuroprotective and neurotrophic activity in primary culture of rat embryo motoneurons, Neurosci Lett, 2002, 329, 334-338

[105] Oppenheim R. W., Wiese S., Prevette D., Armanini M., Wang S., Houenou L. J., *et al.*, Cardiotrophin-1, a muscle-derived cytokine, is required for the survival of subpopulations of developing motoneurons, J Neurosci, 2001, 21, 1283-1291

[106] Lesbordes J. C., Cifuentes-Diaz C., Miroglio A., Joshi V., Bordet T., Kahn A., *et al.*, Therapeutic benefits of cardiotrophin-1 gene transfer in a mouse model of spinal muscular atrophy, Hum Mol Genet, 2003, 12, 1233-1239

[107] Takeuchi Y., Miyanomae Y., Komatsu H., Oomizono Y., Nishimura A., Okano S., *et al.*, Efficacy of thyrotropin-releasing hormone in the treatment of spinal muscular atrophy, J Child Neurol, 1994, 9, 287-289

[108] Tzeng A. C., Cheng J., Fryczynski H., Niranjan V., Stitik T., Sial A., *et al.*, A study of thyrotropin-releasing hormone for the treatment of spinal muscular atrophy: a preliminary report, Am J Phys Med Rehabil, 2000, 79, 435-440

[109] Bosboom W. M., Vrancken A. F., van den Berg L. H., Wokke J. H., Iannaccone S. T., Drug treatment for spinal muscular atrophy types II and III, Cochrane Database Syst Rev, 2009, CD006282

[110] Kato Z., Okuda M., Okumura Y., Arai T., Teramoto T., Nishimura M., *et al.*, Oral administration of the thyrotropin-releasing hormone (TRH) analogue, taltireline hydrate, in spinal muscular atrophy, J Child Neurol, 2009, 24, 1010-1012

[111] Caruso J. F., Signorile J. F., Perry A. C., Leblanc B., Williams R., Clark M., *et al.*, The effects of albuterol and isokinetic exercise on the quadriceps muscle group, Med Sci Sports Exerc, 1995, 27, 1471-1476

[112] Kindermann W., Do inhaled beta(2)-agonists have an ergogenic potential in non-asthmatic competitive athletes?, Sports Med, 2007, 37, 95-102

[113] Martineau L., Horan M. A., Rothwell N. J., Little R. A., Salbutamol, a beta 2-adrenoceptor agonist, increases skeletal muscle strength in young men, Clin Sci (Lond), 1992, 83, 615-621

[114] Angelozzi C., Borgo F., Tiziano F. D., Martella A., Neri G., Brahe C., Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells, J Med Genet, 2008, 45, 29-31

[115] 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, 609-610

[116] Pane M., Staccioli S., Messina S., D'Amico A., Pelliccioni M., Mazzone E. S., *et al.*, Daily salbutamol in young patients with SMA type II, Neuromuscul Disord, 2008, 18, 536-540

[117] Tiziano F. D., Lomastro R., Pinto A. M., 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, J Med Genet, 2010, 47, 856-858

[118] Franz D.N., Tudor C.A., Samaha F.J., A phase I trial of recombinant human ciliary neurotrophic factor in spinal muscular atrophy, Ann Neurol,1995, 38, 546

[119] Lee S. J., Quadrupling muscle mass in mice by targeting TGF-beta signaling pathways, PLoS One, 2007, 2, e789

[120] Rose F. F., Jr., Mattis V. B., Rindt H., Lorson C. L., Delivery of recombinant follistatin lessens disease severity in a mouse model of spinal muscular atrophy, Hum Mol Genet, 2009, 18, 997-1005

[121] Sumner C. J., Wee C. D., Warsing L. C., Choe D. W., Ng A. S., Lutz C., *et al.*, Inhibition of myostatin does not ameliorate disease features of severe spinal muscular atrophy mice, Hum Mol Genet, 2009, 18, 3145-3152

[122] Stavarachi M., Apostol P., Toma M., Cimponeriu D., Gavrila L., Spinal muscular atrophy disease: a literature review for therapeutic strategies, J Med Life, 2010, 3, 3-9

[123] Gao J., Coggeshall R. E., Chung J. M., Wang J., Wu P., Functional motoneurons develop from human neural stem cell transplants in adult rats, Neuroreport, 2007, 18, 565-569

[124] Wichterle H., Lieberam I., Porter J. A., Jessell T. M., Directed differentiation of embryonic stem cells into motor neurons, Cell, 2002, 110, 385-397

[125] Deshpande D. M., Kim Y. S., Martinez T., Carmen J., Dike S., Shats I., *et al.*, Recovery from paralysis in adult rats using embryonic stem cells, Ann Neurol, 2006, 60, 32-44

[126] Harper J. M., Krishnan C., Darman J. S., Deshpande D. M., Peck S., Shats I., *et al.*, Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats, Proc Natl Acad Sci U S A, 2004, 101, 7123-7128

[127] 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, J Clin Invest, 2008, 118, 3316-3330

[128] Flax J. D., Aurora S., Yang C., Simonin C., Wills A. M., Billinghurst L. L., *et al.*, Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes, Nat Biotechnol, 1998, 16, 1033-1039

[129] Lee J. P., Jeyakumar M., Gonzalez R., Takahashi H., Lee P. J., Baek R. C., *et al.*, Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease, Nat Med, 2007, 13, 439-447

[130] Park K. I., Teng Y. D., Snyder E. Y., The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue, Nat Biotechnol, 2002, 20, 1111-1117

[131] Nayak M. S., Kim Y. S., Goldman M., Keirstead H. S., Kerr D. A., Cellular therapies in motor neuron diseases, Biochim Biophys Acta, 2006, 1762, 1128-1138

[132] 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, 2010, 133, 465-481

[133] Azzouz M., Le T., Ralph G. S., Walmsley L., Monani U. R., Lee D. C., *et al.*, Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy, J Clin Invest, 2004, 114, 1726-1731

[134] Baughan T., Shababi M., Coady T. H., Dickson A. M., Tullis G. E., Lorson C. L., Stimulating full-length *SMN2* expression by delivering bifunctional RNAs via a viral vector, Mol Ther, 2006, 14, 54-62

[135] Baughan T. D., Dickson A., Osman E. Y., Lorson C. L., Delivery of bifunctional RNAs that target an intronic repressor and increase SMN levels in an animal model of spinal muscular atrophy, Hum Mol Genet, 2009, 18, 1600-1611

[136] Madocsai C., Lim S. R., Geib T., Lam B. J., Hertel K. J., Correction of *SMN2* Pre-mRNA splicing by antisense U7 small nuclear RNAs, Mol Ther, 2005, 12, 1013-1022

[137] Geib T., Hertel K. J., Restoration of full-length SMN promoted by adenoviral vectors expressing RNA antisense oligonucleotides embedded in U7 snRNAs, PLoS One, 2009, 4, e8204

[138] Meyer K., Marquis J., Trub J., Nlend Nlend R., Verp S., Ruepp M. D., *et al.*, Rescue of a severe mouse model for spinal muscular atrophy by U7 snRNA-mediated splicing modulation, Hum Mol Genet, 2009, 18, 546-555

[139] Valori C. F., Ning K., Wyles M., Mead R. J., Grierson A. J., Shaw P. J., *et al.*, Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy, Sci Transl Med, 2010, 2, 35ra42

[140] Bevan A. K., Hutchinson K. R., Foust K. D., Braun L., McGovern V. L., Schmelzer L., *et al.*, Early heart failure in the SMNΔ7 model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery, Hum Mol Genet, 2010, 19, 3895-3905

[141] Passini M. A., Bu J., Roskelley E. M., Richards A. M., Sardi S. P., O'Riordan C. R., *et al.*, CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy, J Clin Invest, 2010, 120, 1253-1264

[142] Foust K. D., Wang X., McGovern V. L., Braun L., Bevan A. K., Haidet A. M., *et al.*, Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN, Nat Biotechnol, 2010, 28, 271-274

[143] Liu X., Jiang Q., Mansfield S. G., Puttaraju M., Zhang Y., Zhou W., *et al.*, Partial correction of endogenous DeltaF508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing, Nat Biotechnol, 2002, 20, 47-52

[144] Rodriguez-Martin T., Garcia-Blanco M. A., Mansfield S. G., Grover A. C., Hutton M., Yu Q., *et al.*, Reprogramming of tau alternative splicing by spliceosome-mediated RNA trans-splicing: implications for tauopathies, Proc Natl Acad Sci U S A, 2005, 102, 15659-15664

[145] Coady T. H., Shababi M., Tullis G. E., Lorson C. L., Restoration of SMN function: delivery of a trans-splicing RNA re-directs *SMN2* pre-mRNA splicing, Mol Ther, 2007, 15, 1471-1478

[146] Ning K., Drepper C., Valori C. F., Ahsan M., Wyles M., Higginbottom A., *et al.*, PTEN depletion rescues axonal growth defect and improves survival in SMN-deficient motor neurons, Hum Mol Genet, 2010, 19, 3159-3168

[147] Kirkinezos I. G., Hernandez D., Bradley W. G., Moraes C. T., Regular exercise is beneficial to a mouse model of amyotrophic lateral sclerosis, Ann Neurol, 2003, 53, 804-807

[148] Mahoney D. J., Rodriguez C., Devries M., Yasuda N., Tarnopolsky M. A., Effects of high-intensity endurance exercise training in the G93A mouse model of amyotrophic lateral sclerosis, Muscle Nerve, 2004, 29, 656-662

[149] Hsieh-Li H. M., Chang J. G., Jong Y. J., Wu M. H., Wang N. M., Tsai C. H., *et al.*, A mouse model for spinal muscular atrophy, Nat Genet, 2000, 24, 66-70

[150] Butchbach M. E., Rose F. F., Jr., Rhoades S., Marston J., McCrone J. T., Sinnott R., *et al.*, Effect of diet on the survival and phenotype of a mouse model for spinal muscular atrophy, Biochem Biophys Res Commun, 2010, 391, 835-840

[151] Bertini E., Burghes A., Bushby K., Estournet-Mathiaud B., Finkel R. S., Hughes R. A., *et al.*, 134th ENMC International Workshop: Outcome Measures and Treatment of Spinal Muscular Atrophy, 11-13 February 2005, Naarden, The Netherlands, Neuromuscul Disord, 2005, 15, 802-816

[152] Hirtz D., Iannaccone S., Heemskerk J., Gwinn-Hardy K., Moxley R., 3rd, Rowland L. P., Challenges and opportunities in clinical trials for spinal muscular atrophy, Neurology, 2005, 65, 1352-1357

[153] Iannaccone S. T., Hynan L. S., Reliability of 4 outcome measures in pediatric spinal muscular atrophy, Arch Neurol, 2003, 60, 1130-1136

[154] Main M., Kairon H., Mercuri E., Muntoni F., The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation, Eur J Paediatr Neurol, 2003, 7, 155-159

[155] Mercuri E., Messina S., Battini R., Berardinelli A., Boffi P., Bono R., *et al.*, Reliability of the Hammersmith functional motor scale for spinal muscular atrophy in a multicentric study, Neuromuscul Disord, 2006, 16, 93-98

[156] Merlini L., Bertini E., Minetti C., Mongini T., Morandi L., Angelini C., *et al.*, Motor function-muscle strength relationship in spinal muscular atrophy, Muscle Nerve, 2004, 29, 548-552

[157] De Gruttola V. G., Clax P., DeMets D. L., Downing G. J., Ellenberg S. S., Friedman L., *et al.*, Considerations in the evaluation of surrogate endpoints in clinical trials. summary of a National Institutes of Health workshop, Control Clin Trials, 2001, 22, 485-502

[158] Lesko L. J., Atkinson A. J., Jr., Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies, Annu Rev Pharmacol Toxicol, 2001, 41, 347-366

[159] Sproule D.M., Kaufmann P., Therapeutic developments in spinal muscular atrophy, Ther Adv Neurol Disorders, 2010, 3, 173-185

Table 1. Evaluation of effects of different treatment strategies on cell cultures (C), SMA mouse models (M) or patients suffering from SMA (P). Compounds, stem cell therapy, delivery systems and other approaches are graded based on their degree of positive (++/+), negative (--/-) or no effects (0).

* note that some compounds exerted positive (increased FL-SMN protein levels, increased motoneuron number, improved motor function, increased survival rate) as well as negative (cytotoxicity, poor blood-brain barrier penetration, short half-life, certain difficulties related to culturing and transplation of stem cells) or no effects in different studies or in different patients.

THERAPEUTIC STRATEGY		Name of compound, delivery system or approach	Effect of treatment	System tested	References
non-compound related evaluation	compounds that activate *SMN2* gene and increase full length *SMN2* transcript level	Sodium butyrate	+/-*	C	Chang, 2001
		Phenylbutyrate (PB)	++/-	C, M, P	Andreassi, 2004; Mercuri, 2004; Brahe, 2005; Kernochan, 2005; Wirth, 2006; Darras, 2007; Kaufmann, 2007; Mercuri, 2007
		Valproic acid (2-propylpentanoic acid)	+/0/-	C, M, P	Brichta, 2003; Sumner, 2003; Brichta, 2006; Weihl, 2006; Kim, 2007; Tsai, 2007; Swoboda, 2009; Swoboda, 2010
		Trichostatin A	++	M	Avila, 2007
		Suberoyl anilide hydroxamic acid (SAHA)	++	M	Hahnen, 2006; Lunke, 2009; Riessland, 2010
		M344 benzamide	+	C	Riessland, 2006
		Hydroxyurea	++	C	Chang, 2002; Grzeschik, 2005; Liang, 2008
		Vorinostat and romidepsin	+	C	Hauke, 2009
		5-(N-ethyl-N-isopropyl)-amiloride	+	C	Yuo, 2008
		Salbutamol (albuterol)	+	P	Tiziano, 2010
		LBH589 (Panobinostat)	+	C, M, P	Garbes, 2009
	compounds that modulate *SMN2* splicing	(E)-resveratrol	+	C	Dayangaç-Erden, 2009
		Sodium vanadate	+	C	Zhang, 2001
		Aclarubicin	+/--	C	Andreassi, 2001
		Synthetic antisense (including bifunctional) oligonucletides	++	C, M	Cartegni, 2003; Skordis, 2003; DiMatteo, 2008; Baughan, 2009; Williams, 2009; Coady, 2010; Hua, 2010
		Curcumin	+	C	Ramassamy, 2006; Sakla,2008
		NMDA	++?	M	Biondi, 2010
		PTK-SMA1	+	C, M	Hastings, 2009
	compounds that stabilize SMN mRNA and SMN protein	Aminoglycosides	++/0/-	C, M	Wolstencroft, 2005; Mattis, 2006; Heier, 2009; Mattis, 2009
		Indoprofen	+/-	C, M	Lunn, 2004
		Drugs that inhibit ubyquitin-proteasome system	+	C	Chang, 2004
		D156844 (piperidine 2,4-diaminoquinazoline derivative)	+	M	Butchbach, 2009
	neurotrophic, neuroprotective and anabolic compounds	Riluzole	+/-	M, P	Bryson, 1996; Haddad, 2003; Merlini, 2003; Russman, 2003; Swoboda, 2007; Bosboom, 2008
		Creatin	0	P	Bessman, 1981; Tarnopolsky, 1999; Ellis, 2004; Wong, 2007
		Gabapentin	0	P	Greensmith, 1995; Taylor, 1998; Miller, 2001; Merlini, 2003
		L-carnitine	+/0	P	Bresolin, 1984; Bigini, 2002
		Cardiotrophin-1	+	M	Oppenheim, 2001; Lesbordes, 2003; Sumner, 2006
		Thyrotropin releasing hormone (TRH)	+/0	P	Tzeng, 2000; Takeuchi, 2004; Bosboom, 2008; Kato, 2009
		Salbutamol	++	C, P	Kinali, 2002; Angelozzi, 2008; Pane, 2008
		Ciliary neurotrophic factor (CNTF)	0	P	Franz, 1995
		Follistatin	+/0	M	Lee, 2007; Rose, 2009; Sumner, 2009
	Stem cell therapy		++/-	M	Flax, 1998; Park, 2002; Wichterele, 2002; Harper, 2004; Deshpande, 2006; Nayak, 2006; Gao, 2007; Lee, 2007; Corti, 2008; Oskoui, 2008; Corti, 2010
	Gene therapy	Adenoviral vectors	+	C, M	Lesbordes, 2003; Geib, 2009
		Lentiviral vectors	+	M	Azzouz, 2004; Meyer, 2009
		AAV vectors	++	M	Bevan, 2010; Foust, 2010; Passini, 2010;Ning, 2010; Valori, 2010
	Other approaches	Supportive care	++	P	Oskoui, 2007; Wang, 2007; Oskoui, 2008
		Forced exercise	++	M	Grondard, 2005; Biondi, 2008
		Diet	+	M	Butchbach, 2010