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Review

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Spinal Muscular Atrophy: From Gene Discovery to Clinical Trials

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Summary

Spinal muscular atrophy (SMA) is a common neuromuscular disorder with autosomal recessive inheritance, resulting in the degeneration of motor neurons. The incidence of the disease has been estimated at 1 in 6000–10,000 newborns with a carrier frequency of 1 in 40–60. SMA is caused by mutations of the *SMN1* gene, located on chromosome 5q13. The gene product, survival motor neuron (SMN) plays critical roles in a variety of cellular activities. *SMN2*, a homologue of *SMN1*, is retained in all SMA patients and generates low levels of SMN, but does not compensate for the mutated *SMN1*. Genetic analysis demonstrates the presence of homozygous deletion of *SMN1* in most patients, and allows screening of heterozygous carriers in affected families. Considering high incidence of carrier frequency in SMA, population-wide newborn and carrier screening has been proposed. Although no effective treatment is currently available, some treatment strategies have already been developed based on the molecular pathophysiology of this disease. Current treatment strategies can be classified into three major groups: *SMN2*-targeting, *SMN1*-introduction, and non-*SMN* targeting. Here, we provide a comprehensive and up-to-date review integrating advances in molecular pathophysiology and diagnostic testing with therapeutic developments for this disease including promising candidates from recent clinical trials.

Keywords: Spinal muscular atrophy (SMA), survival motor neuron (SMN), diagnosis, clinical trials

Introduction

Spinal muscular atrophy (SMA; OMIM 253300) is an autosomal recessive neuromuscular disorder characterized by the degeneration of motor neurons in the spinal cord. The incidence of the disease has been estimated at 1 in 6000–10,000 newborns, with an expected carrier frequency of 1 in 40–60 (Prior et al., 2010). SMA is clinically heterogeneous and can be classified into three subtypes depending on the age of onset and achievement of motor milestones: SMA type 1 (severe type with the onset before the age of 6 months, unable to sit without support), SMA type 2 (intermediate type with the

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onset before the age of 18 months, unable to stand or walk without support), and SMA type 3 (mild type with the onset after the age of 18 months, able to stand and walk independently until the disease progresses) (Zerres & Davies, 1999). Additionally, two other forms of the disease with the most severe phenotype with prenatal onset and the mildest phenotype manifesting after 20 years of age, have been reported as SMA type 0 and SMA type 4, respectively (Kolb & Kissel, 2011).

Genetic linkage studies have mapped all disease subtypes to chromosome 5q13 and the survival motor neuron genes (*SMN*) were identified as the disease-causing genes in SMA (Brzustowicz et al., 1990; Gilliam et al., 1990; Melki et al., 1990a, b; Lefebvre et al., 1995). The cloning and characterization of *SMN1* (OMIM 600354) and its homologue *SMN2* (OMIM 6001627) have led to an improved understanding of the molecular basis of SMA and have facilitated the development of techniques for molecular diagnosis of this disease.

Although the pathogenesis of SMA remains to be fully understood, there have been active investigations into pharmacological agents and other novel therapeutic strategies for the treatment of SMA. An in-depth understanding of disease pathophysiology is necessary to direct design of therapeutic strategies. Elucidation of mechanisms and efficacies of the therapeutic approaches is also essential to guide clinical application. Here, we discuss advances in diagnostic procedures, molecular pathophysiology, and therapeutic strategies in SMA. In this review, information representing significant findings in SMA was collected from scientific articles published between 1990 and 2013 retrieved from PubMed and MEDLINE databases.

The *SMN* Genes

Discovery of the SMA Causative Gene

The SMA locus contains multiple repetitive and inverted sequences resulting in two highly homologous copies of *SMN*, namely *SMN1* (telomeric *SMN*) and *SMN2* (centromeric *SMN*) (Lefebvre et al., 1995). Both genes differ by only five nucleotides. *SMN 1* is an SMA-causing gene, due to its homozygous deletion in ~95% of SMA patients (Hahnen et al., 1995). Among the remaining patients, some may retain both *SMN1* alleles carrying intragenic mutations or they may be compound heterozygotes for a deletion and an intragenic mutation in one allele of *SMN1* (Rochette et al., 1997). On the contrary, *SMN2* is a modifier for SMA phenotype with an inverse relationship between *SMN2* copy number and disease severity. High copy number of *SMN2* ameliorates the clinical severity in some patients (McAndrew et al., 1997). However, complete loss of *SMN2* has not been observed in any SMA patients with homozygous *SMN1* deletion (Lefebvre et al., 1995), suggesting that its complete loss may show embryonic lethality (Schrank et al., 1997; Hsieh-Li et al., 2000).

Splicing Regulation of the SMN Genes

Of the five nucleotide differences between the two *SMN* genes (Lefebvre et al., 1995), only one is present in the coding region at position +6 of exon 7 in *SMN1* (c.840C) and *SMN2* (c.840T). Although this mutation is translationally silent, the C-to-T transition alters the splicing pattern in *SMN2* exon 7 (Lorson et al., 1999). *SMN1* exclusively produces full-length (FL) *SMN1* transcripts, while *SMN2* produces ~90% of exon 7-lacking (Δ7) *SMN2* transcripts and ~10% of FL-*SMN2* transcripts (Jodelka et al., 2010). SMN protein produced by *SMN1* transcript including exon 7 (FL-SMN) oligomerizes by means of self-association via a domain encoded by exon 7 (Lorson et al., 1998) and interacts with other proteins to form a multimeric complex (Burnett et al., 2009). However, SMN protein produced by *SMN2* transcript lacking exon 7 (Δ7-SMN) is unable to oligomerize because of the absence of the domain encoded by exon 7. The instability of Δ7-SMN may be explained by protein conformation and/or incompetency of oligomerization and complex formation (Burnett et al., 2009). Cho and Dreyfuss also showed that the splicing defect of exon 7 creates a potent degradation signal (degron) at Δ7-SMN's C-terminal 15 amino acids which target Δ7-SMN to the proteasomal degradation pathway, making it unstable and vulnerable (Cho & Dreyfuss, 2010).

SMN exon 7 has weak 3′- and 5′-splice sites (Lim & Hertel, 2001; Singh et al., 2004b). Thus, to be correctly spliced, additional splicing elements are required: *cis*-elements and *trans*-acting splicing proteins. The *cis*-elements include exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs), and intronic splicing silencers (ISSs). These *cis*-elements are recognized by trans-acting splicing proteins. In the central region of *SMN* exon 7, there is an ESE which binds a positive splicing protein, Htra2-β1 (Hofmann et al., 2000). Together with other proteins such as SR$_P$30c, hnRNP-G, and RBM, Htra2-β1 facilitates the inclusion of exon 7 (Hofmann et al., 2000; Hofmann & Wirth, 2002). However, these *cis*-elements are not sufficient to explain the differential splicing of exon 7 in *SMN1* and *SMN2*.

Cytosine at position +6 of exon 7 may be essential for inclusion of the exon into mRNA, while thymine (or uracil in the pre-mRNA) at this position may cause exclusion of the exon (exon skipping). Cartegni and Krainer (2002) presented an enhancer model in which the C-to-T transition abrogates an essential ESE associated with positive splicing protein SF2/ASF. On the other hand, Kashima and Manley (2003) proposed a silencer model whereby the C-to-T transition creates a new ESS associated with a negative splicing protein, hnRNPA1. According to the extended inhibitory context (Exinct) model by Singh's group, the C-to-T transition strengthens an inhibitory context that covers a larger sequence than SF2/ASF and hnRNP-A1 binding sites

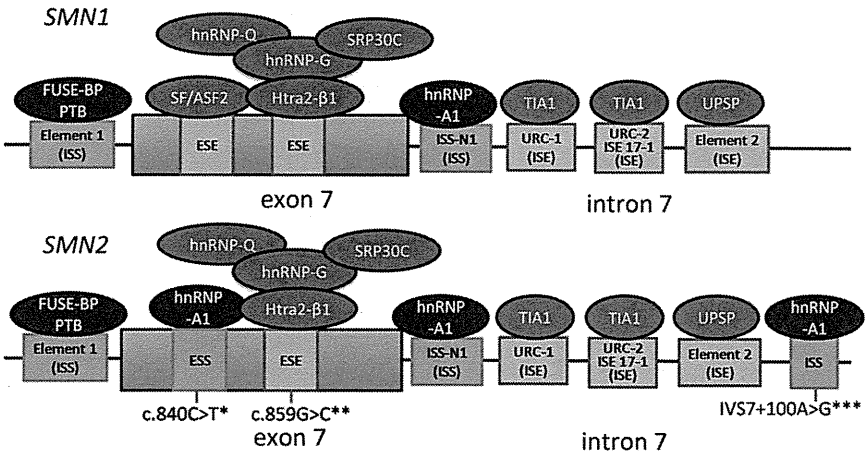

Figure 1 Splicing regulation of the *SMN* genes. Red boxes represent splicing enhancer motif sequences, and blue boxes represent splicing silencer motif sequences. UPSP denotes an unidentified positive splicing protein. *: The c.840C>T mutation (an *SMN2*-specific nucleotide) creates an hnRNP-A1 binding site (Kashima & Manley, 2003). **: The c.859G>C mutation disrupts an unforeseen hnRNP-A1 binding site, resulting in creation of a strong ESE (Vezain et al., 2010). ***: The IVS7+100A>G mutation (an *SMN2*-specific nucleotide) creates an hnRNP-A1 binding site (Kashima et al., 2007).

(Singh et al., 2004a). More recently, another new splicing regulator has been reported by Pedrotti et al. (2010): one of the multifactorial RNA-binding proteins, Sam68, binds to the C-to-T transition site in *SMN2* pre-mRNA exon 7 and triggers exclusion or skipping of the index exon. Collectively, the C-to-T transition at position +6 of exon 7 could create one or a combination of several situations including disruption of an enhancer, creation of a silencer, weakening of a stimulatory RNA structure, and strengthening of an inhibitory RNA structure (Singh et al., 2007).

However, a nucleotide change other than C-to-T transition at position +6 can also alter the splicing pattern of exon 7. It has been recently reported that a variant c.859G>C (at position +25 of exon 7), located in a composite splicing regulatory element in the center of *SMN2* exon 7, induces inclusion of exon 7 into *SMN2* transcript (Prior et al., 2009; Vezain et al., 2010). Besides exonic splicing motif sequences, intronic splicing motif sequences are involved in the regulation of alternative splicing in the *SMN* genes: one ISS has been found in intron 6 of *SMN*, three ISEs and two ISSs in intron 7 of *SMN* (Fig. 1). The ISS in intron 6 is known as element 1 (Miyajima et al., 2002). The ISEs identified in intron 7 are URC-1, URC-2 (or ISE I7–1), and element 2 (Miyajima et al., 2002; Miyaso et al., 2003). The ISSs in intron 7 are ISS-N1 (Singh et al., 2006) and *SMN2*-specific A-to-G transition at position +100 (Kashima et al., 2007). Thus, intron 7, especially the region in the vicinity of exon 7, may play a critical role in regulating *SMN* exon 7 splicing. Splicing proteins bound to the splicing motif sequences are shown in Figure 1. Splicing of *SMN* exon 7 with weak 3′- and 5′-splice sites is regulated in delicate balance among ESEs, ESSs, ISEs, ISSs, and their positive and negative splicing proteins.

Here, we mainly describe the alternative splicing behavior of *SMN2* exon 7. However, other alternative splicing patterns of *SMN1* and *SMN2* pre-mRNAs have been reported. Early studies showed that there are several isoforms generated by the *SMN* genes in muscle cells, indicating that exon 5 can be excluded in *SMN1* and *SMN2* pre-mRNAs (Gennarelli et al., 1995). Most recently, Singh et al. reported that the *SMN1* gene also generates surprising diversity of splice isoforms in some cell types, and that oxidative-stress can induce alternative splicing (Singh et al., 2012). An understanding of these alternative splicing mechanisms is important as strategies based on splicing correction of *SMN2* exon 7 may lead to novel treatment strategies for patients.

Molecular Diagnostics

Methods for Mutation Screening and Gene Dosages Analysis

To confirm the diagnosis of SMA, molecular genetic analysis to detect *SMN1* mutation is essential. Current methods for mutation screening in SMA are summarized in Table 1.

Table 1 Molecular diagnostic methods for SMA.

Methods	Applications	References
Single strand conformation polymorphism (SSCP)	1	(Lefebvre et al., 1995)
Restriction fragment length polymorphism (RFLP)	1	(van der Steege et al., 1995)
Competitive PCR		
Radioisotope method	2	(McAndrew et al., 1997)
Nonradioisotope method	2, 3	(Chen et al., 1999, Wirth et al., 1999, Scheffer et al., 2000)
Real-time PCR		
Absolute quantifications		
Probe method	2, 2s	(Feldkötter et al., 2002)
Nonprobe method	2, 2s	(Feldkötter et al., 2002)
Relative quantifications		
Probe method	2, 2s	(Anhuf et al 2003, Gómez-Curet et al 2007)
Nonprobe method	2, 2s	(Cuscó et al., 2002, Tran et al., 2008, Abbaszadegan et al., 2011)
Denaturing high performance liquid chromatography (DHPLC)		
	1	(Sutomo et al., 2002)
	1s, 2, 2s	(Su et al., 2005)
	3	(Kotani et al., 2007)
High-resolution melting analysis (HRMA)		
Probe method	1, 1s	(Chen et al., 2009, Dobrowolski et al., 2012)
Nonprobe method	1, 1s, 2, 2s, 3	(Chen et al., 2009, Morikawa et al., 2011)
Multiplex ligation probe amplification (MLPA)	1, 1s,	(Arkblad et al., 2006, Scarciolla et al., 2006)
	2, 2s	(Passon et al., 2010, Su et al., 2011)
Liquid microbead array		
Tag-IT protocol	1, 1s	(Pyatt et al., 2007)
Multicode-PLx protocol	1, 1s	(Pyatt et al., 2007)

1: *SMN1* deletion screening, 1s: *SMN1* deletion test for newborn screening, 2: Quantification of *SMN* gene dosage, 2s: Quantification of *SMN* gene dosage for carrier screening, and 3: Detection of intragenic mutation in *SMN1* gene. It should be noted that to identify the intragenic mutation, nucleotide sequencing and assignment of the mutation to *SMN1* or *SMN2* are essential.

In order to detect *SMN1* deletion, single strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) were initially used (Lefebvre et al., 1995; Van der Steege et al., 1995).

To detect *SMN* gene dosage or copy number analysis, a competitive PCR method was first described by McAndrew et al. (1997). This method used exogenous in vitro synthesized DNA as internal standards and radioisotope-labeled primers for autoradiograph analysis of the amplified products. This was later replaced with fluorescence-labeled primers and the amplified labeled products were analyzed on the auto-sequencer (Chen et al., 1999; Wirth et al., 1999; Scheffer et al., 2000; Harada et al., 2002).

Several quantitative real-time PCR approaches have been adopted for *SMN* gene dosage since then. These include absolute quantitative real-time PCR methods using *SMN1* or *SMN2* gene-specific primers (Cuscó et al., 2002; Feldkötter et al., 2002). However, a more convenient approach based on relative-quantification methods was later introduced utilizing an intrinsic gene existing in two copies as a reference (Anhuf et al., 2003; Gómez-Curet et al., 2007; Tran et al., 2008; Abbaszadegan et al., 2011; Chen et al., 2011). To ensure *SMN1*-specific and *SMN2*-specific detection, mismatched designed primer and/or probes (hybridization probes like FRET probes or hydrolysis probes like TaqMan probes) were used in the experimental procedures.

Several other technologies have also been introduced for *SMN* analysis: denaturating high-performance liquid chromatography (DHPLC) (Sutomo et al., 2002; Su et al., 2005; Chen et al., 2007), multiplex ligation probe amplification (MLPA) (Arkblad et al., 2006; Scarciolla et al., 2006) and high resolution melting analysis (HRMA) (Chen et al., 2009; Morikawa et al., 2011), and liquid microbead array (Pyatt et al., 2007). DHPLC, HRMA, and liquid microbead array can be applied for high throughput *SMN1* exon 7 deletion screening (Chen et al., 2007; Pyatt et al., 2007; Su et al., 2011; Dobrowolski et al., 2012) while DHPLC, MLPA, and HRMA techniques allow dosage analysis of *SMN* genes (Su et al., 2005; Scarciolla et al., 2006; Passon et al., 2010; Morikawa et al., 2011). Among these methods, only DHPLC and HRMA can facilitate both dosage and intragenic point mutation analysis (Kotani et al., 2007; Morikawa et al., 2011).

Homozygous deletion screening of *SMN1* represents the first-tier in diagnostic testing since around 95% of SMA patients carry homozygous gene deletion (Hahnen et al., 1995). The majority of the remaining 5% of SMA patients retaining *SMN1* are compound heterozygotes with one *SMN1* allele deletion and one intragenic mutation in the other *SMN1* allele. In addition, some patients may retain two *SMN1* alleles, each carrying a subtle sequence mutation (Bussaglia et al., 1995; Rochette et al., 1997). Thus, for the diagnosis of SMA patients retaining *SMN1*, it is necessary to determine *SMN1* copy number, screen for point mutations, and assign the mutation location to either *SMN1* or *SMN2*. For the latter, long-range PCR for genomic DNA (Clermont et al., 2004) or reverse-transcription PCR for mRNA is performed (Harada et al., 2002).

Based on our experience, the RFLP method (van der Steege et al., 1995) is well suited for those hospitals or laboratories that deal with a small population of subjects with SMA or with a high index of suspicion for SMA because this method does not require any specialized laboratory equipment. Based on this method, homozygous deletion of *SMN* exon 7 can be detected by a simple combination of a conventional PCR machine with a gel-electrophoresis detection apparatus.

Although the RFLP method for detecting homozygous deletion of *SMN1* is currently used in many laboratories, dosage analysis using MLPA is being increasingly adopted for first-tier diagnosis of SMA. The latter enables both *SMN1* deletion screening and *SMN2* copy number analysis to be carried out simultaneously. However, for population screening, the HRMA method may be better because of its low cost, rapid turn-around reporting time for results and ability to process high throughput samples.

SMN2 Gene Dosage and Disease Severity

Several studies have reported a phenotype–genotype relationship among the SMA patients suggesting that increased *SMN2* copy number is related to improved survival outcomes and maintenance of motor function (Velasco et al., 1996; Coovert et al., 1997; McAndrew et al., 1997; Taylor et al., 1998; Harada et al., 2002). Usually, type 1 SMA patients have one or two *SMN2* copies, type 2 patients have three *SMN2* copies, type 3 patients have three or four *SMN2* copies, and type 4 patients have four or more *SMN2* copies (Feldkötter et al., 2002; Wirth et al., 2006a). Individuals carrying 5 or more *SMN2* copies were observed to develop very mild SMA symptoms (Wirth et al., 2006b). Higher *SMN2* copy number in the patients with milder phenotype can be explained by the gene-conversion theory. Gene-conversion events in which *SMN1* is replaced by its centromeric counterpart, *SMN2*, results in higher *SMN2* copies in type 2 and type 3 patients as compared with type 1 patients (Bussaglia et al., 1995; Campbell et al., 1997).

Although the phenotype–genotype relationship may allow us to predict disease severity or prognosis by *SMN2* copy number to some extent in a research setting, specific correlation between disease severity and *SMN2* copy number on an individual level has not been proven. Our own experience also showed that a high *SMN2* copy number did not always guarantee complete protection against SMA (Harada et al., 2002). The correlation between SMA phenotype and *SMN2* copies is not absolute; other factors may also modify the SMA clinical phenotypes (Prior, 2007). Exceptional cases include SMA patients with zero copies of *SMN1* and two copies of *SMN2* who may show a milder phenotype than expected because of the presence of a single mutation in one of the *SMN2* alleles (Prior et al., 2009; Vezain et al., 2010). A single base substitution in *SMN2*, c.859G>C, was identified in exon 7 in these patients. This nucleotide change creates a new ESE element and increases the amount of full-length transcripts, thus resulting in less severe phenotypes. In addition, it may be impossible to predict clinical severity from gene dosage of *SMN2* alone in SMA patients retaining SMN1. Some SMA patients with one copy of mutated *SMN1* (with p.W92S mutation in the Tudor domain of SMN) and three copies of *SMN2* showed the severest phenotype (Kotani et al., 2007). The presence of a single mutation affecting the Tudor domain of SMN may hamper the formation of the SMN complex with other proteins. Recently, it has been reported that HuD binds to the Tudor domain of SMN (Fallini et al., 2012). HuD is a neuron-specific RNA-binding protein that interacts with mRNAs, which play a crucial role in axonal transport. Thus, Tudor domain mutations may deteriorate motor neuron growth and the residual functions of mutated *SMN1* may determine the prognosis of the patients.

Carrier Screening and Prenatal Diagnosis

Advances in methodologies for *SMN1* gene testing have allowed carrier testing and prenatal diagnosis to be offered to families with an affected child (Matthijs et al., 1998). However, prenatal diagnosis is more complicated in a family with an affected child heterozygous for a gene deletion and an intragenic subtle mutation, because it requires both the assessment of *SMN1* gene dosage and sequencing for subtle nucleotide mutations.

Prior to prenatal diagnosis, it is recommended that SMA carrier status be confirmed in both parents based on *SMN1* gene dosage (Wirth et al., 1999). Having one *SMN1* copy confirms carrier status (carrier with "1+0" genotype) (Ogino et al., 2002), whereas the presence of two *SMN1* copies generally excludes carrier status. However, false negative

Table 2 Population carrier frequencies of SMA.

Nation/Ethnic group	Carrier frequency	Subject number	Analytical method	Reference
Australia	1 in 49	146	Real-time PCR	(Smith et al., 2007)
China	1 in 63	569	Real-time PCR	(Chan et al., 2004)
China	1 in 42	1712	DHPLC	(Sheng-Yuan et al., 2010)
Germany	1 in 35	140	Real-time PCR	(Feldkotter et al., 2002)
Germany	1 in 25	100	Real-time PCR	(Anhuf et al., 2003)
Israel	1 in 62	9.037	MLPA	(Sukenik-Halevy et al., 2010)
Korea	1 in 47	326	Real-time PCR	(Lee et al., 2004)
Korea	1 in 50	100	MLPA	(Yoon et al., 2010)
Taiwan	1 in 48	107,611	DHPLC & MLPA	(Su et al., 2011)
USA				
Caucasian	1 in 35	1028	Real-time PCR	(Hendrickson et al., 2009)
Askhenawi Jewish	1 in 41	1002		
Asian	1 in 53	1027		
African America	1 in 66	1015		
Hispanic	1 in 117	1030		
USA				
Pan-ethnic	1 in 54	68,471	Real-time PCR	(Sugarman et al., 2012)
Caucasian	1 in 47	24,471		
Askhenawi Jewish	1 in 67	5806		
Asian	1 in 59	4647		
Hispanic	1 in 68	7655		
Asian Indian	1 in 52	4883		
African America	1 in 72	976		
Not provided	1 in 54	17,235		

exclusion can occur when a minority of carriers possess two *SMN1* copies on one chromosome and zero copies on the other chromosome (carrier with "2+0" genotype) (Ogino et al., 2002). Dosage analysis is also unreliable for carrier status prediction in germline mosaicism cases unless DNA samples from both gametes and peripheral blood are analyzed (Ogino & Wilson, 2002). Similarly, the rare occurrence of somatic mosaicism can also lead to ambiguous results in *SMN1* genotype analysis (Eggermann et al., 2005). In addition, individuals who carry an *SMN1* point mutation may be falsely identified as non-carriers based on deletion screening alone. Thus, genetic counseling for SMA families should always take these situations into consideration.

According to the Practice Guidelines of the American College of Medical Genetics (ACMG), routine SMA-carrier testing is recommended not only for SMA-affected families but also for population-based screening (Prior, 2008). This is due to the severity of the disease and the high carrier frequency in many countries (Table 2) (Feldkötter et al., 2002; Anhuf et al., 2003; Chan et al., 2004; Lee et al., 2004; Smith et al., 2007; Hendrickson et al., 2009; Sheng-Yuan et al., 2010; Sukenik-Halevy et al., 2010; Yoon et al., 2010; Su et al., 2011; Sugarman et al., 2012). However, the American College of Obstetricians and Gynecologist (ACOG) (ACOG, 2009) has expressed caution for preconception and prenatal screening of SMA for the general population due to logistics, education and counseling issues. Factors such as the wide phenotypic variation ranging from mild to severe disease forms in SMA, technical limitations of current routine screening methods which may not detect non-SMN deletion patients (Prior et al., 2010), limited cost-effectiveness of carrier screening (Little et al., 2010) and the absence of curative treatment for SMA (Gitlin et al., 2010), all contribute toward the lack of consensus in implementing a population screening program in many countries. Although such carrier testing would be voluntary and made available in conjunction with genetic counseling services, the implementation of such screening, whether offered only for couples-at-risk in affected families or for large-scale healthy populations, requires an understanding of the sensitivity and limitations of the tests so that individuals can make informed choices on the uptake of such screening. It should be noted that the purpose of carrier testing for couples is to identify risks for conceiving an affected child and that the carrier status, if undiagnosed, does not pose a threat to the health of the couples themselves or others in the community.

Newborn Screening

The main purpose of newborn screening is to identify affected children prior to the development of clinical symptoms for early treatment interventions. The most suitable technology for newborn screening, in our opinion, may be HRMA, which is a simple and rapid but low-cost test for detecting human disease-associated mutations, especially for samples with mutations of low incidence in the population (Li et al., 2011).

Regarding the implementation of newborn screening, there should be availability of an accepted treatment for patients with a recognized disease as a prerequisite for such screening, following the Wilson-Junger criteria (Orzalesi & Danhaive, 2009). There are a number of genetic conditions for which newborn screening is routinely carried out in a number of countries. An example is that for phenylketonuria (PKU) deficiency, which was the first newborn screening test implemented in the USA in the 1960s. PKU is a condition in which early treatment will make a significant difference to clinical outcome, thus meeting the Wilson-Junger criteria. This is in contrast with SMA which is still an incurable disease.

There are two challenges in recommending newborn screening for SMA, namely cost-effectiveness and psychosocial issues. In principle, any newborn screening program should achieve maximum public health benefits with economic savings to costs ratio. A pilot population-based carrier screening for SMA showed it not to be cost effective (Little et al., 2010), suggesting that this may be true for newborn screening too. The second issue with such newborn screening relates to the psychosocial effects affecting the relationship between parents and child (Kerruish & Robertson, 2005), resulting in emotionally draining experiences. In addition, current diagnostic methods can identify SMA mutations but cannot ascertain the SMA subtype or accurately predict disease onset or severity. In particular, we cannot identify the SMA subtype in pre-symptomatic children with any of the current methods. Early diagnosis of SMA in a presymptomatic child may have a negative impact on bonding with the parents and bring about uncertain psychosocial effects. Raising such a presymptomatic offspring with an incurable disease and uncertain prognosis may be a traumatic experience for many parents.

However, Swoboda and her colleagues pointed out that newborn screening may be important even in the absence of a curative treatment for SMA (Swoboda et al., 2005). Citing the experience in cystic fibrosis, they argued that newborn screening can lead to significant improvements in quality of life for patients as supportive interventions can be implemented early. According to them, the prerequisite for newborn screening is the presence of early supportive care. A recent study showed that presymptomatic treatment of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) at the earliest postnatal time was the most effective, lending further support to the usefulness of early neonatal screening in humans (Porensky et al., 2012). In this context, such newborn screening can identify appropriate patient cohorts for enrolment into clinical trials at the earliest possible time before disease onset (Prior, 2010). However, this needs to be balanced against the ethical viewpoint that without a known cure for the disease, the implementation of newborn screening to accelerate clinical trials cannot be justified.

In theory, newborn screening is useful for providing a framework in place to identify children as early as possible when better treatments become available. At this point in time, it may be a rational decision to accept the statement of Wirth et al. (2006b) that "Neonatal screens will be crucial for a successful SMA therapy. Children at risk to develop SMA should be recognized as soon as possible before first symptoms occur. However, as long as we do not have a clear answer whether any drug is sufficiently beneficial to SMA patients, a neonatal screening for SMA should not be offered." Newborn screening may be indicated if an effective treatment is found to cure or slow disease progression.

Molecular Pathophysiology

Biogenesis of Small Nuclear Ribonucleoprotein

SMN is a 38 kDA protein that is ubiquitously expressed in both neuronal cells and non-neuronal cells. SMN interacts and forms a complex with binding partner proteins in a variety of cellular activities; including pre-mRNA splicing (Fischer et al., 1997; Pellizzoni et al., 1998), biogenesis of small nuclear ribonucleopoteins (snRNPs) (Burghes & Beattie, 2009), transcription (Strasswimmer et al., 1999), stress responses (Zou et al., 2011), apoptosis (Iwahashi et al., 1997), axonal transport (Pagliardini et al., 2000) and cytoskeleton dynamics (Bowerman et al., 2007).

In 1996, Dreyfuss' group first reported the presence of SMN in both the nucleus and cytoplasm of HeLa cells, an immortalized cell line derived from cervical cancer cells. In the nucleus, SMN is localized within a nuclear structure, so called "Gems," or gemini of coiled bodies (Cajal bodies), interacting with RNA-binding proteins (Liu & Dreyfuss, 1996). In any type of cell, SMN exists as a part of a stable multiprotein complex in cytoplasm and nuclear Gems. SMN, together with Gemin2-Gemin8 and UNRIP, plays an essential role in the assembly of snRNPs and their transport from the cytoplasm into the nucleus. In the cytoplasm, the SMN complex functions as an assemblyosome in the formation of snRNP. The SMN complex facilitates the assembly of a small nuclear RNA (snRNA) with RNA binding proteins (known as Sm proteins) to form snRNP

(Gubitz et al., 2004). The SMN complex re-enters the nucleus with the snRNP-cargo through the help of snurportin and importin (Pellizzoni, 2007). In the nucleus, the SMN complex is liberated from its snRNP-cargo and then shuttles back to the cytoplasm to help assemble the new snRNPs (Matera et al., 2007; Burghes & Beattie, 2009; Cauchi, 2010). In addition, snRNPs join the spliceosome to participate in splicing. According to Zhang et al. (2008), SMN-deficient mouse tissues show alteration in the stoichiometry of snRNAs as well as widespread pre-mRNA splicing defects in numerous transcripts of diverse genes. These findings highlight the role of the SMN complex in RNA metabolism and splicing regulation, suggesting that SMA is a general splicing disease that is not restricted to motor neurons (Zhang et al., 2008).

Even so, the main pathological finding of SMA is the loss of motor neurons. It remains to be resolved whether loss of function in spliceosomal assembly, resulting in widespread defects in mRNA splicing, is directly responsible for motor neuron death. Bäumer et al. (2009) assessed the degree of altered splicing in the spinal cord of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$), using exon-specific microarrays. According to them, the vast majority of splicing changes are a late feature of the disease and are therefore unlikely to contribute to early disease pathogenesis. These findings noted that splicing defects may not be a primary effect of the loss of SMN. However, the authors could not fully rule out the presence of significant early changes in a small number of transcripts crucial to motor neuron survival (Bäumer et al., 2009).

Two hypotheses have been presented to explain the basis of the motor neuron defects in SMA (Burghes & Beattie, 2009). The first suggests that disturbed snRNP synthesis due to decrease of SMN affects the splicing of genes that are important for the circuit formation of motor neurons. Although defects in these genes may not cause the same SMA phenotype, it is thought that they affect motor neuron functioning that contributes toward the clinical symptoms in SMA (Jablonka et al., 2002). Even though the identities of these genes have yet to be fully clarified, it was recently reported by Lotti and colleagues that SMN deficiency perturbs splicing and decreases the expression of a subset of U12 intron-containing genes in animal models, including a protein called Stasimon (Lotti et al., 2012). Restoration of Stasimon expression in the motor circuit corrects defects in neuromuscular junction (NMJ) transmission and muscle growth in Drosophila SMN mutants, and corrects aberrant motor neuron development in SMN-deficient zebrafish (Lotti et al., 2012). These findings link defective splicing of critical neuronal genes induced by SMN deficiency to motor circuit dysfunction, contributing toward further understanding on the role of the snRNP complex in SMA pathogenesis.

The second hypothesis suggests that SMN has some critical role, independent of snRNP synthesis, in the motor neuron function. SMN deficiency has been reported to produce defects in β-actin mRNA axonal transport, neurofilament dynamics, neurotransmitter release, and synapse maturation (Torres-Benito et al., 2011). Some of the SMN functions in the motor axon outgrowth may be independent of the functions required for snRNP synthesis, because SMN oligomerization or Sm binding does not correlate with the motor axon growth (Carrel et al., 2006).

Axonal Transport of Motor Neurons and NMJ Maturation

Rossoll and colleagues demonstrated that a complex of Smn (the ortholog of human SMN) with its binding partner hnRNP-R has been found to interact with β-actin (Rossoll et al., 2002; Rossoll et al., 2003). In Smn-deficient motor neurons from an SMA mouse model ($Smn^{-/-}SMN2^{+/+}$), reduced axon elongation and small-sized growth cones correlated with reduced β-actin protein and mRNA levels in distal axons and growth cones (Rossoll et al., 2003). The model for pathogenesis based on their findings was that defects in dynamic processes in axons may hamper axonal elongation, synapse formation, and presynaptic function of the motor endplate in SMA.

Zhang and colleagues also showed that SMN is localized in granules that are actively transported into neuronal processes and growth cones (Zhang et al., 2006). According to them, in cultured motor neurons, SMN granules co-localize with ribonucleoprotein Gemin proteins but not with spliceosomal Sm proteins that are required for snRNP assembly. SMN–Gemin complex containing granules are distributed to both axons and dendrites of differentiated motor neurons. In addition, high-speed dual channel imaging of live neurons depicted rapid and bidirectional transport of the SMN–Gemin complex.

Recently, it has been reported that SMN also interacts with RNA-binding protein, HuD (Hubers et al., 2010; Fallini et al., 2011; Akten et al., 2011). HuD is a neuron-specific RNA-binding protein. The complex of SMN and HuD regulates localization of poly(A) mRNA (Fallini et al., 2011). It also binds to the candidate plasticity-related gene, *cpg15* (Akten et al., 2011). The *cpg15* protein is highly expressed in the developing ventral spinal cord and can promote motor axon branching and neuromuscular synapse formation, suggesting a crucial role in the development of motor axons and NMJs. All these findings support the model for pathogenesis mentioned above, and imply that motor neuron degeneration could begin before the formation of NMJs.

However, the deteriorating mechanisms of motor neurons in SMA are more complicated than expected. Cifuentes-Diaz et al. (2002) reported that SMA mice ($Smn^{F7}/Smn^{\Delta 7,NSE\text{-}Cre+}$) display a drastic and progressive loss of motor axons, consistent with the skeletal muscle denervation process in SMA. Interestingly, they also found accumulation of neurofilaments in terminal axons of the remaining NMJs, associated with a defect of axonal sprouting and postsynaptic apparatus formation. These findings suggested that loss of motor neuron cell bodies results from a "dying-back" axonopathy in SMA. Such denervation likely resulted from defects in synapse maintenance rather than defects in the initial formation of nerve-muscle contact (Kong et al., 2009; Ling et al., 2012). Based on these findings, motor neuron degeneration may begin after NMJ formation. Such understanding on the timing of the beginning of motor neuron degeneration may be critical for effective treatment.

Cytoskeleton Dynamics Regulated by SMN Downstream Signaling

Recent observations have also revealed an association between cytoskeleton dynamics and the pathogenesis of SMA. Axonogenesis of motor neurons, as well as axonogenesis of other neurons, is mediated by changes in cytoskeletal dynamics, i.e., assembly and disassembly of cytoskeleton proteins including actin and tubulin. Thus, much of the research in this area has been focused on the relationship between SMN and cytoskeleton dynamics.

It has been reported that two SMN-binding proteins, profilin IIa and plastin 3, are closely related to actin dynamics (Bowerman et al., 2007, 2005; Oprea et al., 2008). Bowerman's group showed that Smn knockdown in neuronal cells increased profilin IIa isoform, resulting in an increased formation of Rho-associated kinase (ROCK)/profilin IIa complex (Bowerman et al., 2007). In the activated RhoA/ROCK pathway, Rho (a well-characterized member of the family of Rho GTPases) and its effector ROCK mediate enhancing signals to the downstream proteins. The increased ROCK/profilin IIa complex activated the RhoA/ROCK pathway inappropriately, resulting in altered cytoskeletal integrity and a subsequent defect in axonogenesis (Bowerman et al., 2007). Meanwhile, Oprea et al. (2008) found that overexpression of *PLS3*, a gene encoding plastin 3, rescued the axonal growth defect in culture motor neurons. They also reported that overexpression of *PLS3* was found in unaffected siblings that shared the same SMN genotype as children with SMA. In addition, a decrease in plastin 3 levels was observed in the brain and spinal cord of SMA mice ($Smn^{-/-}SMN2^{+/+}$) and this is now considered to be related to the pathophysiology of SMA (Bowerman et al., 2009).

In the context of dysregulation of cytoskeleton in SMA, stathmin, a microtubule-destabilizing protein, should also be considered. Upregulated stathmin has been shown to correlate with a decrease in polymerized tubulin level in distal axons of SMA mice ($Smn^{-/-}SMN^{2/-}$) and in Smn-deficient cells (Wen et al., 2010). It was observed that knockdown of stathmin restored the microtubule network defects of Smn-deficient cells, and promoted axonal growth in motor neurons of SMA mice.

Potential Treatment Strategies and Clinical Trials for SMA

Classification of SMA Treatment Strategies

Current SMA therapeutic strategies can broadly be classified into three major groups. We summarize the therapeutic strategies and the compounds that have been used as candidate drugs for SMA in Table 3. Some compounds in each group have already been tested while others are still in the test phase. Table 3 also provides the information on the different test phases of the compounds: in vitro, in vivo, and human trial (HT) phases. HT phases include case series (CS), open label study (OL), and randomized controlled trial (RCT).

The first group which we term "*SMN2*–targeting strategies" involves strategies to increase FL-SMN protein either using pharmacological compounds or through splicing correction of *SMN2* mRNA by antisense oligonucleotides (ASO), and to produce a stable form of Δ7-SMN protein with additional C-terminal peptides by a translational read-through method.

The second group, "*SMN1*-introduction strategies," involves strategies to introduce exogenous *SMN1* gene copies using vector-mediated gene delivery methods (gene therapy), and stem cell transplantation methods (stem cell therapy)

The third group, "Non-*SMN*-targeting strategies," involves strategies to protect motor neurons or improve the pathological conditions of non-neuronal tissues including muscles. This group also includes modulation of SMN-downstream signaling systems including the RhoA/ROCK pathway.

In this review, we put stress on the new treatment candidates. Some clinical trials have already been conducted for SMA based on new strategies for increasing FL-SMN protein or protection of motor neurons.

SMN2-Targeting Strategies

Increasing FL-SMN protein using pharmacological compounds

In 2001, Chang and colleagues reported that a histone deacetylase (HDAC) inhibitor, sodium butyrate, increased FL-SMN protein in SMA lymphoid cells and prolonged the

Table 3 Current advances in SMA therapeutic strategies.

1. *SMN2*-targeting strategies					
Treatment categories	Compounds/Drugs	Clinical trial phase	Subjects	Outcome	References
(1) Increase in FL-SMN levels by pharmacological compounds					
HDAC inhibitors	Benzamide M344	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Riessland et al., 2006)
	Hydoroxamic acid LBH589(panobinostat)	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Garbes et al., 2009)
	Sodium butyrate	In vitro	SMA lymphoblastoid cells	Increase in SMN protein	(Chang et al., 2001)
		In vivo	mice ($Smn^{-/-}SMN2^{+/+}$)	Improvement in lifespan and motor functions	(Chang et al., 2001)
	Sodium phenylbutyrate (PBA)	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Andreassi et al., 2004)
		HT-CS Phase 0	4 patients (type 2) and 2 patients (type 3)	Increase in full-length *SMN2* transcript of leukocytes and in muscle strength	(Brahe et al., 2005)
		HT-RCT Phase 2	107 patients (type 2)	No benefit in motor function	(Mercuri et al., 2007)
	Suberoylanilide hydroxamic acid (SAHA)	In vitro	Neuroectodermal tissues	Increase in SMN protein	(Hahnen et al., 2006)
		In vivo	mice ($Smn^{-/-}SMN2^{+/+}$ and $Smn^{-/-}SMN2^{+/-}$)	Improvement in lifespan, motor function Increase in weight gain	(Riessland et al., 2010)
	Trichostatin A (TSA)	In vivo	SMA mice ($Smn^{-/-}SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan, and motor function Increase in weight gain	(Avila et al., 2007)
	Valproic acid (VPA)				
	a. VPA only	In vitro	SMA fibroblasts	Increase in SMN protein	(Brichta et al., 2003)
	b. VPA only	In vitro	SMA fibroblasts	Increase in SMN protein	(Sumner et al., 2003)
	c. VPA only	HT-CS Phase 0	7 patients (type 3 and 4)	Improvement in muscle strength and subjective functions	(Weihl et al., 2006)
	d. VPA only	HT-OL Phase 2	42 patients (type1,2 and 3)	Improvement of motor function in patients with SMA type 2	(Swoboda et al., 2009)
	e. VPA + Carnitine	HT-OL Phase 2	61 patients (type 2 and 3)	No benefit in motor function	(Swoboda et al., 2010)
	f. VPA + Carnitine	HT-OL Phase 2	33 patients (type 3)	No benefit in motor function	(Kissel et al., 2011)
Non-HDAC inhibitors	Aclarubicin	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Andreassi et al., 2001)
	Hydroxyurea (HU)	In vitro	SMA lymphoblastoid cells	Increase in SMN protein and gem numbers	(Grzeschik et al., 2005)
		HT-RCT Phase 2	28 patients (type 2) and 29 patients (type 3)	No benefit in motor function	(Chen et al., 2010)

Continued

Table 3 Continued

Treatment categories	Compounds/Drugs	Clinical trial phase	Subjects	Outcome	References
	Indoprofen	In vitro	SMA fibroblasts	Increase in SMN protein and and gem numbers	(Lunn et al., 2004)
		In vivo	SMA mice embryos ($Smn^{-/-}$ $SMN2^{+/-}$)	Improvement in viability and lifespan	
	Prolactine	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Increase in SMN protein, weight gain, improvement in lifespan, and motor function	(Farooq et al., 2011)
	Salbutamol (Albuterol)	HT-CS Phase 0	5 patients (type 2) and 8 patients (type 3)	Increase in muscle mass and strength	(Kinali et al., 2002)
		HT-CS Phase 0	12 patients (type 2 and 3)	Increase in full length *SMN2* transcripts of leucocytes	(Tiziano et al., 2010)
		In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Angelozzi et al., 2008)
		HT-OL Phase 0	23 patients (type 2)	Increase in muscle mass and strength	(Pane et al., 2008)
	Tetracycline-like compound PTK-SMA1				
		In vitro	SMA fibroblasts	Increase in SMN protein	(Hastings et al., 2009)
		In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$, and $Smn^{-/+}$ $SMN2^{+/+}$)	Increase in SMN protein and improvement in motor function	
	Quinazolines				
	a. D156844	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan and motor function	(Butchbach et al., 2010b)
	b. Quinazoline-495 (or RG 3039)	In vivo	SMA Δ7-mice	Improvement in lifespan and motor function	(Van Meerbeke et al., 2011)
		HT-RCT Phase 1	patients (type 2 and 3)	Ongoing	
	Triptolide (PG490)	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Hsu et al., 2012)
		In vivo	SMA Mice ($Smn^{-/-}$ $SMN2^{+/+}$)	Increase in SMN protein, improvement in weight loss and improvement in motor function	
(2) Increase in FL-SMN levels by antisense oligonucleotides					
Splice-site targeting	Antisense oligonucleotide against 3′ splice-site	In vitro	In-vitro splicing assay	Increase in exon 7 inclusion into *SMN2* mRNA	(Lim & Hertel, 2001)
Bifunctional peptide nucleic acid	Peptide nucleic acid with splicing factor mimic peptides (ESSENCE)	In vitro	In-vitro splicing assay	Increase in exon 7 inclusion into *SMN2* mRNA	(Cartegni & Krainer, 2003)

Continued

Table 3 Continued

Treatment categories	Compounds/Drugs	Clinical trial phase	Subjects	Outcome	References
Bifunctional oligonucleotide	Oligonucleotide with ESE mimic sequence	In vitro	SMA fibroblasts	Increase in SMN protein	(Skordis et al., 2003)
		In vivo	SMA mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *U7-ESE-B+*)	Increase in weight gain Improvement in lifespan and motor neuron	(Meyer et al., 2009)
Trans-splicing	a. Oligonucleotide with *SMN1* exon 7 sequence	In vitro	SMA fibroblasts	Increase in SMN protein	(Coady et al., 2007)
		In vivo	SMA Mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Improvement in lifespan and phenotype	(Coady & Lorson, 2010)
	b. Oligonucleotide with *SMN1* exon 7 in sinergy with *IGF1* expressed vector	In vivo	SMA Mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Improvement In lifespan Increase in weight gain	(Shababi et al., 2011)
ISS masking	Antisense oligonucleotide				
	a. Oligomer against ISS-N1	In vitro	SMA fibroblasts	Increase in SMN protein	(Singh et al., 2006)
		In vivo	SMA Mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Increase in weight gain, Improved motor function	(Porensky et al., 2012)
	b. ASO-10—27 (or ISIS-SMNRx)	In vivo	SMA mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Improvement in lifespan and motor function Increase in weight gain	(Hua et al., 2010, Hua et al., 2011, Passini et al., 2011)
	c. ASO-10—27 (or ISIS-SMNRx)	HT-RCT Phase 1	28 Patients (type 1,2 and 3)	The compound was well tolerated Improvement in HFMSE, MUNE and CMAP score	http://www.isisph.com/pdfs/AAN_Isis_Investor-Event.pdf
(3) Stabilization of Δ7-SMN protein via read-through strategy					
Stop-codon read-through technology	Aminoglycosides				
	a. TC007 (PTC-X)	In vitro	SMA fibroblasts	Increase in SMN protein and gem numbers	(Mattis et al., 2006)
	b. TC007 (PTC-X)	In vitro	SMA mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Improvement in lifespan and phenotype	(Mattis et al., 2009)
	c. G418 (Geneticin)	In vitro	SMA fibroblasts	Increase in SMN protein	(Heier & DiDonato, 2009)
		In vivo	SMA mice (*Smn*$^{-/-}$ *SMN2*$^{+/+}$ *SMN*Δ*7*$^{+/+}$)	Increase in motor function, no significant benefit in lifespan and body weight	

Continued

Table 3 Continued

2. *SMN1*-introduction strategies					
Treatment categories	Vector (administration route)/ Stem cells	Clinical trial phase	Subjects	Outcome	References
(1) Gene therapy					
Vector mediated gene delivery	EIAV- SMN (muscle injection and retrograde axonal transport)	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan, increase in weight gain, reduction in motor neuron death	(Azzouz et al., 2004)
	AAV8-SMN, scAAV8-SMN (cerebral lateral ventricle injection)	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan and motor function. Increase in weight gain. Histological improvement in NMJ formation	(Passini et al., 2010)
	scAAV9-SMN(intravenous injection)	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan, increase in weight gain, rescue in motor function.	(Foust et al., 2010)
	scAAV9-SMNopti (intravenous injection)	In vitro In vivo	SMA astrocyte cells SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$) ($Smn^{-/-}$ $SMN2^{+/+}$ $SMNA2G^{+/-}$)	Increase in SMN protein Improvement in lifespan, increase in weight gain, rescue of motor neuron function	(Dominguez et al., 2011)
(2) Stem cell therapy					
Cell transplant technology	Neuronal stem cells				
	a. Spinal cord-derived neuronal stem cells	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in lifespan, locomotor activity and exploratory behavior. Increase in weight gain	(Corti et al., 2008)
	b. Embryonic stem cell-derived neural stem cells	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta7^{+/+}$)	Improvement in muscle innervation, lifespan, and behavior endpoint. Increase in weight gain.	(Corti et al., 2010)

Continued

Table 3 Continued

3. Non-*SMN*-targeting strategies					
Treatment Categories	Compounds/Drugs	Clinical trial phase	Subjects	Outcome	References
1. Neuroprotection therapy					
Reduction in glutamate mediated excitotoxicity	Gabapentin	HT-RCT Phase 2	84 patients (type 2 and 3)	No benefit in motor function	(Miller et al., 2001)
		HT-RCT Phase 2	120 patients (type 2 and 3)	Slight improvement in muscle strength	(Merlini et al., 2003)
	Riluzole	HT-CS phase 1	10 patients type 1	No adverse event, possible benefit in lifespan	(Russman et al., 2003)
		HT-RCT phase 2	141 patients (type 2 and 3)	Ongoing	
	Beta-lactam antibiotics	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$)	Improvement in lifespan, muscular phenotype, & neuromuscular function Increase in SMN protein in spinal cord	(Nizzardo et al., 2011)
Neurotrophic effect	Thyrotropin releasing hormone (TRH)	HT-CS Phase 0	3 patients SMA type 1 and	No benefit in SMA patients type 1 Increase in muscle strength for patient type 2	(Takeuchi et al., 1994)
		HT-CS Phase 0	1 patient	Increase in muscle strength	(Kato et al., 2009)
		HT-RCT Phase 0	9 patients (type 2 and 3)	Improvement in motor function	(Tzeng et al., 2000)
	rhIGF1-rhIGF1BP-3 (IPLEX™)	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$)	No improvement in lifespan and weight gain. Improvement in motor function, increase in muscle fiber size	(Murdocca et al., 2012)
Protection of mitochondria	Olesoxime (TRO-19622)	HT-RCT Phases 2 and 3	150 patients (type 2 and 3)	On going	

Continued

Table 3 Continued

3. Non-*SMN*-targeting strategies					
Treatment Categories	Compounds/Drugs	Clinical trial phase	Subjects	Outcome	References
2. Improvement of pathological conditions of non-neuronal tissues					
Amendment of affected muscles	Inhibition of myostatin				
	a. follistatin	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$)	No improvement in lifespan	(Sumner et al., 2009)
	b. ActRIIB-Fc	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$)	Slight improvement in motor function, but no improvement in lifespan	
	Expression of IGF-1	In vivo	SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$ $mIGF\text{-}1^{+/-}$) ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$ $mIGF\text{-}1^{-/-}$)	Increase in muscle fiber size and body weight gain. Improvement in lifespan	(Bosch-Marcé et al., 2011)
Inhibition of ROCK pathway	Y-27632(ROCK inhibitor)	In vivo	SMA mice ($Smn^{2B/-}$ mice, $Smn^{2B/+}$mice and $Smn^{-/-}$ $SMN2^{+/+}$)	Improvement in lifespan, muscle size and motor end plate maturation. Increase in weight gain	(Bowerman et al., 2010)
	Fasudil	In vivo	SMA mice ($Smn^{2B/-}$ mice and $Smn^{2B/+}$mice)	Improvement in lifespan, muscle size and motor end plate maturation. Increase n weight gain	(Bowerman et al., 2012)

NB. Olexosime clinical trial was recently completed and showed no significant improvement in patients with ALS; HT: human-trial phases (CS: case series; RCT: randomized controlled trial; OL: open label trial).

lifespan of the SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$) (Chang et al., 2001). In the same year, a cancer drug, aclarubicin, was also reported to increase FL-SMN protein in SMA fibroblasts (Andreassi et al., 2001). These reports led to a number of studies investigating potential therapeutic candidates for SMA.

Several HDAC inhibitors were found to activate *SMN2* transcription and correct splicing of *SMN2* exon 7, leading to a significant increase in FL-SMN. These compounds include sodium butyrate (Chang et al., 2001), valproic acid (VPA) (Brichta et al., 2003; Sumner et al., 2003), sodium phenylbutyrate (PBA) (Andreassi et al., 2004; Brahe et al., 2005; Mercuri et al., 2007), suberoylanilidehydroxamic acid (SAHA) (Hahnen et al., 2006; Riessland et al., 2010), benzamide M344 (Riessland et al., 2006), trichostatin A (Avila et al., 2007), and hydoroxamic acid LBH589 (panobinostat) (Garbes et al., 2009). Among them, VPA, a drug that is widely used for epilepsy patients, was first shown to increase SMN protein level in SMA fibroblasts via upregulation and correction of exon 7 splicing of *SMN2* (Brichta et al., 2003; Sumner et al., 2003).

Weihl et al. (2006) reported that VPA was also able to increase muscle strength and subjective function of seven adult patients with SMA type 3–4. A Phase 2 open label study conducted by Swoboda et al. (2009) also showed that VPA improved the motor function in SMA type 2 patients. According to them, significant improvement was limited to the patients who were under 5 years of age.

However, a double-blind, randomized placebo controlled trial demonstrated no benefits from 6 to 12 months treatment with VPA and carnitine in a cohort of non-ambulatory subjects with SMA type 2–3 (sitters, 2–8 years of age) (Swoboda et al., 2010), with similar results from another prospective single-armed trial on a cohort of ambulatory subjects with SMA type 3 (standers and walkers, 3–17 years of age) (Kissel et al., 2011). In both studies, carnitine was given to the patients for two reasons: SMA patients may have a limited carnitine synthetic capacity due to reduced skeletal muscle mass, and VPA itself may inhibit carnitine transport and deplete carnitine levels. Thus, the combination therapy of VPA and carnitine was chosen in order to avoid concerns about a confounding effect of carnitine depletion (Swoboda et al., 2010).

Inconsistent data relating to VPA effects may be explained by the coexistence of responders and non-responders to the drug, suggesting the necessity of pre-selecting potential responders (Pruss et al., 2010). A recent study has reported that an increase in fatty acid translocase CD36 expression may account for VPA non-responsiveness (Garbes et al., 2013). Pretreatment analysis of genetic background including CD36 expression may be useful for identification of potential responders.

The change in *SMN* transcript levels or SMN protein levels in the blood cells or cultured fibroblasts treated with VPA could be a measurable and informative biomarker for the biochemical/pharmacological effect of VPA treatment. However, it should be noted that the increase in *SMN* transcript levels or SMN protein levels would not necessarily guarantee the amelioration of SMA disease progression. Any valid biomarkers for SMA disease progression have yet to be identified or validated. Hence, currently there are no useful biomarkers to predict the outcome of the clinical trials.

Non-HDAC inhibitor drugs may also activate *SMN2* transcription and correct splicing of *SMN2* exon 7, leading to a significant increase in FL-SMN protein. These drugs include aclarubicin (Andreassi et al., 2001), hydroxyurea (HU) (Grzeschik et al., 2005; Chen et al., 2010), salbutamol (Kinali et al., 2002; Angelozzi et al., 2008; Pane et al., 2008; Tiziano et al., 2010), indoprofen (Lunn et al., 2004), PTK-SMA1 (Hastings et al., 2009), quinazolines (Singh et al., 2008), and triptolide (Hsu et al., 2012).

Hydroxyurea has been reported to increase FL-*SMN2* transcripts and SMN protein levels, but without changing total *SMN* mRNA, suggesting that it promotes the inclusion of exon 7 during *SMN2* transcription in SMA fibroblast cell lines (Grzeschik et al., 2005). However, a clinical trial of HU in 2007 showed no significant clinical improvements in motor function (Chen et al., 2010).

Among the β-adrenergic agonists, only salbutamol has been identified as a candidate drug for SMA. Early studies had shown that salbutamol enhanced muscle strength in patients with SMA type 2–3 (Kinali et al., 2002; Pane et al., 2008), and it was recently proven that it increases FL-*SMN2* mRNA in fibroblasts and leukocytes from SMA patients (Angelozzi et al., 2008; Tiziano et al., 2010).

Indoprofen has been used as a non-steroidal anti-inflammatory drug and cyclooxygenase inhibitor. This therapeutic candidate was selected from a high-throughput screen using a splicing reporter mini-gene and was found to selectively increase *SMN2* exon 7 inclusion and therefore increase the amount of FL-SMN protein produced from transfected cells (Lunn et al., 2004). Recently, NINDS (National Institute of Neurological Disorders and Stroke, USA) announced the start of clinical trials of indoprofen derivatives for SMA (http://www.ninds.nih.gov/news).

PTK-SMA1, a tetracycline-like compound, stimulates *SMN2* exon 7 inclusion and increases SMN production in vitro and in vivo (Hastings et al., 2009). There is a structural similarity between tetracyclines and aclarubicin which also activates FL-*SMN2* transcription. It is notable, that the former is far less toxic compared to the latter. According to Paratek Pharmaceuticals, clinical trials of PTK-SMA1 could begin in 2013 (http://www.ricercasma.it/).

Quinazolines have also been reported to be potent *SMN2* promoter activators (Jarecki et al., 2005; Thurmond et al., 2008). These compounds work by binding to the scavenger decapping enzyme, DcpS, and potently inhibit its decapping activity. DcpS is a nuclear shuttling protein that binds and hydrolyzes the m7GpppN mRNA cap structure and is a modulator of RNA metabolism. The potency of DcpS inhibition correlates with potency for *SMN2* promoter activation (Singh et al., 2008; Butchbach et al., 2010b; Van Meerbeke & Sumner, 2011). A clinical trial (phase 1) of a C5-substituted quinazoline called quinazoline495 (or RG3039) is currently ongoing (Van Meerbeke et al., 2011)

Another promising candidate drug for SMA is Triptolide (PG490) that was reported to increase FL-*SMN2* transcript and SMN protein levels in fibroblast cells derived from SMA patients (Hsu et al., 2012). In addition, injection of the drug improved survival in SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$). Triptolide is a diterpene triepoxide antibiotic isolated from extracts of the herb *Tripterygium wilfordii* Hook F (TWHF). TWHF has been used as an herbal drug for rheumatoid arthritis in traditional Chinese medicine because of its immunosuppressive and anti-inflammatory properties.

Recently, prolactin (PRL) was shown to increase SMN expression PRL is a 199–amino acid 23-kDa polypeptide hormone that binds to the PRL receptor and activates the JAK2/STAT5 pathway, resulting in SMN upregulation in both SMA mouse models ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) and human motor neuron-derived cell lines (Farooq et al., 2011). PRL may be a good candidate for SMA therapy; however, clinical experience with PRL is still limited, and there are some potential side effects associated with high levels of PRL. Hyperprolactinaemia can lead to precocious puberty, infertility, and osteoporosis (Advis et al., 1981; Aguilar et al., 1988; Farooq et al., 2011). Thus, careful monitoring for hyperprolactinaemia-related symptoms is essential if long-term PRL treatment is given to the patients.

Charbonnier and colleagues reported that NMDA receptor activation leads to an increase in SMN expression through AKT/cAMP response element-binding protein (CREB) pathway (Biondi et al., 2010; Branchu et al., 2013). According to them, because of the reciprocal crosstalk in the level of extracellular signal-regulated kinase (ERK) and AKT kinases, pharmacological inhibition of the MEK/ERK/Elk-1 pathway using experimental compound UO126 or selumetinib may efficiently activate the AKT/CREB pathway, resulting in an increase in SMN expression (Branchu et al., 2013).

Once SMN is expressed, the problem of the SMN degradation comes next. Makhortova and colleagues carried out an image based screen to identify regulators of SMN levels, and found that glycogen synthase kinase (GSK)-3 is a key regulator of SMN degradation. Its activity is also controlled by certain neurotransmitter ligands. Here, certain sets of kinase inhibitors may be able to promote motor neuron survival (Makhortova et al., 2011). It is conceivable that some drugs increasing SMN stability might provide an adjunctive effect in addition to *SMN2*-targeting strategies.

Splicing correction of FL-SMN2 mRNA by oligonucleotides

Correction of *SMN2* splicing (incorporation of *SMN2* exon 7) may increase FL-SMN protein production. In 2001, Lim and Hertel reported that an antisense oligonucleotide (ASO) targeting the 3′ splice site of exon 8 was able to incorporate exon 7 into *SMN2* mRNA. Since then, at least five methods, including that of Lim and Hertel have been developed to modulate *SMN2* mRNA. The first strategy of Lim and Hertel (2001) involves blocking splice sites in the exon-intron boundaries (Table 3).

The second method promotes exon-specific splicing enhancement using bifunctional peptide nucleic acid as chimeric effectors (ESSENCE). The "nucleic acid" part of the synthetic compound binds to exon 7 sequences and the "peptide" part with serine-arginine repeats exercises the ESE-dependent function of positive splicing proteins, thus facilitating inclusion of the index exon (Cartegni & Krainer, 2003).

The third method uses bifunctional oligonucleotides, such that one half of the oligonucleotide binds to exon 7 sequences and the second half contains ESE motifs which facilitates the index exon to be included (Skordis et al., 2003; Meyer et al., 2009).

The fourth is a *trans*-splicing method incorporating an exogenous RNA sequence of SMN1 exon 7 into the FL-SMN transcript (Coady et al., 2007; Coady & Lorson 2010). The *trans*-splicing RNA containing *SMN1* exon 7 sequence binds to endogenous *SMN* pre-mRNA at the intron 6 region by complementary base-pairing. The mRNA product includes *SMN1* exon 7 sequence followed by a poly-adenylation signal. More recently, Shababi and colleagues reported the synergistic effect of *trans*-splicing RNA and a neurotrophic factor, insulin-like growth factor (IGF)-1. Intracerebroventricular injection of the *trans*-splicing/IGF-1 vector significantly increased SMN protein levels in brain and spinal cord of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$), extended lifespan, and increased the body weight (Shababi et al., 2011). However, it should be noted that a vector with IGF-1 alone has similar efficacy as one containing the splicing modulator that promotes exon 7 inclusion and IGF-1. We will discuss the effect of IGF-1 again in the section on "protection of motor neurons."

The fifth is an ISS-masking method to facilitate the inclusion of exon 7 into *SMN2* mRNA since a target ISS has been found in *SMN2* intron 7 (Singh et al., 2006; Hua et al., 2008; Porensky et al., 2012). Singh et al. (2006) showed that an antisense oligonucleotide (ASO) against ISS-N1 in

intron 7, Anti-N1, facilitated the inclusion of exon 7 into *SMN2* mRNA leading to increased SMN production in SMA cell lines. Krainer's group also reported that an ASO against the ISS, named ASO-10–27, effectively corrected SMN2 splicing (Hua et al., 2010) and demonstrated that it restored SMN expression in motor neurons of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) after intracerebroventricular injection (Passini et al., 2011). They also clarified that systemic administration (subcutaneous injection) of ASO-10–27 to neonates extended the median lifespan of SMA mice, although distribution of the ASO was limited and the *SMN2*-splicing changes were moderate in the CNS (Hua et al., 2011).

ISIS Pharmaceuticals recently announced the results of a phase 1 clinical trial of an ASO-10–27 delivering system, ISIS-SMNRx, using intrathecal administration. In the trial, a single dose (1, 3, 6, and 9 mg) was given intrathecally as a lumbar puncture (LP) bolus injection in male and female SMA patients 2–14 years old who are medically stable. According to their report with a total of 28 patients enrolled in the trial (http://www.isisph.com/pdfs/AAN_Isis_InvestorEvent.pdf), (1) ISIS-SMNRx was well tolerated, (2) the LP injection procedure was shown to be feasible in SMA children, (3) Improvement in Hammersmith Functional Motor Scale Examination (HFMSE) scores and electrophysiology measurements (motor unit number estimation (MUNE) with stable compound muscle action potential (CMAP)) were observed at the highest dose level. They are now planning controlled phase 2/3 registration-enabling studies in infants and children with SMA.

Producing a stable form of Δ7-SMN

It has been reported that the Δ7-SMN is unable to oligomerize or self-associate as well as FL-SMN (Lorson et al., 1998). The exon 7 domain is also necessary for localization of SMN into the cytoplasm (Zhang et al., 2003). However, interestingly, Δ7-SMN itself was reported to be capable of extending survival of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Le et al., 2005). It was postulated that Δ7-SMN may produce such phenotypic improvement either through partial functionality or by "seeding" oligomerization with functional FL-SMN. In this context, stabilization of Δ7-SMN may present a viable therapeutic strategy for SMA (Heier & DiDonato, 2009).

Aminoglycosides are an FDA-approved class of drug that acts within cells by binding to ribosomes to affect the translation of proteins from mRNA transcripts, i.e., by misreading stop codons (Wolstencroft et al., 2005). Aminoglycosides can lessen the severity of the SMA mouse model ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) via a Δ7-SMN translational read-through mechanism which enhances the stability of the Δ7-SMN protein with additional C-terminal peptides (Heier & DiDonato, 2009). Recently, two aminoglycosides, G418 and TC007, have been reported as candidate dugs for SMA (Mattis et al., 2006; Heier & DiDonato, 2009). G418 improved motor function of SMA mice, but did not extend their lifespan. On the contrary, TC007 demonstrated improved phenotypic measures and prolonged the lifespan of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Mattis et al., 2009).

SMN1-Introduction Strategies

Introduction of exogenous SMN1 by gene or stem cell therapies may prevent or alleviate the symptoms associated with motor neuron defects in SMA. With regards to gene therapy, researchers devoted their ingenuity and resources to developments in vector-gene construction, delivery systems and maximization of treatment effect. In 2004, Azzouz and colleagues first reported successful rescue of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) using a vector-mediated gene delivery approach (Azzouz et al., 2004). They injected SMN-expressing lentivector [Equine Infectious Anemia Virus (EIAV) vector] in various muscles of SMA mice on postnatal day 2. The vector reached the motor neurons by retrograde axonal transport and restored SMN levels. This gene therapy resulted in body weight gain and extension of the lifespan of SMA mice.

In 2010, Passsini and colleagues published a report on gene therapy using a self-complementary adeno-associated virus (scAAV) 8 vector expressing SMN (Passini et al., 2010). The scAAV vector is a recombinant virus defined as having a double-stranded DNA genome resulting in earlier onset of gene expression compared with regular single-stranded AAV. In their study, scAAV 8-SMN was injected on postnatal day 0 into the CNS (cerebral lateral ventricle and upper lumber spinal cord) of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$). This resulted in increase in body weight gain and muscle strength as well as in lifespan extension in the treated SMA mice. Interestingly, they also demonstrated that the CNC-directed gene therapy partially resolved the abnormal architecture of the NMJ. This rescue may have been achieved by improved axonal transport and/or efficient spliceosomes modifying gene expression related to NMJ function.

In the same year, Foust and colleagues reported successful rescue of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) using an intravenous injection approach with scAAV-9 vector (Foust et al., 2010). They injected scAAV-9 carrying SMN1 into the facial vein of mice pups on postnatal days 1, 5, and 10. According to them, scAAV9-mediated vascular gene delivery at postnatal day 1 successfully introduced SMN into SMA pups and rescued motor function, neuromuscular physiology and lifespan. Treatment on postnatal day 5 resulted in partial correction, whereas postnatal day 10 treatment had little effect. These experimental data with SMA mice suggested the

presence of a critical period when a sufficient amount of SMN protein is required during motor neuron development. In addition, the maturation of the blood brain barrier may hamper the transport of the SMN-expressing vectors to the target neurons, suggesting a finite period for efficient gene therapy.

In 2011, Dominguez and colleagues reported that they used postnatal day 1 systemic injection of self-complementary adeno-associated virus (scAAV9) vectors carrying a codon-optimized *SMN1* sequence and a chimeric intron placed downstream of the strong phosphoglycerate kinase (PGK) promoter (SMNopti) to overexpress the human SMN protein in a mouse model of severe SMA ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Dominguez et al., 2011). Codon optimization is a gene optimization technology that can alter both naturally occurring and recombinant gene sequences to achieve the highest possible levels of productivity in any given expression system. This treatment increased life expectancy from 27 to over 340 days (median survival of 199 days) in mice that normally survive about 13 days. The systemic scAAV9 therapy mediated complete correction of motor function, prevented motor neuron death and rescued the weight loss. This study also showed sex differences in the responsiveness to the treatment. Male SMA mice displayed a lower body weight gain than age-matched control mice, whereas the body weight of females was not statistically different from the controls.

As for stem cell therapy, it has been shown that "spinal cord-derived stem cells" and "embryonic stem cell-derived neural stem cells" can differentiate into motor neurons in vivo (Corti et al., 2008; Corti et al., 2010). In addition, following intrathecal transplantation into SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$), the administrated neural stem cells survived and migrated extensively to appropriate areas. Here, the transplanted embryonic stem cells were found to work by secreting soluble neuroprotection factors, such as glial-cell derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), and tumor growth factor (TGF)-α. These growth factors were confirmed to play a role in the improved functional recoveries of SMA mice following transplantation, showing an increase in myofiber number and size, axon length and body weight gain, suggesting that neural stem cell transplantation resulted in successful amelioration of behavioral end points and life span extension in SMA mice (Corti et al., 2010).

Gene and stem cell therapies may be very promising treatments for SMA patients, especially for pre-symptomatic patients. However, the efficacy of such therapies should further be tested in non-human primates before such approaches are applied to the patients, especially in considering if blood brain barrier function may hinder the delivery of therapeutic agents to the neurons (Tsai, 2012). Foust and colleagues investigated whether scAAV9 can traverse the blood–brain barrier in a nonhuman primate, *cynomolgus macaque* (Foust et al., 2010). They intravenously injected scAAV9 carrying the green fluorescent protein (GFP) gene on postnatal day 1, and demonstrated that scAAV9 crossed the blood brain barrier and reached motor neurons in the nonhuman primate model, suggesting that gene therapy targeting motor neurons can also be done in human.

Non-*SMN*-Targeting Strategies

Protection of motor neurons

Neuroprotection therapy with riluzole and gabapentin, which had originally been used for the patients with amyotrophic lateral sclerosis (ALS), was also applied to SMA patients (Russman et al., 2003; Merlini et al., 2003). Glutamate excitotoxicity may be an important factor in the pathogenesis of ALS since the cell bodies of motor neurons receive afferent innervation from glutamate neurons. Hence, pharmacologic agents that rescue glutamate excitotoxicity may be effective in slowing disease progression in ALS (van den Bosch, 2006). Similarly, glutamate excitotoxicity may also be an important factor in the pathogenesis of SMA. Riluzole inhibits the presynaptic release of glutamate, while gabapentin reduces the pool of releasable glutamate in the pre-synaptic neurons. Russman et al. (2003) reported the outcome of a clinical trial of riluzole in 10 patients with SMA type 1 (phase 1 trial with randomization of 2:1, i.e., 2 riluzole to 1 placebo). None of the subjects in this study experienced adverse effects. Even though the study sample size was small, some benefits of riluzole were suggested in the treated patients: three of the seven patients taking riluzole lived to more than 5, 4, and 2 years of age respectively with only BiPAP respiratory assistance at night. On the contrary, the placebo-controlled trials of gabapentin showed no significant benefit in motor function of the patients with SMA type 2/3 (Miller et al., 2001; Merlini et al., 2003).

Another group of compounds, β-lactam antibiotics, can also provide neuroprotection against glutamate-mediated excitotoxicty by increasing the expression level of the glutamate transporter EAAT2/GLT-1. However, to date these observations have only been demonstrated using model mice. A treated ALS mouse model showed a delay in loss of neurons and muscle strength, and increase in survival rate (Rothstein et al., 2005). Nizzardo and colleagues demonstrated that a β-lactam antibiotic, ceftriaxone, also ameliorated the neuromuscular phentotype in SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Nizzardo et al., 2011). Treatment with ceftriaxone increased general weight, muscle size, motor neuron numbers and NMJs, which are likely the reasons for the increased life span and muscle strength of the SMA mice. According to them, the neuroprotective effect of the β-lactam antibiotic in the SMA mice seems to be mediated not only through the process of increasing

EAAT2/GLT-1, but also by other mechanisms that increase transcription factor Nrf2 and SMN.

Thyrotropin-releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide) has trophic effects on spinal motor neurons, and it has also been tried for ALS and SMA patients. TRH was administered intravenously (Takeuchi et al., 1994; Tzeng et al., 2000) or orally (Kato et al., 2009) to SMA patients in small clinical trials but only transient improvement was observed in some patients.

Olesoxime (TRO19622), a small molecule with a cholesterol-like structure, has protective properties for motor neurons. It targets proteins associated with the mitochondrial permeability pore (Bordet, 2007). Olesoxime has been granted orphan drug status for the treatment of ALS and SMA, and clinical trials for ALS and SMA have been started in the US and Europe (http://clinicaltrials.gov/ct2/show/NCT01285583; http://clinicaltrials.gov/ct2/show/NCT01302600). In December 2011, Trophos SA announced the results from the phase 3 study of olesoxime in 512 patients with ALS; olesoxime was well tolerated but did not demonstrate a significant increase in survival of patients receiving riluzole (Rilutek®). Olesoxime trials for SMA are still ongoing and the results are to be expected in 2013 (http://www.trophos.com/news/pr20111213.htm).

IGF-1 is reported to modulate multiple fundamental cellular processes, such as cellular growth, proliferation, and survival (Vardatsikos et al., 2009). Most recently, Tsai and colleagues reported that CNS-directed IGF-1 delivery could reduce motor neuron death in SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/-}$) (Tsai et al., 2012). Murdocca and colleagues also reported the effects of IPLEX™ [recombinant human insulin-like growth factor 1 (rhIGF-1) combined with recombinant human IGF-1 binding protein 3 (rhIGFBP-3)] on a severe SMA mouse model (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMN*Δ*7*$^{+/+}$) (Murdocca et al., 2012). According to them, perinatal administration of IPLEX™ results in reduced degeneration of motor neurons, increased muscle fiber size and in amelioration of motor functions in SMA mice, suggesting this compound as a plausible therapeutic candidate to hinder the progression of the neurodegenerative process in SMA. However, it should be noted that CNS-directed IGF-1 delivery could not improve motor function in SMA mice (Tsai et al., 2012) and that IPLEX™ did not improve lifespan and body weight gain of the treated mice (Murdocca et al., 2012). IGF-1 may provide at least some beneficial effects on the survival of motor neurons. However, it is necessary to further study the systemic effect of IGF-1 administration before clinical application, because IGF-1 has multiple functions in various organs.

Protection of non-neuronal tissues

Nutrition may be critical for the care of SMA patients, especially SMA type 1 patients (Oskoui et al., 2007). The improved survival of SMA type 1 patients observed in recent years can be attributed to noninvasive pulmonary support and aggressive nutrition with gastrostomy feedings. Butchbach and colleagues observed that maternal diet can significantly modify survival and the motor neuron disease phenotype in SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMN*Δ*7*$^{+/+}$). According to them, SMA mice from dams that were fed a higher fat diet survived longer than those from dams on a lower fat diet (Butchbach et al., 2010a). The effect of nutritional support on survival of SMA patients and model animals indicates that improvement of pathological conditions of non-neuronal tissues including muscles should be considered in SMA therapy.

Treatments that directly target muscles and improve muscle mass have been reported: inhibition of myostatin by overexpression of follistatin (Sumner et al., 2009) and expression of IGF-1 (Bosch-Marcé et al., 2011). Although inhibition of myostatin did not ameliorate motor function or survival of severe SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMN*Δ*7*$^{+/+}$) (Sumner et al., 2009), overexpression of IGF-1 resulted in enlarged myofibers, but not in improvement of motor function (Bosch-Marcé et al., 2011). Murine IGF-1 administration had been proven to give different positive effects when it was expressed locally in muscle of SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMN*Δ*7*$^{+/+}$*mIGF-1*$^{+/-}$) (Bosch-Marcé et al., 2011) or in motor neurons (Murdocca et al., 2012).

Recently, there has been emerging evidence that the RhoA/ROCK pathway may play an important role in the pathogenesis of SMA (Bowerman et al., 2009; Nölle et al., 2011) since SMN depletion leads to an increased activation of ROCK, a major regulator of actin dynamics. Bowerman et al. (2010) reported that ROCK inhibitors, Y-27632 and Fasudil, dramatically improved the survival of the Smn$^{2B/-}$ mice, an intermediate SMA mouse model. They emphasized that lifespan extension in SMA mice with ROCK inhibitors was accompanied by an improvement in the maturation of NMJs and an increase in muscle fiber size (Bowerman et al., 2010; Bowerman et al., 2012). However, Bowerman and colleagues showed that administration of Y-27632 had no beneficial effect on the Smn$^{-/-}$ mouse model with the most severe SMA phenotype. Here, they suggested that there may be a need for differential therapies for the different types of SMA severities and that the "one size fits all" approach may not be tenable (Bowerman et al., 2010).

Challenges in Clinical Trials

Despite the large number of candidate compounds evaluated, there has yet to be any effective drug treatment reported for all types of SMA (Wadman et al., 2012a; Wadman et al., 2012b). A number of clinical trials for SMA have already been conducted in the past decade, some of which are still ongoing (Miller et al., 2001; Mercuri et al., 2007; Pane et al., 2008;