REVIEW ARTICLE

Novel challenges in spinal muscular atrophy – How to screen and whom to treat?

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Abstract

In recent years, disease-modifying and life-prolonging therapies for spinal muscular atrophy (SMA) have been developed. However, patients are currently diagnosed with significant delay and therapies are often administered in advanced stages of motor neuron degeneration, showing limited effects. Methods to identify children in presymptomatic stages are currently evaluated in newborn screening programs. Yet, not all children develop symptoms shortly after birth raising the question whom to treat and when to initiate therapy. Finally, monitoring disease progression becomes essential to individualize management. Here, we review the literature on screening approaches, strategies to predict disease severity, and biomarkers to monitor therapy.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease affecting one in 10,000 live births with a carrier frequency of 1 in 50 unaffected individuals. Clinically, SMA has been divided into four subtypes based on age at onset, phenotypic severity, and the highest motor milestone achieved, which can be lost later upon disease progression: Type I (“nonsitters”), type II (“sitters”), type III (“walkers”), and type IV (adult-onset). The underlying genetic causes of SMA are homozygous deletions or loss-of-function mutations in the survival motor neuron 1 gene (SMN1) with retained function of at least one copy of the paralogous gene SMN2, both located on chromosome 5q13. Due to a nucleotide substitution, the majority of SMN2 pre-mRNA transcripts undergo alternative splicing resulting in exclusion of exon 7. The resulting truncated SMN protein is rapidly degraded and the overall lack of full-length SMN protein ultimately leads to degeneration of alpha motor neurons in the spinal cord.

In recent years, novel causal therapies for SMA have been developed. Following successful approval by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), a large number of SMA patients has recently been treated with antisense oligonucleotides (Nusinersen, Spinraza). Large phase 3 trials have shown improvement in motor function and higher event-free and overall survival in infantile-onset SMA as well as significant improvement in HFMSE (Expanded version of the Hammersmith Functional Motor Scale) scores in SMA patients with disease onset after 6 months of age. Recently, Biogen™, the market authorization holder of Nusinersen, released interim results of a phase 2 trial evaluating the effects of Nusinersen in presymptomatic SMA patients types I-III (NURTURE, see ClinicalTrials.gov: NCT02386553), arguing that preemptive treatment led to the achievement of

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age−expected World Health Organization motor milestones, such as sitting without support in SMA type I and stable or improved motor function in SMA type II-III. Taking into account the molecular pathways affected in SMA pathology and the complex canonical and non-canonical roles that SMN protein plays in neuronal metabolism, further promising approaches including adenovirus-mediated SMN1 gene replacement therapies, modulators of SMN2 splicing, neuroprotective agents that modify mitochondrial pathways, compounds acting on muscles and neuromuscular junctions, and modifiers of endocytosis, actin dynamics, and ubiquitin homeostasis have been developed.5–7 Amongst these novel approaches, gene replacement therapies hold particularly great potential to change the course of SMA and are currently investigated in advanced stages of clinical trials. Preliminary data have already been published and show very promising results.8

However, the advent of disease-modifying therapies raises important questions: How do we identify affected children in the pre-symptomatic stage and how can we predict disease severity to choose the optimal therapeutic window for initiation of treatment? Further, currently all SMA patients receive the same absolute dose of Nusinersen irrespective of age, body weight, and residual motor function, raising another crucial question: How do we monitor patients under therapy and on what grounds can we adjust treatment doses to individual needs?

In this review, we will discuss the literature on pre- and neonatal screening approaches for SMA, provide an overview about current strategies to predict disease severity and summarize potential candidate biomarkers to monitor therapeutic response.

Newborn Screening and Prenatal Diagnosis

Preliminary data published on the preemptive treatment of SMA patients have shown a significant increase in motor function and quality of life corroborating data from mouse models that point out that early restoration of SMN levels, preferably within the first three postnatal days, can rescue phenotypes, whereas administration of therapies beyond postnatal day 5 only showed attenuated effects and initiation of treatment after postnatal day 10 failed to improve motor function and survival.9,10 Along these lines, different Expanded Access Programs for Nusinersen worldwide have clearly identified an early age at treatment initiation as the major determinant for therapeutic success.11–13 However, in the vast majority of cases, SMA patients are still diagnosed with significant delay, ranging from 4 months after onset of symptoms in SMA type I to over 10 months in SMA type III.14,15 As a result, available life-prolonging and life-saving therapies are often administered in a stage of advanced alpha motor neuron degeneration, therefore only showing limited effects.

Newborn screening programs for SMA hold tremendous potential for identifying affected children at an asymptomatic stage, allowing presymptomatic initiation of therapy before irreversible motor neuron damage occurs. Nationwide, genetic newborn screening programs for SMA have been widely discussed in the context of the novel life-prolonging therapies. A recent study convincingly demonstrated that homozygous SMN1 mutations can be detected with high accuracy in dried blood spots, proving a feasible and practical genetic newborn screening method for SMA.16 In the U.S., SMA newborn screening is already implemented in screening programs in a number of states and further clinical trials are currently evaluating the applicability and economic challenges implicated in a nationwide genetic newborn screening for SMA in Taiwan and Belgium (ClinicalTrials.gov: NCT03217578 and NCT03554343). Limitations to these methods include the fact that point mutations in the SMN1 gene, accounting for approximately 5% of patients, cannot be detected. Furthermore, SMN has been shown to play a role in neuronal differentiation and formation of the neuromuscular junction in murine cell models, highlighting the demand of SMN during neurodevelopment and synaptogenesis17 and raising the question whether treatment in neonatal SMA (sometimes referred to as SMA type 0) should already be initiated in the prenatal period.18 These considerations gain particular importance in the context of promising results in the field of gene replacement therapies that can theoretically be administered in utero. Prenatal screening methods offer the chance to identify affected children during early pregnancy, allowing prenatal therapeutic intervention. Prenatal testing is possible and widely available. Chorionic villus sampling or amniocentesis can be performed at 10–14 or 15–20 weeks of gestation respectively and can determine a child’s risk for SMA with high accuracy. However, these techniques are invasive and carry significant risks for the mother and the unborn child and are therefore only carried out in high-risk pregnancies with proven carrier status of the parents. Interestingly, noninvasive prenatal diagnosis techniques have been reported in the literature. By isolating circulating fetal trophoblastic cells19 or cell-free fetal DNA from maternal blood,20 it is possible to detect SMA in unborn children with 100% diagnostic sensitivity and specificity, thus holding great potential to further change the field of SMA. However, besides technical limitations, such as isolation of fetal cells and cell-free fetal DNA from maternal blood, these techniques are costly and require laboratories.
with special expertise and are thus not likely to be implemented on a nationwide scale. Finally, significant ethical concerns are implicated with genetic screening methods that have to be discussed in detail by the community before making these tests available.\textsuperscript{21}

\textbf{Approaches to Predict Disease Severity}

Screening methods for SMA are important tools to detect affected children at an early stage. However, not all patients will develop clinical signs shortly after birth, and many SMA patients show a very mild disease course with late onset of symptoms and only minimal muscle weakness. In regard of the costly and invasive therapies, the question arises how we can reliably predict disease severity and derive clinical decisions from these predictions.

Recently, the \textit{SMA NBS Multidisciplinary Working Group} released a treatment algorithm for SMA children identified through newborn screening based on \textit{SMN1} deletion analysis in dried blood spots.\textsuperscript{15} Recommendations for treatment decisions were based on the correlation of \textit{SMN2} copy numbers and clinical phenotype. The group argues that all children with 2–3 copies should receive immediate treatment, patients with one copy should be treated if asymptomatic at birth and therapy should be delayed in patients with four or more copies due to a usually milder disease course. However, how closely are \textit{SMN2} copy numbers correlated with SMA phenotype and is this quantification enough to decide whether a child should receive therapy?

A number of groups investigated the correlation of \textit{SMN2} copy number and disease severity (Table 1). The majority of studies conclude that a correlation exists, but is not absolute, since there is significant overlap between different SMA types. The most compelling evidence comes from Caluchu et al. who attempted to correlate \textit{SMN2} copy number with clinical data in a cohort of almost 3500 SMA patients. The study concluded that while one and four copies were associated with a severe and mild phenotype respectively, there was significant overlap between patients with two and three copies, showing any possible phenotype.\textsuperscript{22} Unfortunately, around 80\% of individuals in the cohort carried 2–3 \textit{SMN2} copies, reflecting the common problem in clinical practice, where in most cases, 2–3 \textit{SMN2} copies are detected rendering predictions about disease severity extremely difficult. The situation becomes even more complicated considering that, in SMA families, siblings with identical \textit{SMN2} copy numbers can have different phenotypes\textsuperscript{23,24} and even five \textit{SMN2} copies in the context of homozygous \textit{SMN1} mutations were found in both SMA type I patients and asymptomatic individuals.\textsuperscript{22,25,26}

Taking into account that some lack of correlation between \textit{SMN2} copy number and phenotype may lie in the accuracy of \textit{SMN2} copy number quantitation, which becomes technically demanding with more than three copies, one might argue that the discordance between \textit{SMN2} copy number and phenotype could also lie in the fact that \textit{SMN2} is not equally transcribed among different individuals. Indeed, many studies have shown that \textit{SMN2} transcripts do not correlate with \textit{SMN2} copy number.\textsuperscript{27–30} \textit{SMN2} full-length (\textit{SMN2-fl}) mRNA and \textit{SMN2} mRNA lacking exon 7 (\textit{SMN2A7}) as downstream readouts of \textit{SMN2} copy number have been studied in SMA patients. Interestingly, some studies identified a correlation between SMN expression and SMA phenotype.\textsuperscript{26,31–32} Tiziano et al., for instance, report that SMA type III patients have significantly higher \textit{SMN2-fl} transcript levels than SMA type II patients and unraveled a correlation between motor function scores and \textit{SMN2-fl} transcripts levels in the SMA type II population.\textsuperscript{31} Similarly, SMA type III patients with higher transcript levels were associated with more advanced age at disease onset, and a dosage of \textit{SMN2-fl} levels $\geq$58 mol/ng predicted a threefold lower risk of disease onset below the age of 3 years, thus discriminating between SMA IIIA and IIIB. Along these lines, Tiziano et al. report that in ambulant SMA type III patients, \textit{SMN2-fl} mRNA levels correlated with motor performance, thus predicting disease severity.\textsuperscript{28} Further evidence comes from families with several SMA children. In siblings with discordant phenotypes, the more severely affected sibling showed significantly lower \textit{SMN2-fl} mRNA levels, while in phenotypically similar siblings, both showed similar \textit{SMN2-fl} transcripts.\textsuperscript{31} By contrast, Sumner et al. found relatively normal \textit{SMN2-fl} mRNA in SMA type II and III patients compared to healthy children\textsuperscript{33} and Vezain et al. state that in SMA patients with three \textit{SMN2} copies and different phenotypes, \textit{SMN2-fl} and \textit{SMN2A7} mRNA levels did not differ.\textsuperscript{32} Thus, conclusions about the use of \textit{SMN2} transcripts in predicting disease severity cannot be made with certainty based on the current literature. Nevertheless, despite discordant results, \textit{SMN2} transcript measurements might be helpful in predicting SMA disease severity and should not be completely left out of considerations when it comes to therapeutic decisions. Interestingly, no robust correlations between \textit{SMN2} copy number and SMN protein levels could be established. However, SMN expression seems to be tissue dependent, since unlike in peripheral blood, quantification of SMN levels in fibroblasts did correlate with \textit{SMN2} copy number arguing that blood might not be the adequate biomaterial to monitor SMA.\textsuperscript{30} Moreover, the significant overlaps between \textit{SMN2} copy numbers and phenotype, as well as the lacking correlation between \textit{SMN2} copy numbers and \textit{SMN2} transcript levels...
highlight the importance of genetic and environmental modifiers. To date, a number of modifiers attenuating or exacerbating SMA phenotype have been reported. Amongst genetic modifiers, a rare polymorphism in \( \text{SMN2} \) (c.859G > C, p.Gly287Arg), acting as an exonic splicing enhancer element and increasing the amount of \( \text{SMN2} \)-fl transcripts has been identified as disease attenuating variant. Similarly, an A-44G transition in \( \text{SMN2} \) intron 6 has recently been reported, a variant that results in enhanced exon 7 inclusion and a milder phenotype. Upregulation of plastin 3 and neuritin 1 as well as reduction of neurocalcin delta have been reported as protective genetic modifiers. Concerning epigenetic modifiers, Hauke et al. report that hypermethylation of \( \text{SMN2} \) results in gene silencing and consequently in disease aggravation. These findings were confirmed by Cao et al. who found 13 differentially methylated units in \( \text{SMN2} \), eight of which were associated with disease severity, thereby showing higher methylation levels in SMA type I compared to SMA type III. In line with these findings, Zheleznyakova et al. carried out genome-wide methylation analysis of SMA patients of all types uncovering several differentially methylated gene loci involved in actin cytoskeleton dynamics, neuronal metabolism, transcriptional regulation, and cell death.

Thus, the exact pathophysiological mechanisms in SMA, especially those determining disease severities, are currently not well understood and the contribution of a number of genetic and epigenetic disease modifiers has been shown in the literature, raising the question whether therapeutic decisions solely based on \( \text{SMN2} \) copy number will suffice in clinical practice.

### Candidate Biomarkers for Therapeutic Monitoring

Currently, all SMA patients receive an absolute dose of 12 mg Nusinersen, which is administered via intrathecal administration in 4-month intervals following a loading phase of five intrathecal injections within the first 180 days of treatment. These recommendations are based on phase 1 trials and pharmacological studies that pointed out a half-life of Nusinersen in the cerebrospinal fluid (CSF) of around 163 days and further showed that

| # SMA patients | SMA types | Correlation of \( \text{SMN2} \) copy number and disease severity | Reference |
|---------------|-----------|----------------------------------------------------------|-----------|
| Strong correlation | I/II | Good correlation | Mailman et al. 61 |
| 50 | I/II | Good correlation | Kesari et al. 62 |
| 87 | I | Good correlation to HFMS in SMA type II | Tiziano et al. 63 |
| 143 | I/II | 1–2 \( \text{SMN2} \) copies predict early disease onset and poor survival | Taylor et al. 64 |
| 26 | I | Correlation with risk of death or permanent invasive ventilatory support | Kolb et al. 65 |
| 3 | asymptomatic | Five \( \text{SMN2} \) copies are protective in case of homozygous \( \text{SMN1} \) deletion | Prior et al. 66 |
| 115 | III/IV | Strong correlation of 1–2 copies with severe phenotype and four or more copies with mild phenotype, strong overlap in cases of three copies | Wirth et al. 67 |
| NA | III/IV | Modifying role in MUNE and CMAP and overall functional status | Swoboda et al. 68 |
| 36 | III/IV | Modest correlation | Czech et al. 69 |
| 42 | III/IV | Correlation exists, but better predictor when combined with \( \text{NAIP} \) mutation analysis | Watihayati et al. 70 |
| 375 | III/IV | Correlation exists, but great overlap between groups | Feldkotter et al. 71 |
| 27 | III/IV | Correlation exists, but great overlap between groups | Harada et al. 72 |
| 51 | III/IV | Correlation exists, but great overlap between groups | Tiziano et al. 73 |
| 144 | III/IV | Correlation exists, but great overlap between groups, especially in cases of 2–3 copies | Medrano et al. 74 |
| 3459 | III/IV | Correlation exists, but great overlap between groups, especially in cases of 2–3 copies | Calucho et al. 75 |
| 45 | III/IV | Correlation exists, but siblings with different phenotypes show identical \( \text{SMN2} \) copy numbers | Cusco et al. 76 |
| Poor correlation | I/II | No correlation | Vezain et al. 77 |
| 45 | III | No correlation | Tiziano et al. 78 |
| 61 | I/II | Four \( \text{SMN2} \) copies in a family member with SMA type III and unaffected sibling and five \( \text{SMN2} \) copies in unaffected family member | Zheleznyakova et al. 79 |
| 108 | I/II | SMA type I patients with four or five copies exist | Crawford et al. 80 |

CMAP, compound muscle action potential; HFMS, Hammersmith Functional Motor Scale; MUNE, motor unit number estimation; \( \text{NAIP} \), neuronal apoptosis inhibitory protein; SMA, spinal muscular atrophy; \( \text{SMN} \), survival motor neuron.
no correlations between age, body weight, and CSF concentration of Nusinersen exist.\textsuperscript{44,45} However, while Luu et al. argue that age-based dosing produced more comparable median exposures of Nusinersen in the CSF, no dose-limiting toxicity was reported, leading to consensus of using fixed-dosing schemes across all age groups. Following these results, Finkel et al. carried out a phase 2 dose-escalation study demonstrating that an absolute dose of 12 mg of Nusinersen was superior to 6 mg.\textsuperscript{46} Outcome measures included achievement of motor milestones, motor function tests, dependence on permanent ventilation, electrophysiological measurements, and overall survival. However, so far only 20 patients were studied and all patients were under 1 year of age and below 10 kg body weight. Ultimately, the question arises if these pharmacologic investigations and the small cohorts studied in phase 2 clinical trials can accurately reflect clinical practice. Considering the broad variability of SMA patients concerning age at disease onset, body weight, and residual motor function, a uniform dose and equal dosing intervals across all patients seem highly inaccurate. But how can we monitor therapies and on what grounds can we base decisions for adjusting therapeutic doses and dosing intervals to optimize therapeutic success? In order to answer these questions, reliable biomarkers dynamically reflecting disease progression under pharmacotherapy are needed. Candidate biomarkers for SMA have therefore been extensively studied in the past.

Recently, the NeuroNEXT study evaluated different instrumental and molecular biomarkers.\textsuperscript{47} Amongst others, compound muscle action potential (CMAP) responses and SMN protein blood levels have been identified as possible biomarkers to monitor therapies.

Electrophysiological measurements have been previously proposed as possible biomarkers for SMA. These measurements include CMAP, motor unit number estimation (MUNE) responses, and electrical impedance myography (EIM). Different studies have shown that both proximal and distal muscles can be used as sites for electrophysiological measurements in SMA patients.\textsuperscript{48,49} Importantly, Swoboda et al. point out that electrophysiological measurements hold prognostic value for an individual SMA patient’s expectations toward clinical improvement and response to therapy.\textsuperscript{50} Indeed, Arnold et al. showed that electrophysiological measurements dynamically change in mice under antisense oligonucleotide therapy providing a potential tool for future treatment stratification.\textsuperscript{51} Similarly, SMN protein levels in peripheral blood have been investigated in the past. Measurements of SMN levels in SMA patients have produced stable readouts over the first 2 years of life with little intra-individual variability and have thus been proposed as potential biomarker to monitor disease progression.\textsuperscript{27,33,47} Recently, Otsuki et al. published a reliable flow cytometry-based approach to quantify SMN protein levels in peripheral blood. Besides reliably differentiating between SMA subjects and healthy controls, SMN quantification could be correlated to some extent to clinical phenotypes and motor scores, therefore providing a promising candidate biomarker.\textsuperscript{52} Meanwhile, approaches for the establishment of radiological biomarkers that are already utilized in neuromuscular disorders such as Duchenne muscular dystrophy, the limb-girdle muscular dystrophies, and others are scarce in SMA and only few studies investigated the correlation between motor function tests and radiologic markers.\textsuperscript{53,54} Further studies evaluating the dynamic changes of electrophysiological measurements, SMN protein levels, or even high-resolution magnetic resonance imaging signals in response to pharmacotherapy are needed to confirm the use of these biomarkers to monitor SMA therapies.

Along these lines, the current lack of reliable biomarkers for SMA has fostered the search for novel candidate substrates. Promising methods to detect novel biomarkers are unbiased “omics” approaches. By using proteomics technologies, Matsaers et al. identified Calreticulin and GRP75/Mortalin, proteins associated with neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson’s disease and Alzheimer’s disease, as potential biomarkers for SMA in muscle samples of SMA patients.\textsuperscript{55} The “Biomarkers for SMA” (BforSMA) project used a combined proteomics, metabolomics, and transcriptomics approach and detected 97 plasma proteins, 59 plasma metabolites, and 44 urine metabolites correlating with motor function tests.\textsuperscript{56} Kobayashi et al. analyzed plasma samples from the BforSMA study in detail and found 12 candidate SMA biomarkers significantly associated with motor function and further analytes associated with nonmotor SMA outcome measures, which were included into a commercial plasma protein panel.\textsuperscript{57} Several analytes deriving from the findings of Kobayashi et al. were subsequently investigated in the SMN.A7 mouse model before and under treatment with antisense oligonucleotides (ASO). Osteopontin, dipeptidyl-dipeptidase 4 (DPPIV), tetranectin, fetuin A and vitronectin were identified to significantly correlate with motor function and some of these candidate biomarkers normalized in ASO-treated mice. However, the group concludes that these candidate biomarkers are not disease-specific and the observed results most likely show compensatory changes rather than being directly attributable to SMA pathology.\textsuperscript{58} Currently, the “NatHis-SMA study” is evaluating the value of a number of biomarkers for predicting SMA disease severity and progression and to determine their use as outcome measures for further therapeutic trials (ClinicalTrials.gov: NCT02391831). Recently published baseline results demonstrate that muscle strength and
motor function tests, measurements of upper limb function, respiratory function tests, as well as electrophysiologic and radiographic studies were able to differentiate among non-sitter and sitter SMA type II, non-ambulant SMA type III and ambulant patients. Longitudinal evaluation during the 2-year observation period will unravel if these candidate biomarkers will be able to dynamically reflect disease progression determining their value as therapeutic outcome measures.  

Thus, the search for biomarkers for SMA has identified a number of candidates. However, none of the designated substrates has yet proven to dynamically reflect SMA disease progression. Further studies are needed to evaluate the clinical benefits of current potential candidates as well as to isolate novel disease-specific biomarkers in order to allow accurate monitoring and recommendations for individual adjustments of therapy.

**Conclusion**

SMA is a devastating neuromuscular disease associated with high morbidity and mortality. In light of novel therapies profoundly changing disease course and prolonging survival, reliable biomarkers and screening methods for early diagnosis and therapeutic monitoring are needed more than ever. Here, we provide a critical review about current pre- and neonatal screening approaches, strategies to predict disease severity, and potential biomarkers for therapeutic monitoring.

Screening methods are currently being evaluated in clinical trials and offer tremendous opportunities for early diagnosis. However, technical and ethical issues are yet to be discussed. Predictions of disease severity remain a challenging topic. Taking into account the discordance between phenotype and SMN2 copy numbers, as well as the many exceptions ranging from asymptomatic to severely-affected patients with high copy numbers and mildly-affected individuals with low copy numbers in combination with the many genetic and epigenetic modifiers contributing to disease severity, the decision upon initiation of costly and invasive SMA therapies based solely on SMN2 copy numbers appears highly unsatisfactory. In addition, SMN2 copy number, to date, holds no prognostic value concerning response to therapy as recently published interim results of the NUTrURE trial demonstrate for presymptomatic SMA individuals corroborating the previously shown data for symptomatic patients. In the absence of reliable predictive biomarkers, assessment of SMN protein levels and genetic and epigenetic modifiers, providing at least some information about disease severity, should in our opinion be considered in cases with more than three SMN2 copies before denying affected children potentially life-saving therapies. Further, the question arises how to monitor untreated individuals to catch the optimal time point for treatment initiation. The SMA NBS Multidisciplinary Working Group suggests regular clinical follow-up visits for individuals with four and more SMN2 copies with age-dependent assessment of EMG, CMAP, myometry, physical exam, and motor function tests such as the HFMSE and the 6-Minute Walk Test (6MWT). Indeed, recently Montes et al. demonstrated high sensitivity of the 6MWT in predicting phenotypic severity in ambulant SMA patients, reliably capturing small prognostic changes. Similarly, sensitive changes in quantitative MRI of the thighs have been reported as powerful monitoring tool. Additional studies are needed to optimize medical care and develop more sensitive tests for untreated individuals with SMA to avoid missing the critical window of opportunity for treatment initiation with potentially devastating consequences for the affected individuals.

Finally, therapeutic monitoring is essential to adjust treatment and optimize outcome. Electrophysiological studies and measurements of SMN protein levels provide possibilities for therapeutic monitoring. Further studies need to evaluate the benefits of these biomarkers. Due to the discordant findings regarding the value of currently available biomarkers, the search for novel candidates is more pressing than ever. This point becomes even more important in light of ongoing and upcoming clinical trials to evaluate novel therapeutics such as the orally administered small molecules risdiplam (FIREFISH, RAINBOW-FISH) and branaplam, intravenous and intrathecal adenovirus-mediated SMN1 gene replacement therapies (SPRINT, STRIVE-EU, REACH), and others. Monitoring and clinically comparing patient cohorts treated with these novel therapies will become particularly challenging since subtle subclinical changes have to be detected to evaluate the benefits of the specific therapeutics and different routes of administration, adjust therapeutic management in case of disease progression and define cutoffs for considering add-on and combination therapies to maximize clinical outcomes. Promising approaches to identify novel biomarkers could come from “omics” studies, providing a useful untargeted tool to screen for candidate biomarkers. However, results are often difficult to interpret. To date, disease-specific biomarkers could not be identified.

In conclusion, the exciting field of SMA is currently being transformed due to novel disease-modifying therapies. Novel screening methods, strategies to predict disease severity and candidate biomarkers allowing therapeutic monitoring are being identified and developed, however, as yet all of these techniques show limitations, and none of them accurately reflects the complexity of SMA pathology. Further research is needed to identify more accurate...
methods for diagnosis, disease prediction, and therapeutic monitoring in SMA and to establish individualized dosing recommendations. This will be the next major step in the transition of a recently untreatable rare neuromuscular disease to precision medicine and satisfactory long-term therapy and outcome.

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All the authors designed the manuscript. A.S. and A.Z. wrote the initial draft. All the authors read, amended, and approved the final manuscript.

**Conflicts of Interest**

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**References**

1. Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. Eur J Hum Genet 2012;20:27–32.

2. Lunn MR, Wang CH. Spinal muscular atrophy. Lancet 2008;371:2120–2133.

3. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. N Engl J Med 2017;377:1723–1732.

4. Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. N Engl J Med 2018;378:625–635.

5. Groen EJN, Talbot K, Gillingwater TH. Advances in therapy for spinal muscular atrophy: promises and challenges. Nat Rev Neurol 2018;14:214–224.

6. Farrar MA, Park SB, Vucic S, et al. Emerging therapies and challenges in spinal muscular atrophy. Ann Neurol 2017;81:355–368.

7. Sumner CJ, Crawford TO. Two breakthrough gene-targeted treatments for spinal muscular atrophy: challenges remain. J Clin Invest 2018;128:3219–3227.

8. Mendell JR, Al-Zaidy S, Shell R, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med 2017;377:1713–1722.

9. Le TT, McGovern VL, Alwine IE, et al. Temporal requirement for high SMN expression in SMA mice. Hum Mol Genet 2011;20:3578–3591.

10. Foust KD, Wang X, McGovern VL, 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.

11. Pechmann A, Langer T, Schorling D, et al. Evaluation of children with SMA Type 1 under treatment with nusinersen within the expanded access program in Germany. J Neuromuscul Dis 2018;5:135–143.

12. Pane M, Palermo C, Messina S, et al. Nusinersen in type 1 SMA infants, children and young adults: preliminary results on motor function. Neuromuscul Disord 2018;28:582–585.

13. Farrar MA, Teoh HL, Carey KA, et al. Nusinersen for SMA: expanded access programme. J Neurol Neurosurg Psychiatry 2018;89:937–942.

14. Lin CW, Kalb SJ, Yeh WS. Delay in diagnosis of spinal muscular atrophy: a systematic literature review. Pediatr Neurol 2015;53:293–300.

15. Glascock J, Sampson J, Haidet-Phillips A, et al. Treatment algorithm for infants diagnosed with spinal muscular atrophy through newborn screening. J Neuromuscul Dis 2018;5:145–158.

16. Chien YH, Chiang SC, Weng WC, et al. Presymptomatic diagnosis of spinal muscular atrophy through newborn screening. J Pediatr 2017;190:e1.

17. Fan L, Simard LR. Survival motor neuron (SMN) protein: role in neurite outgrowth and neuromuscular maturation during neuronal differentiation and development. Hum Mol Genet 2002;11:1605–1614.

18. Govoni A, Gagliardi D, Comi GP, Corti S. Time is motor neuron: therapeutic window and its correlation with pathogenetic mechanisms in spinal muscular atrophy. Mol Neurobiol 2018;55:6307–6318.

19. Mouawia H, Saker A, Jais JP, et al. Circulating trophoblastic cells provide genetic diagnosis in 63 fetuses at risk for cystic fibrosis or spinal muscular atrophy. Reprod Biomed Online 2012;25:508–520.

20. Parks M, Court S, Bowns B, et al. Non-invasive prenatal diagnosis of spinal muscular atrophy by relative haplotype dosage. Eur J Hum Genet 2017;25:416–422.

21. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. N Engl J Med 2018;379:464–473.

22. Calucho M, Bernal S, Alias L, et al. Correlation between SMA type and SMN2 copy number revisited: an analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. Neuromuscul Disord 2018;28:208–215.

23. Cusco I, Barcelo MJ, Rojas-Garcia R, 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.

24. Zheleznjakova GY, Kiselev AV, Vakharlovsky VG, et al. Genetic and expression studies of SMN2 gene in Russian patients with spinal muscular atrophy type II and III. BMC Med Genet 2011;15:96.

25. Prior TW, Swoboda KJ, Scott HD, Hejmanowski AQ. Homozygous SMN1 deletions in unaffected family...
members and modification of the phenotype by SMN2. Am J Med Genet A 2004;130A:307–310.

26. Crawford TO, Paushkin SV, Kobayashi DT, et al. Evaluation of SMN protein, transcript, and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. PLoS ONE 2012;7:e33572.

27. Simard LR, Belanger MC, Morissette S, et al. Preclinical validation of a multiplex real-time assay to quantify SMN mRNA in patients with SMA. Neurology 2007;68:451–456.

28. Tiziano FD, Lomastro R, Di Pietro L, et al. Clinical and molecular cross-sectional study of a cohort of adult type III spinal muscular atrophy patients: clues from a biomarker study. Eur J Hum Genet 2013;21:630–636.

29. Czech C, Tang W, Bugawan T, et al. Biomarker for spinal muscular atrophy: expression of SMN in peripheral blood of SMA patients and healthy controls. PLoS ONE 2015;10:e0139950.

30. Wadman RI, Stam M, Jansen MD, et al. A comparative study of SMN protein and mRNA in blood and fibroblasts in patients with spinal muscular atrophy and healthy controls. PLoS ONE 2016;11:e0167087.

31. Tiziano FD, Pinto AM, Fiori S, et al. SMN transcript levels in leukocytes of SMA patients determined by absolute real-time PCR. Eur J Hum Genet 2010;18:50–58.

32. Vezain M, Saugier-Weber P, Melki J, et al. A sensitive assay for measuring SMN mRNA levels in peripheral blood and in muscle samples of patients affected with spinal muscular atrophy. Eur J Hum Genet 2007;15:1054–1062.

33. Sumner CJ, Kolb SJ, Harmison GG, et al. SMN mRNA and protein levels in peripheral blood: biomarkers for SMA clinical trials. Neurology 2006;66:1067–1073.

34. Wirth B, Garbes L, Riessland M. How genetic modifiers influence the phenotype of spinal muscular atrophy and suggest future therapeutic approaches. Curr Opin Genet Dev 2013;23:330–338.

35. Bernal S, Elias L, Barcelo MJ, et al. The c.859G>C variant in the SMN2 gene is associated with types II and III SMA and originates from a common ancestor. J Med Genet 2010;47:640–642.

36. Vezain M, Saugier-Weber P, Goina E, et al. A rare SMN2 variant in a previously unrecognized composite splicing regulatory element induces exon 7 inclusion and reduces the clinical severity of spinal muscular atrophy. Hum Mutat 2010;31:E1110–E1125.

37. Wu X, Wang SH, Sun J, et al. A-44G transition in SMN2 intron 6 protects patients with spinal muscular atrophy. Hum Mol Genet 2017;26:2768–2780.

38. Oprea GE, Krober S, McWhorter ML, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science 2008;320:524–527.

39. Yener IH, Topaloglu H, Erdem-Ozdamar S, Dayangac-Erden D. Transcript levels of plastin 3 and neurtin 1 modifier genes in spinal muscular atrophy siblings. Pediatr Int 2017;59:53–56.

40. Riessland M, Kaczmarek A, Schneider S, et al. Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis. Am J Hum Genet 2017;100:297–315.

41. Hauke J, Riessland M, Lunke S, 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.

42. Cao YY, Qu YJ, He SX, et al. Association between SMN2 methylation and disease severity in Chinese children with spinal muscular atrophy. J Zhejiang Univ Sci B 2016;17:76–82.

43. Zheleznyakova GY, Voisin S, Kiselev AV, et al. Genomewide analysis shows association of epigenetic changes in regulators of Rab and Rho GTPases with spinal muscular atrophy severity. Eur J Hum Genet 2013;21:988–993.

44. Chiriboga CA, Swoboda KJ, Darras BT, et al. Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. Neurology 2016;86:890–897.

45. Luu KT, Norris DA, Gunawan R, et al. Population pharmacokinetics of nusinersen in the cerebral spinal fluid and plasma of pediatric patients with spinal muscular atrophy following intrathecal administrations. J Clin Pharmacol 2017;57:1031–1041.

46. Finkel RS, Chiriboga CA, Vajzar J, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet 2016;388:3017–3026.

47. Kolb SJ, Coffey CS, Yankey JW, et al. Natural history of infantile-onset spinal muscular atrophy. Ann Neurol 2017;82:883–891.

48. Galea V, Fehlings D, Kirsch S, McComas A. Depletion and sizes of motor units in spinal muscular atrophy. Muscle Nerve 2001;24:1168–1172.

49. Bromberg MB, Swoboda KJ, Lawson VH. Counting motor units in chronic motor neuropathies. Exp Neurol 2003;184 (Suppl 1):S53–S57.

50. Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: relation to age, SMN2 copy number, and function. Ann Neurol 2005;57:704–712.

51. Arnold W, McGovern VL, Sanchez B, et al. The neuromuscular impact of symptomatic SMN restoration in a mouse model of spinal muscular atrophy. Neurobiol Dis 2016;87:116–123.

52. Otsuki N, Arakawa R, Kaneko K, et al. A new biomarker candidate for spinal muscular atrophy: identification of a peripheral blood cell population capable of monitoring the level of survival motor neuron protein. PLoS ONE 2018;13:e0201764.

53. Bonati U, Holiga S, Hellbach N, et al. Longitudinal characterization of biomarkers for spinal muscular atrophy. Ann Clin Transl Neurol 2017;4:292–304.

54. Chabanon A, Seferian AM, Daron A, et al. Prospective and longitudinal natural history study of patients with Type 2...
and 3 spinal muscular atrophy: baseline data NatHis-SMA study. PLoS ONE 2018;13:e0201004.
55. Mutsaers CA, Lamont DJ, Hunter G, et al. Label-free proteomics identifies Calreticulin and GRP75/Mortalin as peripherally accessible protein biomarkers for spinal muscular atrophy. Genome Med 2013;5:95.
56. Finkel RS, Crawford TO, Swoboda KJ, et al. Candidate proteins, metabolites and transcripts in the Biomarkers for Spinal Muscular Atrophy (BforSMA) clinical study. PLoS ONE 2012;7:e35462.
57. Kobayashi DT, Shi J, Stephen L, et al. SMA-MAP: a plasma protein panel for spinal muscular atrophy. PLoS ONE 2013;8:e60113.
58. Arnold WD, Duque S, Iyer CC, et al. Normalization of patient-identified plasma biomarkers in SMNDelta7 mice following postnatal SMN restoration. PLoS ONE 2016;11:e0167077.
59. Aragon-Gawinska K, Seferian AM, Daron A, et al. Nusinersen in patients older than 7 months with spinal muscular atrophy type 1: a cohort study. Neurology 2018;91:e1312–e1318.
60. Montes J, McDermott MP, Mirek E, et al. Ambulatory function in spinal muscular atrophy: age-related patterns of progression. PLoS ONE 2018;13:e0199657.
61. Mailman MD, Heinz JW, Papp AC, et al. Molecular analysis of spinal muscular atrophy and modification of the phenotype by SMN2. Genet Med 2002;4:20–26.
62. Kesari A, Idris MM, Chandak GR, Mittal B. Genotype-phenotype correlation of SMN locus genes in spinal muscular atrophy patients from India. Exp Mol Med 2005;37:147–154.
63. Tiziano FD, Bertini E, Messina S, et al. The Hammersmith functional score correlates with the SMN2 copy number: a multicentric study. Neuromuscul Disord 2007;17:400–403.
64. Taylor JE, Thomas NH, Lewis CM, 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.
65. Wirth B, Brichta L, Schrank B, et al. Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number. Hum Genet 2006;119:422–428.
66. Watihayati MS, Fatemeh H, Marini M, et al. Combination of SMN2 copy number and NAIP deletion predicts disease severity in spinal muscular atrophy. Brain Dev 2009;31:42–45.
67. Feldkotter M, Schwarzer V, Wirth R, et al. 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.
68. Harada Y, Sutomo R, Sadewa AH, et al. Correlation between SMN2 copy number and clinical phenotype of spinal muscular atrophy: three SMN2 copies fail to rescue some patients from the disease severity. J Neurol 2002;249:1211–1219.
69. Medrano S, Monges S, Gravina LP, et al. Genotype-phenotype correlation of SMN locus genes in spinal muscular atrophy children from Argentina. Eur J Paediatr Neurol 2016;20:910–917.