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Combinatorial treatment for spinal muscular atrophy

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ABSTRACT

Spinal muscular atrophy (SMA) is a severe autosomal recessive motor neuron disease caused by loss of *SMN1*, which encodes a protein essential for motor neuron survival. SMA patients have one or more copies of an alternate *SMN* gene, *SMN2*, which is nearly identical to *SMN1*. *SMN2* differs at a single nucleotide from *SMN1* which results in the skipping of exon 7 in the mRNA and produces an unstable protein (SMNΔ7). Therapeutic approaches that have been undertaken include i) replacement of *SMN1* by gene delivery mediated by adeno-associated virus serotype 9 (AAV9) (Zolgensma), ii) correction of the aberrant *SMN2* splicing using an antisense oligonucleotide (ASO) or small molecule (nusinersin, risdiplam), and iii) increased expression of *SMN2* mediated by histone deacetylase (HDAC) inhibitors. Two of these three approaches have given rise to successful treatments for SMA, but they are very expensive, and their long-term safety is not well known. In addition, the ability of ASOs and viral vectors to reach their targets in the CNS with peripheral administration is limited. Small molecules may cross the brain blood barrier when orally delivered and can be discontinued if needed to mitigate adverse effects. This Editorial highlights the current

study by Pagliarni et al. in which they used combined treatment of cell models of SMA with an ASO and an orally delivered HDAC inhibitor (panobinostat) to overcome the limitations of a single therapeutic approach. Panobinostat enhanced the expression of *SMN2*, increasing the amount of *SMN2* mRNA available for splicing correction mediated by the ASO. In addition, panobinostat increased exon 7 retention in the *SMN2* mRNA. This combinatorial treatment might allow lower or less frequent ASO doses, reducing the need for repeated intrathecal administration. The combined effects of panobinostat and nusinersen can now be tested in SMA animal models to determine whether this approach will be translatable to patients.

ABBREVIATIONS: AAV9= adeno-associated virus serotype 9; ASO = antisense oligonucleotide; EMA = European Medicine Agency; FDA = Food and Drug Administration; HDAC = histone deacetylase; SMA = Spinal muscular atrophy; *SMN1*= survival motor neuron 1; *SMN2*= survival motor neuron 2; SMNΔ7 = survival motor neuron 2 *lacking* exon 7

EDITORIAL

Spinal muscular atrophy (SMA) is a severe autosomal recessive motor neuron disease characterized by muscle weakness and atrophy, with a variable loss of motor function. The deficit is a direct consequence of the degeneration of motor neurons in the anterior horns of the spinal cord. These motor neurons directly connect the nervous system with skeletal muscle fibers to control voluntary movements. Different SMA forms have been described (types I, II and III), which are clinically characterized by age of onset and severity. Most SMA cases appear as the severe form in the first few months of age, while in other cases the disease shows later onset of symptoms, after the first year of age. The third form of SMA occurs in children and adolescents and is characterized by a less severe deficit with a slow rate of progression. In addition to these, other SMA forms are embryonically lethal (type 0) or occur with a mild phenotype in early adulthood (type IV).

Despite the differing manifestations, all SMA forms are due to homozygous deletions and other mutations in the survival motor neuron 1 (*SMN1*) gene, which encodes a protein essential to maintain motor neuron viable, thus preventing their death (Lefebvre *et al.* 1995). The differences in SMA phenotype from type 0 to type IV are due to variable copies of a second SMN gene (*SMN2*), which is highly homologous to *SMN1* (figure 1). *SMN2* has five nucleotide differences compared to *SMN1*, one of which, a substitution of a cytidine with a thymine (C → T) in exon 7 (Monani *et al.* 1999; Lorson *et al.* 1999) causes the *SMN2* primary transcript to undergo alternative splicing which causes the loss of exon 7 in the mature mRNA. The encoded protein (SMNΔ7) is shorter and unstable, but small amounts of normally spiced mRNA, and consequently full-length active protein (FL-SMN), are also produced from the *SMN2* gene. The severity of SMA is thus due to the combination of the *SMN2* copy number and its ability to produce an active SMN protein, which partially rescues the defect associated with loss of *SMN1* in patients.

Therapeutic approaches for SMA can thus be based on a) restoration of normal *SMN1*, b) induction of a proper *SMN2* splicing to allow exon 7 retention in mature mRNA, or c) transcriptional or translational stimulation of the *SMN2* gene product (figure 1). Over the past 20 years, these experimental and clinical approaches have resulted in precise therapeutic strategies that are now approved by regulatory agencies worldwide.

Restoration of normal SMN1 levels in affected cells has been achieved using adeno-associated virus serotype 9 (AAV9) vector to deliver the cDNA encoding SMN1 (onasemnogene abeparvovec-xioi, Zolgensma®) (Mendell *et al.* 2017). The AAV9 vector is able to cross the brain-blood barrier, and this strategy is based on a single dose, administered intravenously, of the viral vector expressing SMN1. Clinical trials have shown a marked improvement of the clinical signs in the SMA-affected infants, as measured with the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale of motor function. The motor functions were significantly better than expected on the basis of the natural history of children affected by the same type of SMA, and were maintained and improved during the follow-up period.

Zolgensma is now approved by the Food and Drug Administration (FDA), European Medicine Agency (EMA), and other regulatory agencies for intravenous administration in early onset SMA. In addition, intrathecal administration in older patients has been under investigation in a clinical trial (STRONG, NCT03381729).

Correction of SMN2 splicing is now possible with an antisense oligonucleotide (ASO) designed to block the alternative splicing that deletes exon 7 from the transcript. The ASO blocks the intronic splicing silencer element located in intron 7, and directs the splicing to exon 7 instead of exon 8 (Hua *et al.* 2010). The ASO binds specifically to this site, sterically inhibiting the binding of the splicing factor and thus repairing the mRNA of SMN2 and allowing the synthesis of a fully functional SMN2 protein.

Phase II and III clinical trials performed in SMA-type I, II and III-affected showed impressive efficacy (Finkel *et al.* 2017). Patients who were administered the ASO had improvement in clinical signs, with increased time to death or need for permanent ventilatory support when compared to sham-control group. These results were confirmed in other trials, which were terminated early to allow treatment for all study participants. The ASO, developed by Ionis and known as nusinersen, was further developed by Biogen with the commercial name of Spinraza®, and readily approved by the FDA, EMA, and other regulatory agencies. A disadvantage of nusinersen compared to Zolgensma, is that the ASO must be administered intrathecally repeatedly, three times per year after the first year of treatment. A comparison between the viral mediated delivery of *SMN1* and the spicing correction approach mediated by ASO is described in recent commentaries (Ridler 2018; Whittaker & Michell-Robinson 2018).

A similar approach based on the restoration of a proper splicing of the *SMN2* transcript has been pursued with the small molecules risdiplam (from Roche, currently under review for approval by the FDA and EMA) and branaplam (from Novartis), which have the advantage of being orally bioavailable and brain-penetrant. Both compounds ameliorate motor function in mouse studies, and in clinical trials risdiplam may be as effective as intrathecal nusinersen.

Other attempts to enhance and restore the expression of a functional SMN protein have been based on the use of histone deacetylase (HDAC) inhibitors, including sodium butyrate, valproic acid, trichostatin, and panobinostat (Chang *et al.* 2001; Sumner *et al.* 2003; Avila *et al.* 2007; Garbes *et al.* 2009). Of these drugs, only valproic acid has been tested in SMA patients, and without positive results (Swoboda *et al.* 2009).

The new therapeutic approaches often incur a high cost that may limit access for many patients worldwide. In addition, the long-term tolerability and safety are not yet fully known. Moreover, it is unknown at present how many motor neurons are targeted by an ASO or a viral vector, and how

long these agents will exert their action in each cell. Neither approach can yet be considered a complete cure for SMA (Finkel *et al.* 2017; Mercuri *et al.* 2018).

A small molecule that can be delivered to all target cells may help to sustain or potentiate the action of a genetic intervention for SMA. Small molecules may be developed to cross the brain blood barrier and to be administered orally to maintain therapeutic levels and also readily discontinued in case of adverse effects. Small molecule pharmacokinetics can be optimized for penetration into skeletal muscle, which is may be affected by SMN deficiency in SMA, reaching each muscle fiber, which is difficult go attain with viral vectors and ASOs.

To circumvent potential limitations of the therapeutic approaches now in clinical use, in Pagliarini *et al.* (Pagliarini *et al.* 2019), in a recent study reported in The Journal of Neurochemistry, have assessed combinatorial treatment for SMA, using an ASO (ASO_ISSN1, to mimic the effects of nusinersen) and panobinostat, a small molecule that can be orally administered, crosses the blood-brain barrier, and is active in the nanomolar range to increase the SMN protein levels in SMA models. Of note, panobinostat has FDA approval for multiple myeloma and is generally well tolerated in patients.

Pagliarini *et al.* used fibroblasts from type I SMA patients and murine neural stem cells (NSCs) isolated from SMA mice and showed that panobinostat has selective effects on SMN2 exon 7 splicing. They found that panobinostat is more active than other known HDAC inhibitors in restoring the splicing of exon 7 of the SMN2 transcripts, acting at doses of 25 nM. These concentrations may be achievable in the brain, as demonstrated in a clinical trial for glioma, and this may be an advantage compared to other treatments (NCT00848523). It is possible that the dose needed for this effect may be lower than is used for treatment of multiple myeloma.

The authors also tested the potential additive effects of panobinostat and the ASO_ISSN1, using as readout the splicing of exon 7 of SMN2 transcript (Pagliarini *et al.* 2019). They demonstrated that

in the combinatorial treatment panobinostat increases the effects of the ASO on exon 7 retention in the *SMN2*. It is still unclear how this additive (or synergistic) effect is obtained in cells, but the authors found that panobinostat also enhances the expression of the *SMN2* gene by inducing histone acetylation and consequently chromatin relaxation at the *SMN2* locus. This increases the amount of the template *SMN2* transcript that is available for splicing repair by the ASO. One relevant aspect in the study, is that the ASO_ISSN1 was given at an otherwise subtherapeutic dose (20 nM), suggesting that the combinatorial treatment might allow patients to be treated with a splice-switching ASO at a lower dose or the same dose with a longer duration of effect, thus reducing the need for repeated intrathecal administration (Pagliarini *et al.* 2019). It is now necessary to test the combinatorial effects of panobinostat and nusinersen in preclinical animal models of SMA, before drawing conclusions about whether this approach will be translatable to patients.

Since nusinersen does not cross the brain blood barrier, at least three intrathecal injections per year are required to obtain the full pharmacological effects in patients, and this may need to be maintained for the entire life of the patient. If the combinatorial treatment is proven to be effective in preclinical and clinical studies, then this may allow less frequent invasive therapeutic intervention in SMA patients. Combinatorial treatment may also help to reduce the high cost of the treatment and perhaps avoid side effects of long term ASO treatment.

The same strategy of combinatorial treatment could also be evaluated in SMA patients treated with Zolgensma. Adjunctive HDAC treatment might help to sustain the efficacy of gene replacement or extend the benefit to motor neurons that have not been transfected with the viral vector. This combinatorial approach could also be assessed for small molecules that restore *SMN2* exon 7 splicing, such as risdiplam, where clinical trials are showing benefit in SMA patients.

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This manuscript is dedicated to the memory of our friend and colleague Giorgio Battaglia, who spent his scientific career in studying Spinal muscular atrophy.

Figure legend:

Figure. Targets for spinal muscular atrophy (SMA) treatment. SMA is caused by loss of functional *SMN1*. SMA patients have one or more copies of *SMN2*, which is nearly identical to *SMN1*, but has a single nucleotide difference that causes skipping of exon 7 in the mRNA. The mRNA lacking exon 7 (SMNΔ7) is unstable and relatively non-functional. Therapeutic approaches include replacing *SMN1* by gene delivery (Zolgensma), correcting the splicing of the *SMN2* mRNA (nusinersin, risdiplam), and increasing expression of *SMN2* (histone deacetylase inhibitors). Combinatorial treatments based on enhanced *SMN2* expression and splicing correction have been found promising in cell models of SMA.