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Spotlight

Augmenting the SMN Protein to Treat Infantile Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a common and oft-fatal pediatric neuromuscular disorder caused by insufficient SMN protein. Now, two clinical trials (Mendell et al., 2017; Finkel et al., 2017) demonstrate that restoring the protein is therapeutic, offering new treatment options and renewed hope to SMA patients.

To those of us who have witnessed the spinal muscular atrophy (SMA) story unfold over the last quarter century, the outcome of two recent clinical trials (Mendell et al., 2017; Finkel et al., 2017) is especially heartening. No longer does a diagnosis of severe SMA have to be inextricably linked to the inexorable deterioration of voluntary movement and loss of life during infancy. It is now shown in humans that, through timely repletion, the protein deficient in SMA can stall—and might permanently arrest—the onset of disease. This is undeniably a development to cheer. Yet there are caveats associated with the treatments in the reports, some noted by the authors and others that are not so apparent but are articulated here. A consideration of these will be important as we attempt to build on the current developments and optimize treatments for what remains the most common inherited cause of infant mortality.

SMA is a common (1 in 10,000 newborns), frequently fatal neurodegenerative disease triggered by homozygous loss of the Survival Motor Neuron1 (*SMN1*) gene and thus paucity of its namesake SMN protein (Lefebvre et al., 1995). A paralog, *SMN2*, is invariably present in SMA patients but aberrantly spliced, producing a mostly truncated and unstable protein that renders the gene unable to adequately compensate for mutations in *SMN1* (Monani and De Vivo, 2014). Fewer *SMN2* copies are generally associated with the most common (~60%) and severe (type I) form of the disease. Incremental increases in *SMN2* copy number result in intermediate (type II) and mild (type III) SMA. The SMN protein is best known as a master regulator in the assembly of ribonucleoprotein (RNP) particles and specifically for its housekeeping role in orchestrating the splicing cascade (Eggert et al., 2006). This aspect of SMN biology has been recognized for almost two decades yet has failed to yield a unifying explanation for the especially detrimental effect of low protein on spinal motor neurons. Still, the inability to find a mechanistic link between SMN and SMA has not hampered the development of at least one therapeutic strategy—restoring protein—for the disease. In part, this is owed to the monogenic and recessive nature of SMA, the invariable presence of *SMN2* gene in patients, the delineation of the mechanism underlying aberrant *SMN2* splicing, and the early recognition that there is a general inverse relationship between SMN protein from *SMN2* and disease severity. "Humanized" SMA model mice also facilitated the pre-clinical development of the SMN repletion strategies. The results in the rodents have now translated into clinically meaningful findings in human patients.

Although each of the recently developed treatments is based on raising SMN levels, the manner in which such repletion was effected differs (Figure 1). The treatment described by Finkel et al. (2017) relies on the presence of at least one copy of the *SMN2* gene in all patients and the identification, in 2006, of an intronic element, ISS-N1, immediately downstream of exon 7 of the two *SMN* genes. ISS-N1 collaborates with other *cis* and *trans* elements to induce exon 7 skipping (Singh et al., 2006). Such exon skipping especially manifests in *SMN2* owing to a c.840C→T transition in the exon that creates a binding site for the splicing repressor hnRNP A1 (Kashima and Manley, 2003). Singh et al. (2006) showed that the skipping can be overcome and the full-length (FL) transcript from *SMN2* restored by sterically blocking ISS-N1. The antisense moiety that was used to rectify the aberrant splicing of the *SMN2* gene was eventually developed into Nusinersen, the biologic employed in the study by Finkel et al. (2017).

More than 100 patients divided in a 2:1 ratio between those receiving active drug and placebo were eventually enrolled in the randomized, double-blind, sham-controlled Nusinersen trial. Eligible participants harboring two copies of the *SMN2* gene and exhibiting the most severe (type 1) form of SMA were intrathecally administered drug or subject to a sham procedure. A regimen of four injections in the first 2 months of treatment was followed by maintenance doses at the 6 and 10 month time points. Efficacy of treatment was determined by motor milestones achieved and event-free survival, the latter defined as time to death or the use of permanent assisted ventilation. Considering the severity of disease in the patients, the outcome of the trial is both significant and encouraging. In an interim analysis, infants receiving Nusinersen were twice as likely as the

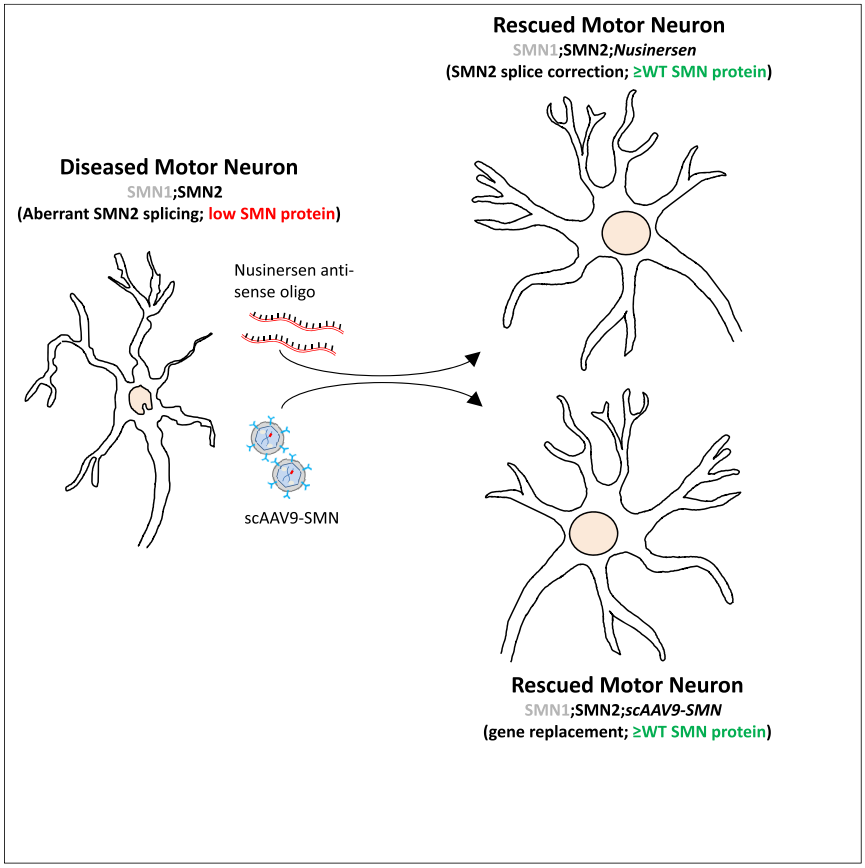

Figure 1. Strategies to Restore SMN as a Means to Treat SMA
SMN repletion is effected either by using an antisense moiety such as Nusinersen or by transducing cells with an AAV9 vector harboring a copy of the *SMN1* gene. Each approach raises SMN in the cell and rescues/prevents the SMA phenotype. Note: text in gray denotes homozygous mutations in *SMN1*.

placebo-administered controls to be alive and free of ventilatory support; severely affected SMA patients rarely live beyond the age of two, frequently succumbing to respiratory distress. Drug treatment also resulted in ~41% of the infants meeting a pre-defined motor milestone. In contrast, motor performance did not improve in any of the placebo-treated controls. The differences in outcome between the groups are especially notable given the somewhat earlier onset of symptoms and thus greater burden of disease in patients in the Nusinersen group. Indeed, such was the extent of the benefit that the trial was concluded early and placebo-treated patients offered the active drug.

The approach adopted by Mendell et al. (2017) to restore SMN and treat patients is, in some respects, more straightforward. The strategy—gene replacement—employed a self-complementary adeno-associated vector (scAAV9) to deliver an SMN-expressing construct to severely affected SMA patients. However, in comparison to the Nusinersen study, the gene replacement trial enrolled relatively few (15) patients, all of whom received a single, intravenous dose of the therapeutic vector. Three were administered a low (6.7×10^{13} vg/kg) dose of scAAV9-SMN and the remainder a relatively high (2×10^{14} vg/kg) dose of the vector. Efficacy of treatment was assessed by comparing the health of the patients to those of similarly affected individuals in a natural history study. In some respects, the patients in this study responded even better to SMN repletion than those administered Nusinersen, although the most parsimonious explanation for the difference could be the somewhat younger age (3.4 months versus 5.3 months) of the patients treated with scAAV9-SMN. All 15 patients survived to 20 months of age without the need for permanent assisted ventilation; survival in an age-matched historical cohort was a mere 8%. Patients in the high-dose cohort benefited to a greater extent than those in the low-dose group, and a majority receiving the more concentrated amount achieved the ability to sit unassisted for ≥30 s. Two of the patients even gained the ability to walk independently. These milestones were never documented in the historical cohort. Akin to the Nusinersen study, patients undergoing gene replacement relatively early during the course of the disease derived maximum benefit. This may have contributed to the better outcome of patients in the high- versus low-dose group; infants in the former cohort were, on average, 3 months younger than those in the latter when treatment was commenced.

Notwithstanding their differences, the overall outcome of the two trials is clearly one to celebrate. Indeed, Nusinersen, sold under the brand name Spinraza, is now approved in the US, as well as Europe, for the treatment of SMA. It is expected that gene replacement will also receive FDA approval soon. Still, neither the scAAV9 treatment nor Nusinersen constitutes an outright cure for SMA. There are plenty of caveats associated with each. There are also key lessons to be learned and important conclusions to be drawn from the two trials. Of perhaps greatest import is the timing of a treatment. Early SMN repletion clearly has the most favorable outcome. The youngest patients who had lived with the disease for the shortest periods of time benefited most. This was predicted from mouse studies (Lutz et al., 2011) and likely stems from a limited window of opportunity within which a full complement of motor neurons that are rapidly turning dysfunctional can nevertheless be resuscitated. Death of the cells marks a point of no return. Accordingly, instituting routine newborn screening for SMA as a means of quickly diagnosing the disease and promptly initiating treatments is not only prudent but will also likely be paramount to the success of the repletion therapies. While universal implementation of such a strategy will undoubtedly require time, local efforts to treat patients as early as possible, even pre-symptomatically are

already underway. One notable example is the Nurture trial in which 25 patients, identified through newborn screens, are being treated with Nusinersen before symptom onset (Chiriboga, 2017). Initial results from the trial are even more promising than those reported by Finkel et al. (2017). The final outcome of the Nurture trial is eagerly anticipated.

Equally important to the success of SMN repletion as a treatment strategy is to ensure that protein is restored to all tissues where it is required to prevent disease. Intrathecal delivery of SMN-restoring agents is clearly beneficial. However, if many of the documented adverse effects of low SMN in the periphery are cell autonomous, it is possible that defects outside the CNS will eventually be unmasked in patients administered drug intrathecally. In this regard, the gene replacement protocol, which involves systemic delivery of scAAV9-SMN, may be superior to intrathecal Nusinersen administration. Still, the gene therapy is not without its own caveats. Chief among these is the notion of neutralizing antibodies to the vector. This will almost certainly limit gene replacement to a one-time treatment and, as reported by Mendell et al. (2017), could preclude certain patients from being treated altogether. A third concern has to do with how SMA patients will respond to the SMN repletion therapies in the long term. Questions associated with turnover of virally transduced cells or toxicity that accrues following repeated doses of biologics such as Nusinersen will invariably have to be addressed. Indeed, if studies in model mice are any indication, early acute treatments to restore SMN will be insufficient to ensure the long-term health of the individual (Kariya et al., 2014). Rather, a strategy to maintain a certain minimum (~50% wild-type levels) amount of protein, perhaps involving chronic treatment, will be required to keep the ill effects of low SMN at bay. Considering the relatively long lifespan of humans versus rodents wherein the temporal requirements for the SMN protein were assessed, questions of how best to maintain a disease-free state are especially relevant. A fourth consideration—stemming from the optimal timing of an SMN repletion therapy—involves older, symptomatic patients. If, as suggested by experiments in rodents, there is truly a point of no return for the diseased motor unit, SMN repletion may be futile for older patients that have lived with SMA for protracted periods of time. Such a prospect, particularly in the context of what appears to be the first truly promising treatments for SMA, is upsetting even to contemplate and underscores the continued need for basic research that might spawn alternative treatments and thus offer succor to the fully symptomatic SMA patient. Finally, there is the question of cost. A year's worth of Nusinersen treatment costs $750,000. Gene replacement may be even dearer in the first instance. Such costs limit affordability to bespoke treatments of the sort discussed here and may eventually warrant reasonable price controls if they are to be made widely available. Nevertheless, despite the numerous issues raised here, the progress marked by the two studies we highlight in this article is to be commended. Severe SMA need no longer be an early death sentence. A newly diagnosed infant with the disease can expect at least some recourse from the SMN repletion treatments.

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