FDA Approval of Nusinersen for Spinal Muscular Atrophy Makes 2016 the Year of Splice Modulating Oligonucleotides

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With the Food and Drug Administration (FDA) approval of nusinersen (tradename Spinraza) for treatment of spinal muscular atrophy patients on December 23 [1], 2016 brought us not one, but two approved splice modulating oligonucleotides to treat neuromuscular disorders. Eteplirsen, an oligonucleotide to treat a subset of Duchenne muscular dystrophy (DMD) patients, was approved earlier this year. However, where the approval of eteplirsen was highly controversial [2], this one seemed to be crystal clear: the drug was approved only 91 days after BioGen filed the new drug application (NDA) with FDA, and the application was not referred to an FDA advisory committee because "outside expertise was not necessary and there were no controversial issues that would have benefited from advisory committee discussion" [3].

Spinal muscular atrophy is an autosomal recessively inherited disease, that is characterized by progressive loss of motorneurons, leading to muscle atrophy and paralysation. Most patients (60%) suffer from the most severe form of the disease, type I SMA. Symptoms start in infants before 6 months of age, patients never achieve independent sitting, need intensive supportive care, including ventilation, and seldom live beyond two years of age. Type II and type III SMA are milder forms of the disease. For type II onset is after 6-18 months, patients are able to sit independently but never able to walk and can live 20-40 years with good supportive care. Type III SMA patients are able to walk, but generally loose this ability when disease progresses. Survival is normal. It should be noted that all types of SMA are severe and debilitating and that the term "milder" is only relative to the very severe type I SMA. There is a type IV SMA, with onset in adulthood (after 30 years of age) and a slowly progressive course, but this is a very rare form [4].

Type I-IV SMA are all caused by loss of function mutations involving the *SMN1* gene, precluding the production of the protein survival of motor neurons (SMN). As the name suggests, this protein is crucial for the survival of motorneurons. SMN is ubiquitously expressed and is a component of the SMN complex, which has a role in assembling the small nuclear RNA proteins (snRNPs) involved in the splicing process [5]. It is not yet fully elucidated why lack of SMN protein primarily appears to have an impact on motorneurons.

Complete lack of SMN protein is embryonic lethal. However, humans have an almost identical copy of the *SMN1* gene, *SMN2*. Transcripts of this gene are unfortunately mostly improperly spliced because exon 7 of SMN2 is poorly recognized by the spliceosome due to single nucleotide changes. As such exon 7 is spliced out in 90% of SMN2 transcripts, leading to only ~10% of full-length SMN2 transcripts and low levels of SMN protein, which are sufficient to prevent lethality before birth, but not enough to prevent the loss of motorneurons in infancy (type I) or childhood (type II and III) [4,5]. The reason that some patients suffer from a more severe disease than others, is that there is copy number variation of the *SMN2* gene. Type I patients generally have 2 *SMN2* copies, while type II and type III patients generally have 3 and 3-4 copies, respectively [4]. While for each SMN2 transcript only 10% contains exon 7, the absolute amount of full-length transcripts – and thus SMN protein - of course increases when patients have more gene copies, leading to a less severe disease.

Since all SMA patients have at least two copies of the *SMN2* gene, and since more SMN protein is associated with a less severe disease, it stands to reason that increasing the amount of full-length SMN2 transcripts would be therapeutic. This is where nusinersen comes into play. The 2'-*O*-methoxyethyl phosphorothioate antisense oligonucleotide targets a region in intron 7 that harbours an intronic splicing inhibitor, which is one of the main reasons why exon 7 is skipped in SMN2 transcripts [6]. Blocking this site prevents splicing inhibitory factors from binding and leads to better recognition of exon 7 by the splicing machinery and therefore more inclusion into SMN2 mRNA, allowing increased translation of SMN protein. This approach has been proposed first by Adrian Krainer's group, who have performed preclinical tests in cell and animal models in collaboration with Ionis Pharmaceutics. In severe animal models nusinersen treatment resulted in increased levels of SMN protein on a molecular level, and increased survival and improved function [7].

The clinical development was coordinated by Ionis Pharmaceuticals, with support from Biogen. Since antisense oligonucleotides do not cross the blood brain barrier, intrathecal delivery was required to target the motorneurons. The feasibility of this approach was tested in a Phase I trial in type II and type III SMA patients (2-14 years old) [8]. This revealed that the procedure was well tolerated. Furthermore, an increase in function as measured by the Hammersmith Motor Function Scale Expanded was observed for patients treated with the highest dose (9 mg), leading to cautious optimism. Several trials were then initiated for type I and type II/III SMA [9, 10, 11]. An open label study in type I SMA showed significant divergence from natural history for survival and age of permanent ventilation, and incremental achievements of motor milestones and increases in motor function as measured with the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) scale [9]. Interim analysis of a placebo-controlled trial for type I patients revealed a significant improvement in the achievement of motor milestones for nusinersen treated patients compared to sham-treated patients, thus meeting the pre-specified primary endpoint for interim analysis [10]. Furthermore, the interim analysis of a placebo-controlled trial in type II SMA patients revealed that nusinersen treated patients had a significantly higher increase in motor

function than placebo-treated patients, thus also meeting the pre-specified primary endpoint [11]. Both trials revealed a good safety profile and were stopped after interim analysis, enrolling all patients into open label trials pending regulatory approval, and an expanded open access programme was initiated for type I SMA [10,11].

The anecdotal evidence of the very good responders presented at scientific conferences and highlighted in social media has truly surprised everyone. This includes type I SMA patients learning to walk and ride bicycles at an age they would normally have died or be on permanent ventilation, and wheelchair-dependent type II and type III patients regaining the ability to walk. Biogen and Ionis filed their NDA on September 23 2016 with the FDA and shortly after with the European Medicines Agency (EMA) as well [12]. FDA approved the drug just before Christmas on December 23, while EMA evaluation is pending. Biogen will be in charge of commercializing nusinersen and conducting future trials.

With 2016 bringing us two approved splice modulating antisense oligonucleotides (nusinersen and eteplirsen, a phosphorodiamidate morpholino oligomer for DMD) as well as one that was deemed not ready for approval by FDA (drisapersen, a 2-*O*-methyl phosphorothioate oligonucleotide for DMD) [2], one cannot but look back and compare. The preclinical development of these three antisense oligonucleotides was done in parallel. It was long thought that developing an antisense therapy for SMA would be more challenging than DMD, because for DMD the target tissue (skeletal muscle) can be reached by systemic treatment, while for SMA it cannot, because antisense oligonucleotides do not cross the blood-brain-barrier. Thus, repeated intrathecal delivery is required, thought to be very invasive and challenging, especially in young and fragile infants.

It has become clear, however, that targeting the central nervous system is actually easier than targeting skeletal muscle. Upon intrathecal delivery, neurons take up antisense oligonucleotides efficiently and oligonucleotides with a phosphorothioate backbone distribute throughout the brain and the spinal cord, as revealed by studies in primates and posthumous analysis of nusinersen treated SMA patients who died [9,13]. While intrathecal delivery was at first thought to be very invasive, the trials show that this is well tolerated. An advantage of local delivery to the central nervous system is that it allows high, local dosing at the target tissue, with a low load for liver and kidney. By contrast, for DMD the target tissue makes up 30-40% of the human bodyweight, and high systemic doses are required to obtain sufficient levels of oligonucleotides in the muscle to achieve minimal levels of exon skipping and dystrophin restoration [2]. Furthermore, the central nervous system is a benign environment for antisense oligonucleotides, and the half-life is months rather than days-weeks for systemic organs [8, 9,13]. As such the dosing frequency can be much lower, with a maintenance dose required every 4 months for nusinersen compared to every week for eteplirsen [14, 15].

Another advantage of nusinersen over eteplirsen and drisapersen is that while eteplirsen/drisapersen apply to only a subset of DMD patients with a specific mutation (13-14% of all patients), nurisersen applies to all SMA patients, across the different types. As such nusinersen is anticipated to be the largest commercial success in the antisense field to date. Similarly to eteplirsen, costs are high,: an estimated $750,000 for the first year, followed by $375,000 annually for nusinersen, compared to $400,000-$500,000 annually per patient for eteplirsen. While for eteplirsen several health insurance companies have indicated not to reimburse the drug, or to only

reimburse for ambulant patients [2], no such statements have as yet been made for nusinersen. However, eteplirsen received accelerated approval, based on minor increases in dystrophin levels in a very small number of patients, with the FDA clearly stating that functional effects will need to be shown in additional, confirmatory trials [2]. Nusinersen, by contrast, received full approval based on clinical evaluation in 173 patients, with confirmed therapeutic effects [14]. Furthermore, eteplirsen aims to slow down disease progression in DMD patients, while nusinersen has been shown to improve motor function for type II/III SMA patients and to have a lifesaving effect in type I patients.

So far nusinersen is only approved in the USA. EMA evaluation is pending and expected to take place in 2017. It is as yet unknown whether the drug will be approved in Europe (though this seems likely) and if so, whether the EMA indication will be for all SMA types or only for type I SMA. There are more outstanding questions: it is not yet known what the long term effects of intrathecal antisense oligonucleotide treatment will be. Given the certainty of the severe, debilitating nature of SMA without treatment, this is an uncertainty that patients and parents find acceptable. However, post marketing studies will need to be done to investigate both the positive and potentially negative effects after years and decades of treatment. Another outstanding issue is the fact that SMN is expressed ubiquitously and that local treatment results in increased levels of SMN protein only in the neurons. In mouse models, full recovery or the life span was only observed when SMN protein was restored both in the central nervous system and systemically [16]. It is as yet unknown whether this finding translates also to humans, or whether it is an artifact of the model, which carries human *SMN2* genes in the absence of mouse *Smn* (mice only possess only one *Smn* gene). Again, time will tell whether upon restoration of the deficit in neurons, systemic symptoms will become more apparent later in life.

An ethical issue that parents and clinicians have to deal with now, is when to treat and when not to treat. For older type I SMA patients, who are already on permanent ventilation, treatment may prolong survival, but it is currently unknown how much improvement in muscle function will occur. This is a very difficult and emotional decision to make by those involved, but one that hopefully will not exist in the future if SMA patients are treated from an early age.

Finally, more drugs are in development for SMA, with the gene therapy approach from Avexis for type I SMA having received breakthrough designation by FDA. This approach involves an adeno-associated viral vector 9 containing the SMN1 cDNA (AVXS-101), and is being evaluated in an open label phase 1 trial in 15 type I SMA infants. Patients have received a single intravenous dose of AVXX-101, at a low (n=3) or high (n=12) dose. For the higher dose group, results are encouraging, with 8/12 patients achieving the ability to sit independently and 2/10 patients able to walk independently. The advantage of this approach is that in theory it requires a single, intravenous treatment. However, thus far the approach has only been tested in a very small group of patients and it is not yet known how long the beneficial effects will persist. It is also unclear whether the current trial results will be sufficient for drug approval. At the same time, obtaining more information in clinical trials will be challenging for Avexis, and also other companies working in the SMA field. Now that nusinersen is approved, it is unethical to perform placebo-controlled trials. Even doing head to head comparisons between nusinersen and investigational drugs is questionable, because it is highly unlikely that parents would willingly participate in a trial, and accept the possibility that their child will receive an investigational drug with unconfirmed therapeutic potency, rather than nusinersen. As such, an add-on approach would probably be most realistic, where both

arms receive nusinersen and one arm also receives the trial drug. This has of course practical implications if both drugs have the same mechanism of action. However, for a disease as fatal and debilitating as SMA, the fact that it is difficult to study new drugs because the current drug appears to be working well, is of course a good problem to have.

Disclosures:

AAR discloses being employed by LUMC which has patents on exon skipping technology. As co-inventor of some of these patents AAR is entitled to a share of royalties. AAR further discloses being ad hoc consultant for PTC Therapeutics, BioMarin Pharmaceuticals Inc., Global Guidepoint and GLG consultancy, Grunenthal and BioClinica, being a member of the Duchenne Network Steering Committee (BioMarin) and of the scientific advisory boards of ProQR and Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC. LUMC also received speaker honoraria from PTC Therapeutics and BioMarin Pharmaceuticals.

References

1. FDA press release on nusinersen approval http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm
2. Aartsma-Rus A, Krieg AM. FDA Approves Eteplirsen for Duchenne Muscular Dystrophy: The Next Chapter in the Eteplirsen Saga. Nucleic Acid Ther. 2016 Dec 8.
3. FDA letter on nusinersen approval to BioGen http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2016/209531Orig1s000ltr.pdf
4. Briefing document to an SMA Europe, European Medicine Agency and TREAT-NMD stakeholder workshop on SMA therapy development: http://www.treat-nmd.eu/downloads/file/meetings/2016/SMA_stakeholder_workshop_briefing_document_11.11.2016.pdf
5. Gubitz AK, Feng W, Dreyfuss G. The SMN complex. Exp Cell Res. 2004 May 15;296(1):51-6.
6. Singh NK, Singh NN, Androphy EJ, Singh RN. Splicing of a critical exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. Mol Cell Biol. 2006 Feb;26(4):1333-46.
7. Kole R, Krainer AR, Altman S. RNA therapeutics: beyond RNA interference and antisense oligonucleotides. Nat Rev Drug Discov. 2012 Jan 20;11(2):125-40.
8. Chiriboga CA, Swoboda KJ, Darras BT, Iannaccone ST, Montes J, De Vivo DC, Norris DA, Bennett CF, Bishop KM. Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. Neurology. 2016 Mar 8;86(10):890-7.
9. Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, Norris DA, Xia S, Bennett CF, Bishop KM. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet. 2017 Dec 17;388(10063):3017-3026
10. Press release type I SMA placebo-controlled trial: http://ir.ionispharma.com/phoenix.zhtml?c=222170&p=irol-newsArticle&ID=2191319
11. Press release type II/III SMA placebo-controlled trial: http://ir.ionispharma.com/phoenix.zhtml?c=222170&p=irol-newsArticle&ID=2220037

12. Press release FDA and EMA regulatory status of nusinersen http://ir.ionispharma.com/phoenix.zhtml?c=222170&p=irol-newsArticle&ID=2217083
13. Rigo F, Chun SJ, Norris DA, Hung G, Lee S, Matson J, Fey RA, Gaus H, Hua Y, Grundy JS, Krainer AR, Henry SP, Bennett CF. Pharmacology of a central nervous system delivered 2'-O-methoxyethyl-modified survival of motor neuron splicing oligonucleotide in mice and nonhuman primates. J Pharmacol Exp Ther. 2014 Jul;350(1):46-55
14. Label nusinersen: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/209531lbl.pdf
15. Label eteplirsen: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206488lbl.pdf
16. Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett CF, Krainer AR. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature. 2011 Oct 5;478(7367):123-6.