Title

Antisense oligonucleotides for neuromuscular disorders

Authors

Kevin Talbot[1,3] and Matthew JA Wood[2,3,4]

Affiliations

1. Nuffield Department of Clinical Neurosciences, University of Oxford, OX3 9DU, Oxford, UK
2. Department of Paediatrics, [3]MDUK Oxford Neuromuscular Centre, [4]Oxford Harrington Rare Disease Centre, University of Oxford, OX1 3QX, Oxford, UK

OVERLINE: 10TH ANNIVERSARY SERIES

Abstract

Effective treatment of spinal muscular atrophy with antisense oligonucleotide therapy opens the door to treating other neurologic disorders with this approach.

Neurological diseases pose unique problems for medical therapy. Neurons are post-mitotic, have limited capacity for regeneration, are vulnerable to age-related degeneration, and function as part of complex and precisely configured neuronal networks mostly laid down

during development. Moreover, the nervous system is essentially sequestered from the systemic circulation behind the blood-brain barrier. A major challenge in advancing promising pre-clinical treatments towards clinical translation is that the neurological pathways underpinning voluntary movement have intrinsic reserve capacity, so that considerable 'silent' and potentially irreversible damage is likely to have occurred in the pre-clinical phase before a patient presents to a physician with weakness. Another reason why therapeutic progress in neurological disorders has been slow compared to areas such as oncology, is that cellular pathophysiology is less well defined. However, we are now entering an era where the first steps are being taken in precision medicine for inherited neurological diseases, based on a detailed mechanistic understanding of the molecular basis of genetic mutations and how these can be manipulated.

Early successes in animal models of spinal muscular atrophy (SMA) short single-stranded DNA-like nucleic acid compounds known as antisense oligonucleotides (ASOs) (1), that target genes via specific Watson–Crick base pairing, have led to trials and regulatory approval. Altering the natural history of neurological disease using ASOs through modulating gene splicing or expression is now a clinical reality, with inherited neuromuscular diseases at the forefront of this new era of therapeutic intervention. In this instalment of the *Science Translational Medicine* anniversary Focus series, we discuss the hurdles that need to be overcome to expand ASO therapy for other diseases.

ASO therapy for SMA

SMA is due to loss of the *SMN1* gene, and reduction in the widely expressed survival motor neuron (SMN) protein, resulting in selective loss of spinal cord motor neurons. Its

commonest and most severe form (SMA type 1) results in lethal infantile paralysis, but all forms of SMA lead to severe disability. Because cells have an obligate requirement for SMN, a key factor in the essential cellular process of small nuclear riboprotein assembly, complete loss of *SMN1* is incompatible with cell viability but a copy gene, SMN2, which is differentially spliced, excluding exon 7 in the majority of transcripts, produces small amounts of full-length SMN protein, sufficient for normal function in most cells, but below the threshold required for spinal motor neurons. The number of *SMN2* copies varies between individuals and correlates with disease severity in SMA. Crucially, the absolute difference in SMN protein concentrations between patients with severe or mild disease is small, suggesting minor increases in SMN protein could have a profound clinical impact. While neuroscientists are still vigorously debating which of the several functions of SMN explain selective motor neuron vulnerability, an effective treatment for SMA has arrived.

A systematic analysis of regulatory sequences in *SMN2* that modulate exon 7 splicing using ASOs showed that blocking the intronic splicing silencer (ISS-N1) of the 5' splice site led to the greatest increase in exon 7 inclusion. Although the evolutionary *SMN1* duplication is not present in rodents, a series of mouse models (with human *SMN2* expressed on a null *Smn* background) replicated the genomic architecture of the human disease and produced robust animal models of SMA, which have provided the essential confirmation that intrathecal injection of the anti-ISS-N1 ASO resulted in restoration of neuromuscular function (1). Subsequent work in nonhuman primates to establish dose and safety led to the first open-label and subsequent sham-controlled clinical trials of nusinersin (Spinraza) in infants with SMA, with encouraging evidence of benefit (2).

Coupled with a newborn screening program enabling ASO treatment to be started soon after birth, SMA, a previously fatal disorder, has become treatable. The development

of Spinraza for SMA establishes the principle that modulating mRNA splicing can be effective therapeutically. In many ways, however, SMA is unique as a disease and translation of ASO therapy to other neurological disorders will require substantial refinements.

Challenges of ASO therapy for other neuromuscular disorders

In contrast to the rapid and impressive development of Spinraza for SMA, progress towards development of an effective ASO therapy for other neuromuscular disorders such as the fatal X-linked disease Duchenne muscular dystrophy (DMD) has been much slower. The *DMD* gene was cloned in the 1980s and its essential function encoding the structural protein dystrophin is well understood. A wide range of deletion/duplication and nonsense mutations cause DMD by disrupting the open reading frame resulting in the absence of full-length functional dystrophin protein. The first evidence that the effects of such mutations could be abrogated through use of splice site modifying ASOs to correct an aberrant reading frame emerged in the mid-1990s. In the early 2000s, in vivo efficacy of such ASOs was demonstrated in the dystrophic *mdx* mouse model of DMD (3) in which therapeutic benefit of a morpholino phosphorodiamidate ASO that rescued muscle dystrophin expression was shown. This ultimately led to the 2016 approval by the US Food and Drug Administration (FDA) of an ASO (eteplirsen) that resulted in skipping of exon 51 in the transcript encoding dystrophin and restoration of protein (4,5). However, the approval of eteplirsen was not without controversy. Although the safety data were supportive, the efficacy of the intravenously administered ASO was in question, especially given the very low amount of dystrophin protein generated (<1% of normal dystrophin protein abundance).

Key differences between ASO development for DMD and SMA must be considered. Given that numerous mutations or deletions in the gene encoding dystrophin lead to DMD,

multiple exon skipping ASOs targeting separate exons will be required in order to treat a majority of patients with DMD (e.g. only approximately 13% of patients would be candidates for eteplirsen treatment). Moreover, as already highlighted, although the fold difference in SMN protein concentrations between SMA patients with severe or mild disease is relatively small, this is not true in the case of DMD, where increasing absolute dystrophin protein to at least 10% of normal expression will be required for therapeutic efficacy. Further, although both diseases could be regarded as systemic in nature, the major cellular target in SMA is the spinal cord motor neuron, which can conveniently be targeted via the local intrathecal route. In the case of DMD, all skeletal muscle groups (especially those relevant to respiration) and cardiac muscle should be targeted given that cardiorespiratory failure is the primary cause of premature death in patients with DMD. Achieving this challenging goal requires substantially higher ASO drug doses administered through a systemic route. Notably, there was one exon 51 skipping ASO (drisapersen) that was rejected by the FDA, principally on the basis of safety concerns, before approval was finally granted to eteplirsen. Despite encouraging clinical trial data on related exon skipping ASOs (e.g. Sarepta Therapeutics' golodirsen and NS Pharma's viltolarsen, both targeting exon 53), most likely due to improved ASO length, target sequence and dose (over eteplirsen), accelerated approval for golodirsen was very recently declined by FDA, highlighting the continued challenges in the field.

Emerging antisense treatments for other neurological disorders

Most genetically determined neurodegenerative disorders are late-onset autosomal dominant conditions in which the gene mutation acts through altering the protein product in a way that leads to acquired toxicity. The task for therapy is therefore to antagonize the

aberrant gene product, at the RNA or protein level, without driving toxicity through loss of function of the normal protein.

Amyotrophic lateral sclerosis (ALS) is an aggressive neurodegenerative disease of motor neurons in which the average survival is 2-3 years from onset and for which significant disease-modifying treatments are currently lacking. Approximately 12-15% of ALS patients carry a disease-determining genetic mutation. There appear to be many biological triggers of ALS, given that mutations in more than 20 different genes can be involved. The *SOD1* and *C9orf72* genes, however, account for approximately 60-70% of ALS mutations, and therefore these two genes have become the focus for ASO therapy. Although highly expressed in the nervous system, rodent knockout experiments suggest that ablation of *SOD1* expression is tolerated. This has led to the development of an ASO targeting both mutant and normal *SOD1*, with the primary aim of reducing the accumulation of misfolded mutant SOD1 protein, which is required because of the impracticality of delivering bespoke ASO therapy for the more than 100 separate missense mutations described in ALS cases. Rodent ALS models treated by intrathecal administration of IONIS-SOD1Rx, a 2'MOE gapmer (which induces RNA degradation via the intracellular enzyme RNAse H), resulted in SOD1 protein, and extension of survival. This ASO is now in Phase 2 clinical trials in patients with ALS.

A hexanucleotide expansion mutation in the first intron of the gene *C9orf72* accounts for up to 10% of all ALS cases and also a substantial fraction of cases of the neurodegenerative disorder frontotemporal dementia. The mechanism of disease toxicity is still debated, but most evidence suggests that a rational therapeutic strategy is to block the production of the repeat RNA to mitigate direct RNA toxicity or the production of dipeptide repeat proteins, which arise when intronic repeat RNA is translated via a non-ATG

dependent mechanism. Because concern exists that haploinsufficiency may play a role in *C9orf72*-related neurodegeneration, ASOs have been designed to target the *C9orf72* pre-mRNA. These ASOs reduce the repeat-containing transcript without affecting total C9orf72 protein and have shown positive effects in reducing toxicity in cellular models, notably induced pluripotent stem cell motor neurons, which have been critical due to the lack of ideal rodent models for C9orf72 (6). A clinical trial to assess safety and toxicity of the ASO IONIS-C9Rx is now underway in patients with ALS who carry *C9orf72* mutations.

Similarly, IONIS-HTTRx, a 5-10-5 2'MOE gapmer targeting the *HTT* gene responsible for the neurodegenerative disorder Huntington's disease (HD), at a site distant from the CAG repeat mutation, has undergone initial clinical studies in patients with early manifest HD (7). The early signals from the trial, with reduced mutant huntingtin protein in the cerebrospinal fluid, are encouraging. Initial analysis has not yet demonstrated any difference in clinical outcome related to reduced huntingtin protein. A competing clinical approach in HD, pioneered by Wave Life Sciences, takes an allele-specific strategy, in which the stereochemistry of the oligonucleotides is controlled (see below) with the aim of improved efficiency in mitigatating toxicity and preserving function of the wild-type allele.

The Future

Evidence from SMA in particular, and emerging data from other neuromuscular disorders, indicate that we are entering a new age of precision genetic medicine for neurological disorders, led by a maturing ASO technology. For this approach to progress rapidly beyond a few of the most obvious neuromuscular disease targets will require the development of next-generation ASO technologies. At present, the intrathecal route remains the most practical as it allows local delivery to the CNS, limits the potential for systemic toxicity and

minimises production costs. In order to achieve disease modification of nervous system disease using a systemic route more practical for long term use, these next generation drugs will have to offer improved potency and safety using advanced delivery technologies driving ASO delivery across the blood brain barrier, thus circumventing the need for repeated intrathecal drug administration. A potentially interesting technological advance is the advent of stereopure ASO chemistry, advanced by Wave Life Sciences (8), permitting chirally controlled ASO synthesis (current ASOs are typically chiral mixtures), with improvements for both potency and safety, and offering the potential of allele-specific targeting. A plethora of delivery technologies are emerging for enhanced intracellular ASO delivery to overcome the very poor intracellular bioavailability of nucleic acid drugs, including protein/peptide-based (9) and exosome-based nanotechnologies (10). These, coupled with next generation ASO chemistries, are likely to herald an age of much wider application of ASO medicines. Ultimately, to transform the therapy of neuromuscular and neurodegenerative disorders will necessitate pre-symptomatic treatment (as is now beginning for SMA) requiring appropriate early screening programs and biomarkers to guide effective treatment intervention. While there are many technical challenges ahead, the first steps towards enabling the realization of disease-modifying therapies for currently untreatable neuromuscular and neurodegenerative diseases have been taken.

Figure Legend

Improving ASO therapy for neurological disorders

Future advances in antisense oligonucleotide (ASO) therapy for neuromuscular and neurodegenerative disorders will depend on both technical advances and improved screening and biomarker strategies for identifying patients during the earliest stages of

disease. Development of next generation ASO compounds will arise from advances in oligonucleotide chemistry, including stereoselective synthesis, coupled with technologies that solve the challenge of intracellular delivery and, for neurological disorders, which also address the challenge of ASO delivery across the blood brain barrier. This will unlock broader application of ASO therapies for a wider range of neurological diseases. Technical advances in ASO scale up and manufacturing are also important, particularly innovations and technologies that facilitate solution phase ASO synthesis, which will drive scale up capabilities at reduced cost. Finally, the ability to more rapidly evaluate ASO therapies in patients will be driven not only by pre-symptomatic genetic screening, but also by development of more robust clinical trial outcome measures, including development of digital technologies and devices that will have implications for reductions in clinical trial size, duration and ultimately cost.

Acknowledgements

KT has served on scientific advisory boards for Roche, Cytokinetics and Biogen in relation to SMA and ALS.

MW is co-founder and non-executive director of PepGen and Evox Therapeutics and currently collaborates with Wave Life Sciences. He is also a non-executive director of Oxford University Innovation, and is a named inventor on numerous patents related to oligonucleotides and nucleic acid drug delivery systems.

We are grateful to Tom Roberts for preparing the figure.

References and Notes

1. Passini MA, Bu J, Richards AM, Kinnecom C, Sardi SP, Stanek LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Bennett CF, Cheng SH. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. Sci Transl Med. 2011 Mar 2;3(72):72ra18.
2. Mercuri E, Darras BT, Chiriboga CA, Day JW, Campbell C, Connolly AM, Iannaccone ST, Kirschner J, Kuntz NL, Saito K, Shieh PB, Tulinius M, Mazzone ES, Montes J, Bishop KM, Yang Q, Foster R, Gheuens S, Bennett CF, Farwell W, Schneider E, De Vivo DC, Finkel RS; CHERISH Study Group. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. N Engl J Med. 2018 Feb 15;378(7):625-635.
3. Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, Partridge TA, Lu QL. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med. 2006 Feb;12(2):175-7.
4. Cirak S, Arechavala-Gomeza V, Guglieri M, Feng L, Torelli S, Anthony K, Abbs S, Garralda ME, Bourke J, Wells DJ, Dickson G, Wood MJ, Wilton SD, Straub V, Kole R, Shrewsbury SB, Sewry C, Morgan JE, Bushby K, Muntoni F. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. Lancet. 2011 Aug 13;378(9791):595-605.
5. Mendell JR, Rodino-Klapac LR, Sahenk Z, Roush K, Bird L, Lowes LP, Alfano L, Gomez AM, Lewis S, Kota J, Malik V, Shontz K, Walker CM, Flanigan KM, Corridore M, Kean JR, Allen HD, Shilling C, Melia KR, Sazani P, Saoud JB, Kaye EM; Eteplirsen Study Group. Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann Neurol. 2013 Nov;74(5):637-47. doi: 10.1002/ana.23982.

6. Jiang J, Zhu Q, Gendron TF, Saberi S, McAlonis-Downes M, Seelman A, Stauffer JE, Jafar-Nejad P, Drenner K, Schulte D, Chun S, Sun S, Ling SC, Myers B, Engelhardt J, Katz M, Baughn M, Platoshyn O, Marsala M, Watt A, Heyser CJ, Ard MC, De Muynck L, Daughrity LM, Swing DA, Tessarollo L, Jung CJ, Delpoux A, Utzschneider DT, Hedrick SM, de Jong PJ, Edbauer D, Van Damme P, Petrucelli L, Shaw CE, Bennett CF, Da Cruz S, Ravits J, Rigo F, Cleveland DW, Lagier-Tourenne C. Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron. 2016 May 4;90(3):535-50.
7. Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, Blair NF, Craufurd D, Priller J, Rickards H, Rosser A, Kordasiewicz HB, Czech C, Swayze EE, Norris DA, Baumann T, Gerlach I, Schobel SA, Paz E, Smith AV, Bennett CF, Lane RM. Targeting Huntingtin Expression in Patients with Huntington's Disease. N Engl J Med. 2019 Jun 13;380(24):2307-2316.
8. Iwamoto N, Butler DCD, Svrzikapa N, Mohapatra S, Zlatev I, Sah DWY, Meena, Standley SM, Lu G, Apponi LH, Frank-Kamenetsky M, Zhang JJ, Vargeese C, Verdine GL. Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. Nat Biotechnol. 2017 Sep;35(9):845-851.
9. Hammond SM, Hazell G, Shabanpoor F, Saleh AF, Bowerman M, Sleigh JN, Meijboom KE, Zhou H, Muntoni F, Talbot K, Gait MJ, Wood MJ. Systemic peptide-mediated oligonucleotide therapy improves long-term survival in spinal muscular atrophy. Proc Natl Acad Sci U S A. 2016 Sep 27;113(39):10962-7.
10. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011 Apr;29(4):341-5.