Development of tailored splice-switching oligonucleotides for progressive brain disorders in Europe: development, regulation, and implementation considerations

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ABSTRACT

Splice-modulating antisense oligonucleotides (ASOs) offer treatment options for rare neurological diseases, including those with very rare mutations, where patient-specific, individualized ASOs have to be developed. Inspired by the development of milasen, the 1 Mutation 1 Medicine (1M1M) and Dutch Center for RNA Therapeutics (DCRT) aim to develop patient-specific ASOs and treat eligible patients within Europe and the Netherlands, respectively. Treatment will be provided under a named patient setting. Our initiatives benefited from regulatory advice from the European Medicines Agency (EMA) with regard to preclinical proof-of-concept studies, safety studies, compounding and measuring benefit and safety in treated patients. We here outline the most important considerations from these interactions and how we implemented this advice into our plan to develop and treat eligible patients within Europe.

Keywords: Europe; N = 1; antisense oligonucleotides; regulators; treatment

OPPORTUNITIES TO TREAT INDIVIDUAL PATIENTS WITH ANTISENSE OLIGONUCLEOTIDES

With the advent of gene and genetic therapies, patients with rare genetic diseases with previously unmet medical needs are now faced with the prospect of potential treatment options that directly target the cause of their disease. Gene therapy examples include zolgensma for spinal muscular atrophy (SMA) and luxturna for inherited retinal dystrophy caused by *RPE65* mutations (Patel et al. 2016; Hoy 2019). Genetic therapies use antisense oligonucleotides (ASOs) or siRNAs to target gene transcripts, which can either restore production of a missing protein (e.g., nusinersen for SMA, Box 1) or decrease the production of a toxic protein (e.g., patisiran and inotersen for transthyretin-mediated amyloidosis) (Aartsma-Rus 2017; Gorevic et al. 2021).

Therapy development for rare diseases is challenging due to the small patient numbers and a scarcity or even absence of outcome measures to detect clinically meaningful therapeutic effects of an intervention in a reasonable time frame (e.g., <3 yr), given the small sample size. As such, it is

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Box 1: Introduction to nusinersen

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by the progressive loss of motor neurons, resulting in muscle atrophy and loss of muscle function (Mercuri et al. 2022). SMA is caused by mutations in the *SMN1* gene that abolish production of survival motor neuron (SMN) protein. Complete loss of SMN protein is embryonically lethal. However, humans also possess an *SMN2* gene that can produce low levels of functional SMN protein. There is copy number variation for *SMN2* and the number of *SMN2* copies correlates inversely with the severity of SMA. The reason *SMN2* produces only low amounts of SMN protein is that exon 7 is poorly recognized and not included in the spliced mRNA for the majority of SMN2 transcripts, due to a silent nucleotide substitution in exon 7 (Singh et al. 2017). As the variant in exon 7 is silent, SMN2 transcripts that do include exon 7 produce an SMN protein that is identical to the protein produced from *SMN1* genes.

Based on the finding that there is an inverse correlation between SMN2 copies and SMN protein levels and disease severity, increasing the amount of SMN protein should have therapeutic effects in SMA. Nusinersen is an antisense oligonucleotide (ASO), containing 18 2′-*O*-methoxy ethyl RNA nucleotides, with 5-methyl C nucleotides and a uniform phosphorothioate backbone (Bennett et al. 2019). Nusinersen targets a splicing silencer in intron 7, causing exon 7 to be better recognized by the splicing machinery and therefore resulting in increased levels of full-length SMN2 spliced transcripts and increased levels of functional SMN protein.

As ASOs do not cross the blood-brain barrier, nusinersen needs to be delivered intrathecally. It has been shown that this method allows successful ASO delivery throughout the central nervous system, including the spinal cord, brain, and motor neurons in animal models and SMA patients (Rigo et al. 2014; Finkel et al. 2016). Clinical trials in type 1 SMA patients showed increased survival for nusinersen-treated compared to sham-treated patients. Furthermore, nusinersen-treated infants gained motor skills never observed in the natural history of type 1 SMA patients (Finkel et al. 2017). Clinical trials in type 2 SMA patients showed an increase in motor function compared to a decrease or no change in sham-treated patients (Mercuri et al. 2018). Based on these results, nusinersen was approved by the Food and Drug Administration in 2016 and by the European Medicines Agency (EMA) in 2017 for the treatment of all types of SMA.

not surprising that most approved gene and genetic therapies target the more common rare diseases (RD) (Aartsma-Rus et al. 2021). Nevertheless, of the less common RD, certain diseases or mutation types uniquely qualify for gene or genetic therapies. The most notable example involves patients with progressive neurodegenerative disorders with cryptic splicing variants. For these RD patients, compartment-specific intrathecal injection with splice-modulating ASOs would restore the normal protein (Fig. 1). Although ASOs do not cross the blood-brain barrier, after intrathecal injection they are taken up efficiently by cells throughout the central nervous system (CNS) and the spinal cord (Rigo et al. 2014; Finkel et al. 2016). This allows treatment with low doses (e.g., 21–42 mg) to achieve high exposure in the CNS compartment with very limited body-wide exposure and much reduced risks for liver and kidney toxicity. Furthermore, after an initial loading regimen, maintenance dosing for intrathecal ASO treatment is only once every 3–4 mo. However, although these patients are well suited for ASO treatment, due to the rarity of the genetic variants, which can be (close to) unique, there is little commercial interest and no well-established drug development path to develop these individualized ASOs. For these patients, ASO development has to proceed quickly (ideally < 18 mo) due to the progressive nature of the disease. This is particularly important for severely debilitating and life-threatening diseases (SDLT) without alternative treatment options, which are the key focus of individualized ASO development.

A team at the Boston's Children Hospital, coordinated by Tim Yu, has shown it is possible to develop an ASO targeting a cryptic splicing variant for a patient with *CLN7* Batten disease within an academic setting (Kim et al. 2019). Treatment with this individualized ASO resulted in a reduction of frequency and duration of epileptic seizures in the patient. However, as anticipated given the advanced disease stage, it was unable to halt the underlying progression of the already accumulated neurodegeneration and the patient passed away in 2021. Nevertheless, this effort showed that it is possible to develop patient-specific ASOs and inspired academic groups globally to follow this example. Furthermore, it led to the establishment of the n-Lorem Foundation, which aims to produce ASO treatments for very rare diseases in the United States (https://www.nlorem.org) (Crooke 2022). Currently, the academic efforts are aligned under the umbrella of the N = 1 collaborative (N1C), which is a global nonprofit initiative to promote transparency and data-sharing and aims to facilitate N = 1 therapy development with high scientific rigor (https://www.n1collaborative.org/). It has become clear that in different regulatory jurisdictions, different regulations and opportunities exist. In this paper we highlight the European experience and route forward based on interactions of the European N = 1 network: 1 Mutation 1 Medicine (1M1M) (https://www.1mutation1medicine.eu) and the Dutch Center for RNA Therapeutics (DCRT) with the Scientific Advice Working Party (SAWP) and the Innovation Task Force (ITF) of the European Medicines Agency.

INDIVIDUALIZED ASO DEVELOPMENT IN EUROPE

Whereas in the United States, N = 1 treatment requires submitting an investigational new drug application (IND)

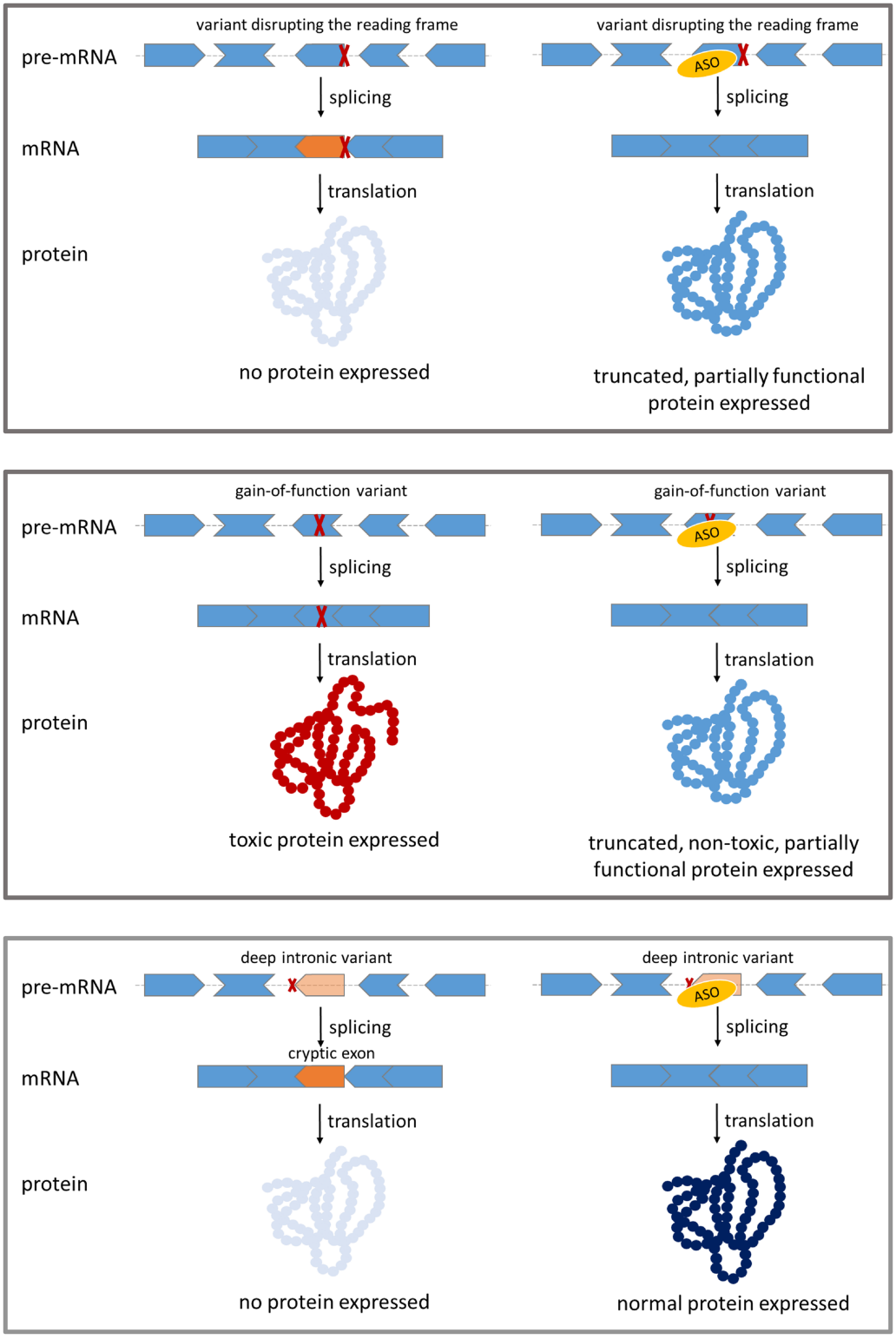

FIGURE 1. Approaches to tackle different genetic variants with splice-modulating ASOs. (*Top* panel) Nonsense variants and indels can lead to the generation of an early stop codon (disruption of open reading frame) within the transcript, and no functional protein being produced. ASO-mediated exon skipping can be used to restore the reading frame and results in the production of a truncated protein with (partial) function. (*Middle* panel) Toxic gain-of-function variants lead to the production of toxic proteins. ASOs can be used to skip exons to result in a shorter, non-toxic protein. (*Bottom* panel) Deep intronic variants can result in parts of the intron being included in the mRNA transcript, causing a shift in the reading frame and abolishing protein function. This intron integration can be prevented using ASOs leading to the canonical transcript being expressed and a restoration of the reading frame. (ASO) Antisense oligonucleotide.

to the Food and Drug Administration (FDA), in Europe individual patients can be treated in a *named patient* setting (Aartsma-Rus 2021), which allows provision of unlicensed medicinal products to single patients to "fulfill special needs" [article 5 (1) of (EC) 2001/83] (for more detailed description of the different regulatory frameworks, including the differentiation of compassionate use vs. hospital exemption vs. named patient use, see Synofzik et al. 2022). In the *named patient* setting a clinician can prescribe an experimental treatment for a patient, which can ethically be justified if no proven therapies for the patient's condition exist (cf. Declaration of Helsinki #37; see Synofzik et al. 2022). The treatment will remain experimental, without an intention to apply for marketing authorization. As such, no dossier has to be submitted to the competent authorities, reducing the administrative burden compared to the situation in the United States. However, the treating clinician bears full responsibility for the experimental treatment and this type of treatment involves a continuous dialogue with the patient and family about parameters to continue and to stop the treatment. As there is no direct control group, efficacy is mainly evaluated intraindividually, comparing a subject's individual pretreatment natural history baseline with the post-treatment disease course. Ideally, collected natural history includes outcome measures that the patient indicates are relevant to them leading up to the treatment to assess a change in trajectory (Aartsma-Rus 2021). Notably, the steps involved in initiating treatment in a named patient setting vary for different European countries.

While regulatory approval and interaction is not required for ASO treatment under a named patient setting, a general framework will greatly facilitate and align named patient uses across Europe. To pave the way, the DCRT and 1M1M initiated interactions with the regulators to ask for advice and input regarding patient-specific development of individualized splice-switching ASOs that use the same backbone and chemistry as nusinersen (see Box 1).

The DCRT received advice from the ITF, a multidisciplinary group organized by EMA, which serves as a discussion platform for early dialogue with applicants, in particular micro, small and medium-sized enterprises (SMEs), academics and researchers, to proactively identify scientific, legal, and regulatory issues of emerging therapies and technologies. The 1M1M network asked for scientific advice—a more formal process to provide nonbinding development advice that is available for both commercially and academically led drug development programs. Whereas normally a fee applies for the scientific advice process, for academically led initiatives, such as 1M1M, fees are waived for orphan-designated medicines or medicines targeting diseases where there is a clear need to stimulate drug development.

LESSONS FROM REGULATORY INTERACTIONS FOR INDIVIDUALIZED ASO DEVELOPMENT IN THE EUROPEAN UNION

Discussion topics included how to optimally develop ASOs preclinically, how to perform toxicity studies, how to compound the ASOs for clinical treatment, how to select eligible patients, and how to measure the treatment effect in an individualized treatment setting for neurological conditions requiring intrathecal delivery. The discussions and advice were given in the context of ASO development for individual patients with a nano-rare SDLT or mutations, that is, where no classical drug development program is possible and where time-urgency might partly justify nonclassical pragmatic approaches.

Pharmacodynamic proof-of-concept in cell models

Cryptic splicing variants cause part of an intron to be included in the mRNA of the affected transcript, or alternatively part of an exon to be identified as intronic and not included in the mRNA. In both cases this abolishes production of functional protein (Fig. 1). ASO development for cryptic splicing variants requires patient-derived cell models, since these cryptic exons are generally not present in transcripts from healthy individuals. A pragmatic approach is supported, using fibroblasts when they are informative, that is, express the target transcript and protein, and ideally downstream pathology is recapitulated (e.g., missing enzyme, metabolic changes, etc.). More laborious and time-consuming studies with patient-derived induced pluripotent stem cell (IPSC)–derived neuronal cells are only warranted when this is considered reasonable, for example, when the transcript is exclusively expressed in neurons or when pathology is only present in these cells. Initial studies are required to assess the optimal ASO, but when possible, additional studies to show protein restoration or reduction of cellular pathology have to be performed. It was acknowledged that this is not always feasible, for example, when no antibodies are available for protein analysis or when no assays are available to measure pathology or protein function. However, the regulators did stress that these assays are highly valuable for the proof-of-concept. We refer the reader to guidelines on preclinical exon skipping ASO development that were produced by the N = 1 collaborative (Aartsma-Rus et al. 2023).

The generation of animal models was not recommended, since it is not deemed feasible to develop an animal model for each individual patient. Well-designed in vitro assays, as described above, are considered adequate to provide proof-of-concept in a timely manner. Furthermore, it will take considerable time to generate new animal models, and any delay in ASO development

will risk irreversible loss of critical function in patients with SDLTs.

Dosing selection for initial treatment of the patient will be calculated based on the dose determined for nusinersen. However, when possible, a concentration series in patient-derived cells was recommended to assess whether the ASO was more or less potent than nusinersen with regard to having a protein restoring effect, to allow optimal dose escalation and prevent underdosing. Note, however, that optimal dosing also depends on the target cells; for example, higher doses are likely required for diseases that affect brain regions than motor neuron diseases.

Safety studies

The advice relating to safety studies was based on the premise that the nusinersen chemistry (2′-*O*-methoxy ethyl RNA, with 5-methyl C nucleotides and a full length phosphorothioate backbone) would be used. Two types of safety issues can be expected: hybridization-dependent (binding of the ASO to nontargeted transcripts) and chemistry-dependent.

For the hybridization-dependent off-target analysis, an in silico analysis in BLAST was deemed suitable to ensure the ASO is unique for the target transcript and to identify potential unintended targets with partial homology. For the latter, it is recommended to assess whether unintended splice modulation is induced when these transcripts are postnatally expressed in the tissues that accumulate most of the ASO after intrathecal delivery (long term for the brain and CNS, and transiently in liver and kidney). Notably, due to differences in transcriptomes of different species, ASO specificity tests can only be properly done in human cell cultures. As the ASOs target transcripts that are expressed under the regular gene promotor, exaggerated pharmacology is not a concern.

The chemistry-dependent toxicity can be sequence-dependent, where specific motifs can elicit an immune response or bind to receptors in an aptamer-like fashion, or sequence-independent due to general binding of proteins by the phosphorothioate backbone. It can be determined whether ASOs are immunogenic through assessing whether the ASO induces cytokine release in peripheral blood mononuclear cells. Performing such a study was highly recommended. Aptamer-like binding to receptors can be assessed in in vitro safety studies in human cells and possibly in in vivo safety studies in a single species (generally Sprague Dawley rats). From the publicly available data for nusinersen, it is known that this ASO chemistry has good tolerability with negligible sequence-independent toxicity.

When using the same chemistry, formulation, and route of administration as nusinersen, no in vivo safety pharmacology or toxicity studies were recommended for a named patient setting in a patient with a SDLT. However, when an ASO with a chemical modification that has not yet been approved for clinical use for intrathecal delivery, or a new formulation is intended to be used, more rigorous safety studies are warranted, including 3 mo studies including multiple intrathecal dosing in a single species (Sprague Dawley rats are most used for intrathecal delivery and accepted as a relevant species) in a GLP-compliant setting. Equivalent dosing is based on CSF volume, and 1 mg ASO in rat relates to a dose of 500 mg in human. The goal of the safety studies is to exclude harm, so rat studies using the equivalent dose of up to 10 times the intended human dose are recommended, while toxicity studies pushing the dose until toxicity is observed are not.

When developing ASOs for a neurogenerative SDLT, "time is neurons," and a balance has to be struck between the time it takes to rule out ASO toxicity and the risk of not treating. Currently, the experience with intrathecal treatment in humans with the fully modified 2′-*O*-methoxy ethyl phosphorothioate chemistry is limited to safety studies with nusinersen, Stoke's STK-001 for Dravet syndrome (Wengert et al. 2022), and four additional ASOs of the same chemistry within the N = 1 collaborative (T Yu, in prep.). They all had comparable safety and biodistribution profiles in Sprague Dawley rats after intrathecal dosing. While the regulators indicated that in vivo safety studies could be omitted in this particular setting, they stressed that this had to be scientifically justified. As yet the individuals involved in the DCRT and 1M1M intend to perform ad hoc safety studies in rats in GLP-like settings to rule out acute toxicity, as we feel the number of compounds is currently insufficient to conclude that ASOs of this chemistry generally are well tolerated. Our concern is primarily for acute toxicity, for example, it is possible that certain sequence motifs in the ASOs can bind to unintended targets in an aptamer-like fashion. After acute safety studies using up to 10 times the intended clinical dose, spontaneous animal behavior observations for acute adverse effects, provoked behavior (like a modified Irwin test), and histopathology of the target organs (brain, and due to leakage to the periphery, liver and kidney) will be studied. ASO levels in the target organs will be assessed as well to confirm the anticipated low exposure in liver and kidney (Monine et al. 2021), which is important for patient monitoring after clinical treatment, as was stressed by the regulators. Furthermore, the CSF ASO levels allow in vitro and in vivo modeling of pharmacokinetic and pharmacodynamic parameters of the ASO, that is, the likely concentrations in different CNS regions, as well as liver and kidney (Monine et al. 2021).

If performing in vivo acute safety studies to exclude harm, the advice from the regulators was to only test the lead candidate, to reduce the number of animals used. Additionally, ASOs tested in an in vivo setting should be systematically compared side-by-side in in vitro safety studies. If in vitro safety studies are predictive of in vivo safety, as has been shown for RNase H inducing ASOs (Hagedorn

et al. 2022), and if biodistribution levels are similar for larger sets of ASOs, eventually in vitro safety studies can replace the in vivo studies in light of the 3Rs (replacement, reduction, and refinement of animal studies). Studies in nonhuman primates are not required, as also indicated by the FDA guidance for individualized ASOs (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/ind-submissions-individualized-antisense-oligonucleotide-drug-products-severely-debilitating-or-life).

Whereas generally, safety studies are conducted in a GLP-compliant setting, it is understood that performing GLP-like studies allows a quicker performance of these studies, as waiting times at contract research organizations (CROs) able to perform GLP-compliant intrathecal injections in rats are close to 12 mo. A GLP-like setting offers more flexibility, thus reducing the time to clinical treatment of an SDLT patient with the individualized ASO. Notably, GLP-like studies are still conducted with scientific rigor, where high levels of control and regulation are in place and where results are witnessed and archived and can become part of a dossier should this be required, in cases where the ASO does not apply solely to an individual case, but unexpectedly applies to a larger group of individuals, and a marketing authorization application is considered necessary.

Compounding

Producing the ASO under GMP conditions will delay treatment of the SDLT patients significantly due to waiting times for GMP production. As such, it might be possible to use GMP-like conditions. However, applying quality criteria needs to be discussed with the responsible local authorities as there is no clear definition of what GMP-like means and requirements may vary in different EU countries.

It is clear that modifications are needed from the general GMP criteria, as within an N = 1 setting the scale at which ASOs are produced is much smaller, and under current criteria >95% of the produced ASO would be used for meeting the GMP quality assessments. However, as the ASOs are delivered intrathecally, several aspects will have to be confirmed, such as the sterility, purity (>99.8%), and identity of the ASO. When stored, stability data and shelf lifetime under specified conditions will have to be generated. Notably, due to the low doses used in an intrathecal setting, in theory a patient could be treated for 100 yr with a single 10 gram batch of ASOs. Discussions are needed on when to replace a batch (e.g., on the intervals and criteria of ASO stability testing).

Clinical treatment and monitoring safety and efficacy

Regulators stressed that individualized intrathecal ASO treatment under a named patient setting should only be considered when no approved treatment is available, it concerns a severely debilitating progressive disease, and restoration of the missing protein is reasonably likely to result in a clinical benefit, based on the understanding of the pathogenesis of the disease. Furthermore, it is important to have clear start and stop criteria in place before treatment commences and to only include patients who are eligible for ASO treatment and for whom it is anticipated that a treatment effect can be measured within a 24–48 mo time frame. Unless these criteria are met, it is ethically difficult to justify initiation of the treatment, especially in light of the burden of having a severely progressive, debilitating disease. The start and stop criteria have to be discussed with the patients and families, be revisited and, if needed, adjusted regularly.

For treatment, it is recommended to start with doses known to be safe for nusinersen and then to escalate, also using the CSF ASO levels as a pharmacokinetic marker to decide whether to increase the dosing further or not. The maintenance dose should be based on safety and tolerability. Regulators appreciate the unique challenges of treating patients within an N = 1 setting and acknowledge that "plan as you go" is the only way forward to make this work.

To evaluate safety in the patient after treatment, general safety monitoring using physical exams and CSF analysis should be performed. Based on the known side effect profile of nusinersen, one should be vigilant for development of hydrocephalus and arachnoiditis. In addition, safety monitoring may have to be tailored based on disease pathology, as this may put certain patients at risk for developing specific side effects.

To evaluate efficacy, patient relevant outcomes that can show efficacy (or lack thereof) in a reasonable time frame (ideally 24 mo with a maximum of 48 mo) must be selected. Monitoring of patients using these outcomes prior to treatment start (run-in baseline study) allows the evaluation of a change in progression trajectory before versus after treatment ("notch-in-slope concept," see Fig. 2). However, classic statistical analysis of such models often assumes an underlying linear disease course, which may not be the case for most individual patients. Rather, nonlinear disease changes are common, for example, due to development, functional and biological thresholds in the neurodegenerative disease, etc. Thus, for modeling such (treatment-induced) intraindividual disease changes, nonlinear models are required. Here the members of 1M1M propose that probabilistic Bayesian modeling of individual-subject disease trajectories—and simulated response thresholds to detect (treatment-induced) deviations thereof—might be helpful (for an example of such a Bayesian modeling, see Fouarge et al. 2021). If available, such an intraindividual comparison can be complemented by an interindividual comparison with a historical disease control group—ideally with the same mutation or mutation type—

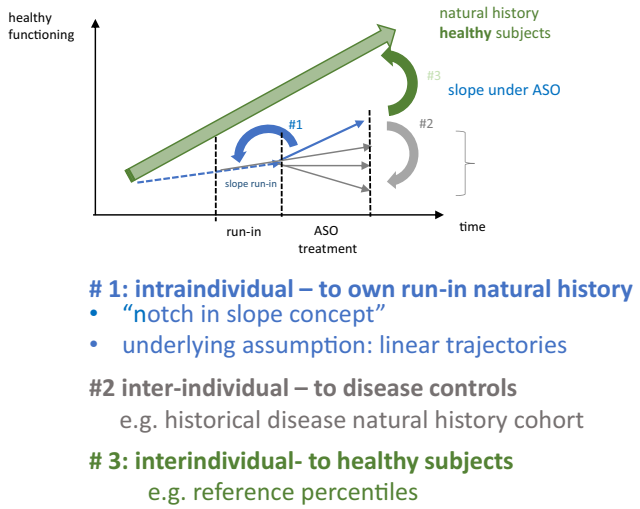

FIGURE 2. 1M1M proposed options for capturing change in an n-of-1 treatment setting. To allow detection of change, a subject's disease course under treatment can be compared: (#1) intraindividually to the slope of his/her own run-in natural history disease course prior to treatment (see scenario #1); (#2) interindividually to a cohort of (historical or concurrent) disease controls (see scenario #2); or (#3) interindividually to healthy control reference ranges, for example, reference percentiles (see scenario #3).

or relative to functional percentiles from healthy subjects (see Fig. 2). Note that for two individuals with the same disease at a different disease stage, it is possible that different outcome measures have to be selected, and what is perceived as benefit can vary. This is similar to the case of nusinersen where depending on the type of SMA, different outcome measures were used in clinical trials (see Box 1).

Measurable clinical outcomes show advantages over patient reported outcomes as they may be less affected by placebo effect and other biases, which might present a major confounder in outcome evaluation, particularly in settings of open label single-subject treatments. However, patient reported outcomes should be included in the wider set of "secondary" outcomes to provide additional evidence of a treatment effect, and in particular to corroborate patient meaningfulness of the treatment effect. Given the long latency until clinical outcomes show robust signals of efficacy, they should ideally be complemented by molecular pharmacodynamic outcomes, which might capture a response earlier. For example, detection of protein restoration in CSF or blood confirms that treatment is working at a molecular level for proteins that are excreted. Reduction of neurofilament light chain protein may be useful as a marker for reduced neurodegeneration when levels are elevated before treatment (Wang et al. 2019).

To substantiate more granular dose-response outcome analysis, measuring ASO levels in the CSF will not only provide information during the dose escalation but will also allow comparison between patients and between ASOs as well as to correlate ASO levels with efficacy, safety, and particular adverse events.

DISCUSSION AND FORWARD LOOKING MESSAGES

Whereas for the named patient setting, approval of national or European regulatory bodies is not required, it is important to mention that regulators are willing to discuss these developments and provide input and expertise, leveraging the available regulatory tools (ITF, PRIME scheme, qualification and scientific advice platforms, etc.). Based on this input and our own internal communications, we propose the following blueprint for treatment of a patient in the Netherlands or Germany and hopefully in time, in other parts of Europe as well.

First, patient eligibility needs to be assessed. This assessment involves four aspects: (i) The mutation must be eligible for splice modulation where a (partially) functional protein is produced; (ii) the disease needs to be a severely progressive and debilitating disorder (SDLT) where the symptoms most impacting patient quality of life are due to pathology of the central nervous system; (iii) it must be possible to treat the patient with intrathecal injections; (iv) a positive benefit-risk ratio should be expected from ASO treatment and the benefit must be measurable ideally within a time frame of 48 mo.

To compensate for the fact that a common regulatory framework for patient-specific development of individualized splice-switching ASOs in EU is still not in place, the 1M1M network has put in place a stringent quality assuring process including Gene Groups and a Treatment Board. When a candidate patient is identified and deemed eligible based on the patient dossier, first the gene group is convened. This group consists of experts on exon skipping, of the particular gene, related protein, and the disease (including in particular natural history trajectories and suitable outcome measures). This group meets to discuss whether sufficient patient and disease information is available to make a well-informed decision on treatment-eligibility and -suitability of the candidate patient. Here in particular the four criteria above are checked and discussed with the clinician proposing N = 1 ASO treatment. When a patient meets all criteria, a treatment board is convened, which contains patient representatives and ethicists, to make the final decision whether treatment development should or should not be initiated.

Once a patient is formally selected, preclinical ASO development starts in cultured cells, a lead compound is selected and studied in an acute rat safety study and in vitro cell culture based safety studies, and finally a GMP or GMP-like batch is ordered. In parallel, the "run-in natural history" is obtained with a selected broader pool of

outcome measures, which then allows to prioritize the three to four individually most promising outcomes at the end of the run for the treatment phase. In addition, start and stop criteria are discussed with the patient and family, prior to initiation of treatment. Treatment is initiated starting with a dose known to be tolerable for nusinersen, and dosing is escalated based on predetermined safety and pharmacodynamic parameters. Monitoring of safety and efficacy is performed regularly and the maintenance dosing is based on pharmacodynamic and safety parameters. Notably, patients and families are heavily involved in the decision making, setting start and stop criteria and making decisions based on realistic assessments during the maintenance-dosing regimen.

It is clear that the individualized ASO development approach operates in uncharted waters. In this regard, it is important to mention the collaborative efforts of the N = 1 collaborative that provides support to the European networks when preparing for individualized ASO treatment in Europe. As there are expected to be thousands of eligible cases, the numbers of clinically used ASOs will significantly increase. To make sure that we can learn from successes and failures and be able to align globally, we need to ensure that data are shared between N = 1 initiatives such as 1M1M and N1C. Only then can N = 1 ASO development be streamlined to benefit as many patients as possible in a timely fashion.

Finally, in principle, there is a lot of flexibility within a named patient setting for a single case. When, however, moving from N = 1 to N = 2 or N = 5, GLP-compliant safety studies and GMP-produced ASOs might be required. This topic was not yet discussed with the regulators of DCRT and 1M1M. Based on currently accumulated knowledge, DCRT and 1M1M would argue that even for cases of N = 50, the current conventional drug development regulation—requiring phase one to two trials and often taking >15 yr until completion—is not fit for purpose. Performing clinical trials for this patient population is extremely challenging as different patients will be in different disease stages, making selection of a single relevant outcome measure impossible. At present, we will focus on the N = 1 challenge, which is sufficiently complex. However, once an individual ASO has been developed, we anticipate that additional eligible cases may present themselves. Thus, the discussion of going from N = 1 to N = few will naturally be initiated.

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