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Review

Joining forces to develop individualized antisense oligonucleotides for patients with brain or eye diseases: the example of the Dutch Center for RNA Therapeutics

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***Abstract*:** Antisense oligonucleotides (ASOs) offer versatile tools to modify the processing and expression levels of gene transcripts. As such, they have a high therapeutic potential for rare genetic diseases, where applicability of each ASO ranges from thousands of patients worldwide to single individuals based on the prevalence of the causative pathogenic variant. It was shown that development of individualized ASOs was feasible within an academic setting, starting with Milasen for the treatment of a patient with CLN7 Batten's disease in the USA. Inspired by this, the Dutch Center for RNA Therapeutics (DCRT) was established by three academic medical centers in the Netherlands with a track record in ASO development for progressive, genetic neurodegenerative, neurodevelopmental, and retinal disorders. The goal of the DCRT is to bundle expertise and address national ethical, regulatory, and financial issues related to ASO treatment, and ultimately to develop individualized ASOs for eligible patients with genetic diseases affecting the central nervous system in an academic, not-for-profit setting. In this perspective, we describe the establishment of the DCRT in 2020 and the achievements so far, with a specific focus on lessons learned: the need for processes and procedures, the need for global collaboration, the need to raise awareness, and the fact that N-of-1 is N-of-a-few.

Plain language summary

Joining forces to develop individualized antisense oligonucleotides for patients with brain or eye diseases: the example of the Dutch Center for RNA Therapeutics

Many rare diseases have a genetic cause. Antisense oligonucleotides (ASOs) are short pieces of modified DNA that have therapeutic potential for some patients with rare diseases. However, often this is in a patient-specific setting, meaning individualized therapy development is required, which has little commercial opportunity for pharmaceutical companies. It was shown however that individualized ASOs can be developed by academics, starting with Milasen, which was developed for a unique DNA variant found in a child with Batten's disease in the USA. Following in the footstep of these academic pioneers we established the Dutch Center for RNA Therapeutics (DCRT), which aims to develop individualized ASOs for eligible patients with eye or brain diseases in a not-for-profit setting. Our goal is to bundle expertise and address national, ethical, regulatory and financial issues related to individualized ASO development. In this perspective review we outline the achievements since establishing the DCRT in 2020,

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with a focus on lessons learnt along the way: the need for processes and procedures, the need for global collaboration, the need to raise awareness and the fact that very often ASOs developed for a single person, could be applied also to a few other patients with the same DNA variants.

Keywords: antisense oligonucleotide, individualized treatment, N-of-1, RNA therapy

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Historical context

Antisense oligonucleotides (ASOs, also known as AONs) are small pieces of chemically modified DNA or RNA that can specifically bind to gene transcripts via Watson-Crick base pairing.[1] They can be utilized to reduce the level of gene transcripts via RNase H1-mediated cleavage of ASO-transcript hybrids. This will reduce the production of the encoded protein, which has therapeutic potential for diseases caused by toxic gain-of-function variants or by targeting key players in pathological pathways. Alternatively, ASOs can be used to modulate splicing.[1] Here ASOs can hide a target exon from the splicing machinery, so it is skipped and not included in the mature mRNA transcript. This approach was originally pioneered by Ryszard Kole to prevent the inclusion of a cryptic exon for a common cryptic splicing variant in the gene encoding beta-globin that was associated with beta-thalassemia.[2] It was then adopted as a therapeutic option for Duchenne muscular dystrophy (DMD).[3] This disease is caused by pathogenic variants that disrupt the open reading frame of the dystrophin transcript. Variants that maintain the open reading frame allow the production of an internally deleted, but partially functional dystrophin protein. These latter variants are found in Becker muscular dystrophy (BMD), a disease with a later onset and slower disease progression than DMD. The goal of ASO-mediated exon skipping is to allow DMD patients to produce partially functional BMD-type dystrophins rather than non-functional dystrophins. Most DMD patients have a deletion involving one or more exons. Counterintuitively, enlarging the deletion on transcript level through skipping of a specific exon is therapeutic, provided the skipping restores the open reading frame and the resulting protein is functional. Exon skipping for DMD is a variant-specific approach, as depending on the size and location of the deletion, different exons have to be skipped to restore the reading frame.[4]

DMD exon skipping was pioneered among others by researchers of the Leiden University Medical Center (LUMC), who were the first to unequivocally show dystrophin restoration in patient-derived cell cultures in 2001[5] and in a clinical trial setting in DMD patients in 2007[6] (Figure 1). We also developed ASOs to induce the skipping of many different dystrophin exons and provided design guidelines based on retrospective analysis of effective and ineffective ASOs.[7,8]

Another application for splice-switching ASOs has been developed for polyglutamine (polyQ) disorders at the LUMC. PolyQ disorders form a group of nine autosomal dominant neurological diseases that are caused by a CAG repeat expansion in the coding region of a gene. The resulting expanded repeats in the transcripts are then translated into an expansion of glutamine amino acids in the protein. Although the CAG repeat expansion is located in a different gene for each polyQ disease, they all share clinical and pathological characteristics, such as adult-onset of a movement disorder and protein aggregates that are formed in the brain.[12] Most of these nine polyQ proteins have important wild-type functions in the brain, making downregulation of RNA expression with an RNase H1-activating ASO a less favorable approach. For the polyQ disease spinocerebellar ataxia type 3 (SCA3), removal of the CAG repeat-containing exon with a splice-switching ASO results in a shorter, in-frame, ataxin-3 transcript and a truncated protein. This truncated protein lacks the toxic repeat expansion but retains most of its wild-type functions and showed a phenotypic improvement in cell- and

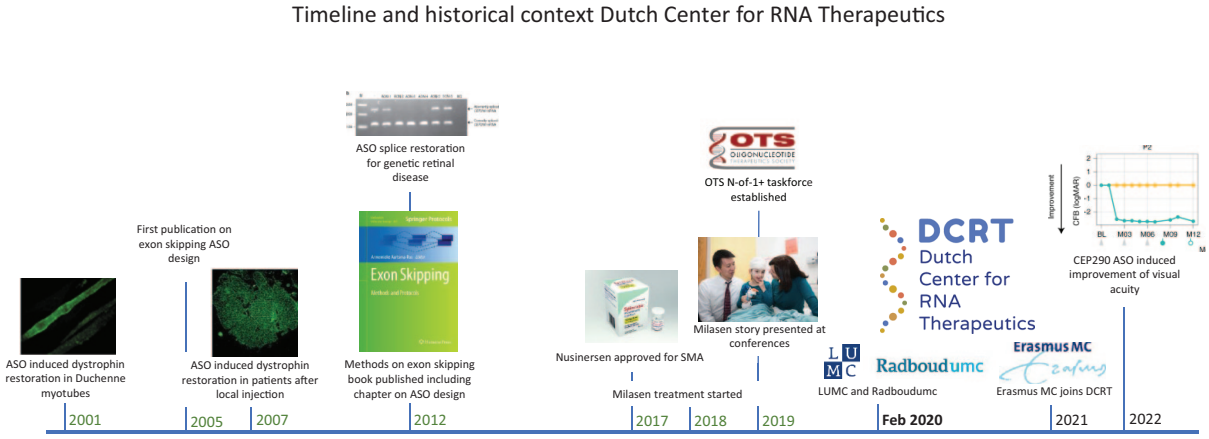

Figure 1. Timeline and context for establishing the Dutch Center for RNA Therapeutics. Schematic depiction of key publications and occurrences for the development of exon skipping antisense oligonucleotides for muscle, eye, and brain disorders and for the development of individualized antisense oligonucleotides.
Source: Belgrad et al.,[9] Synofzik et al.,[10] Lauffer et al.[11]
ASO, antisense oligonucleotide; SMA, spinal muscular atrophy.

animal models.[13,14] For another polyQ disease, Huntington disease (HD), the CAG repeat expansion is located in the first exon, making it impossible to remove the CAG-containing exon from the transcript. However, proteolytic cleavage of the mutant huntingtin protein is an important step in the disease pathology, and removing exon 12 of the huntingtin transcript, where an important caspase cleavage is located, reduces toxicity of the mutant protein and improves the HD phenotype in cells and mice.[13,15]

At the Radboudumc, the use of ASOs to treat inherited retinal diseases (IRDs) was pioneered. Following the identification of a common deep-intronic variant in *CEP290* (NM_025114.4: c.2991+1655A>G) underlying early-onset severe IRD[16] that causes the insertion of a cryptic exon to *CEP290* transcripts, preclinical efficacy of ASOs to rescue the splicing defect caused by this variant was demonstrated in vitro and in vivo.[17–19] In a subsequent phase I/IIa clinical trial sponsored by ProQR Therapeutics, intravitreal administration of ASOs to patients harboring the *CEP290* variant in homozygous or compound heterozygous state led to improvement of visual function in some subjects.[20,21] Unfortunately, clinical endpoints were not met in the subsequent phase III clinical trial. Other examples of IRDs in which a subset of genetic defects have been successfully targeted with ASOs include Usher syndrome type 2A (up to and including phase I/IIa clinical testing),[22,23] Stargardt disease (preclinical)[24,25] and choroideremia (preclinical).[26]

There has been a long-standing interest in developing treatments for neurodevelopmental disorders and bringing these treatments to clinical trials at the *ENCORE* Expertise Center for Neurodevelopmental Disorders within Erasmus MC. The interest in ASO development started with the realization that RNase H1 ASO-mediated targeting of the *UBE3A-ATS* transcript that represses the expression of the paternal *UBE3A* allele, might offer a potential treatment for Angelman syndrome.[27,28] Although this project started entirely within an academic setting, it eventually moved into a collaboration with pharmaceutical partners, resulting in a proof-of-concept study that early ASO treatment could rescue many of the behavioral phenotypes in a mouse model of Angelman syndrome.[29] Currently, several clinical trials are underway to test the therapeutic efficacy of ASO treatment for this disorder.

From clinical developments for nusinersen, a splice-modulating ASO for the treatment of spinal muscular atrophy (SMA), it became clear that while ASOs do not cross the blood-brain-barrier, they are taken up efficiently by most cells in the central nervous system, after penetrating this barrier through local intrathecal injection.[30,31] This allows injection of low doses of ASOs (21 mg for nusinersen), with low systemic exposure at a maintenance dosing frequency of once every

4 months. Nusinersen was evaluated in clinical trials in severe SMA patients, where it increased survival and allowed patients to reach milestones such as sitting, standing, and walking, which are normally never achieved for these individuals.[30] In milder SMA patients, nusinersen treatment improved motor function and decreased disease progression.[32] Nusinersen was approved by the Food and Drug Administration (FDA, US) in 2017 and by the European Medicines Agency (EMA) in 2018.[33]

As described, there was a track record of ASO development in the Netherlands, where the initial proof-of-concept work was done within an academic setting and the clinical development was then furthered by pharmaceutical companies: Prosensa/GlaxoSmithKline/Biomarin for Duchenne, ProQR for Leber Congenital Amaurosis, Usher syndrome type 2A and HD, Ionis/Biogen for Angelman syndrome and Ionis for SCA3. These efforts focused on the more common rare diseases or more common subgroups for variant-specific approaches. However, as researchers, we also encountered extremely rare variants that were excellent candidates for ASO treatment, but for which there was no commercial interest, as they only existed in a single family. Then, in 2018, Tim Yu showed that it is possible to develop ASOs for a single individual within an academic setting. He discovered that a patient with CLN7 Batten's disease had a causative retrotransposon element insertion on one allele of the *MFSD8* gene that resulted in cryptic splicing.[34] Based on the nusinersen evidence and approval, and published guidelines for ASO exon skipping design and development, he embarked on developing a patient-specific ASO (Milasen) for this patient (Mila). Within a year after finding the genetic cause, the patient was treated for the first time. While the treatment could not undo the neurological damage and blindness that had developed, it did reduce the frequency and duration of the epileptic seizures, which improved the quality of life of both the patient and the family before the patient succumbed to her disease in 2021.

Inspired by this story in 2019 and looking at our respective track records, we realized that we had the expertise to achieve something similar in the Netherlands. Rather than establishing local centers, we decided to join forces and establish a Dutch Center for RNA Therapeutics (DCRT). The goal of the DCRT is to develop individualized ASOs for eligible patients with genetic eye and brain diseases in a not-for-profit setting. The DCRT was launched on February 29, 2020 by the LUMC and Radboudumc, while Erasmus MC joined in 2021. LUMC mainly focuses on progressive neurodegenerative diseases, Radboudumc on progressive retinal diseases, and Erasmus MC on neurodevelopmental disorders. The researchers collaborate on paving a way to implement individualized ASO development and clinical treatment within the Netherlands.

This perspective review focuses on national developments, specifically the individualized ASO treatment development coordinated by the DCRT. For a recent review of different oligonucleotide modalities and an overview of oligonucleotide therapies approved and in clinical development we refer the reader to an excellent review paper by Belgrad et al.[9]

Establishing the DCRT

A few weeks after the start of the DCRT, the COVID pandemic closed most of the universities in The Netherlands, and in our first few years we established proper regulatory requirements to introduce the development of individualized ASOs in Europe. The Milasen developmental pipeline was taken as blueprint, however, it soon became apparent that the European route from ASO design to first in human dosing was very different from the one used in the United States. While an Investigational New Drug (IND) application was needed and granted for treatment with Milasen by the FDA, in Europe this individualized ASO treatment would fall under named patient use. This allows the use of unlicensed medicinal products to single patients to "fulfill special needs" (article 5 (1) of (EC) 2001/83). The direct responsibility would lie with an authorized health care professional and the drug should be given on the basis of purely therapeutic considerations[10] and would not result in marketing authorization. On the one hand, this seems favorable and would suggest a shorter route from designing an individualized ASO to administration to the patient, however, it also requires an improbable high level of expert knowledge within local institutes and a large responsibility for the authorized healthcare professional. The DCRT decided early after its instigation to pursue

standardized protocols and procedures tailored to the European regulatory and health insurance reimbursement situation. Furthermore, designing these procedures so they could be performed as much as possible within an academic setting, will keep the cost of each treatment as low as possible.[35] This would increase the chances of reimbursement by health care insurance and making this a sustainable treatment modality. Based on the white paper that was released by the Oligonucleotide Therapeutic Society (see below) and the FDA draft guidance published in December of 2021 [FDA-2021-D-1140], the DCRT had an initial Innovative Task Force meeting with experts from EMA. Discussed was a European-tailored preclinical and clinical pipeline for assessing ASO efficacy and safety.[36]

Current activities

The DCRT directors have monthly progress meetings to update each other on local projects and to discuss joint efforts. So far, the DCRT has produced a tool to visualize the exon structure of transcripts (van den Berg et al., manuscript in preparation) and a list of candidate genes that are known to be associated with progressive brain or eye diseases (https://www.rnatherapy.nl/our-work). Until now, the focus is on diseases that (primarily) affect the brain and the eye and result in progressive pathology as this allows local treatment and benefits can be expected (for more detail see Dominski and Kole[2]). ASOs target transcripts and thus address the genetic cause of a disease, in contrast to symptomatic treatment that addresses one or a few symptoms. In this regard, ASOs are expected to address all aspects of downstream pathology and comorbidities. For instance, both seizures and behavioral disturbances are expected to improve after a missing protein is restored by ASOs. There are some caveats here, however, as ASOs cannot return neurons or retinal cells that have been lost or reverse anatomical malformation that occurred *in utero*. Furthermore, when there are comorbidities affecting tissues outside of the central nervous system or the eye, these will not be addressed with local ASO treatment. DCRT has published on these considerations with international collaborators (see below).[2,3]

Furthermore, guidelines for the *in vitro* efficiency testing of exon skipping ASOs were published by the DCRT within the $N=1$ Collaborative (see below).[37] DCRT is focusing on developing and optimizing in silico, in vitro, and in vivo platforms for designing ASOs and establishing their efficiency and toxicity with the aid of a ZonMW Psider consortium grant (called Tailored) from the Dutch government to Erasmus MC and LUMC.

At the LUMC, the focus is on two aspects of the development of individualized ASO treatments. First, we are developing and building computational tools and experimental pipelines to facilitate the treatments and provide resources to the N-of-1 community globally. This includes the development of tools to enhance and accelerate the identification of eligible candidates and variants for genetic treatments. It further encompasses the establishment of a neurotoxicity pipeline using human-derived cell models to evaluate ASO toxicity in vitro as well as establishment of standardized protocols for testing ASO efficacy and their effects on restoration of protein function in vitro. For example, we are developing ASOs for *PLP1*-associated Hypomyelination of Early Myelinating Structures (HEMS) for which we try to increase the production of full-length *PLP1* transcripts, an approach that would be applicable to multiple patients. We are further working on different genes associated with neurometabolic disorders in which (deep) intronic variants have been identified to cause cryptic splicing and where we aim to correct splicing.

At the Radboudumc, efforts to establish N-of-1 treatments are mainly focused on *ABCA4*-associated retinal disease (i.e., Stargardt disease). Stargardt disease is a progressive retinal disorder mainly affecting central vision, and is caused by biallelic variants in *ABCA4*. It is characterized by an unprecedented allelic heterogeneity. To date, more than 1200 different causative *ABCA4* variants have been described[38] https://databases.lovd.nl/shared/genes/ABCA4. Whereas some of these variants are highly recurrent and present in several thousands of individuals worldwide, others are ultrarare, down to being present in a single individual. Besides molecular eligibility (the effect of the mutation should be rescuable with an ASO), the clinical status should also be carefully checked, as retinal cells do not regenerate. At this moment, we have identified two cases with Stargardt disease potentially eligible, as the

splicing defect caused by one of their pathogenic *ABCA4* variants can be rescued with an ASO, and there is sufficient remaining integrity of the retina anticipating that treatment will have benefit for the patient. In more general terms, we are establishing a routine procedure to determine, at an early stage, whether an IRD patient who enters the clinic and in whom the causative genetic defect has been identified, is a candidate for a future N-of-1 intervention, regardless of the causative gene.

At Erasmus MC, the focus is on developing ASOs for treating ultrarare and private variants causing neurodevelopmental disorders. In particular, the lab explores the value of using extracellular electrophysiological measurements via multi-electrode arrays with induced pluripotent stem cell (iPSC)-derived neurons or mouse primary neurons as an in vitro proxy for toxicity and efficacy. The lab is also investigating ASO-associated toxicity in the developing (mouse) brain, and determining the critical time window for reversing phenotypes in mouse models.[39]

Lessons learned

The DCRT celebrated its fourth birthday in 2024. It is clear that a lot of work still has to be done to make individualized ASO development and treatment a recognized and sustained option to treat patients with eligible pathogenic variants. As this is a pioneering effort we also did not anticipate to have all the answers within 4 years. However, we would like to share several lessons learned along the way as this may be helpful for other researchers in the N-of-1 development field.

The need for procedures and processes

The first lesson is that procedures and processes are needed to facilitate application of challenging new treatment paradigms. While we stress that involving the patient in the N-of-1 development from an early stage, we struggle with aspects like how to select a patient, when to best inform the patient, and how to optimize informed consent. Also, guidance is lacking on how much preclinical evidence is needed: when is an ASO efficient enough in patient-derived cells to expect benefit in patients? Which safety studies are sufficient? Which manufacturing processes best fit ASO quality in a personalized treatment setting, selecting the essential quality assessments that would then not require production of large amounts of ASOs, while 10 g is sufficient to treat an individual with a brain or eye disease for life? How to monitor safety and efficacy and how to make sure the DCRT but also the wider N-of-1 field learn from our work for effective and ineffective ASOs, but also safe and unsafe ASOs? Obviously, these are questions that are asked by N-of-1 ASO developers around the world and not just by the DCRT. As such, we appreciate the international umbrella organizations that try to streamline the development of the procedures (see below).

Within the DCRT, we are trying to find the right balance between establishing certainty on the efficiency and safety of the compound and its quality, while also keeping costs and development time low. GMP-grade (Good Manufacturing Practice) ASOs and GLP-compliant (Good Laboratory Practice) toxicity studies are not only much more expensive, but they also generally mean a 12–18 month delay due to waiting time at the companies involved. However, the increase in development time is a larger challenge as we aim to treat patients with progressive diseases and these delays will mean irreversible loss of function. The current struggle we are facing as researchers is to balance these components.

International links and positioning

The second lesson is that one cannot operate on a national level for individualized ASO development. While the DCRT focuses on developing individualized ASOs for Dutch patients, similar efforts are established around the world. Giving the pioneering aspects and many unknowns of developing individualized therapies, it is important not to work in isolation but to collaborate and share. That way, we can learn from each other's successes and failures and we avoid duplicating efforts. Furthermore, if there are a few patients that carry the same ultrarare pathogenic variant, the chances are high that they live in different countries or even on different continents. This strengthens the urgency to collaborate and share information and knowledge.

The DCRT is involved in three global efforts: (i) the $N=1$ task force of the International Rare Disease Research Consortium (IRDiRC); (ii) the

N-of-1+ task force of the Oligonucleotide Therapeutics Society (OTS) and, (iii) the *N*=1 Collaborative. The IRDiRC task force ran in 2023 to do a landscaping exercise of *N*=1 treatments (not ASO specific), to establish a roadmap for individualized treatment development and to identify gaps and needs. Reports are expected to be published this year (Jonker et al., manuscript submitted). The OTS N-of-1+ task force was established in 2019, in recognition of the fact that guidance and coordination were needed for the individualized ASO development after the Milasen story, with AAR as one of the founders and leaders. A stakeholder meeting with FDA, patient representatives, researchers, and industry representatives was planned for April 2020 at the FDA by this task force. However, due to the COVID-19 pandemic, the meeting was canceled. With support from OTS, AAR coordinated the production of a briefing document with guidance and considerations for those interested in individualized ASO development (https://www.oligotherapeutics.org/rare-disease-task-force/rare-disease-briefing-document/). The *N*=1 Collaborative (N1C) was established in 2021 as an international hub for individualized therapy development with Tim Yu and Julia Vitarello (Mila's mother) and key members of the N-of-1+ OTS task force. N1C for now focuses on ASOs, but works under the premise that many of the tools and processes developed will also be usable for other individualized therapeutic approaches, such as genome editing. Members of the DCRT are actively participating in N1C as directors (AAR), are part of the scientific advisory board (AAR), and are involved in several working groups: preclinical development (AAR and WvRM co-chairs), safety (WvRM), and patient identification (MCL co-chair and AAR member). In the USA several initiatives were established, for example, n-Lorem, a foundation to develop ASOs for nano-rare patient groups in the USA and making them available for free for life to these patients.[41]

The need to raise awareness

The third lesson is the lack of familiarity with the named patient setting for N-of-1 ASOs within our clinical institutes. Local ethics boards lack expertise on ASO development and do not feel comfortable with making an assessment. However, as the goal is to treat individuals with an experimental treatment rather than to do clinical research the approval is not done by regulatory competent authorities such as the EMA, or the National Committee for Human Research (centrale commissie voor mensgebonden onderzoek CCMO), who may have more experience with ASOs.

As a pragmatic solution, the DCRT is currently working on a master protocol to standardize the essential steps and processes for clinical development of an individualized ASO in collaboration with expert panels and CCMO. Each individual ASO could then be provided as an amendment to the local ethics board, which hopefully will feel supported by the master protocol approval at a national level.

N-of-1 is often N-*of-1+*

The fourth lesson is that N-of-1 often is not truly N-of-1, but N-of-few. This can be as simple as multiple patients being affected within the same family. However, cases where unrelated individuals presented with the same variant have been reported as well.[40] While originally the DCRT only wanted to focus on true N-of-1 cases, we have come to realize that this is not realistic. Furthermore, the developmental challenges that apply to single individuals, still apply to 2 or 3 cases spread around the world. These individuals will likely be in a different stage of disease, thus requiring a different approach for monitoring treatment effects. Also, each individual will have its own tolerance for risk and uncertainty. As such, even for the N-of-1+ cases, an individualized approach is required. This lesson does flag the importance of data sharing, however, when an ASO is already developed for a specific pathogenic variant, this information should be shared to avoid duplication of efforts and delay of treatment.

Regulatory considerations

From a regulatory perspective, it has become clear that the clinical implementation of individualized ASOs differs between different regulatory jurisdictions.[35] As mentioned, within the European Union treatment can be done under a named patient setting. The logistic and administrative aspects of named patient treatment, however, vary in European countries. To streamline development and treatment of ASO for patients

with neurodegenerative diseases, the 1 Mutation 1 Medicine (1M1M) network was established. This is coordinated by researchers from Tübingen University Hospital, Heidelberg University, and the LUMC. The 1M1M network will be able to develop tools and processes via the recently launched Medicine Made to Measure (MMM) Innovative Training Network. Notably, the N-of-1 ASO field may use part of the model for personalized advanced therapeutic medicinal products (ATMPs). ASOs are synthetic and therefore are not ATMPs. However, clinical implementation of N-of-1 ASO treatment can benefit from processes for the implementation of (personalized) ATMPs, such as hospital exemption.

Toward the future

When we started the DCRT, the focus was on N-of-1 and unique cases. However, as outlined, we have learned that a pathogenic variant is seldom unique. A concrete example relates to atipeksen, a splice-modulating ASO that Tim Yu developed for a patient with ataxia telangiectasia with a cryptic splicing variant in the *ATM* gene.[40] In 2021, our colleagues in Tübingen reached out to us for help with preclinical ASO development for an ataxia telangiectasia patient with a cryptic splicing variant. After cross-referencing with Tim Yu, it turned out that the patient from Germany had the same variant as the patient Tim Yu was treating already, and that atipeksen treatment should be applicable to this individual. Currently, the patient from Germany is also being treated with atipeksen. This example confirms the importance of dialogue and data sharing as it avoids duplication of effort and unnecessary delays.

It is clear that there is still a lot of work to be done nationally and internationally to allow a streamlined development of individualized ASO treatment of eligible patients. However, after considering and discussing the processes and tools needed, the way forward is more clear. We will have to shift our mindset on how to approach drug development as the traditional model does not apply to the individualized setting where placebo-controlled trials are not possible and where extensive preclinical studies to confirm efficiency and safety mean that by the time the patient can be treated, there is no treatable patient.[42] Obviously, some preclinical studies will still be required to show that the ASO has the intended effect on transcript and protein levels and is safe. The challenge will be to find the right balance between what is needed from a safety and efficiency data aspect and what risks and uncertainties are acceptable in light of the disease burden and progression.

While the development of individualized ASOs and aspects involved in confirming efficacy of treatment in a patient differs for each indication, there are many processes and procedures that apply to all cases, such as assessing eligibility, confirming preclinical efficiency and safety, informed consent, manufacturing, regulatory compliance, clinical implementation and statistical tools to assess efficacy on an *N*-of-1 level. It is one of the aims of the DCRT to formalize these general processes and procedures to be used by Dutch institutes as a general guideline that can then be amended for every individual patient. This general guideline will capture the worldwide experience and expertise and will aid local ethical boards in assessing and approving individualized ASO treatments. Furthermore, it is clear that every individualized case can teach us something for future development of other individualized ASOs, both when the patient was successfully treated and when unexpected complications became apparent. Thus, tools to share data are required. All these processes, procedures, and tools are needed at a global level and this work is currently coordinated by N1C.

For now, the efforts to develop individualized ASOs are funded primarily through institutes involved, grants, or crowdfunding. The hope is that the development of these ASOs will become cheaper and easier in the future based on learnings from each individualized ASO development. Furthermore, we hope that in the future, it will be possible to sustain the development of individualized ASOs financially through healthcare funds. As the DCRT we envision a transparent development cost model, where each ASO is reimbursed by the payers once it reaches the clinical stage with a minor markup to recover costs for ASOs that failed to make it to the clinical stage. The recovered funds will then be used to develop the next individualized ASO. Should additional patients be eligible for treatment with the same ASO, this would only involve the clinical costs of injection and management by the clinician. While

there is still a lot to be done until that stage is reached, progress has been made toward making individualized ASO treatment a reality in the Netherlands.

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Not applicable.

Consent for publication
Not applicable.

Author contributions
Annemieke Aartsma-Rus: Conceptualization; Funding acquisition; Supervision; Writing – original draft; Writing – review & editing.

Rob W. J. Collin: Conceptualization; Funding acquisition; Writing – original draft; Writing – review & editing.

Ype Elgersma: Conceptualization; Funding acquisition; Writing – original draft; Writing – review & editing.

Marlen C. Lauffer: Conceptualization; Writing – original draft; Writing – review & editing.

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