# Applying lessons learned from developing exon skipping for duchenne to developing individualized exon skipping therapy for patients with neurodegenerative diseases

Aartsma-Rus, A.

Applying Lessons Learned from Developing Exon Skipping for Duchenne to Developing Individualized Exon Skipping Therapy for Patients with Neurodegenerative Diseases

Annemieke Aartsma-Rus * ⓘ

Dutch Center for RNA Therapeutics, Department of Human Genetics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands
a.m.rus@lumc.nl

Antisense oligonucleotides (ASO)
Restore protein production by manipulating splicing
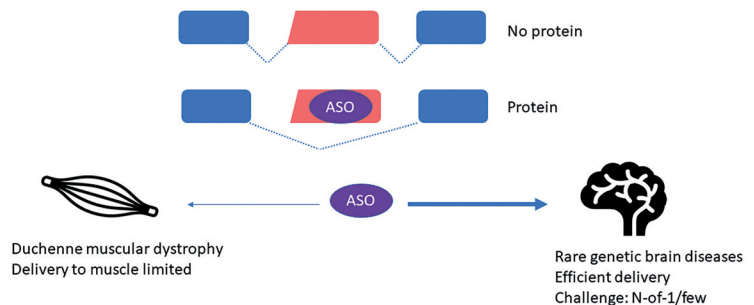
Abstract Antisense oligonucleotides (ASOs) are short, modified pieces of DNA that are chemically modified. They can be used to induce exon skipping and treat Duchenne muscular dystrophy (DMD) patients by interfering with the splicing process so mutated dystrophin transcripts become readable allowing production of partially functional dystrophin proteins, rather than nonfunctional dystrophins. After over 2 decades of research, 4 ASOs are FDA approved for DMD, but clinical effects are suboptimal due to limited delivery to skeletal muscle. At the same time, ASOs for brain diseases result in much more functional impact, because local delivery allows higher exposure to the target tissue at a low dose and infrequent treatment regimen. This has opened the way to develop ASOs in an individualized setting, as was exemplified by the development of Milasen to treat a patient with CLN7 Batten disease. In this perspective paper I will share my personal journey as one of the pioneers of ASO-mediated exon skipping development for DMD, currently applying expertise gained and lessons learned along the way to develop exon skipping ASOs for eligible patients with genetic brain diseases in a national and international setting.

1 Duchenne and Antisense-Mediated Exon Skipping

Antisense oligonucleotides (ASOs) are short synthetic pieces of DNA that are chemically modified to give them druglike properties.[1] ASOs can bind to gene transcripts in a sequence specific manner. They have been employed to reduce the production of toxic proteins or to modulate the splicing process to restore production of missing proteins. The latter is used in Duchenne muscular dystrophy (DMD) to restore production of dystrophin.[2] DMD is a severe muscle-wasting disease resulting in loss of ambulation before the age of 12, the need for assisted ventilation by the age of 20, and premature death in the 2nd to 4th decade of life.[3] The disease is caused by mutations (mostly deletions of one or more exons) in the *DMD* gene that disrupt the reading frame and thus prevent the production of functional dystrophin. Splice modulating ASOs can induce exon skipping to restore the reading frame, allowing the production of shorter, but partially functional dystrophin proteins, such as those found in the later onset and less severely progressive Becker muscular dystrophy.[2]

The idea for ASO-mediated exon skipping to restore the dystrophin transcript reading frame was posed over 25 years ago and the approach was pioneered at different locations around the world. Proof-of-concept studies showing ASO-mediated exon skipping and reading frame restoration in patient-derived cell cultures and DMD mouse models were published by groups in Australia, Japan, the Netherlands, and the UK.[4–9] The approach is mutation specific, as different exons have to be targeted based on the size and lo-

cation of the deletion.[10] However, ASOs inducing the skipping of all internal dystrophin exons were identified.[11]

As DMD affects most of the skeletal muscles, systemic treatment is required. It was shown that ASO uptake by dystrophic muscles is more efficient than by healthy muscles,[12] which facilitated systemic treatment. Clinical trials in DMD patients were then conducted in Japan and Europe and later also in the USA with ASOs targeting exon 44, 45, 51, and 53.[13–16] These revealed that systemic ASO treatment can result in exon skipping and dystrophin restoration in skeletal muscles. However, efficiency is very low with dystrophin restoration levels usually being less than 1% after a year of weekly intravenous ASO treatment.

Currently, 4 exon skipping ASOs are approved by the Food and Drug Administration of the USA (FDA) targeting exon 45 (casimersen), 51 (eteplirsen), and 53 (golodirsen and viltolarsen).[2] One exon skipping ASO is approved by the Japanese Ministry of Health, Labor and Welfare (viltolarsen). The approvals were only based on dystrophin protein restoration at low levels (0.4% for eteplirsen, ca. 1% for casimersen and golodirsen, and ca. 5–6% for viltolarsen). Clinical trials to assess whether the low amounts of dystrophin are sufficient to slow down disease progression are currently ongoing.[2]

2　Opportunities for Treating Central Nervous System Diseases and Developing Individualized ASOs for Central Nervous System Diseases

From over 2.5 decades of research into dystrophin exon skipping, we can conclude that delivery of ASOs to skeletal muscles is challenging.[17] Delivery to the central nervous system, however, is relatively straightforward using intrathecal injections.[18] After local delivery, ASOs are distributed throughout the central nervous system and efficiently taken up by most residing cells.[19] The advantages of local delivery are that it requires a low dose, so systemic exposure is low, and that the treatment frequency is low (3–6 times per year). This is exemplified in nusinersen, an ASO for the treatment of spinal muscular atrophy that is approved since 2016 in the USA[20] and currently marketed in many countries. Intrathecal treatment with nusinersen in patients with the severe type 1 form of spinal muscular atrophy allows treated patients to achieve milestones that are by definition not achieved by these individuals. Furthermore, in a phase 3 clinical trial there was a significant reduction in death or permanent ventilation for treated patients.[21]

As such, splice modulating ASOs offer great potential to treat brain diseases for eligible genetic conditions.[22] The most notable eligible pathogenic variant would be the intronic 'cryptic splicing mutation'. This is a variant where a noncoding part of a gene is aberrantly included into the

---

Biographical Sketches

Prof. Dr. Annemieke Aartsma-Rus is a professor of Translational Genetics at the Department of Human Genetics of the Leiden University Medical Center (LUMC, the Netherlands). She played an important role in the development of antisense-mediated exon skipping for Duchenne muscular dystrophy during her PhD research at the LUMC. In 2020 she co-founded the Dutch Center for RNA Therapeutics (DCRT), a non-for-profit academic collaboration aiming to develop clinical treatment with exon skipping therapies for eligible patients with unique mutations. In 2022 she became a board member of the N-of-1 collaborative (N1C), a global umbrella organization aiming to facilitate development of antisense oligonucleotide therapies for patients with very rare, eligible mutations. She has published over 250 peer-reviewed papers, 11 book chapters, and 15 patents. She has given many invited lectures at scientific conferences and patient organization meetings, where she is known for her ability to present science in a clear and understandable way. In 2011 she received the Duchenne Award from the Dutch Duchenne Parent Project in recognition of her dedication to the Duchenne field. In 2020 she received the Black Pearl Science Award from Eurordis for her work in educating patients. In 2021 she received the Ammodo Science Award for her contribution to developing exon skipping therapies for Duchenne, the outstanding achievement award from the Dutch Society of Gene and Cell Therapy for her work and the Rosalind Franklin in Science award for her work for the journal Nucleic Acid Therapeutics. From 2015 to 2023 she was the most influential scientist in Duchenne muscular dystrophy in the past 10 years 8 times in a row (https://expertscape.com/ex/muscular+dystrophy%2C+duchenne).

messenger RNA transcript, thus abolishing protein production. ASOs can prevent this aberrant inclusion thus restoring normal protein expression (Figure 1).
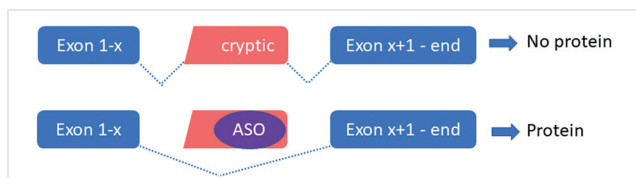
Figure 1 Cryptic splicing variants cause the inclusion of part of an intron (cryptic exon) into the messenger RNA, thus abolishing protein production. Antisense oligonucleotides (ASOs) can target cryptic exons, thus preventing their inclusion into the messenger RNA during the splicing process and restoring production of a missing protein.

However, these cryptic splicing variants are incredibly rare, often even identified in single individuals. Therefore, there is no commercial incentive to develop these ASOs for eligible patients. Tim Yu (Boston Children Hospital) showed it is possible to develop an individualized ASO for a patient with a cryptic splicing variant with Batten disease, a severe disease characterized by blindness, frequent epileptic seizures, and early onset dementia. This ASO was delivered intrathecally and significantly reduced the number and duration of the seizures. The ASO was called milasen after the patient, Mila.[23] This group later developed an ASO for an ataxia telangiectasia patient with a cryptic splicing variant and further showed that probably ca. 15% of disease causing variants in ataxia telangiectasia might be amenable to ASO-induced splicing modulation.[24]

3 Collaborative Spirit to Develop Individualized Treatments Globally

The milasen story inspired many academics with expertise in oligonucleotide therapy to embark on similar efforts to develop ASOs for very eligible candidates within an academic setting. In the Netherlands, the Leiden University Medical Center (LUMC), Radboudumc Nijmegen, and Erasmus Medical Center Rotterdam established the Dutch Center for RNA Therapeutics.[25] This is a collaborative effort of institutes with expertise in ASO development and treatment. The goal is to develop individualized ASOs for eligible patients with genetic brain or eye diseases and to provide them to patients without a profit.[26] Similar initiatives were set up, such as the N-lorem foundation.[27] Furthermore, umbrella initiatives were started to align efforts in Europe (1 Mutation 1 Medicine, 1M1M)[28] and globally (N-of-1 collaborative, N1C).[29] Recently, also a taskforce on N-of-1 therapy development in general (so not ASO specific) was launched by the International Rare Disease Research Consortium (IRDIRC).[30]

These initiatives are crucial as individualized treatment development is a pioneering effort, where the traditional

drug development pathway does not apply. Most obviously, the development time needs to be much quicker than the >10 years it generally takes from target validation to approval, as the individual for whom individualized treatment is an option, generally cannot wait 10 years. Either the disease by then will have progressed so much that no treatment benefit is to be expected, or the patient will have passed away. Still, it is pertinent that the individualized treatments show efficacy and safety in preclinical studies before treatment of a patient in a clinical setting is initiated. Another difference is that for individualized therapies, placebo-controlled clinical trials cannot take place. Instead, the treatment will be provided as an experimental treatment in a patient care setting. However, monitoring benefit and side effects will be crucial, so collecting natural history data before the treatment, while the individualized treatment is in preclinical development, will be imperative to allow comparison of trajectories before and after treatment on top of comparison of the individual trajectory of the treated patient with the natural history. Here, outcome measures and start and stop criteria will have to be discussed by the clinician and the patient/family.

A roadmap covering all the steps involved in individualized treatment development, from assessing patient eligibility to clinical implementation is being constructed by the N-of-1 IRDIRC Taskforce.[31] Furthermore, the DCRT has produced educational papers in collaboration with N1C on which ASO modality apply to which genetic disease,[22] including also considerations and caveats, as well as guidance on preclinical assessment of efficiency for exon skipping ASOs.[32,33] The 1M1M network has produced a guidance document on eligibility criteria from a genetic, clinical, and ethical perspective.[34] Notably, in Europe individualized treatment can be done in a named patient setting and does not need approval from the European Medicines Agency (EMA), while in the USA an investigational new drug (IND) application has to be done to FDA even for individualized treatments. While regulatory approval is not required in Europe, 1M1M and DCRT has approached EMA for advice and have shared these experiences.[33]

4 Global Implementation

Current efforts are ongoing to develop individualized ASOs. In the USA, Tim Yu's group has started treatment of 6 patients with 4 different ASOs, 1 in collaboration with N-lorem and Children's hospital in Colorado. N-lorem has initiated treatment of 5 patients, 2 are pending institutional review boards (IRB) approval, and 2 INDs are pending (presented at N-lorem colloquium Oct 12, 2023). In Europe, ASOs are in preclinical development. One individual with AT is being treated with atipeksen. Notably, after cross referencing ASO amenable AT variants the PIs involved in the N-of-1 collaborative, it became clear that an AT patient in

Germany carried the same variant as the individual already treated by Tim Yu with atipeksen. The patient moved to Boston to initiate treatment there and is currently being treated and monitored in Germany. Additional individuals with the same pathogenic variant have been identified in Turkey.

This example highlights several aspects. First, that ASOs developed for what is thought to be a unique patient, can turn out to apply to additional individuals. Furthermore, due to different regulations in different jurisdictions, it is not always straightforward to initiate treatment of a second patient with such an ASO. Especially in Europe, routes for clinical implementation within a named patient setting vary per country and sometimes even per region or hospital. Ideally, in the future treating patients in an "N-of-1-at-a-time" fashion is streamlined better to facilitate quick implementation of treating additional patients. Notably, treating a second patient with an ASO reduces the uncertainty with regards to safety and efficacy, as some extrapolation can be made from the first treated individual.

With the rapid development of N-of-1 ASOs, many patients will be the first to be treated with a given ASO. While these are tested in vitro to confirm efficacy and in vivo to confirm safety, there may be unexpected side effects. Valeriasen was developed for a patient with a severe infantile onset epilepsy syndrome caused by variants in *KCNT1*.[35] This RNase H activating ASO was effective in reducing seizure frequency in a first patient, Valeria, as well as a second patient with the same disease for whom treatment was initiated later. However, it became apparent that the ASO also induced hydrocephalus, leading to a decision pursue hospice for the first patient and placement of a shunt for the second patient. Shunt placement relieved the hydrocephalus, but after pausing the ASO treatment, the epileptic seizures returned in this individual. None of the safety studies hinted at the toxicity. Note that safety studies are conducted in healthy rats, and it is conceivable that an interaction between the ASOs in a pathological brain of the patient induced the hydrocephalus. Indeed, it has also been reported in several nusinersen-treated patients and increased intracranial pressure and enlarged ventricles were reported in a clinical trial for Huntington disease patients treated with tominersen.[36,37] Now that hydrocephalus has been reported for valeriasen in individuals with KCNT1-variant-induced pathology, reverse translation experiments have been initiated to develop preclinical safety models that can predict it, so that this information can be used to avoid this toxicity for future ASOs.

A challenge for the N-of-1 treatment development is the lack of infrastructure at several fronts. First, at diagnosis, it is generally not flagged that a pathogenic variant might be treatable by ASOs, and some treatable variants such as cryptic splicing variants, are not routinely screened for. Secondly, processes are needed to ensure eligible patients are identified justly and ethically, based on eligibility. Thirdly,

processes for ASO development and efficacy and safety testing can be further optimized and streamlined. Fourthly, to measure clinical benefit, a toolkit of patient-relevant outcome measures is needed to allow individualized measurements of treatment effects. Fifthly, ideally, catalogues of ASOs are shared – both those that are safe and effective, and those that are not safe or not effective. Sixth, processes to facilitate and streamline clinical implementation within a hospital setting are required. Currently, the administrative burden and the clinical monitoring comes down mainly on the clinician who will treat the patient. Within each center, getting approval to start treatment of the first individual will be the most challenging, as many IRBs are not familiar with the N-of-1 treatment setting. Regardless, support for the clinicians for the time invested and the administrative burden would lighten the workload. The N-of-1 collaborative is working on providing guidance on development and implementation of this missing infrastructure.

Finally, a model to cover the costs of development of individualized ASOs is required. N-lorem currently develops ASOs for free and provides them for free for life. However, this does not cover the costs made by the hospitals and families to allow treatment and management. In other areas, development is currently funded via crowd funding, funding from the government or by individual institutes. While existing efforts is sufficient to provide proof-of-concept for a few patients, there has yet to emerge a clearly sustainable or scalable solution. The individualized ASO development requires innovation and pioneering of the entire drug development and reimbursement model and the IRDRIC N-of-1 taskforce will discuss possible solutions.

5   Concluding Remarks

There is a long way to go to make individualized ASOs and individualized treatments a reality around the world, and likely the process will not be identical in different regulatory jurisdictions and for different therapeutic modalities. It is clear this can only be achieved through sharing knowledge and expertise, successes and failures, and only through the combined effort of academics, patients, and regulators can we make this a reality.

Conflict of Interest

inica. AAR also reports having been a member of the Duchenne Network Steering Committee (BioMarin) and being a member of the scientific advisory boards of Eisai, hybridize therapeutics, silence therapeutics, Sarepta therapeutics. Past SAB memberships: ProQR, Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC. LUMC also received speaker honoraria from PTC Therapeutics, Alnylam Netherlands, Pfizer and BioMarin Pharmaceuticals and funding for contract research from Italfarmaco, Sapreme, Eisai, Galapagos, Synnaffix and Alpha Anomeric. Project funding is received from Sarepta Therapeutics and Entrada.

Funding Information

Acknowledgment

References

(1) Kuijper, E. C.; Bergsma, A. J.; Pijnappel, W.; Aartsma-Rus, A. *J Inherited Metab. Dis.* **2021**, *44*, 72.

(2) Aartsma-Rus, A. *Hum. Gene Ther.* **2023**, *34*, 372.

(3) Duan, D.; Goemans, N.; Takeda, S.; Mercuri, E.; Aartsma-Rus, A. *Nat. Rev. Dis. Primers* **2021**, *7*, 13.

(4) van Deutekom, J. C.; Bremmer-Bout, M.; Janson, A. A.; Ginjaar, I. B.; Baas, F.; den Dunnen, J. T.; van Ommen, G. J. *Hum. Mol. Genet.* **2001**, *10*, 1547.

(5) Pramono, Z. A.; Takeshima, Y.; Alimsardjono, H.; Ishii, A.; Takeda, S.; Matsuo, M. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 445.

(6) Mann, C. J.; Honeyman, K.; Cheng, A. J.; Ly, T.; Lloyd, F.; Fletcher, S.; Morgan, J. E.; Partridge, T. A.; Wilton, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 42.

(7) Wilton, S. D.; Lloyd, F.; Carville, K.; Fletcher, S.; Honeyman, K.; Agrawal, S.; Kole, R. *Neuromuscular Disord.* **1999**, *9*, 330.

(8) Dunckley, M. G.; Manoharan, M.; Villiet, P.; Eperon, I. C.; Dickson, G. *Hum. Mol. Genet.* **1998**, *7*, 1083.

(9) Lu, Q. L.; Mann, C. J.; Lou, F.; Bou-Gharios, G.; Morris, G. E.; Xue, S. A.; Fletcher, S.; Partridge, T. A.; Wilton, S. D. *Nat. Med.* **2003**, *9*, 1009.

(10) Aartsma-Rus, A.; Fokkema, I.; Verschuuren, J.; Ginjaar, I.; van Deutekom, J.; van Ommen, G. J.; den Dunnen, J. T. *Hum. Mutat.* **2009**, *30*, 293.

(11) Wilton, S. D.; Fall, A. M.; Harding, P. L.; McClorey, G.; Coleman, C.; Fletcher, S. *Mol. Ther.* **2007**, *15*, 1288.

(12) Heemskerk, H.; de Winter, C.; van Kuik, P.; Heuvelmans, N.; Sabatelli, P.; Rimessi, P.; Braghetta, P.; van Ommen, G. J.; de Kimpe, S.; Ferlini, A.; Aartsma-Rus, A.; van Deutekom, J. C. *Mol. Ther.* **2010**, *18*, 1210.

(13) Goemans, N. M.; Tulinius, M.; van den Akker, J. T.; Burm, B. E.; Ekhart, P. F.; Heuvelmans, N.; Holling, T.; Janson, A. A.; Platenburg, G. J.; Sipkens, J. A.; Sitsen, J. M.; Aartsma-Rus, A.; van Ommen, G. J.; Buyse, G.; Darin, N.; Verschuuren, J. J.; Campion, G. V.; de Kimpe, S. J.; van Deutekom, J. C. *N. Engl. J. Med.* **2011**, *364*, 1513.

(14) Cirak, S.; Arechavala-Gomeza, V.; Guglieri, M.; Feng, L.; Torelli, S.; Anthony, K.; Abbs, S.; Garralda, M. E.; Bourke, J.; Wells, D. J.; Dickson, G.; Wood, M. J.; Wilton, S. D.; Straub, V.; Kole, R.; Shrewsbury, S. B.; Sewry, C.; Morgan, J. E.; Bushby, K.; Muntoni, F. *Lancet* **2011**, *378*, 595.

(15) Takeshima, Y.; Yagi, M.; Wada, H.; Ishibashi, K.; Nishiyama, A.; Kakumoto, M.; Sakaeda, T.; Saura, R.; Okumura, K.; Matsuo, M. *Pediatr. Res.* **2006**, *59*, 690.

(16) Mendell, J. R.; Rodino-Klapac, L. R.; Sahenk, Z.; Roush, K.; Bird, L.; Lowes, L. P.; Alfano, L.; Gomez, A. M.; Lewis, S.; Kota, J.; Malik, V.; Shontz, K.; Walker, C. M.; Flanigan, K. M.; Corridore, M.; Kean, J. R.; Allen, H. D.; Shilling, C.; Melia, K. R.; Sazani, P.; Saoud, J. B.; Kaye, E. M. *Ann. Neurol.* **2013**, *74*, 637.

(17) Hammond, S. M.; Aartsma-Rus, A.; Alves, S.; Borgos, S. E.; Buijsen, R. A. M.; Collin, R. W. J.; Covello, G.; Denti, M. A.; Desviat, L. R.; Echevarría, L.; Foged, C.; Gaina, G.; Garanto, A.; Goyenvalle, A. T.; Guzowska, M.; Holodnuka, I.; Jones, D. R.; Krause, S.; Lehto, T.; Montolio, M.; Van Roon-Mom, W.; Arechavala-Gomeza, V. *EMBO Mol. Med.* **2021**, *13*, e13243.

(18) Rigo, F.; Chun, S. J.; Norris, D. A.; Hung, G.; Lee, S.; Matson, J.; Fey, R. A.; Gaus, H.; Hua, Y.; Grundy, J. S.; Krainer, A. R.; Henry, S. P.; Bennett, C. F. *J. Pharmacol. Exp. Ther.* **2014**, *350*, 46.

(19) Mortberg, M. A.; Gentile, J. E.; Nadaf, N. M.; Vanderburg, C.; Simmons, S.; Dubinsky, D.; Slamin, A.; Maldonado, S.; Petersen, C. L.; Jones, N.; Kordasiewicz, H. B.; Zhao, H. T.; Vallabh, S. M.; Minikel, E. V. *Nucleic Acids Res.* **2023**, *51*, 7109.

(20) Aartsma-Rus, A. *Nucleic Acid Ther.* **2017**, *27*, 67.

(21) Finkel, R. S.; Mercuri, E.; Darras, B. T.; Connolly, A. M.; Kuntz, N. L.; Kirschner, J.; Chiriboga, C. A.; Saito, K.; Servais, L.; Tizzano, E.; Topaloglu, H.; Tulinius, M.; Montes, J.; Glanzman, A. M.; Bishop, K.; Zhong, Z. J.; Gheuens, S.; Bennett, C. F.; Schneider, E.; Farwell, W.; De Vivo, D. C. *N. Engl. J. Med.* **2017**, *377*, 1723.

(22) (a) Lauffer, M. **2023**, in press. (b) Zardetto, et al., **2023**, in press.

(23) Kim, J.; Hu, C.; Moufawad El Achkar, C.; Black, L. E.; Douville, J.; Larson, A.; Pendergast, M. K.; Goldkind, S. F.; Lee, E. A.; Kuniholm, A.; Soucy, A.; Vaze, J.; Belur, N. R.; Fredriksen, K.; Stojkovska, I.; Tsytsykova, A.; Armant, M.; DiDonato, R. L.; Choi, J.; Cornelissen, L.; Pereira, L. M.; Augustine, E. F.; Genetti, C. A.; Dies, K.; Barton, B.; Williams, L.; Goodlett, B. D.; Riley, B. L.; Pasternak, A.; Berry, E. R.; Pflock, K. A.; Chu, S.; Reed, C.; Tyndall, K.; Agrawal, P. B.; Beggs, A. H.; Grant, P. E.; Urion, D. K.; Snyder, R. O.; Waisbren, S. E.; Poduri, A.; Park, P. J.; Patterson, A.; Biffi, A.; Mazzulli, J. R.; Bodamer, O.; Berde, C. B.; Yu, T. W. *N. Engl. J. Med.* **2019**, *381*, 1644.

(24) Kim, J.; Woo, S.; de Gusmao, C. M.; Zhao, B.; Chin, D. H.; DiDonato, R. L.; Nguyen, M. A.; Nakayama, T.; Hu, C. A.; Soucy, A.; Kuniholm, A.; Thornton, J. K.; Riccardi, O.; Friedman, D. A.; El Achkar, C. M.; Dash, Z.; Cornelissen, L.; Donado, C.; Faour, K. N. W.; Bush, L. W.; Suslovitch, V.; Lentucci, C.; Park, P. J.; Lee, E. A.; Patterson, A.; Philippakis, A. A.; Margus, B.; Berde, C. B.; Yu, T. W. *Nature* **2023**, *619*, 828.

(25) https://www.rnatherapy.nl

(26) Aartsma-Rus, A. *Nat. Med.* **2021**, *27*, 939.

(27) Gleeson, J. G.; Bennett, C. F.; Carroll, J. B.; Cole, T.; Douville, J.; Glass, S.; Tekendo-Ngongang, C.; Williford, A. C.; Crooke, S. T. *Nat. Med.* **2023**, *29*, 1302.

(28) https://www.1mutation1medicine.eu/ (accessed Dec. 13, 2023)

(29) https://www.n1collaborative.org/ (accessed Dec. 13, 2023)

(30) https://irdirc.org/preparing-for-genetic-n-of-1-treatments-of-patients-with-ultra-rare-mutations/ (accessed Dec. 13, 2023)

(31) Aartsma-Rus, A.; manuscript in preparation.

(32) Aartsma-Rus, A.; Garanto, A.; van Roon-Mom, W.; McConnell, E. M.; Suslovitch, V.; Yan, W. X.; Watts, J. K.; Yu, T. W. *Nucleic Acid Ther.* **2023**, *33*, 17.

(33) Aartsma-Rus, A.; van Roon-Mom, W.; Lauffer, M.; Siezen, C.; Duijndam, B.; Coenen-de Roo, T.; Schüle, R.; Synofzik, M.; Graessner, H. *RNA* **2023**, *29*, 446.

(34) Synofzik, M.; van Roon-Mom, W. M. C.; Marckmann, G.; van Duyvenvoorde, H. A.; Graessner, H.; Schüle, R.; Aartsma-Rus, A. *Nucleic Acid Ther.* **2022**, *32*, 83.

(35) https://www.nytimes.com/2022/10/26/health/gene-treatment-epilepsy-antisense-brain.html (accessed Dec. 13, 2023)

(36) Becker, L. L.; Weiß, C.; Tietze, A.; Martiny, V.; Kaindl, A. M. *Neuropediatrics* **2021**, *52*, 219.

(37) Tabrizi, S. J.; Estevez-Fraga, C.; van Roon-Mom, W. M. C.; Flower, M. D.; Scahill, R. I.; Wild, E. J.; Muñoz-Sanjuan, I.; Sampaio, C.; Rosser, A. E.; Leavitt, B. R. *Lancet Neurol.* **2022**, *21*, 645.