Leids Universitair Medisch Centrum

Antisense oligonucleotide therapeutics Opportunities for rare brain disorders

Annemieke Aartsma-Rus

September 2023

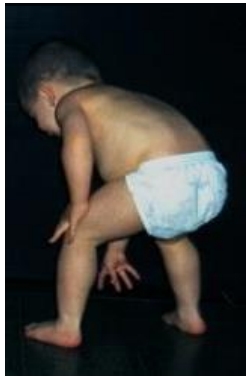
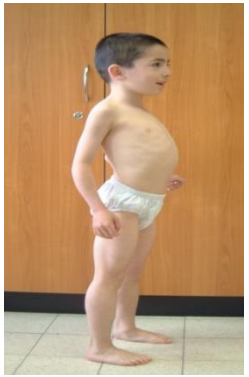
Disclosures

- Employed by LUMC, which has patents on exon skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As co-inventor of some of these patents, I am entitled to a share of royalties
- Ad hoc (past) consultant for PTC Therapeutics, BioMarin Pharmaceuticals Inc., Eli Lilly, Astra Zeneca, Eisai, Sarepta Therapeutics, Takeda, Regenxbio, Splicesense, Dyne, Galapagos, (Alpha Anomeric, Global Guidepoint and GLG consultancy, Grunenthal, Wave and BioClinica). Remuneration paid to LUMC
- Member of the scientific advisory boards of ProQR, Sarepta Therapeutics, Hybridize Therapeutics and Silence Therapeutics. Remuneration paid to LUMC
- LUMC received speaker honoraria from PTC Therapeutics, Alnylam and BioMarin Pharmaceuticals

Learning objectives

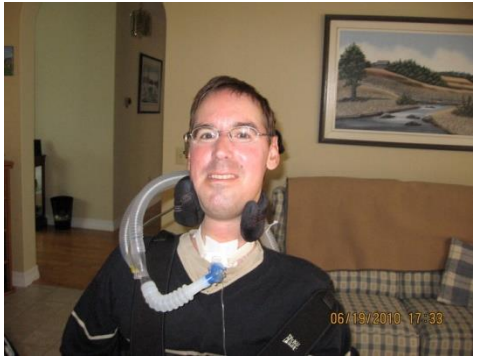
- Showcase that for the most rare cases (individualized treatment) the traditional drug development route is not fit for purpose
- Therapy development for these cases is possible but
    - International collaboration will be crucial to make it work
    - Collaboration with all stakeholders (especially patients) will be crucial
    - This is work in development: pioneering
        - Learn from successes and failures
        - Risk of not treating vs uncertainty of an experimental treatment
        - There are many unsolved issues yet
        - Payment/sustainability

Duchenne Muscular Dystrophy

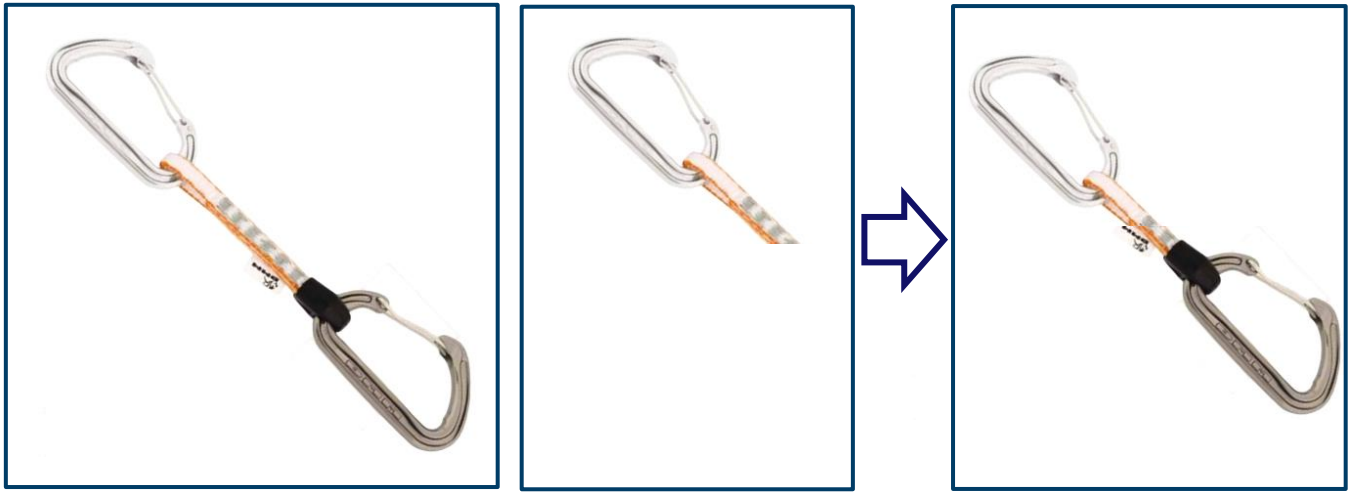










Dystrophin



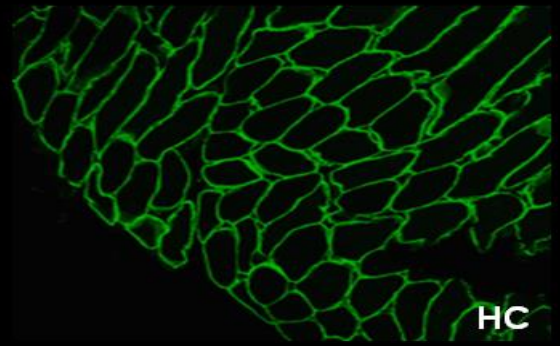
Genetic code unreadable: Duchenne (severe)

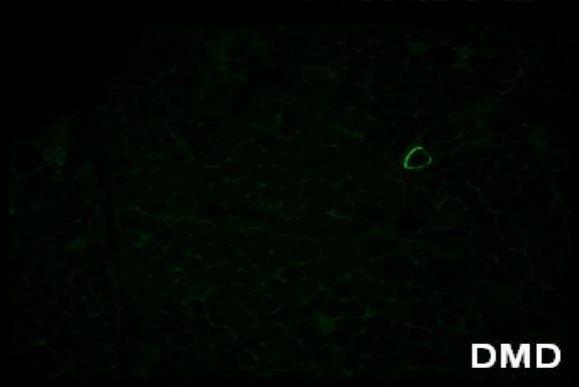
Genetic code readable: Becker (milder)

Exon skipping: allowing Duchenne patients to make Becker-like dystrophins using antisense oligonucleotides (AONs)

Antisense oligonucleotides (AONs or ASOs)

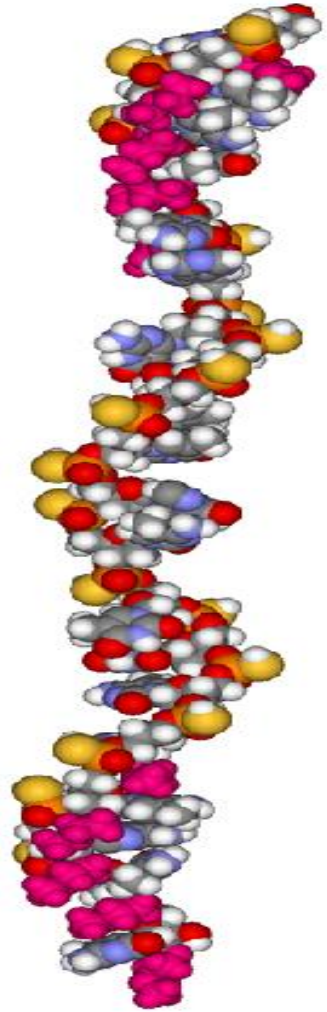
- Small pieces of modified DNA or RNA
  - Chemically modified nucleobases and backbone
  - Synthetically produced
- Bind to RNA in a sequence specific manner via Watson-Crick base-pairing
- Use to
  - Reduce production of toxic protein
  - **Modulate processing of RNA: restore protein production**


HC


DMD

AON delivery

- AON very small (8-12 kDa)
- Filtered out by kidney
- Phosphorothioate modification
  - Serum protein binding
  - Less clearance by kidney
  - Uptake by liver
- Uptake muscle poor



After 23 years of development

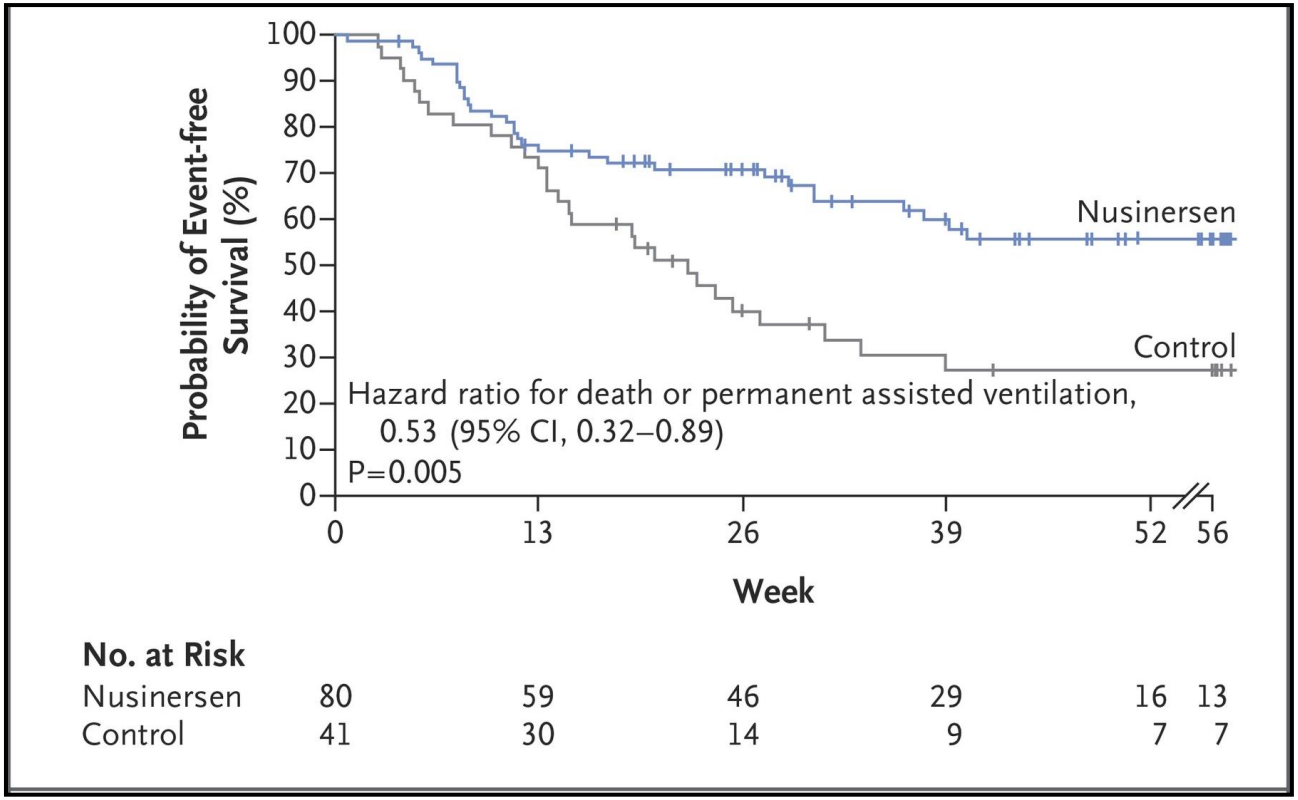
- 4 AONs approved for Duchenne (USA/Japan, not Europe)
  - Exon 51, 53 and 45
- Based on dystrophin restoration (<1-5%)
- Weekly intravenous infusion
- Functional effects not yet clear
- Confirmatory trials ongoing to evaluate this
- Largest challenge: efficient delivery to skeletal muscle and heart

Delivery to brain

- Delivery to CNS and eye (local treatment)
  - Low doses
  - Systemic exposure low, local exposure high
- Treatment frequency low

Spinal muscular atrophy: nusinersen

Type 1 SMA: event free survival



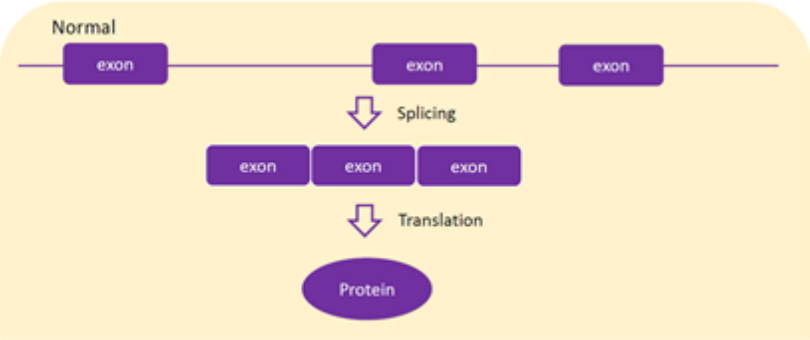
Summary RNA therapy for muscle vs brain

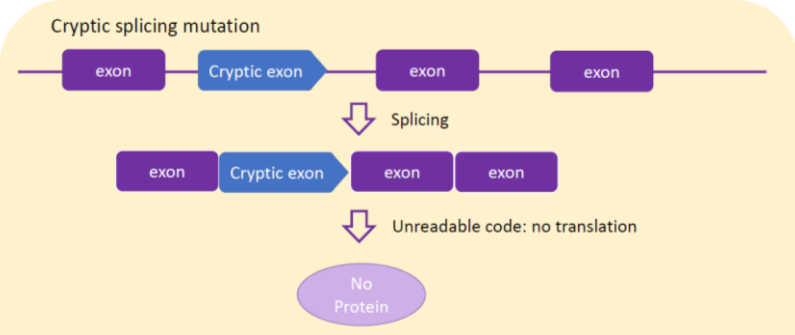
- AONs for skeletal muscle very ineffective
  - Weekly intravenous infusions
  - High dose
- Delivery to central nervous system
  - 3 maintenance doses per year, intrathecal
  - Very low dose

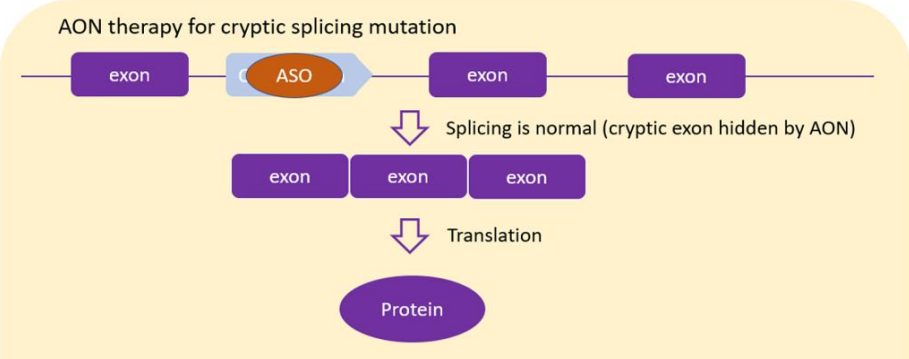
Opportunities for exon skipping with local treatment

- Delivery to CNS and eye (local treatment)
  - Low doses
  - Systemic exposure low, local exposure high
- Treatment frequency low
- One mutation type perfect candidate: cryptic splicing mutation
  - Restore normal protein
- But unattractive to Industry
  - Numbers too low, sometimes even single individuals

Cryptic splicing mutations







Milasen: the ultimate personalized medicine

1 year

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

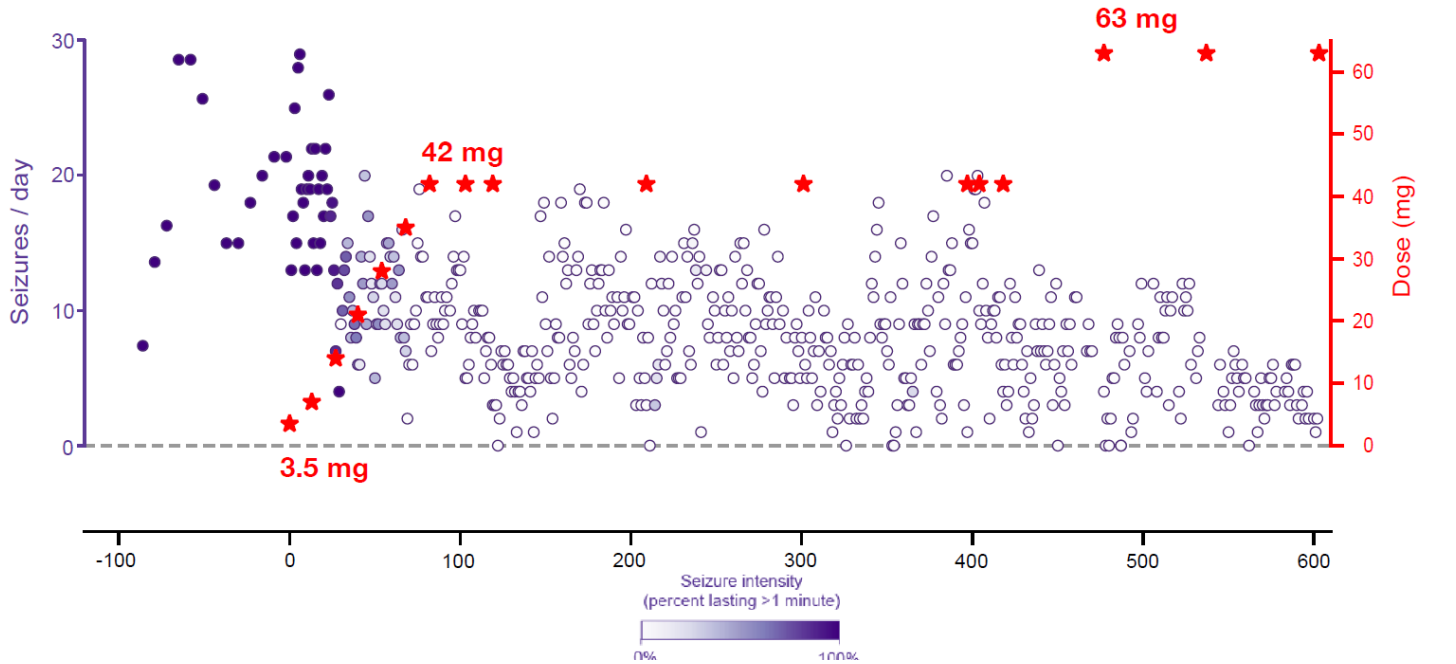
Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease

- Genetic diagnosis
- AON design
- Tests in fibroblasts
- FDA discussion: rat tox
- Investigational new drug application
- First treatment

Milasen treatment effects

DCRT Dutch Center for RNA Therapeutics

Milasen treatment: first two years



The Dutch Center for RNA Therapeutics

- The Dutch Center for RNA Therapeutics (DCRT) is a non-profit consortium
- Aim is to develop tailor-made RNA therapy for patients with ultrarare genetic mutations focused on eye and central nervous system disorders
- Please reach out to DCRT@lumc.nl for any questions.

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For whom and how?

- Mutations must be very rare ((close to) unique)
- Exon skipping must restore protein function
- Target tissue: brain or eye
- Patient are expected to have benefit of treatment
- Patient must be willing to undergo experimental treatment
- Launched Feb 29 2020

Interactions

- European Medicines Agency and MEB (Dutch EMA)
- Zorginstituut Nederland (ZIN, health technology agency)
- Patients and patient organisations
- Hospital pharmacy/ Academic Pharma
- Ministry of Health
- Many others

Lessons from many zoom meetings

- Named patient setting: no regulatory approval

  →We are communicating with regulators for advice

- Hospital pharmacy preparation of GMP-like compound
- Preclinical studies and safety studies as much as possible in-house in GLP-like setting
- Keep costs as low as possible and be much faster
- Collect natural history during development of ASO

Traditional drug approval process

Drug design and discovery

Clinical trials

Target validation

~1.5 year

Compound screening

~1.5 year

Lead optimization

~1.5 year

Pre-clinical test

~1 year

Phase I

~1.5 years

Phase II

~2.5 years

Phase III

~2.5 years

Approval to launch

~1.5 year

Patient specific N-of-1 treatment

Pre-clinical test

N-of-1 design

0.1 year

***In vitro* Efficacy studies**

~0.5 year

***In vivo* & *in vitro* safety studies**

<0.5 year

Manu-facturing

<0.5 year

Treatment and monitoring

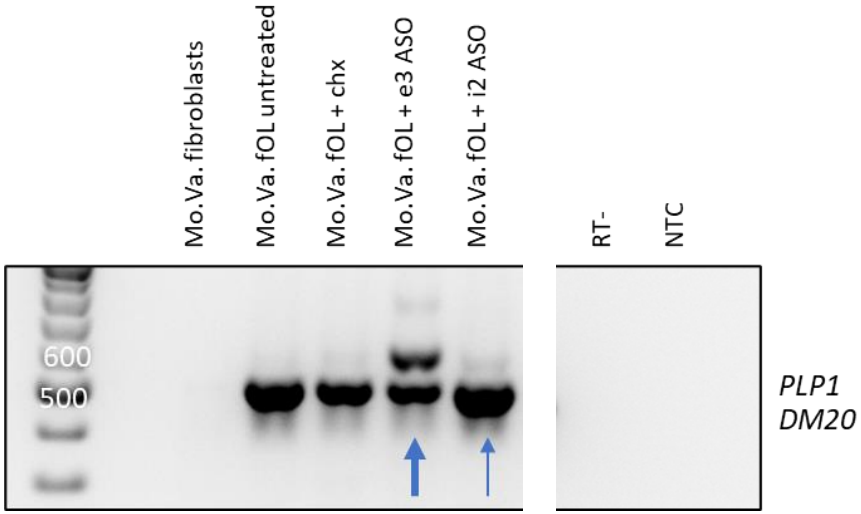
Natural history

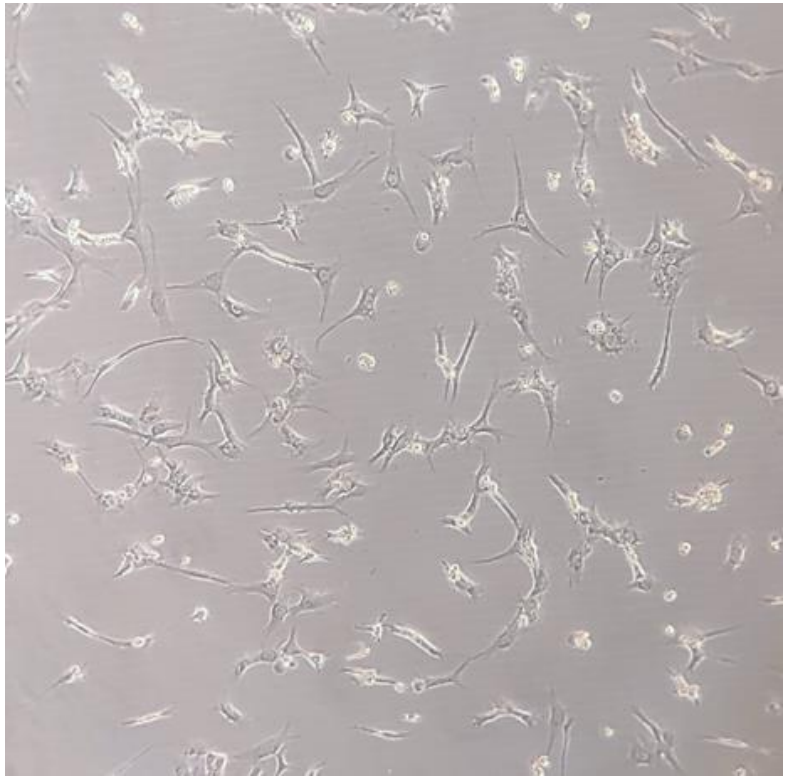
Treatment effect

Examples of currently ongoing projects

- Studying a patient with a demyelinating disease
  - *PLP1* mutation: change in normal splicing
  - AONs designed
  - Currently being tested in patient cells
- Studying 2 patients with Stargardt disease (Radboudumc), 11 more candidates identified
  - Lead candidate identified
  - Preparing for treatment (manufacturing and clinical implementation)

PLP1 exon skipping





Global Collaborations

DCRT Netherlands

1 Mutation 1 Medicine Europe

N=1 collaborative (www.n1collaborative.org/) worldwide

Preparing for genetic N-of-1 treatments of patients with ultra-rare mutations

IRDiRC - Preparing for genetic N-of-1 treatments of patients with ultra-rare mutations

- Annemieke Aartsma-Rus – Leiden University Medical Center, The Netherlands (Co-Chair)
- Anneliene Jonker – University of Twente, The Netherlands (Co-Chair)
- Daniel O'Connor – Medicines and Healthcare products Regulatory Agency (MHRA), UK (Co-Chair)
- PJ Brooks – NCATS/NIH, USA
- Timothy Yu – Harvard Medical School, USA
- Erika Augustine – The Johns Hopkins University School of Medicine, USA
- Adam Jaffe – UNSW Sydney, Australia
- Alison Bateman-House – NYU Grossman School of Medicine, USA
- Julie Douville – n-Lorem Foundation, Canada
- David Dimmock – Creyon Bio, USA
- Larissa Lapteva – FDA, USA
- Marjon Pasmooij – Dutch Medicines Evaluation Board, The Netherlands
- Matthis Synofzik – Hertie Institute for Clinical Brain Research and Center of Neurology, University of Tubingen, Germany
- Oxana Iliach – Certara, Canada
- Ralf-Dieter Hilgers – Medizintechnisches Zentrum (MTZ), Germany
- Virginie Hivert – EURORDIS, France
- Gareth Baynam – Rare Care Centre/Perth Children's Hospital, Australia
- Holm Graessner – European Reference Network for Rare Neurological Diseases (ERN-RND), Germany
- James Davies – Weatherall Institute of Molecular Medicine, Oxford University, UK
- Jill Morris – NINDS/NIH, USA
- Rich Horgan – Cure Rare Disease, Inc., USA
- Shruti Mitkus – Global Genes, USA

Eligible diseases: Gene list

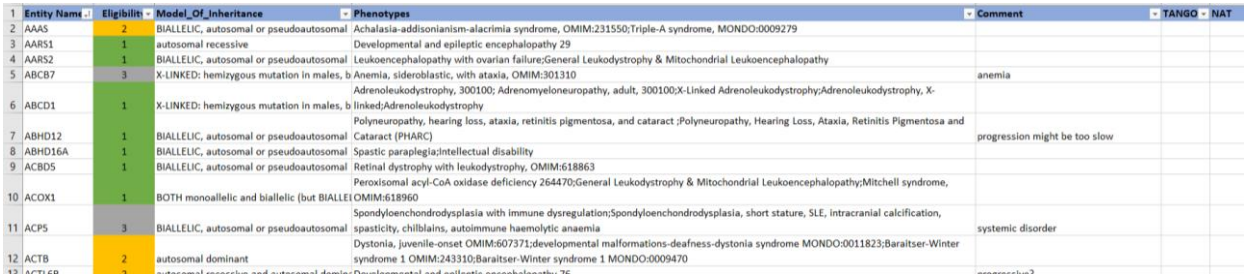
DCRT Dutch Center for RNA Therapeutics

Which diseases are eligible for RNA therapy at DCRT?

rnatherapy.nl

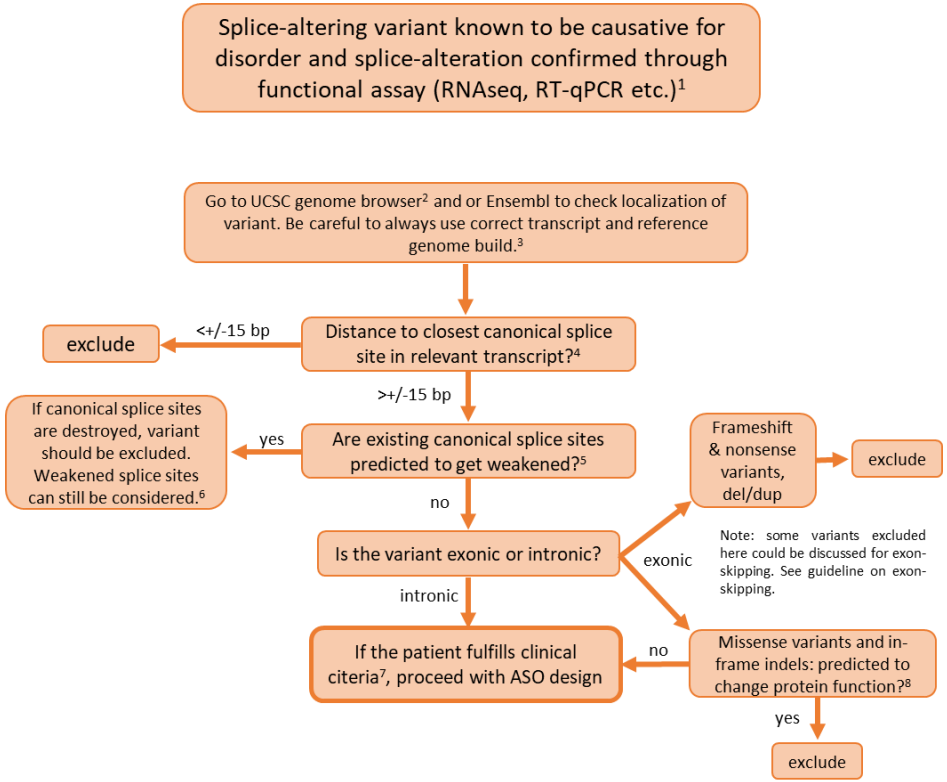
We focus on progressive genetic diseases that affect tissues of the brain or the eye.
In this list, you will find the genes of interest that we have identified so far. The list is non-exhaustive so please contact us if there are other mutations that we are not aware of, or if you want to know more about RNA therapy. Feel free to send your questions to DCRT@LUMC.nl.

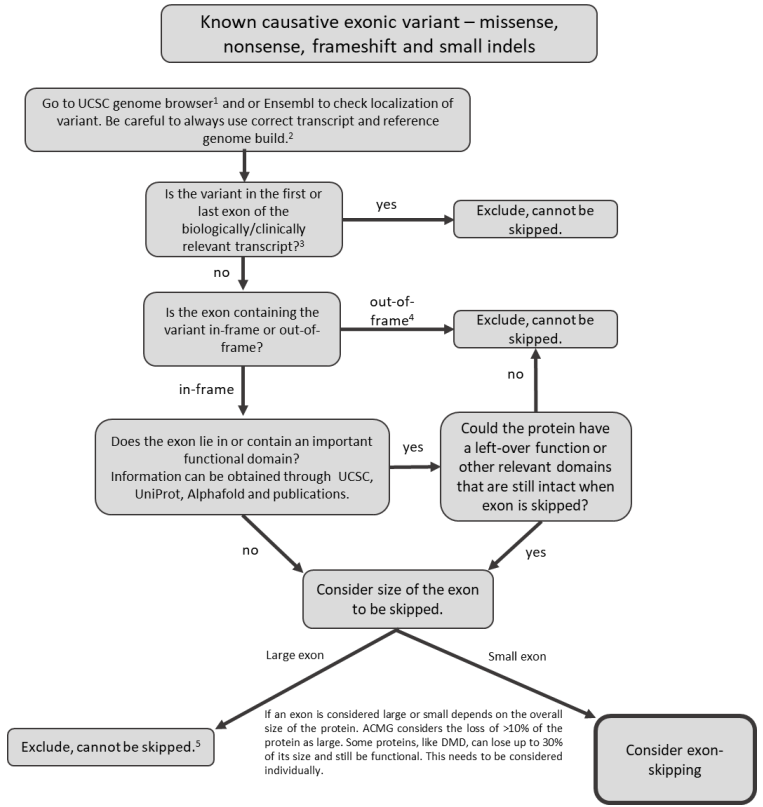
- Initially evaluated ca. 700 genes for their eligibility
- Currently expanding to >2,000 genes
- Selected genes and diseases are based on the Genomics England Panel App and HPO terms



	Entity Name	Eligibility	Model_Of_Inheritance	Phenotypes	Comment	TANGO	NAT
2	AAAS	2	BIALLELIC, autosomal or pseudoautosomal	Achalasia-addisonianism-alacrimia syndrome, OMIM:231550;Triple-A syndrome, MONDO:0009279			
3	AARS1	1	autosomal recessive	Developmental and epileptic encephalopathy 29			
4	AARS2	1	BIALLELIC, autosomal or pseudoautosomal	Leukoencephalopathy with ovarian failure;General Leukodystrophy & Mitochondrial Leukoencephalopathy			
5	ABCB7	3	X-LINKED: hemizygous mutation in males, b	Anemia, sideroblastic, with ataxia, OMIM:301310	anemia		
6	ABCD1	1	X-LINKED: hemizygous mutation in males, b	Adrenoleukodystrophy, 300100; Adrenomyeloneuropathy, adult, 300100;X-Linked Adrenoleukodystrophy;Adrenoleukodystrophy, X-linked;Adrenoleukodystrophy			
7	ABHD12	1	BIALLELIC, autosomal or pseudoautosomal	Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract ;Polyneuropathy, Hearing Loss, Ataxia, Retinitis Pigmentosa and Cataract (PHARC)	progression might be too slow		
8	ABHD16A	1	BIALLELIC, autosomal or pseudoautosomal	Spastic paraplegia;Intellectual disability			
9	ACBD5	1	BIALLELIC, autosomal or pseudoautosomal	Retinal dystrophy with leukodystrophy, OMIM:618863			
10	ACOX1	1	BOTH monoallelic and biallelic (but BIALLEI	Peroxisomal acyl-CoA oxidase deficiency 264470;General Leukodystrophy & Mitochondrial Leukoencephalopathy;Mitchell syndrome, OMIM:618960			
11	ACP5	3	BIALLELIC, autosomal or pseudoautosomal	Spondyloenchondrodysplasia with immune dysregulation;Spondyloenchondrodysplasia, short stature, SLE, intracranial calcification, spasticity, chilblains, autoimmune haemolytic anaemia	systemic disorder		
12	ACTB	2	autosomal dominant	Dystonia, juvenile-onset OMIM:607371;developmental malformations-deafness-dystonia syndrome MONDO:0011823;Baraitser-Winter syndrome 1 OMIM:243310;Baraitser-Winter syndrome 1 MONDO:0009470			

Eligible variants: challenging → trying to automate





1M1M – 1 mutation, 1 medicine

Annemieke Aartsma-Rus

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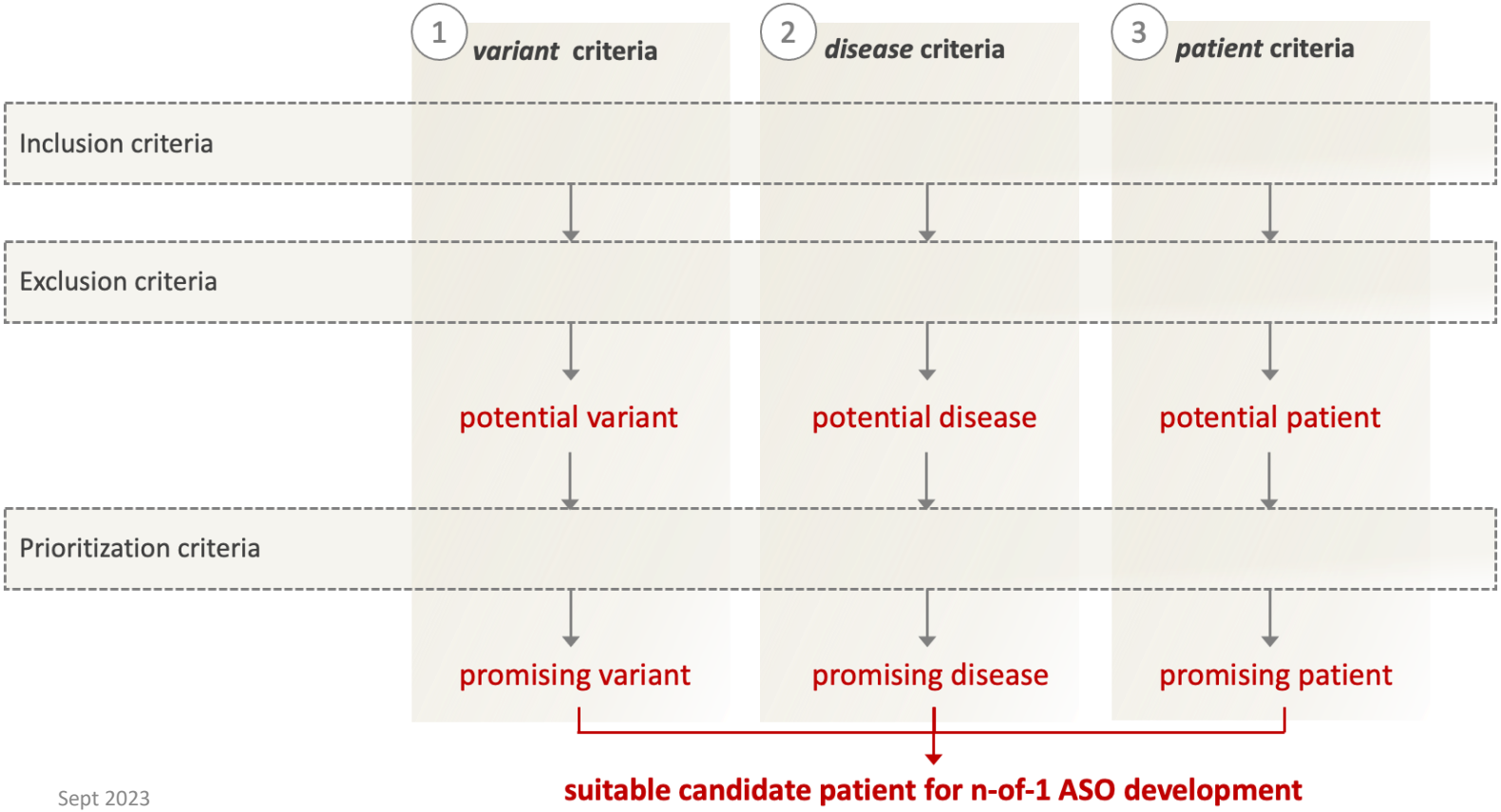
Rebecca Schüle

Lessons from 1M1M: Patient selection



1M1M processes:
The 1M1M pathway towards individual patient selection and treatment decisions

1. Case Dossier

Patient data sufficient for gene group?

- Submitted by clinician
- Completeness check secretariat UT

2. Gene Group Meeting

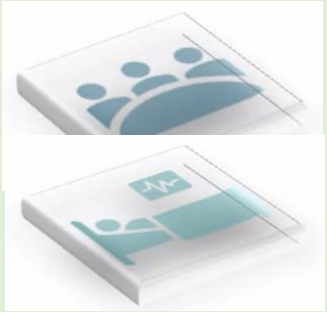
Patient and disease information enough to decide on treatment readiness?

Meeting with

- Submitting clinician
- Clinical and research leads UT & LUMC
- Clinical disease experts
- ASO biologists
- Ad hoc domain experts as needed
- 1M1M secretariat UT

3. Treatment Board meeting

Decide on start/stop development
Decide on start/stop treatment



Meeting with

- Submitting clinician
- Clinical and research leads UT & LUMC
- PI representatives of gene group (1-2)
- External experts (N1C)
- Patient organisation representative
- Ethicist
- Disease domain experts as needed
- Independent clinical experts as needed
- ASO biologists as needed
- 1M1M secretariat UT

Yes/
No

PERSPECTIVE

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

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10200189/

Global collaboration

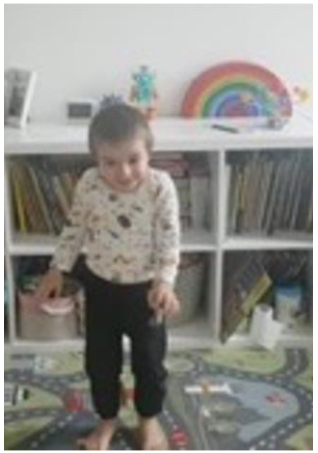

Tim Yu


Matthis Synofzik


Rebecca Schüle

Example AON design pipeline: ATM - already *first-in-human*

Fig. 2. Strategy for rescuing abnormal splicing induced by the c.7865C>T mutation, employing steric blockade by a splice-switching antisense oligonucleotide.



1. subject #1: Ipek: 3 years treated in Boston since 2018, on AON maintenance dose
2. subject #2: P.K: 4 years, transferred from Tübingen to Boston in 2021 for treatment, AON dose escalation phase, treatment continuation in Tübingen from Sept 2022 on

Sept 2023

Target ATM mutation: c.7865 C>T, p. Ala2622Val⇒ generates a cryptic exonic splice donor site

The New York Times

Gene Treatment for Rare Epilepsy Causes Brain Side Effect in 2 Children

The side effect, a buildup of fluid in the brain, led to the death of one of the children and presents a grave setback for a class of personalized medicine.

Give this article

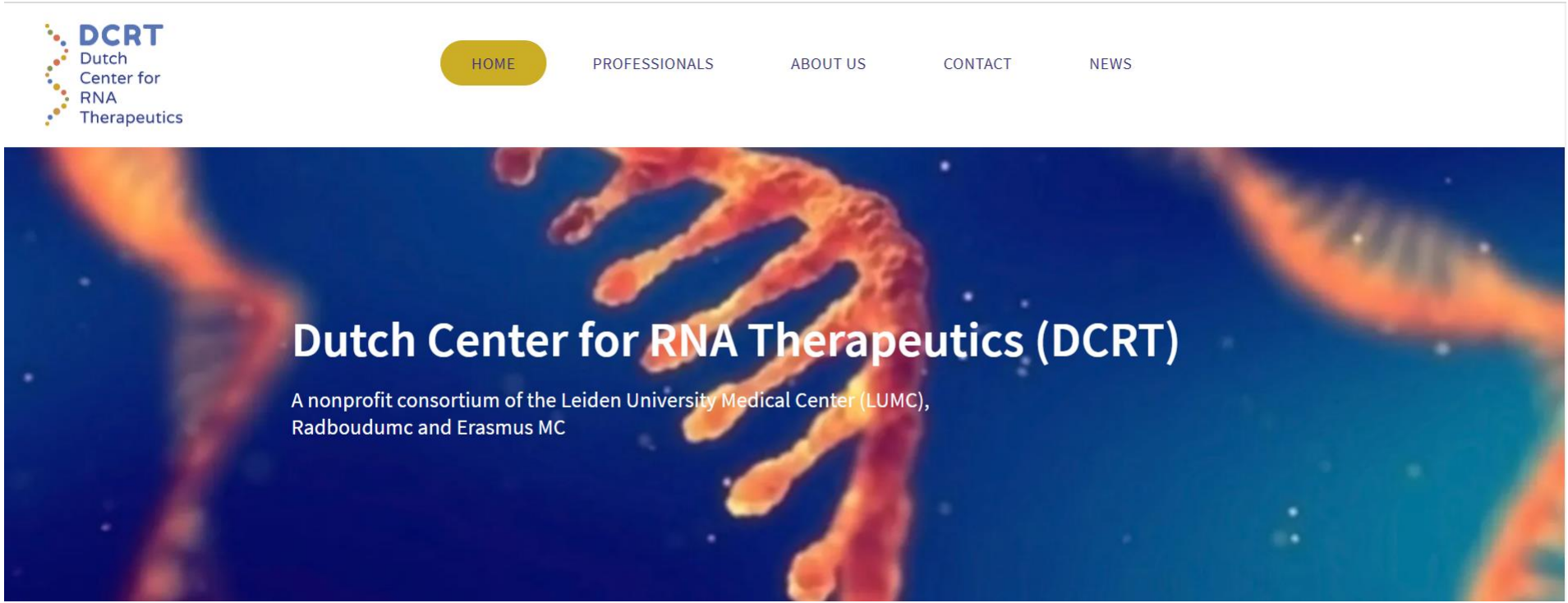
N=1 COLLABORATIVE

Tim Yu

Summary

- AONs offer unique opportunities for mutation specific treatments
- N-of-1 AON development and treatment is feasible for brain diseases (and likely eye diseases)
- While the scientific context is different for each mutation/disease/patient, there are common processes
- National, European and global efforts to align and facilitate as much as possible for AONs and other treatments
- Need multistakeholder involvement and transparency

Want to know more? www.rnatherapy.nl



Acknowledgements

Annemieke-Aartsma Rus
Willeke van Roon-Mom
Bianca Zardetto
Anouk Spruit
Pauline de Graaf
Frank Baas
Camiel Boon
Tineke Coenen-de Roo
Hermine van Duyvenvoorde
Teun van Gelder
Henk-Jan Guchelaar
Gre Luyten
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Erik Niks
Gijs Santen
Jan Verschuuren

Rob Collin

Carel Hoyng
Alejandro Garanto
Erwin van Wijk
Jeroen Pas
Ronald Pennings
Edwin van Oosten
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Franca Hartgers
Frans Cremers
Susanne Roosing

Ype Elgersma

Edwin Mientjes
Annelot van Esbroeck-Weevers
Claudia Milazzo
Emilio Harris-Mostert
Rosan Lechner