Leiden University Medical Center

DCRT Dutch Center for RNA Therapeutics

Developing mutation-specific splice switching ASOs for ultra-rare and private ataxias (EU)

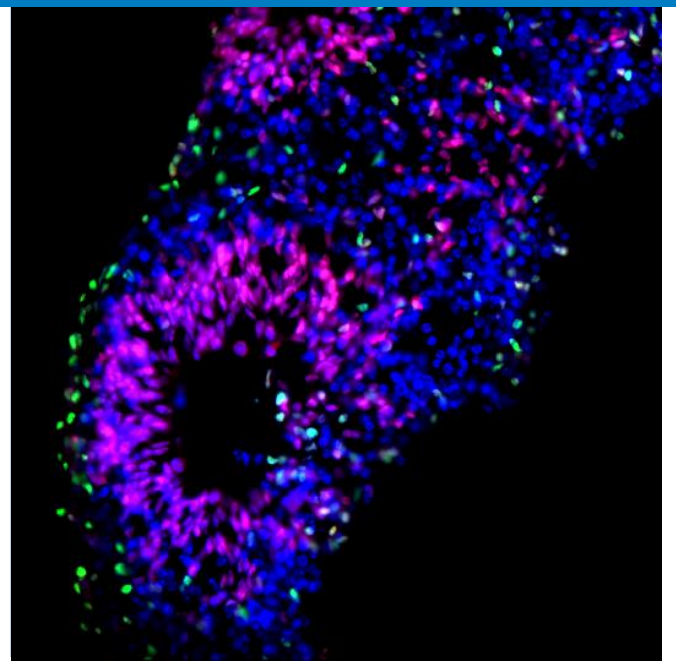
Prof.dr. Willeke van Roon-Mom

Department Human Genetics

Leiden University Medical Centre

The Netherlands

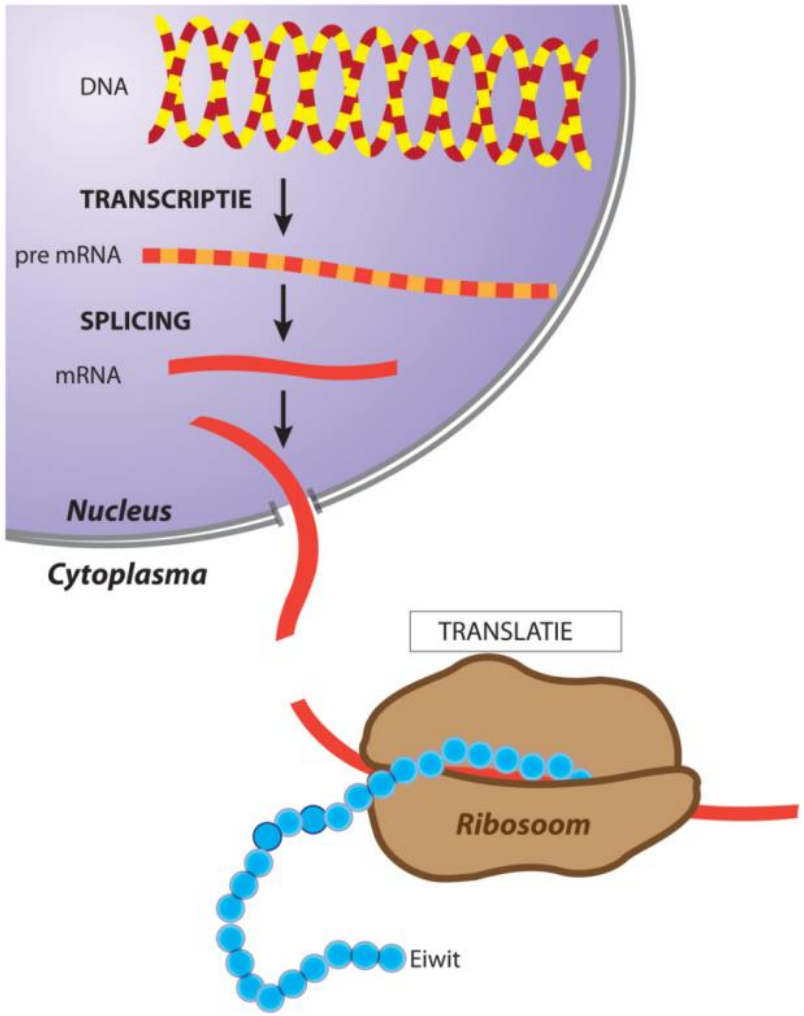
Co-director DCRT (RNAtherapy.nl)



www.neurodlableiden.com

www.RNAtherapy.nl

From genes to proteins – RNA targeting therapies



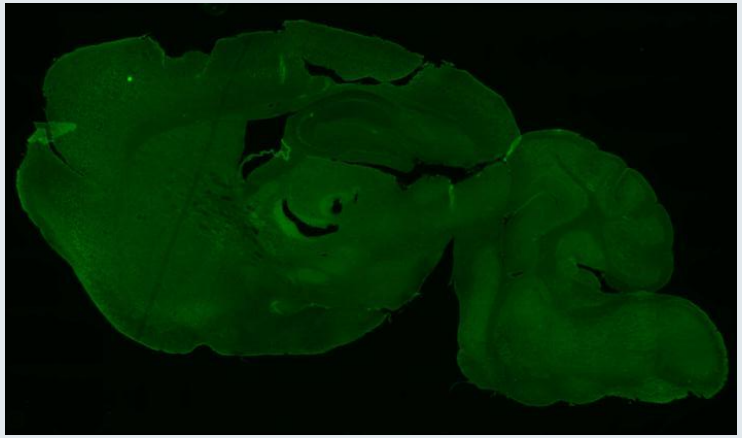
RNA targeting therapies

- ssRNA/DNA molecules
- Transient modification
- Repeated delivery
- Very efficient uptake in all brain cells
- High target specificity

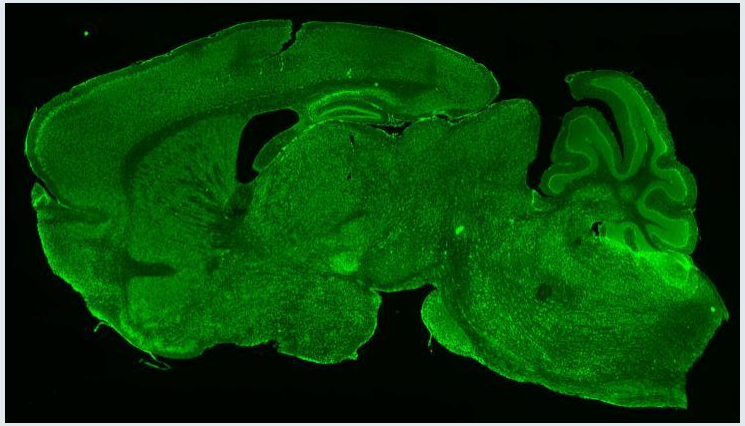
ASO distribution in mouse brain and brain organoids

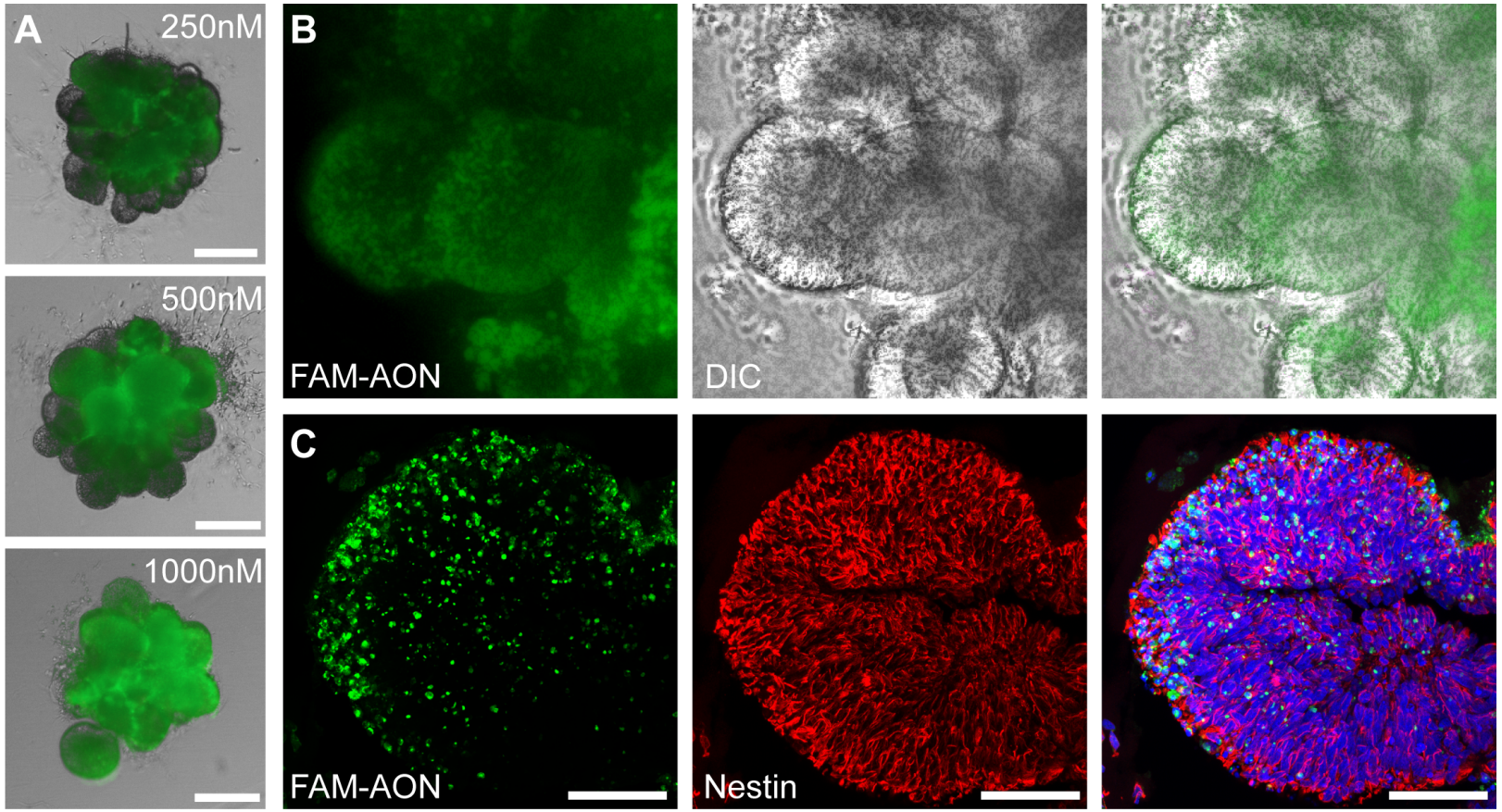
Local delivery - Local high dose -Effect long lasting - Months

PBS treated:

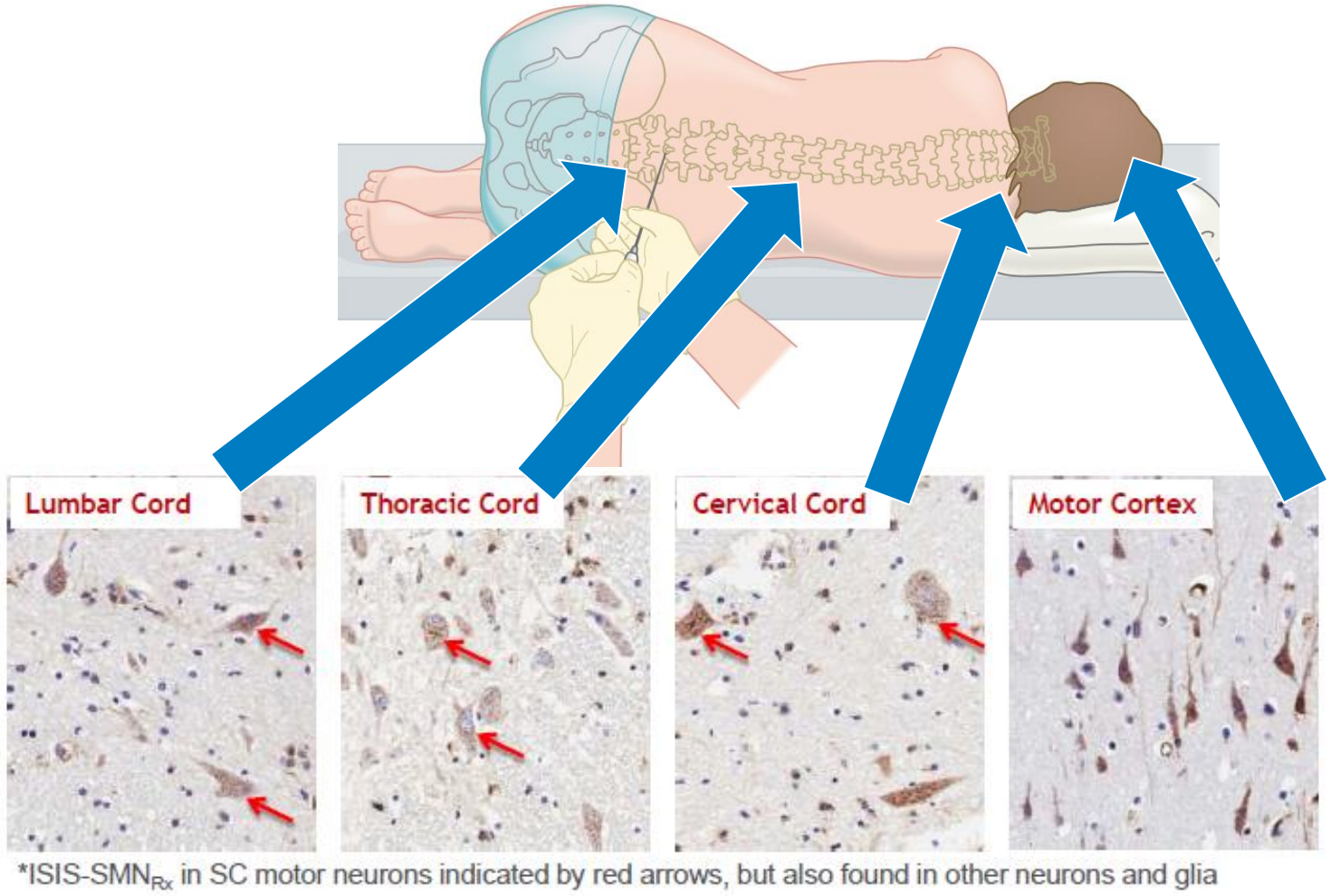


Ataxin-3 AON treated:





ASO distribution after intrathecal delivery humans



*ISIS-SMN$_{Rx}$ in SC motor neurons indicated by red arrows, but also found in other neurons and glia

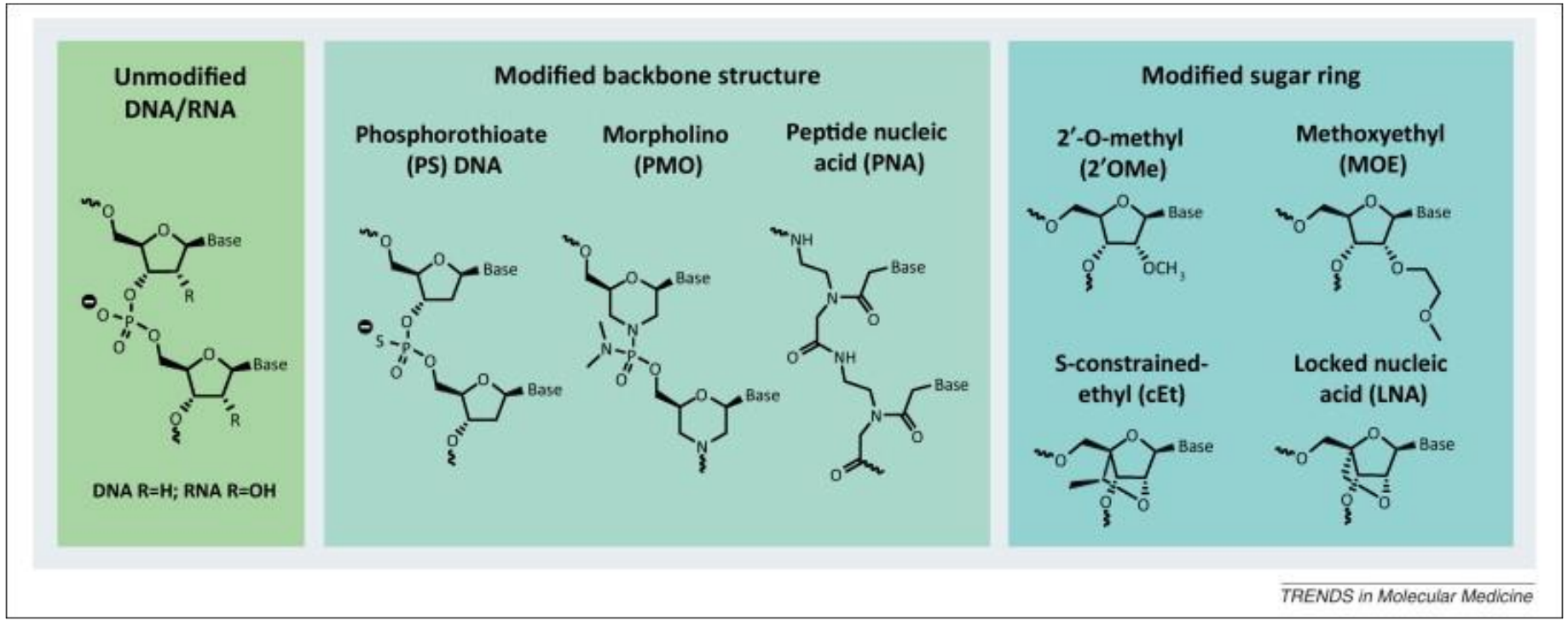
Repeated intrathecal bolus injections in children with SMA type I

R. Finkel, Nemours Children's Hospital

Antisense oligonucleotide modifications

Target RNA cleaved by RNAse H

Non-degradation mechanisms

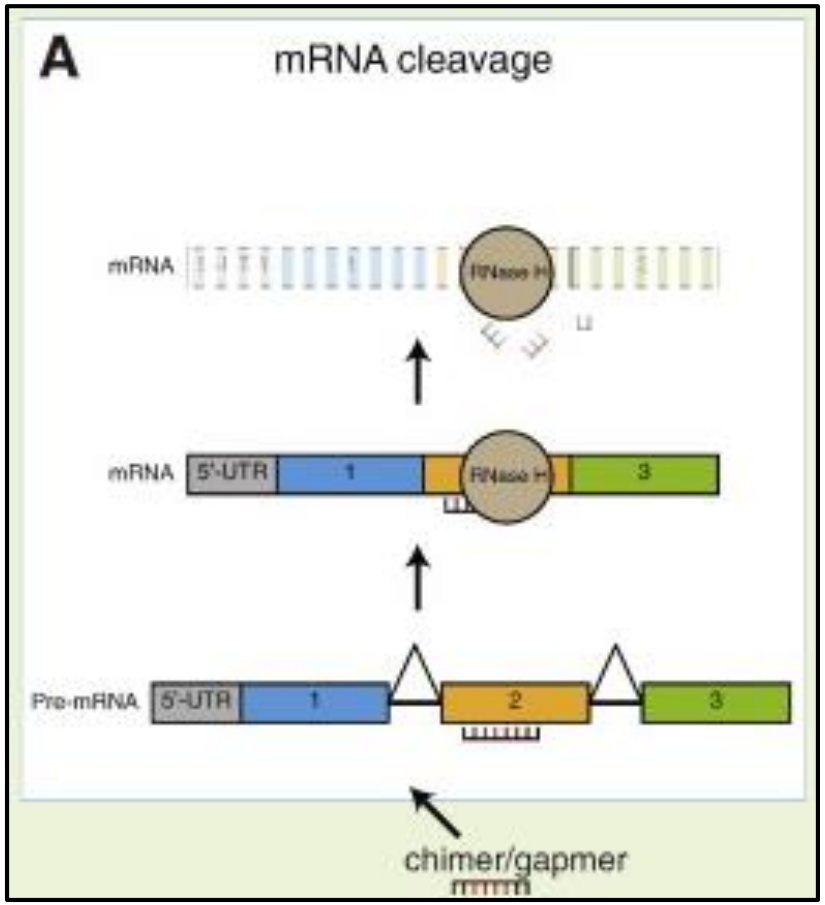


increased tissue half-life

increased target binding affinity

Trends Mol Med. 2012,18(11):634-43

Gapmer antisense oligonucleotide – RNA degradation



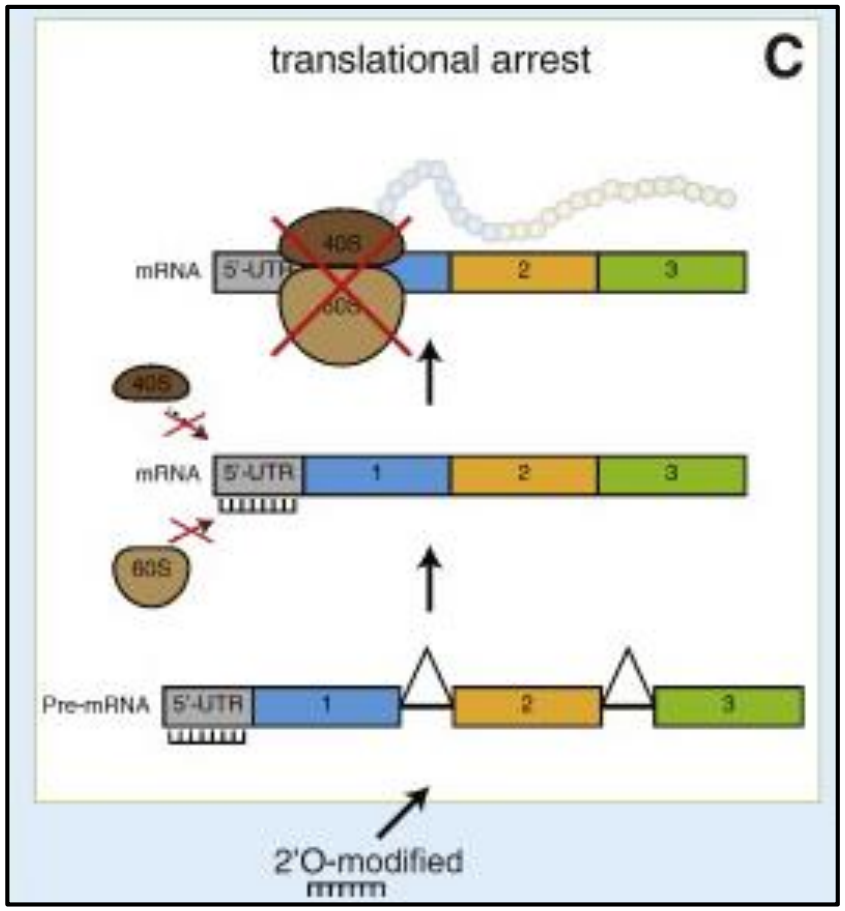
SCA3 NCT05160558 Phase I clinical study Biogen

For all PolyQ SCAs this has been shown to be effective in preclinical studies

Reduction of both wild type and mutant transcripts

No FDA/EMA approved Gapmer ASOs for neurological disorders

Allele ‘specific’ reduction of mutant protein



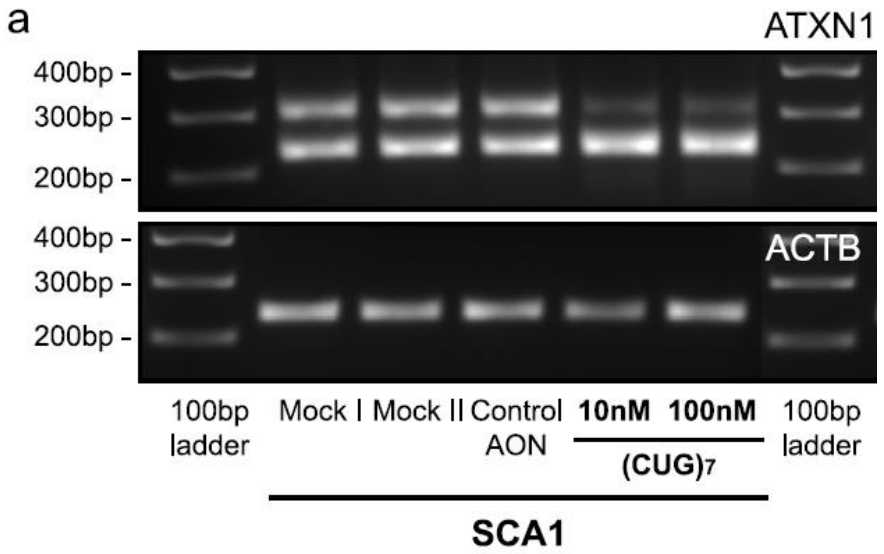
OPEN ACCESS Freely available online

PLoS one

Targeting Several CAG Expansion Diseases by a Single Antisense Oligonucleotide

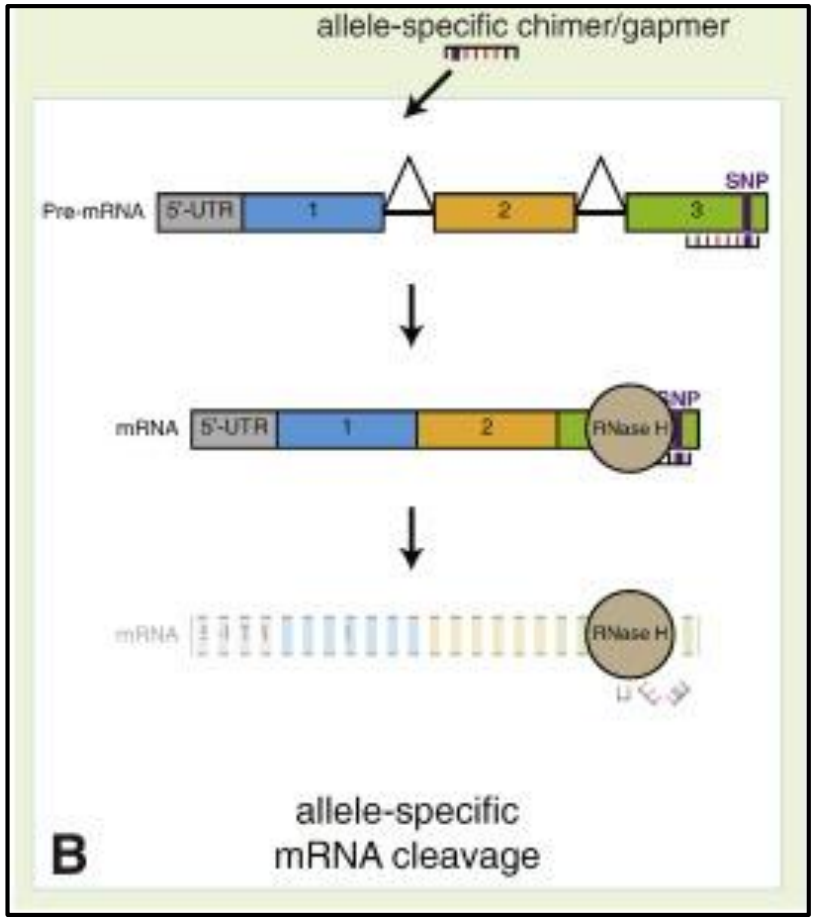
Melvin M. Evers[1], Barry A. Pepers[1], Judith C. T. van Deutekom[2], Susan A. M. Mulders[2], Johan T. den Dunnen[1,3], Annemieke Aartsma-Rus[1], Gert-Jan B. van Ommen[1], Willeke M. C. van Roon-Mom[1]*

1 Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, **2** Prosensa Therapeutics B.V., Leiden, The Netherlands, **3** Leiden Genome Technology Center, Leiden University Medical Center, Leiden, The Netherlands



VICO Therapeutics

Allele specific reduction of mutant RNA

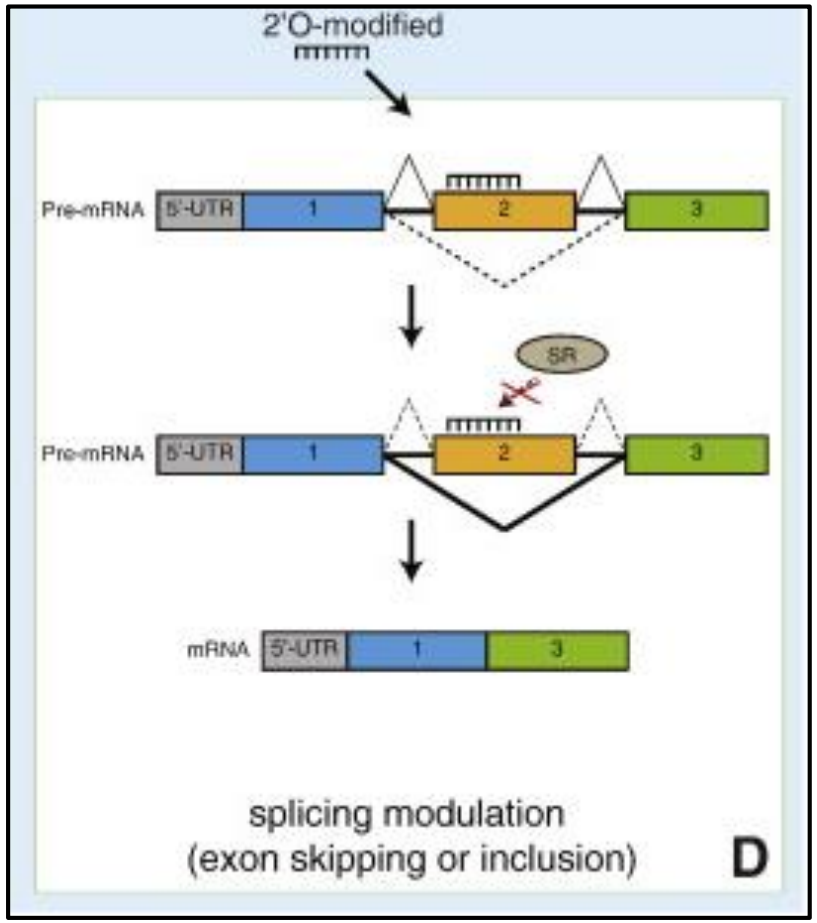


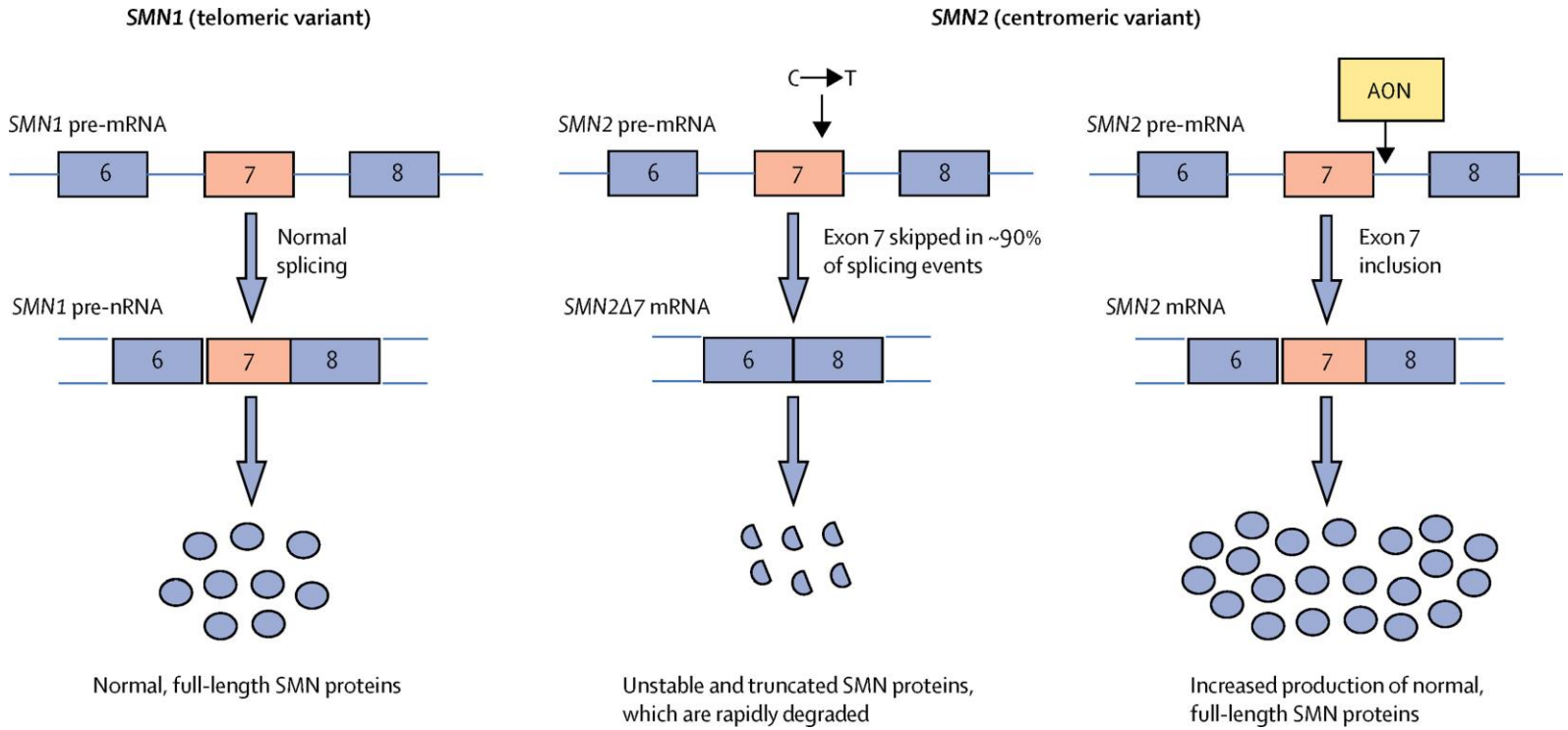
Allele specific knock down of mutant ataxin-3 through SNP specific Gapmer ASO (Hauser et al 2022)

Wave Life Science – preclinical stages for SCA3

No FDA/EMA approved Gapmer ASOs for neurological disorders

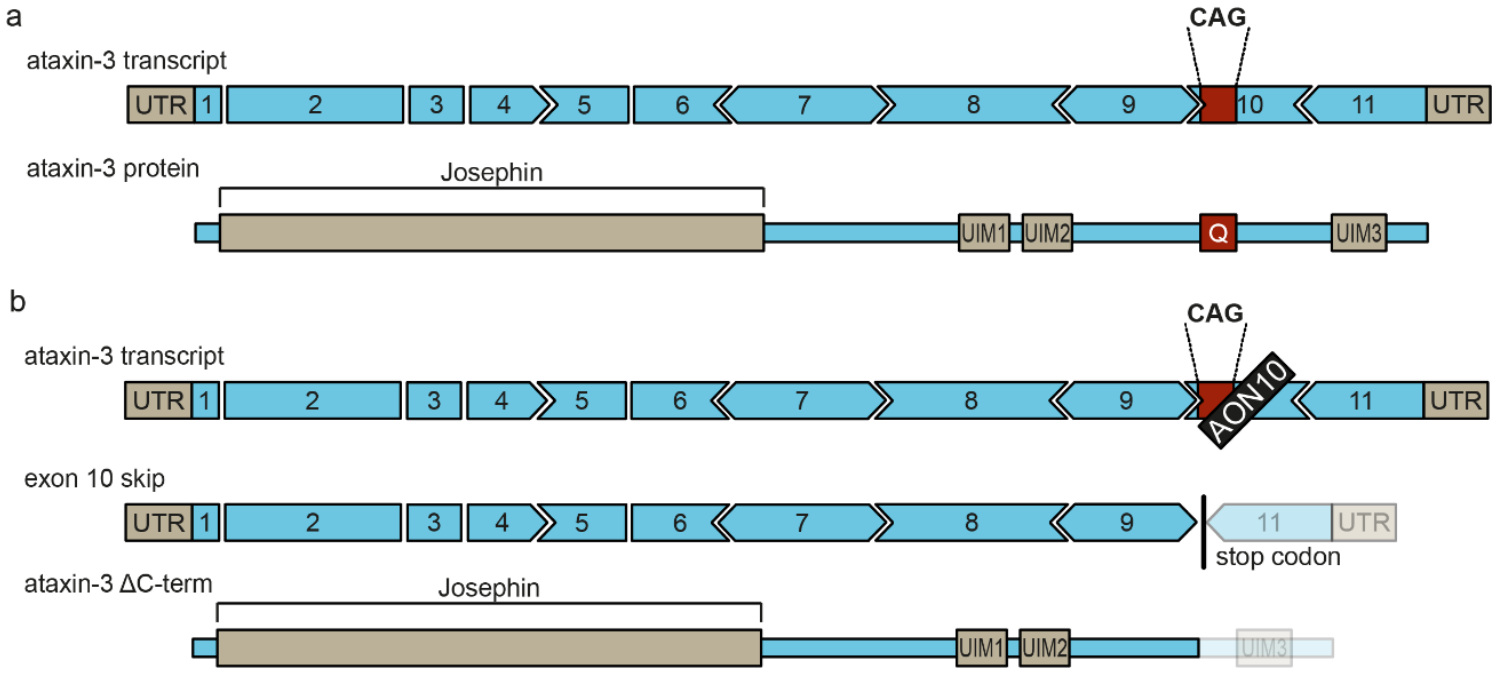
Antisense oligonucleotide mechanism of action



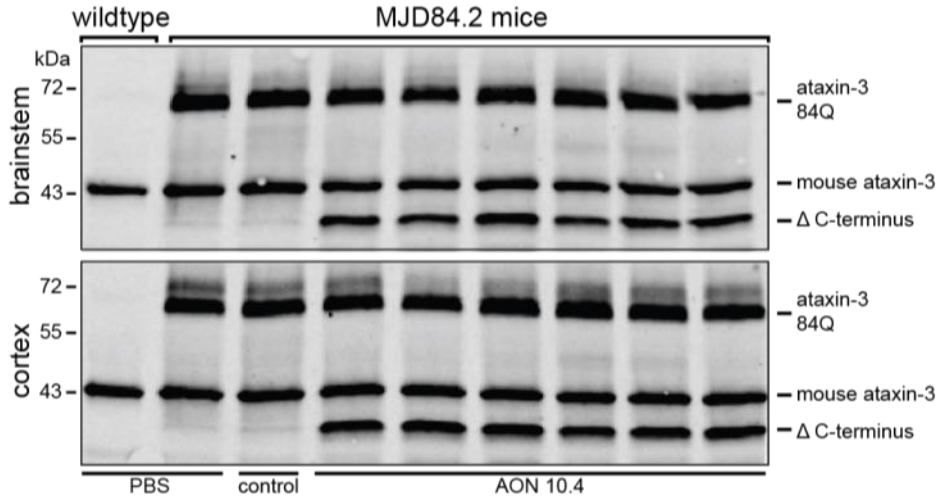


Splice modulating ASO, only approved AON by FDA and EMA for neurological disorders

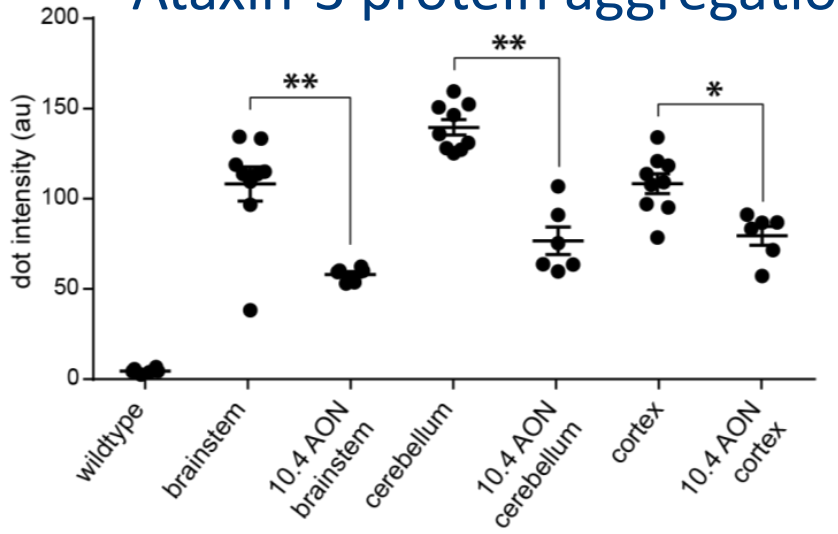
Ataxin-3 splice modulation in MJD84.2 mice



Ataxin-3 protein modification



Ataxin-3 protein aggregation



Toonen et al Mol Ther Nucleic Acids. 2017

Milasen: the ultimate personalized medicine

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease

1 year

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

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 the central nervous system disorders
- DCRT@lumc.nl for any questions.

Annemieke Aartsma-Rus

LUMC

Rob Collin

Radboudumc

Willeke van Roon-Mom

LUMC

Ype Elgersma

Erasmus MC

Anouk Spruit

LUMC

Marlen Lauffer

LUMC

1M1M – 1 mutation, 1 medicine

Academically driven European platform for the development and implementation of RNA Therapies for ultra-rare diseases

coordination: University of Tübingen (DE) + University of Heidelberg (DE) + University Medical Center Leiden (NL)

Annemieke Aartsma-Rus

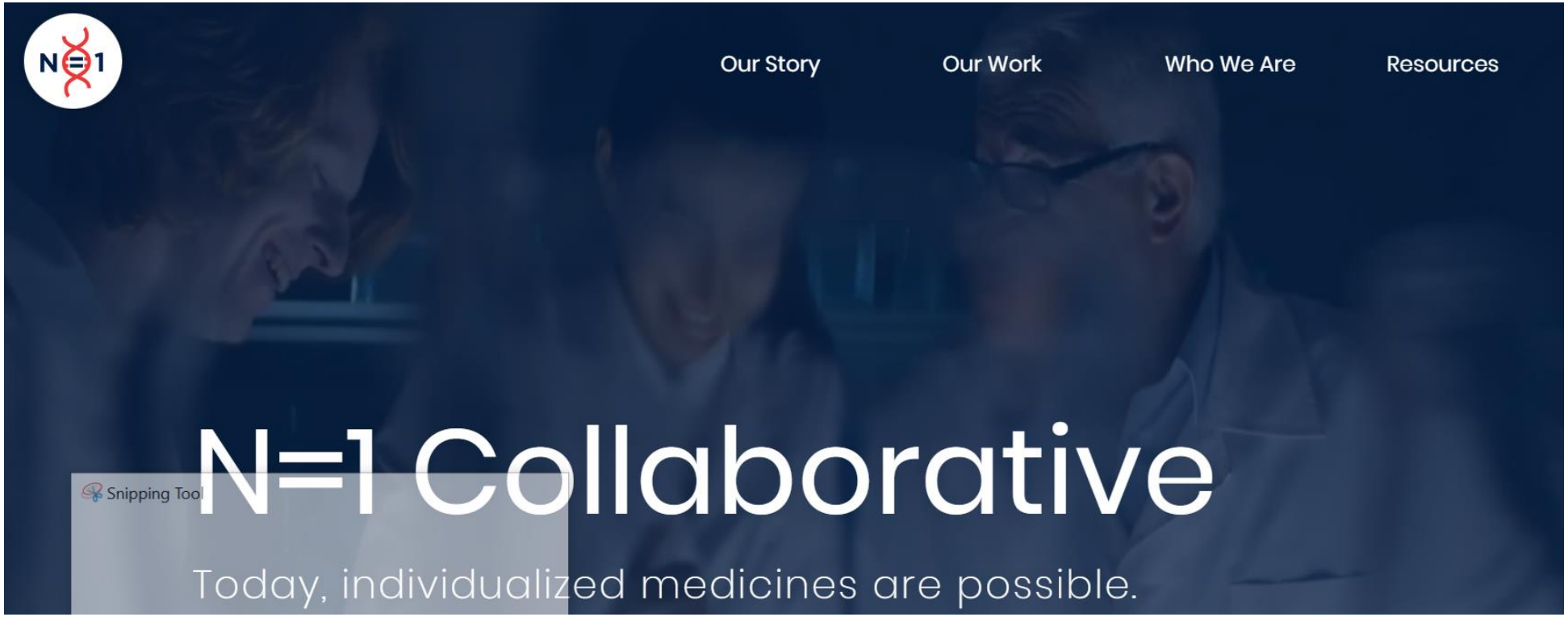
Willeke van Roon-Mom

Holm Graessner

Matthis Synofzik

Rebecca Schüle

N1C – n-of-1 collaborative



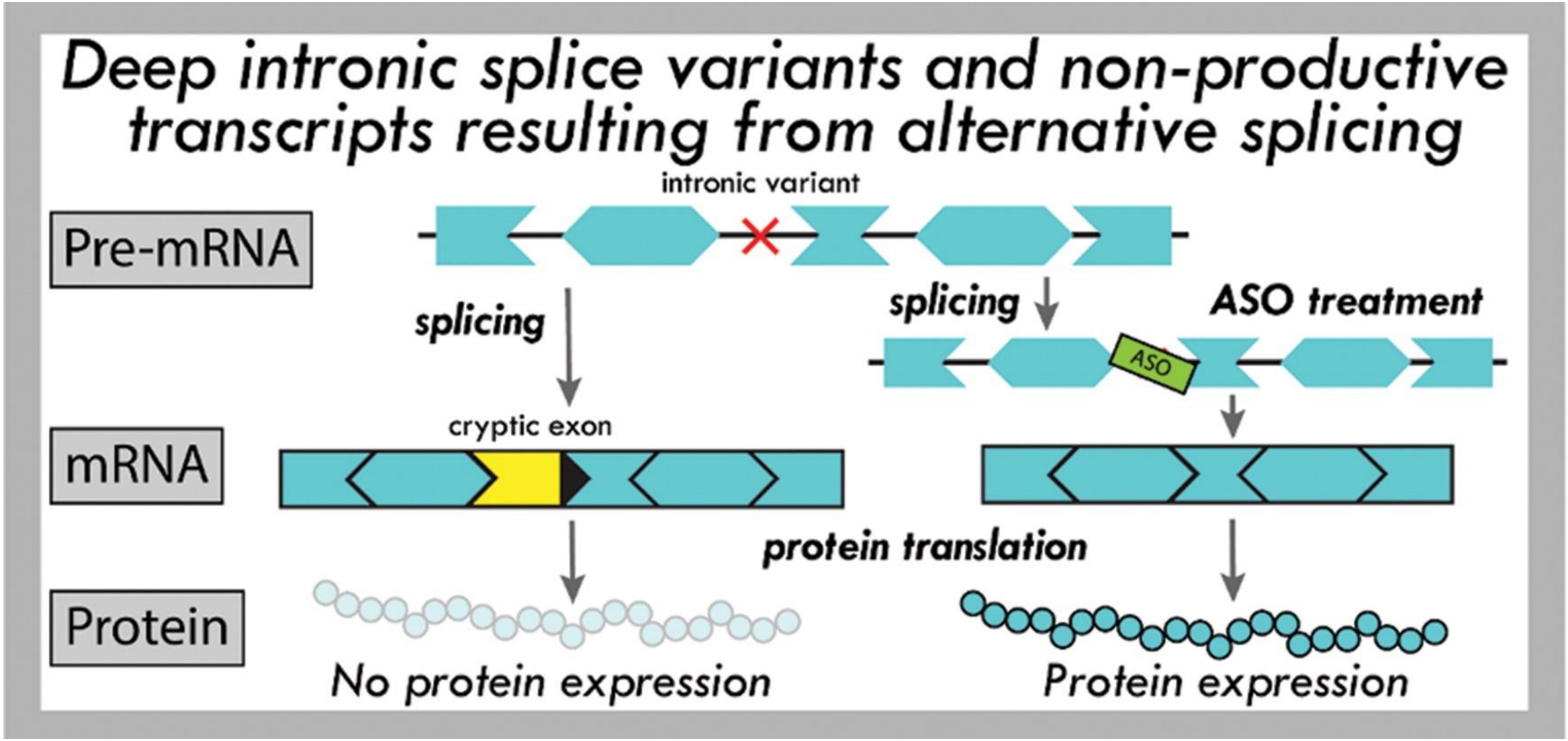
Patient specific ASO treatment in the EU

- ASO design: use backbone and chemistry of Nusinersen as a lot of data on pharmacokinetics and pharmacodynamics is known
- Targeting mutation types rather than individual diseases (→ splice-modulating ASOs)
- Target tissue: CNS allows standardization of application → intrathecal
- No clinical trial/no EMA registration
- Named patient setting
- Standardization and quality control framework for all relevant processes (scientific, preclinical, clinical, regulatory, logistical, legal, ethical, financial) in close contact with regulators
- New ethical framework (patient selection, cost, research vs. named-patient application, ….)
- European network of 1M1M clinical sites qualified to conduct ASO treatments for rare neurological diseases

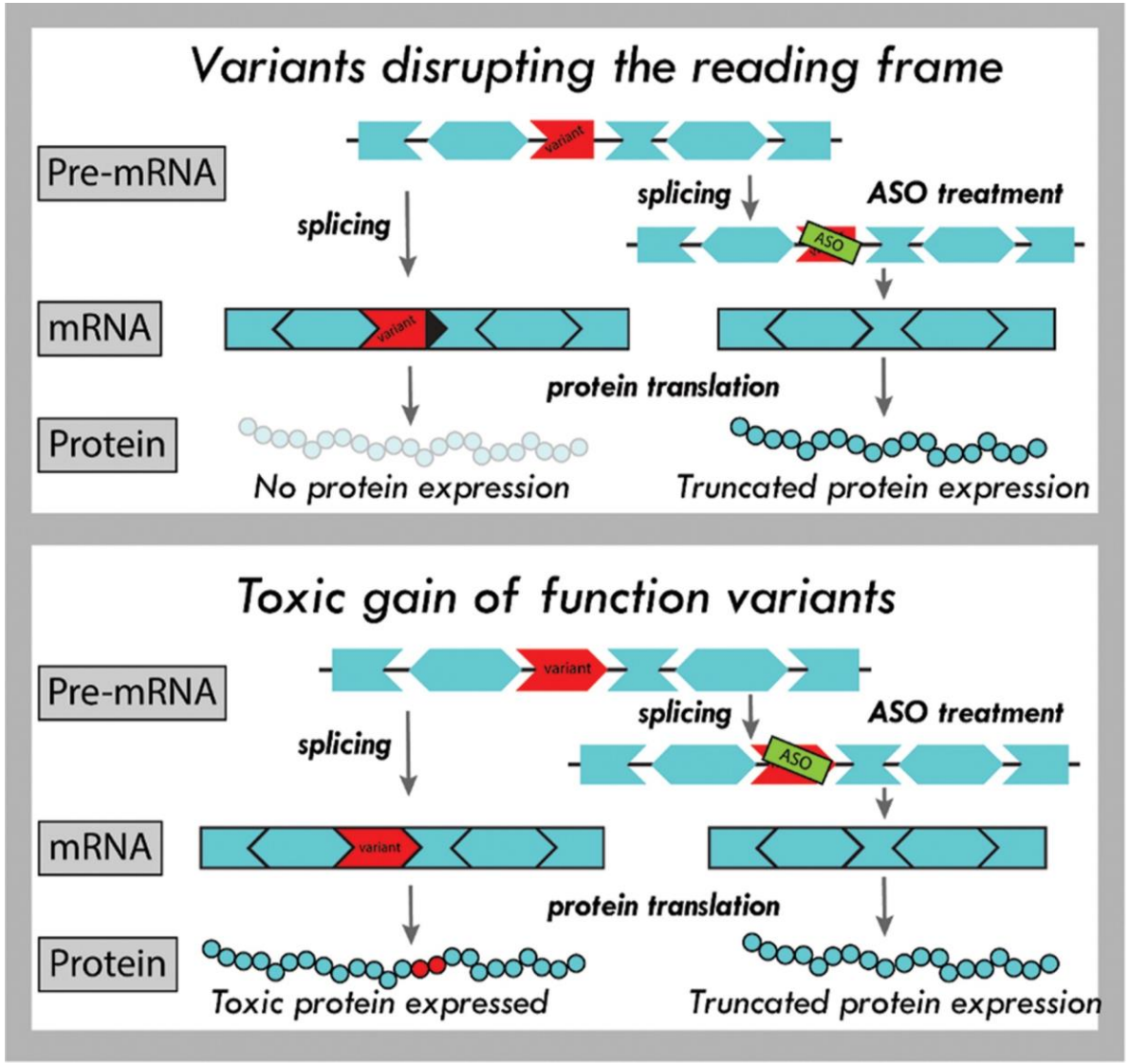
ASO design	***In vitro* tox study**	***In vivo* safety study**	***Treatment and monitoring***
~0.5 year	~0.5 year	~0.5 year	

Natural history → **Treatment effect**

ASO-mediated splice modulation



ASO-mediated splice modulation

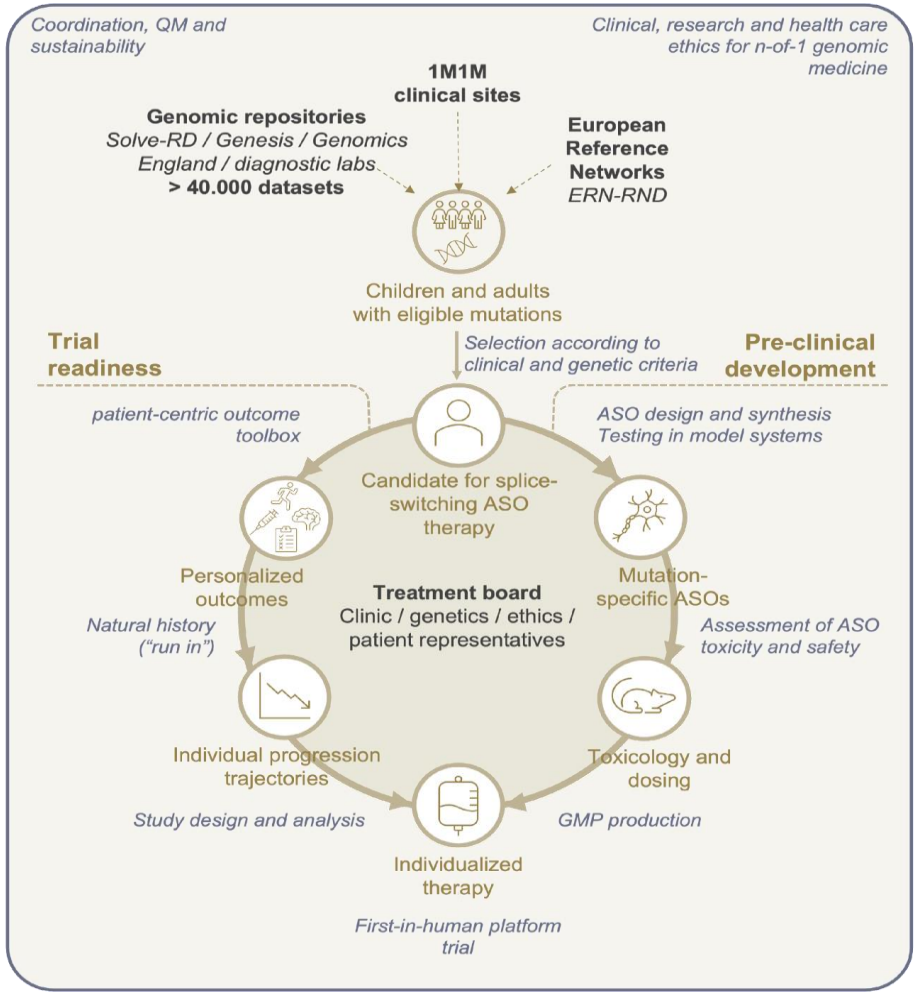


Synofzik et al NAT 2022

1M1M network – treatment development pipeline

Patient-specific tailored treatment development requires a novel, non-standard, but still systematic treatment development process, which requires specific cross-disciplinary expertise in all steps from patient identification to translation into clinics.

Pharma approach	**Academic network approach:**
	• Patients/families, • Preclinical and clinical researchers, • Hospital pharmacies, ASO manufacturing • Experts in safety studies, • Regulators, • Payers, and • Policy makers
• 12-12 years • Preclincal development including efficacy and toxicity studies • Phase 1/2/3 trials • On average 2.6 billion Euro costs • Market authorization	• Maximum 18 months • No animal models for the respective mutations, etc. • No or very reduced in-vivo tox studies • No phase 1/2/3 trials possible; standard trial designs and analyses models not appropriate; • Standard outcomes not appropriate to capture change on an n-of-1 level • Comparable small costs (< 0.5 million) • No market authorization



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

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- External experts (N1C)
- Patient organisation representative
- Ethicist
- Disease domain experts as needed
- Independent clinical experts as needed
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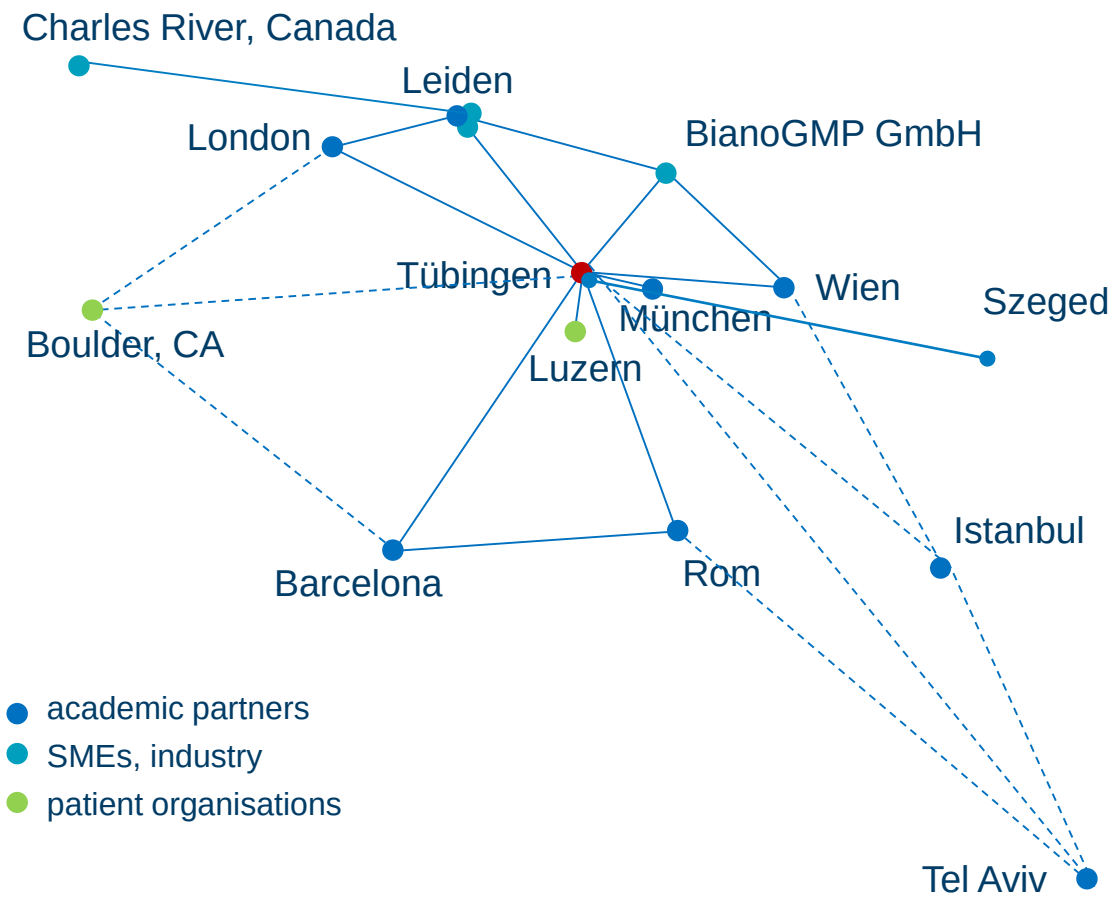
- 1M1M secretariat UT

Yes/
No

1M1M partners



Academic partners:

- Eberhard-Karls Universität Tübingen, Germany
- Leiden University Medical Centre, Netherlands
- University College London, UK
- Medical University Vienna, Austria
- Ludwig Maximilians University Munich, Germany
- Pediatric Hospital Bambino Gesù, Italy
- Koc University, Turkey
- Sheba Medical Center, Israel
- Vall d'Hebron Barcelona Hospital, Spain

Industry partners:

- BianoGMP GmbH, Germany
- Biotalentum, Szeged, Hungary
- Charles River, Canada

Patient advocacy organisations:

- European Leukodystrophies Association
- EuroAtaxia
- Valeria Association
- Mila's Miracle Foundation

Associated partners and additional collaborations:

- CONSILIUM Salmonson & Hemmings
- N=1 ASO collaborative
- European Reference Network for Rare Neurological Diseases

1M1M – 1 mutation, 1 medicine



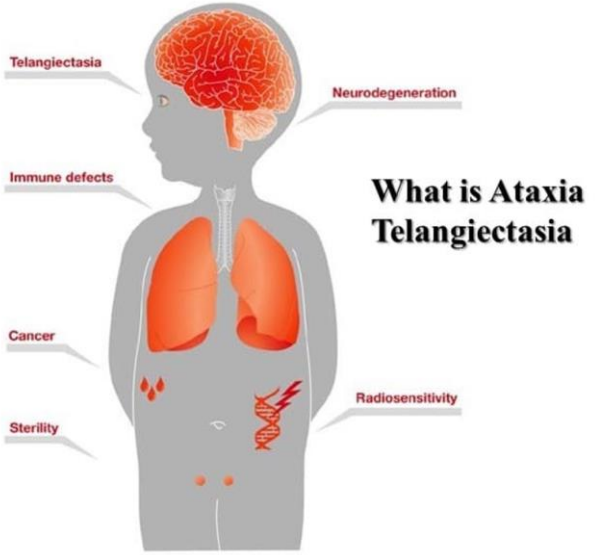
Tim Hu Matthis Synofzik Rebecca Schüle

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

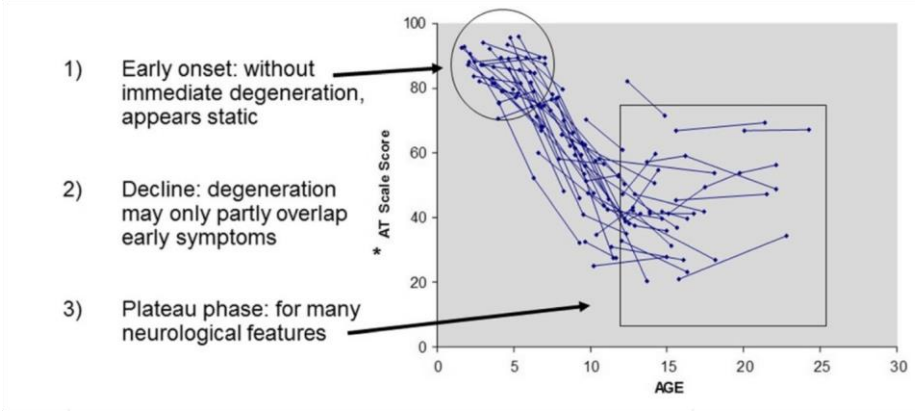
Example ASO design pipeline: already *first-in-human*

ATM (ataxia teleangiectasia)

- autosomal-recessive disease
- *ATM* mutations
- multi-systemic early-onset ataxia



- classic AT: loss of ambulation between age 10-20yrs



Rothblum-Oviatt et al, 2016, OJRD

1M1M – 1 mutation, 1 medicine



Tim Hu Matthis Synofzik Rebecca Schüle

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

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

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, already on ASO maintenance dose
2. **subject #2: P.K: 4 years, transferred from Tübingen to Boston in 2021 for treatment, ASO dose**

   escalation phase, treatment continuation inTübingen from Sept 2022 on

   - c.7865 C>T, p. Ala2622Val; in trans with c.829G>T,p. Glu227*

Acknowledgements

LUMC – Neuro-D group

- Willeke van Roon-Mom
- Barry Pepers
- Ronald Buijsen
- Elena Daoutsali
- Tom Metz
- Linda van der Graaf
- Linde Bouwman
- Elsa Kuijpers
- Laura Kerkhof
- Mariana Ramos
- Bas Voesenek
- Hannah Bakels
- Eleni Mina
- Daniel Bijster
- Sean Visser

DCRT - LUMC

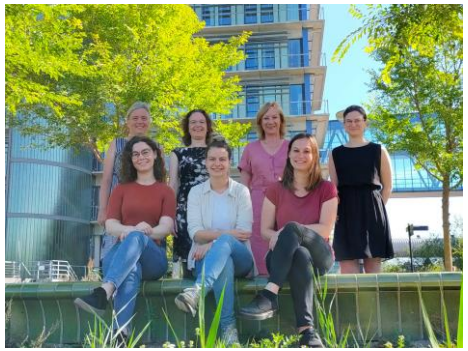
- Annemieke Aartsma-Rus
- Anouk Spruit
- Marlen Lauffer
- Pauline v/d Graaf
- Bianca Zardetto

DCRT - radboudumc

- Rob Collin

DCRT - ErasmusMC

- Ype Elgersma



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Name	Site
Marc Engelen	Amsterdam
Nicole Wolf	Amsterdam
Juan dario Ortigoza Escobar	Barcelona
Alfons Macaja	Barcelona
Maria Judit Molnar	Budapest
Marina Koning-Tijssen	Groningen
Emil Ylikallio	Helsinki
Nazli Basak	Istanbul
Jørgen Erik Nielsen	Kopenhagen
Annemieke Aartsma-Rus	Leiden
Willeke van Roon	Leiden
Rik Vandenberghe	Leuven
Kristl Claeys	Leuven
Damjan Osredkar	Ljubjana
Paul Gissen	London
Bart van de Warrenburg	Nijmegen
Manuel Menéndez	Oviedo
Lucie Stovickova	Prag
Nicita Francesco	Rome
Enrico Bertini	Rome
Andrea Bevot	Tübingen
Ludger Schoels	Tübingen
Matthis Synofzik	Tübingen
Rebecca Schüle	Tübingen
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Zanni Ginevra	Rome
Nofar Mor	Tel Aviv
Matias Wagner	Munich

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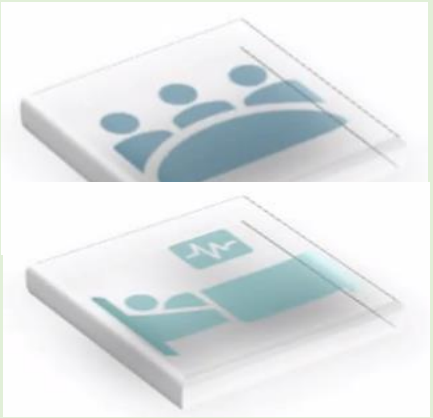

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Yes/
No

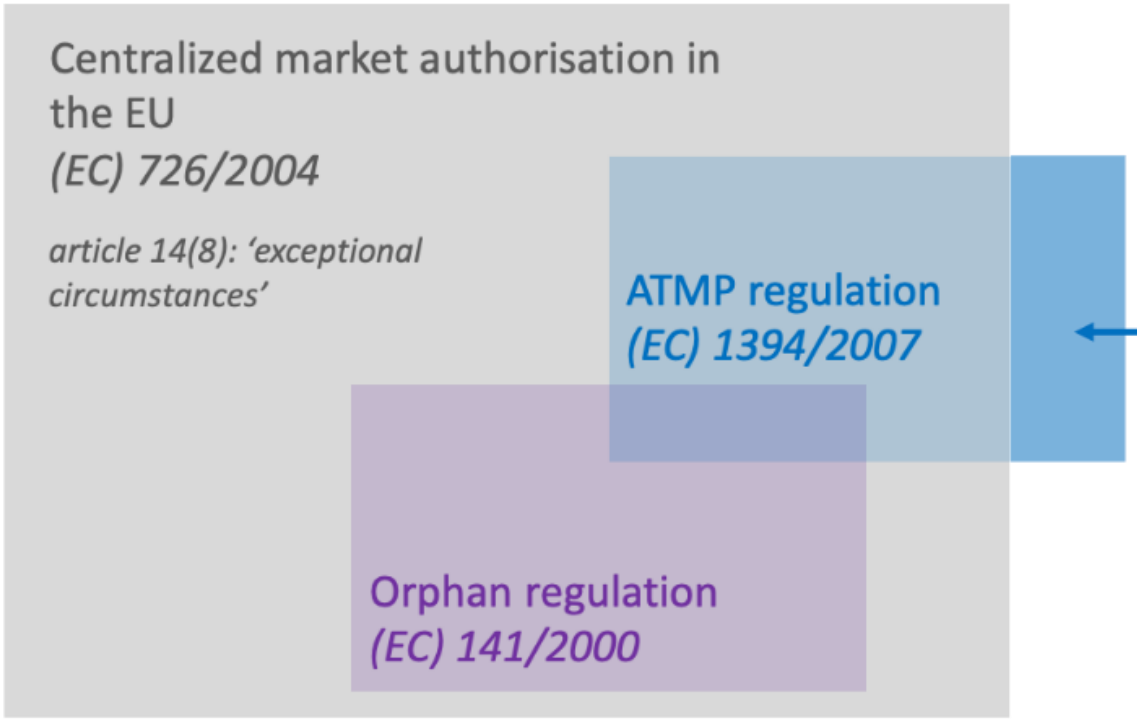
Access options for mutation-specific ASOs

Unlicensed access options for groups of patients

Clinical trial
(EC) 2001/83 Article 3(3)

Compassionate use
(EC) 726/2004 Article 83

- groups of patients with chronically or seriously debilitating or life-threatening disease
- medicinal product is undergoing clinical trial or subject of a marketing authorization application



Unlicensed access options for single patients

Hospital exemption
(EC) 1394/2007 Article 28(2)

- ATMP custom-made for an individual patient; not routinely produced
- administered in a hospital setting under exclusive responsibility of a medical practitioner
- produced and administered in the same member state
- manufacturing authorised by a competent authority of the member state
- quality standards equivalent to (EC) 726/2004

Named patient use
(EC) 2001/83 Article 5(1)

- to fulfill special needs
- purely therapeutic considerations