Leiden University Medical Center

DCRT Dutch Center for RNA Therapeutics

ASO therapies for genetic brain disorders

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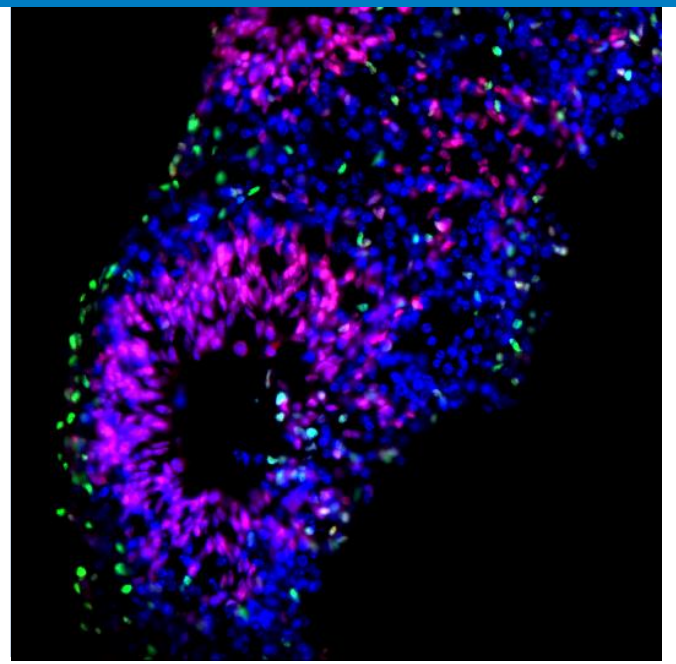
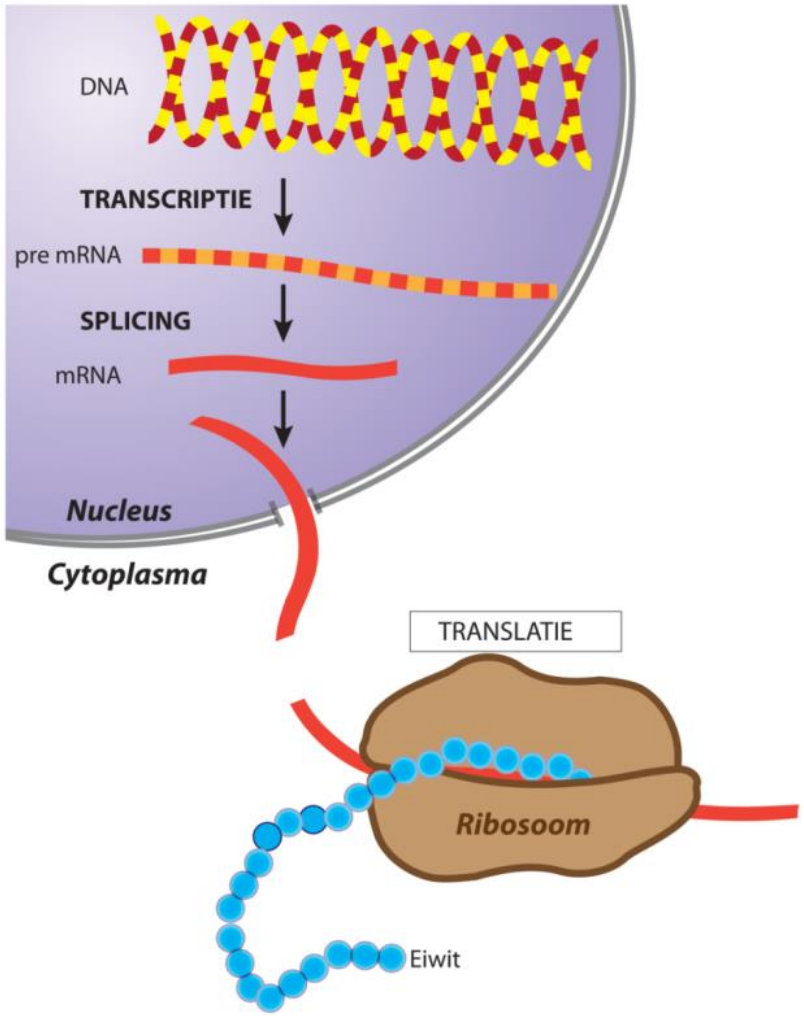


www.neurodlableiden.com

www.RNAtherapy.nl

From genes to proteins



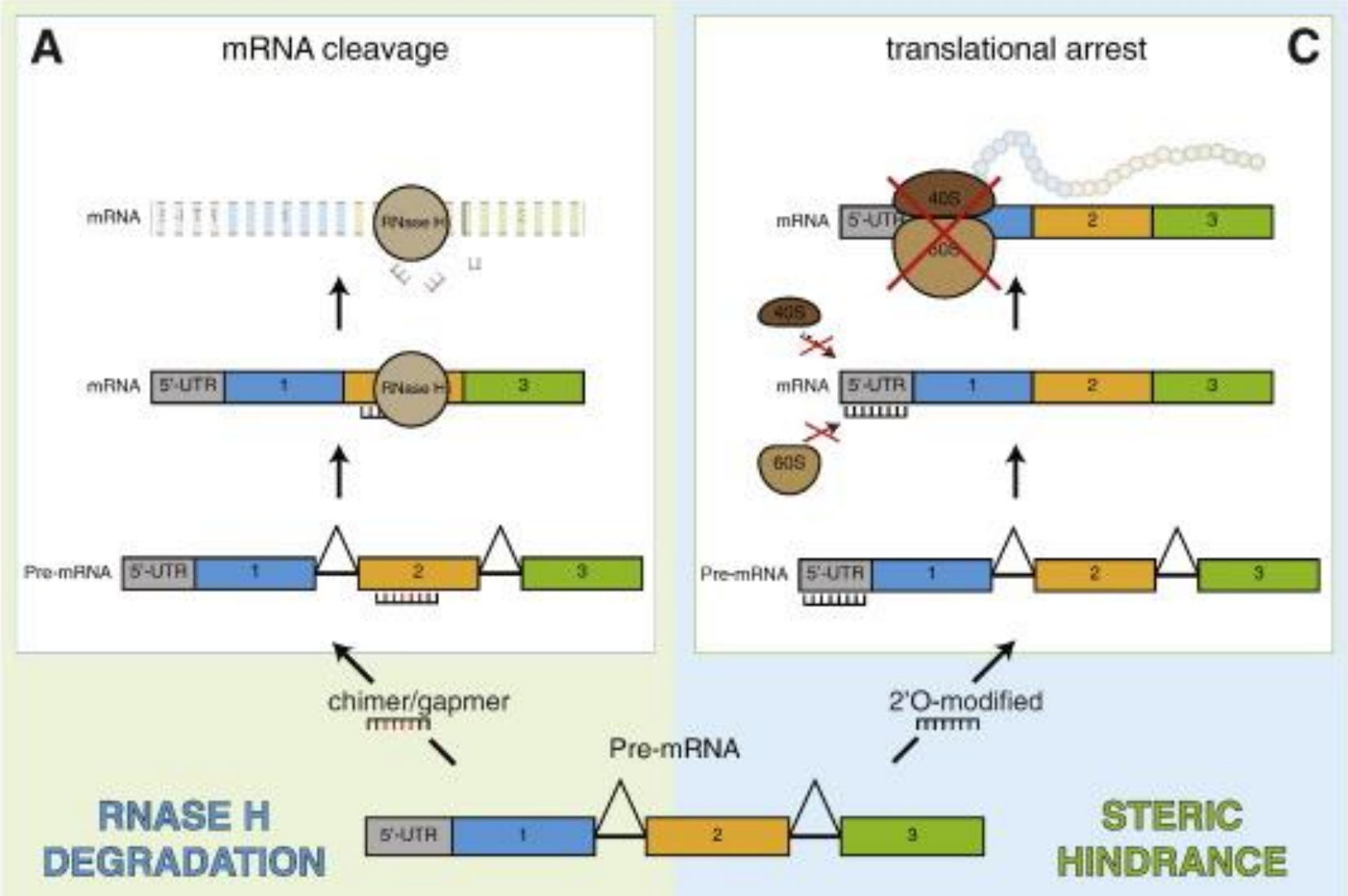
DNA targeting therapies

- CRISPR/Cas9
- Permanent modification
- Single administration
- Delivery to all brain cells problematic

RNA targeting therapies

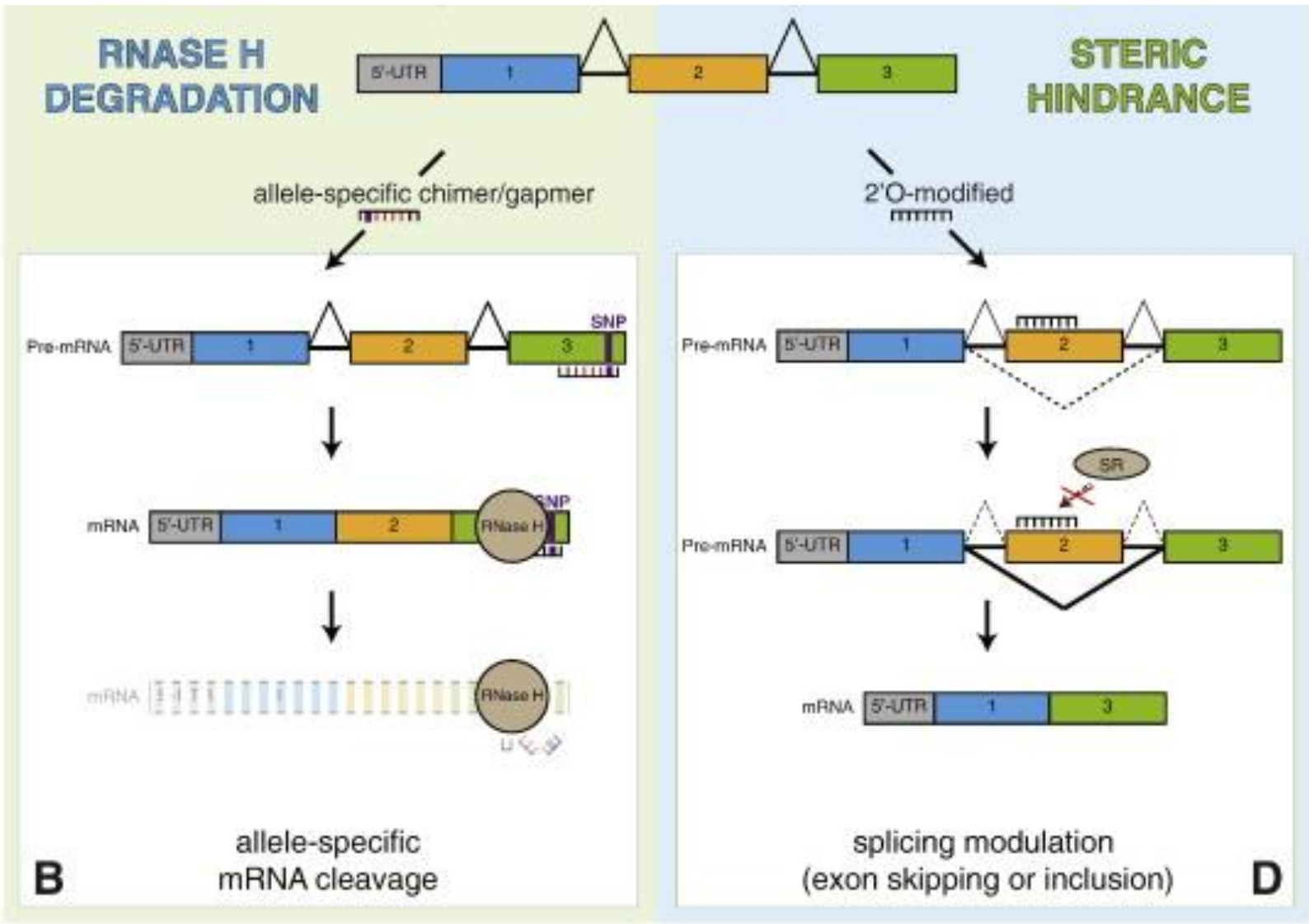
- ssRNA/DNA molecules
- Transient modification
- Repeated delivery
- Very efficient uptake in all brain cells

Antisense oligonucleotide mechanism of action



Evers *et al.* 2015 ADDR

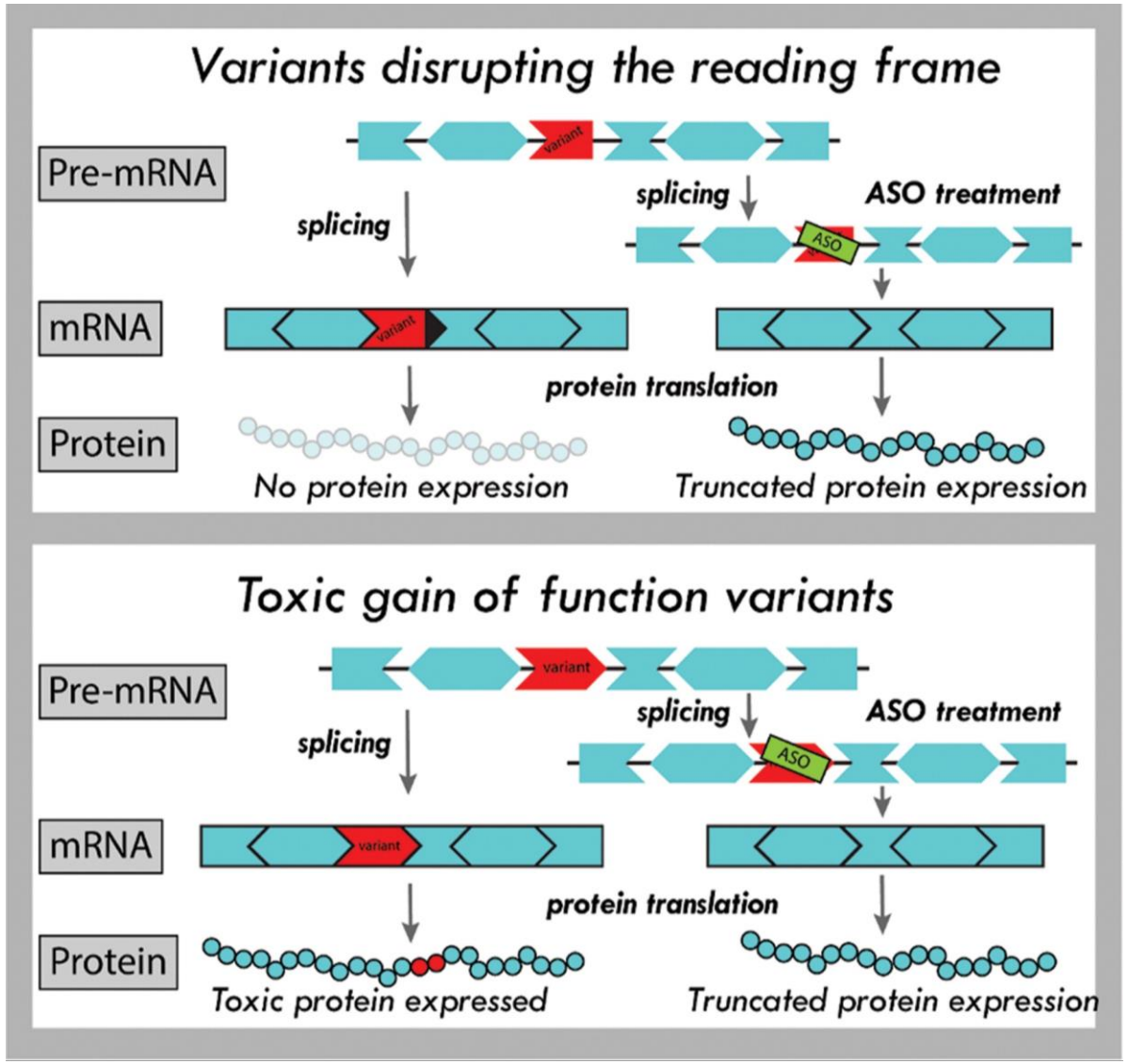
Antisense oligonucleotide mechanism of action



Approved oligonucleotide drugs (FDA/EMA)

- Modalities
  - Translation block: formivirsen
  - Aptamer: pegaptanib
  - Rnase H: mipomirsen, inotersen, valonesorsen
  - Splice switching: eteplirsen, nusinersen, golodirsen, viltolarsen, casimersen
  - SiRNA: patisiran, givosiran, inclisiran, lumasiran
  - mRNA vaccines: corminaty, Moderna COVID-19 vaccines
- Target tissues
  - Eye
  - Liver
  - Muscle
  - CNS

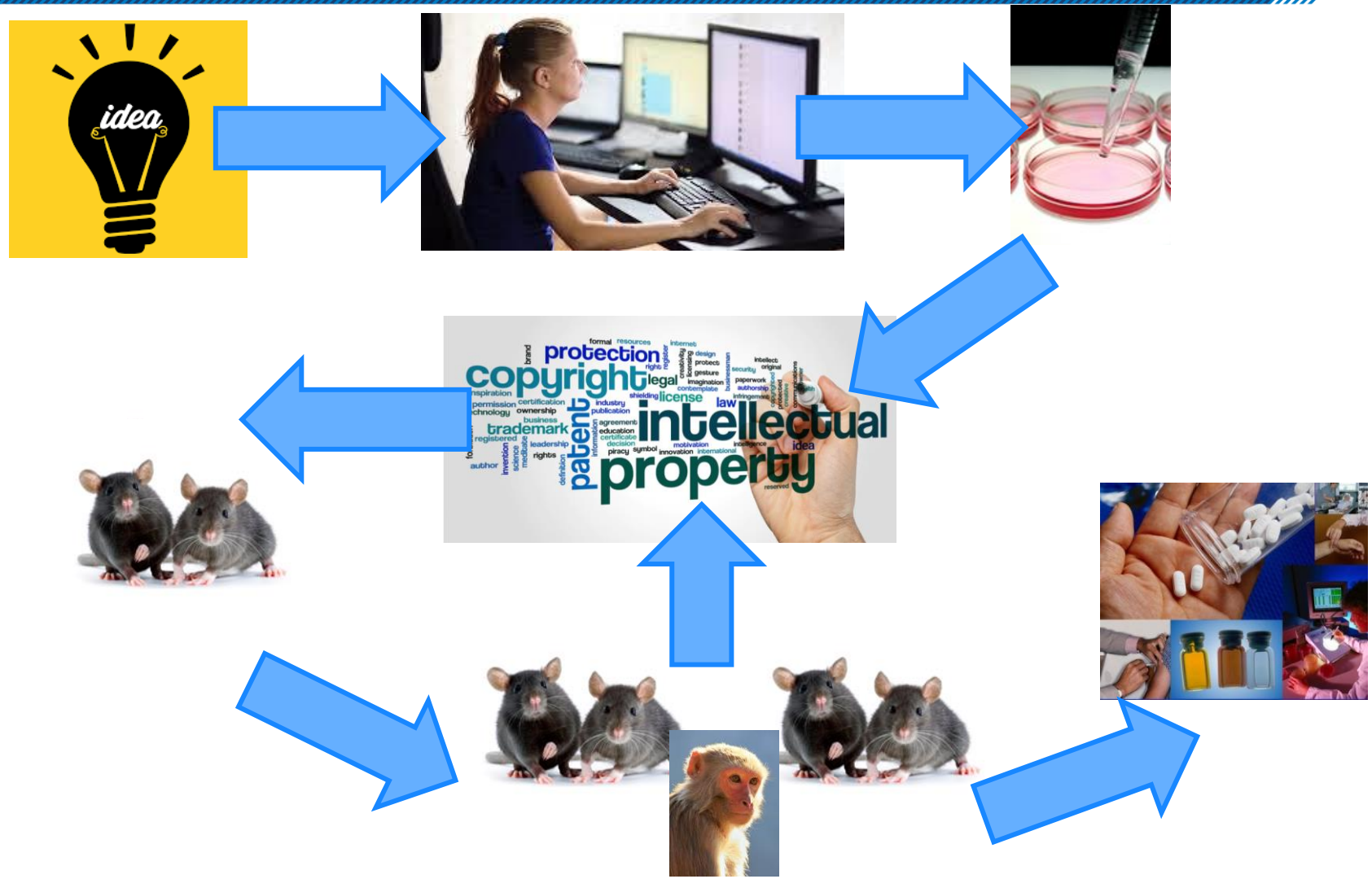
Splice modulation comes in different flavors



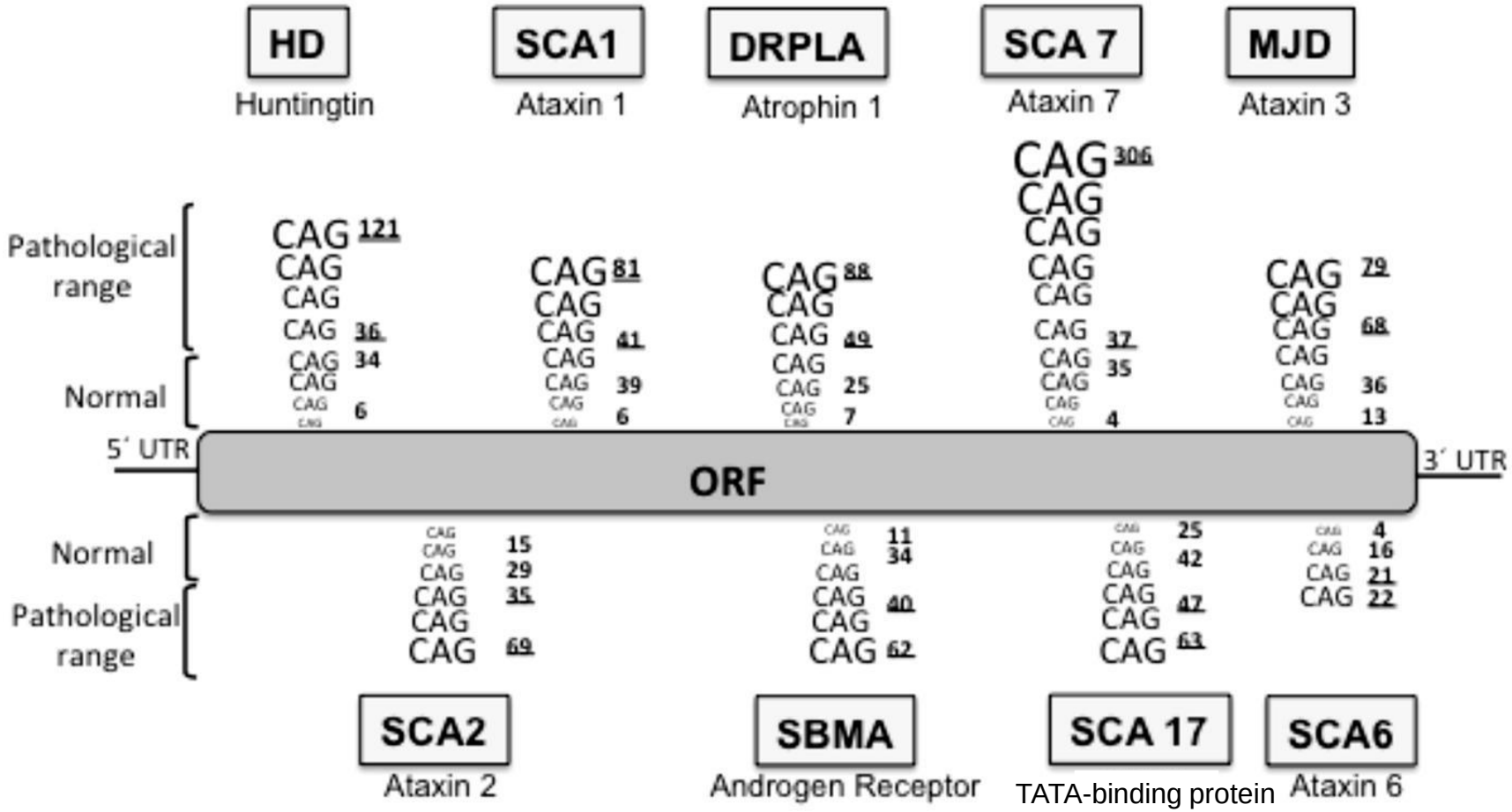
Eteplirsen - DMD

Spinocerebellar ataxia type 3

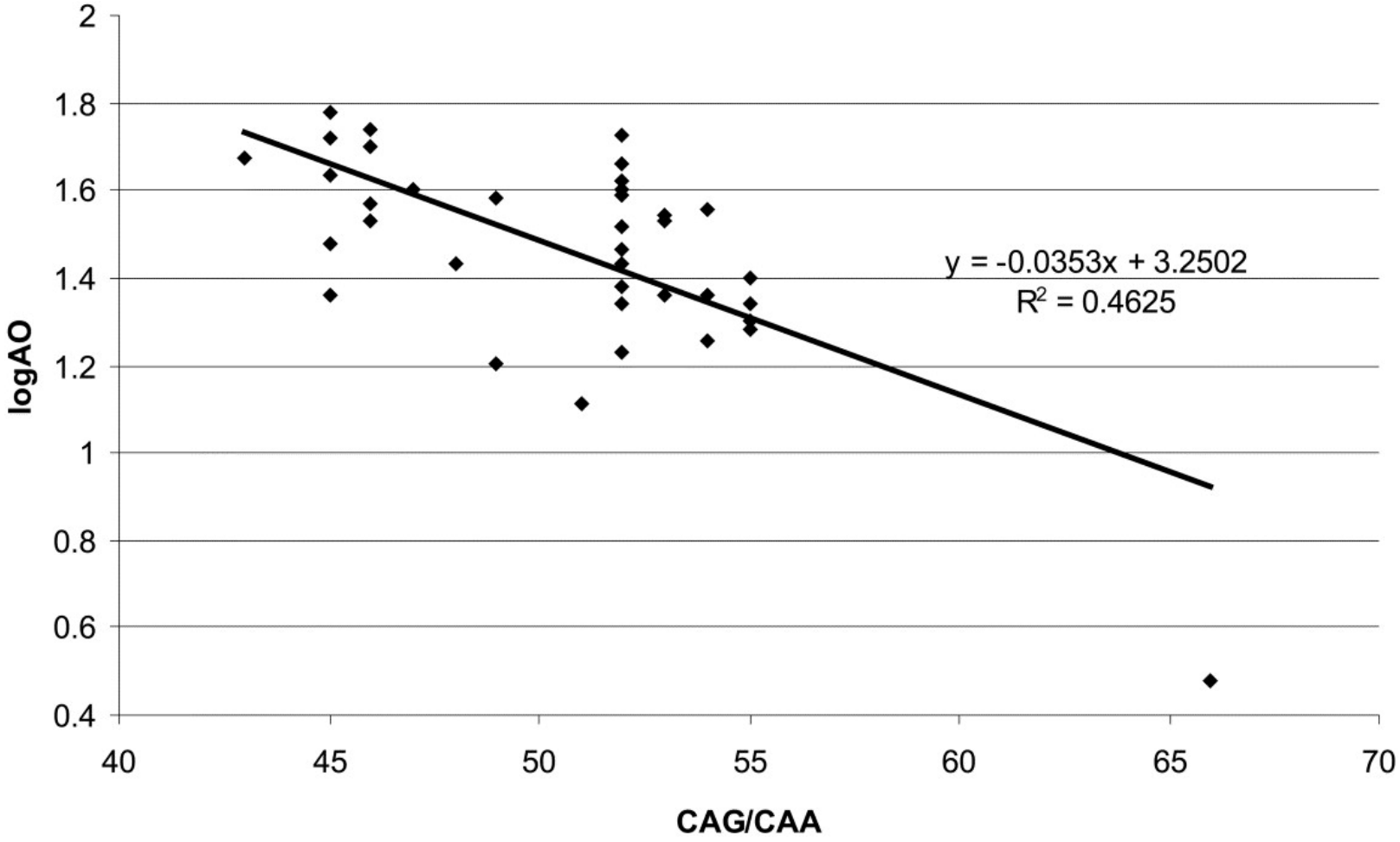
Development of a therapeutic idea into preclinical studies



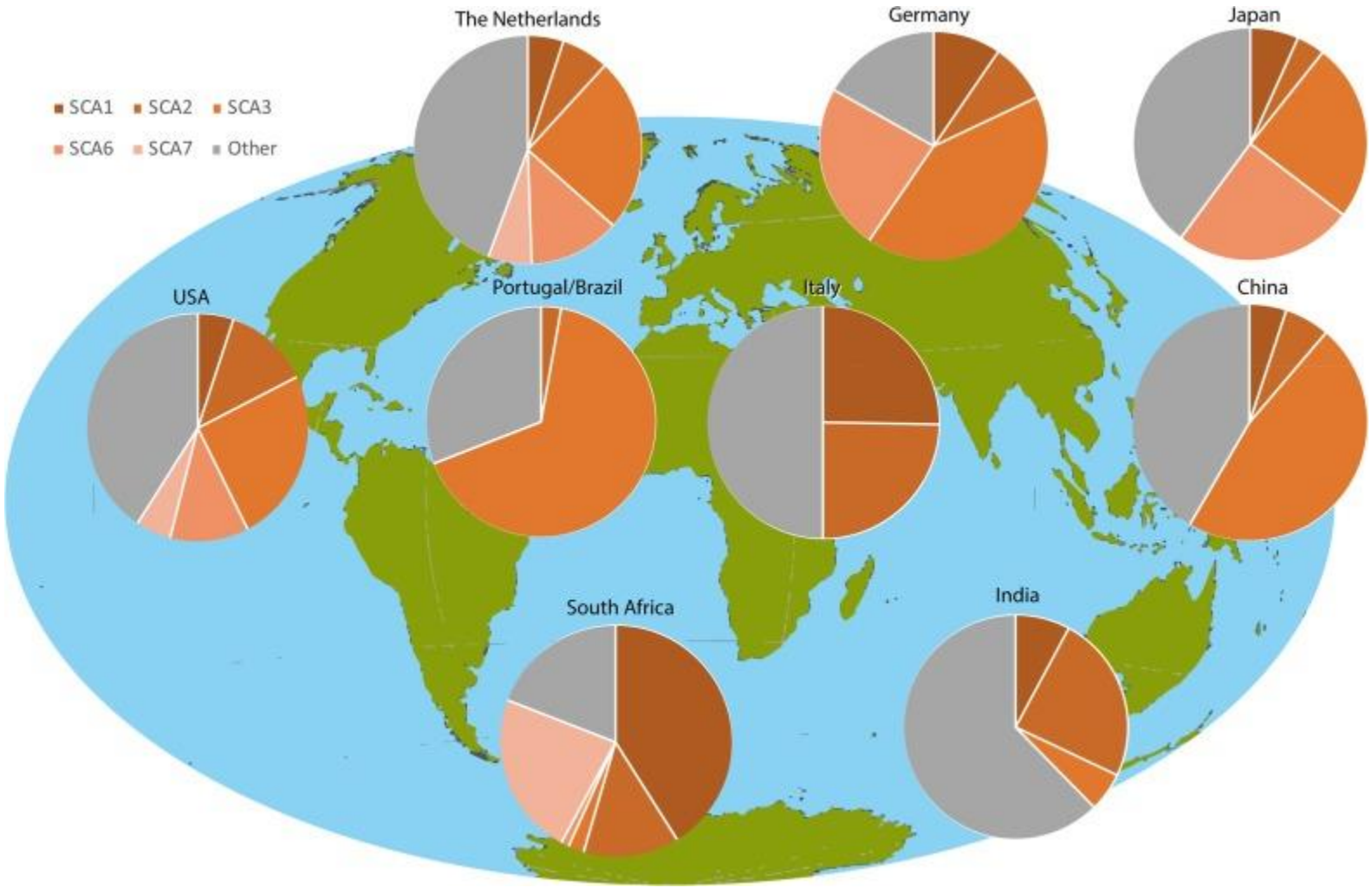
Polyglutamine disorders



More is worse – example SCA17



Worldwide prevalence of polyglutamine SCAs



Buijsen *et al* Neurotherapeutics2019

Spinocerebellar ataxia type 3 (SCA3)

SCA3:

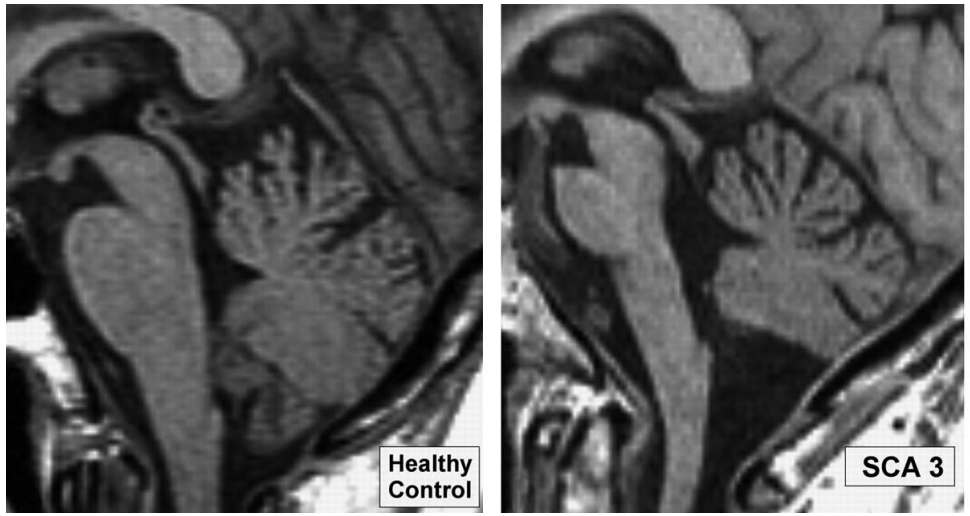
- Autosomal dominant
- Prevalence ~ 1 : 100.000
- Onset age: 37 years
- Neurodegenerative

Symptoms:

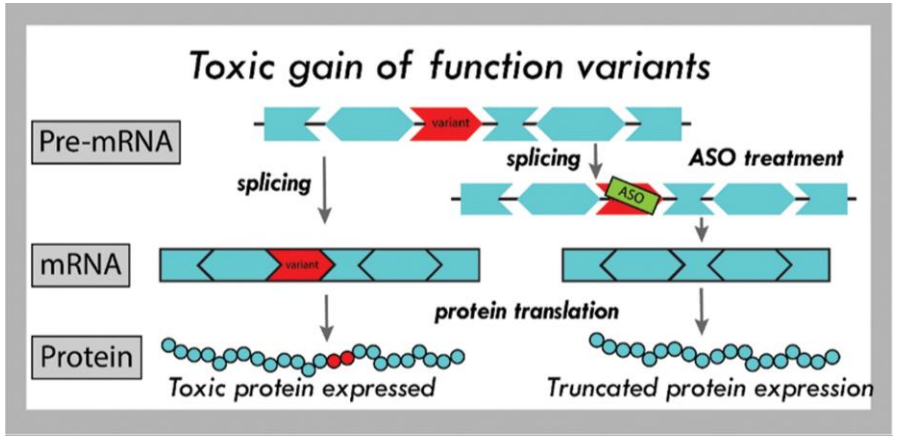
- Ataxia
- Distal muscular atrophy
- Paralysis

Cause:

- CAG expansion in *ATXN3* (>50 repeats)
- PolyQ expansion in ataxin-3 protein

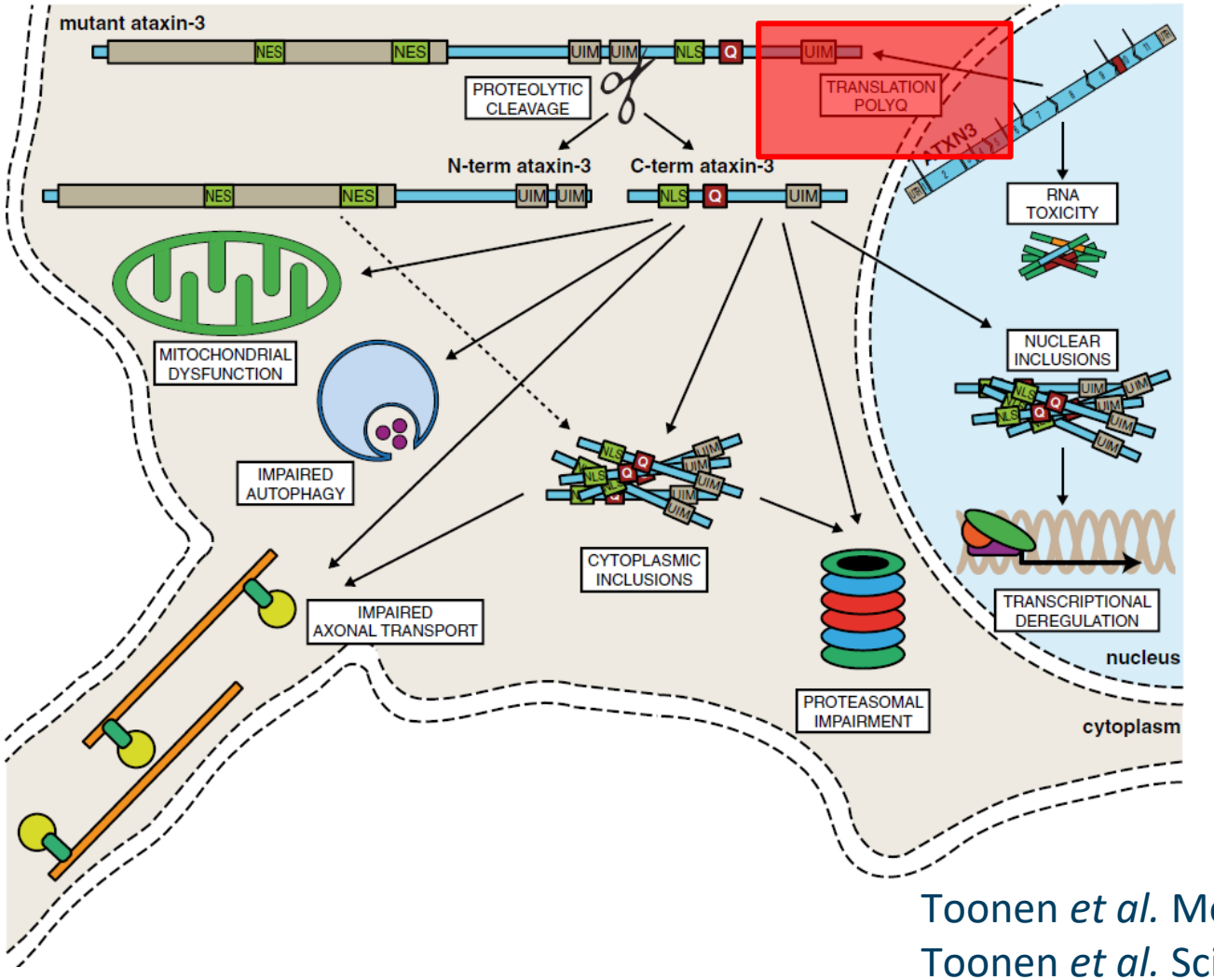


Eichler L et al. Am J Neuroradiol (2011)



SCA3 disease mechanism

Antisense oligonucleotides



Toonen *et al.* Mol Ther Nucleic Acids. 2017

Toonen *et al.* Sci Rep. 2016

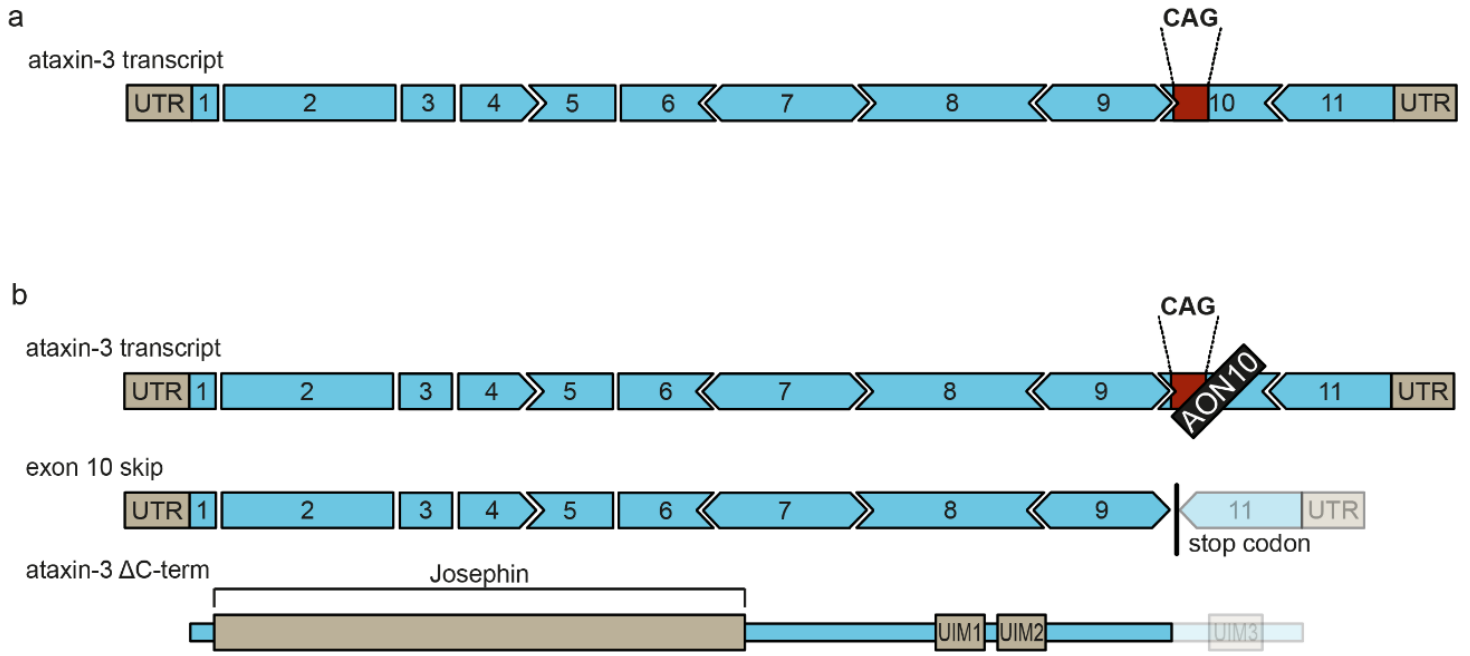
Ataxin-3 protein

Ataxin-3 protein:

- 42 kDa
- Highly conserved
- PolyQ repeat in C-terminus

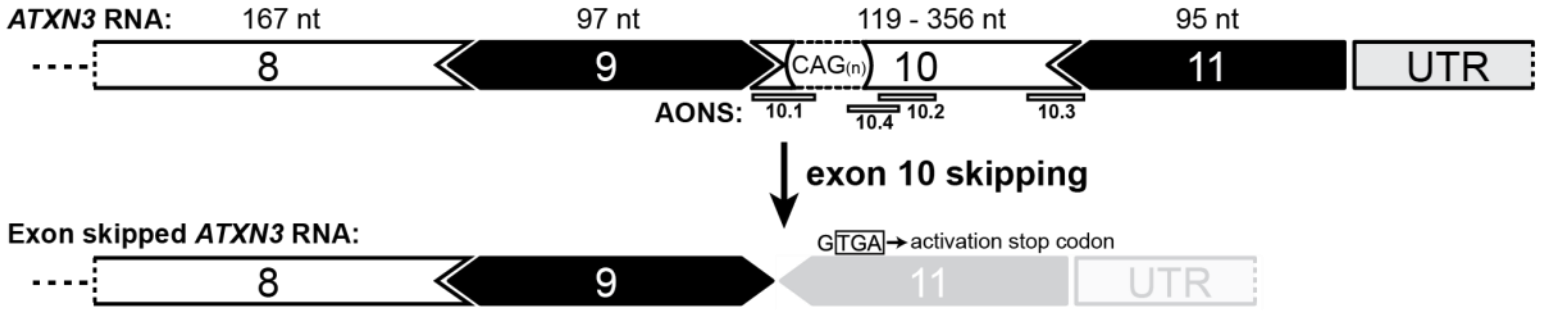
Function:

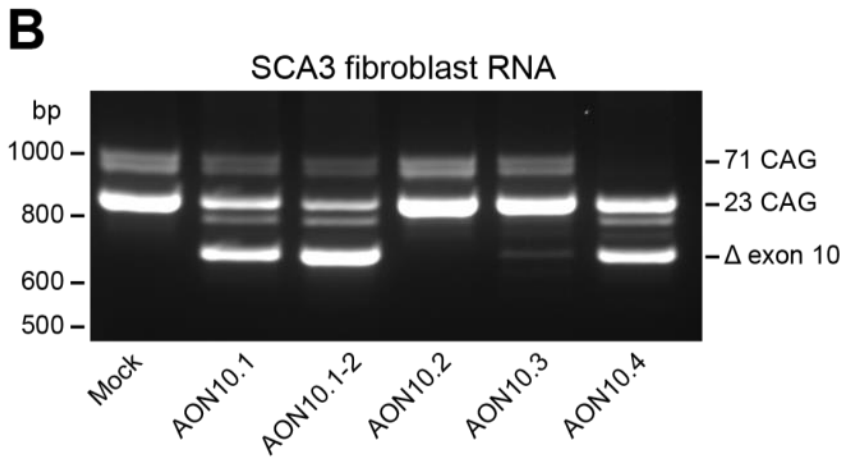
- Involved in proteasomal protein degradation
- Cleavage ubiquitin chains
- Many protein interactors + transcriptional activity
- Essential protein for cellular function?

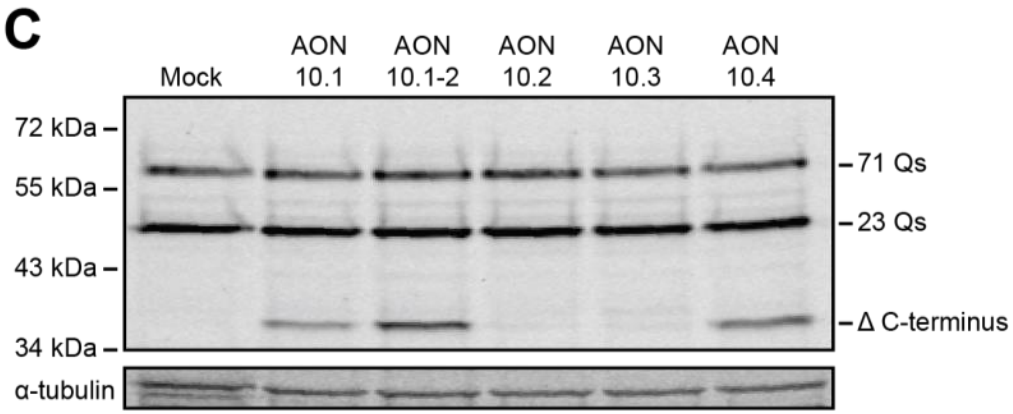


AON screening in cell culture

4 AONs against splicing enhancer sites:







Conclusion: 3 AONs capable of inducing exon skip

Truncated ataxin-3 ubiquitin binding

Ataxin-3 ubiquitin interaction:

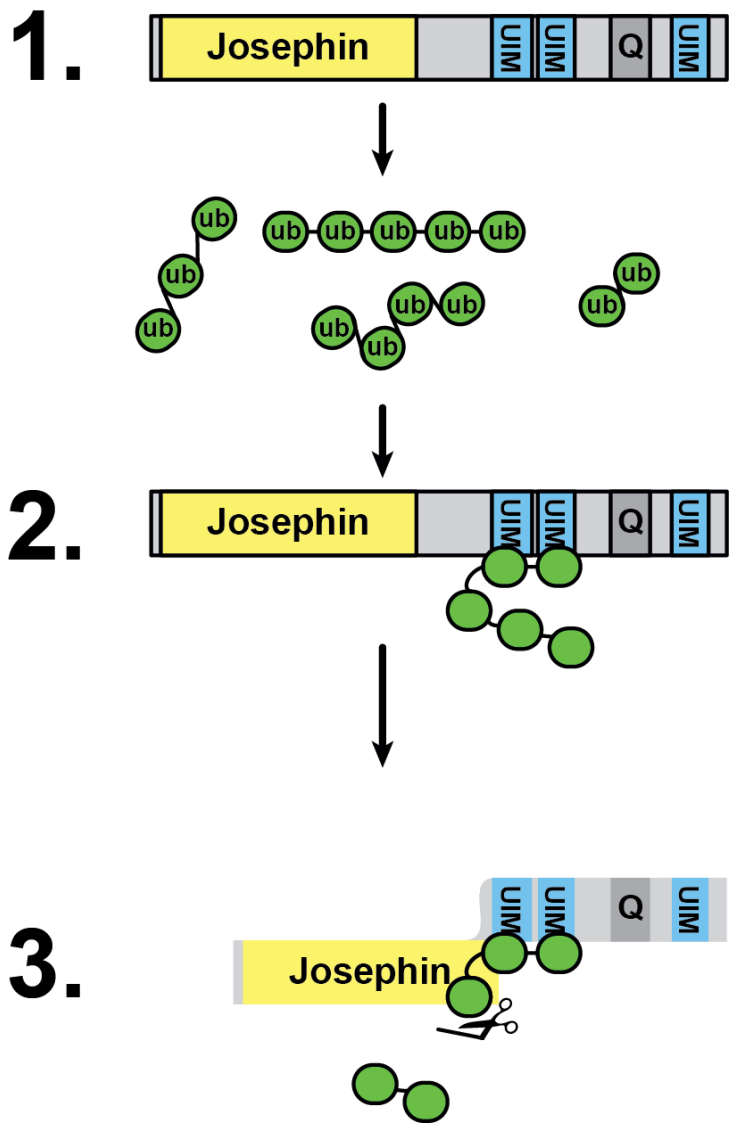
Binding of ub > 4

UIMs 1 and 2 position chain

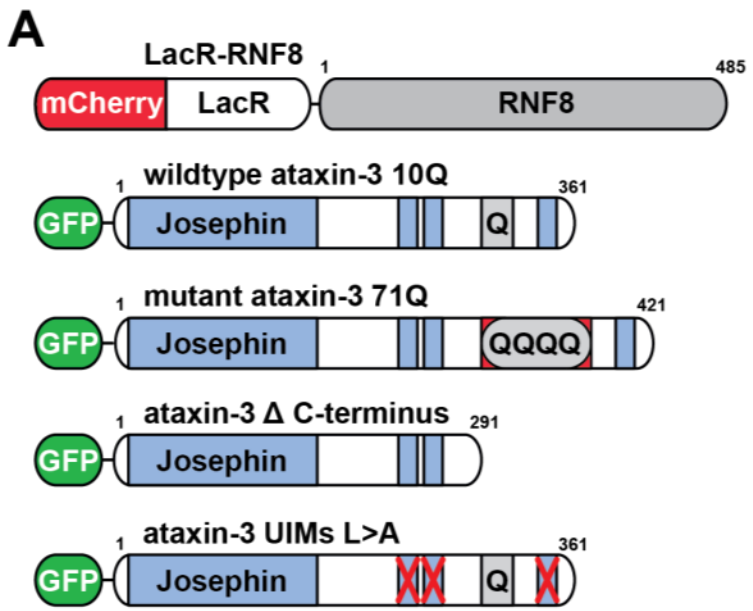
Josephin cleaves

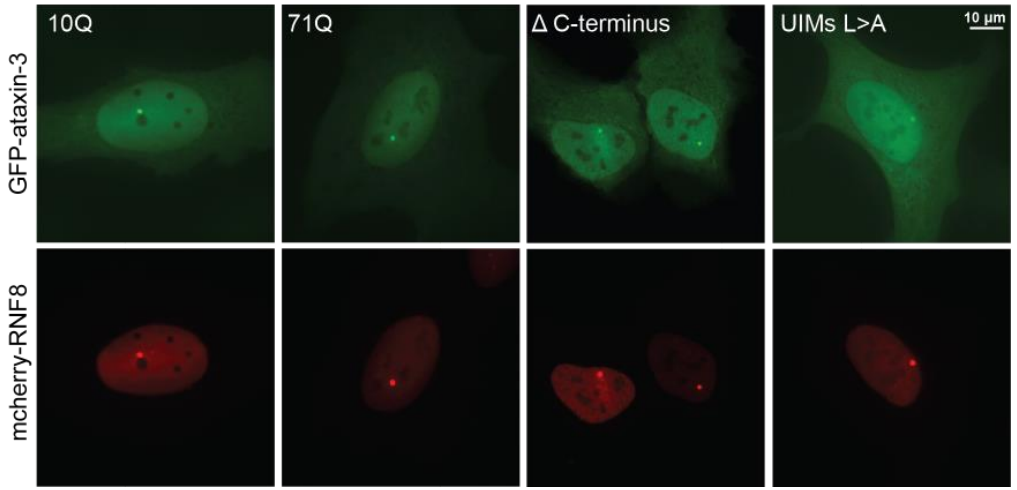
De-ubiquitination of substrate

UIM3 not required?

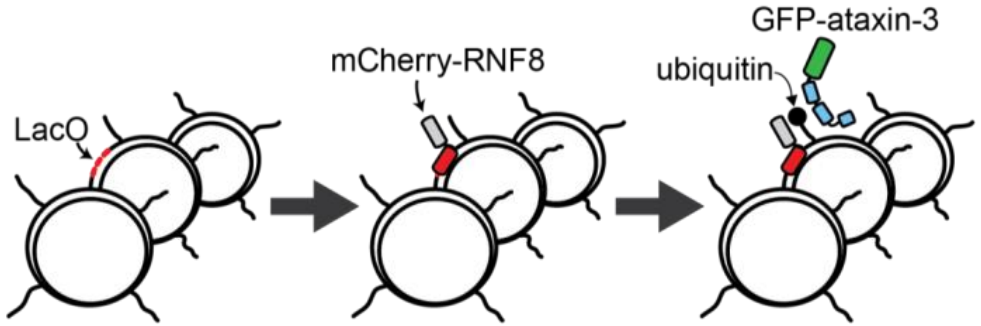


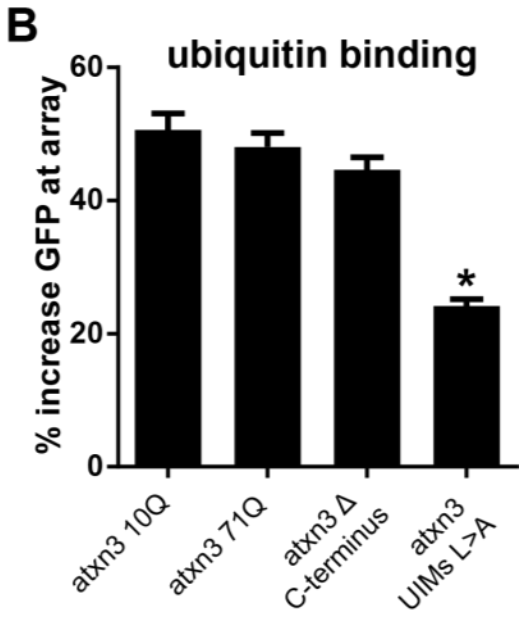
Truncated ataxin-3 ubiquitin binding





Chromatin ubiquitination:





AON distribution in brain

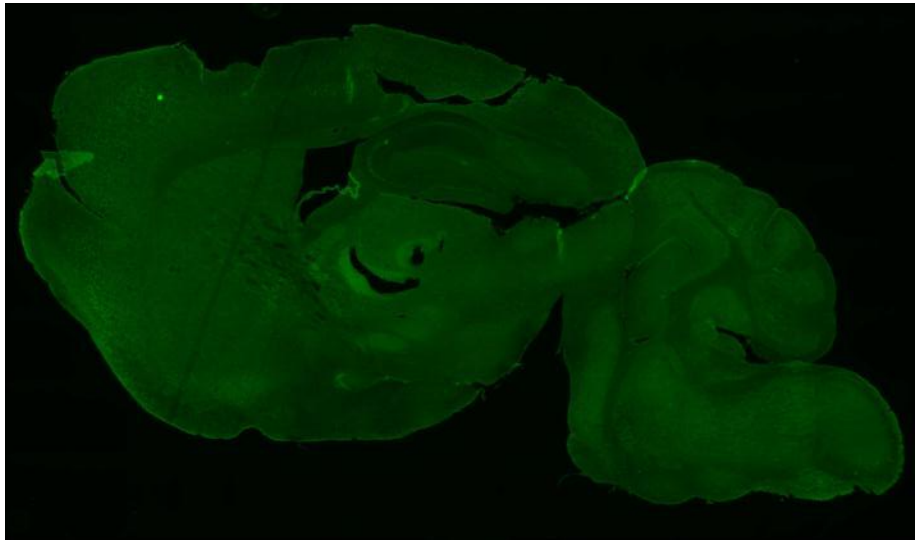
Histology:

Mice sacrificed 2.5 months after last AON injection

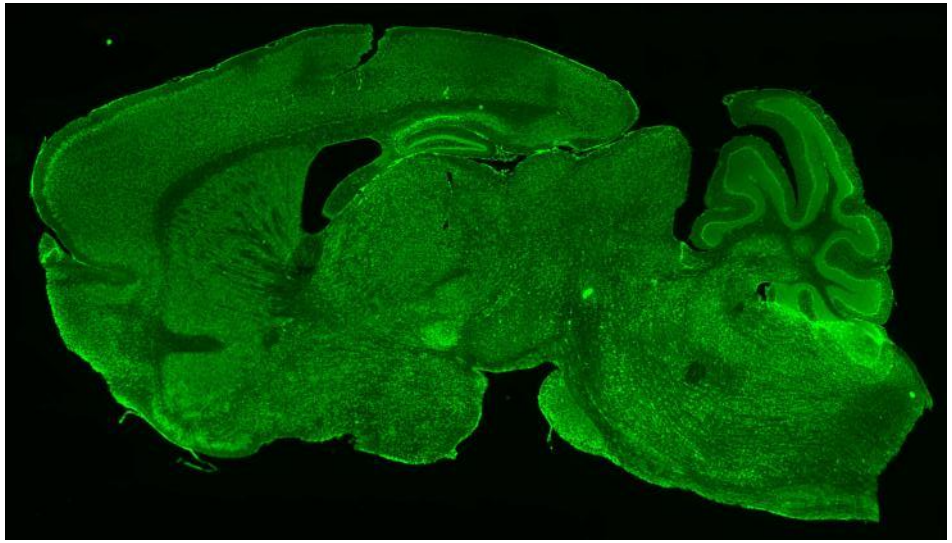
Right hemisphere for histology

Antibody against AON backbone

PBS treated:

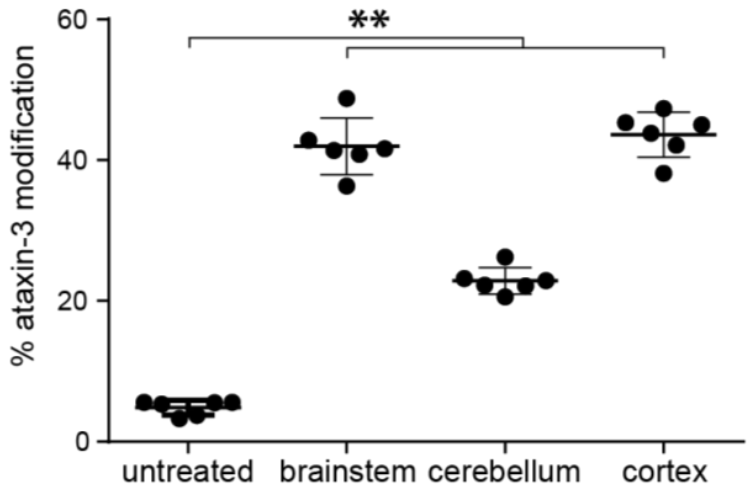


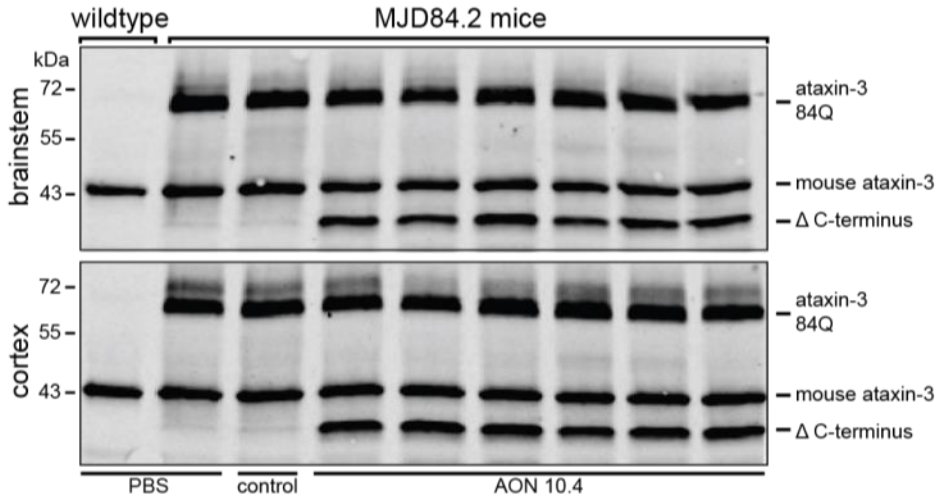
AON 10.4 treated:



Analysis of exon skipping

Analysis of exon skipping:
Isolated RNA and protein
Brainstem, cerebellum and cortex
RT-PCR and western blotting





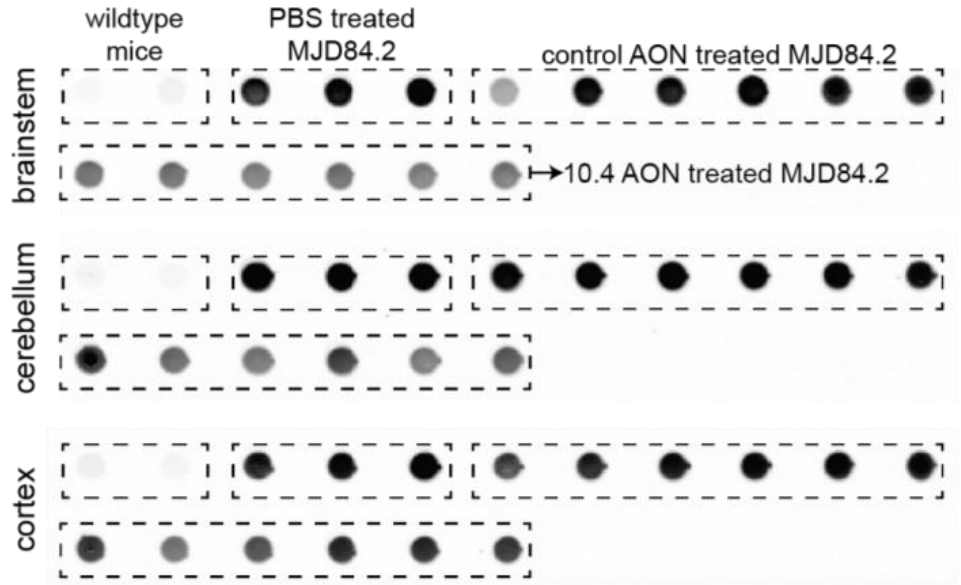
Conclusions:

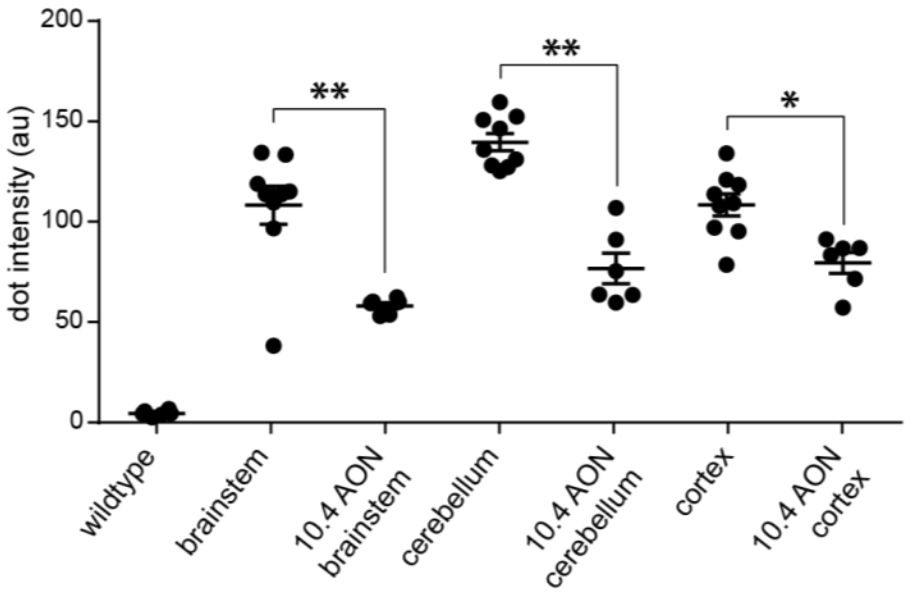
- Exon skip + ataxin-3 truncation in all tested brain regions
- Up to 40% of ataxin-3 truncated after 2.5 months

Reduction of ataxin-3 aggregation

Determine ataxin-3 aggregates:

- Mutant ataxin-3 forms aggregates in brain
- Filter trap assay brain lysates → trap insoluble protein





Reduction of ataxin-3 nuclear localisation

Histological analysis:

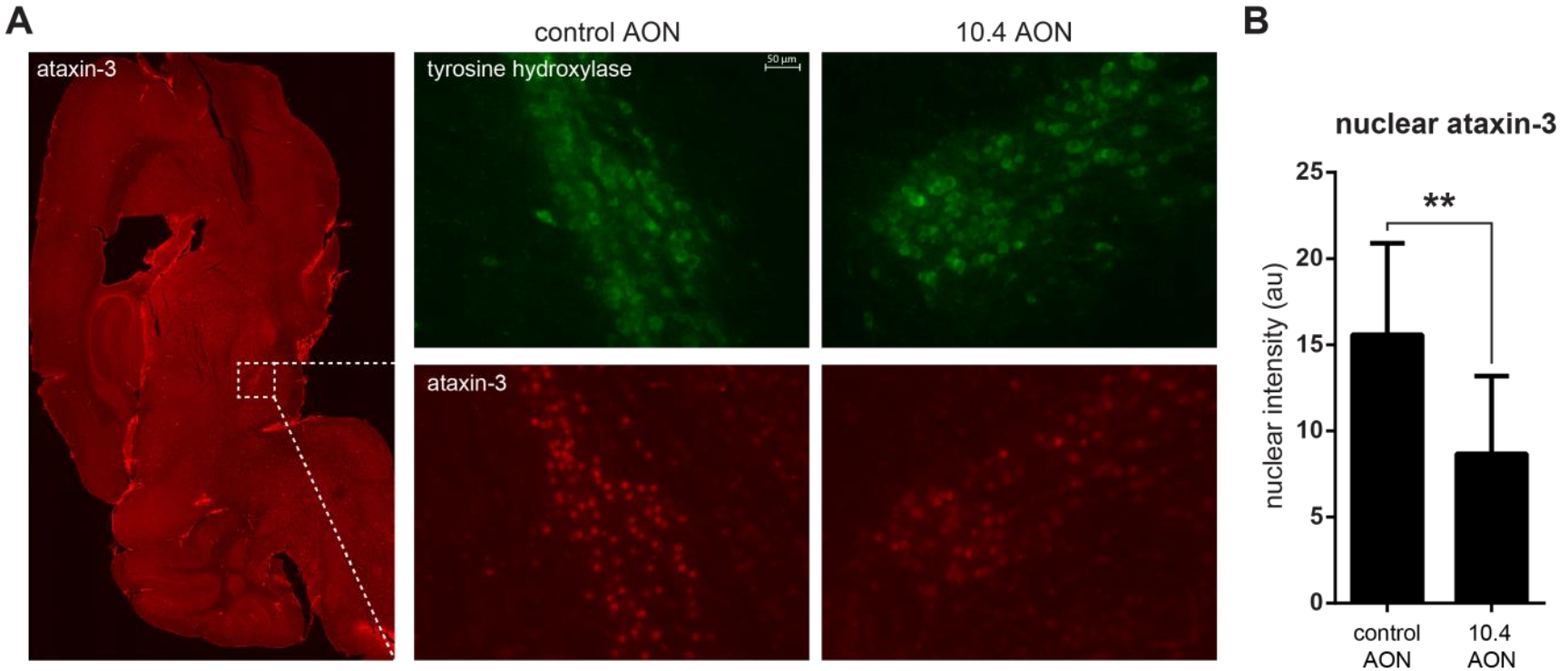
No ataxin-3 aggregates visible → mice too young?

Increase in ataxin-3 nuclear localization

J Neurosci. 2007 Jul 11;27(28):7418-28.

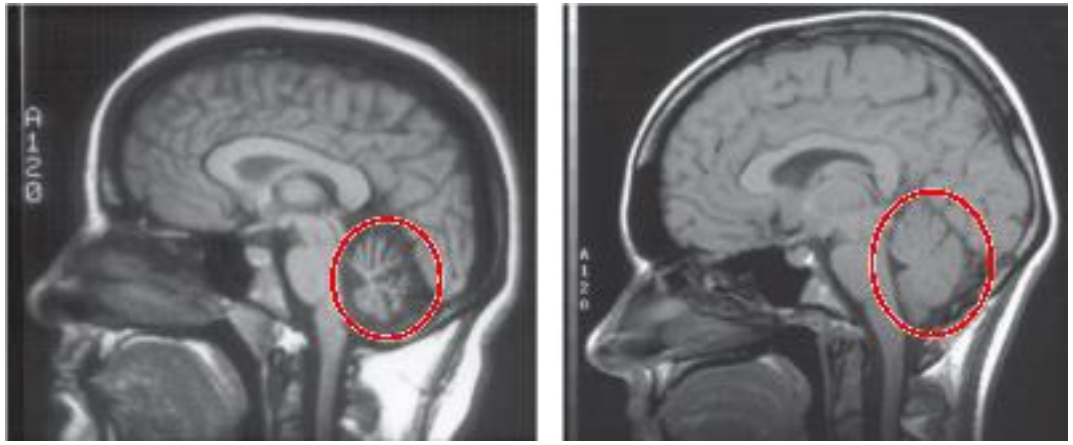
Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence.

Bichelmeier U[1], Schmidt T, Hübener J, Boy J, Rüttiger L, Häbig K, Poths S, Bonin M, Knipper M, Schmidt WJ, Wilbertz J, Wolburg H, Laccone F, Riess O.



Spinocerebellar ataxia type1 (SCA1)

- Ataxia
- Speech and swallowing difficulties
- Spasticity
- Ophthalmoplegia
- Cognitive impairment



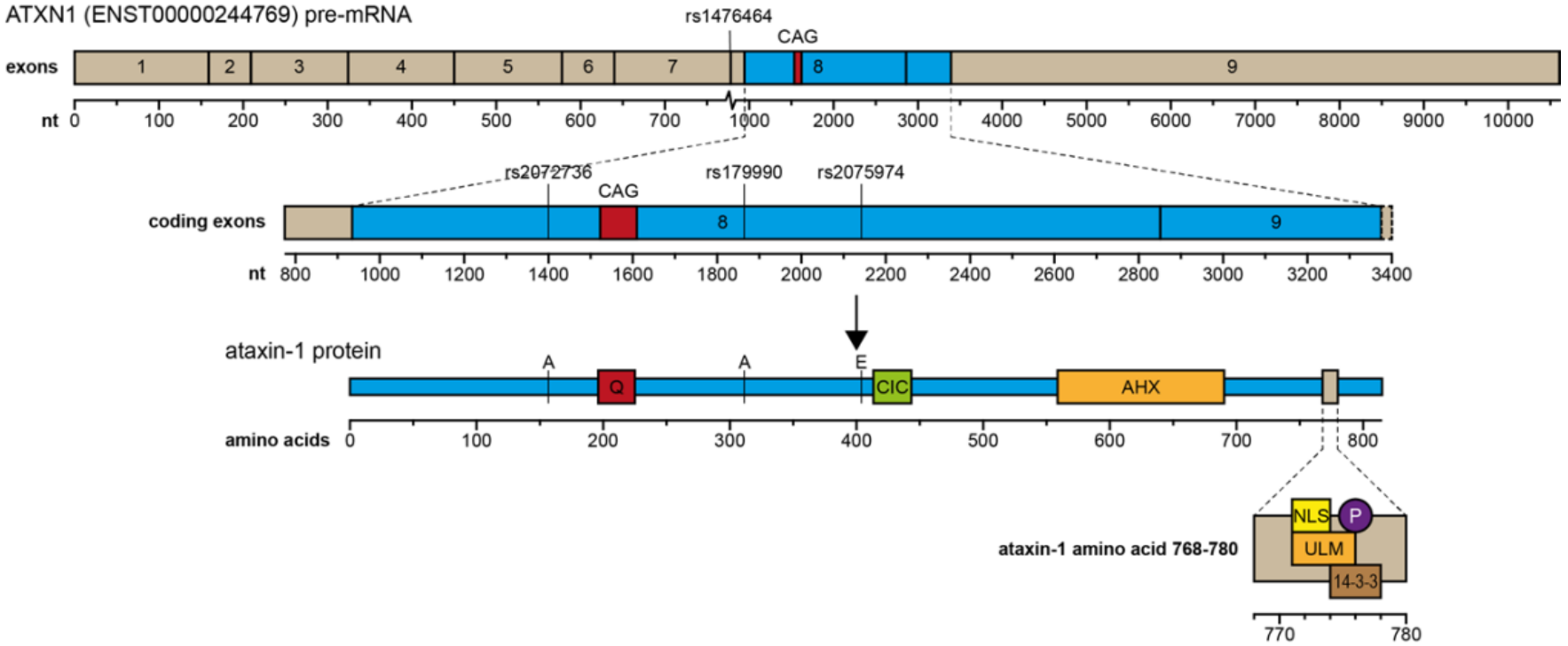
Dell'Orco et al., 2015

First symptoms usually begin in the 3th or 4th decade of life

Progressive loss of cerebellar neurons, particularly Purkinje neurons

Prevalence <1 :100,000

ATXN1 gene

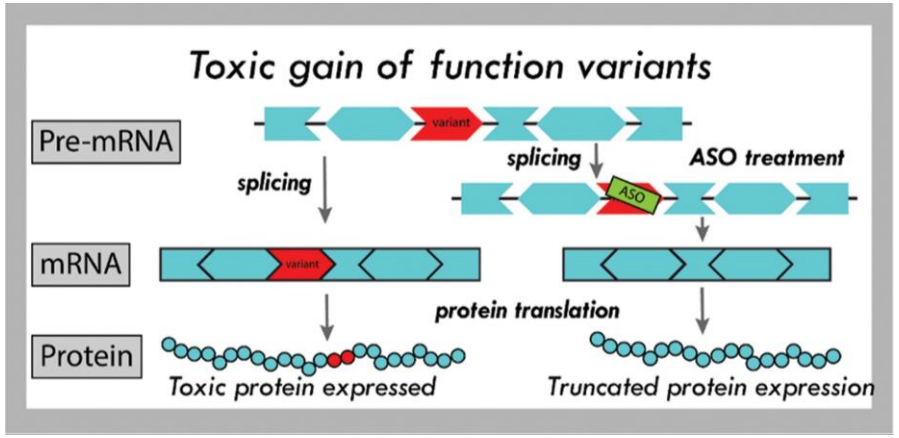


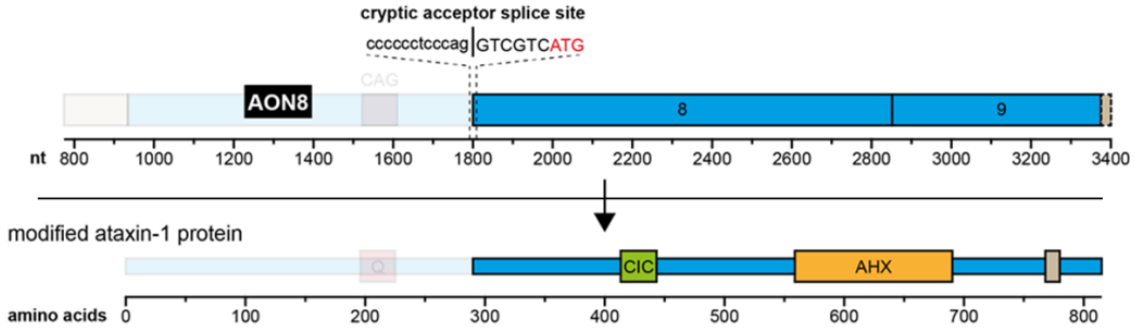
CAG repeat expansion in exon 8 of the *ATXN1* gene

Function: Regulating gene expression:
Interactions with transcriptional regulators and involved in RNA splicing machinery

phosphorylation site regulates the protein's stability and interactions with its binding partners

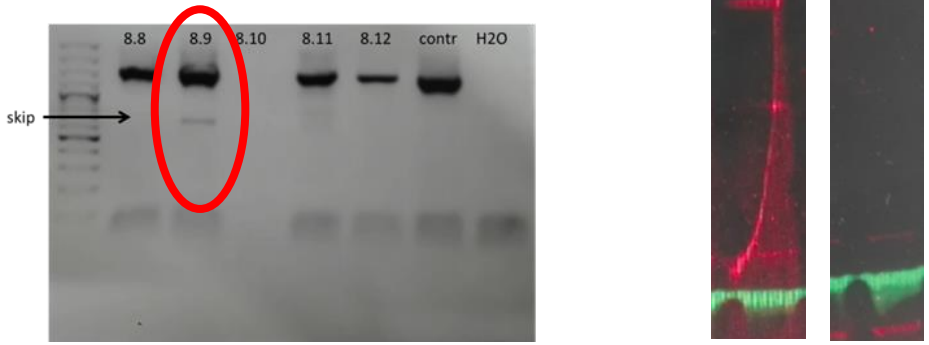
RNA/Protein modification – exon skipping

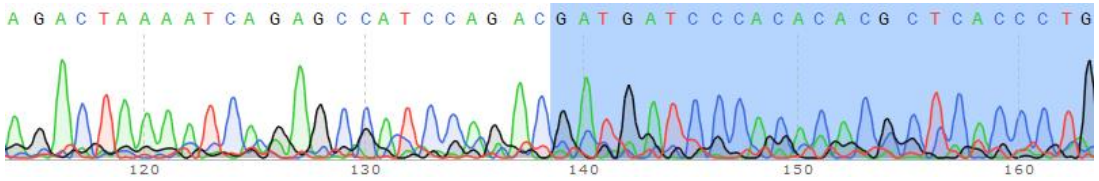




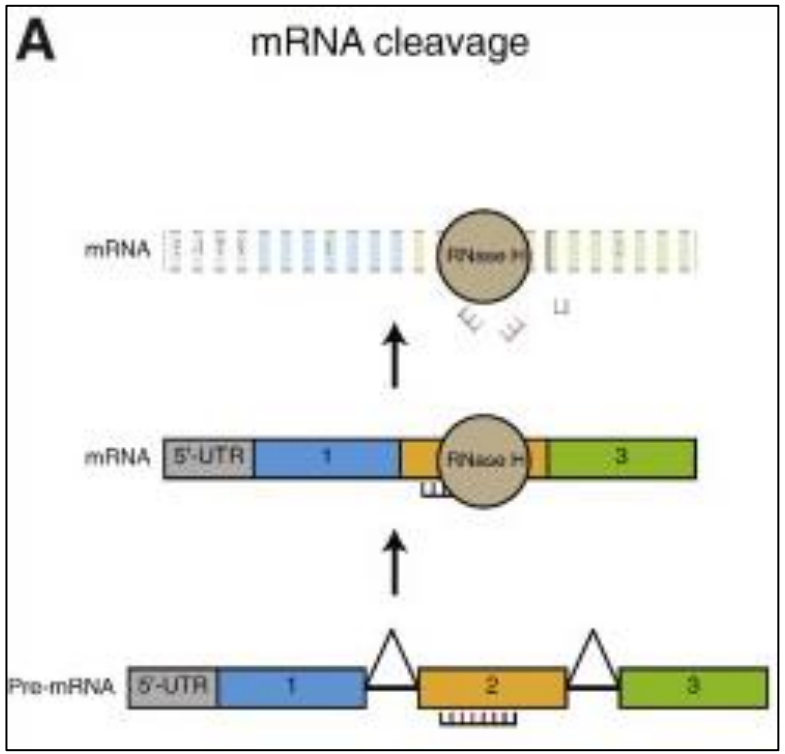
AON control

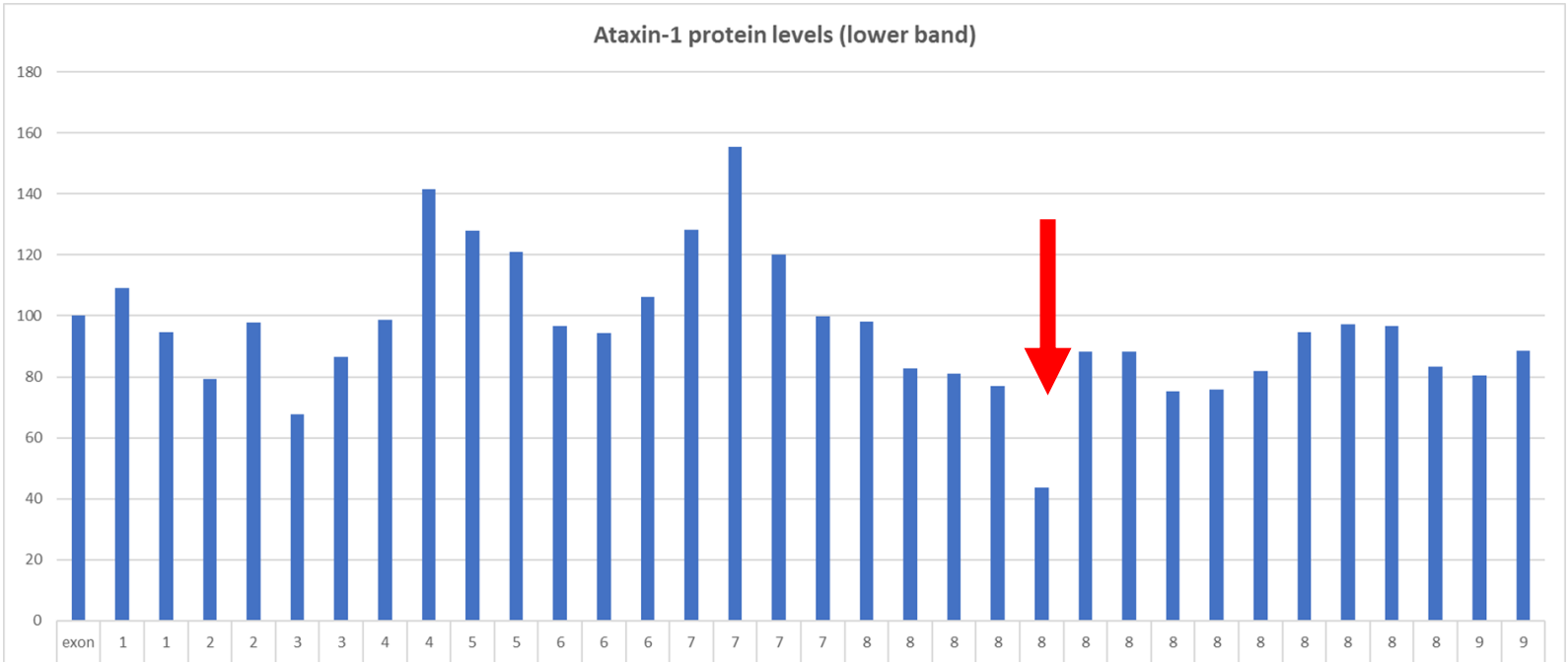
20 nM AON



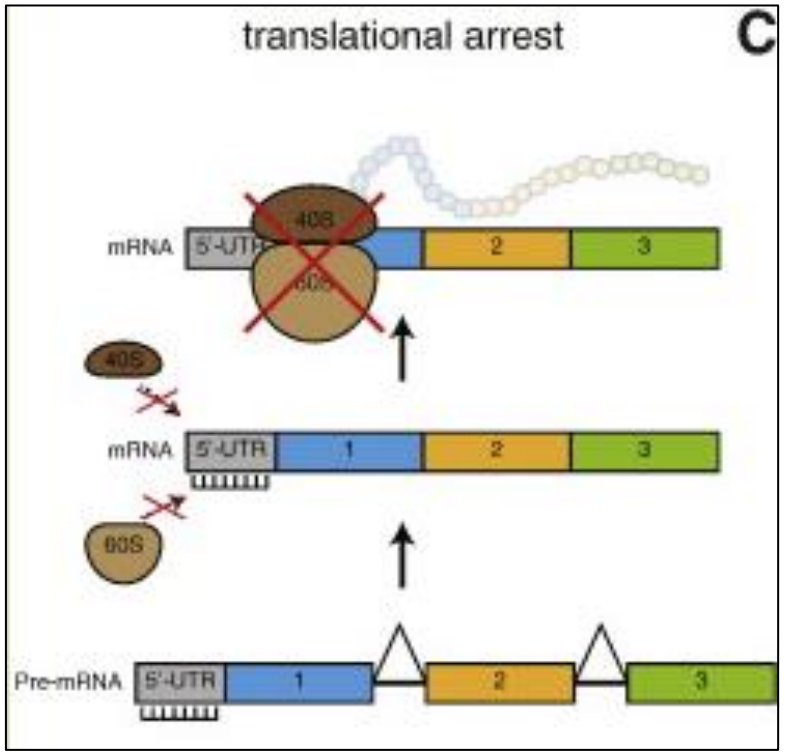


Non allele-specific reduction: Gapmers





Allele specific reduction



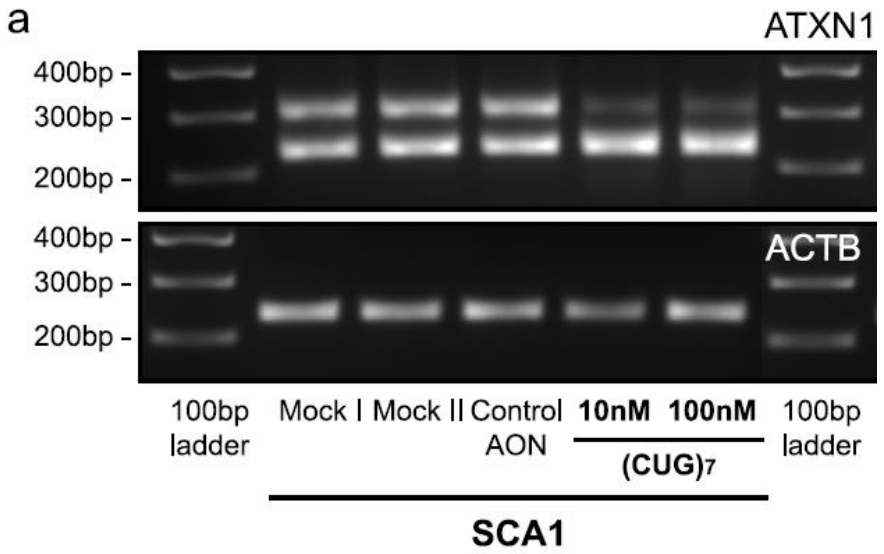
OPEN ACCESS Freely available online

PLoS one

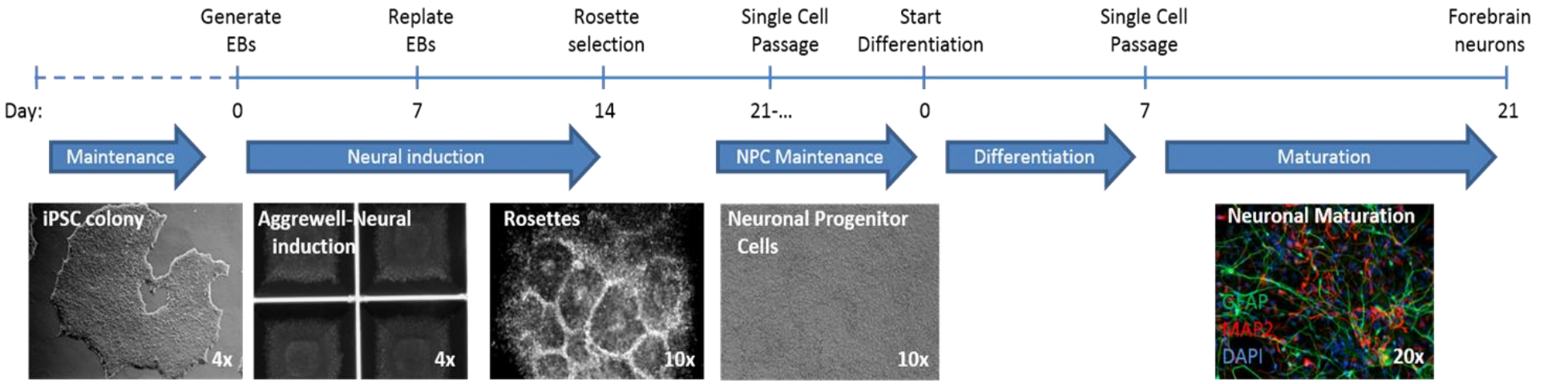
Targeting Several CAG Expansion Diseases by a Single Antisense Oligonucleotide

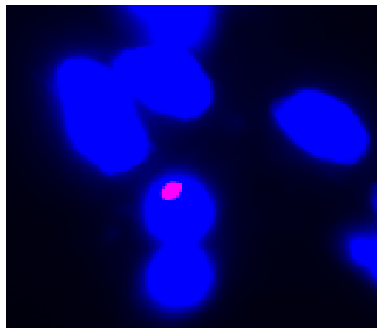
Melvin M. Evers[1], Barry A. Pepers[1], Judith C. T. van Deutekom[2], Susan A. M. Mulders[2], Johan T. den Dunnen[1,3], Annemieke Aartsma-Rus[1], Gert-Jan B. van Ommen[1], Willeke M. C. van Roon-Mom[1]*

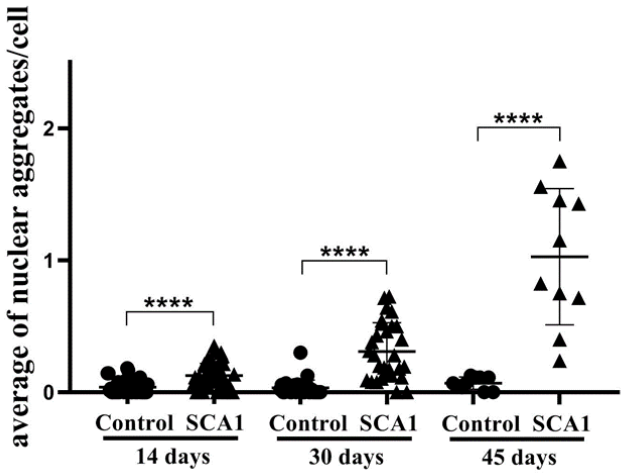
1 Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, **2** Prosensa Therapeutics B.V., Leiden, The Netherlands, **3** Leiden Genome Technology Center, Leiden University Medical Center, Leiden, The Netherlands

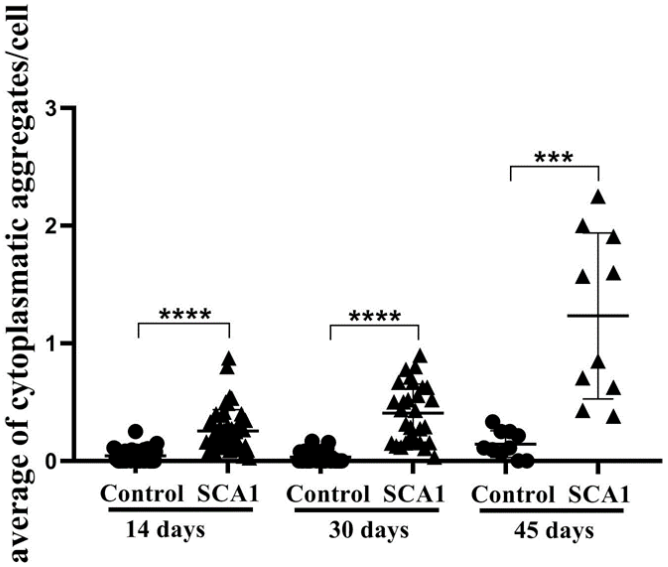


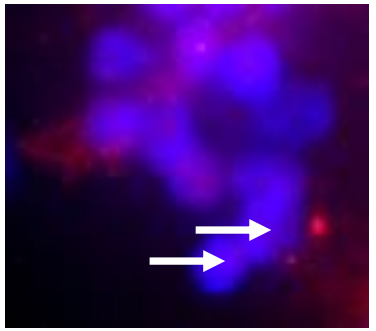
Cellular models: iPSC to Neurons



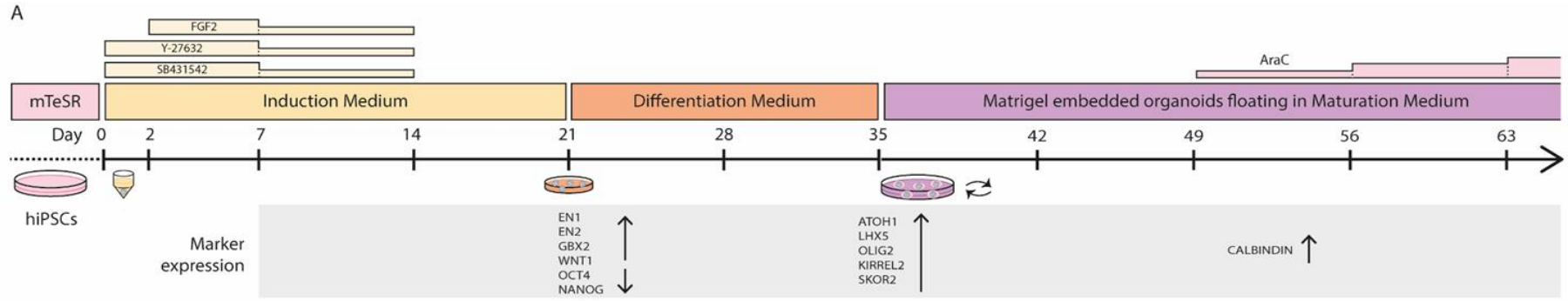


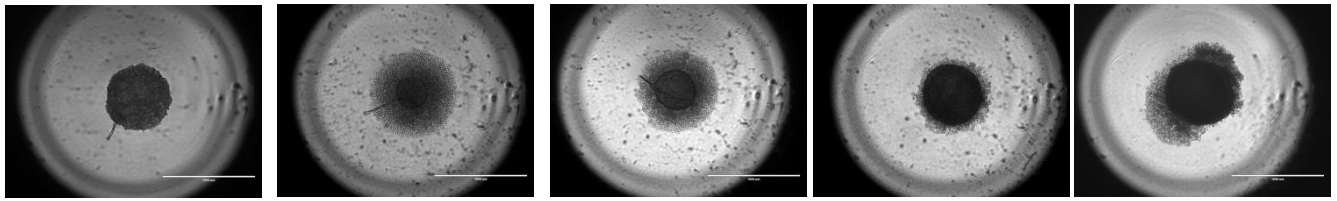




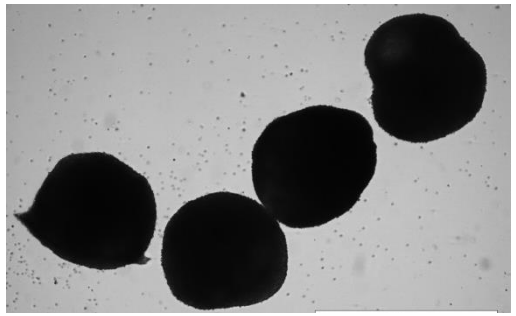


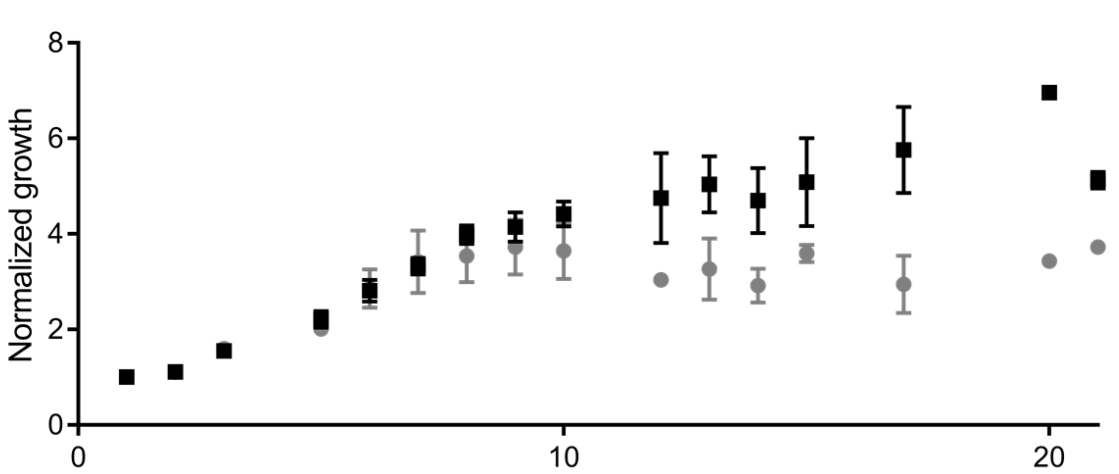
Hindbrain organoids



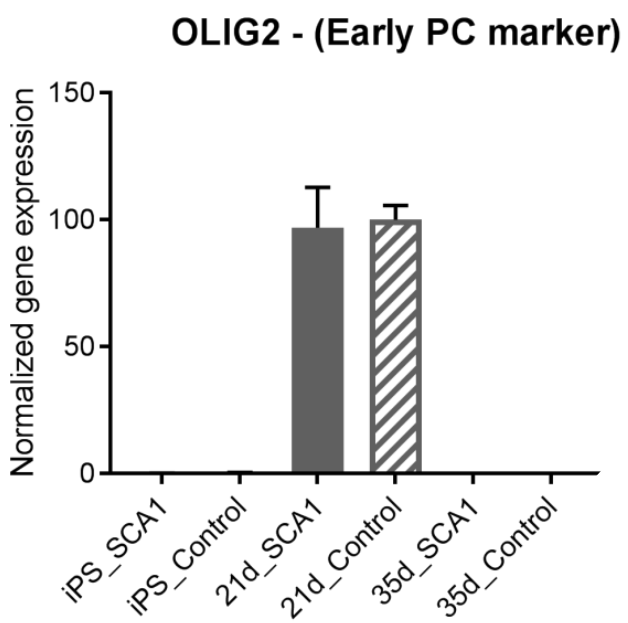


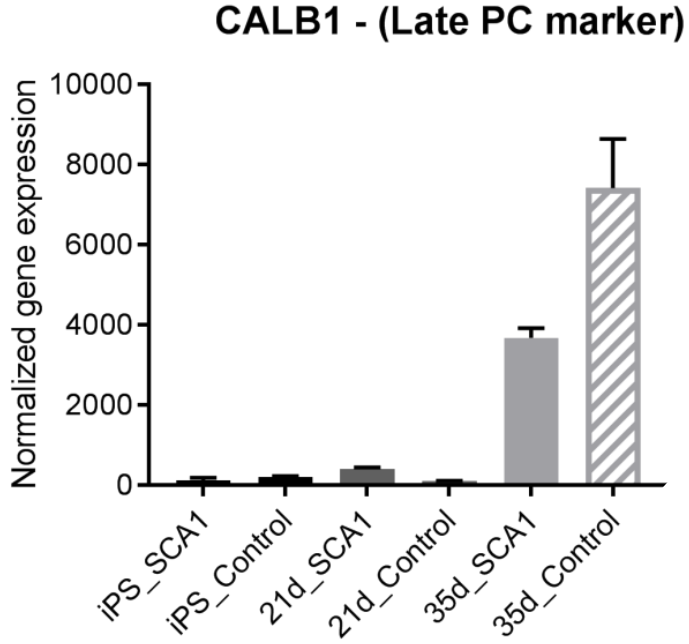
D0-21

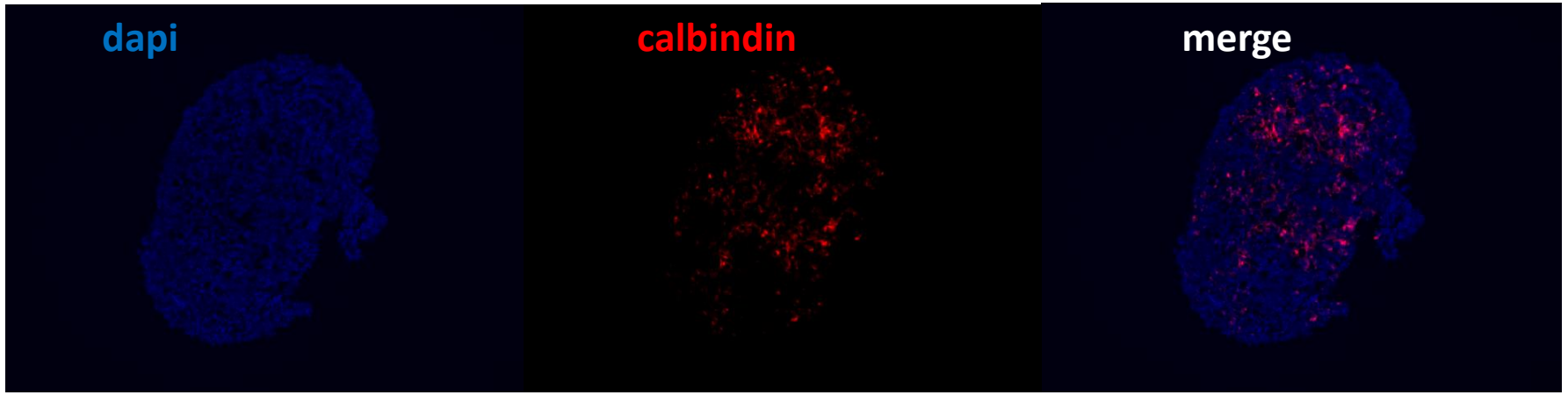




Purkinje cell markers in cerebellar organoids







Milasen: the ultimate personalized medicine

DCRT Dutch Center for RNA Therapeutics

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

Kim *et al. N.Engl.J.Med.* 2019

Milasen: monitoring after treatment

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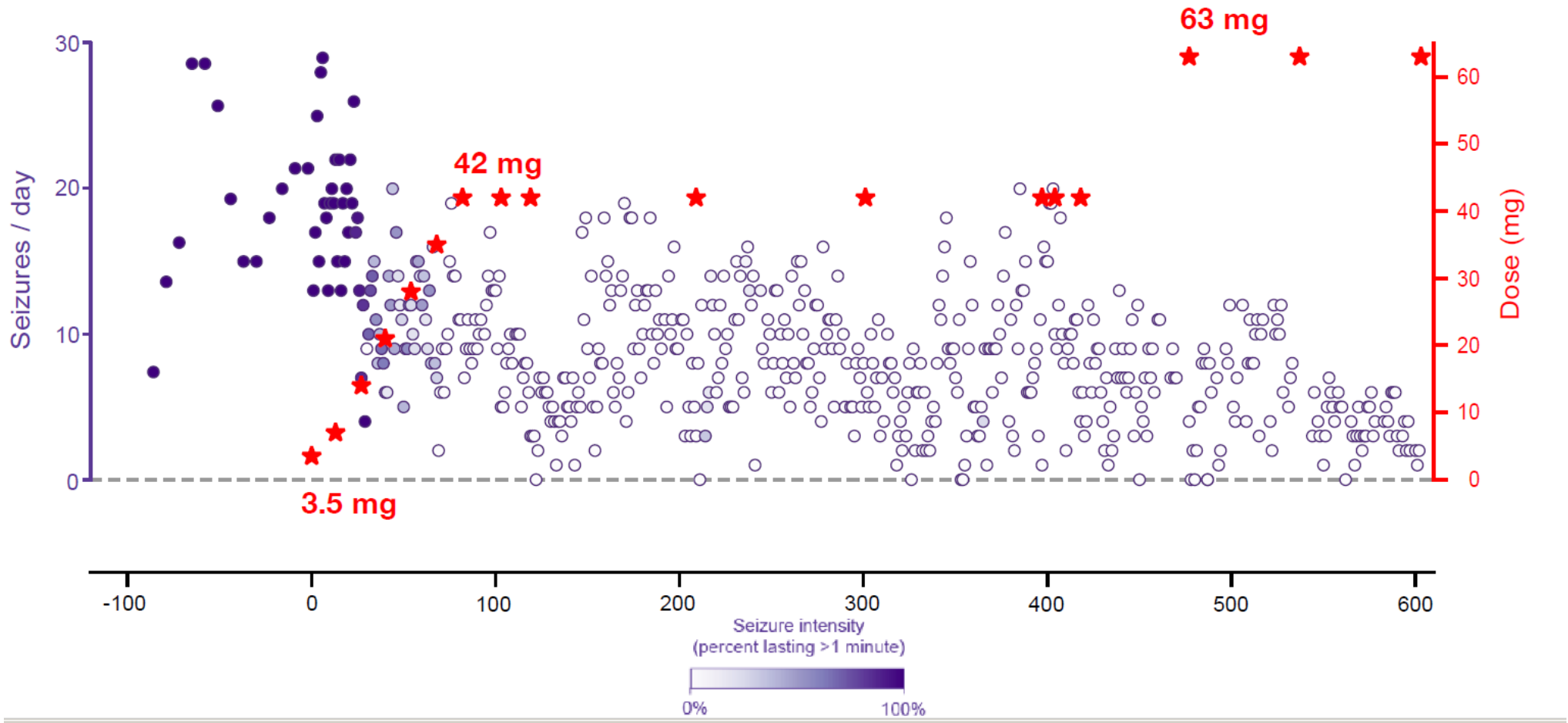
Milasen treatment: first two years



The Dutch Center for RNA Therapeutics

DCRT Dutch Center for RNA Therapeutics

What if a patient has ataxia that is caused by a DNA mutation that is found in less then 5 patients world-wide?

Such a disease is so rare, that if a company would develop a therapy it is not possible to get a return on its investment

It is not possible to perform classical clinical trials

In Europe, it is not possible to get such drugs registered by the European Medicine Agency

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
- Please reach out to DCRT@lumc.nl for any questions.


Annemieke Aartsma-Rus


Rob Collin

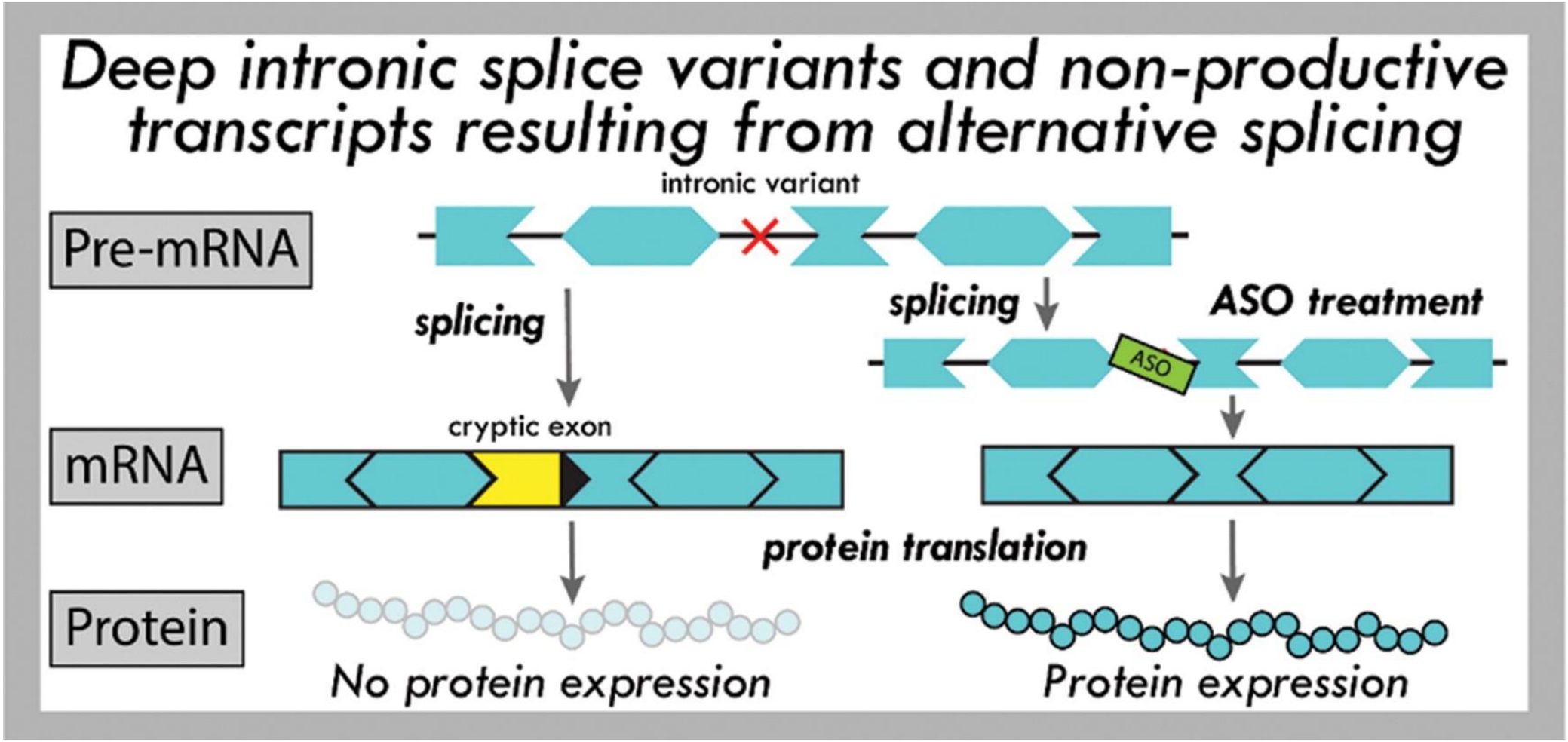

Willeke van Roon-Mom


Ype Elgersma


Anouk Spruit


Marlen Lauffer

Potential for cryptic splicing mutations



Opportunities for eye and brain diseases

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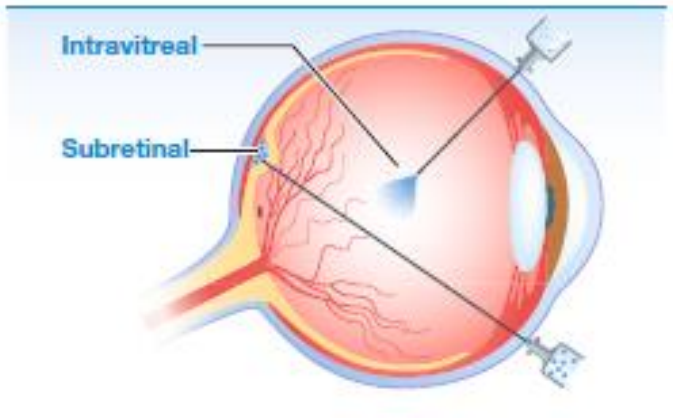

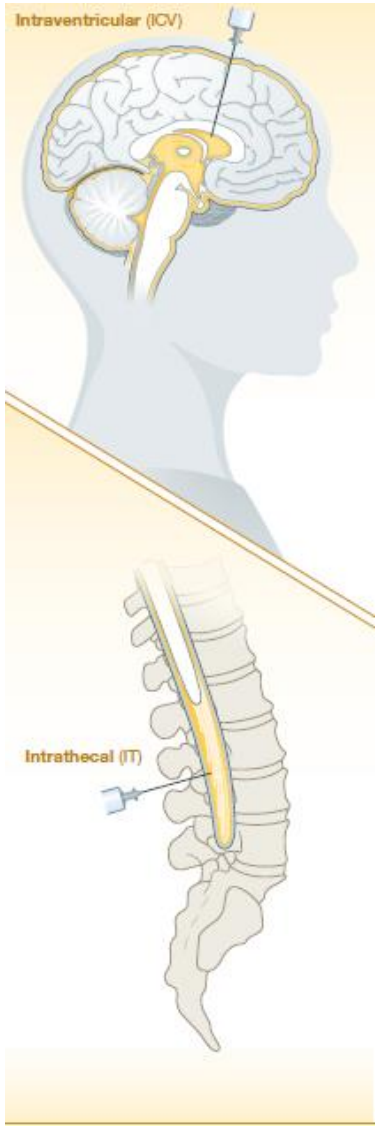

- Local treatment
  - Low dose, high local exposure
  - Systemic exposure low
- Low treatment frequency

For whom?

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

Development of a therapy for one person

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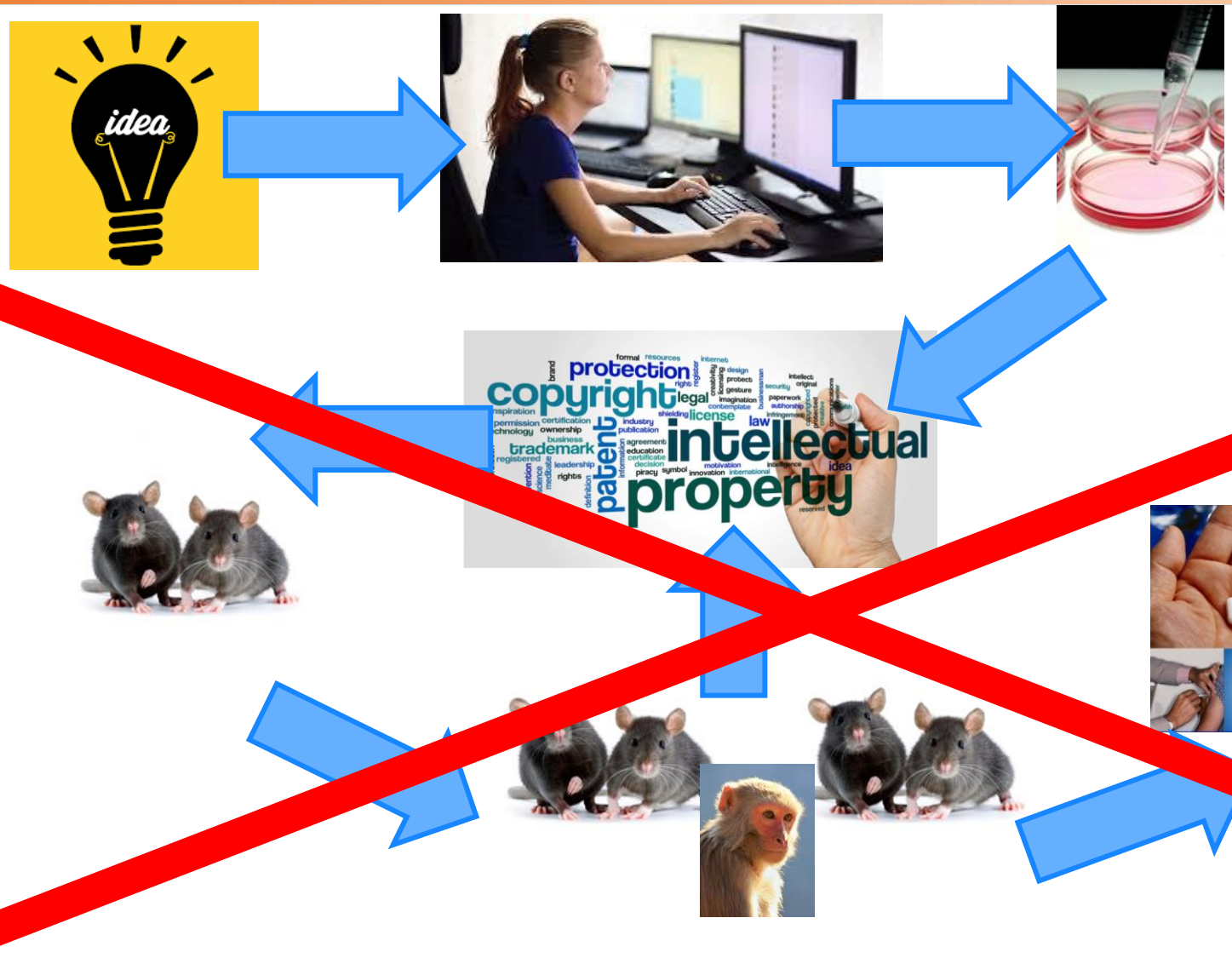

European laws and regulations

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- Named patient setting: no regulatory approval

  - We are communicating with regulators for advice

- Hospital pharmacy preparation of GMP or GMP-like compound
- Preclinical studies and safety studies as much as possible in house
- For now: funding from University Medical Centers involved
- Future: reimbursement through Health Institute of The Netherlands of development costs

  - Already discussing options

Preliminary timeline DCRT

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In house fibroblasts/iPSC cells	Animal facility/academic pharma: Study in rats In vitro- neuronal iPSC	Clinic: Natural history, treatment, monitoring

AON design & testing → **Functional studies** → **Preparing for treatment/safety pharmacology profile** → **Treatment** → **Deposit sequence**

50k → 50k → 500k → administration of drug

6 months → 6 months → 6 months → 4/6x year lifelong

Aligning with other initiatives

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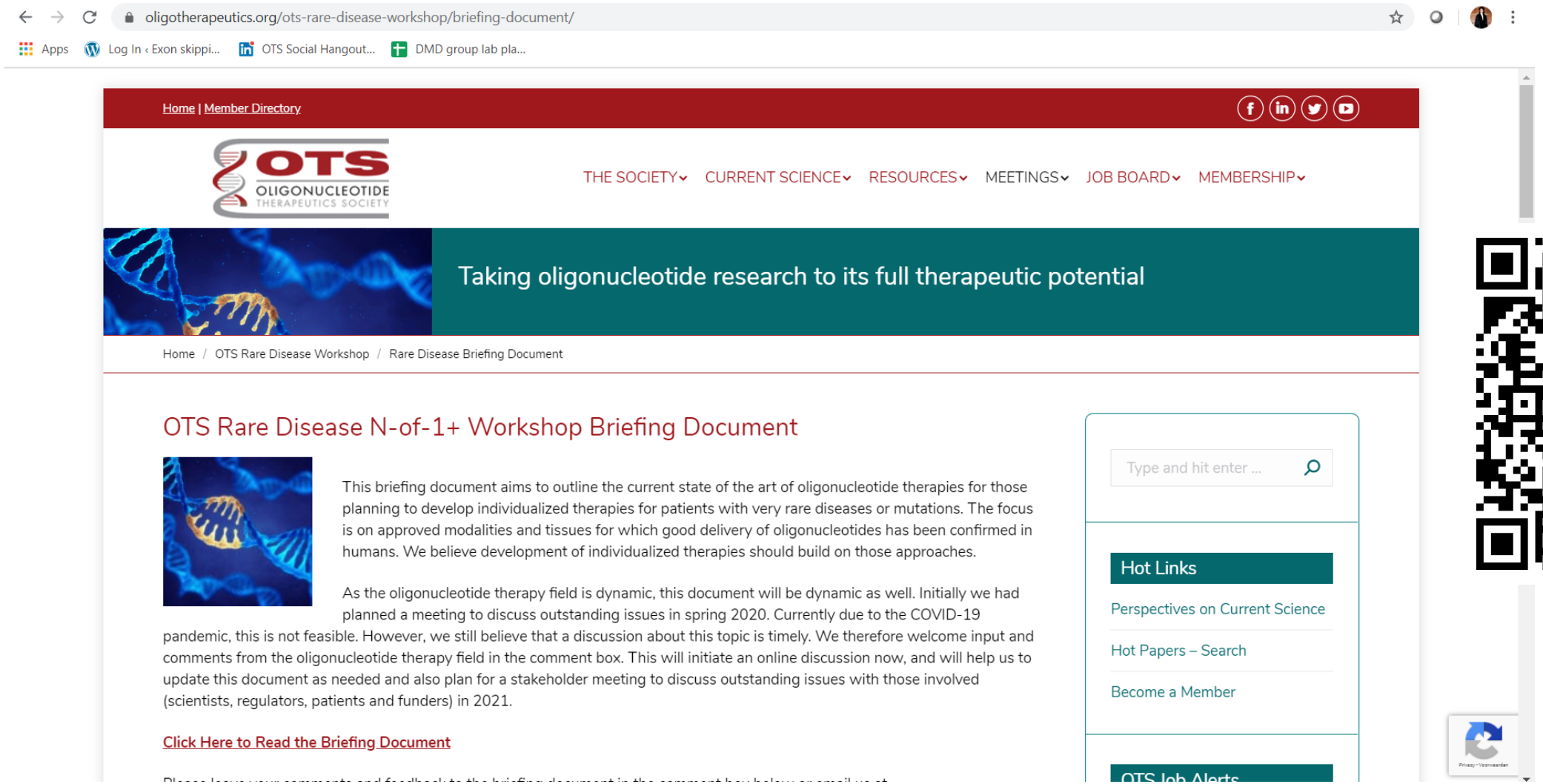

From The Netherlands to Europe

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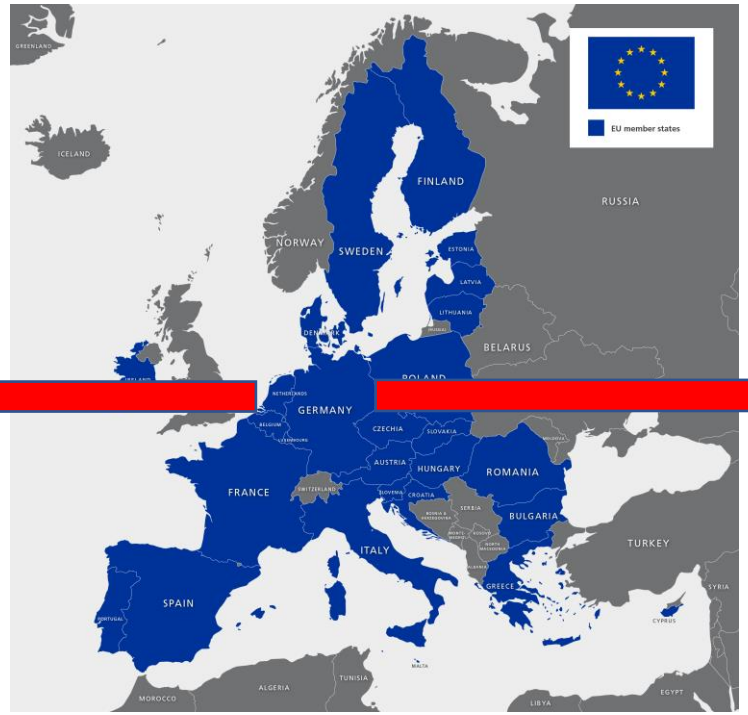

L U M C

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

Annemieke Aartsma-Rus

Willeke van Roon-Mom

Holm Graessner

Rebecca Schule

Matthis Synofzik

Where are we now? - Regulation

- Meeting with European Medicine Agency Innovation Task Force to get advice on appropriate in vitro and in vivo safety and toxicity studies (December 2021)
- Follow-up Scientific Advice meeting with European Medicine Agency start 2022
- Once we have treated first patients we will discuss reimbursement strategies with Zorg Instituut Netherlands

Where are we now? - Research

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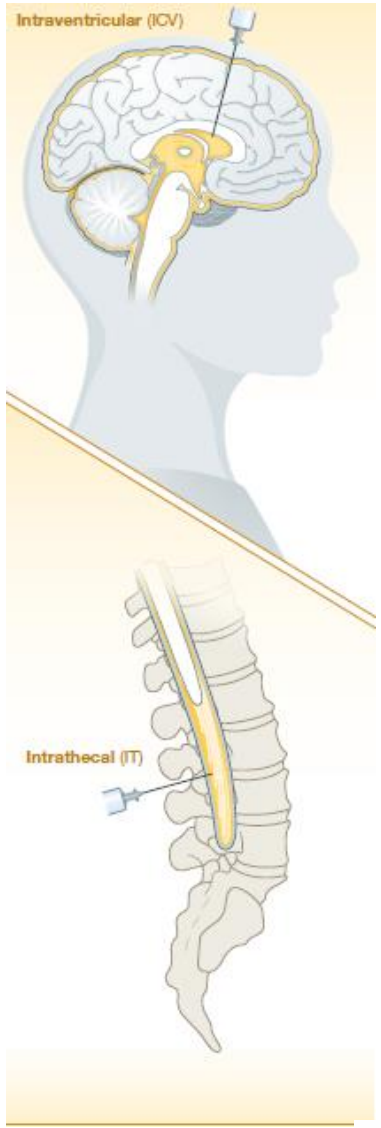
- Studying 2 patients with BPAN (LUMC/University Tubingen)
  - WDR45 mutations, both likely exon skipppable
  - AONs designed
  - Currently being tested in vitro
- Studying 1 patient with Stargardt disease (Radboudumc) 12 more identified
  - Effective AON identified in vitro
  - Preparing for clinical treatment



BPAN

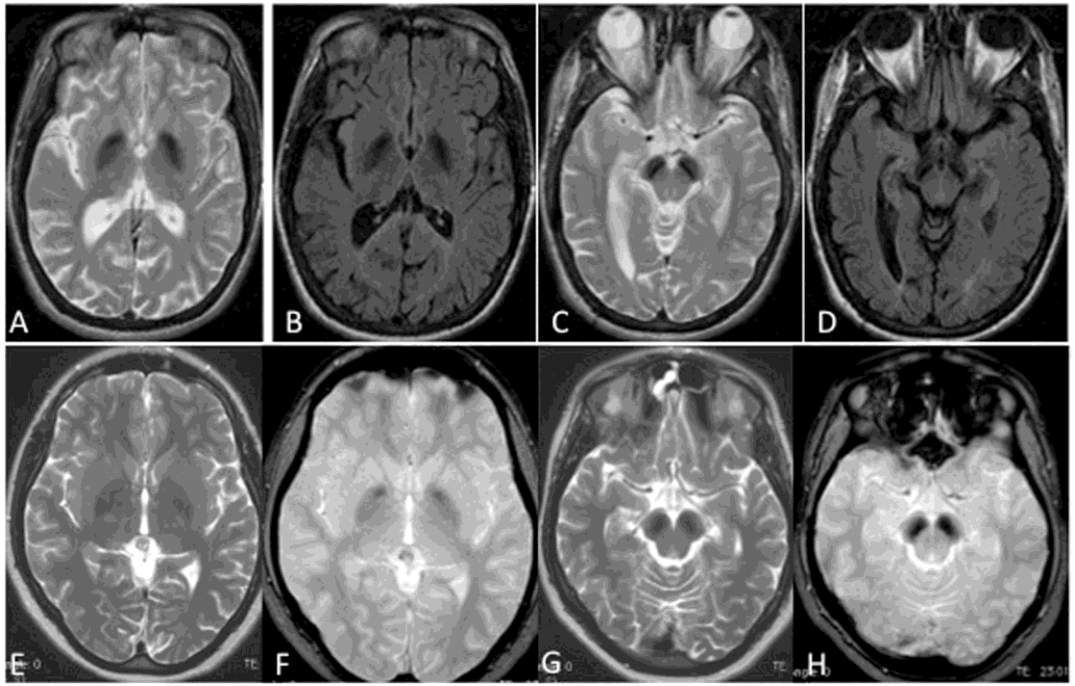
Matthis Synofzik

Tassula Proikas-Cezanne

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

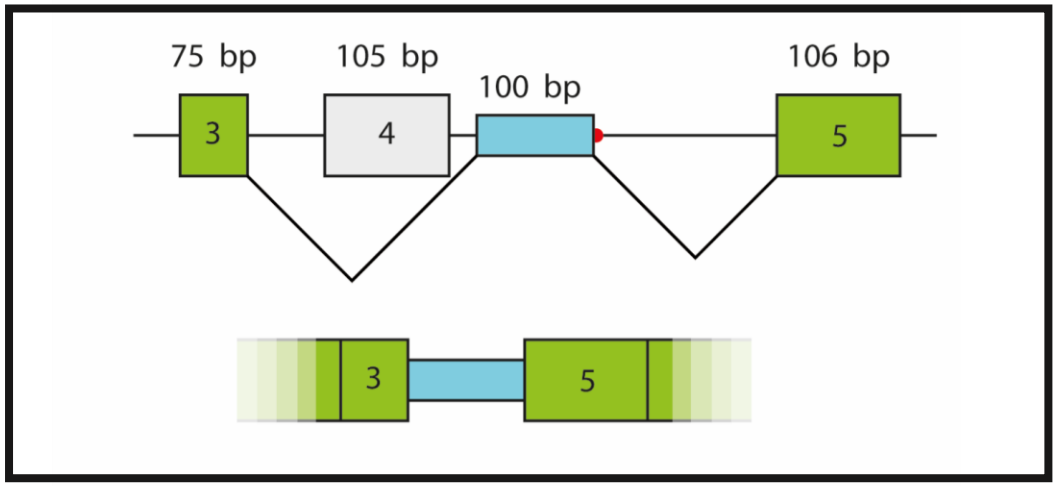
BPAN (beta-propeller protein-associated neurodegeneration

- Autosomal dominant X-linked disease
- *WDR45* mutations
- early developmental delay
- Regression and Parkinsonism in adulthood
- MRI high iron in basal ganglia
- WDR45 = WIPI4 essential function in autophagy

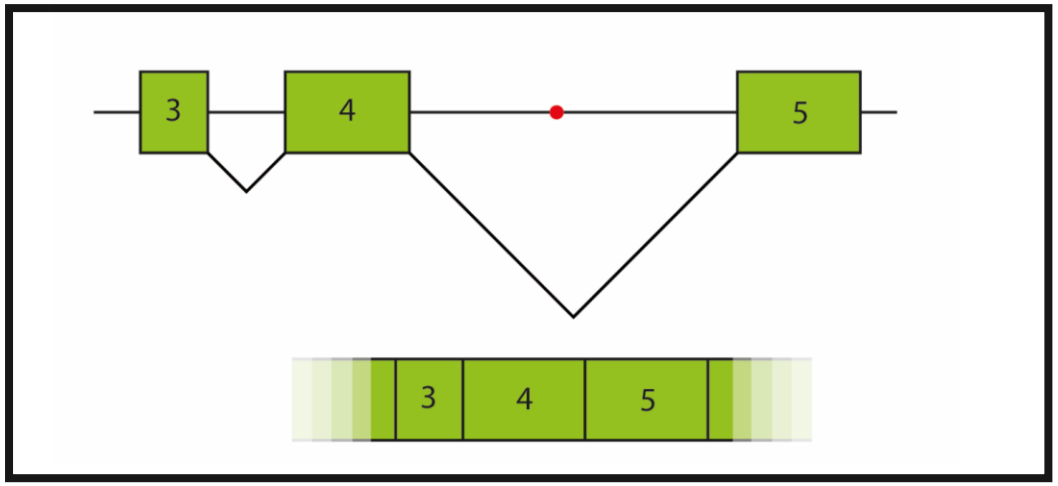


Brain 2013 Jun:1708-17. doi: 10.1093

WDR45 mutation

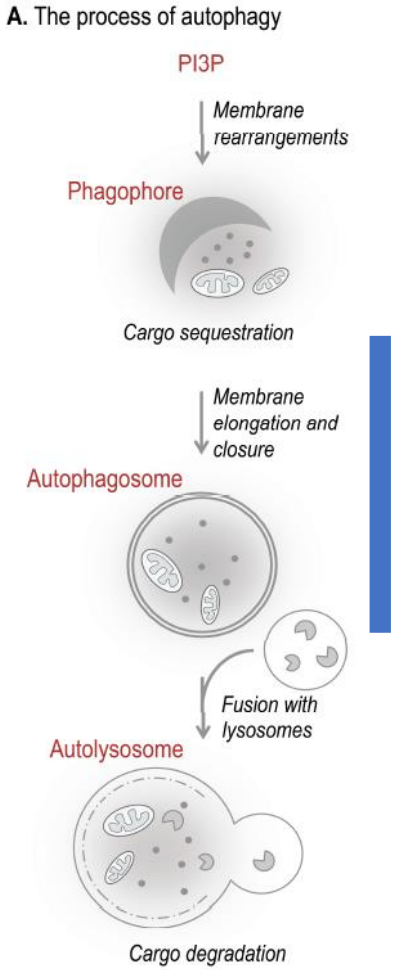


Deep intronic mutation
Exclusion of exon 4
Inclusion of cryptic exon
RNA out of frame – no protein expressed

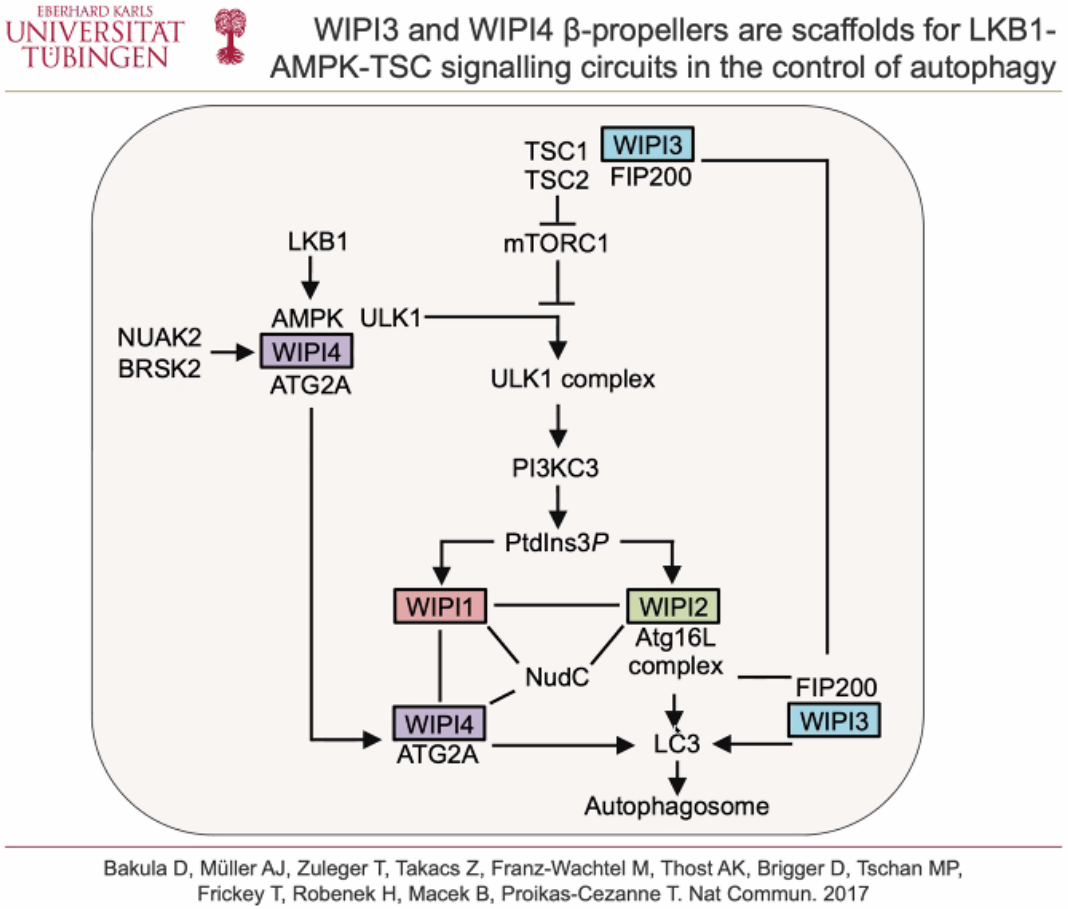


Splice modulation
Restore normal splicing
Restore normal protein expression

WIPI4 controls the size of the phagophore

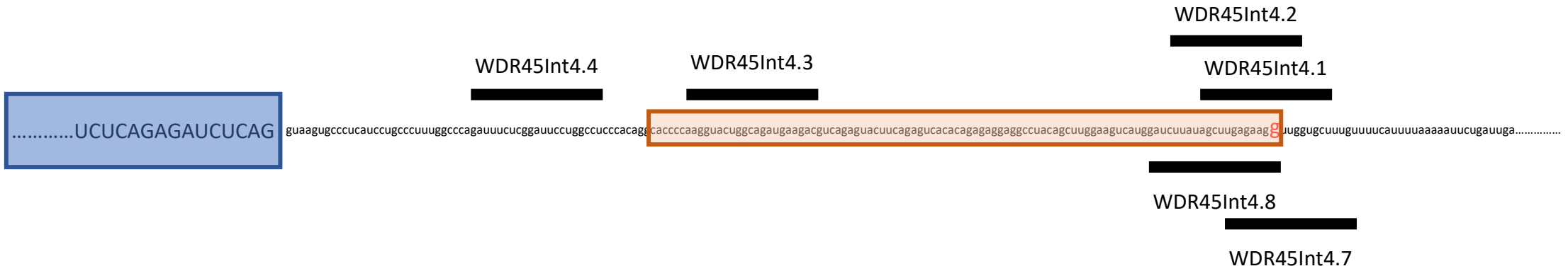


WIPI4/WDR45



Autophagosome formation requires WIPI3 and 4

WDR45 AONs


WDR45Int4.2
WDR45Int4.4
WDR45Int4.3
WDR45Int4.1
……UCUCAGAGAUCUCAG
guaagugcccucauccugcccuuuggcccagauuucucggauuccuggccucccacaggcacccaagguacuggcagaugaagacgucagaguacuucagagucacacagagaggaggccuacagcuuggaagucauggaucuuauagcuugagaaggguggugcuuuguuuucauuuuaaaaauucugauuga……
WDR45Int4.8
WDR45Int4.7

WDR45 splice correction

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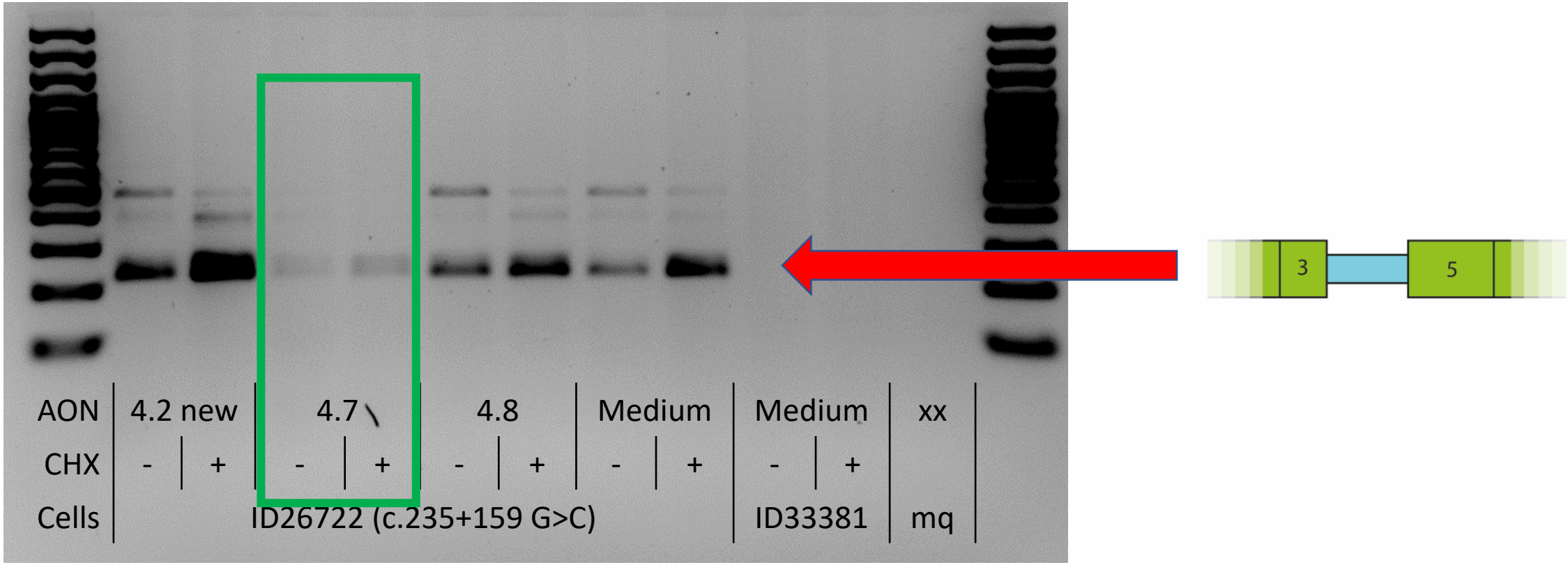

Next steps

- No antibody available, cannot examine restoration of protein expression

- Test for normalization of autophagy
  * Optimize concentration
  * Safety / pharmacology study

- Clinical work:
  * Patient eligibility
  * Patient/parent approval
  * Natural history

- Regulatory:
  * iPSC toxicity studies
  * Rodent safety studies
  * Ethical approval
  * ???

Room for improvement in diagnostics?

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Deep intronic mutations

- Often genetic diagnosis does not look for deep intronic mutations
- If you identified or know of a deep intronic mutation causing or likely causing a progressive brain or eye disorder please let us know

Want to know more?

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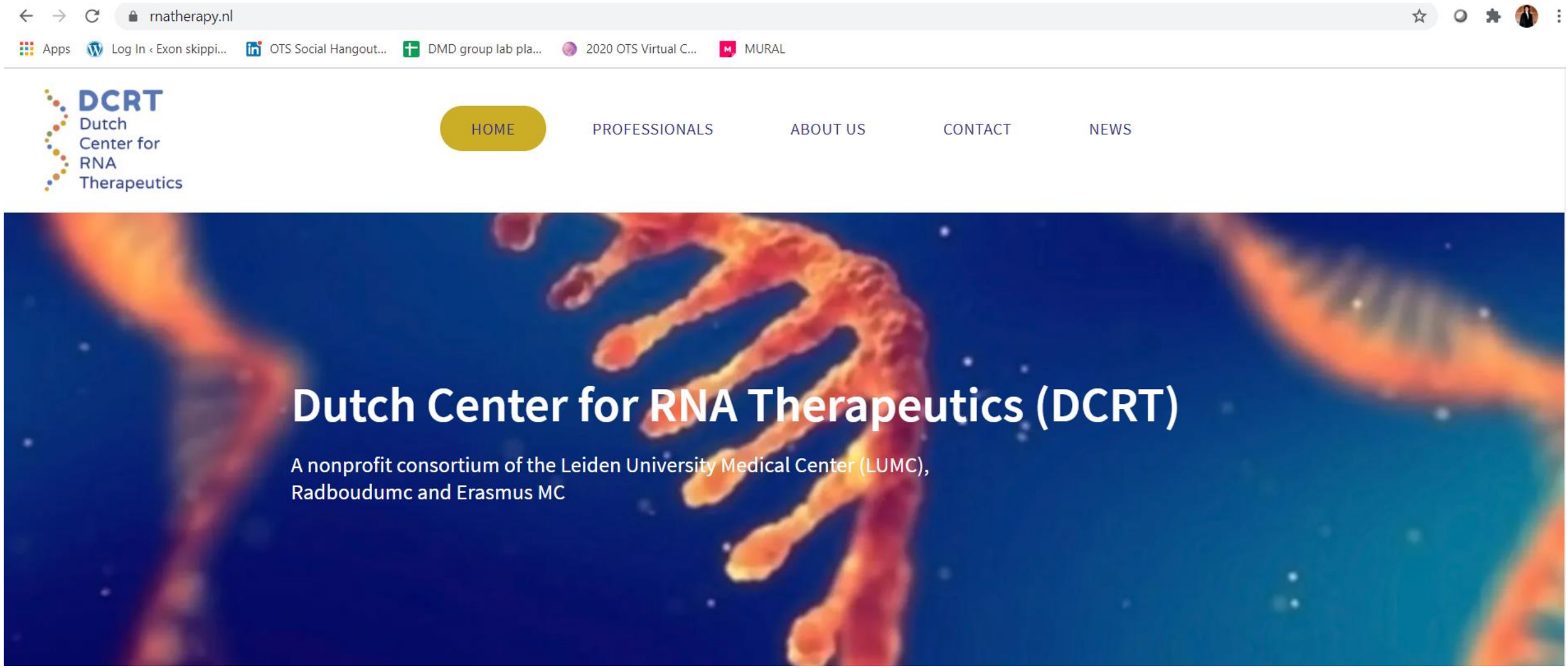

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- Annemieke Aartsma-Rus
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- Rob Collin

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- Ype Elgersma

VICO Therapeutics

CURE RARE DISEASE

IONIS

Brugling Fonds

Hersenstichting Nederland

AFMTÉLÉTHON CURE THROUGH INNOVATION

SCA1 Families Fund

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