Leids Universitair Medisch Centrum

Oligonucleotide therapies: a new calss of drugs that allow precise genetic targeting

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October 2021

European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

Learning objectives

By the end of this webinar you will be able to:

- Describe what makes oligonucleotide therapies unique
- Explain what is needed to turn oligonucleotides into drugs
- Differentiate between different type of oligonucleotide modalities and explain how they work
- Apply this knowledge to your own use cases

Outline

- What are oligonucleotide drugs?
- What is needed to turn oligonucleotides into drugs?
- Considerations for delivery
- Which modalities are there?
- Considerations for safety
- Oligonucleotide therapies for very small groups

- Will illustrate these aspects with examples from approved oligonucleotides and those in clinical development

What are antisense oligonucleotide drugs?

- Small pieces of modified DNA or RNA
  - Single or double stranded
  - Synthesized from chemically modified nucleotides
- Target RNA in a sequence specific manner
- Aim: therapeutic effect
  - Knockdown of toxic protein
  - Restoration of missing protein
- Intermediate MW (5-12 kDa)

What makes them so unique?

- Watson-Crick basepairing to target transcript
- Very specific and targeted effects
  - Side effects mainly driven by chemical modifications
- Can intervene at a genetic level

Requirements to make them drugs

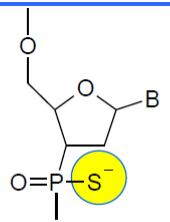
- Improve stability (nuclease resistant)
  - 2’-O modification
  - Phosphorothioate
- Pharmacokinetic properties/bioavailability
  - Phosphorothioate
  - Delivery: systemic vs local (intrathecal)
- Many modifications available
  - Which one you can use depends on therapeutic approach

Common chemical modifications

First Generation

Modified Phosphate backbone

Greater Resistance to nucleases

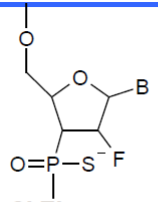


phosphorothioate

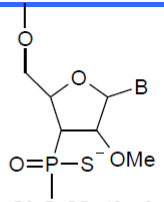
Second Generation

2' Substituted

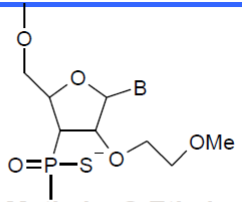
Tighter target binding



2' Fluro phosphorothioate



2' O Methyl (OMe PS)

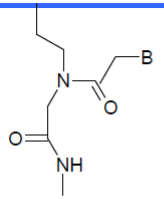


2' O Methyl – O Ethyl (O-MOE PS)

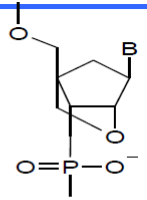
Third Generation

Non-Ribose

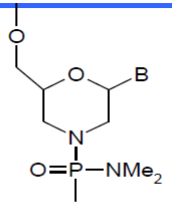
Altered charge and flexibility



Peptide Nucleic Oligomer (PNOs)



Locked Nucleic Acids (LNAs)



Phosphoamidite Morpholino Oligomers (PMOs)

Question

- What do oligonucleotides target?

  a. Specific regions in DNA

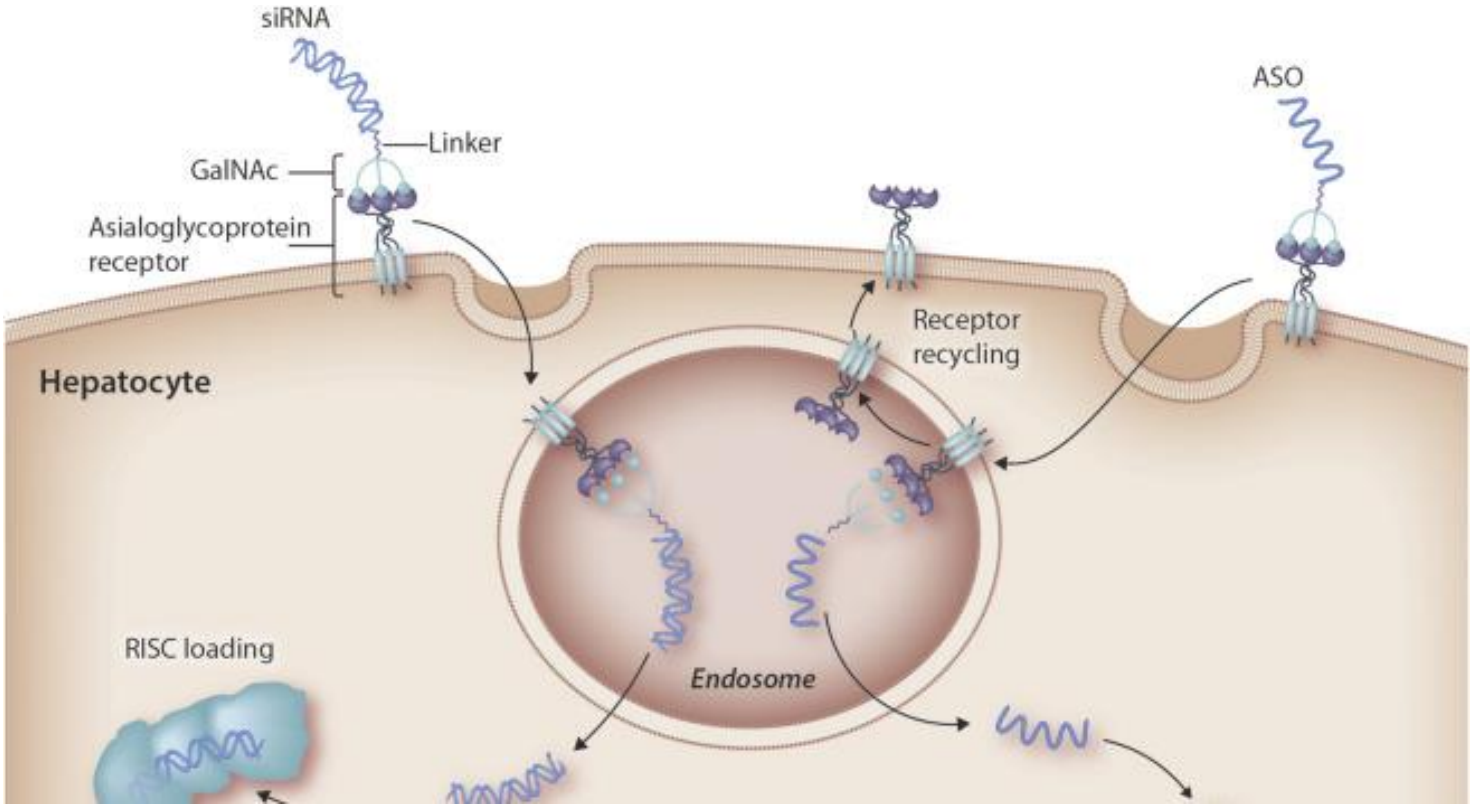
  b. Specific regions in RNA

  c. Toxic proteins

Delivery considerations

- Bioavailability single stranded oligos better than double stranded
- PS modified single stranded oligos
  - Liver and kidney, liver improved with GalNac conjugate
  - Most other tissues
  - Not nervous system (local delivery!)
- Double stranded oligos
  - Tissue uptake much reduced
  - Lipid nanoparticles: liver uptake (patisiran)
  - GalNac conjugate: very efficient liver uptake

Delivery considerations



Currently 15 approved oligonucleotide drugs (FDA/EMA)

- Modalities
  - Translation block: formivirsen
  - RNase H: mipomirsen, inotersen, valonesorsen
  - Splice switching: *eteplirsen*, nusinersen, *golodirsen, viltolarsen, casimersen*
  - SiRNA: patisiran, givosiran, inclisiran, lumasiran
  - mRNA vaccines: corminaty, Moderna COVID-19 vaccine

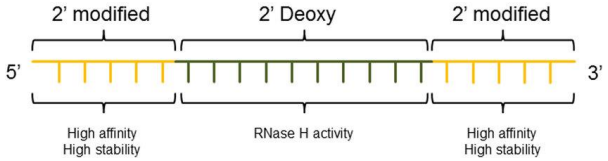
- Target tissues
  - Eye (local injection)
  - Liver (GalNac, LNPs)
  - Muscle (LNP: local injection, systemic very poor uptake)
  - CNS (intrathecal injection)

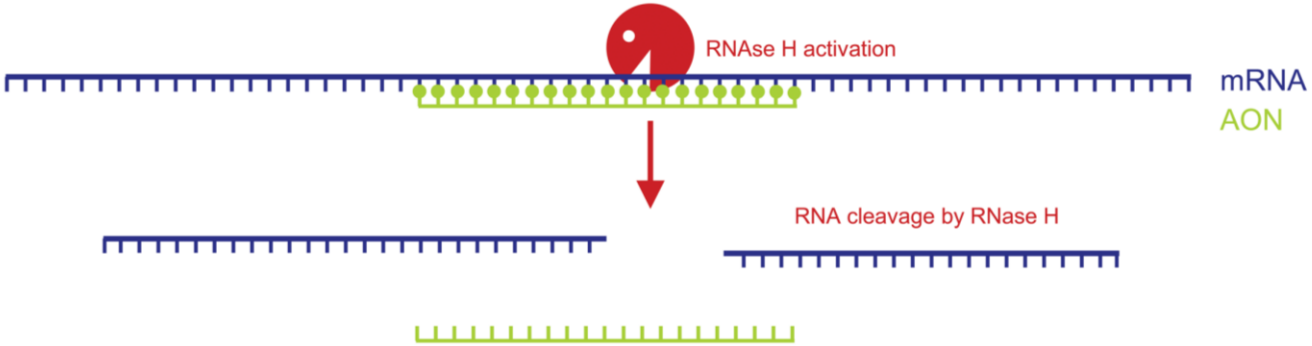
What can we use them for?

- **Protein knockdown**
  - Toxic gain of function
  - Key protein in pathological pathway
  - RNase H and siRNA
- Protein restoration (splice modulation)

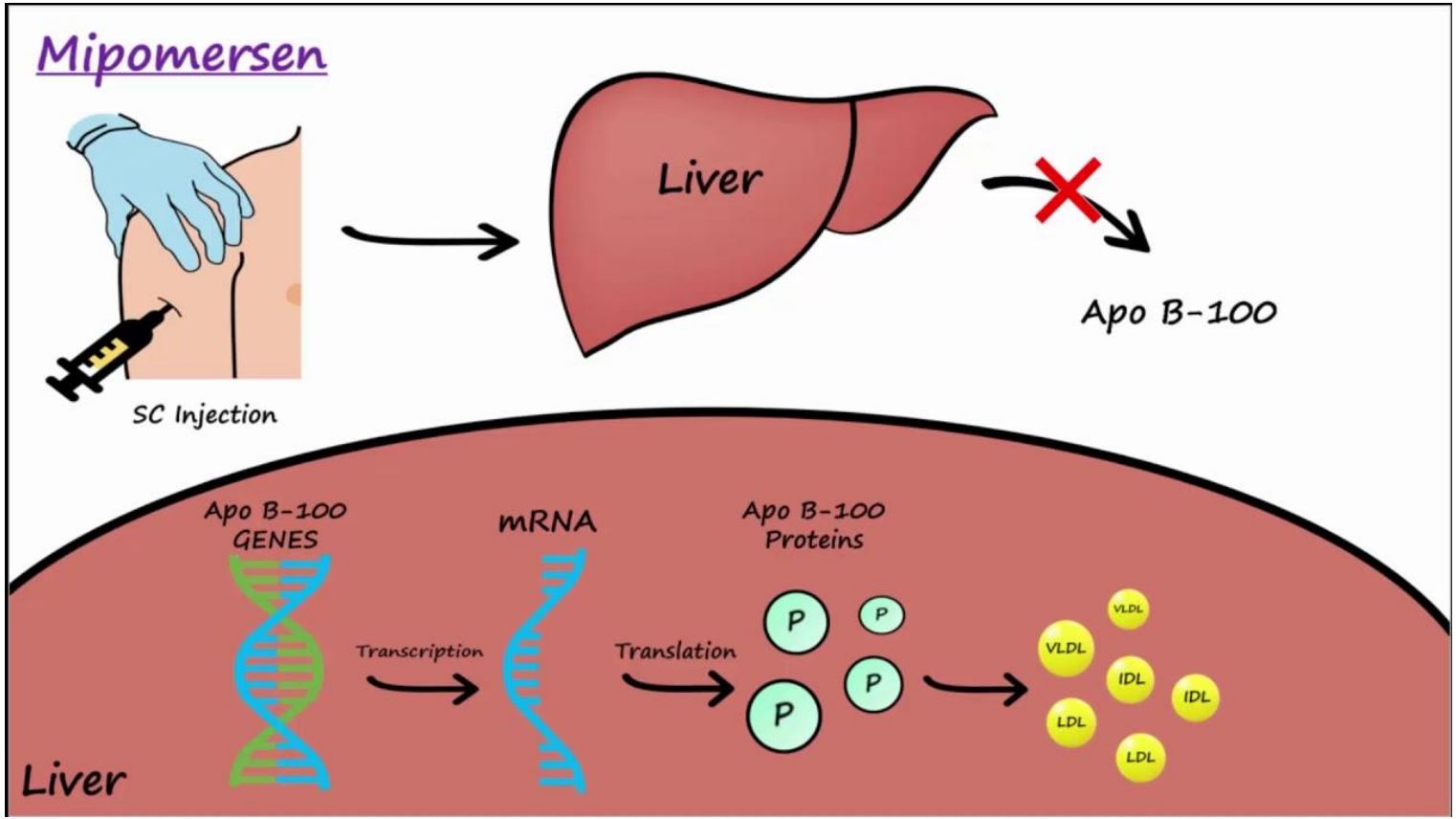
Protein knockdown 1/2: RNase H

- RNase H cleaves RNA in RNA/DNA hybrids
- Targeted knockdown of transcript
- Modifications: gapmers with PS backbone
- E.g.: mipomersen (Ionis), familial hypercholesterolemia (FDA approved

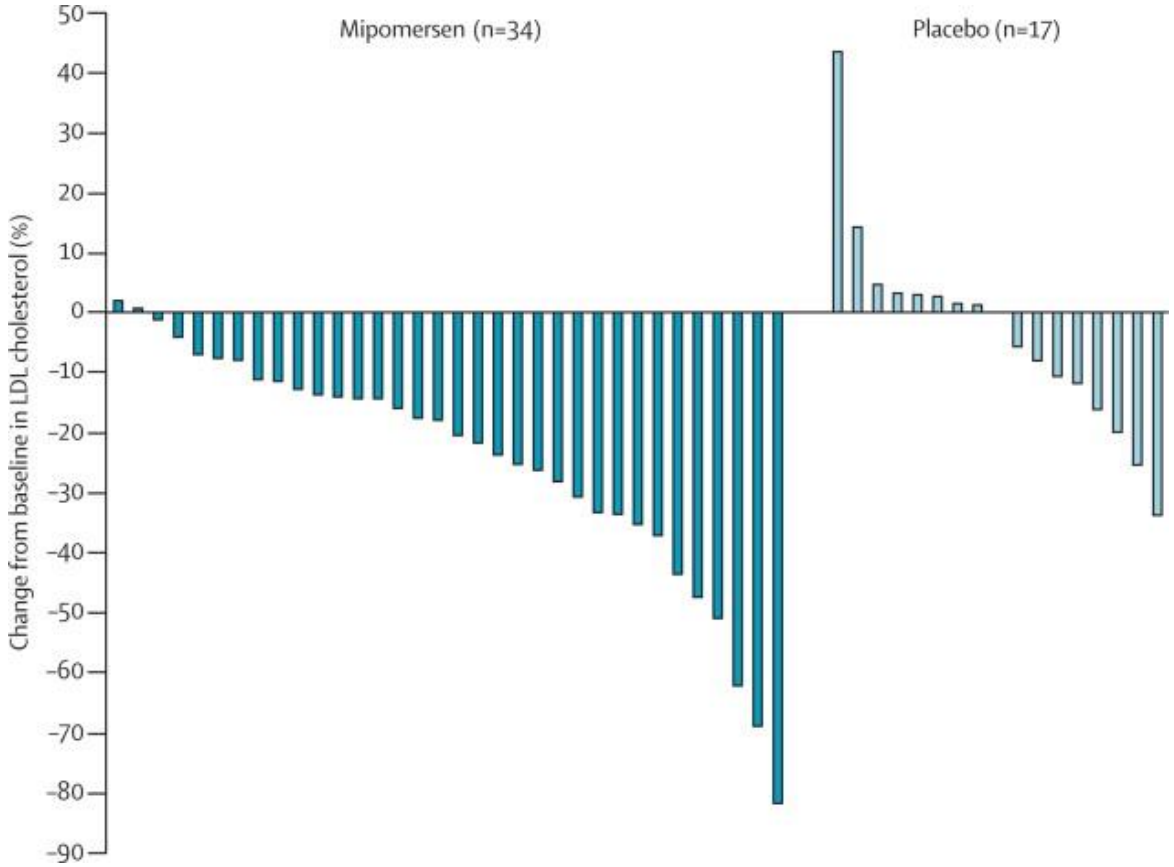




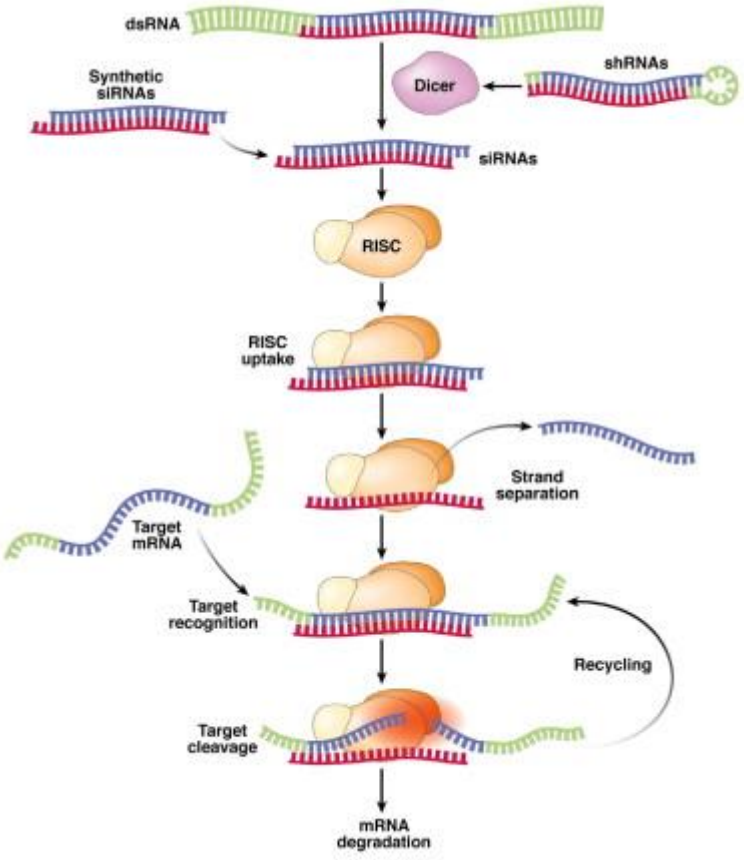
Protein knockdown 1/2: RNase H



Protein knockdown 1/2: RNase H

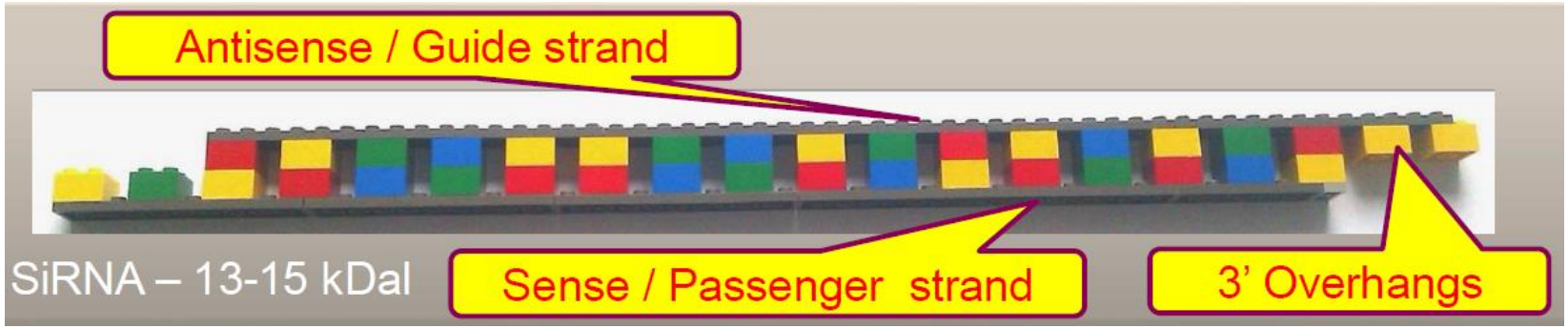


Protein knockdown 2/2: siRNA



Protein knockdown 2/2: siRNA

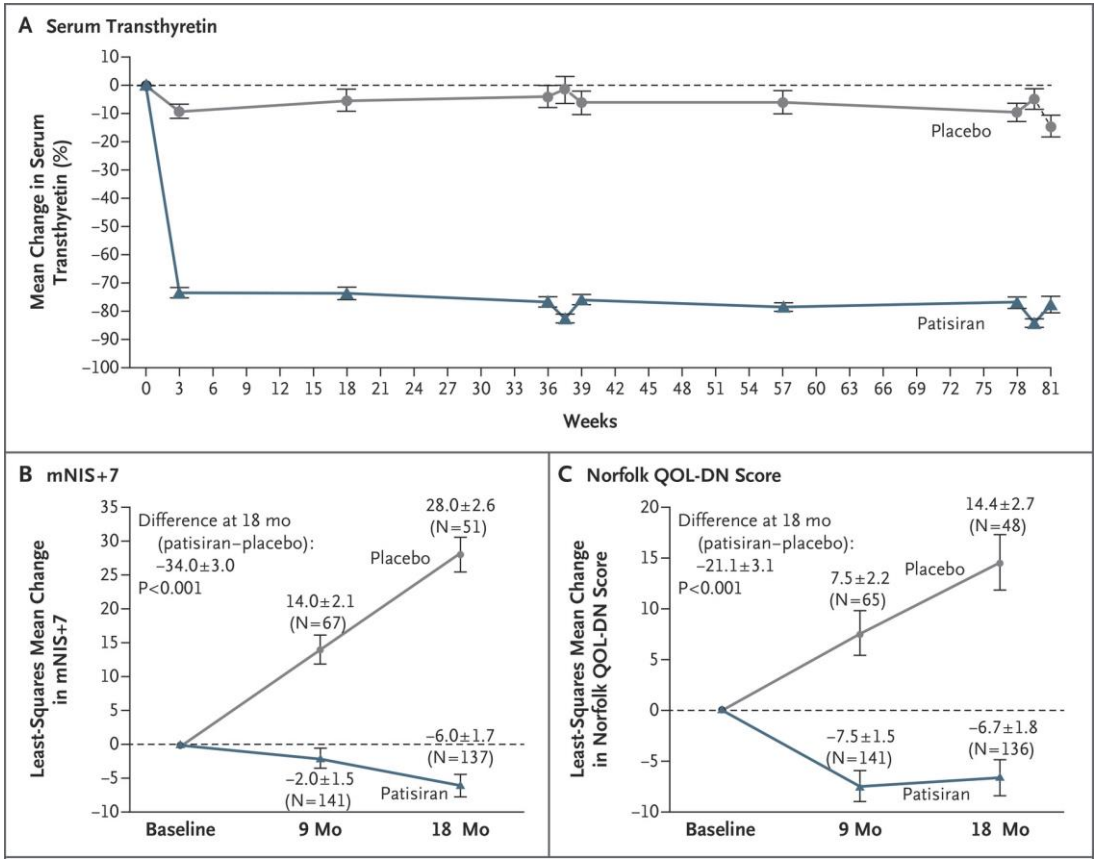
- Catalytic process → very efficient
- Tolerates some mismatches
- Modifications: limited for guide (antisense) strand (goes into RISC complex), possible for passenger (sense) strand



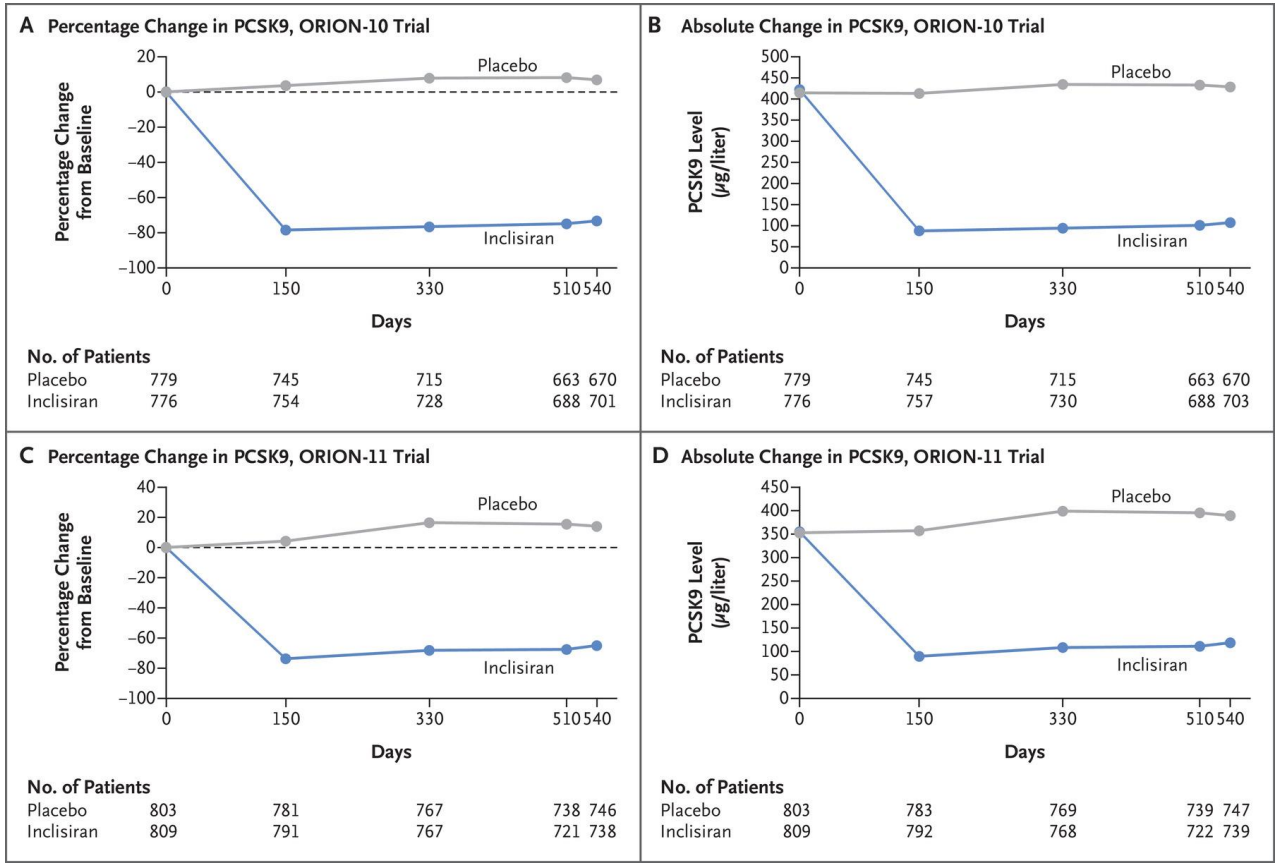
Protein knockdown 2/2: siRNA

- Example: patisiran for hereditary transthyretin-mediated amyloidosis (hATTR Amyloidosis): toxic protein
- Patisiran is an si-RNA targeting transthyertin transcripts → reduced expression
- Delivery via LNPs
- Phase 3 clinical trial primary endpoint met (modifies polyneuropathy)
- FDA and EMA approved
- Inotersen (Ionis): same indication (RNase H mech)
- Givosoran and inclisiran also approved (GalNac conjugate)

Patisiran


A Serum Transthyretin
Mean Change in Serum Transthyretin (%)
Placebo
Patisiran
Weeks
B mNIS+7
Least-Squares Mean Change in mNIS+7
Difference at 18 mo (patisiran–placebo): −34.0±3.0
P<0.001
28.0±2.6 (N=51)
Placebo
14.0±2.1 (N=67)
−6.0±1.7 (N=137)
−2.0±1.5 (N=141)
Patisiran
Baseline
9 Mo
18 Mo
C Norfolk QOL-DN Score
Least-Squares Mean Change in Norfolk QOL-DN Score
Difference at 18 mo (patisiran–placebo): −21.1±3.1
P<0.001
14.4±2.7 (N=48)
Placebo
7.5±2.2 (N=65)
−7.5±1.5 (N=141)
−6.7±1.8 (N=136)
Patisiran
Baseline
9 Mo
18 Mo

Inclisiran: single dose results in long term effects



Question

- Which diseases are currently good targets for RNase H oligonucleotides but not for siRNA?

  a. Brain disease caused by loss of function mutations

  b. Brain diseases caused by toxic gain of function mutations

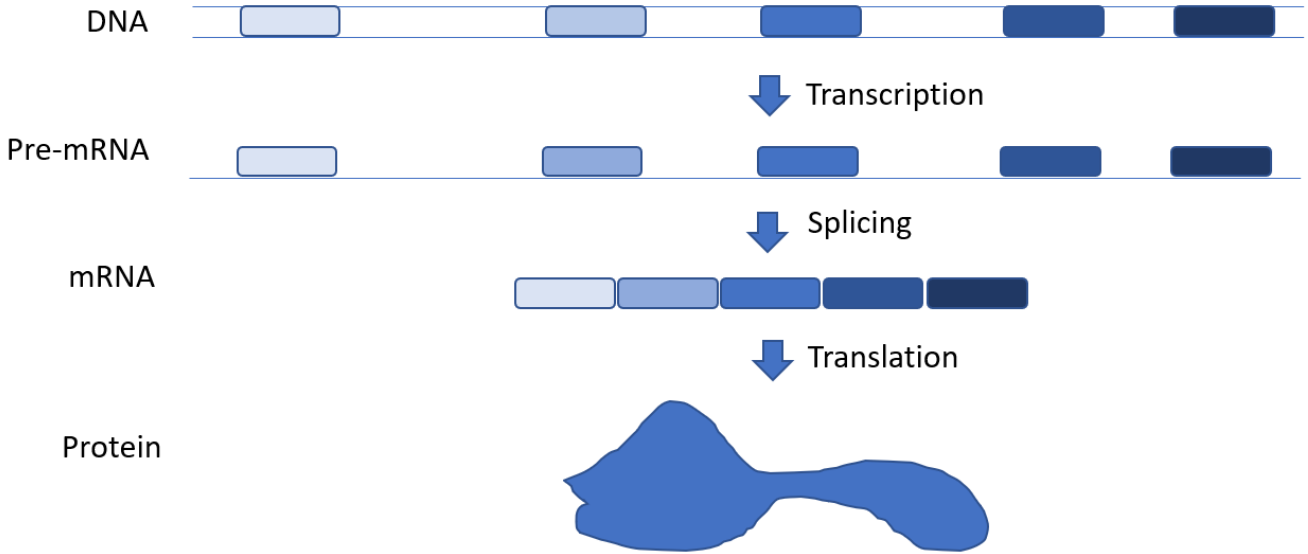
  c. Liver diseases caused by loss of function mutations

  d. Liver diseases caused by toxic gain of function mutations

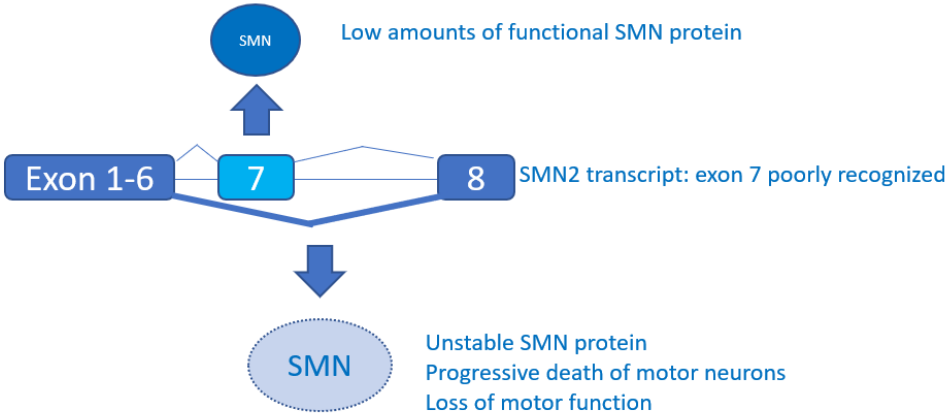
What can we use them for?

- Protein knockdown
  - Toxic gain of function
  - Key protein in pathological pathway
- **Protein restoration**
  - Splicing modulation
  - Oligonucleotides fully modified (no RNase H activation)

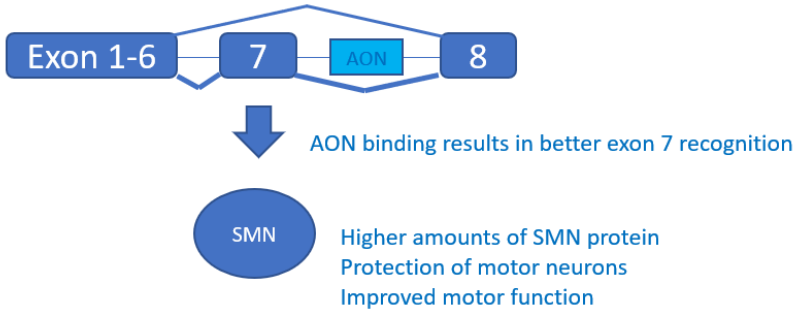
Splicing modulation



Splicing modulation: spinal muscular atrophy



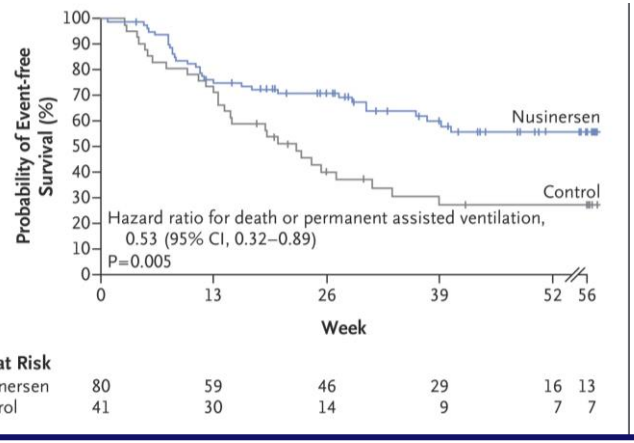
Splice modulation



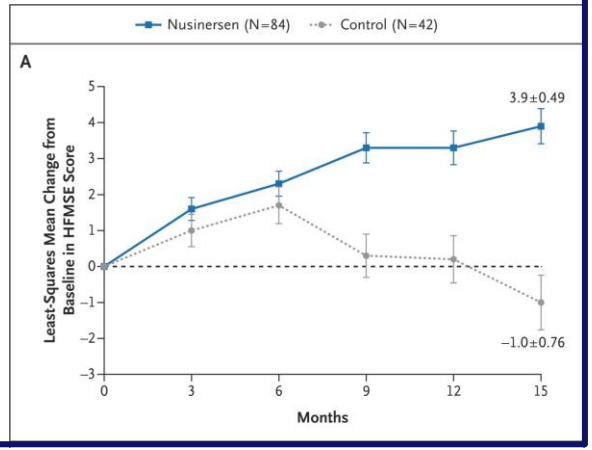
Protein restoration: exon inclusion

- Example: nusinersen (Ionis/BioGen, FDA and EMA approved for all types of SMA)
- Modification: 2'-*O*-methoxyethyl PS
- Delivery intrathecal (every 3-6 months)

Type 1 SMA: event free survival

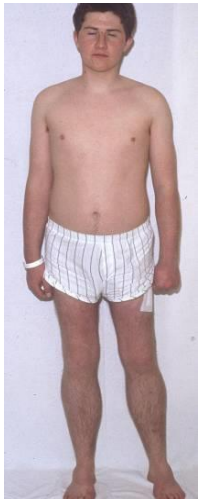


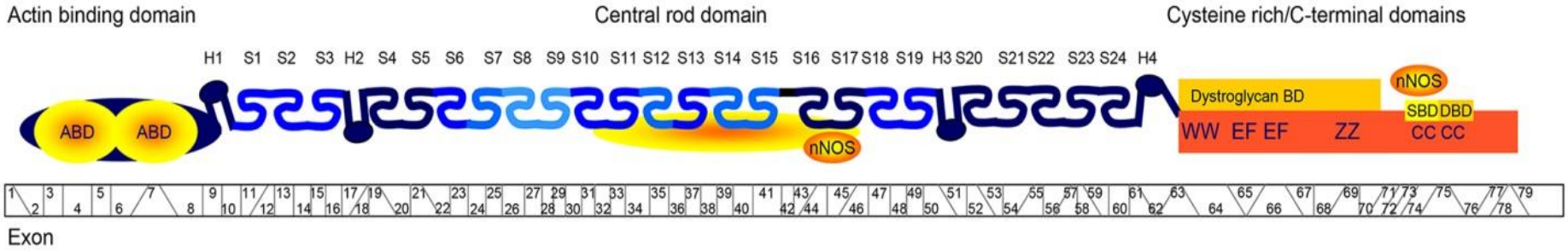
Type 2 SMA: motor score



Duchenne: produce partially functional proteins

- Out-of-frame mutation: no functional protein, Duchenne
- In-frame mutation: partially functional protein, Becker
- Allow Duchenne patients to make Becker-type dystrophins





Duan, et al Nat Rev Dis Primers 2021, 7:13

Exon skipping to restore reading frame

Reading frame restored

Partially functional dystrophin

Duchenne exon skipping

- Mutation specific
- Applies to groups of patients (8/14/8% for exon 45/51/53)
- Challenge: efficient delivery to muscle
- Examples
  - Eteplirsen (Sarepta, phosphoridiamidate morpholino, FDA approved, EMA did not approve)
  - Golodirsen (Sarepta, PMO, FDA approved, exon 53 skip)
  - Casimersen (Sarepta, PMO, FDA approved, exon 45 skip)
  - Viltolarsen (Nippon Shinyaku, PMO, FDA, JMHLW approved, exon 53 skip)
  - Approvals based on very low levels of dystrophin in biopsies

Question

- Will exon skipping to restore the reading frame work for Duchenne patients with small deletions or insertions within an exon.

  a. Yes, always

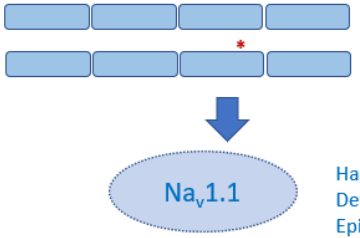
  b. Yes, but only for mutations in in-frame exons

  c. Yes, but only for mutations in out-of-frame exons

  d. No

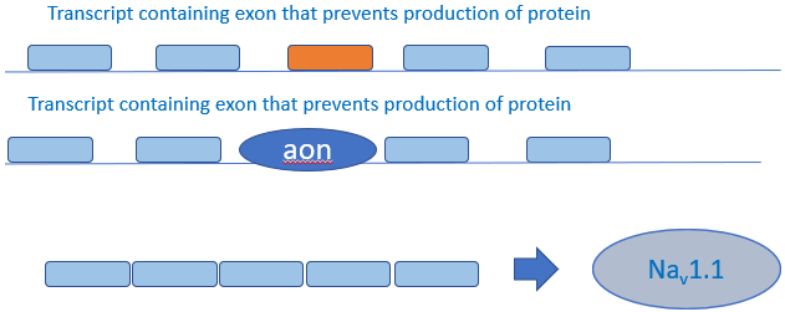
Increasing protein amounts (haploinsufficiency)

E. Dravet syndrome



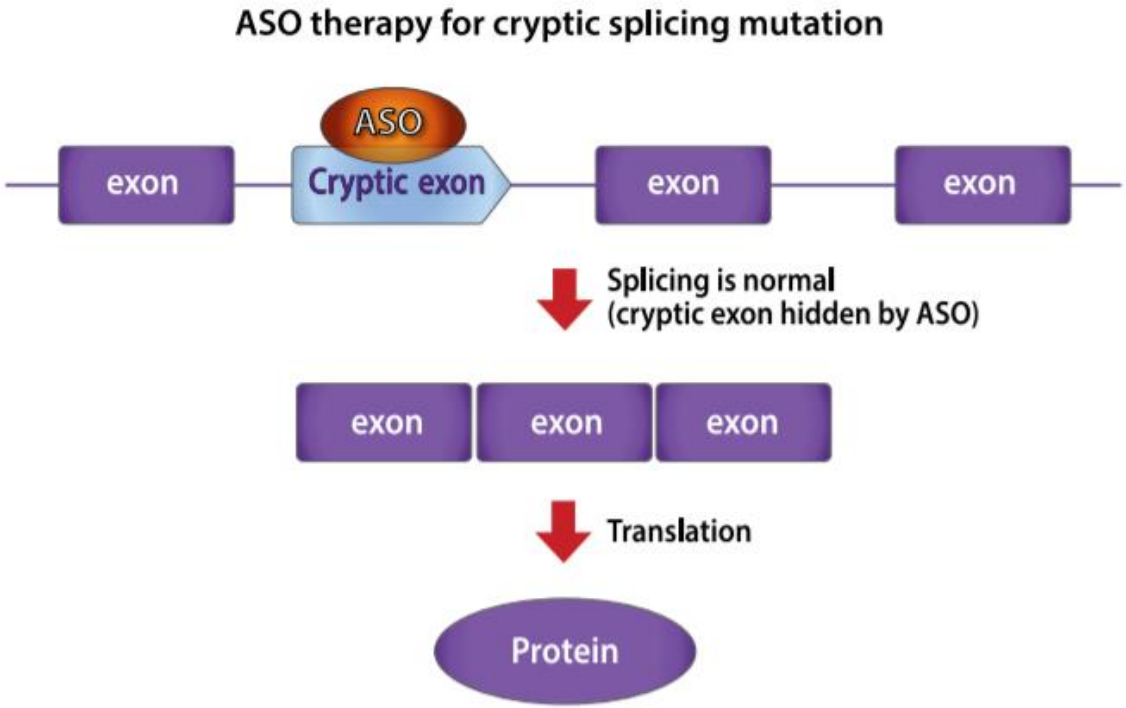
Haploinsufficiency in voltage-gated sodium channel alpha subunit 1.1
Developmental encephalopathy
Epileptic seizures

D. Splice modulation

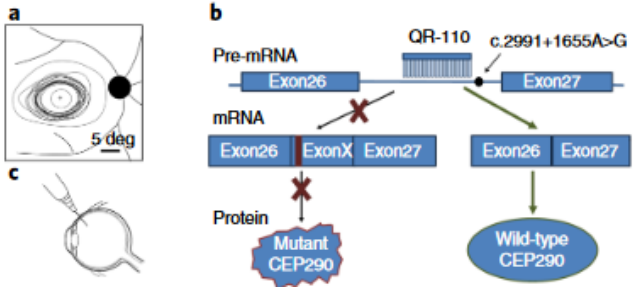


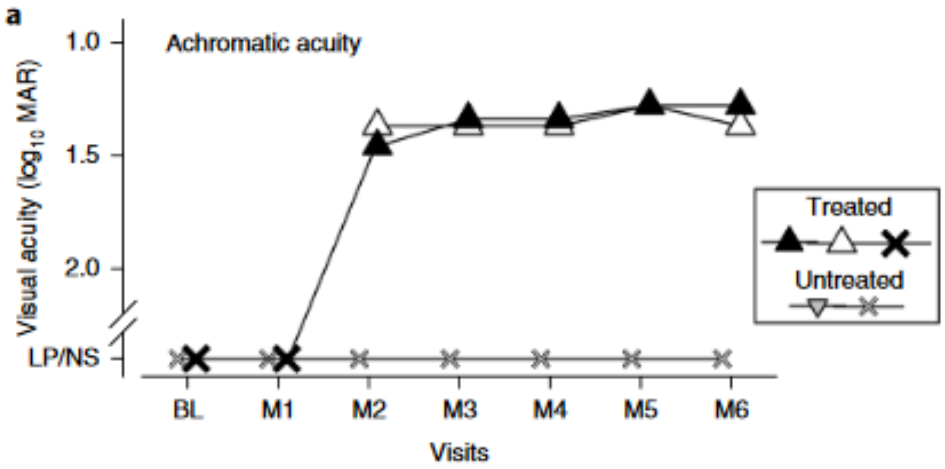
Normal allele produces many non productive transcripts
AON blocks non-productive exon, allowing increased production of functional transcripts and increased amounts of functional protein

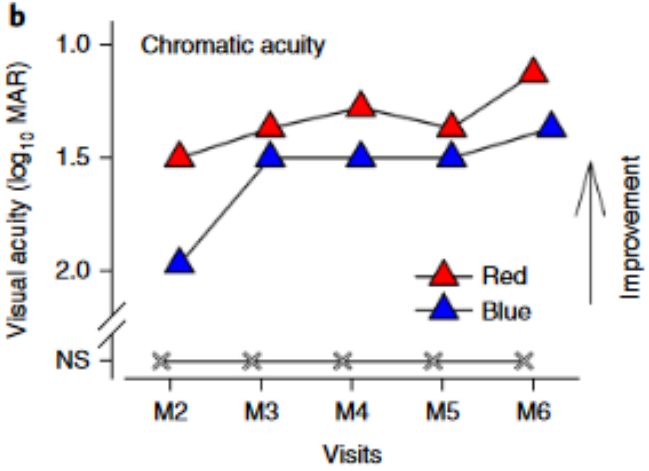
Exon skipping for cryptic splicing mutations



Protein restoration: exon skipping







Summary

- Antisense oligonucleotide drugs are coming of age
- They are very target specific
- Different ways they can be exploited, allowing protein knockdown and restoration
- Broad clinical applicability
- Chemical modifications are needed, but depend on modality
- Delivery is challenging for some tissues

Safety considerations

- On target
  - Exaggerated pharmacology
  - Depends on where target gene is expressed
  - Not an issue for protein restoring approaches
- Off target
  - Specificity varies per chemistry
  - Check for uniqueness target (in silico)

Safety considerations

- Aptameric effect (PS modification)
  - Effects on coagulation (inhibition tenase pathway)
  - Complement activation (Factor H binding)
- Sequence (motifs)
  - Immunogenic (TLR activation)
  - Can screen in vitro
- Injection site reactions for PS (SC delivery)
  - Mechanism?
- Dose dependency
  - E.g. siRNAs side effect profile; PS effects

The field has a society

- Oligonucleotide Therapeutics Society
- www.oligotherapeutics.org

OTS's Mission

- Foster academia and industry-based **research** and development of oligonucleotide therapeutics and young researchers (**we focus on the science and our future**)

OTS keeps growing

- 677 members and counting
- >1000 participants at meetings
- Next meeting: Oct 2-7 2022, Montreal

What about very rare mutations?

- Delivery to CNS and eye (local treatment)
  - Low doses
  - Systemic exposure low, local exposure high
- Treatment frequency low
- Cryptic splicing mutations excellent candidates
  - Restore normal protein
- But unattractive to Industry
  - Numbers too low

Milasen: Individualized treatment

The NEW ENGLAND JOURNAL of MEDICINE

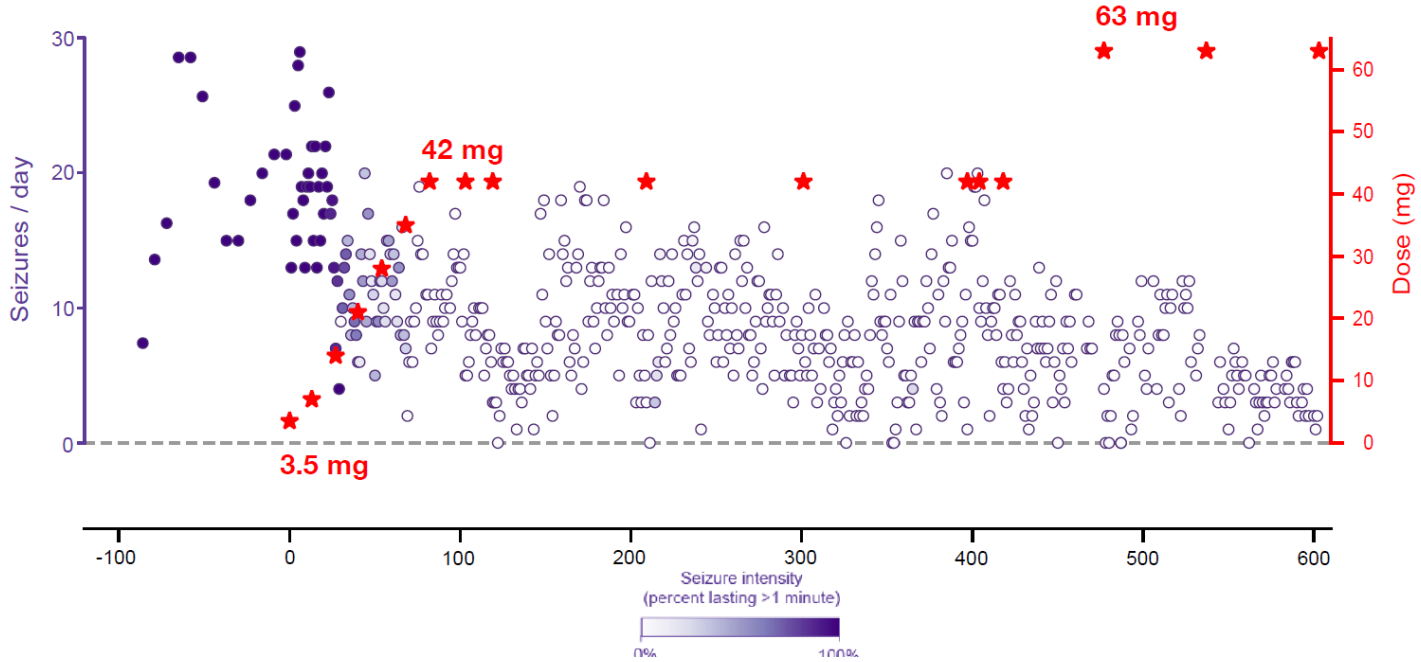
BRIEF REPORT

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

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 the central nervous system disorders
- Please reach out to DCRT@lumc.nl for any questions. Go to our website through QR code

Annemieke Aartsma-Rus

Rob Collin

Willeke van Roon-Mom

Ype Elgersma

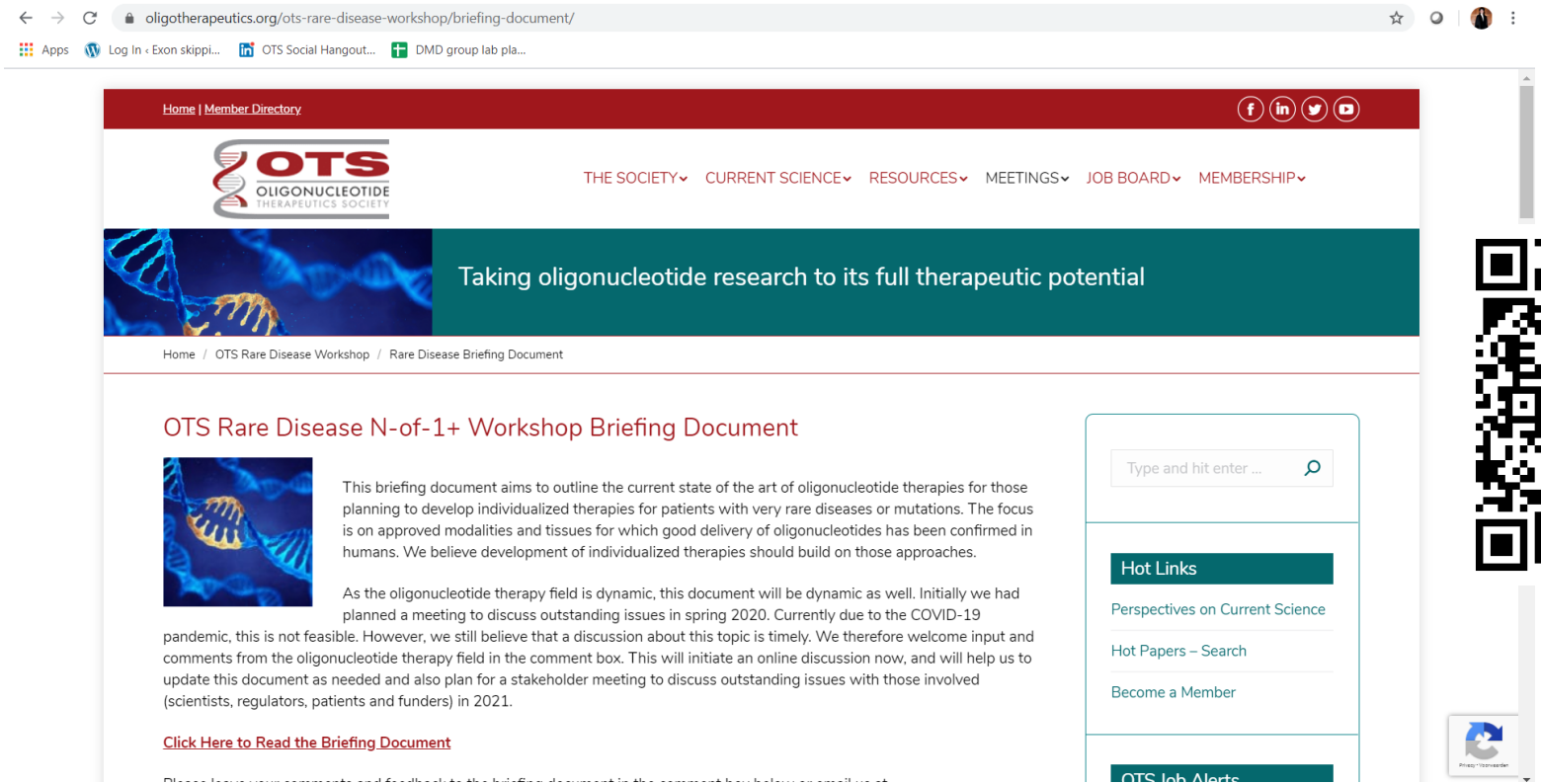
Linda van der Graaf

How?

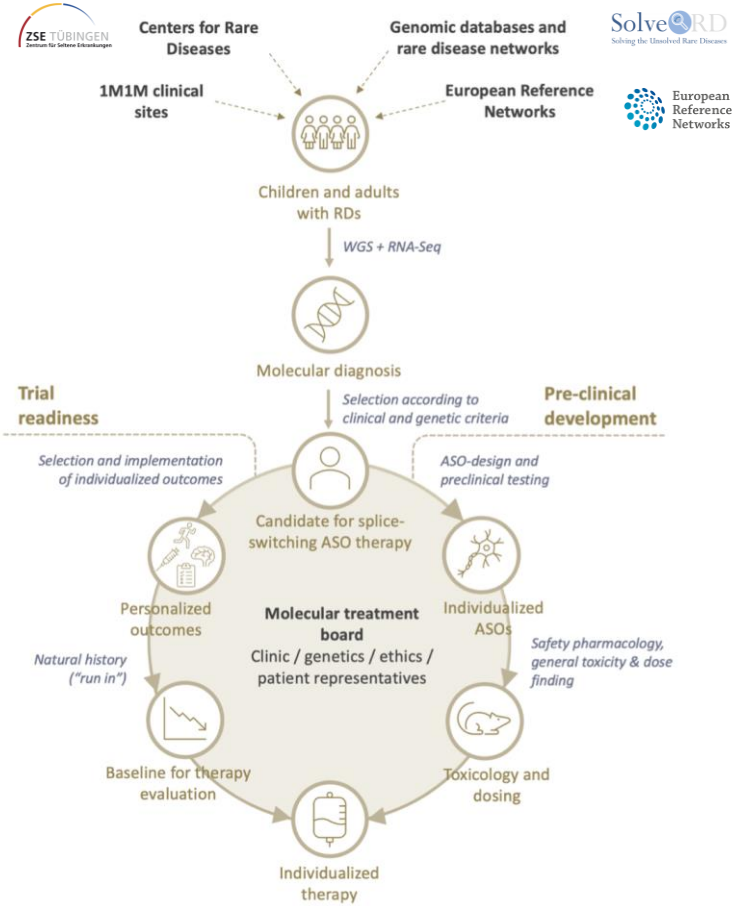
- Named patient setting: no regulatory approval
  - We are communicating with regulators for advice
- Design and optimization in house
- Hospital pharmacy preparation of GMP or GMP-like compound
- Preclinical studies and safety studies in house
- For now: funding from UMCs involved
- Future: reimbursement through ZIN of development costs

Aligning with other initiatives

DCRT Dutch Center for RNA Therapeutics



1 Mutation 1 Medicine (1M1M):
an n-of-1 ASO therapy platform for ultra-individualized mutation-specific therapies



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Volume 00, Number 00, 2021
Mary Ann Liebert, Inc.
DOI: 10.1089/nat.2021.0039

Preparing n-of-1 Antisense Oligonucleotide Treatments for Rare Neurological Diseases in Europe: Genetic, Regulatory, and Ethical Perspectives

Matthis Synofzik,[1,2,i] Willeke M.C van Roon-Mom,[3,*] Georg Marckmann,[4,*] Hermine A. van Duyvenvoorde,[5,*] Holm Graessner,[6,7,*] Rebecca Schüle,[1,2,*] Annemieke Aartsma-Rus,[3,*,ii] on behalf of the 1M1M consortium

How can you help us?

DCRT Dutch Center for RNA Therapeutics

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

Thank you