Leids Universitair Medisch Centrum

A brief introduction into oligonucleotide therapies

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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 consultant for PTC Therapeutics, BioMarin Pharmaceuticals Inc., Alpha Anomeric, Astra Zeneca, Eisai, Sarepta Therapeutics, Takeda, Regenxbio, Splicesense, Galapagos, 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 and BioMarin Pharmaceuticals

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 (Watson-Crick base pairing → intervene at genetic level)
- Aim: therapeutic effect
  - Knockdown of toxic protein
  - Restoration of missing protein

Requirements to make them drugs

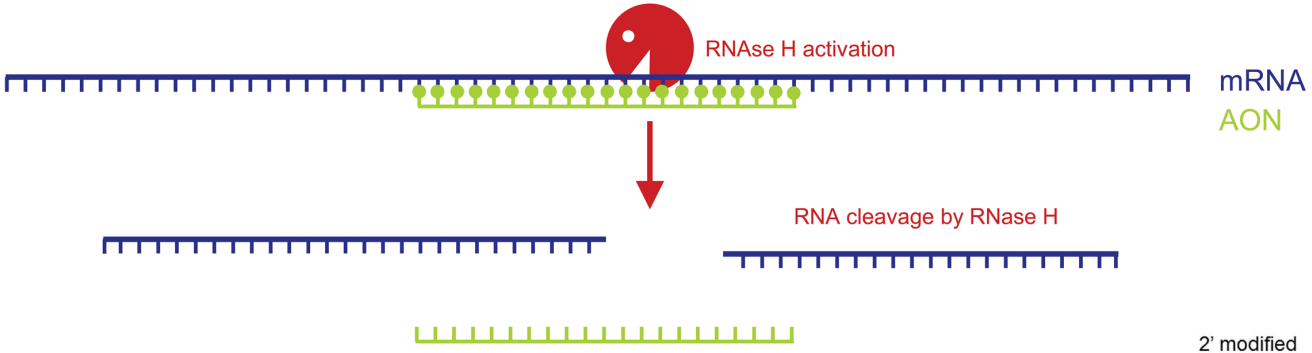
- Improve stability (nuclease resistant)
  - Sugar modification
  - Backbone modification
- Pharmacokinetic properties/bioavailability
  - Phosphorothioate
  - Delivery: systemic vs local (intrathecal/intraocular)
- Many modifications available
  - Which one you can use depends on therapeutic approach

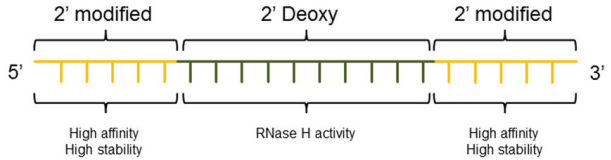
What can we use them for?

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

Protein knockdown: RNase H

- RNase H cleaves RNA in RNA/DNA hybrids
- Targeted knockdown of transcript
- Modifications: gapmers with PS backbone





RNase H approved oligonucleotides

- Mipomersen (Familial hypercholesterolemia, FDA only)
- Inotersen (Transthyretin mediated amyloidosis)
- Volonesorsen (High cholesterol)

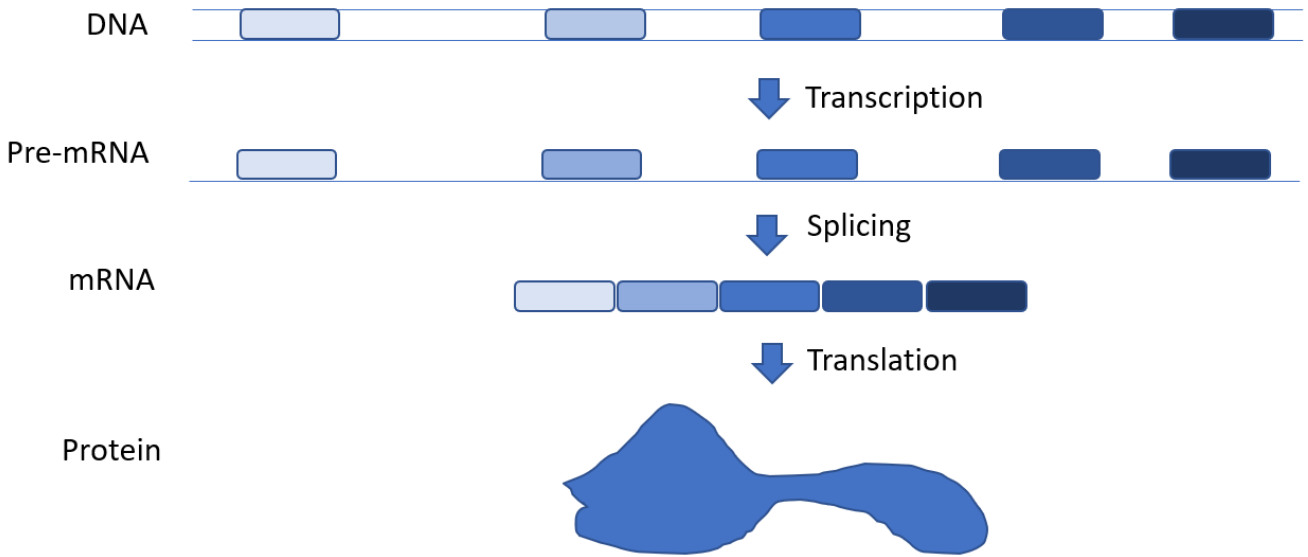
- NB: Approved ASOs are not allele specific

  Knockdown of wild type and mutated protein

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 (in or exclude exon)



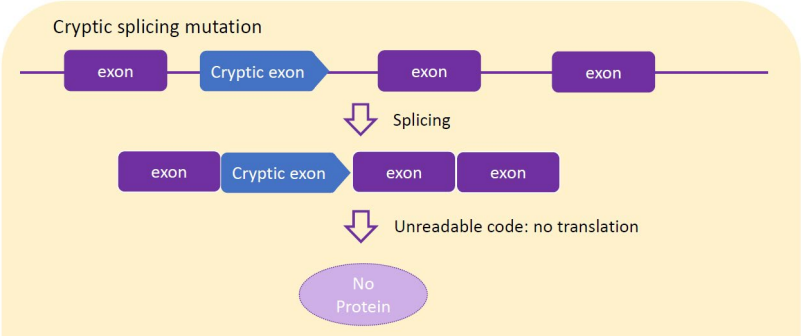
Aartsma-Rus et al unpublished

Approved splice modulating ASOs

- Nusinersen (Spinal muscular atrophy)
- Eteplirsen, Golodirsen, Casimersen, Viltolarsen (Duchenne exon 51, 53, 45, 53 skipping, FDA only)

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

Kim, et al, NEJM 2019;381: 1644-52

Safety considerations (target related)

- 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)

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

Thank you