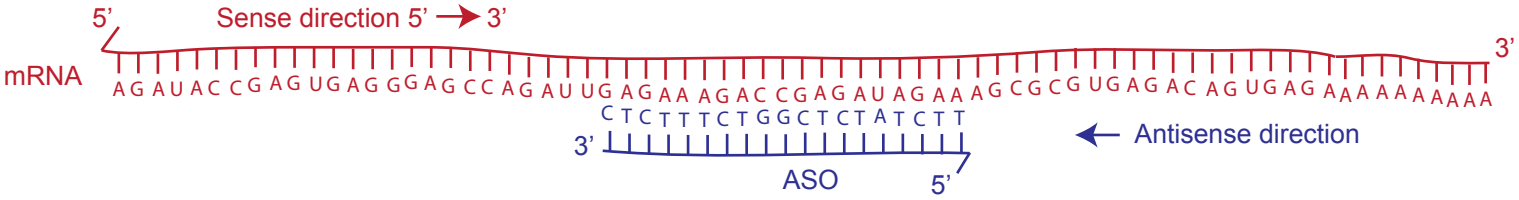
FSHD Therapeutic Approaches: ASO (Pt 1)

ASO (antisense oligonucleotide; aka ANO or AO) approaches can target the processing of specific RNAs, block their translation into protein, or target them for degradation.

Antisense technology has been around since 1977 (Paterson *et al. PNAS USA* 74:4370-74).
ASO technology has been around since 1978 (Stephenson and Zamecnik, *PNAS USA* 75:285-8).

What is antisense technology?

Delivery of small pieces of RNA or DNA (~20-25nt) that can bind to specific RNA molecules to affect pre-mRNA splicing or polyadenylation, mRNA translation, or mRNA stability.



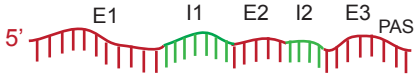
How does antisense technology work?

First, the molecule needs to get to the tissue and cells of interest. In FSHD, this is all of one's skeletal muscles. Once there, the ASO binds the DUX4 mRNA.

FSHD conditions

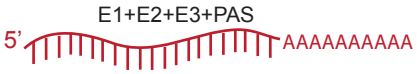
1) Transcription of the *DUX4* gene

DUX4 Pre-mRNA



2) Processing of the DUX4 pre-mRNA -splicing and polyadenylation

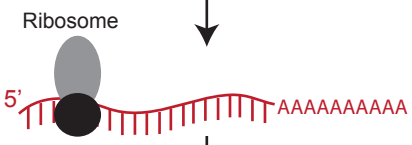
Mature DUX4 mRNA with exons 1, 2, and 3 5' cap and polyA



Cell Nucleus

Cell Cytoplasm

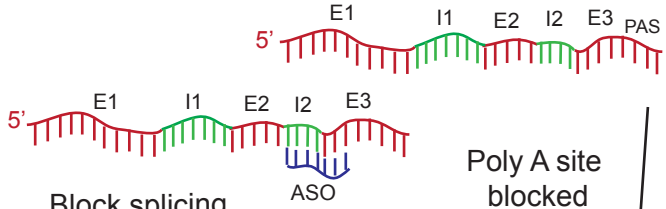
3) Translation of the DUX4 mRNA into DUX4 protein



DUX4 protein

FSHD pathogenic cascade

Treatment with ASO



No polyA → degraded

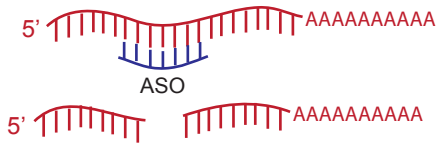
No polyA → degraded

Block translation of the DUX4 mRNA

No DUX4 protein

RNase H cleavage of the DUX4 mRNA

The DUX4 mRNA is destroyed



No DUX4 protein

FSHD Therapeutic Approaches: ASO (Pt 2)

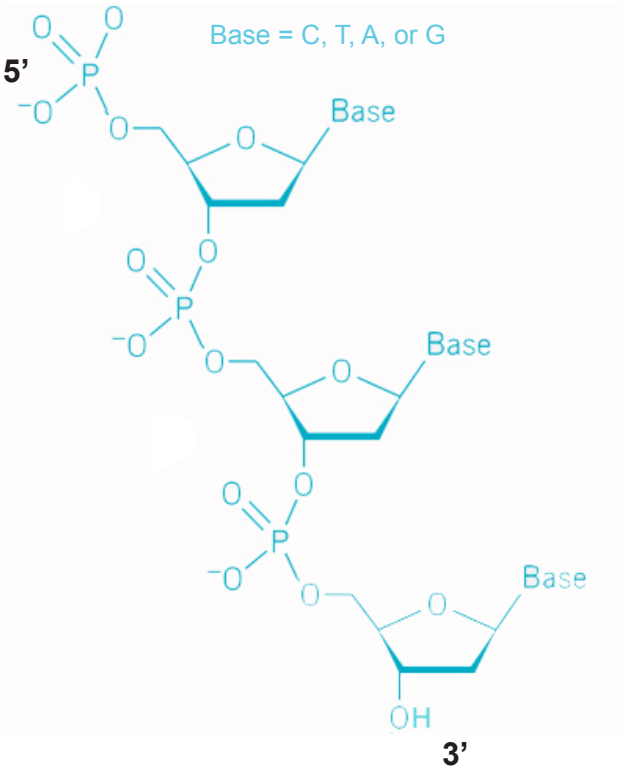
ASO (antisense oligonucleotide; aka ANO or AO) approaches can target the processing of specific RNAs, block their translation into protein, or target them for degradation.

What is antisense technology?

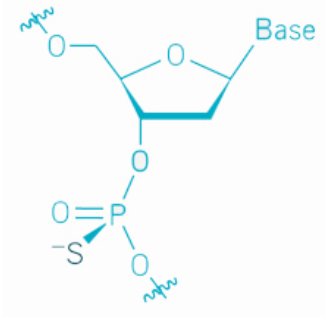
Delivery of small pieces of RNA or DNA (~18-25nt) that can bind to specific RNA molecules to affect pre-mRNA splicing or polyadenylation, mRNA translation, or mRNA stability.

There are many ways to modify and synthesize molecules to increase stability.

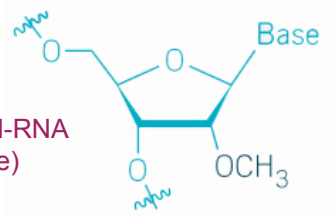
DNA with phosphodiester linkage



1) Modified phosphorothioate backbone to be resistant to cellular nucleases.

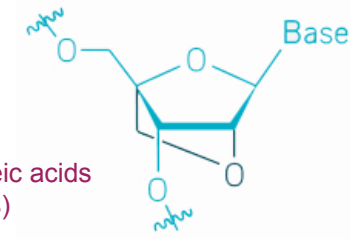


2) Modified ribose sugar increases target binding and resistance to cellular nucleases.



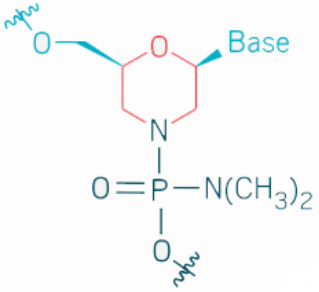
2'-O-Methyl-RNA (2'-OMe)

3) Bridged nucleic acids greatly increase target binding affinity.



Locked nucleic acids (LNAs)

4) Nucleotide analogs using a morpholino ring and a phosphorodiamidate linkage.



Phosphorodiamidate morpholino oligomers (PMOs)

Delivery can be improved by linking ASO with modifications to affect:

- Stability
- Tissue specificity/delivery
- Solubility/cell permeability

FSHD Therapeutic Approaches: ASO (Pt 3)

Therapeutic ASO approaches are already in clinic (and many more are in the pipeline) for a variety of diseases.

First ASO to gain FDA approval was Vitravene (Novartis Pharmaceuticals) for CMV in HIV patients.

There are now multiple FDA-approved ASO therapeutics for other diseases:

2016 - Spinraza for spinal muscular atrophy (Ionis and Biogen)
2016 - Exondys 51 for Duchenne muscular dystrophy (Sarepta Therapeutics)
2018 - Tegsedi for familial amyloid neuropathies (Akcea Therapeutics)
2019 - Vyondys 53 for Duchenne muscular dystrophy (Sarepta Therapeutics)

ASO technology against the DUX4 mRNA for FSHD was first shown by the Alexandra Belayew lab in 2011 (Vanderplanck *et al. PLoS One* 6:e26820).

Advantages of the ASO approach included:

- Can be chemically modified to improve pharmacokinetis
  - for higher target affinity and increased specificity, thus few off-targets.
  - for higher stability.
  - for better biodistribution.
- Potential for decreasing toxicity.
- Potential for tissue-targeted delivery.
- Personalized medicine, make a “drug” specific of a person’s specific mutation.

Potential concerns with ASO therapy for FSHD include:

- Systemic delivery to all skeletal muscles.
  - FSHD is a few cells expressing lots of toxic DUX4, thus you need to target most, if not all, skeletal muscle cells to shut down the few DUX4 expressing cells.
  - This is different from most other AON therapies, which “fix” a defect and where even a small fix has therapeutic benefit.
- Effective dosing without toxicity.
  - The *DUX4* gene is very GC-rich, can pose problems for stimulating immune response to certain antisense sequences.
- Durability of knockdown effect.
  - Does not turn off *DUX4*, thus DUX4 mRNA is continually made.
- Cost of synthesis for toxicology studies to get through FDA (multiple molecules?) and ultimately repeated systemic treatment throughout one’s lifetime.

Multiple companies with publically disclosed FSHD AON programs, including:

Dyne Therapeutics https://www.dyne-tx.com/
MiRecule RNA Therapeutics http://www.mirecule.com/

Overall, the technology in the ASO field is evolving at breakneck speed and holds great promise in the clinic for FSHD.

FSHD Therapeutic Approaches: ASO (Pt 4)

There are two main approaches to target specific mRNAs for therapeutic knockdown:

1) RNA interference (RNAi), which uses double stranded RNA with homology against the target mRNA.

2) Antisense oligonucleotides (ASO), which use a single stranded RNA/DNA-based synthetic molecule with antisense sequence to the target mRNA.

ASO (antisense oligonucleotide) approaches in FSHD target the *DUX4* mRNA for degradation or inhibition.

ASO technology against the DUX4 mRNA for FSHD was first shown by the Alexandra Belayew lab in 2011 (Vanderplanck *et al. PLoS One* 6:e26820), effectively targeting the DUX4 polyadenylation site and disrupting polyadenylation of the DUX4 mRNA.

There are now several groups and companies using different antisense technologies targeting different parts of the DUX4 mRNA for therapeutic knockdown, including:

Antisense targeting of 3′ end elements involved in *DUX4* mRNA processing is an efficient therapeutic strategy for facioscapulohumeral dystrophy: a new gene-silencing approach

Human Molecular Genetics (2016) 25:1468-78.

Anne-Charlotte Marsollier[1], Lukasz Ciszewski[2,†], Virginie Mariot[1,†], Linda Popplewell[2], Thomas Voit[1,‡], George Dickson[2] and Julie Dumonceaux[1,*]

Article

Antisense Oligonucleotides Used to Target the *DUX4* mRNA as Therapeutic Approaches in FaciosScapuloHumeral Muscular Dystrophy (FSHD)

Eugénie Ansseau [1], Céline Vanderplanck [1,†], Armelle Wauters [1], Scott Q. Harper [2,3], Frédérique Coppée [1] and Alexandra Belayew [1,*]

Genes (2017) 8:93.

Inhibition of *DUX4* expression with antisense LNA gapmers as a therapy for facioscapulohumeral muscular dystrophy

Kenji Rowel Q. Lim[a], Rika Maruyama[a], Yusuke Echigoya[a,b], Quynh Nguyen[a], Aiping Zhang[c,d], Hunain Khawaja[c,d], Sreetama Sen Chandra[c,d], Takako Jones[e], Peter Jones[e], Yi-Wen Chen[c,f,1], and Toshifumi Yokota[a,g,1]

PNAS USA (2020) 28:16509-15.