Antisense Oligonucleotide mediated up-regulation of mRNA: Implications for SLC6A1

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The Central Dogma of Molecular Biology

Credit: Jon Watts
The *Central Dogma* of Molecular Biology

Most drugs bind to disease associated proteins e.g. small molecules

Credit: Jon Watts
The Central Dogma of Molecular Biology

DNA → RNA-specific Agent? → Protein

Protein product modulated through RNA targeting

Credit: Jon Watts
The **dianophore** principle

**Traditional, small molecule drug**

*Chemical structure*

**Pharmacophore**
Ensemble of molecular features that determine target recognition and modulation

**Dianophore***
Ensemble of molecular features that determine PK/PD/ADME

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*Dianophore*—from the Greek “διανομή-dianomi” for distribution or delivery

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Credit: Jon Watts

Khvorova & Watts (*Nature Biotechnology*) 2017
The dianophore principle

Traditional, small molecule drug

![Chemical structure of a small molecule drug]

**Pharmacophore**
Ensemble of molecular features that determine target recognition and modulation

**Dianophore**
Ensemble of molecular features that determine PK/PD/ADME

Oligonucleotide drug

![Chemistry of the backbone and ligand in an oligonucleotide drug]

**Dianophore**
Ensemble of molecular features that determine PK/PD/ADME

**Pharmacophore**
Ensemble of molecular features that determine target recognition and modulation

*Dianophore*—from the Greek “διανομή-dianomi” for distribution or delivery

Credit: Jon Watts

Khvorova & Watts (Nature Biotechnology) 2017
Oligonucleotide chemistry and ligand drives durable, potent silencing in a given tissue.

Once chemistry is validated, lead compounds for new diseases can be found and validated with a timescale of months rather than years.
Oligonucleotide Therapies (development & approved)*

* as of March 2019
The *Central Dogma* of Molecular Biology

1. DNA
2. RNA-specific Agent
3. Protein

Transcription

Protein product modulated through RNA targeting

Credit: Jon Watts
Targeting specific RNA through base pairing

Anti-RNA agent utilizing:
- Watson-Crick Base Pairing
- G-C or A-U or A-T
- Highly conserved
- High specificity

Complementary or “antisense” oligonucleotide

Credit: Jon Watts
Two mechanisms of action

### Silencing

- **ASO**
- Finds its target
- **mRNA**
- RNase H-mediated degradation

### Blocking

- **Chemically modified ASO**
- Finds its target
- **mRNA**
- Steric blocking of RNA (splice site, binding site, *et al.*)

Credit: Jon Watts
Correcting mis-splicing with ASOs

SMN2 pre-mRNA

1 2a 2b 3 4 5 6 7 8

SPLICING

Full-length SMN2 mRNA

1 2a 2b 3 4 5 6 7 8

TRANSLATION

Full-length SMN protein

Abnormal MFSD8 Splicing and Translation after SVA Insertion

Exon 5  Exon 6  i6  SVA

Donor  G  P

Accomptor  atagATGAGTAA

Spinraza/nusinersen

milasen
Upregulation by targeting non-productive splicing

- often degraded (NMD, other mechanisms)
- wasted transcriptional output
- NON-PRODUCTIVE isoform

Can we re-direct products of wasted transcription from the healthy allele towards productive mRNA isoforms?

Targeting ASOs to block sites of non-productive splicing

- translated into protein
- PRODUCTIVE isoform

Kole et al. (Nat Rev Drug Discovery) 2012
Non-productive splicing is common...

Pickrell, Pai et al. *PLOS Genetics* 2010
... but hard to find in current RNA profiling datasets

Pickrell, Pai et al. (PLOS Genetics) 2010
Computational prediction of non-productive splicing

maxEnt software: Yeo & Burge (Journal of Comp. Biology) 2004
Experimentally identifying sites of non-productive splicing

RNA-sequencing captures mature, stable mRNA

Instead, capture nascent RNA molecules (before maturation & degradation)

short 4sU labeling periods

10 minutes

20 minutes
Sites of non-productive splicing in nascent RNA

30m 4sU labeling in SH-SY5Y cells

junction reads from nascent RNA-seq data

computational predictions (maxEnt)

annotated splice sites
cryptic splice sites

Schwarzl et al. (Journal of Molecular Biology) 2015
Predicting sites of non-productive splicing in *SLC6A1*

- GABAergic neurons (healthy donor)
- astrocytes (healthy donor)
- GABAergic neurons (iPSCs from SLC6A1 patient)
- GABAergic neurons (CRISPR-corrected SLC6A1)
- SH-SY5Y (neuroblastoma cell line)

1. **4sU labeling (15 minutes)**
2. **targeted sequencing of *SLC6A1***
3. **identification of ubiquitously used cryptic splice sites**
4. **antisense oligonucleotide design and testing**
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