Treatment of Neuromuscular Diseases With Antisense Oligonucleotides

C. Frank Bennett Ph.D.
Isis Pharmaceuticals
To date there are more than 12 different antisense mechanisms that have been characterized.
Antisense Mechanism of Action
- RNase H Oligonucleotides -
Isis’ Second Generation Antisense Drugs
2’-Methoxyethyl Modification

Tissue and Cellular Pharmacokinetics

- Distribute out of plasma and into tissues
- Majority of drug localizes within cells in tissues
- Multiple routes of delivery
- Strong PK/PD correlation demonstrated in tissues such as:
  - Kidney
  - Liver
  - Bone marrow
  - Adipose tissue
  - Spleen, lymph nodes
  - Lung (aerosol)
  - CNS (ICV or IT)
  - Human cancer
  - Sites of inflammation
2’-O-Methoxyethyl Modified ASOs (RNase H Dependent) Broadly Inhibit Gene Expression in Mice

Liver
- SCD-1
  - Saline
  - ASO
- FBP-1
  - Saline
  - ASO
- FKHR
  - Saline
  - ASO

Adipose Tissue
- IKK-β
  - Saline
  - ASO
- p85α (PI-3K)
  - Saline
  - ASO
- PTP-1B
  - Saline
  - ASO

Spleen
- IKK-β
  - Saline
  - ASO

Kidney
- SGLT-2
  - Saline
  - ASO
How do Oligonucleotides Distribute From the Needle to Their Receptor (RNA) inside Cells?
Summary of Data Identifying Mechanism of Oligonucleotide Accumulation in Cells

- Measurement of bulk oligo dynamics is instructive, but not the complete story
- Serum protein binding is important to prevent excretion of Oligos into urine
  - P=S linkage and lipophilic conjugates enhance serum protein binding
- Role of serum proteins in mediating oligonucleotide uptake in tissues/cells is not well defined
  - Some evidence that subsets of serum proteins may enhance delivery to an oligo “sink”
- Oligonucleotides accumulate in at least two cellular compartments
  - Target RNA compartment (cytoplasm/nucleoplasm)
  - Lysosome or other endo-lysosomal structure
- Functional uptake into cytoplasm/nucleoplasm initially involves a vesicular uptake pathway with release from vesicles (oligoportin)
  - Clathrin and caveolin independent
  - Distinct SAR exists for oligonucleotide uptake into cells
    - DNA preferred over RNA
- Blocking specific vesicular transport/maturation pathway blocks antisense effects
- Several genes have been identified that block antisense effects helping to define transport pathways
Mechanism of Oligonucleotide Uptake into Cells:

- High affinity plasma protein
- Oligoportin
- Low affinity membrane protein
- Ap2M1

Lysosomes

Nucleus

BFA

Chloroquine
Examples of Therapeutic Areas and Targets Where We Currently Practice Oligonucleotide Based Therapies

- **Diabetes/obesity** - Liver, adipose tissue, muscle (?)
- **Cardiovascular** – Liver, endothelial cells, macrophages
- **Inflammation** -
  - **Systemic** - APCs, endothelium, granulocytes
  - **Local** - APCs, endothelium, granulocytes, epithelium
- **Oncology** - multiple
- **Neurodegeneration/Neuromuscular** -
  - CSF delivery: neurons, astrocytes, microglial cells
  - **Systemic** - muscle (?)
- **Bone** - osteoclasts, osteoblasts, synovial tissues
- **Hematology** - Progenitor cells in marrow (?)
<table>
<thead>
<tr>
<th>Project</th>
<th>Indication</th>
<th>Target</th>
<th>Preclinical</th>
<th>Phase I</th>
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Antisense Technology - Summary

• Numerous antisense mechanisms work in cultured mammalian cells
  – Exploiting all antisense mechanisms broadens portfolio of human diseases approachable with oligonucleotide technology

• RNase H based antisense drugs are most advanced
  – Attractive potency in man
  – Distribute widely
  – Highly selective
  – Administered via multiple routes

• Further advancements in antisense technology are expected.
  – Medicinal Chemistry
  – Additional Terminating Mechanisms
## Isis Neurodegeneration Drug Discovery And Development Program

<table>
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<th>Project</th>
<th>Exploratory Research</th>
<th>Late Stage Research</th>
<th>Preclinical Development</th>
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<tr>
<td>Myotonic dystrophy</td>
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ISIS 333611 Project Status
SOD-1 Targeting ASO Drug for Familial ALS

- Isis 333611 selected as development candidate
- Manufactured toxicology, phase 1 and phase 2 API
- Pre IND meeting held with FDA
- Granted Orphan Drug Status
- Successfully completed IND enabling toxicology studies
- First trial will begin this year
  - Merit Cudkowicz, Timothy Miller PI’s
- ALS-Association and MDA providing funding for preclinical and clinical studies
Antisense Delivery To CNS Tissues

- Antisense oligonucleotides do not cross an intact blood brain barrier
- Delivery technologies are available which deliver drugs directly into the CSF
- Will ASO distribute into neural tissues following CSF delivery?
Second-Generation ASO Distribute Broadly in Rhesus Monkey CNS Tissues After ICV or IT Infusion

Oligonucleotide Distribution in Rhesus Monkey Brain Following IT Infusion (*Oligo = Brown Staining*)
ICV Infusion of Htt ASO in Primates Decreases Htt Expression in Caudate

<table>
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<tr>
<th>Individual Animals</th>
<th>RTS2617/mkCycloA</th>
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<td>Htt mRNA</td>
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<td>Caudate</td>
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<td>0036</td>
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<tr>
<td>6546</td>
<td>Sham</td>
<td>None</td>
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Immunohistochemistry staining of a coronal brain slide from animal infused with ISIS43689 at 4 mg/day for 28 days stained against a polyclonal antiserum specific for oligonucleotides.
ICV Infusion of Htt ASO Inhibits Expression of Htt mRNA in Spinal Cord Tissue in Primates

<table>
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<th>% Avg. Vehicle/Sham</th>
<th>Cervical Cord</th>
<th>Thoracic Cord</th>
<th>Lumbar Cord</th>
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<td>100</td>
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<td>1.5mg/day</td>
<td>25</td>
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</tr>
<tr>
<td>4mg/day</td>
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<tr>
<td>Sham</td>
<td>100</td>
<td>100</td>
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**Immunolocalization of ASO**

- **Saline**
  - 7274
  - 0270
- **1.5 mg/day**
  - 0036
  - 4F75
  - 2B17
- **4.0 mg/day**
  - 4954
  - 5038
  - 3814
Tolerability of CSF Delivered ASOs

• Sequence dependent inflammation at site of administration
  – Rats > mice>> primates
  – May be avoided by proper sequence selection
• In rodents an increase in body weight observed during CSF infusion
  – Increase in fat mass secondary to increase in food consumption
• Direct administration into striatal tissues results in concentration-dependent seizures and hyper-activity
  – Occurs immediately after injection
  – Last 1 to 2 h
  – Re-challenge does not result in seizure
CNS Delivery Summary

- 2nd generation antisense oligonucleotides distribute broadly into different brain regions when introduced into CSF
- Direct injection into brain parenchyma results in increased potency, but more limited distribution
- 2nd generation ASOs have long duration of actions in CNS tissues
- 2nd generation ASOs are well tolerated when administered into CSF
SMA at The Level of Gene Expression

SMN-2 Gene

SMN-1 Gene

Normal individual

SMA patient

Antisense treatment of SMA Patient

SMN-2 mRNA

SMN-1 mRNA

SMN-2 Gene

SMN-1 Gene

Antisense (ASO)
Identification of ASO Target Sites on SMN-2 Pre-mRNA

~500 ASOs Have Been Screened for Ability to Promote Exon 7 Inclusion

ASO Modulation of SMN2 Splicing

Exon 7

Intron 7

UUAAAUUAAGGAGUAAGUCUGCCAG

CAUUAUGAAAG

UGA

hnRNP A1

hnRNP A2

Exon 6

Exon 7

Exon 8

No ASO

+ ASO

UUAAAUUAAGGAGUAAGUCUGCCAG

Intron 7

GGUCGUAUACUUUUCACUGU

UUAAAUUAAGGAGUAAGUCUGCCAG

CAUUAUGAAAG

UGA

hnRNP A1

hnRNP A2
Treatment of Transgenic Mice with SMN Splicing ASO Increases Production of SMN
(Transcript Missing in Spinal Muscular Atrophy)

Liver (Systemic dosing)

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<td>4 5 6 7 8</td>
<td>9 10 11 12</td>
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<td>% exon 7 incl.</td>
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<td>6 7 8</td>
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<tr>
<td>α-tubulin</td>
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Spinal Cord (ICV Dosing)

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% exon 7 incl.

| 13 10 6 10 11 11 | 89 91 83 96 88 84 94 |
| 13 10 6 10 11 11 | 91 90 84 98 85 97 |
Dose-response study of ASO ICV infusion

(Smn +/−; hSMN2 +/-)

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Standard RT-PCR

Real-Time RT-PCR

(Frank Rigo)
ASO Treatment of Myotonic Dystrophy Type 1

- CUG repeat in 3’-UTR of DMPK
- Repeat length ranges from 50 to 3000 repeats
- DMPK RNA with repeat expansion retained in nucleus
  - Localized in distinct structures
  - Decreased expression of DMPK
  - Binds to splicing factors muscleblind and CUG binding protein, serving as a sink
  - Alternate splicing of various genes noted including troponin C and muscle-specific chloride channel (CIC-1)

Distribution of 2\textsuperscript{nd} Generation ASO in Rat

- 2\textsuperscript{nd} generation ASOs distribute to muscle tissue, but concentration very low
- Modest pharmacology demonstrated in muscle following systemic administration
- Can Distribution to muscle be improved with chemical modifications?
Oligonucleotide Chemistries

2'-O-Methoxyethyl (MOE)

2'-O-Methyl

2'-Fluoro

Phosphorothioate (PS) DNA

Locked Nucleic Acid (LNA)

Bicyclic Nucleic Acid Family (BNA)

DNA and RNA (R=H,OH)

Peptide Nucleic Acid (PNA)

Morpholino

Thiophosphoramidate
Muscle Targeting with Generation 2.0 vs New Generation Chemistry (PTEN as Model Target)
Summary

- Antisense oligonucleotides are showing promise for the treatment of a wide range of neurodegenerative diseases
  - ALS
    - ISIS 333611 is advancing to the clinic
  - Huntington’s disease
    - Demonstrated proof of concept in mice
    - Optimization of the human clinical candidate for huntingtin is ongoing
  - SMA
    - Demonstrated proof of concept in mice
    - Identified human clinical candidate
  - Myotonic dystrophy
    - Encouraging data for increased muscle delivery with novel modified oligonucleotides
Acknowledgements

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- Sanay Pandey
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- Ed Wancewicz
- Andy Watt

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- Holly Kordasiewicz

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- Yimin Hua

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- Thurman Wheeler

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- Carol Nelson
- Andrew Ledger

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