Use of Antisense Oligonucleotides for the Treatment of Inheritable Rare Disorders

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Agenda

- Review different antisense strategies
- Delivery of oligonucleotides to the skin, lessons learned and challenges
- FALS a Case Study for Academia/Industry Partnership in Developing Therapies for Rare Diseases
Antisense Technology
- Integration of Target Validation and Drug Discovery -

- Represents one of the fastest ways to go from target identification to drug in man
  - Predictable pharmacokinetics (same from drug to drug)
  - Predictable chemical class-related toxicology (same from drug to drug)
  - Identical manufacturing procedures

- Antisense technology opens up the target space by being able to inhibit expression of “non-druggable” targets
Examples of Antisense Mechanisms of Action for Oligonucleotides Drugs

- Transcription
  - 5'-cap formation
  - Polyadenylation

- Splicing modulation
- RNase H

- Inhibit 5'-cap formation

- dsRNase

- Inhibition of polyadenylation

- Transport

- Nucleus

- Cytoplasm

- Inhibit Translation

- Ribozymes

- dsRNase/RNAi
Second Generation Chemistry
Delivering on the Promise of the Science

Similar to the evolution that transpired with monoclonal antibodies; oligonucleotide chemistry has evolved.

- **First generation chemistry** was important for proof-of-concept in man. Identified the strengths and limitations of the technology.

<table>
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<tr>
<th>STRENGTHS</th>
<th>LIMITATIONS</th>
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<tbody>
<tr>
<td>Selectivity</td>
<td>Potency</td>
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<tr>
<td>Rapid ID of leads</td>
<td>Duration of action</td>
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<td>Local applications</td>
<td>Chronic toxicities</td>
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<td>I.V. dosing (for systemic)</td>
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- **Second Generation chemistry** brings all the strengths of first generation chemistry and addresses the limitations:
  - 10 – 15 fold increase in potency
  - 5 – 20 fold increase in duration of action
  - Marked decrease in toxicities and increase in therapeutic index
  - S.C. dosing once a week or less frequently (potentially oral)
2nd Generation RNase H Antisense Oligonucleotides are Potent Inhibitors of Gene Expression in Cell Culture

Inhibition of Target mRNA by 2nd Generation RNase H ASOs

lipofectin - 24hrs

Inhibition of Target Gene Expression by 2nd Generation RNase H ASOs

free uptake - 48hrs
2nd Generation MOE ASOs 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
ISIS 301012 Produces a Significant, Dose-dependent and Prolonged Reduction in apoB-100 in Human Subjects

Data as of 09/10/04
34 patients
Modulation of Alternative Splicing Using Antisense Oligonucleotides

- 15% of Inheritable Diseases Can Be Attributable to Splicing Defects
Delivery of Oligonucleotides to The Skin
Distribution of Oligonucleotide in Skin

Oligo follows perfused areas (stained brown);
Topically drug is delivered to all cells (note nuclear localization)
Dose Response of ISIS 2302 in Human Skin Model (ICAM Stained Brown)

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<tr>
<th></th>
<th>Basal</th>
<th>TNF 4000U</th>
<th>ISIS-2302 2% Cream</th>
<th>ISIS-2302 0.5% Cream</th>
<th>ISIS-2302 0.1% Cream</th>
<th>ISIS-8424 2% Cream</th>
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</table>
Distribution of Topically Applied FITC-labeled Oligo in Balb/c Mouse Skin

2% 18073 @ 0 hour

Derm #73 (repeat with new FITC filter no DAPI) 3 hours

2% 18073 @ 24 hours
Delivery of ASOs to the Skin Summary

- Delivery of nucleic acids through the skin to epidermal cells remains a challenge
- Follicular and dermal delivery observed in shaved mouse skin
- A first generation ICAM-1 ASO (alicaforsen) demonstrated modest activity in psoriasis patients
  - Unclear if modest activity was due to potency, delivery, target or combination.
- A second generation ASO targeting IGF receptor is currently in clinical trials for treatment of psoriasis
- Additional investments in formulations warranted
Treatment of Familial ALS with An Antisense Inhibitor to SOD1:
A Case Study for Developing a Drug for a Rare Inheritable Disorder

- Joint collaboration between:
  - Richard Smith - Center for Neurological Studies
    - Clinician highly motivated to find treatment for ALS
  - Don Cleveland – UCSD/Ludwig Cancer Institute
    - Basic researcher interested in molecular mechanisms of neurodegenerative diseases
  - Tim Miller - UCSD
    - MD/PhD Neurology Fellow in Cleveland lab
  - Isis Pharmaceuticals
    - Experts in Antisense Technology
Scientific Rationale

- FALS accounts for approximately 10% of ALS cases
- Mutations in SOD1 are found in about one half the cases of familial ALS
- Mutations result in a gain of function for SOD1 resulting in production of a toxic protein
  - Over 100 mutations have been documented
  - A4V accounts for 50% of cases in U.S.
- Expression of human mutant forms of SOD1 result in ALS-like disease in rats and mice
- Inhibition of SOD1 expression extends life in transgenic rats and mice
Stages in FALS Project

- Technical feasibility
  - Demonstrated delivery of 2nd generation ASO to motor neurons
  - Demonstrated reduction of SOD1 expression in neurons
- Drug Identification
  - Identified human clinical candidate
- Clinical Plans
  - Worked with internationally recognized leaders in ALS clinical research to develop clinical plan. Obtain FDA feedback on plan
- In process of raising funds for toxicology studies and clinical activity
Keys to success

- Motivated investigators who wanted to develop therapies to treat a uniformly lethal disease
  - Investigators reasonably well funded
  - Willing to think creatively about funding and research activities
  - Initially willing to do some of the heavy lifting
Obstacles We Had To Overcome

- Lack of knowledge about opportunity at company
- Developing a research plan that was achievable with constraints at Company and Academic labs
- Money
- Resources to devote to project
- Technology
  - Unknown if technology would work well enough for this application
  - Delivery to CNS
Conclusions

- Antisense technology (including RNAi) is an important drug discovery platform for treatment of rare inheritable disorders.

- There are a variety of strategies available for exploiting antisense technology that can be tailored to specific problems, i.e., RNase H, RNAi, splicing modulation, translation modulation, etc.

- Companies are willing to invest in developing therapies for rare inheritable disorders.
  - Easier to make investment decisions if companies do not have to bear all the risk.