Antisense Therapy: A New Concept in Disease Management

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What Is Antisense Therapy?

Generally refers to oligonucleotides that are complementary for a gene target of interest.

An antisense sequence binds to mRNA and prevents translation.
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An antisense sequence binds to mRNA and prevents translation
History of Antisense Therapy

1978 Stephenson - first report of antisense oligonucleotide (ASO) used to block viral RNA

1990 van der Krol; Napoli. RNA silencing was described (RNAi)


1999 Hamilton et al, Science. siRNA gene silencing in plants

2000 Reinhart et al, Nature. miRNA described


2006 Nobel Prize in Medicine: Andrew Fine, Craig Mello, for discovery of RNA interference - gene silencing by double-stranded RNA
History of Antisense Drug Development

1980 Sarepta Therapeutics (formerly Antivirals; AVI Biopharma)

1989 ISIS Pharmaceuticals founded by Stanley Crooke

1998 FDA approval of first antisense drug: fomivirsen (Vitravene) for treatment of CMV retinitis in immunocompromised patients

1998 Antisense Pharma founded in Munich

2000 Antisense Therapeutics founded in Australia

2000 Gene Signal, Switzerland

2001 Antisense Drug Technology, (ed. S. Crooke) published

2002 Prosensa established in The Netherlands

2002 Alnylam, Cambridge (2012 Genyme alliance)

2012 Genta – Bankrupt after failed cancer drugs

2013 Mipomersen approved by FDA for treatment of homozygous FH
# Antisense Drug Development

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<th>Drug</th>
<th>Company</th>
<th>Disease</th>
<th>Phase</th>
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<td>Kynamro</td>
<td>Isis Pharmaceuticals and Sanofi</td>
<td>Homozygous familial hypercholesterolemia</td>
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<td>Drisapersen</td>
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<td>ATL1102</td>
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<td>ISIS-EIF4EXr</td>
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<td>EXC 001</td>
<td>Pfizer and Isis</td>
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<td>iCo-007</td>
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<td>OGX-427</td>
<td>Oncogenex and Isis</td>
<td>Bladder cancer and prostate cancer</td>
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Concept of Antisense Therapy

Gene → Transcription → mRNA → Translation

Antisense Drug (Oligonucleotide)

Traditional Drug

Proteins

Courtesy of Genzyme
Challenges in Antisense Drug Development

- Need to identify appropriate gene target
- Need to generate antisense oligonucleotide that is specific for the gene of interest
- Molecule needs to be modified to prevent degradation by RNAses and other enzymes
- Drug needs to be deliverable to the organ of interest
The Antisense Platform

- Rapid identification of drug entity
  - Isis has Identified AS inhibitors to over 3000 genes
- No “undruggable” targets
- Short timelines from concept to first human dose. eg, 21 months for apoB

Courtesy of Isis
Generate all ASOs complementary to pre-mRNA and variant mRNAs

Evaluate in silico

Screen in vitro

Confirm in vitro with alternate endpoints and dose response

Screen in vivo at a single dose

Dose response in vivo

Pharmacology in vivo
Distribution of Antisense Oligonucleotides

Oligonucleotide Conc. In Tissue (µg/g)

- Bone Marrow
- Brain
- Heart
- Kidney Cortex
- Kidney Medulla
- Liver
- Lung
- Mesenteric Lymph
- Ovaries
- Spleen
- Testes
- Uterus

Courtesy of Isis
Second Generation Antisense Technology

2'-O-Methoxyethyl Modification

- Generation 0: native DNA
- 1st generation: phosphorothioate
- 2nd generation: MOE

- chimeric oligonucleotides support RNase H activity
- increased affinity for mRNA
- potency increase 10-15 fold
- increased nuclease resistance yields prolonged half life 5-20 fold
- better tolerated

Courtesy of Isis
Mipomersen
Second Generation Apo B Antisense Oligonucleotide

• Developed by ISIS; later partnered with Genzyme/Sanofi
• S.Q. drug given 200 mg weekly
• Five pivotal placebo-controlled double-blind trials in 601 subjects (397 receiving mipomersen)
• LDL-C decreased 25-37%
• Approved by FDA in USA for treatment of homozygous FH January 2013
Mipomersen Mechanism of Action

Mipomersen (Apo B) antisense strand

Hepatocyte cell membrane

DNA

Apo B mRNA

mRNA-antisense duplex

RNase H recognizes duplex

RNA is cleaved

Nucleus

Cytoplasm

VLDL

LDL

Lp(a)

Courtesy of Genzyme
Based on KYNAMRO™ (mipomersen sodium) injection full Prescribing Information. January 2013.
Sustained Reductions in LDL-C, Apo B and Lp(a) During Long-Term Open-Label Treatment of FH with Mipomersen
Mipomersen
Primary Side Effects

- Injection site reactions (ISRs) - 84% vs 33%
- Flu-like reactions - 30% vs 16%
- Transaminase elevations (ALT > 3x ULN) - 17% vs 2%
- Hepatic fat deposition - mean increase of 9.6% vs 0.02%
Mipomersen
Strategies to Mitigate Side Effects

- Patient reassurance
- Maintain close relationship with patient - be available
- For ISRs: Allow drug to warm, inject slowly, identify preferable injection site, inject into S.Q. tissue, possible icing of site, avoid rubbing
- For Flu-like symptoms: icing, take at bedtime, ibuprofen
- For ALT & hepatic fat elevations: monitor ALT/AST monthly for 1st year, avoid alcohol excess and other hepatotoxins, alleviate features of the metabolic syndrome, temporarily stop treatment for marked ALT elevation
New Antisense Targets in Lipid Metabolism

- Apo C-III
- CRP
- Lipoprotein(a)
- PCSK9
Summary

• Antisense technology has great promise for specific suppression of translation of disease associated proteins from target genes

• Mipomersen is an FDA-approved antisense drug for treatment of homozygous familial hypercholesterolemia

• New antisense drugs are being developed to target other aspects of lipid metabolism, diabetes and atherosclerosis