Oligonucleotides: important therapeutic agents

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Conflicts of Interest

Alnylam Pharmaceuticals—co-Founder, Stock holder, Member of Board of Directors, and Chair of Scientific Advisory Board
Biotechnology—new therapeutic modalities

1980–1990 Interferons, protein hormones
1990–2000 Monoclonal antibodies
2000–2010 Oligonucleotides, siRNA
2010–2020 CRISPR, cell therapy

Gene Therapy (1980–2020)
DNA sequencing costs over time

- 2003: Human genome sequence completed
- 2005: Next-generation sequencing introduced
- 2014: "$1,000 genome" sequencer announced
1. ~3,000 Mendelian disease genes known

2. ~8% of live births- genetic disorder by early adulthood

3. Estimated- each child with genetic disorder ~costs $5,000,000

4. Diagnostic rate of genetic disorders: children ~11%, adults ~34%

Emma walks!
Summary of the genetics of SMA

- **SMN1 gene** - survival motor neuron

- **SMA patients** - mutation in SMN1 gene

- **SMN2 gene or genes** - poorly expressed
  - Defect in RNA splicing

ASOs can modify splicing of SMN2

• Antisense Oligonucleotide (ASO) to intron 7-inclusion of exon 7

• Inclusion of exon 7-functional SMN protein

1. Adrian Krainer (CSHL) collaborated with Frank Bennett of Ionis (2008)

2. Ionis Pharmaceutical developed the anti-sense oligonucleotide, founded by Stanley Crooke in 1989

Celebrating the Nobel Prize for RNAi
Drs. Andrew Fire and Craig Mello
Short RNAs silence by mRNA degradation

Drosophila and Mammals

Worms and Plants

mRNA degradation
microRNA pathway

Pol II → miRNA gene

pri-miRNA

Drosha → microprocessor

Nucleus

Ran-GTP/exportin

Dicer

miRNA:miRNA* duplex

Cytoplasm

siRNA Pathway

Ago

miRNA Pathway

RNA Interference

Ago

RNA Cleavage

Cap

AAAAAAA

Ago

Silencing by degradation and translation

Cap

Stop

AAAAAAA
Harness natural pathway
Catalytic mechanism
Silence any gene in genome
Upstream of today’s medicines
Clinically proven approach
Proposed mechanism of iLNP-mediated siRNA delivery

- **Blood Compartment**: Lipoprotein particle
- **pH 7.4**
- **Fenestration**
- **ApoE**
- **Endosoma membrane**
- **pH ~ 5**
- **Apoe-binding cell surface receptor (e.g., LDLR)**
- **Hepatocyte**
- **Risc**
- **Hexagonal H_{II}**
- **Bilayer**
Innovating LNP Delivery: Highlights 2006-2013

- 500X less drug needed/TI increased
- MC3/Patisiran
- TTR02
- PCS02
- TTR01
- VSP

>100 fold potency improvement

Alnylam data on file
Transthyretin (TTR)-mediated amyloidosis (ATTR) program
Unmet Need and Product Opportunity

- ATTR is significant orphan disease
  - ~50,000 Patients worldwide
- Clinical pathology
  - Onset ~40 to >60 yr
  - Peripheral sensorimotor neuropathy, autonomic neuropathy, and/or cardiomyopathy
  - Fatal within 5-15 years
Patisiran Phase 3 APOLLO Study Results
Serum TTR Reduction

87.8% mean max serum TTR reduction from baseline for patisiran over 18 months

Placebo (N=77)
Patisiran (N=148)
Lipid nanoparticle delivery of mRNA

- Encapsulation of mRNA within a lipid nanoparticle
- Nanoparticle enters cell
- mRNA is released
- mRNA instructs cells to produce spike protein
- Presence of spike protein causes immune response leading to the production of antibodies

antibodies
Tissue and Cellular Uptake
Targeting the liver: ASGPR and GalNAc

- **GalNAc-siRNA Conjugate**
  - GalNAc ligand conjugated to chemically modified siRNA to mediate targeted delivery
  - Trivalent GalNAc carbohydrate cluster has nM affinity for ASGPR
  - Administered subcutaneously (SC)

[Diagram showing the targeting mechanism of GalNAc-siRNA conjugate to the liver via the ASGPR receptor with a trivalent carbohydrate cluster.]
Potent and Durable Silencing Supports Quarterly Dosing in Humans

Mean (± SEM) C5 Knockdown Relative to Baseline

Days

Mean (SEM) % PCSK9 Knockdown (Change from Baseline)

Months

Treatment
Placebo
50 mg
200 mg

ALN-CC5

ALN-PCS
No current therapies to prevent or reverse neurodegenerative disease

- Dominantly inherited neurodegeneration
  - Alzheimer’s disease
  - Parkinson’s disease
  - Frontotemporal dementia
  - Huntington’s disease
  - Amyotrophic lateral sclerosis (ALS)
  - Spinocerebellar ataxia
  - Many others
IT Dosing of SOD1 siRNA Conjugate to Evaluate CNS RNAi activity

Durable SOD1 mRNA silencing is seen in all regions of the brain and spinal cord tested.
Robust Silencing of CNS and Ocular Targets

CNS APP mRNA Knockdown (Single Intrathecal Dose in NHP)

- Lumbar Spine
- Cervical Spine
- Prefrontal Cortex
- Temporal Cortex
- Striatum

Control
APP siRNA

Ocular TTR Protein Knockdown (Single Intravitreal Dose in NHP)

- PBS
- 0.003 mg
- 0.03 mg
- 0.1 mg
- 0.3 mg
Thank you for the opportunity to present this lecture.
- Phil Sharp