PCSK9 RNAi Therapeutics

Kevin FitzGerald
PSCK9 RNAi Therapeutics

- The following relationships exist related to this presentation:
  - Kevin Fitzgerald and Alnylam team members:
    - Holds stock options in Alnylam Pharmaceuticals
  - University of Texas Southwestern Medical Center:
    - Engaged in research for Alnylam
  - Tekmira Pharmaceuticals/UBC
    - Engaged in research for Alnylam Pharmaceuticals
RNAi Therapy for Hypercholesterolemia
Rationale for PCSK9

Hypercholesterolemia/PCSK9
- Well validated target
  - Gain and loss of function mutations
    - Rodent and Human (gf) and (lf) mutations
    - PCSK9 expressed in liver (delivery)
    - Early clinical markers of activity possible (PCSK9, LDLc, ApoB)

Alnylam PCSK9 program
- Discovery of pM active PCSK9 siRNAs
- Formulations or conjugates for delivery
- Proof of concept
  - Multiple rodent models, NHP’s
- Collaboration with UT Southwestern
  - Horton, Hobbs, Brown and Goldstein
RNAi Product Platform
Turning siRNAs into Drugs

Bioinformatics, *in vitro* assays

Cholesterol, others

Phosphorothioate 2’OMe, 2’F

• Select
  » *In silico* design
  » *In vitro* assays

• Stabilize
  » Chemistry

• Deliver
  » Formulations
  » Conjugates

Incorporate minimal number of modifications required for appropriate pharmaceutical properties
No Evidence of Off-Target Silencing by PCS-B2

Endogenous Genes at High Concentration of PCS-B2

*in vitro* (*Hep3B, RNAiMax, 1uM*)

- ORMDL2
- BMP6
- TAPT1
- MYEF2
- LOC442252
- RFT1
- PCSK9

PCS-B2 pM IC$_{50}$
Lack of PCS-B2 Cytokine Induction
Panel of 7 Cytokines, IFN-α, IP-10, IFN-γ, TNFα, IL-6, IL-1ra, G-CSF (hPBMC) 13 Donors
PCS-B2 Has No Effect On Cell Proliferation In 4 Different Cell Lines (Hep3B, Cos7 Cells Example)
Lipid Nanoparticles for Systemic RNAi

- Multi-component lipid formulation
  - Cationic lipid
  - Structural lipid
  - PEG lipid
  - Cholesterol

- Highly efficient for liver delivery
  - Hepatocyte-specific gene silencing achieved

- Low surface charge
- Small uniform size particle <100 nm
RNAi Silencing of PCSK9 in Rats

Liver PCSK9 mRNA and serum Tc levels

Predicted PCR band
Seq. Confirmed

5’RACE (Liver)

LNP-siRNA (mg/kg) Day 0 N=6/group

PCSK9 mRNA Total chol. LDLR

RNAi Silencing of PCSK9 Decreases Total Cholesterol in Rats
Duration and Lack of Fatty Liver

PCSK9 mRNA is down modulated in hCETP/hApoB100 Transgenic Mice

LDL particle number lowering

LNPX-PCS siRNA (5mg/kg)

Day 0 3

N=4/group

liver PCSK9 mRNA
NMR LipoProfile: LDL and HDL particle number

PCSK9/GAPDH

Relative to PBS=1

Control PCS-A2

Total Particle Number

Control PCS-A2

LDL HDL LDL HDL
RNAi Silencing of LDLc and PCSK9 Protein Non-Human Primates

- Acute and durable effects after a single 30 minute infusion
- PCSK9 plasma levels reduced by up to 70% of pre-dose levels
- Rapid reductions in LDL cholesterol levels by 40-60%

LNP Progress

Efficacy Improvement Over Time

LNP- formulated FVII siRNA

Day 0 → serum FVII protein

% Residual Factor VII

ED_{50}

FVII siRNA Dose (mg/kg)

C12-200

DLin-KC2-DMA-a

DLin-KC2-DMA-b

DLinDMA

DLinDAP

98N12-5(I)

serum FVII protein

Even Formulated FVII siRNA

Day 0

3

0.01

0.1

1

10

0

20

40

60

80

100

120

ED_{50}

ED_{50}
Dose Sparing Paradigm and Multiple Injections

Rats were bled one day prior to repeated dosing. Maintenance: 1 x wk

- Initial 3mg/kg bolus
- Maintenance: 1 x wk

PBS
- 3mg/kg bolus + PBS once a week
- 3mg/kg bolus + 0.3 mg/kg per week
**Dose Response in Rats**

PCS-A2 ED50 <0.1 mg/kg in Newer Formulations

LNP E-formulated PCS-A2 siRNA

Liver PCSK9 mRNA, total serum cholesterol

Day 0 → 3

Graph showing relative to PBS=1 for PCSK9/GAPDH and Cholesterol levels with LNP E-formulated PCS-A2 siRNA dosage ranging from 0.01 to 10 mg/kg.
ALN-PCS demonstrates potent efficacy in primates
- Employs 2nd generation LNPs
- Rapid and durable dose-dependent reduction in PCSK9 protein
- PCSK9 silencing results in >50% reductions in LDLc
Treatment with LNP-PCS-B2 Does Not Affect HDLc Levels
5 Different siRNAs Formulated Nanoparticles Inhibit 5 Different Hepatic mRNAs in Dose Dependent Manner Mice 72h

**Graph:**
- y-axis: Target mRNA, relative to LNP12-Luc Control
- x-axis: siRNA mg/kg
- Lines represent different target mRNAs: PCSK9, ApoB, FVII, Xbp1, SORT1
- ED50 values: PCSK9 0.07 mg/kg, ApoB 0.05 mg/kg, FVII 0.025 mg/kg, Xbp1 0.03 mg/kg, SORT1 0.08 mg/kg

*PNAS publication in press*
10 Different Formulated siRNAs in One Nanoparticle Inhibit 10 Different Hepatic mRNAs
Mice, 0.1mg/kg of each siRNA, 48h
RNAi therapeutic targeting PCSK9:

- Results in rapid, significant, and sustained lowering of LDLc levels, but not HDLc
- Lead molecules are active in all pre-clinical models tested:
  - pM and specific
- Rats
  - Total cholesterol lowering; RNAi based and LDLR mechanisms
  - <3 weeks duration on single dose
  - Steatosis free
- NHP study:
  - LDLc decreased ~40-60% with no effects on HDL cholesterol at doses >0.1mg/kg
- Dose sparing paradigms may be possible
- Combinations possible to treat metabolic syndrome
  - Up to ten targets in same formulation
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