siRNA Therapeutics: Target Identification, Discovery and Early Development Considerations

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Disclosure: I am an employee of Arrowhead Pharmaceuticals
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Multiple older platforms converging on targeting with direct conjugation of NAG

- Alnylam – Lipid Nanoparticle (LNP) to GalNAc conjugation (GalNAc-ESC/ESC+)
- Dicerna – GalXC™ (tetraloop)
- Arrowhead – Dynamic Polyconjugates (DPC™) (2 molecules with endosomal escape agent) to TRiMTM

(DPC™ (EX-1) and cholesterol-conjugated RNAi trigger are separately directed to the liver)

(1) Sperry BW and Tang Heart 2017 103: 812-817
(2) Dicerna Pharmaceuticals Corporate Overview 2018
TRiM™ has rules and algorithms to optimize trigger sequence

- Limit cross reactivity with off target genes
- Maximize activity
- Maximize innate stability
- Rational use and placement of modifying chemistries
Hepatic siRNA discovery/development

Direct conjugation with NAG allows for binding and endocytosis with highly and specifically expressed Asialoglycoprotein receptor (ASGPr) in hepatocytes.

Binding of NAG to ASGPr initiates endocytosis.

**Key Design Elements in Hepatic Platform**

- Subcutaneous dosing, monthly or less frequency
- Stable and potent sequences
  - No need to use endosome escape moieties
- Suppression of liver target protein production
- Expectation of wide therapeutic index
What makes an optimal hepatic RNAi gene target

RNAi competitively advantageous for targets that are not easily/well targeted with small molecules or mAbs

Examine diseases with limited or no treatment options, where knockdown of protein expression is hypothesized to be beneficial to disease initiation/progression

With hepatocyte-targeted RNAi agents:

- Target is expressed in hepatocytes
  - If not primarily expressed in hepatocytes, hepatocyte expression is key for disease etiology
- Convenient if sequence is cross-reactive with human, NHP, and rodent
- Secreted protein advantageous (blood-based monitoring of knockdown)
- Non-secreted protein knockdown can be monitored through liver biopsy or well characterized secondary biomarker
- Disease-relevant animal models available
  - Proof of Concept studies
  - Can be used to estimate level of knockdown required for beneficial effect

2 examples of targets: Factor XII (F12) for thrombotic disease and Alpha 1 antitrypsin (AAT) for AAT-deficiency (liver)
Hepatic RNAi agent development funnel

Bioinformatic selection of RNAi trigger sequences specific for target gene – filter to identify cross-reactive triggers (human/NHP/rodent, human/NHP)

Cross-reactive RNAi trigger synthesis and *in vitro* testing

Synthesis and *in vivo* testing of select RNAi triggers amenable for subcutaneous (SC) administration

Lead Optimization on RNAi triggers for SC administration with *in vivo* testing

Proof of Concept for disease modification in animal models

Exploratory Toxicology
Targeting factor XII by RNAi as a prophylactic treatment of thrombotic disease

**Factor XII (F12)**
- Key component of contact activation pathway (thrombosis) and kinin-kallikrein (angioedema)
- Predominantly expressed in the liver; circulates in plasma

**F12 inhibition is genetically validated**
- F12-deficient mice:
  - viable and fertile
  - No bleeding defects
  - protected from thromboembolic disease (stroke, pulmonary embolism)
- F12 deficiency in humans is **not** associated with either bleeding or thrombotic disorders

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* Figure modified from Albert-Weissenberger, C., et al. (2014) Front. Cell Neurosci. 8:345
Measuring F12 knockdown and effects – serum/plasma

- F12 levels can be measured in mouse and NHP by ELISA-based methods to monitor knockdown
  - RNAi triggers tested are cross reactive between human, NHP and rodent
  - Mouse F12 protein (total and activated) measured by custom AlphaLISA™ (Perkin Elmer)
  - NHP F12 protein measured by human F12 ELISA (cross-reactive with NHP)
- F12 activity can be measured through a modified version of standard coagulation measure activated Partial Thromboplastin Time (aPTT)
  - aPTT is inversely correlated with F12 levels (ELISA or Activity)

![Graph showing the inverse correlation between aPTT and F12 Activity](graph.png)

Dashes = normal range
Examination of modified RNAi triggers in mice

**First Generation**

*Single 3 mg/kg SC dose*

- n=3/group

**Second Generation**

*Single 0.5 or 1 mg/kg SC dose*

- n=4/group

- Modifications to SC1 to yield SC2 improved knockdown
- 85% at 3 mg/kg vs 91% at 1 mg/kg at nadir
• Achieved ~90% knockdown of F12 in NHP after the second dose at 1.5 mg/kg with >1 month duration
• 90% knockdown of F12 activity correlates with significant increase in aPTT
• No changes in toxicity markers (clin chem, CBC) after dosing
Disease-relevant animal modeling: ferric-chloride study

- Thrombus induced by exposure of carotid artery to FeCl₃
- Measure time to blood flow occlusion (thrombus formation)
- Single SC injection of SC2 or negative control, 2 weeks prior to challenge with FeCl₃, n=7/group

Dose response observed for inhibition of clot formation
- Statistically significant change in occlusion times (p<0.02) observed with >80% knockdown of serum F12

* p<0.02
** p<0.001
Bleeding risk assessment through mouse modeling

- Transverse cut of tail vein, monitor time to clotting
- Single dose SC4, 14 days prior to assessment, n=7/group (saline and SC4), n=10/group (heparin)

- No increased bleeding observed, even with 99% knockdown of F12 levels
- Consistent with F12 (−/−) mice showing no increase in bleeding over wild type controls
Alpha-1 Antitrypsin Deficiency (AATD)

• AATD is a large scale orphan disease
  ➢ Alpha-1 Foundation estimates 100,000+ in the US
  ➢ Approximately 100,000+ in Europe
• Mutation in AAT gene (Z-AAT) leads to mis-folding of the protein and poor export from hepatocytes: low levels in circulation and accumulation in liver

Pathophysiology

Lung
Tissues susceptible to damage by neutrophil proteases: COPD
Treated with AAT enzyme replacement therapy

Liver
Accumulation of mutant Z-AAT protein can cause cirrhosis and HCC
Currently no treatment
RNAi trigger mechanism of action

RNAi trigger designed to stop Z-AAT production by silencing AAT gene to:

- Prevent liver accumulation
- Allow clearance of accumulated protein
- Prevent cycles of cellular damage
- Prevent/Reverse progression of liver fibrosis

Feldmann G et al., Gut 1975
ARO-AAT Provides Durable AAT knockdown: Multi-dose in NHP, dosed subcutaneously

- 92% maximum serum AAT knockdown achieved in cynomolgus monkeys
- Knockdown sustained for 7+ weeks following second dose

Durable knockdown supports once monthly or less frequent dosing
Based on clinical observations, clinical pathology and histopathology evaluations, ARO-AAT was well tolerated in the following non-GLP exploratory toxicity studies:

- A repeated dose study in rats administered 3 weekly subcutaneous doses at dose levels of 30, 60, 120, and 300 mg/kg
- An escalating dose study in two cynomolgus monkeys dosed weekly subcutaneously at doses up to and including 300 mg/kg

ARO-AAT is now entering into clinical trials
Key considerations entering development

• FDA treats RNAi therapeutics like small molecules (CDER)
• Requirements for particular enabling studies may vary based on placement within CDER
• Coordination of required GLP studies can speed transition to clinic
Summary

• Most current RNAi agents specifically target hepatocytes through direct conjugation with NAG (ASGPr1 ligand)

• RNAi agents can be effective in knocking down expression of target protein responsible for rare/orphan diseases (AAT-deficiency) and more common conditions (Factor XII in thrombosis)

• Speed of evaluation of potency/efficacy is increased with human/NHP/rodent cross-reactive RNAi agents

• Knockdown that can be measured by blood biomarker (primary or secondary biomarker) speeds evaluation

• Exploratory toxicology studies of RNAi agents support wide therapeutic index

• RNAi agents are considered small molecules by regulatory agencies, with respect to requirements