# siRNA Therapeutics: Target Identification, Discovery and Early Development Considerations

Stacey Melquist, PhD, PMP

Senior Scientist

Arrowhead Pharmaceuticals

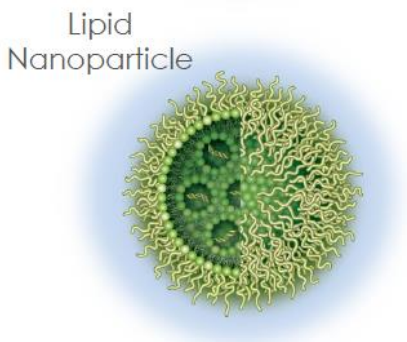
Disclosure: I am an employee of Arrowhead Pharmaceuticals

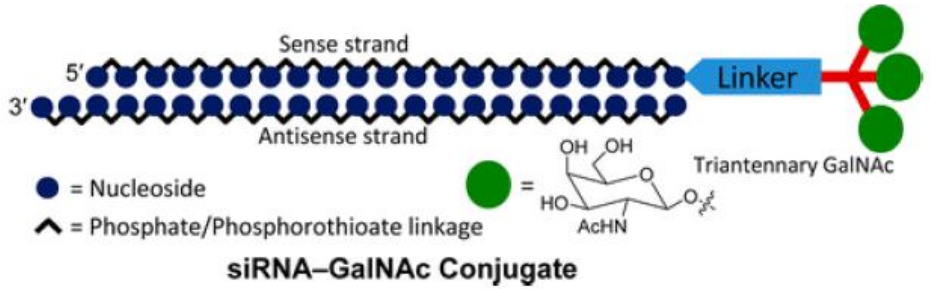
# Safe Harbor Statement

This presentation contains forward-looking statements within the meaning of the "safe harbor" provisions of the Private Securities Litigation Reform Act of 1995. These statements are based upon our current expectations and speak only as of the date hereof. Our actual results may differ materially and adversely from those expressed in any forward-looking statements as a result of various factors and uncertainties, including, without limitation, our developmental stage and limited operating history, our ability to successfully and timely develop products, enter into collaborations and achieve other projected milestones, rapid technological change in our markets, demand for our future products, legislative, regulatory and competitive developments and general economic conditions. Our Annual Report on Form 10-K, recent and forthcoming Quarterly Reports on Form 10-Q, recent Current Reports on Forms 8-K, and other SEC filings discuss some of the important risk factors that may affect our ability to achieve the anticipated results, as well as our business, results of operations and financial condition. Readers are cautioned not to place undue reliance on these forward-looking statements. Additionally, Arrowhead disclaims any intent to update these forward-looking statements to reflect subsequent developments.

# Multiple older platforms converging on targeting with direct conjugation of NAG

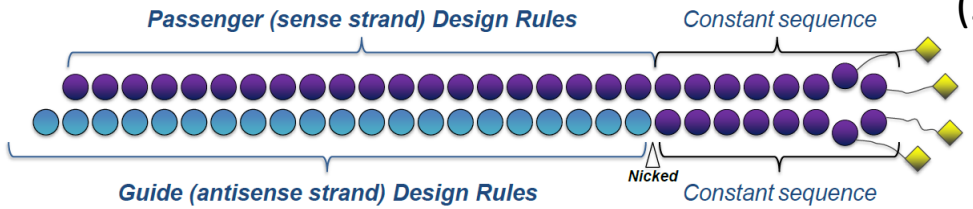
- Alnylam – Lipid Nanoparticle (LNP) to GalNAc conjugation (GalNAc-ESC/ESC+)




(1)

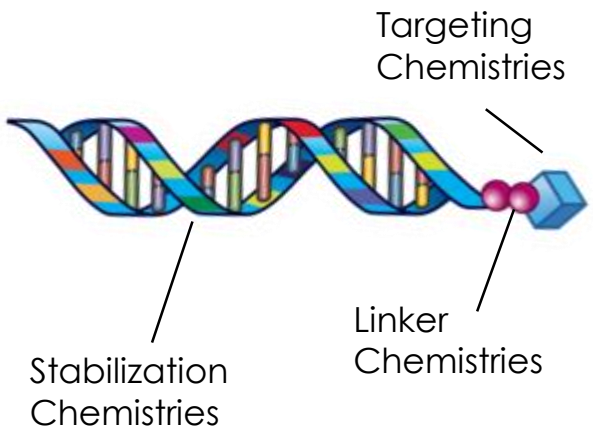
- Dicerna – GalXC™ (tetraloop)




(2)

- Arrowhead – Dynamic Polyconjugates (DPC™ )(2 molecules with endosomal escape agent) to TRiM™


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

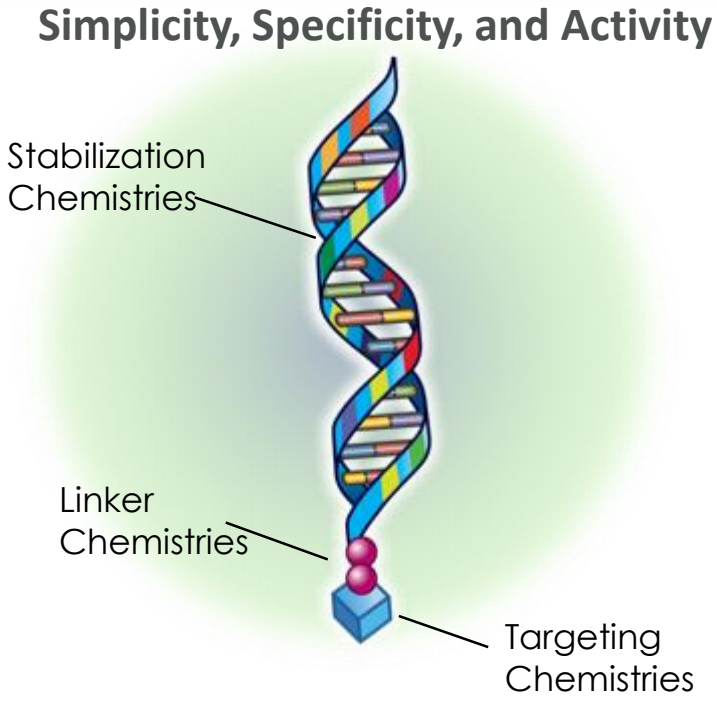



**Targeted RNAi Molecule**

**TRiM™ platform**

(1) Sperry BW and Tang Heart 2017 103: 812-817
(2) Dicerna Pharmaceuticals Corporate Overview 2018

# Arrowhead RNAi platform: TRiM™




**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

**Targeted RNAi Molecule**

**TRiM™ platform**

# 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

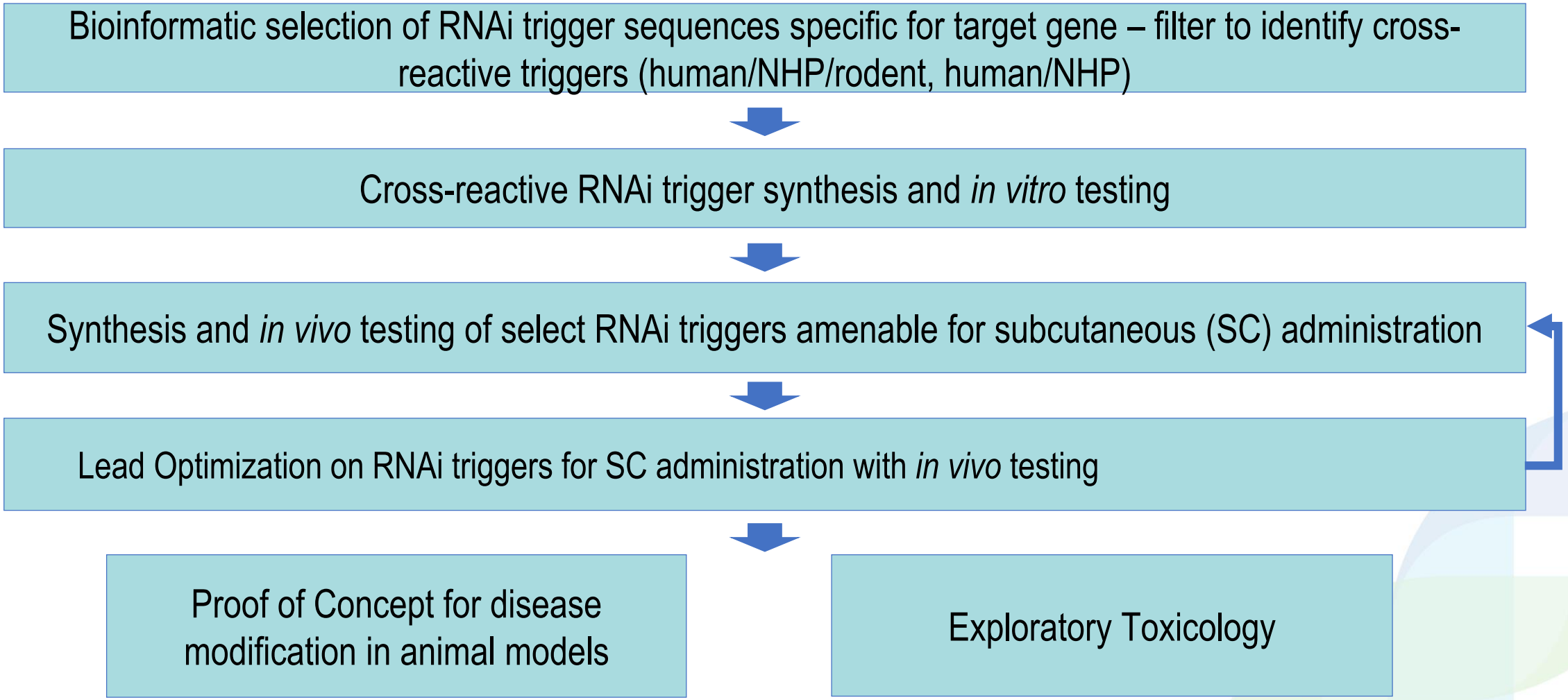
Examine diseases with limited or no treatment options, where knockdown of protein expression is hypothesized to be beneficial to disease initiation/progression

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



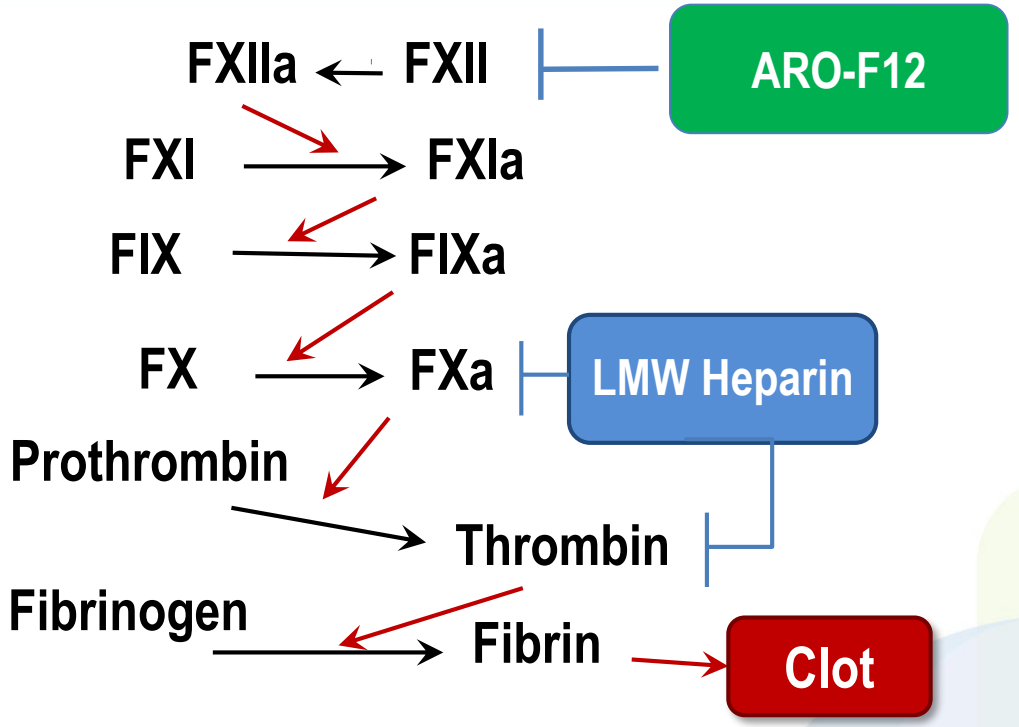
# Targeting factor XII by RNAi as a prophylactic treatment of thrombotic disease

**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**

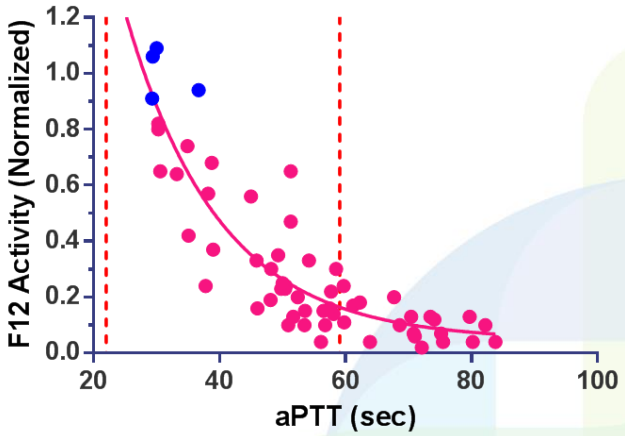
- F12-deficient mice:
  - viable and fertile[4]
  - No bleeding defects[4,5]
  - protected from thromboembolic disease (stroke, pulmonary embolism)[5]
- F12 deficiency in humans is *not* associated with either bleeding or thrombotic disorders[1,2,3]




[1] Girolami A. *et al.* (2004) J. Thromb. Thrombolysis 17:139–143
[2] Koster A. *et al.* (1994) Br. J. Haematol. 87:422–424
[3] Zeerleder S. *et al.* (1999) Thromb. Haemost. 82:1240–1246
[4] Pauer, H. U., *et al.* (2004) *Thromb. Haemost.* 92:503
[5] Renne, T. *et al.* (2005) *J. Exp. Med.* 202:271
* 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)

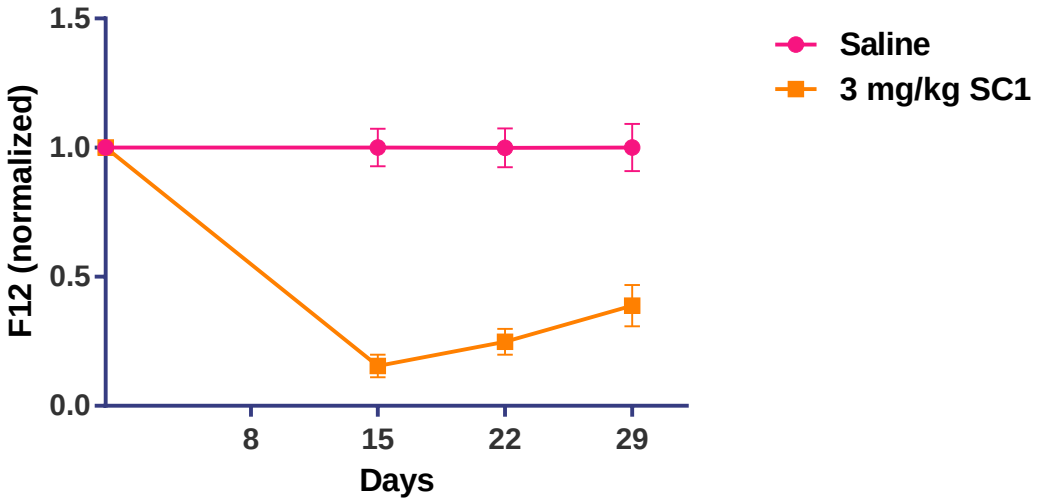



dashes = normal range

# Examination of modified RNAi triggers in mice

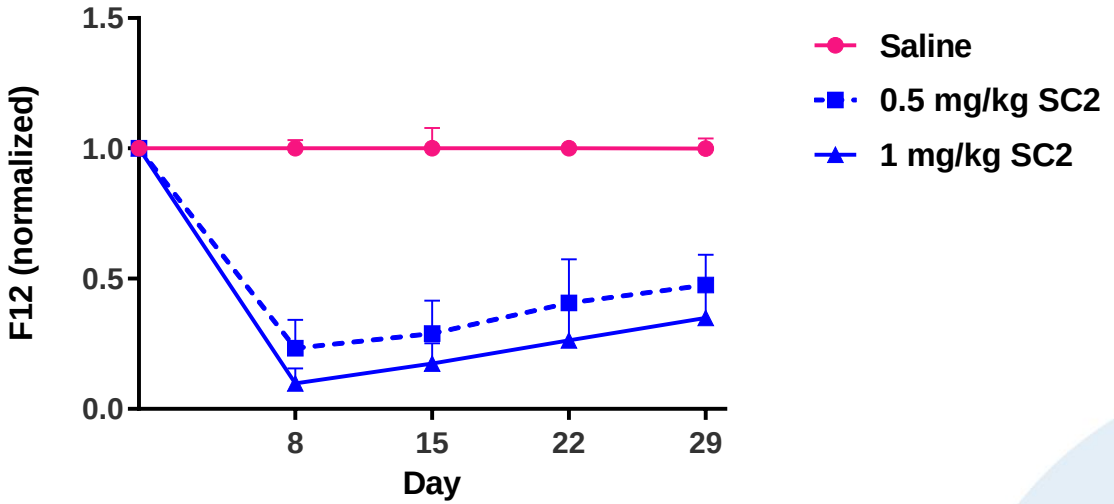
## First Generation

***Single 3 mg/kg SC dose***
***n=3/group***




## Second Generation

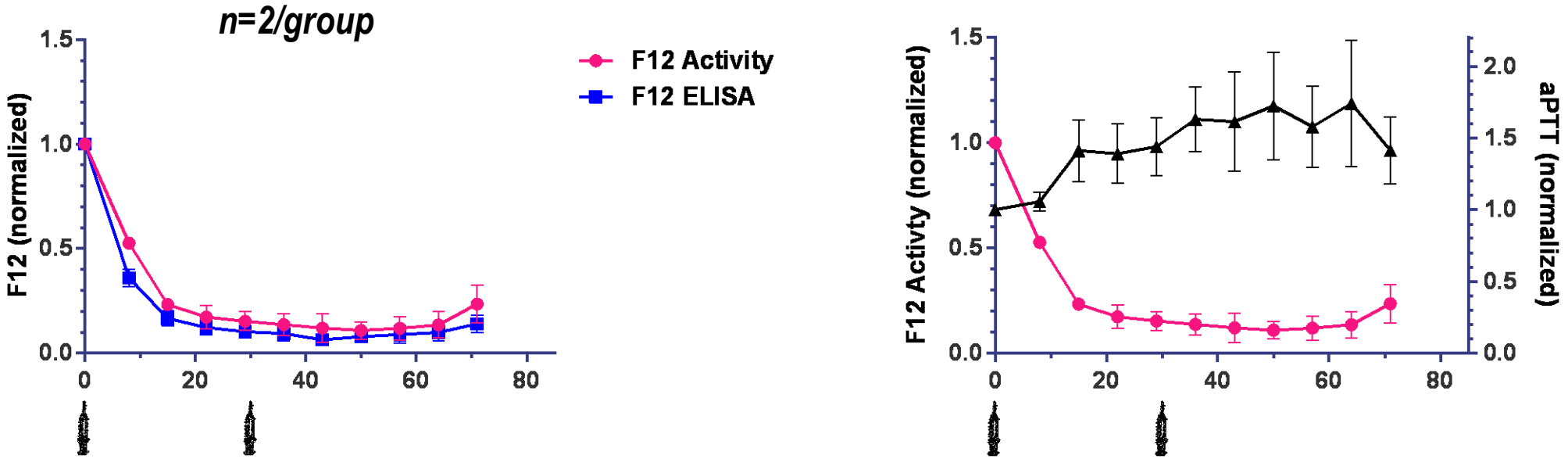
***Single 0.5 or 1 mg/kg SC dose***
***n=4/group***




- Modifications to SC1 to yield SC2 improved knockdown
  - 85% at 3 mg/kg vs 91% at 1 mg/kg at nadir

# Second generation triggers – examination in NHP

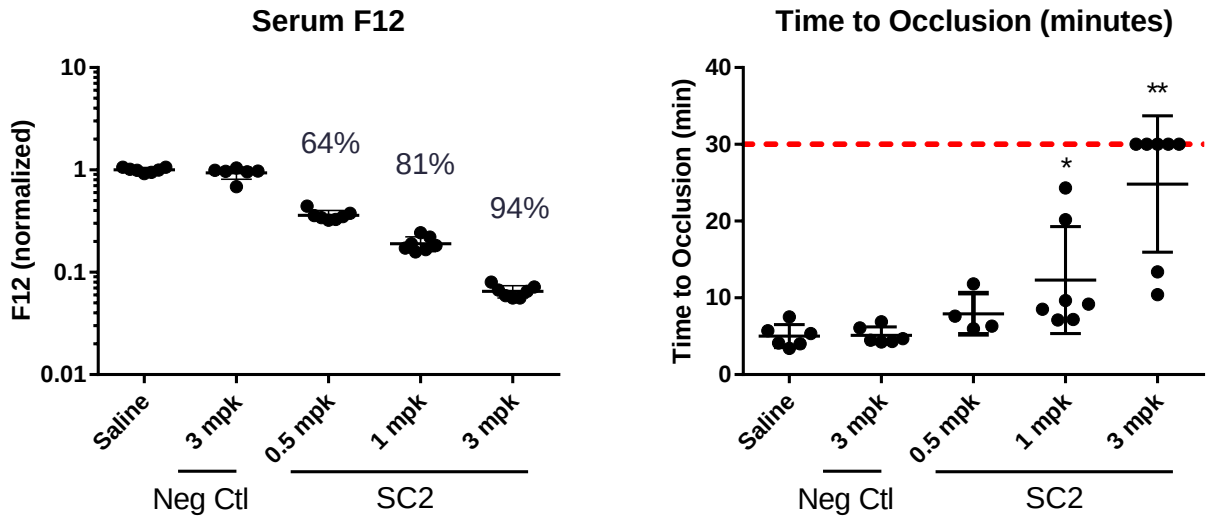
Initial SC dose of 3 mg/kg SC2, followed by 1.5 mg/kg dose on day 29




- 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 $FeCl_3$
- Measure time to blood flow occlusion (thrombus formation)
- Single SC injection of SC2 or negative control, 2 weeks prior to challenge with $FeCl_3$, n=7/group





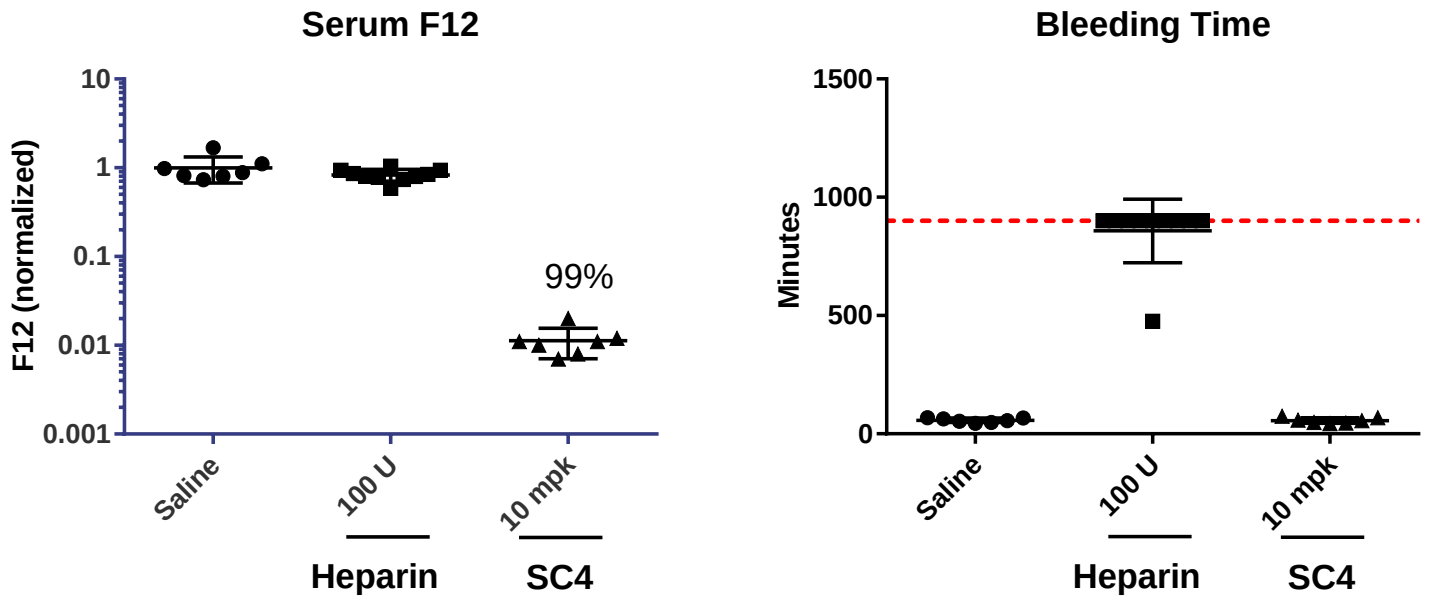


*p<0.02
**p<0.001

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

# 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

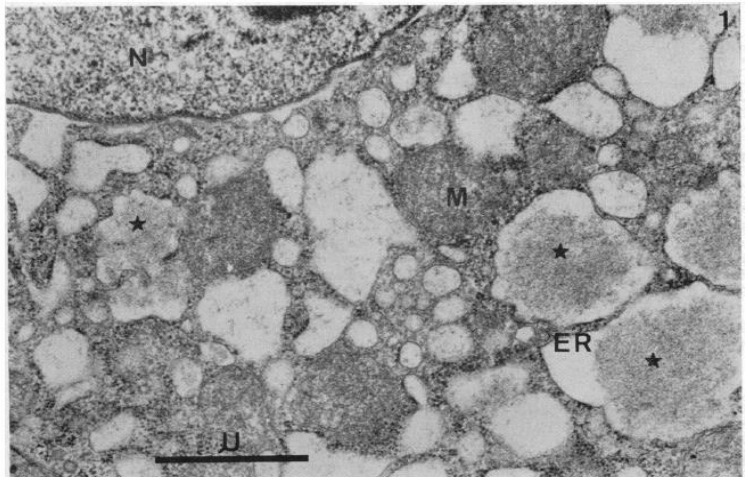
**Currently no treatment**

# RNAi trigger mechanism of action

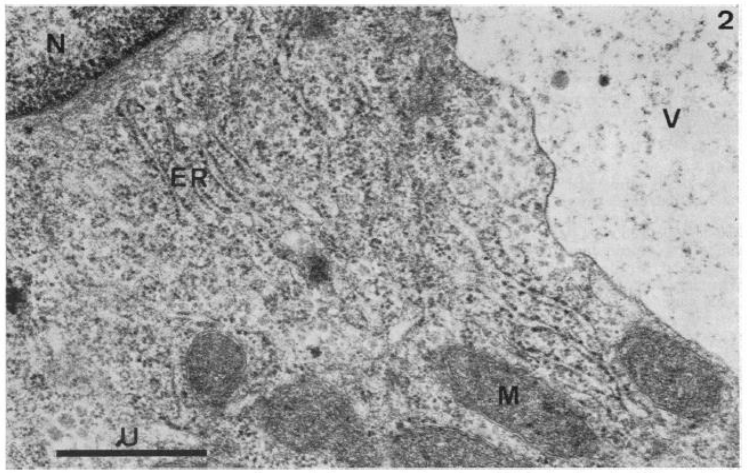
RNAi trigger designed to stop Z-AAT production by silencing AAT gene to:

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

**PiZZ phenotype (diseased)**



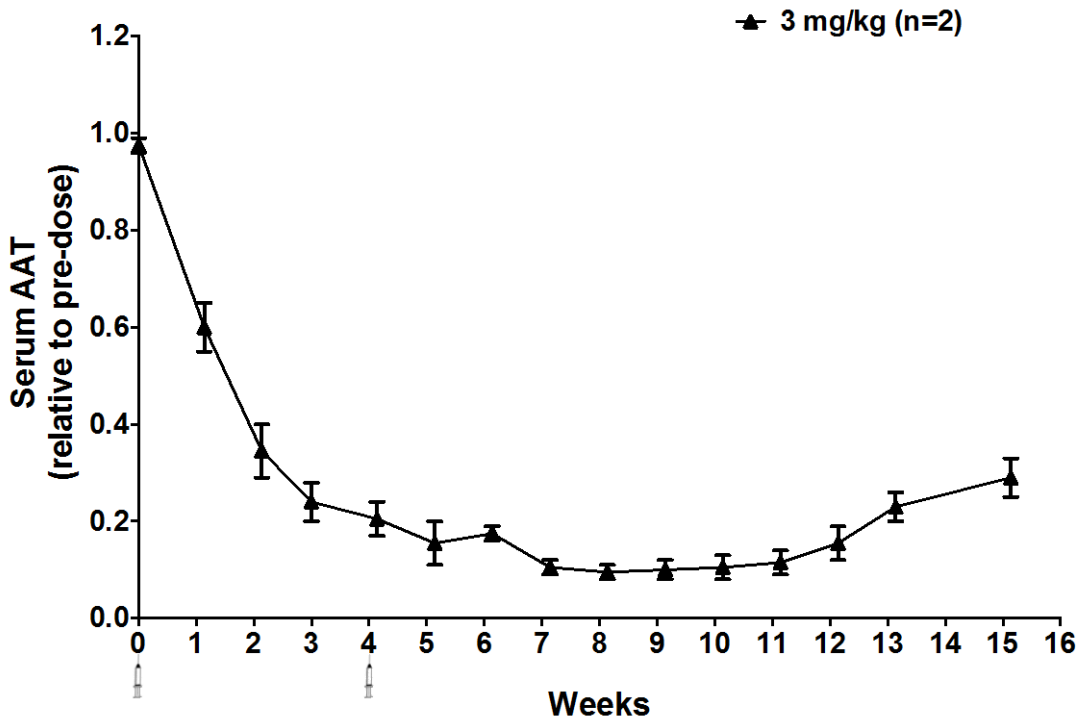

**Pi null phenotype (normal)**




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

# Exploratory Toxicology: ARO-AAT preliminary safety evaluation

- 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

# Arrowhead Team