Factor 12 RNAi-based therapeutic as a prophylactic anti-thrombotic therapy

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Presenter Disclosure Information Elements

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- **FINANCIAL DISCLOSURE:**
  - **Presenter and all abstract authors are employees and stockholders of Arrowhead Pharmaceuticals**

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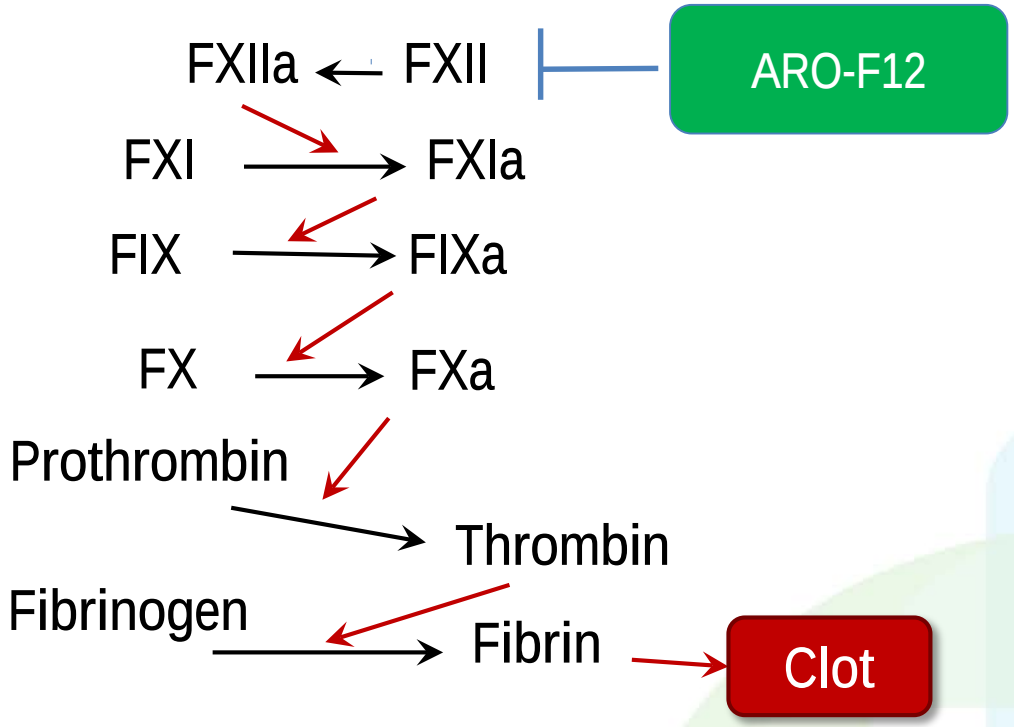
Factor XII is an attractive target for RNAi therapeutics

Factor XII (F12)

- Key component of contact activation pathway
- Predominantly expressed in the liver; circulates in plasma

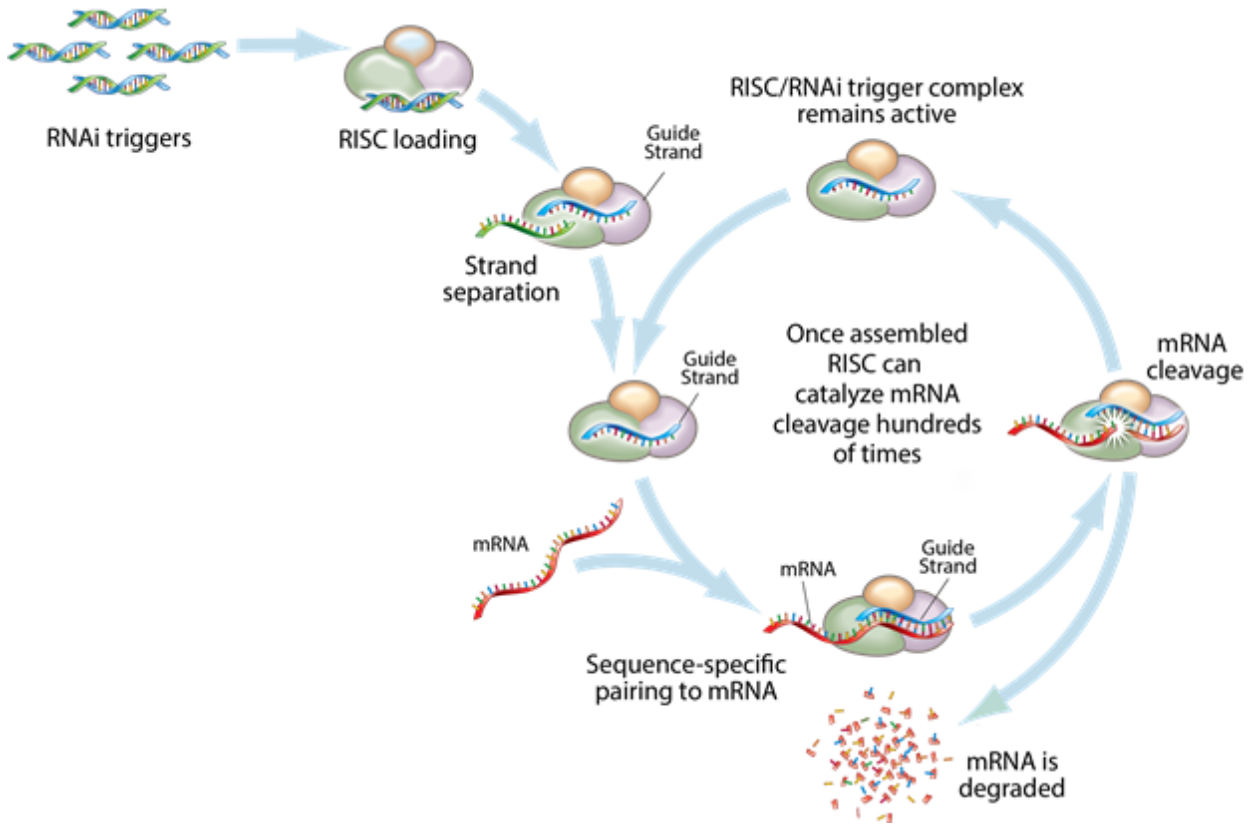
F12 inhibition is genetically validated

- F12-deficient mice:
  - viable and fertile[4]
  - do not show 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]



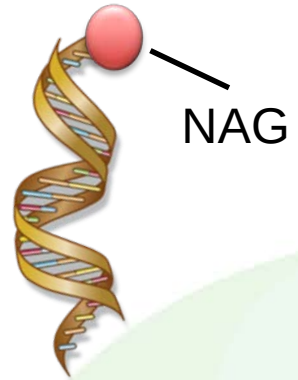
[1] Girolami A. *et al.* (2004) J. Thromb. Thrombolysis 17:139–143
[2] Koster A. *et al.* (1994) Br. J. Haematol. 87:422–424
[3] Zeerleder S. *et al.* (1999) Thromb. Haemost. 82:1240–1246
[4] Pauer, H. U., *et al*. (2004) *Thromb. Haemost*. 92:503
[5] Renne, T. *et al*. (2005) *J. Exp. Med*. 202:271
* Figure modified from Albert-Weissenberger, C., *et al*. (2014) *Front. Cell Neurosci*. 8:345

Gene silencing with RNA interference



ARO-F12 RNAi trigger

- Short dsRNA targeting *F12* mRNA
- 5’ NAG (Liver-tropic targeting ligand)
- Injected SC



ARO-F12 screening funnel

Bioinformatic selection of RNAi trigger sequences specific for F12 – filter to identify mouse/human/NHP cross-reactive triggers

Mouse/human/NHP RNAi trigger synthesis and *in vitro* testing

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

Structure Activity Relationship (SAR) on RNAi triggers for SC administration with *in vivo* testing

Proof of Concept for disease modification in animal models of thrombosis

Pre Clinical Candidate = ARO-F12

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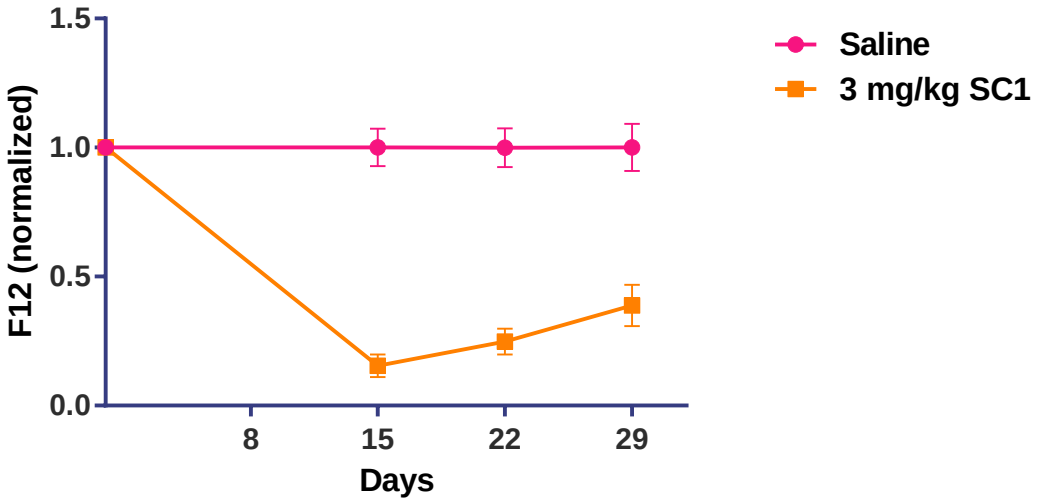
Examination of modified RNAi triggers in mice

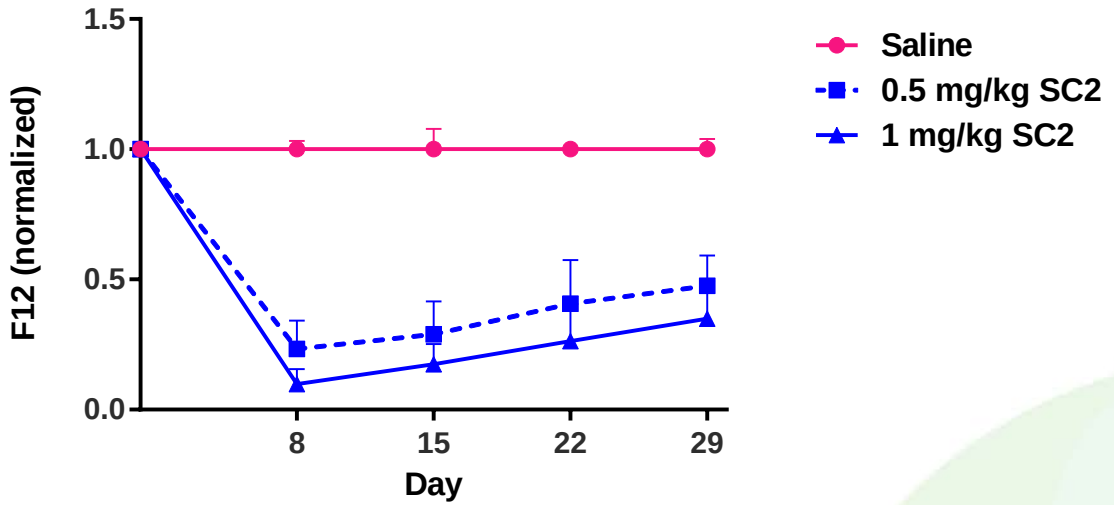
First Generation

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**
- **Dose response observed with SC2**

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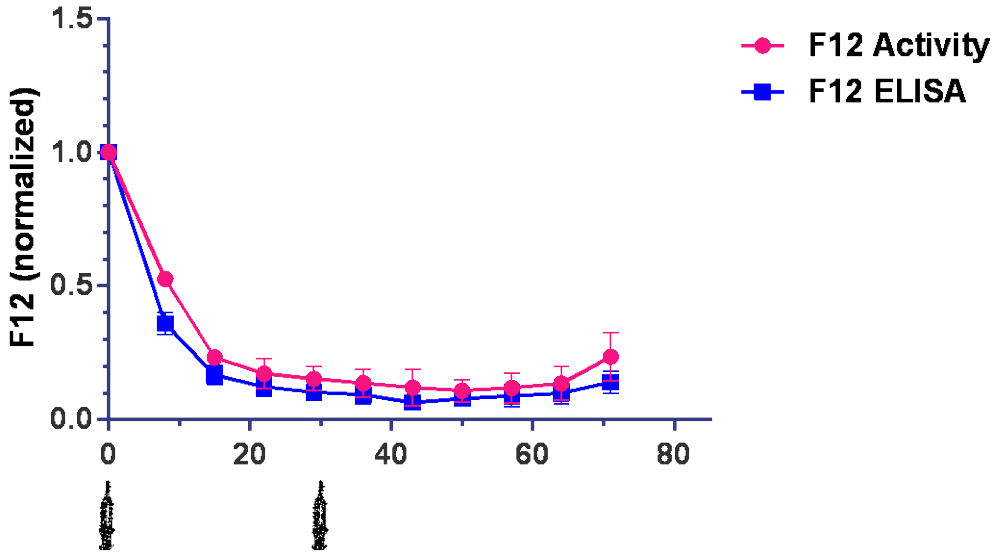
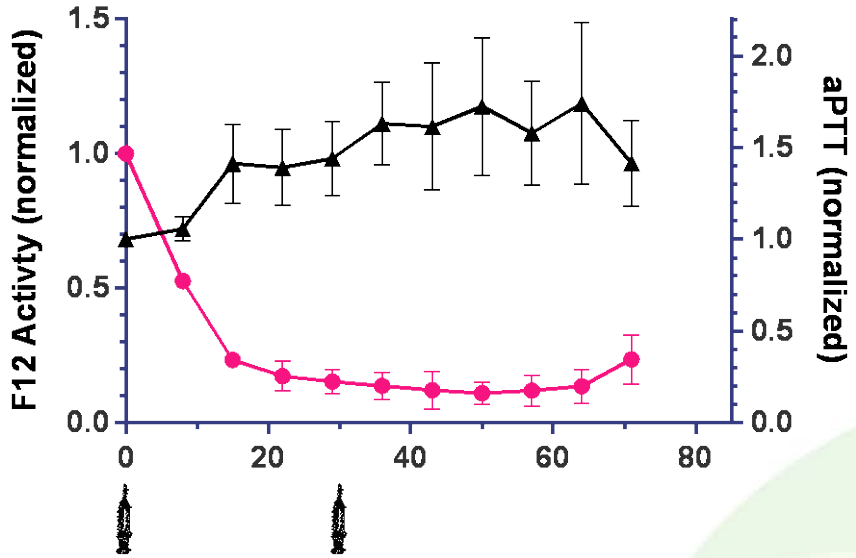
Second Generation Triggers – Examination in NHP

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

n=2/group





- **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**

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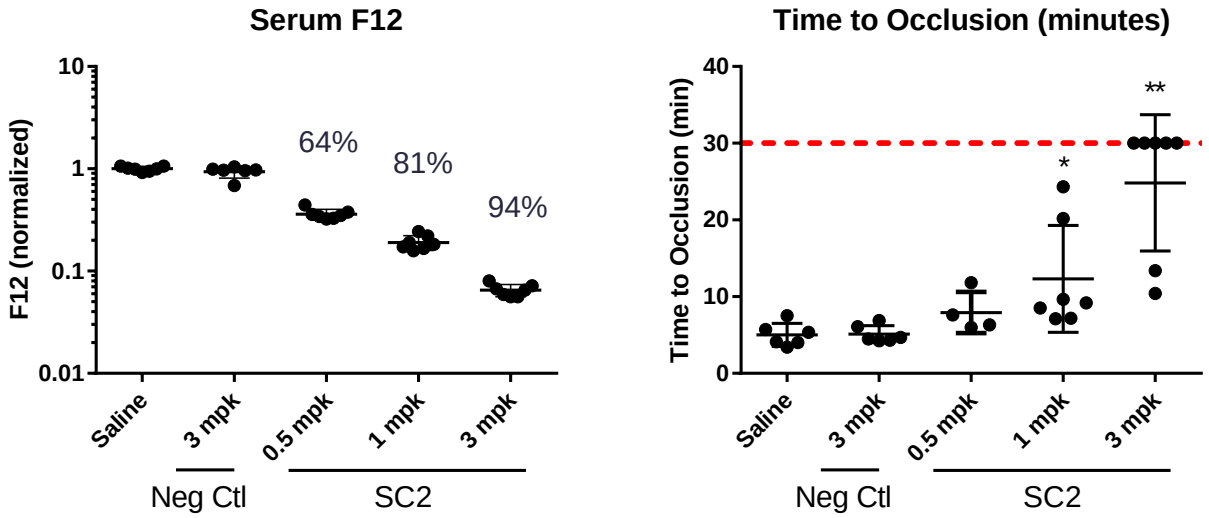
Ferric-chloride model is dose responsive

- *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*

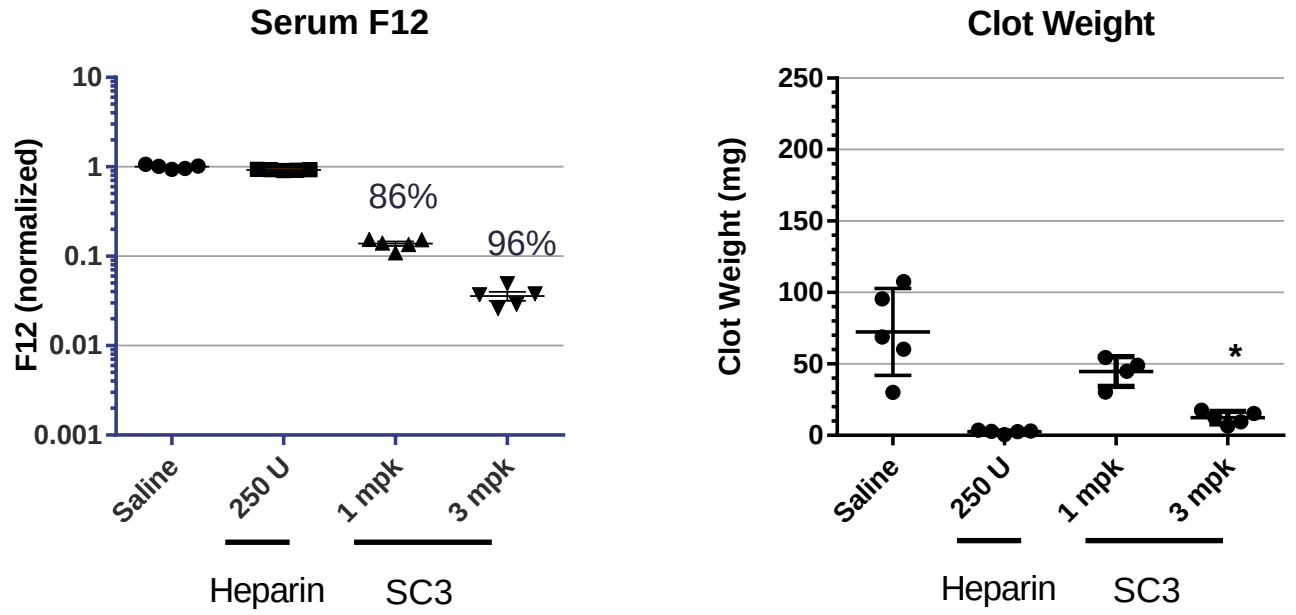


*p<0.02
**p<0.001

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

Rat arterio-venous shunt model is dose responsive

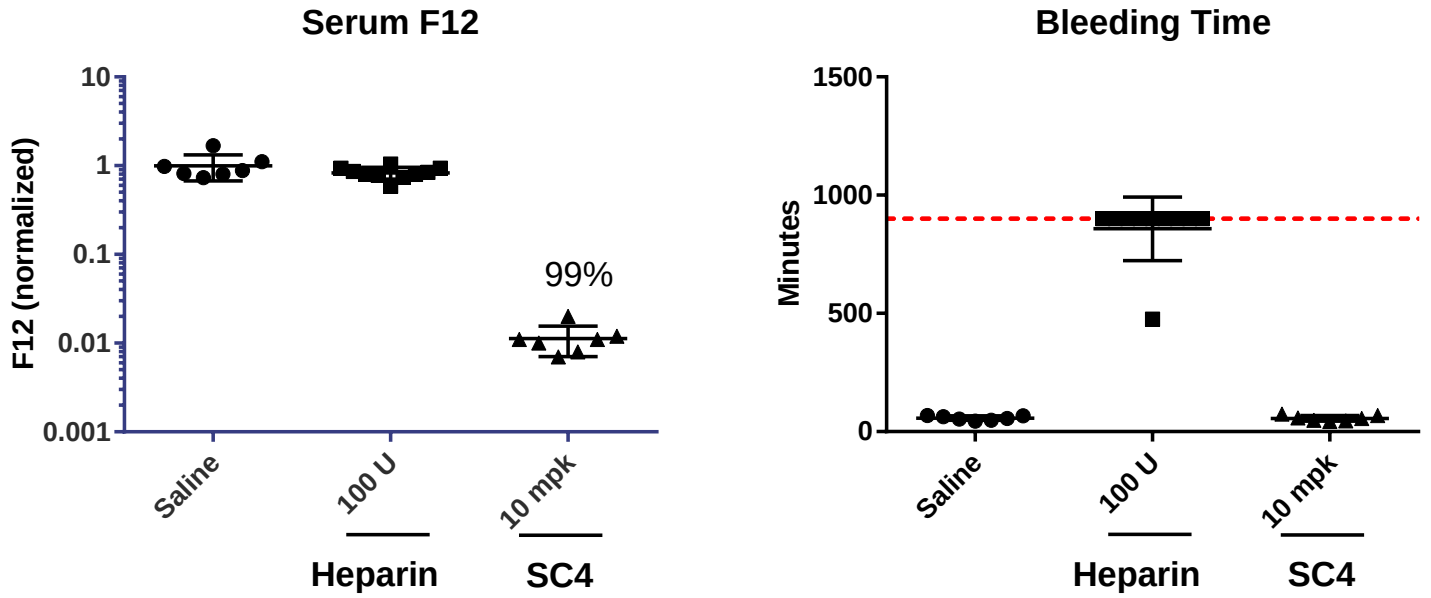
- *Measure thrombus weight by collection from Tygon tubing shunt*
- *Single dose of SC3, 14 days prior to assessment, n=5/group*



- **Dose response observed for serum F12 levels and thrombus weight**
- **Statistically significant reduction in thrombus weight at >95% F12 knockdown (*p=0.002)**

No increase in bleeding risk (mouse model)

- *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**

Conclusions

- **Arrowhead Pharmaceuticals is developing a RNAi-based approach targeting F12 as a prophylactic treatment of thromboembolism**
- **Human/NHP/rodent cross-reactive RNAi triggers exhibited ~90% knockdown of serum F12 levels with up to 5 weeks duration in both mice and NHPs**
- **Ferric chloride and arterio-venous shunt rodent models showed statistically significant reduction in thrombus formation with >95% knockdown of serum F12**
- **No prolonged bleeding in treated mice with >99% knockdown of serum F12**

Acknowledgements

- **Biology Department**
  - Steve Kanner
  - **Bioassays Team**
    - Holly Hamilton
    - Christine Chapman
    - Aaron Andersen
    - Qili Chu
  - **Laboratory Animal Resources**
    - Julia Hegge
    - Tracie Milarch

- **Chemistry Department**
  - Tao Pei
  - Zhen Li
  - **Oligo Synthesis Team**
    - Megan Walters
    - Collin Hagen
    - Casi Schienebeck