818

Targeting Factor 12 (F12) with a novel RNAi delivery platform as a prophylactic treatment for Hereditary Angioedema (HAE)

Stacey Melquist[1], Darren Wakefield[1], Holly Hamilton[1], Qili Chu[1], Aaron Almeida[1], Lauren Almeida[1], Megan Walters[1], Jessica Montez[1], Julia Hegge[1], Jason Klein[1], Christine Hazlett[1], Stephanie Bertin[1], Tracie Milarch[1], Edie Doss[1], Rachael Schmidt[1], Linda Goth[1], Sheryl Ferger[1], David Rozema[1], James Hamilton[2], David Lewis[1] and Steven Kanner[1]

[1]Arrowhead Research Corporation, Madison, WI 53711 [2]Arrowhead Research Corporation, Pasadena, CA 91101

Arrowhead Research CORPORATION

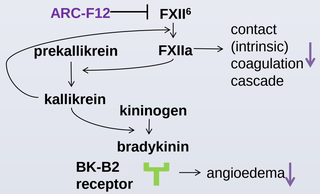
INTRODUCTION

Factor XII (F12)

- Key component of contact activation pathway (thrombosis) and kinin-kallekrein system (angioedema)
  - Cleavage of FXII by kallikrein generates FXIIa: FXIIa generates FXIa (coagulation) and kallikrein (angioedema)
  - Predominantly expressed in the liver; circulates in plasma

F12 inhibition is genetically validated

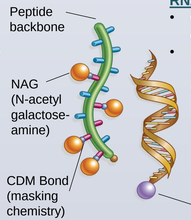
- F12-deficient mice:
  - viable and fertile[4]
  - do not show bleeding defects[4,5]
  - protected from thromboembolic disease (stroke, pulmonary embolism)[5]
- F12 deficiency in humans is *not* associated with either bleeding or thrombotic disorders[1,2,3]



Dynamic PolyConjugate (DPC™) for liver delivery

DPC™

- Amphipathic peptide for endosomal escape
- Peptide amines "masked" with pH-labile moiety, unmasked in endosome
- Targeted to liver with NAG
- Co-injected IV with RNAi trigger
- For ARC-F12, DPC™ is ARC-EX1



RNAi trigger

- Canonical siRNA or other format
- Liver-tropic targeting ligand (eg. cholesterol)

DPC™ and RNAi trigger do NOT form a complex, they are separately targeted to the liver

REFERENCES

[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
[6] Figure modified from Albert-Weissenberger, C. *et al.* (2014) Front. Cell Neurosci. 8:345
[7] Kenniston, J.A. *et al.* (2014) J. Biol. Chem. 289:23596–23608

ARC-F12 screening funnel

Bioinformatic selection of RNAi trigger sequences specific for F12 – filter to identify rodent/human/non human primate (NHP) cross-reactive triggers

↓

RNAi trigger synthesis and *in vitro* screening

↓

Synthesis of chol-RNAi triggers and *in vivo* screening in WT mice

↓

SAR on lead candidate chol-RNAi triggers: synthesis of modified chol-RNAi triggers and *in vivo* screening

***In vivo* screening in NHP** | **Efficacy testing in disease-relevant animal models** | **Non-GLP toxicology**

↓

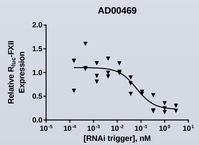
Lead candidate

RESULTS

Two-point *in vitro* screen of *F12* RNAi triggers

- **Huh7 cells transfected with RNAi triggers at 1 nM or 0.1 nM**
- **Data expressed as *Renilla*/firefly luciferase ratio**

Summary of EC_{50} values (*in vitro* screening)



RNAi Trigger ID	EC_{50} (pM)
AD00466	18.6
AD00469	69.5
AD00468	81.5
AD00467	133.4
AD00470	145.0
AD00474	261.0

ACKNOWLEDGEMENTS

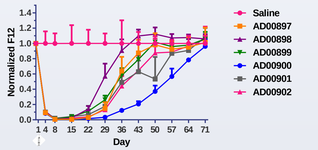
The authors would like to thank Aaron Andersen, Crystal Klas, Molly Zeller, Leah Staley, Felicia Beauprey and Christina Furseth for technical assistance.

This publication was made possible in part by Grant Number P51 RR000167 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), to the Wisconsin National Primate Research Center, University of Wisconsin-Madison. This research was conducted in part at a facility constructed with support from Research Facilities Improvement Program grant numbers RR15459-01 and RR02141-01. This publication's contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.

RESULTS

Initial F12 chol-RNAi trigger *in vivo* screen

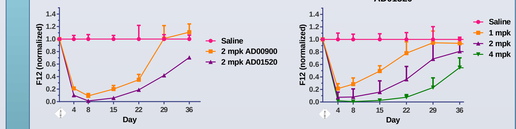
- Serum F12 levels measured by ELISA, values were normalized to pre-treatment and saline control.
- Mice were dosed at 8 mg/kg (mpk) F12 RNAi trigger/ARC-EX1, single dose, IV injection



- **Single high dose of chol-RNAi triggers showed significant and sustained knockdown of F12 levels**
- **AD00900 (AD00469 family) exhibited >1 log_{10} knockdown of F12 for >1 month**

Iteration of RNAi trigger modification

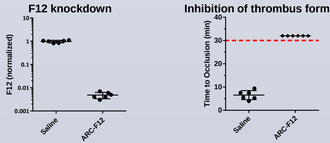
- *F12 RNAi trigger dosed 1:1 with ARC-EX1, single dose, IV injection*



- **Dose-dependent F12 knockdown**
- **4 mpk dose showed >1 log_{10} knockdown at nadir (Day 15), with 80% knockdown for 1 month**
- **ARC-F12 = AD01520 + ARC-EX1 dosed at 1:1 ratio**

$FeCl_3$ thromboembolism mouse model

- Induced by exposure of carotid artery to ferric chloride
- Measure time to blood flow occlusion, thrombi
- Clinically relevant indicator of physiological response to F12 knockdown
- Treatment with 4 mpk ARC-F12 seven days prior to challenge

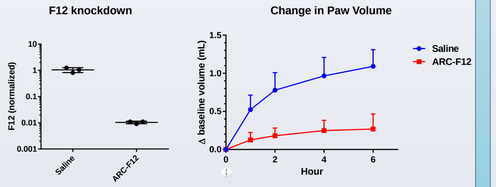


- **Dramatic increase in occlusion times observed in mice receiving ARC-F12 is similar to results obtained in FXII$^{-/-}$ mice[5]**

RESULTS

Carrageenan-induced paw edema in rats

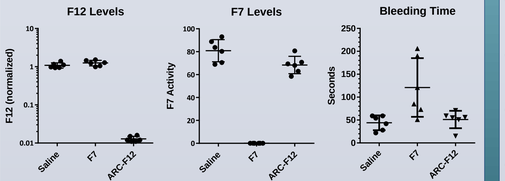
- Inflammation caused by subcutaneous injection of λ carrageenan
- Induced by multiple mediators of inflammation/edema including pro-inflammatory cytokines, histamine, prostaglandins, and others in addition to bradykinin
- Treatment with 8 mpk ARC-F12 seven days prior to carrageenan challenge



- **ARC-F12 treatment significantly reduced edema from carrageenan challenge (p<0.001)**
- **Reduction in swelling in ARC-F12 treated rats is similar to those seen in rats treated with kallikrein-targeted antibody[7]**

Bleeding risk assessment in mouse model

- Transverse cut of tail vein, monitor time to clotting
- Factor 7 (F7) is a key clotting factor in extrinsic pathway, and a F7-targeted RNAi trigger is used as a positive control in this study
- 8 mpk ARC-F12 or 8 mpk F7 trigger + 8 mpk ARC-EX1, single IV dose seven days prior to challenge

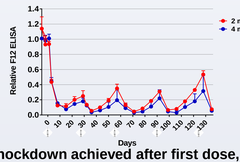


- **Bleeding times do not differ between saline and ARC-F12 treated mice**
- **Lack of bleeding observed in ARC-F12 treated mice is similar to results obtained in FXII$^{-/-}$ mice[5]**

RESULTS

Evaluating ARC-F12 in multi-dose NHP studies

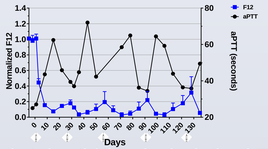
- *2 or 4 mpk ARC-F12, 5xq4w dosing, IV injection*



- **~90% F12 knockdown achieved after first dose, even greater knockdown following subsequent doses**
- **>80% inhibition of F12 levels maintained between dosing**
- **No changes in toxicity markers (clin chem, CBC) after dosing**

Pharmacodynamic effect of F12 knockdown in NHP

- *4 mpk ARC-F12, 5xq4w dosing, IV injection*



- **Increased activated partial thromboplastin time (aPTT) in ARC-F12-treated Cynomolgus monkeys is observed in parallel with decreased serum F12 levels**
- **No changes in Prothrombin Time (PT)**
- **aPTT is elongated in individuals with F12 deficiency**

CONCLUSIONS

- **Screening of in vitro-active F12 RNAi triggers in wild type mice identified those triggers that exhibited significant and sustained knockdown of serum F12 levels**
- **SAR studies allowed identification of a lead RNAi trigger that demonstrated >97% maximum knockdown after a single 2 mg/kg dose**
- **Studies in mice showed reduced $FeCl_3$-induced thromboembolism consistent with the expected physiological effects of F12 knockdown**
- **High dose ARC-F12 treatment showed no evidence of increased bleeding in mice**
- **Carrageenan-induced paw edema is reduced in ARC-F12 treated rats**
- **A multi-dose study in NHPs showed >90% sustained knockdown of serum F12 levels without toxicity for monthly doses up to 4 mg/kg ARC-EX1**
- **NHPs in these studies showed changes in aPTT, a physiological response consistent with F12 deficiency**