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Using DPC Technology in RNAi Therapeutics for Chronic HBV Infection and Factor 12-Mediated Diseases

17th Annual TIDES
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CSO – Arrowhead Research Corporation

DPC technology for delivery of RNAi triggers

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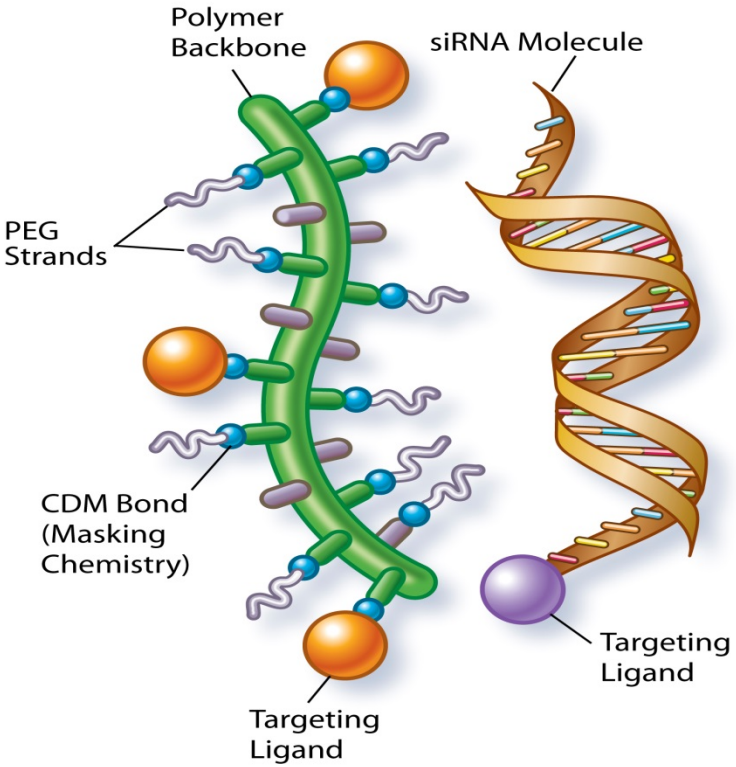
DPC (Dynamic Polyconjugate)

- Amphipathic polymer (or peptide) that promotes endosomal escape of RNAi trigger
- Polymeric amines "masked" with pH-labile moiety, unmasked in endosome
- Slightly negatively charged
- Targetable
- Co-injected with RNAi trigger



RNAi Trigger

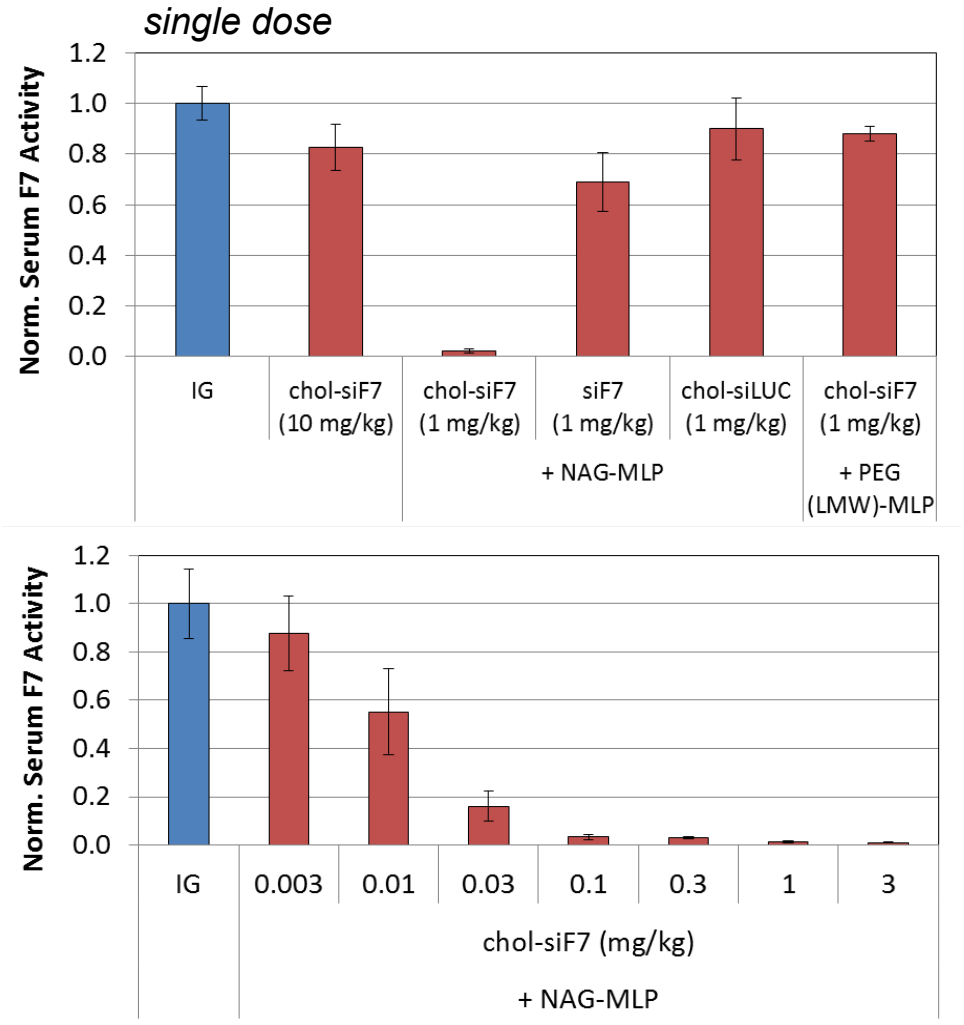
- Canonical siRNA or other format
- Targeting ligand (eg. cholesterol, GalNAc) attached to sense strand

DPC and RNAi trigger do *NOT* form a complex, they are separately targeted to the tissue of interest

Using DPC (NAG-MLP) for delivery of chol-siRNA to liver in mice

Target: Coagulation Factor 7

MLP: melittin-like peptide for endosomal escape
NAG: N-acetyl-galactosamine targeting ligand



Target gene knockdown requires:
Liver-tropic siRNA (cholesterol-siRNA)
and hepatocyte-targeted DPC peptide (NAG-MLP)

Co-injection of NAG-MLP enables highly efficient chol-siRNA delivery

– ED_{80} = 0.03 mg/kg chol-siF7

Mice, 48 hr timepoint
Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

ARC-520

Treatment of chronic hepatitis B virus infection

Chronic hepatitis B virus infection: the need for better therapeutics

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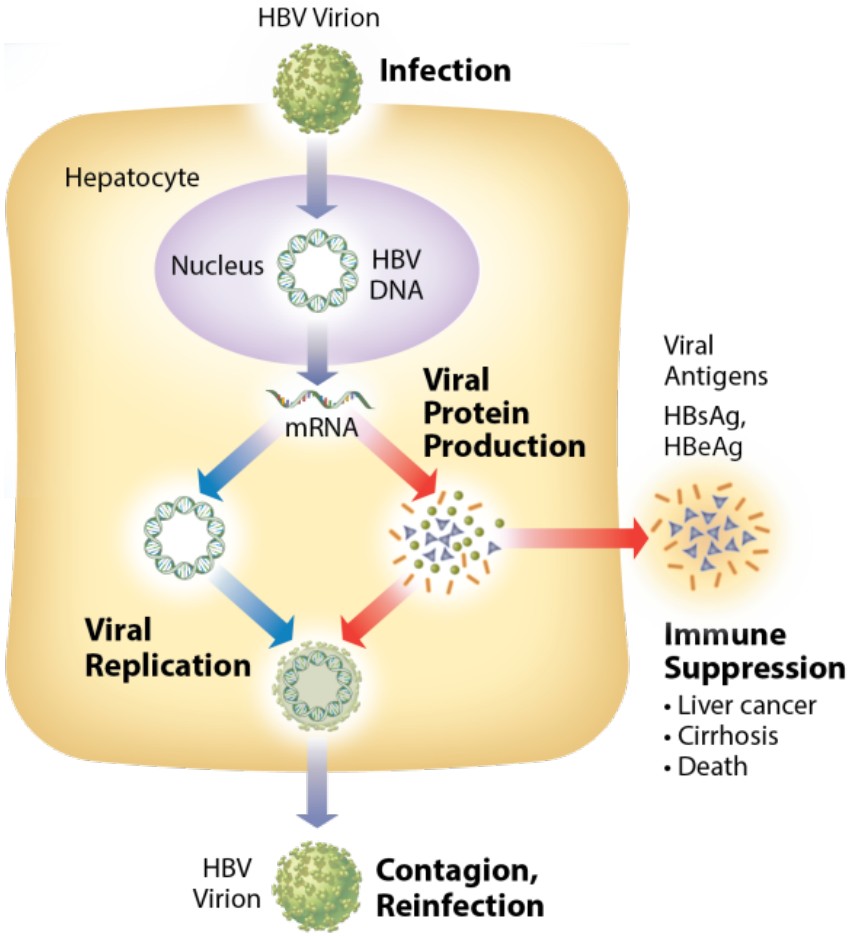
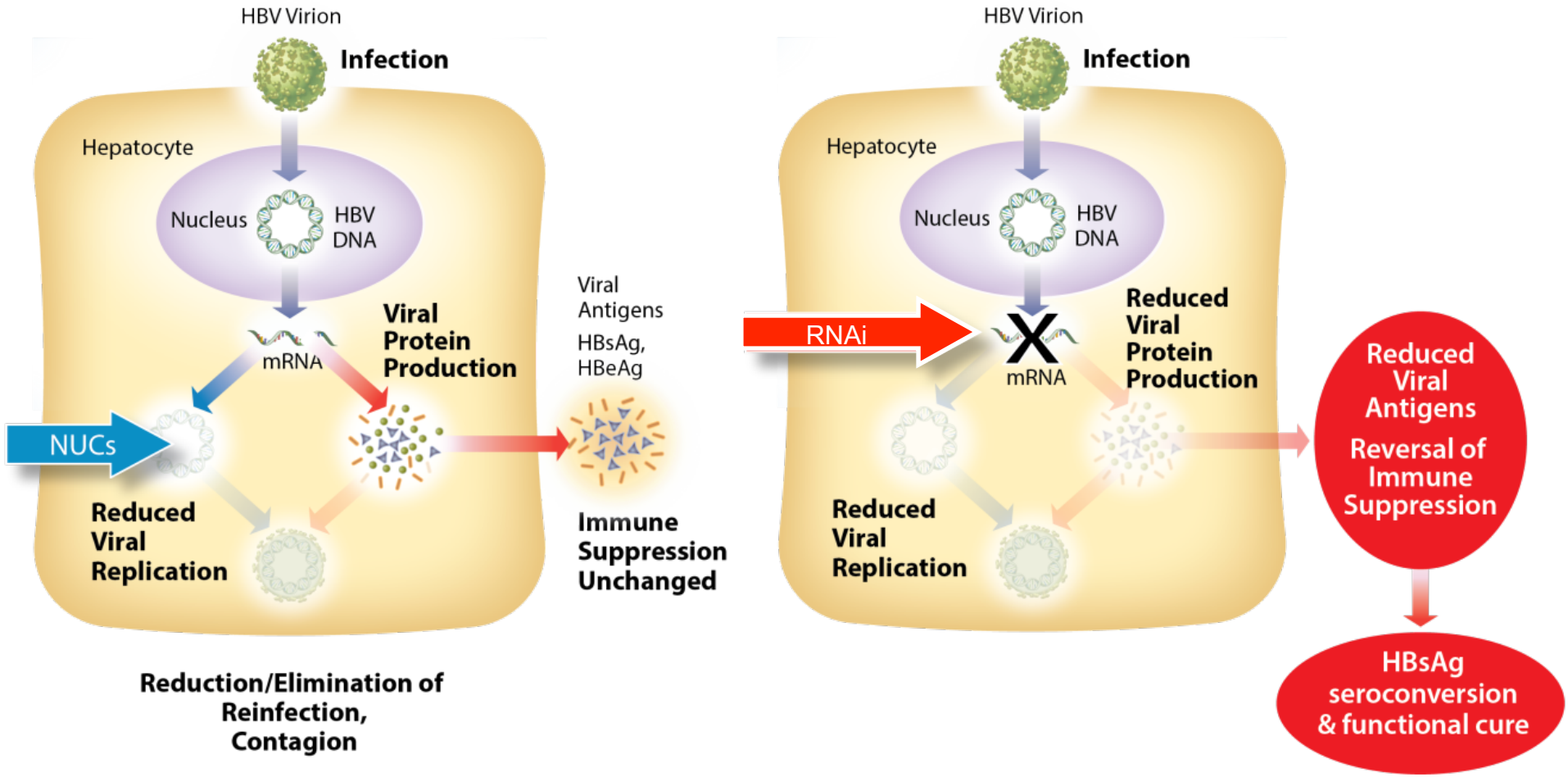
- ~340M people chronically infected worldwide
  - Lifetime risk of developing cirrhosis, liver failure or HCC is 15-40%, leading to ~0.5-1M deaths annually
  - Complex interplay between immune system during chronic infection with levels of viral proteins playing an integral role in immune inhibition ("T-cell exhaustion")
  - Seroclearance of surface antigen (HBsAg) ≈ functional cure (rare)
- Existing drugs (reverse transcriptase inhibitors, PEG-Interferon) are unsatisfactory
  - RT inhibitors "NUCs" (eg. tenofovir, entecavir, lamivudine)
    - Dramatically decreases viral titer and can improve patient outcomes
    - Only marginally decreases HBsAg levels and does not result in significant HBsAg seroclearance → life-long treatment required
  - PEG-Interferon (PEGASYS)
    - 48 week course
    - Can result in HBsAg seroclearance, but only 3-5% /yr (natural conversion rate is ~ 0.5%)
    - Significant side effects (flu-like symptoms, depression)

HBV infection cycle and suppression of the immune response



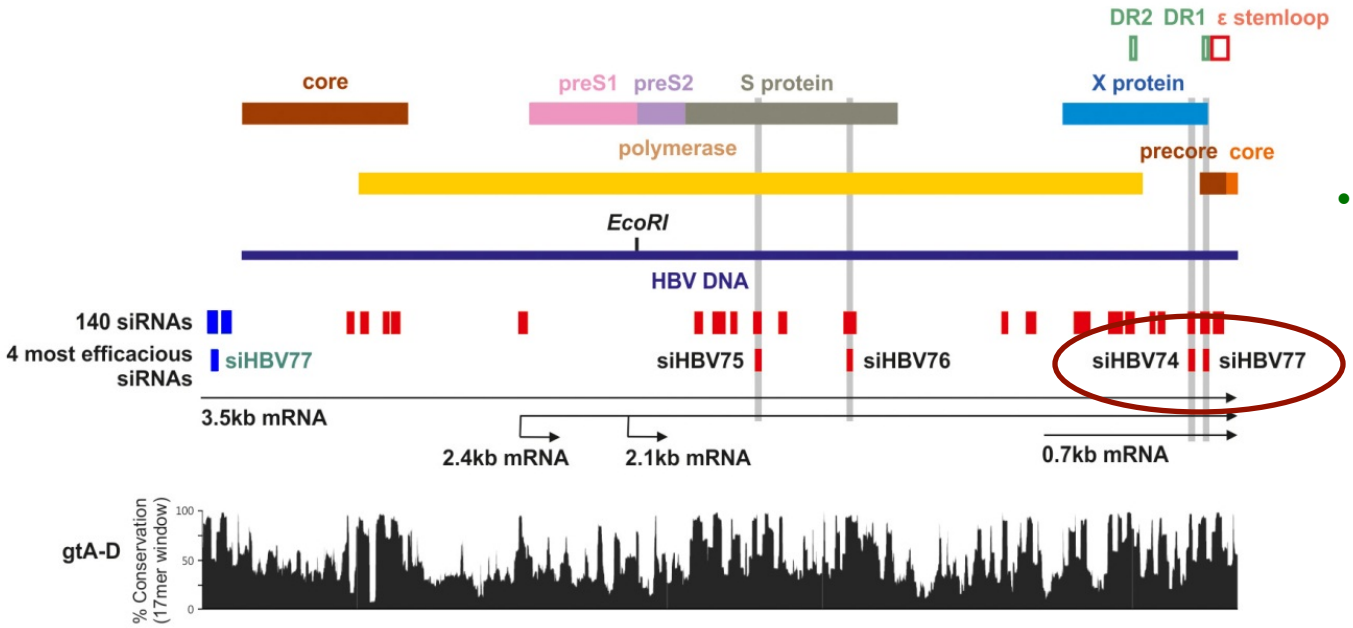
Sustained, high levels of viral protein production leads to immune suppression and chronicity.

RNAi therapeutics vs. reverse transcriptase inhibitors (NUCs) for treatment of chronic HBV

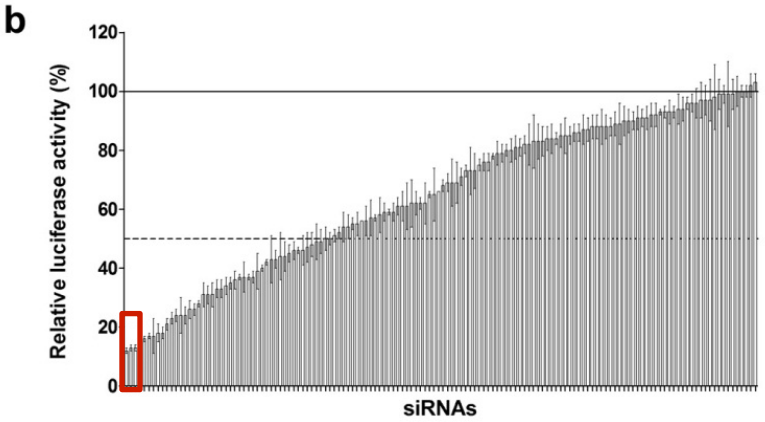


RNAi for treatment of chronic Hepatitis B

siRNA design and in vitro screening



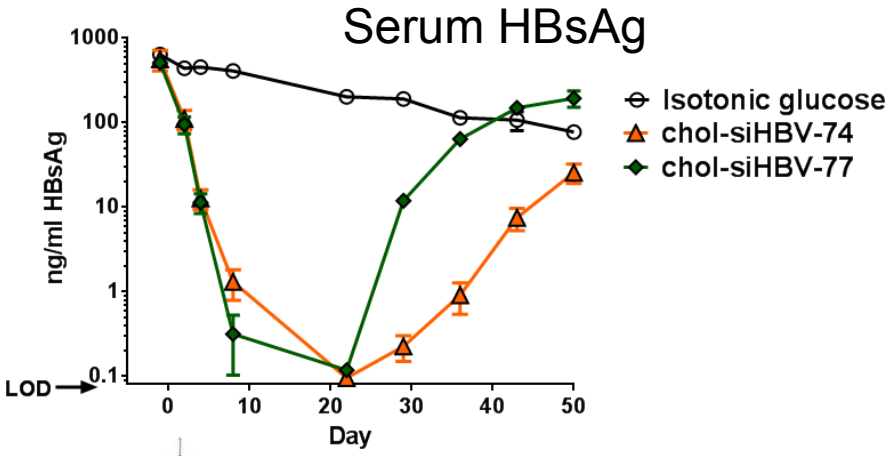
- Designed 140 siRNAs targeting conserved regions in GenBank HBV sequences (2,754)

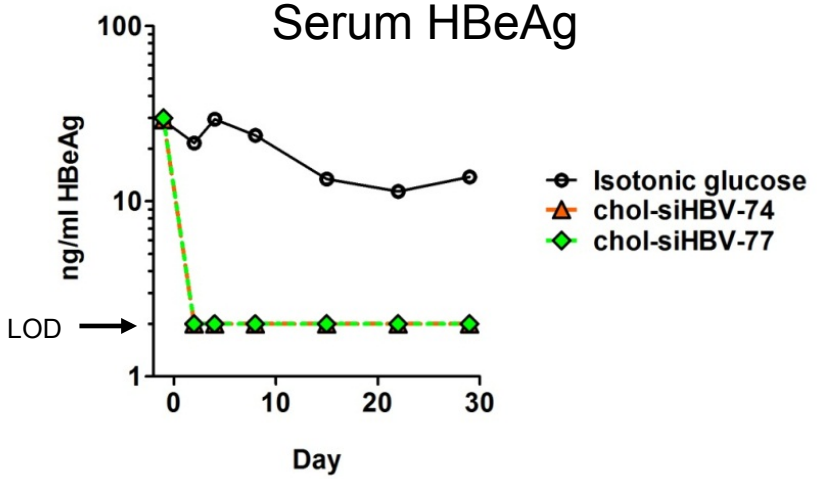


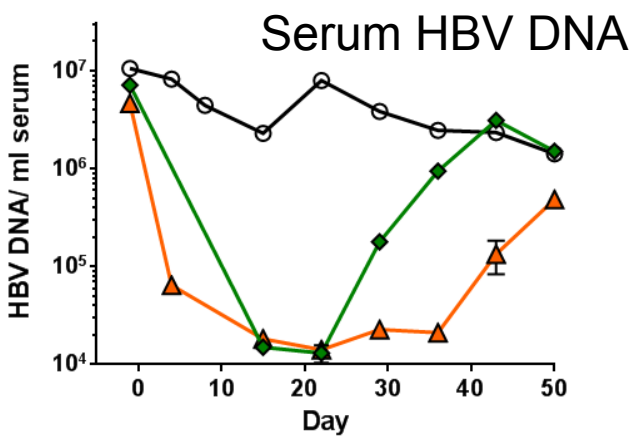
- Screened candidate siRNAs in a cell culture system
- 4 highly potent siRNAs chosen for further testing in animal models
- siHBV-74 and siHBV-77 chosen as leads

Roche-Kulmbach (Axolabs GmbH)

Co-injection of chol-siHBVs with NAG-MLP in HBV mouse model







Strong reduction of serum viral markers using either chol-siHBV-74 or -77 with NAG-MLP after a single dose

Decreased HBsAg

- 3-4 log reduction with both chol-siHBVs
- > 2 log reduction for 1 month

Decreased HBeAg to LOD

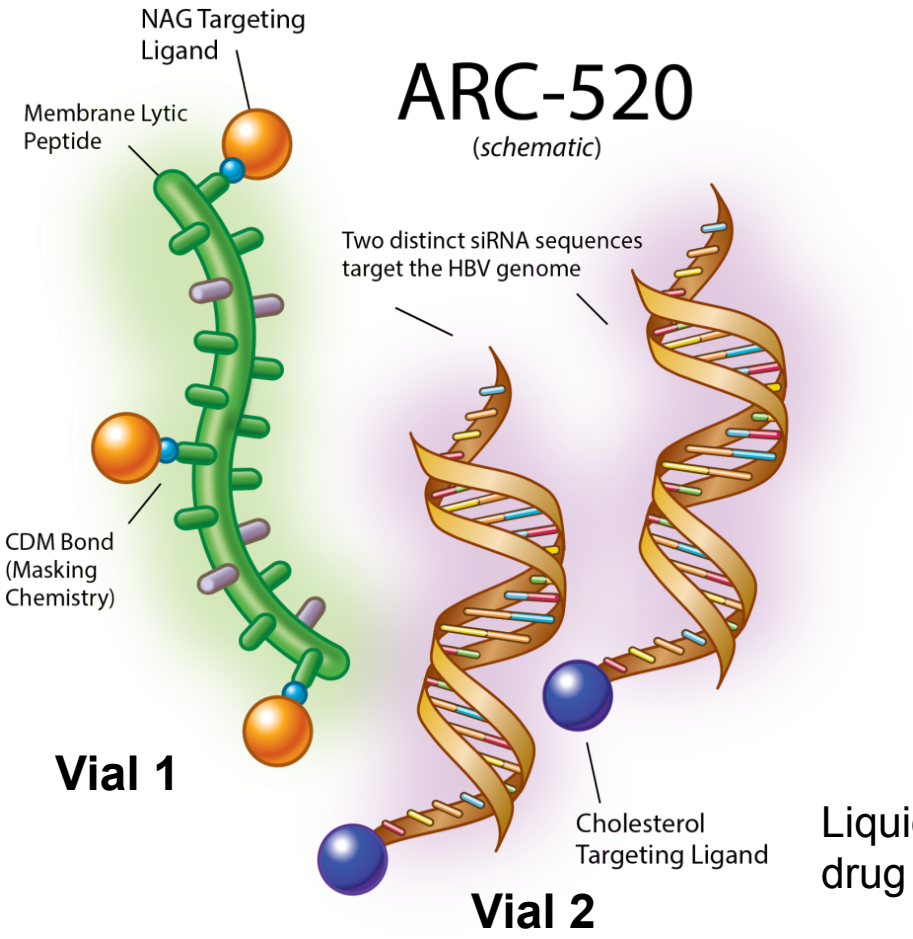
Decreased HBV DNA

- ~ 3 log reduction of HBV DNA for ~ 1 month

6 mg/kg NAG-MLP + 6 mg/kg chol-siRNA

Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

ARC-520 for chronic HBV infection



- **Vial 1: ARC-520 Excipient (ARC-EX1)**
  - Hepatocyte-targeted DPC peptide (NAG-MLP)
  - Lyophilized powder, stable
- **Vial 2: ARC-520 API**
  - Mixture of chol-siHBV-74 and -77 in solution
  - Inclusion of two siRNAs gives broader genotype coverage (>99%)

Liquid in Vial 2 is used to dissolve contents of Vial 1, drug administered IV

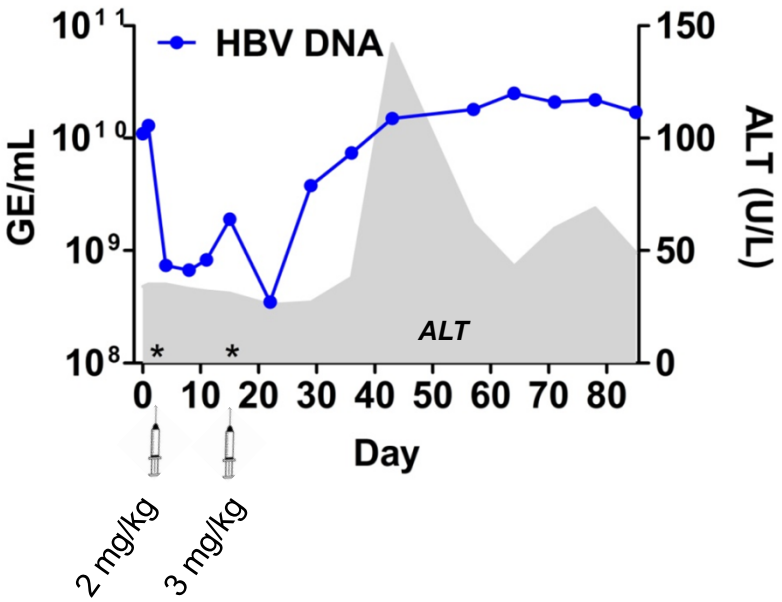
Testing ARC-520 in a chimpanzee chronically infected with HBV

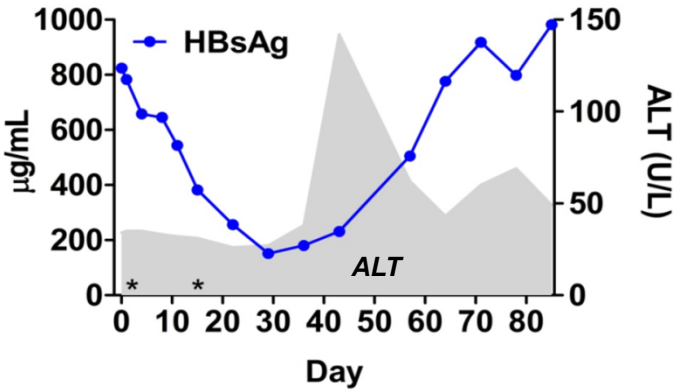
- Key historical attributes
  - 36 year old female, weight 113 pounds
  - Chronic HBV since 1979
  - Liver biopsy shows near 100% staining for HBV
  - Exceptionally high titers of circulating DNA (10^{10} vs. 10^{7} in average patient) and HBsAg
  - Genotype B with mismatch for one of the two siRNAs in ARC-520
    - Only 8% of genotype B sequences in GenBank are mismatches for this siHBV

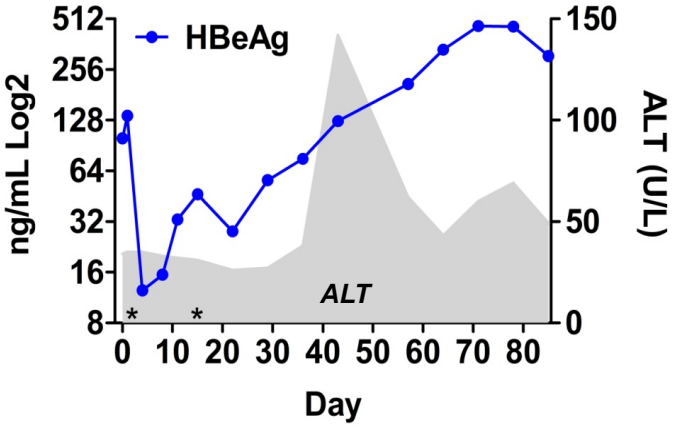
Reduction in HBV after treatment with ARC-520

Elevated liver enzymes observed 4 weeks post-last dose

chimpanzee







- Strong reduction in HBV DNA, HBsAg, HBeAg
- An increase in ALT was observed near the HBsAg nadir, 3 weeks after ARC-520 injection

  → T-cell reactivation and attack of infected hepatocytes?

ARC-520: Phase IIa clinical trial

- Trial design
  - Chronic HBV patients (HBeAg-neg) on the RTI Baraclude (entecavir)
  - Randomized, double-blind, placebo controlled, single IV dose escalation with over the counter oral antihistamine prior to receiving ARC-520
  - Dose levels: 1, 2, 3, 4 mg/kg
  - 8 patients/cohort (2 placebo, 6 ARC-520)
  - Primary objectives: safety in patients; evaluate depth and duration of HBsAg decline
- Interim results
  - 3 and 4 mg/kg cohorts still blinded and final analysis not yet available
  - No clinically significant, treatment emergent changes in measurements of organ toxicity, vital signs, physical exam or ECG. All AEs reported to date (n=4) have been mild or moderate and rated as unrelated to study drug by the investigator
  - HBsAg KD observed: 1 mg/kg mean at nadir = -39% (range -22 to -57)
    2 mg/kg mean at nadir = -51% (range -46 to -59)
- Phase IIb US multi-dose trial expected to start Q2 2015

ARC-F12

Inhibition of coagulation factor 12 for thromboembolic and angioedemic diseases

F12 is an attractive target for RNAi therapeutics

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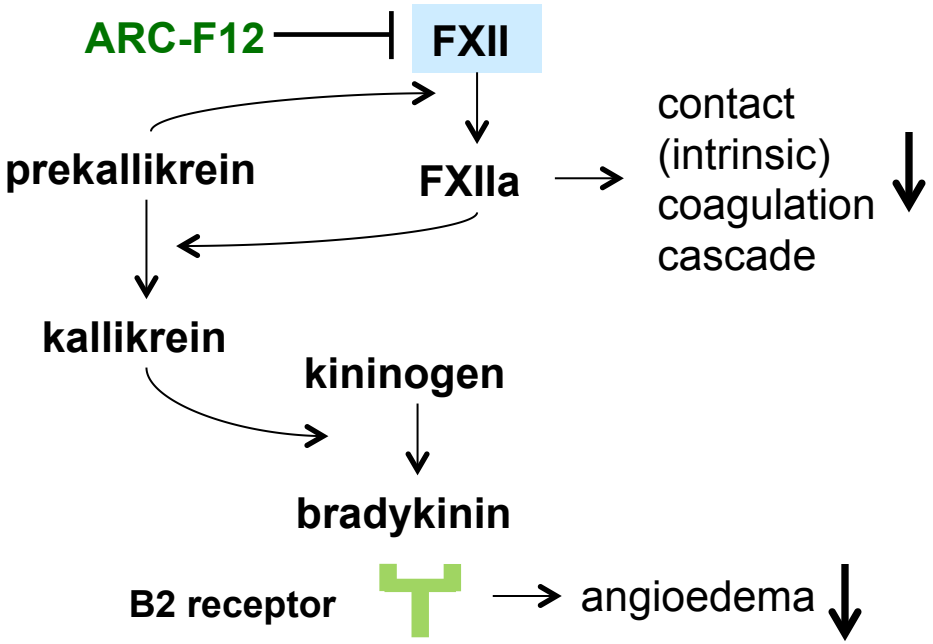
Coagulation Factor XII (F12)

- Key component of contact activation pathway (thrombosis) and kinin-kallekrein system (angioedema)
  - Cleavage of FXII by prekallikrein 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]



F12 represents an attractive target for addressing unmet medical needs in thromboembolic and angioedemic diseases

[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

ARC-F12 vs. ARC-520

Same DPC (ARC-EX1), different RNAi trigger

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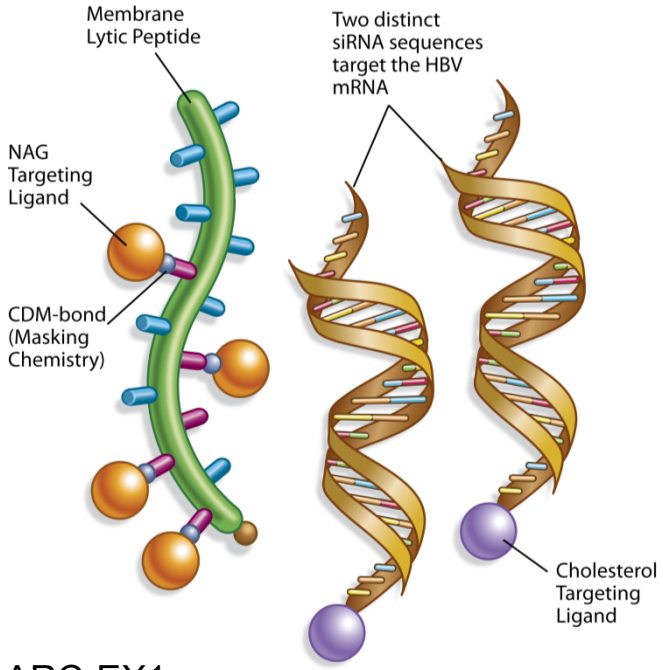
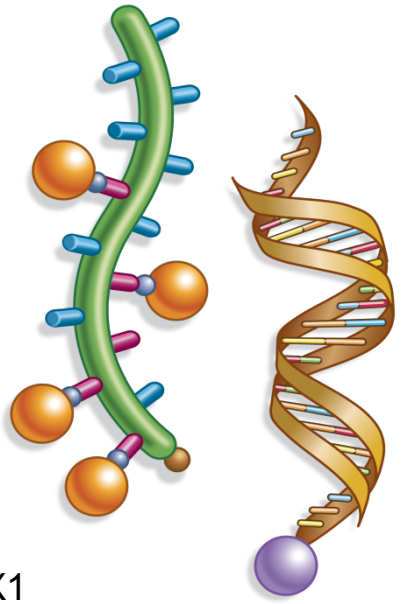
ARC-520 for chronic HBV infection



ARC-EX1

HBV RNAi Triggers

ARC-F12



ARC-EX1

F12 RNAi Trigger

Able to consolidate ARC-EX1 manufacturing and leverage learnings from clinical experience with ARC-520

F12 program screening funnel

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

↓

RNAi trigger synthesis and *in vitro* screening – identify 6 most potent

↓

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

↓

SAR on lead candidate chol-RNAi triggers with *in vivo* screening

In vivo screening of potential leads (NHP)

Efficacy testing in disease-relevant animal models

DRF tox (non-GLP)

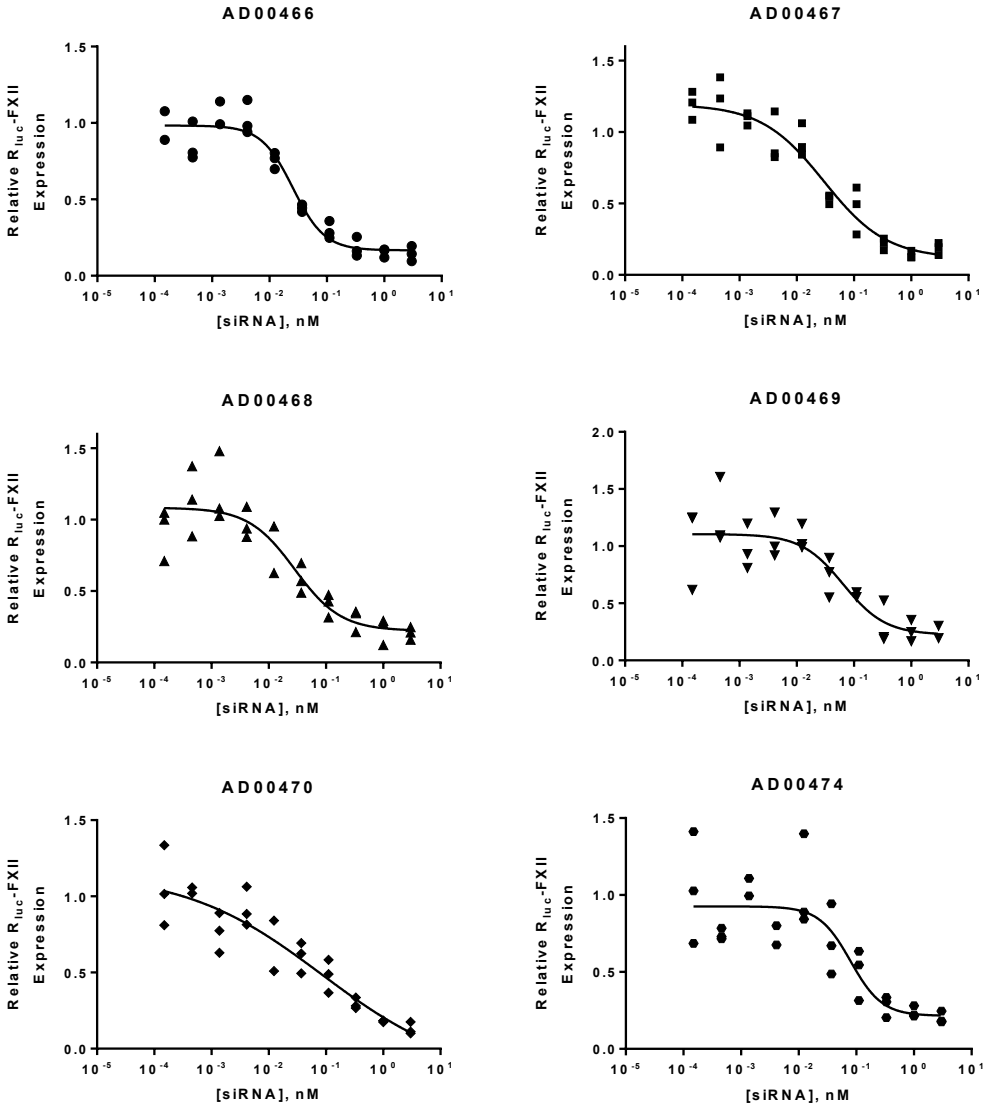
Determine dose, ratio and schedule

↓

Lead candidate

Top six F12 canonical RNAi triggers from round 1:

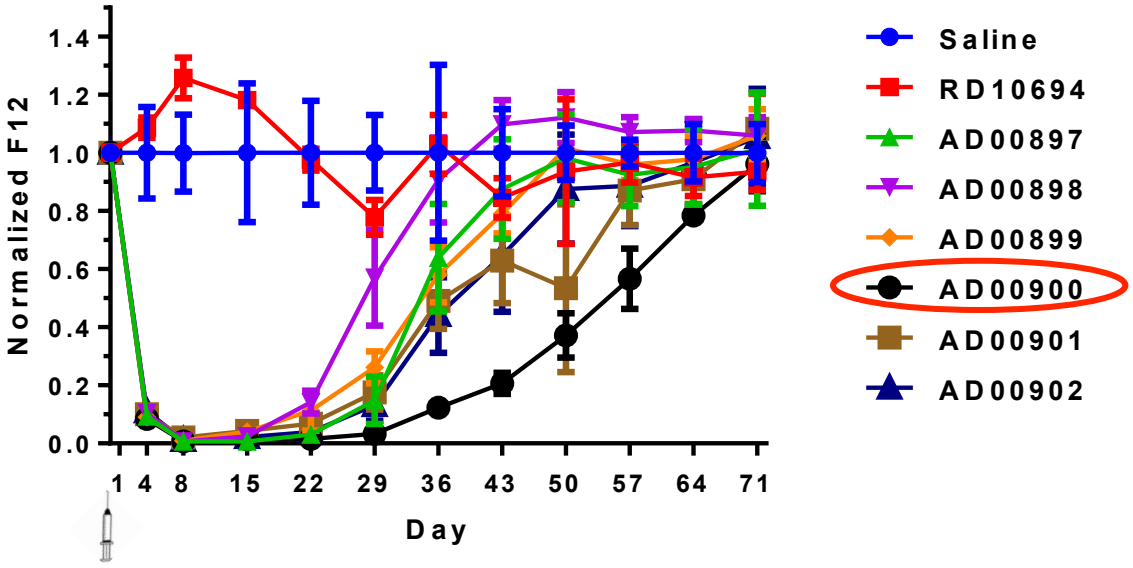
Summary of EC_{50} values (in vitro screening)



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

Initial F12 chol-RNAi trigger *in vivo* screen in mice

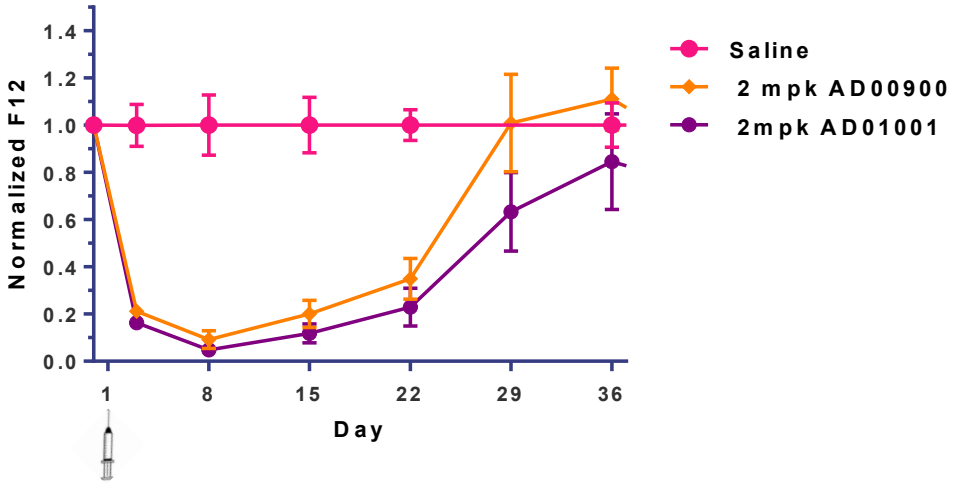
- ***8 mpk F12 RNAi trigger/ARC-EX1, single dose, IV injection***

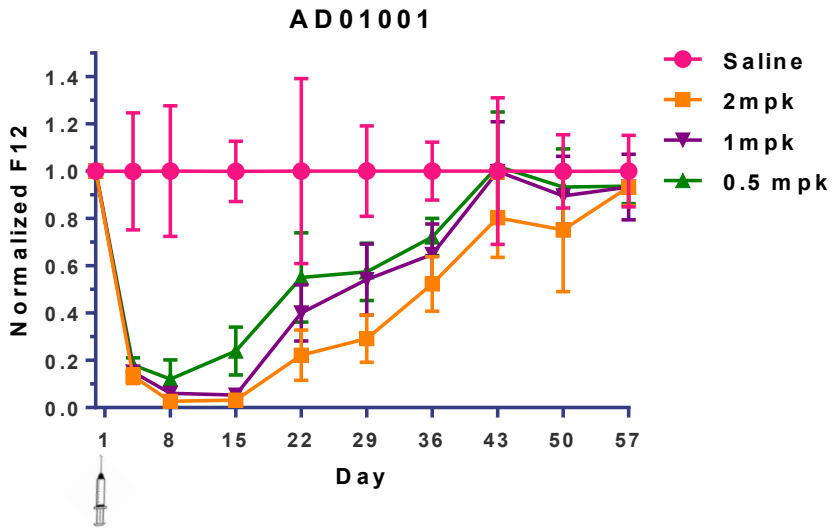


- Single high dose of canonical chol-RNAi triggers showed significant and sustained knockdown of F12 levels (>99% for most triggers at nadir)
- AD00900 (AD00469 family) exhibited >90% knockdown of F12 for 1 month

Modification of AD00900 and dose response

- ***F12 RNAi trigger dosed with 2 mpk ARC-EX1, single dose, IV injection***

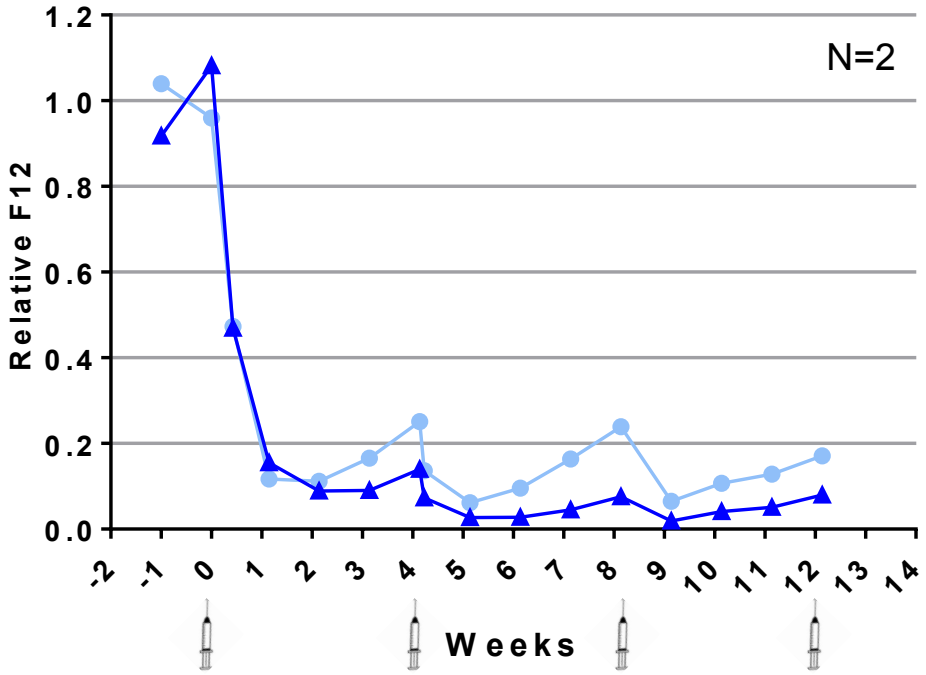




- Strategic incorporation of modifications in AD00900 RNAi trigger increases depth and duration of F12 knockdown
- Dose-dependent F12 knockdown, with >80% KD after a single dose of 0.5 mpk
- 2 mpk dose results in >95% knockdown at nadir (Day 15), with >70% knockdown for 1 month

Evaluating the F12 lead candidate in NHPs

- ***2 mpk AD01001/ARC-EX1, q4w dosing, IV injection***



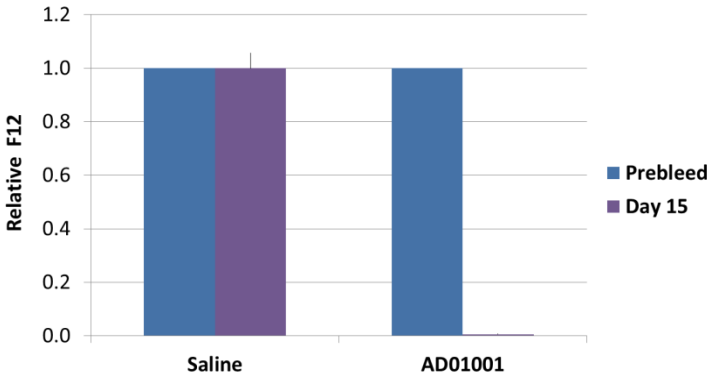
- ~90% F12 KD achieved after first dose, even greater KD following subsequent doses
- >80% F12 KD maintained between monthly dosing
- No changes in toxicity markers (clin chem, CBC) after dosing

Thromboembolism mouse model

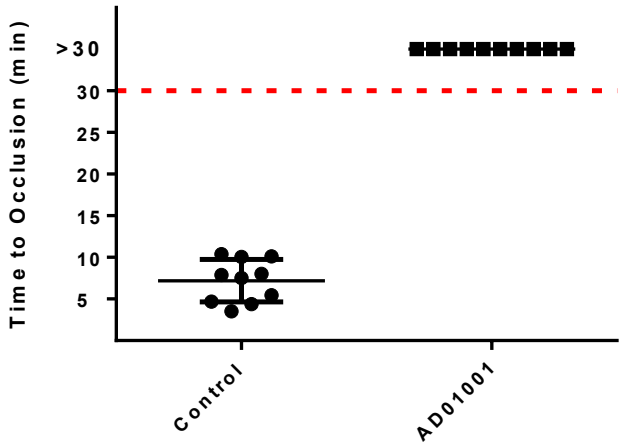
- *Induced by exposure of carotid artery to ferric chloride*
- *Measure time to blood flow occlusion*
- *Clinically relevant indicator of physiological response to F12 knockdown*

8 mpk AD01001/ARC-EX1, single dose, IV injection

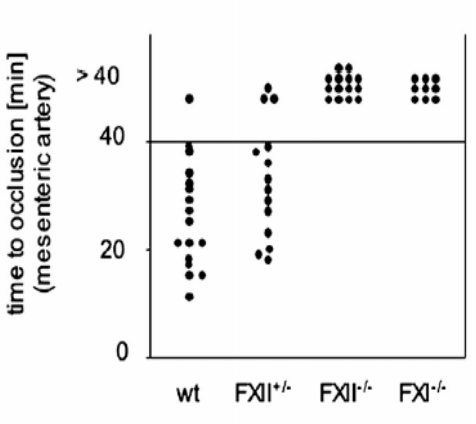
F12 knockdown



Inhibition of thrombus formation



FXII$^{-/-}$ mouse



- Animals treated with saline or AD01001/ARC-EX1 15 days prior to ferric chloride challenge
- Treated animals showed ~99% knockdown in serum F12 levels at Day 15
- Dramatic increase in occlusion times observed in mice receiving AD01001/ARC-EX1. Results are similar to those published using FXII$^{-/-}$ mice.

FXII$^{-/-}$ data in right panel from: Renne, T. *et al*. (2005) *J. Exp. Med*. 202:271

First F12 target indication: Hereditary Angioedema

- **Hereditary Angioedema (HAE)**
  - Orphan disease (prevalence 1/5,000-1/10,000)
  - Most commonly caused by mutations in the complement factor 1 esterase inhibitor (C1INH) gene
  - Attacks induced by trauma, infections, fever, etc.
  - multiple tissues affected, laryngeal edema can be fatal
- **Available prophylactic treatment**
  - Cinryze® (Shire, pdC1INH)
  - Injected IV before trigger events (eg. dental work, etc)
  - Goal of long-term prophylaxis is to reduce frequency, severity and duration of HAE attacks
    - Typically given regularly for weeks to years in an effort to minimize the overall attack burden
- **New therapeutic options are needed**
  - Many patients do not respond adequately to replacement therapies
  - Frequent dosing req'd for prophylaxis (1-3x per week, IV)

Opportunity for F12 targeting

- **Novel mechanism for long-term prophylaxis**
- **IV dosing every 4-6 weeks would be seen as significant advance**

ARC-F12 - conclusions

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- At single high dose of RNAi trigger with ARC-EX1 in wild type mice:
  - Multiple RNAi triggers exhibited >99% knockdown at nadir
  - Top RNAi trigger exhibited >90% of knockdown for 1 month
- Application of modifications to top RNAi trigger resulted in increased depth and duration of KD
- Lead candidate F12 RNAi trigger was highly active in NHP
  - q4w dosing showed ~90% KD after first dose, >90% knockdown after subsequent doses
  - >80% inhibition of F12 levels maintained between monthly dosing
  - No drug-related changes in toxicity markers (clin chem, CBC)
- Single administration of lead candidate F12 RNAi trigger prevented thrombus formation after challenge with ferric chloride in mouse model
  - PoC for F12 KD in a clinically relevant model of disease
- Evaluation in HAE models planned
  - C1INH knockout animals
  - Captopril-induced vascular leak

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