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Lipoprotein(a) targeting with RNAi delivery platforms in transgenic mice and cynomolgus monkeys

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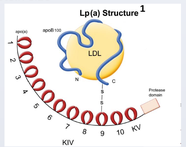
INTRODUCTION

- **Lp(a) is a heterogeneous lipoprotein particle expressed predominantly in liver**
  - Lp(a) is an LDL-like particle is composed of apo(a) protein linked to LDL via disulfide bond to apoB-100
  - Restricted to humans and non-human primates
  - apo(a) length varies dependent on the number of Kringle IV-2 (KIV-2) repeats (2 to >40)
  - Expression is inversely correlated with protein size
  - Half-life in serum: 3 – 4 days

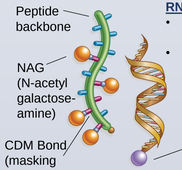


- **Lp(a) levels in humans are genetically defined**
  - Levels do not change significantly with diet, exercise, etc.
  - Normal levels are 0.1 – 25 mg/dL
  - ~25% of US population has >30 mg/dL
- **Lp(a) is an independent risk factor for cardiovascular disease (CVD) through its atherogenic potential**
  - Higher levels of Lp(a) correlate with increased risk of CVD[2-4]
  - Indications include myocardial infarction, stroke, calcific aortic valve stenosis

Dynamic PolyConjugate (DPC™) for liver delivery

• Intravenous (IV) Administration

DPC™ (ARC-EX1)
- 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

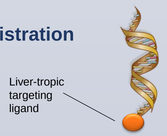


RNAi trigger
- Canonical siRNA or other format
- Liver-tropic targeting ligand (eg. cholesterol)

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

• Subcutaneous (SQ) Administration

RNAi trigger (sole component)
- Canonical siRNA or other format
- Liver-tropic targeting ligand



OBJECTIVES

HYPOTHESIS: Reduction of Lp(a) in patients with elevated Lp(a) levels (>30 mg/dL) by RNAi interference will reduce risk for cardiovascular events

ARC-LPA screening funnel

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

↓

Human/NHP RNAi trigger synthesis and *in vitro* screening by dual luciferase (DLR) assay

↓

Synthesis of chol-RNAi triggers and *in vivo* screening by intravenous (IV) administration

↓

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

↓

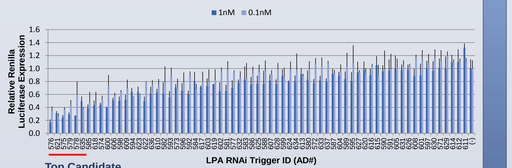
Structure Activity Relationship (SAR) on RNAi triggers for SQ administration with *in vivo* testing

↓

Proof of Concept for disease modification in Lp(a) Tg mice | Lead Candidate = ARC-LPA | *In vitro* tox, dose range finding tox (non-GLP)

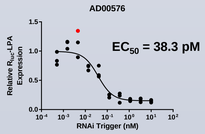
RESULTS

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



- **Huh7 cells co-transfected with a plasmid containing both LPA-Renilla luciferase fusion & constitutively expressed firefly luciferase, and RNAi triggers at 1nM or 0.1 nM**
- **Knockdown measured by Renilla/firefly ratio compared to 'no trigger' control transfections**

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



Trigger ID	EC_{50} (pM)
AD00576	38.3
AD00575	73.5
AD00579	82.8
AD00621	99.6
AD00578	112.1
AD00635	577.4

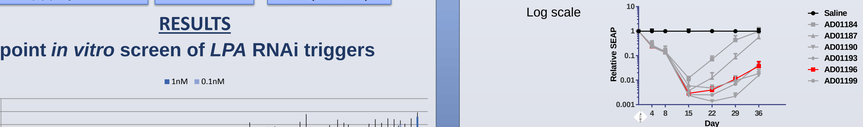
RESULTS

Since *LPA* is not expressed in mice, the following animal models were used for screening :

- **Transiently transgenic mice**
  - Plasmid containing Secreted Embryonic Alkaline Phosphatase (*SEAP*) with *LPA* trigger sequences in the 3'UTR injected into wt mice by hydrodynamic tail vein injection[5]
  - SEAP activity in serum was used to monitor potency of *LPA* RNAi trigger sequences
  - ***Phased out after stably transgenic mice were sufficiently available***
- **Transgenic mice**
  - **apo(a) Tg mice (YAC)[6] and apo(a) Tg mice (cDNA)[7,8]**
    - Measure apo(a) levels
  - **Lp(a) Tg mice (Tg apo(a) x Tg apoB-100)[9]**
    - Measure apo(a) and Lp(a) levels
    - Median pretreatment value (range) = 51.4 mg/dL (15.2-92.4)
- **Non-human primate (NHP) (Cynomolgus monkey)**
  - Measure apo(a) and Lp(a) levels
  - Median pretreatment values (range) =51.6 mg/dL (20.9-108.7)

Initial *LPA* chol-RNAi trigger *in vivo* screen (IV)

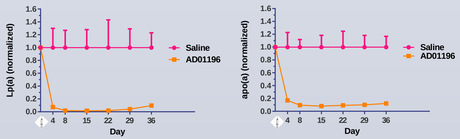
- *Single 8 mg/kg (mpk) LPA RNAi trigger/ARC-EX1 dose, IV injection, n=3 per group*
- *Serum SEAP activity measured and values were normalized to pre-treatment and saline control*



- **Single high dose of chol-RNAi triggers showed substantial knockdown of up to 99.9%**
- **AD01196 (AD00621 family) exhibited >2 log_{10} as measured by SEAP activity for >1 month**

Confirmation of RNAi trigger activity in Lp(a) transgenic mouse model

- *Single 4 mpk AD01196/ARC-EX1 dose, IV injection, n=3 mice per group*
- *Apo(a) levels measured by ELISA*
- *Lp(a) levels measured by clinical chemistry analyzer*



- **Similar reductions achieved for both apo(a) and Lp(a), with maximum apo(a) knockdown of 92% and maximum Lp(a) reduction of 98%**
- **Long duration of effect with ~1 log10 knockdown for >1 month**

RESULTS

Subcutaneous (SQ) RNAi Trigger Development

RNAi trigger relationship map

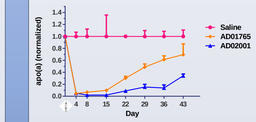
		SQ format			
***In vitro* format**	**IV format**	**basic**	**1º modified**	**2º modified**	**3º modified**
AD00621	AD01196	AD01532	AD01765	AD02001	AD02619

- **Iterative modifications to the backbone pattern (not the sequence) enhanced both depth and duration of knockdown**

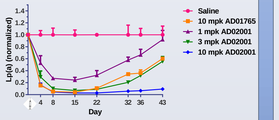
First iterations of SQ RNAi trigger modification

- *n=3 per group*

*Single **10** mpk SQ dose apo(a) Tg mice*



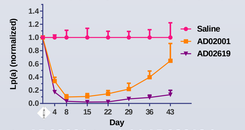
Single SQ dose Lp(a) Tg mice



- **Modifications to AD01765 improved both knockdown (97%) and duration of effect (~90% at 1 month)**

Further iteration of SQ RNAi trigger modification

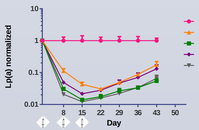
- *Single **3** mpk SQ dose in Lp(a) Tg Mice, n=3 per group*



- **Modifications to AD02001 to yield AD02619 improved knockdown (98%) and duration of effect (>85% at 6 weeks)**

Evaluating ARC-LPA candidates in multiple dose mouse studies

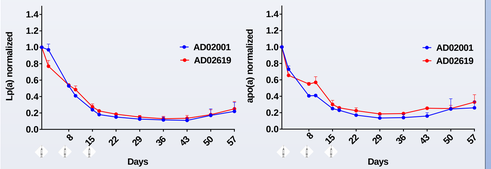
- *Three weekly 3 mpk SQ doses (3xqw) in Lp(a) Tg mice, n=4 per group*



- **Dose response observed for both AD02001 and AD02619**
- **In multiple-dose studies, both AD02001 and AD02619 appear similar in both depth and duration of knockdown**

Evaluating ARC-LPA candidates in non-human primates

- *Three weekly 3 mpk SQ doses (3xqw)*



- **Potency of AD02001 and AD02619 appears similar in both depth and duration of apo(a) knockdown and Lp(a) reduction in NHPs**
- **Lp(a) levels show knockdown of 85-90% between days 29 and 43, with >75% knockdown observed 6 weeks after final dose**

CONCLUSIONS

- **Screening of *in vitro*-active *LPA* RNAi triggers in Tg mice identified those that exhibited substantial and sustained knockdown of serum apo(a) and Lp(a) levels**
- **RNAi trigger sequences were active in both IV and SQ platforms**
- **SAR studies allowed identification of a lead RNAi trigger that demonstrated >98% maximum knockdown after a single 3 mg/kg SQ dose in transgenic mice**
- **In NHPs, 85-90% reduction of serum Lp(a) levels with >1 month duration of effect was observed after three weekly 3 mpk SQ doses**

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