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# Targeting apolipoprotein(a) with a novel RNAi delivery platform as a prophylactic treatment to reduce risk of cardiovascular events in individuals with elevated lipoprotein(a)

Monday, November 14, 2016

Stacey Melquist

arrowhead pharmaceuticals

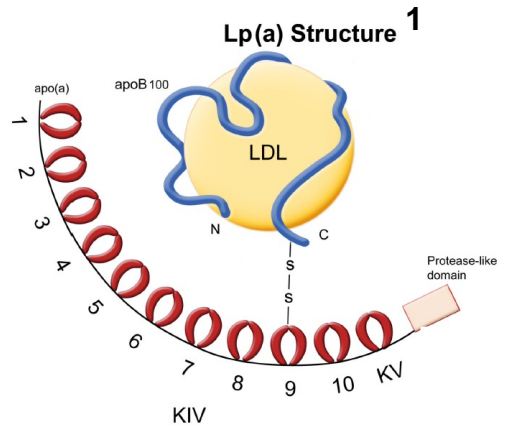
#AHA16

# Disclosures

- Financial Relationships
  - S Melquist, D Wakefield, H Hamilton, C Chapman, C Schienebeck, L Almeida, C Klas, C Hagen, A Almeida, J Hegge, Q Chu, E Doss, V Trubetskoy, D Rozema, D Lewis, and S Kanner
    - Employee and stockholder of Arrowhead Pharmaceuticals
  - J Grondolsky and J. Hoover-Plow
    - Grant support from Arrowhead Pharmaceuticals

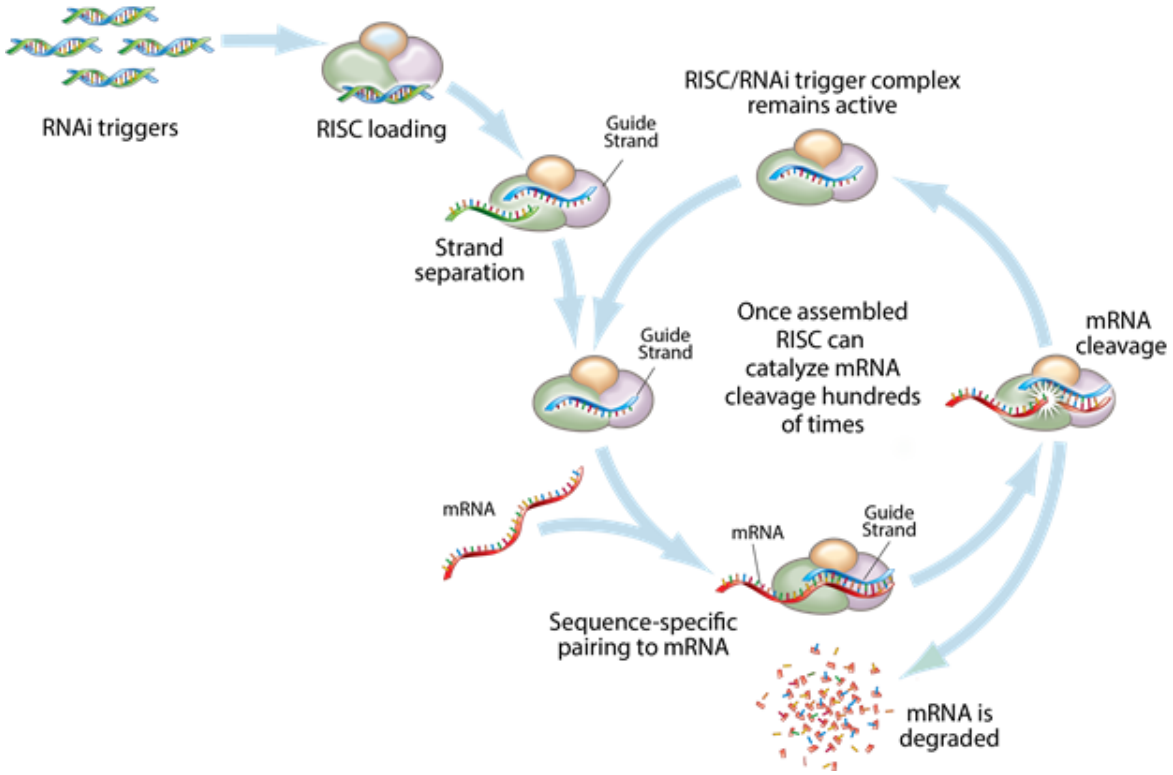
# Lp(a) background

- **Lp(a) is a heterogeneous lipoprotein particle expressed predominantly in liver**
  - Lipid rich particle composed of apolipoprotein(a) linked to LDL via a disulfide bond to apoB-100
  - Restricted to humans and non-human primates
- **Lp(a) levels in humans are genetically defined**
  - Levels not changed significantly with diet, exercise, etc.
  - ~25% of US population has >30 mg/dL (normal levels: 0.1 – 25 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



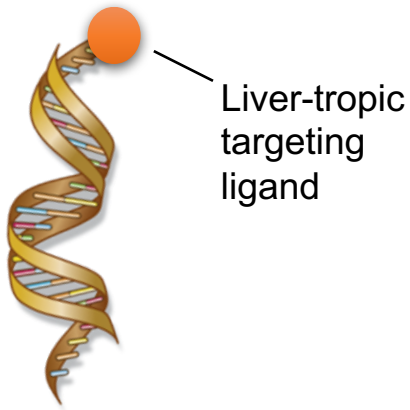

[1]Hoover-Plow J and Huang M (2013) Metabolism 62:479-491
[2]Nordestgaard BG *et al.* (2010) Eur. Heart J. 31:2844-2853
[3]Clarke R *et al.* (2009) N. Engl. J. Med. 361:2518-2528
[4]Kampstrup PR *et al.* (2009) JAMA 310:2331-2339

# Gene silencing with RNA interference

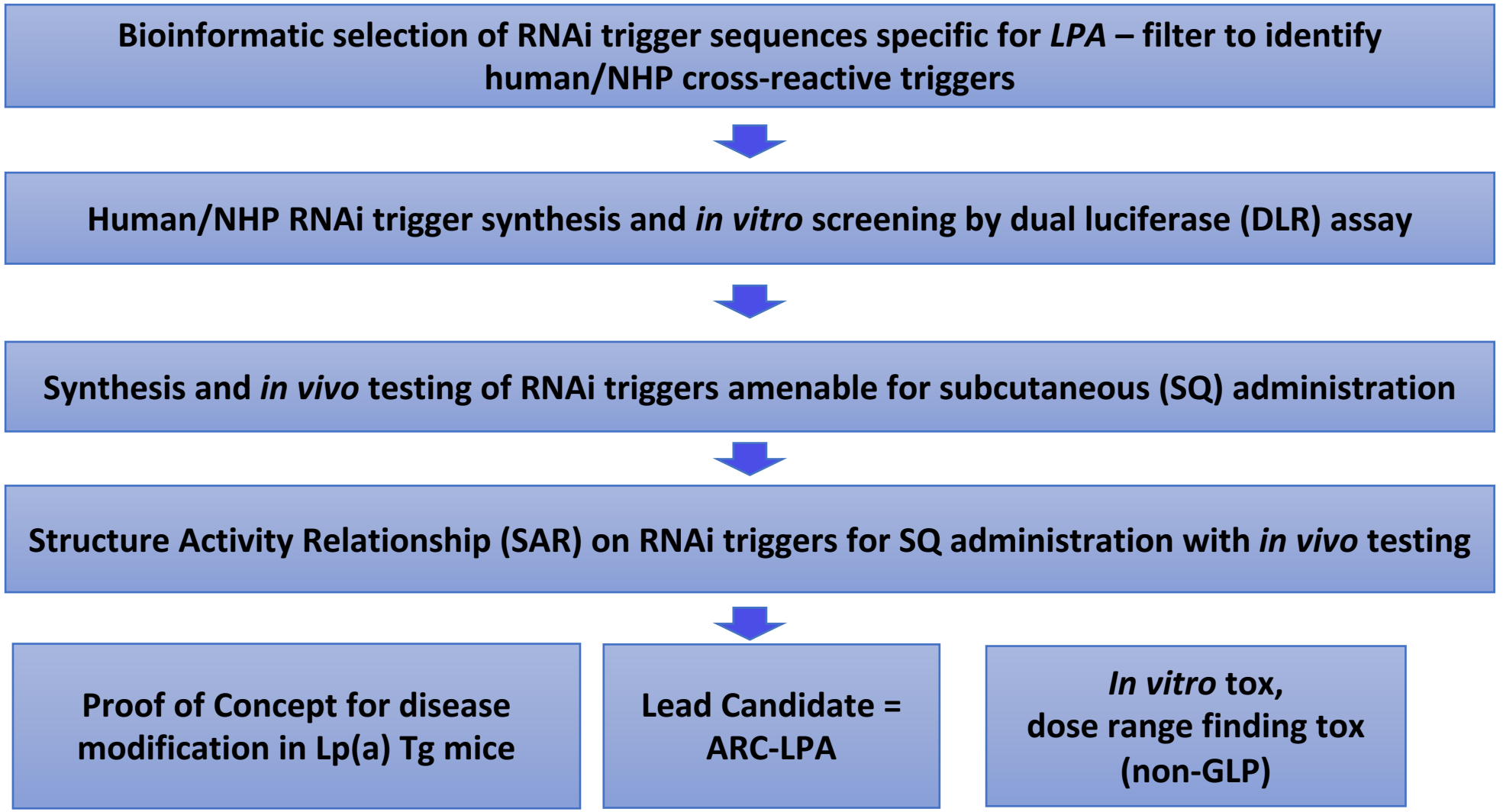



## ARC-*LPA* RNAi trigger

- Short dsRNA targeting *LPA* mRNA
- Liver-tropic targeting ligand
- Injected SQ

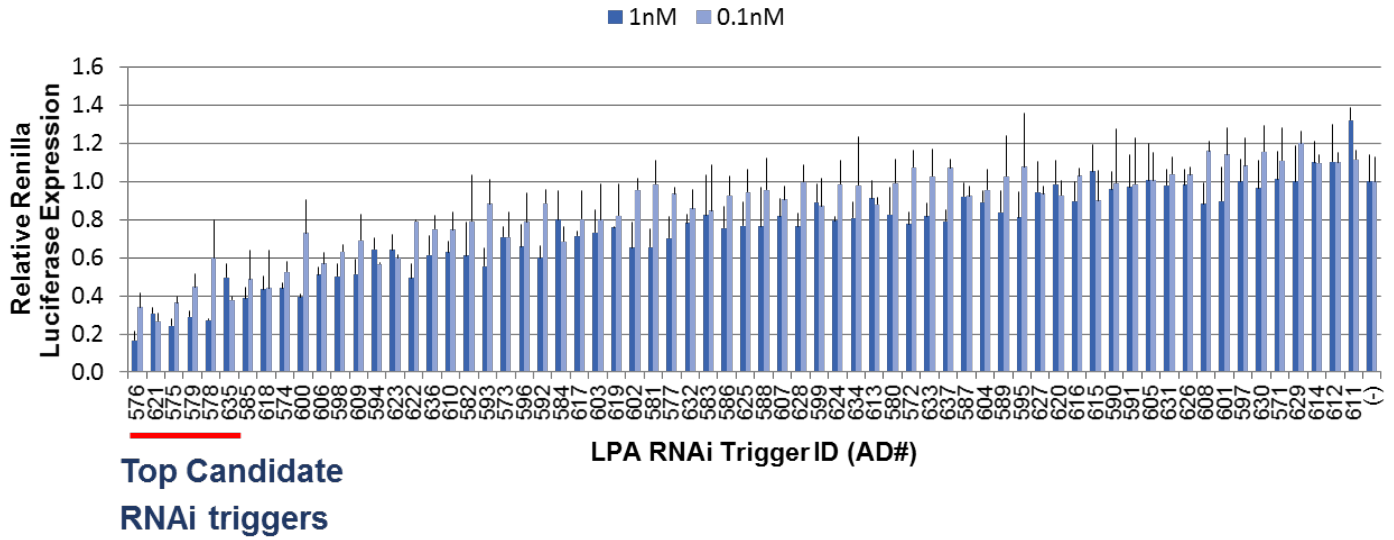


# ARC-LPA Screening Funnel

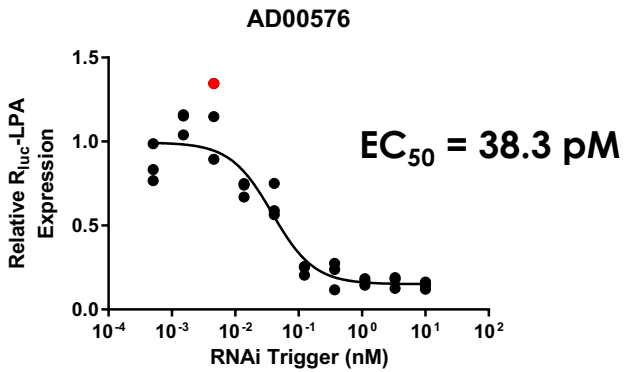


# *In vitro* screening

- Huh7 cells co- transfected with:
  - Dual luciferase plasmid
  - RNAi triggers (0.1 or 1 nM)
- Knockdown measured by Renilla/Firefly luciferase ratio




- $EC_{50}$ determination




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

# *In vivo* screening

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

- **Transgenic mice**
  - **apo(a) Tg mice (YAC)[1] and apo(a) Tg mice (cDNA)[2]**
    - Measure apo(a) levels
  - **Lp(a) Tg mice (Tg apo(a) x Tg apoB-100)**
    - Measure apo(a) and Lp(a) levels
    - Median pretreatment value (range) = 51.4 mg/dL (15.2-92.4)

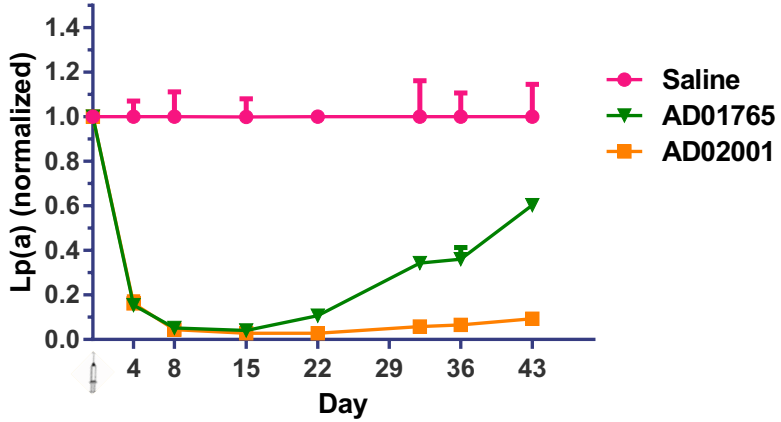
- **Non-human primate (NHP) (Cynomolgus monkey)**
  - Measure apo(a) and Lp(a) levels
  - Median pretreatment values (range) =51.6 mg/dL (20.9-108.7)

[1]Frazer KA *et al.* (1995) Nat. Genet. 9:424-431
[2]Huang M *et al.* (2014) Am. J. Pathol. 184:1503-1517

# Subcutaneous (SQ) RNAi trigger development

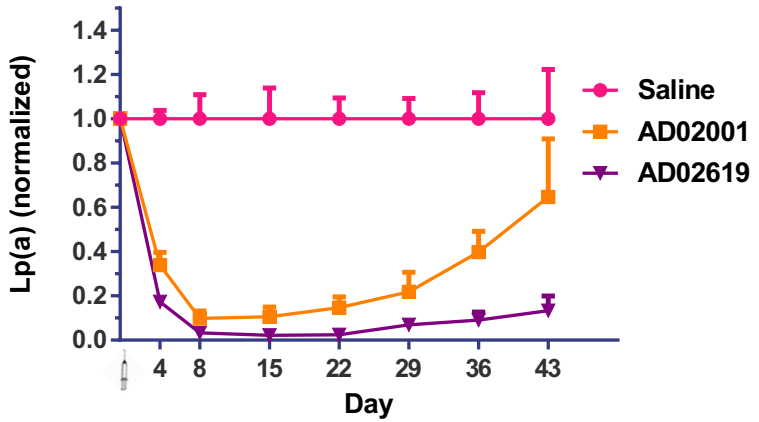
## *First generation*

*Single 10 mg/kg SQ RNAi trigger dose in Lp(a) Tg mice, n=3 per group*




## *Second generation*

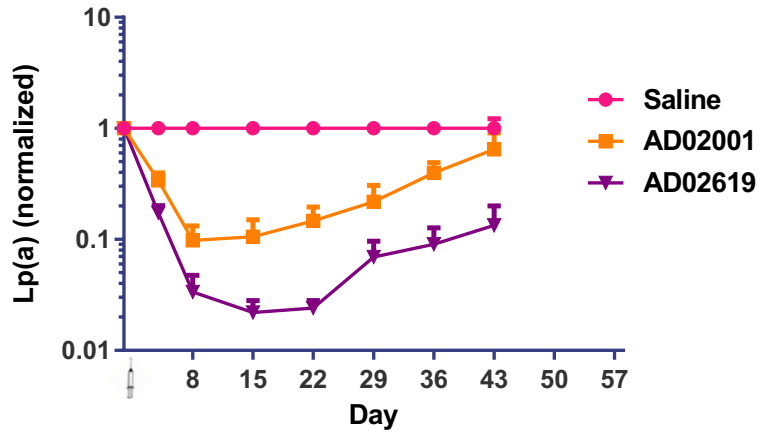
*Single 3 mg/kg SQ RNAi trigger dose in Lp(a) Tg mice, n=3 per group*



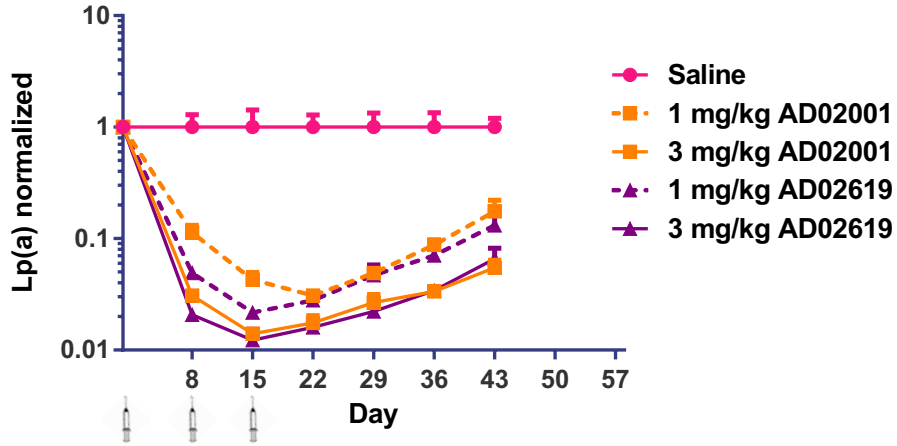

- **Modifications to AD01765 to yield AD02001 improved both knockdown (97% at nadir) and duration of effect (~90% at 1 month)**
- **Modifications to AD02001 to yield AD02619 improved knockdown at reduced dose of 3 mg/kg (98% at nadir) and an extended duration of effect (>85% at 6 weeks)**

# *LPA* SQ RNAi triggers: single vs multiple dose

***Single** 3 mg/kg SQ RNAi trigger dose in Lp(a) Tg Mice, n=3/group*



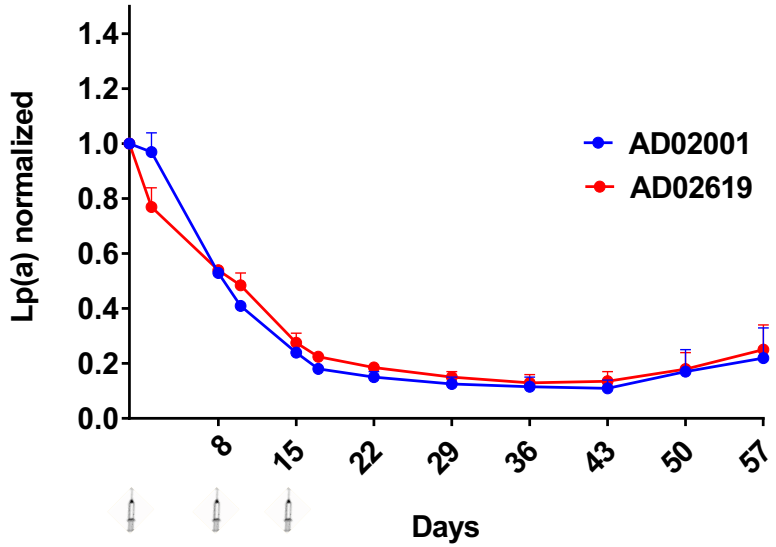

***Three weekly** SQ RNAi trigger doses (3xqw) in Lp(a) Tg mice, n=4 /group*




- **Dose response observed for both AD02001 and AD02619**
- **In multiple-dose studies, both AD02001 and AD02619 exhibit greater depth and duration of knockdown compared to a single dose**

# *LPA* SQ RNAi trigger evaluation in NHP

**Three weekly 3 mg/kg SQ RNAi trigger doses (3xqw), n=2/group**

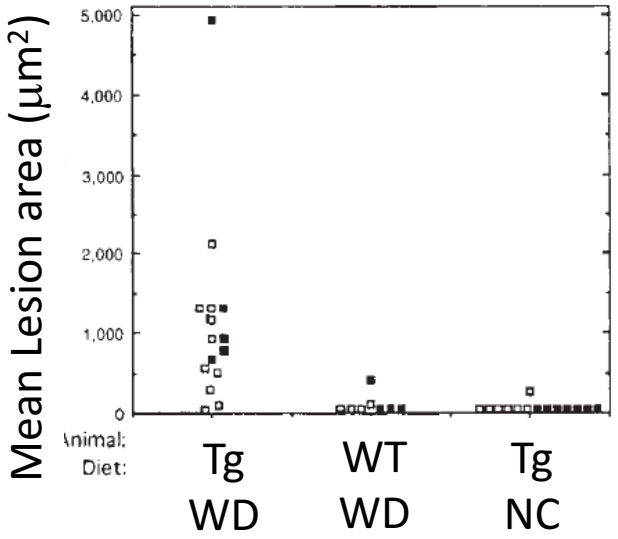



- **RNAi triggers AD02001 and AD02619 exhibit similar depth and duration of Lp(a) reduction in NHP**
- **Lp(a) reduction of 85-90% observed between days 29 and 43, with >75% knockdown at 6 weeks after the final dose**

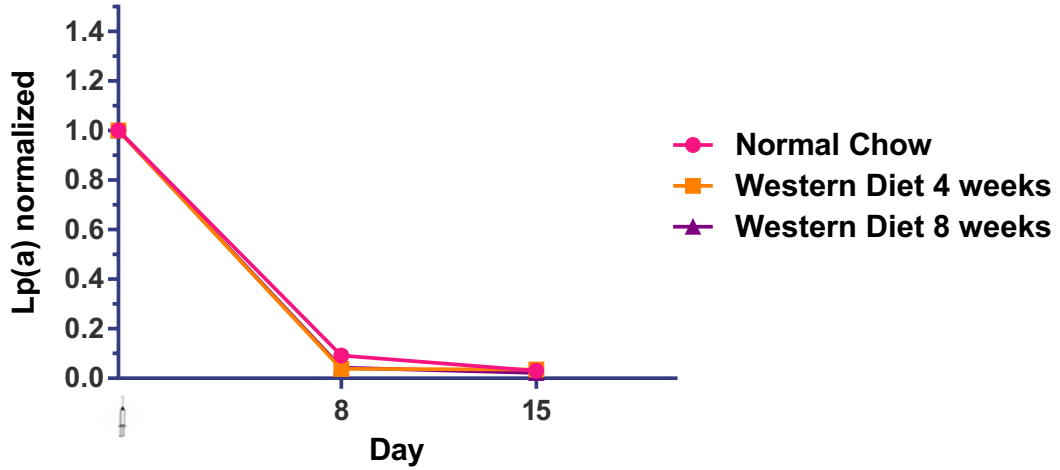
# Atherosclerosis model

- Lipid deposition/atherogenic lesions in aorta of Lp(a) Tg mice fed Western diet
- Can RNAi triggers be effectively delivered to fatty liver?
- After 4 or 8 weeks on Western diet (WD), dose with RNAi trigger

WD = Western Diet
NC = Normal Chow
Tg = apo(a) Tg mice
WT = wt littermate




***Single 10 mg/kg SQ RNAi trigger dose in Lp(a) Tg Mice, n=4/group***




- **No significant difference in Lp(a) knockdown in animals on normal chow vs Western diet for 4 or 8 weeks prior to RNAi trigger injection**



Figure on left: Lawn RM, *et al*. (1992) Nature 360:670-672

# Summary

- Screening of *LPA* RNAi triggers in Lp(a) Tg mice identified several that induced substantial and sustained knockdown of serum Lp(a) levels
- SAR studies identified a lead RNAi trigger that exhibited >98% maximal knockdown after a single 3 mg/kg SQ dose in Tg mice
- NHPs treated with 3 weekly 3 mg/kg SQ RNAi trigger doses resulted in 85-90% reduction in serum Lp(a) with >1 month duration of effect

# Acknowledgements


Cleveland Clinic
Lerner Research Institute

- Jessica Grondolsky
- Jane Hoover-Plow

Wisconsin National Primate Research Center
UNIVERSITY OF WISCONSIN–MADISON

arrowhead pharmaceuticals

- Bioassays Team
- Laboratory Animal Resources
- Holly Hamilton
- Christine Chapman
- Crystal Klas
- Julia Hegge
- Qili Chu
- Edie Doss

arrowhead pharmaceuticals

- Oligo Synthesis Team
- Darren Wakefield
- Lauren Almeida
- Casi Schienebeck
- Collin Hagen
- Aaron Almeida
- Tao Pei
- David Rozema
- Zhen Li
- David Lewis
- Steve Kanner