Abstract ID# 324

Personalized Medicine for Dyslipidemias by RNA Interference-Mediated Reductions in Apolipoprotein C3 or Angiopoietin-Like Protein 3

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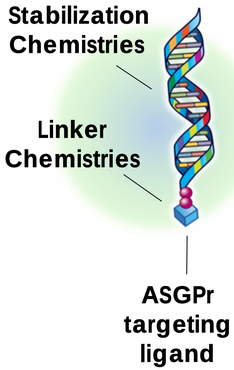
BACKGROUND

Human genetic analysis has identified that individuals with loss-of-function mutations in either apolipoprotein-C3 (APOC3) or angiopoietin-like protein 3 (ANGPTL3) have very low plasma levels of triglycerides (TGs) and in the setting of ANGPTL3 deficiency, low-density lipoprotein (LDL-C). Both conditions are associated with a reduced risk of cardiovascular disease.

OBJECTIVE

ANGPTL3 and APOC3 are primarily expressed in hepatocytes. An RNA interference (RNAi) based therapy using Arrowhead Pharmaceuticals' TRiM™ platform to reduce APOC3 or ANGPTL3 production by gene silencing may be an effective approach to treat dyslipidemias and metabolic diseases (AHA 2018).

TRiM™ Platform



ARO-ANG3 or ARO-APOC3

- Short dsRNA targeting *ANGPTL3* or *APOC3* mRNA
- Hepatocyte ASGPr targeting ligand
- Subcutaneous (SQ) dosing
- Designed to reduce production of ANGPTL3 or APOC3 to potentially treat dyslipidemias
- Specific, catalytic and highly efficient

METHODS

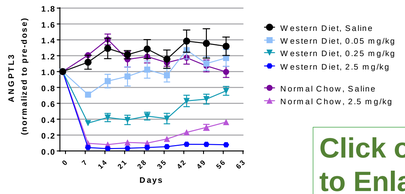
Highly potent and specific RNAi conjugates were identified targeting human and non-human primate (NHP) *APOC3* transcripts (ARO-APOC3) or *ANGPTL3* transcripts (ARO-ANG3). Rodent or NHP (high fructose diet-fed rhesus macaques) dyslipidemic animal models were used to study pharmacodynamic effects in target protein reduction and reductions in TGs and LDL-C.

RESULTS

ARO-ANG3 studies

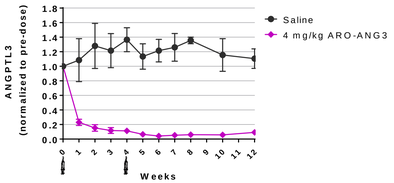
ARO-ANG3 was evaluated in LDL receptor knockout (LDLr KO) mice, diet-induced obese (DIO) mice, as well as a fructose-fed dyslipidemic NHP models. In all animal models, maximum serum reductions in ANGPTL3 of 95% were achieved and persisted for at least 8 weeks. Reductions in TGs and LDL-C were also observed.

Dose response in *LDLr* KO mice



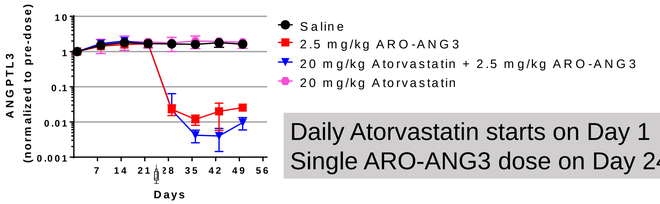
Click on Figures to Enlarge and for Additional Results

Dyslipidemic NHP model



ARO-ANG3 Studies

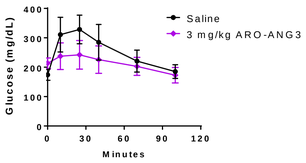
ARO-ANG3 with atorvastatin in *LDLr* KO mice



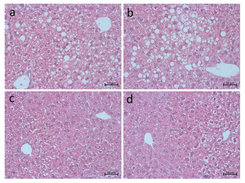
Daily Atorvastatin starts on Day 1
Single ARO-ANG3 dose on Day 24

Improvements in glucose tolerance and reduction in hepatic steatosis in DIO mice

Glucose Tolerance Test



Liver Histology (H&E)

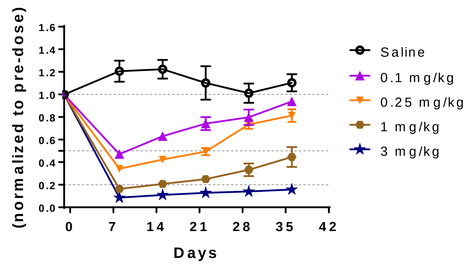


Saline (a, b)

ARO-ANG3 (c, d)

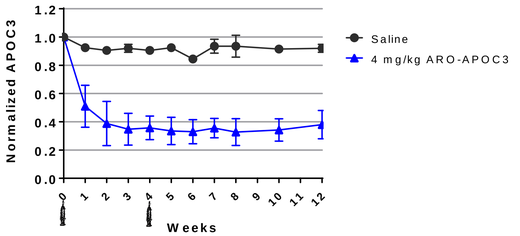
ARO-APOC3 in human APOC3 transgenic mice

- Dose-dependent reductions in serum human APOC3 protein (maximum 91%) and liver mRNA
- Reductions in TGs and LDL-C, increases in HDL-C



ARO-APOC3 in Dyslipidemic Rhesus

- Reductions in serum APOC3 (up to 80%) and TGs (up to 90%)
- Magnitudes of reductions correlated to the severity of dyslipidemia



CONCLUSIONS

- Our results support an RNAi therapeutic targeting *APOC3* or *ANGPTL3* as a treatment for dyslipidemia
- ARO-ANG3 may also provide metabolic benefits in the liver and impact LDL-C in familial hypercholesterolemia
- Both development candidates can be used to target specific patient populations depending on underlying genetic and metabolic profiles
- ARO-APOC3 and ARO-ANG3 have recently entered human clinical trials

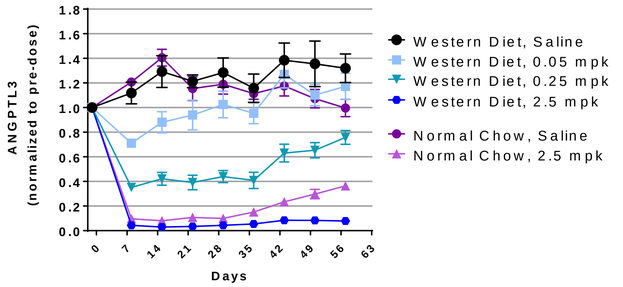
ACKNOWLEDGEMENTS

We thank Vladimir Subbotin, Maria Afrazi and Anna Rowe for their contributions in histopathology evaluations; the Arrowhead Laboratory Animal Research team for their excellent animal care and surgical techniques

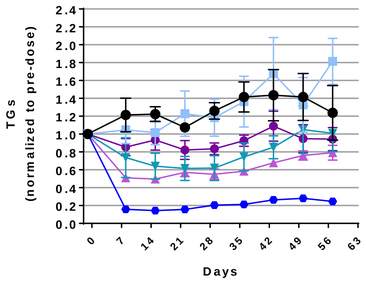
ARO-ANG3 Studies in Mouse Models

Dose response in *LDLr* KO mice, N = 5-6, single SQ dose on Day 1

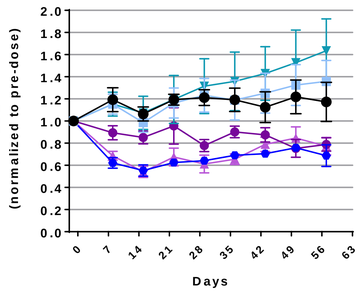
Serum ANGPTL3



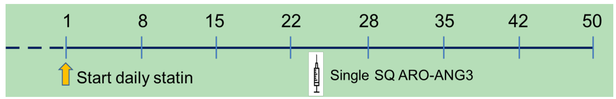
Serum TGs



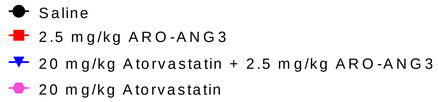
Serum LDL-C



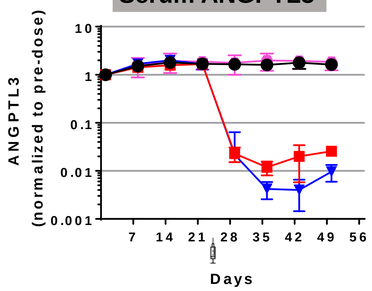
ARO-ANG3 with atorvastatin in *LDLr* KO mice, N = 6-7



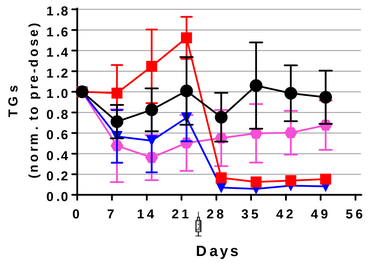
- *LDLr* KO mice on Western diet
- Atorvastatin has no effects on ANGPTL3 expression
- Additive effects of ARO-ANG3 + atorvastatin



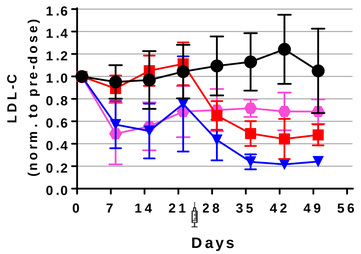
Serum ANGPTL3



Serum TGs

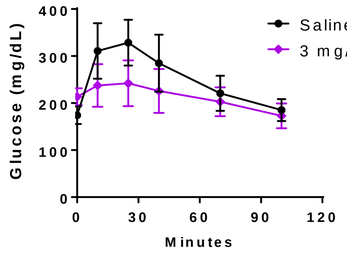


Serum LDL-C

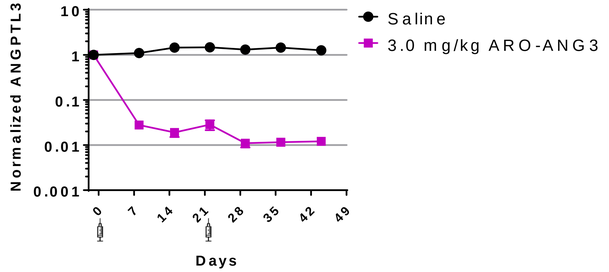


Improvement of glucose tolerance and reduction in hepatic steatosis in DIO mice

Glucose Tolerance Test

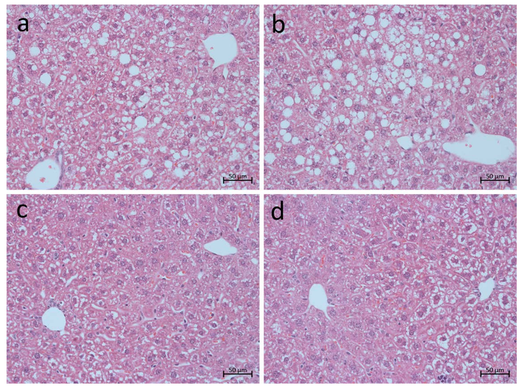


Serum ANGPTL3



- Mice on high fat diet (60% kcal% fat), N = 7
- Two doses of 3 mg/kg ARO-ANG3 on Day 1 and 22
- Glucose Tolerance Test on Day 41, necropsy Day 44

Reduction in hepatic Steatosis (H&E)

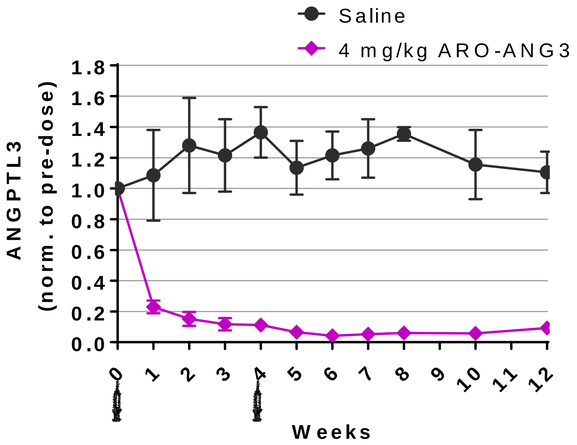


Saline (animals a and b)

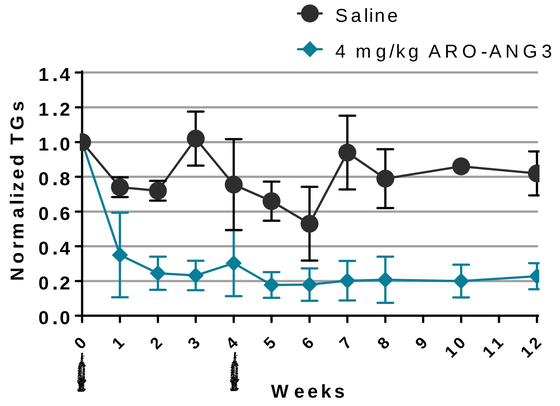
ARO-ANG3 (animals c and d)

ARO-ANG3 in Fructose Diet-Fed Dyslipidemic Rhesus Monkeys

Reductions in Serum ANGPTL3: group average
N = 2 (Saline) or 4 (ARO-ANG3)

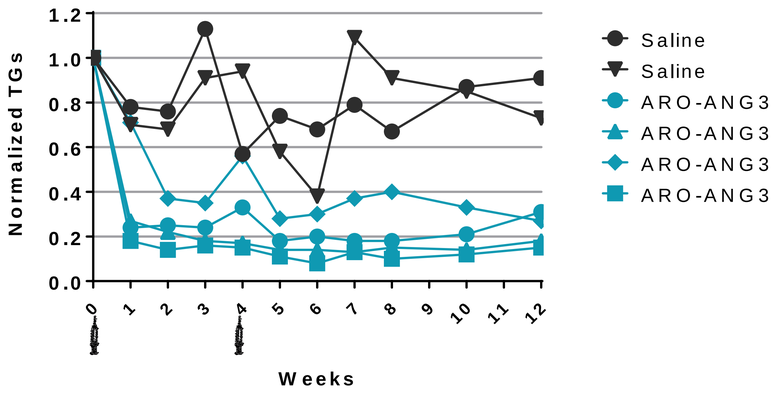


Reductions in Serum TGs: group average
N = 2 (Saline) or 4 (ARO-ANG3)



- Animals on fructose diet for 6 weeks before dosing
- Variable diet-induced dyslipidemia
- Over 95% maximum reductions in serum ANGPTL3 protein levels
- 80% maximum mean reductions in TGs
- 20-60% max reductions in LDL-C (not shown)
- ARO-ANG3 (N=4), Saline (N=2)

Reductions in Serum TGs: individual



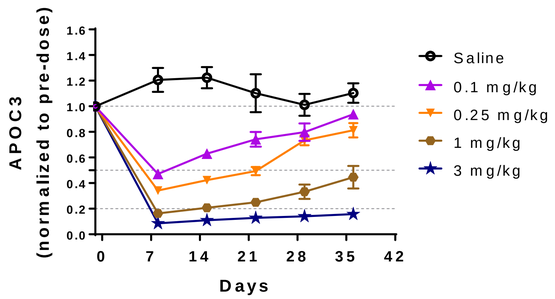
Fructose-diet mediated changes in ANGPTL3 and TGs

Animal	Serum ANGPTL3 (ng/dL)		TGs (mg/dL)	
	Pre-diet	Pre-dose	Pre-diet	Pre-dose
Saline-1	38.8	45.9	87	213
Saline-2	43.4	81.8	73	172
ARO-ANG3-1	50.2	87.5	47	188
ARO-ANG3-2	28.4	61.5	44	250
ARO-ANG3-3	29.5	58.8	36	76
ARO-ANG3-4	53.9	127.4	132	767

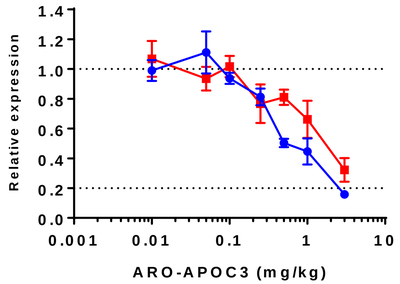
ARO-APOC3 Studies in Mice and Fructose Diet-Fed Dyslipidemic Rhesus Monkeys

Dose response in human APOC3 transgenic mice
N = 5-6, single SQ dose on Day 1

Serum APOC3

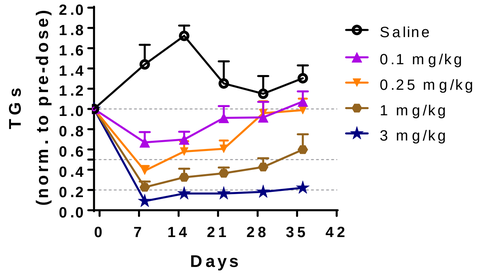


APOC3 mRNA vs Protein (Day 37)

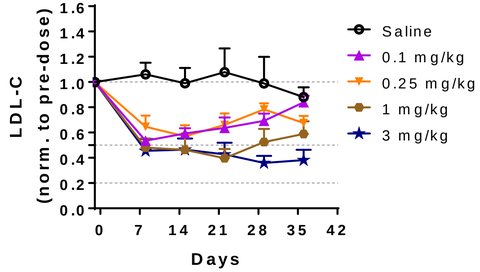


- **Close dose dependent correlation of mRNA and serum APOC3 reduction**

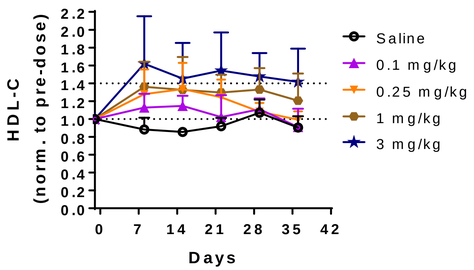
TGs



LDL-C

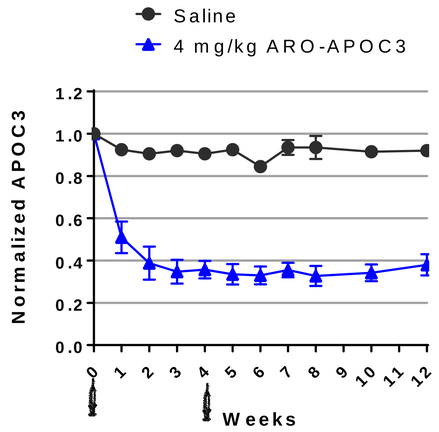


HDL-C

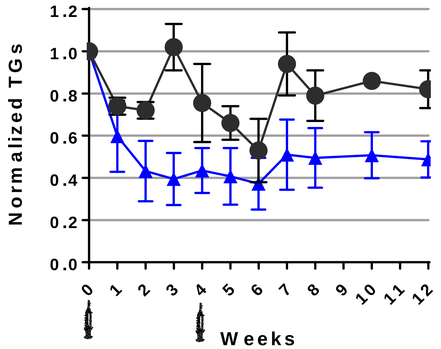


Fructose Diet-Fed Dyslipidemic Rhesus Monkeys
N = 2 (Saline) or 4 (ARO-APOC3)

Serum APOC3



TGs



- **Animals on fructose diet for 6 weeks**
- **Variable diet-induced dyslipidemia**
- **Maximum mean serum APOC3 reduction of 67% (range: 60-80%), which likely represents complete hepatocyte knockdown**
- **Small intestinal production still intact**
- **Maximum mean TGs reduction of 60% (range: 40-90%)**
- **20-60% max reductions in LDL-C (not shown)**
- **ARO-APOC3 (N=4), Saline (N=2)**