Subcutaneous delivery of an effective RNA interference (RNAi) therapeutic candidate silencing angiopoietin-like protein 3 for treatment of hyperlipidemia

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Background

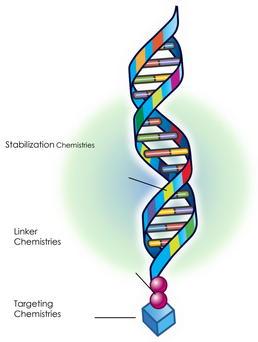
Angiopoietin-like protein 3 (ANGPTL3), a secreted protein predominantly expressed in the liver, has been highlighted from recent human genetic studies for its role in cardiovascular disease. Loss-of-function mutations in ANGPTL3 result in low plasma levels of triglycerides (TG) and low-density lipoprotein (LDL), and genome-wide association studies (GWAS) have suggested a reduced risk of cardiovascular mortality without producing a negative phenotype.

Arrowhead Pharmaceuticals' TRiM™ platform, in this instance targeting hepatocyte delivery, is designed to selectively deliver potent RNA interference (RNAi) therapeutics to specific tissues of interest. It contains a multivalent N-acetylgalactosamine (NAG) as the targeting ligand, which binds to the asialoglycoprotein receptor (ASGR) abundantly expressed on hepatocytes. The platform also includes Arrowhead proprietary RNA stabilization and linker chemistries, to increase potency and minimize off-target activity.

Here we describe a subcutaneous (subQ) RNAi therapeutic, ARO-ANG3, designed with the TRiM™ platform as a potentially effective approach to treat a number of potential lipid disorders by reducing hepatic ANGPTL3 production.

TRiM™ platform

Specificity, Activity and Simplicity



- Rules and algorithms allow selection of optimized RNAi trigger sequences
- Limit cross-reactivity with off-target genes
- Maximize innate stability
- Rational use and placement of modifying chemistries
- Active endosomal escape chemistries ***not*** required
- Targeting ligands and linker chemistries improve delivery to target tissues

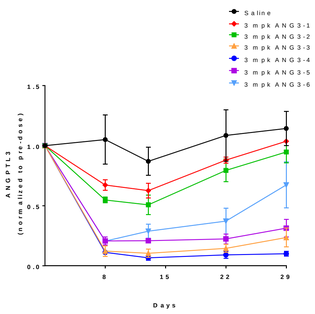
Methods

Animal studies to demonstrate efficacy and safety

- Efficacy of ANGPTL3 RNAi was evaluated in wild type mice (C57bl/6) and cynomolgus monkeys by measuring serum ANGPTL3 protein
- Lead compound, ARO-ANG3, was also evaluated in a dyslipidemic mouse model (LDLr$^{-/-}$, Jackson Laboratory) which allows assessment of lipid responses

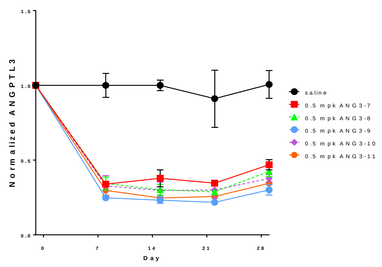
Results

RNAi trigger sequence testing in mice



Candidate RNAi trigger sequences were studied for ANG3 knockdown (KD) activity in female C57bl/6 mice given a single subQ injection of 3 mg/kg (mpk) RNAi trigger on Day 1. Serum ANGPTL3 protein was measured by ELISA, shown with standard error (SEM).

Optimization of RNAi triggers in mice



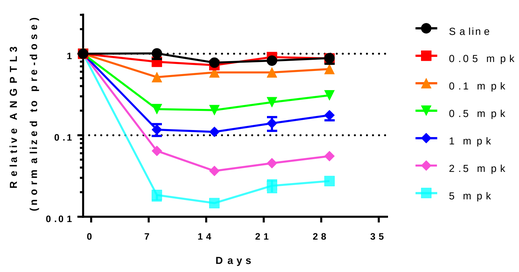
Optimization of lead sequences was performed by measuring serum ANGPTL3 KD activity in female C57bl/6 mice given a single subQ injection of RNAi trigger on Day 1.

Potency was increased with optimized chemical modifications, with up to 80% KD of serum ANGPTL3 was achieved at nadir by a single dose of 0.5 mg/kg RNAi trigger.

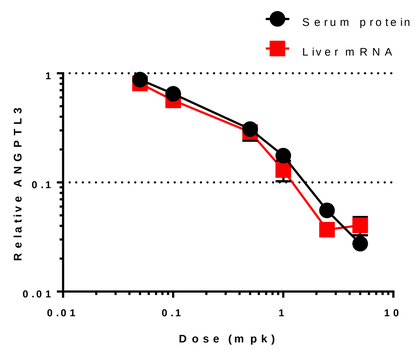
Dose dependent knockdown of serum ANGPTL3 protein and liver mRNA in wild type mice

Female C57bl/6 mice were given various subQ doses of ARO-ANG3 ranging from 0.05 to 5 mpk on Day 1. Blood samples were taken periodically and livers were harvested on Day 29 for mRNA analysis.

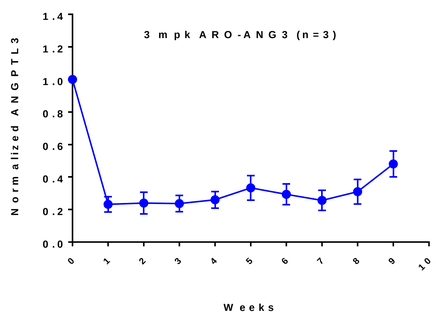
ANGPTL3 serum protein expression



ANGPTL3 mRNA relative to serum protein expression
on day 29 after a single SubQ dose of ARO-ANG3



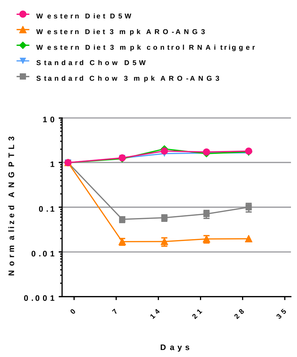
Durable serum ANGPTL3 protein knockdown in non-human primates



Cynomolgus monkeys (n=3) were given a single subQ injection of 3 mg/kg ARO-ANG3 on study Day 1. Serum ANGPTL3 protein was measured by ELISA, shown with standard error (SEM).

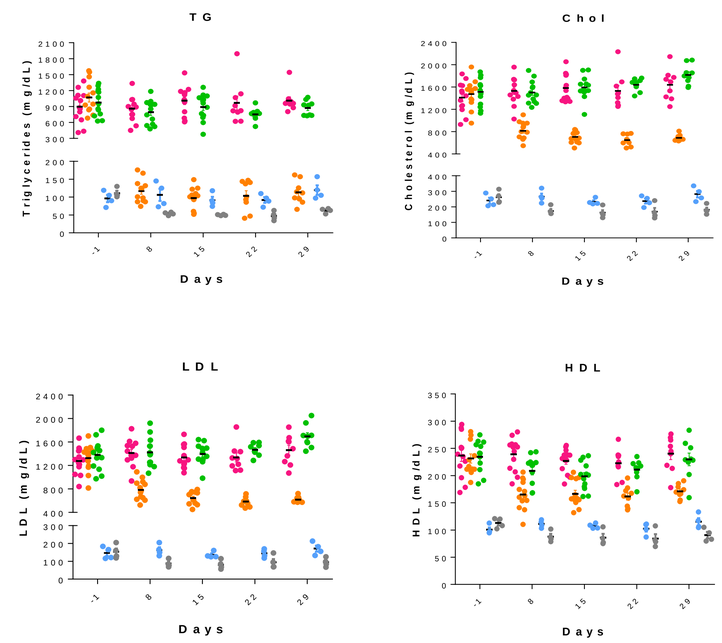
Evaluation of ARO-ANG3 in a dyslipidemic mouse model

ANGPTL3 serum protein expression



- 7-8 week old males LDLr$^{-/-}$ mice were put on a Western diet or standard chow for 3 weeks prior to treatment, and maintained on the same diet throughout the study. A single dose of 3 mg/kg ARO-ANG3, negative control RNAi trigger or D5W was injected on Day 1. ANGPTL3 protein, serum TG, Chol, LDL, and HDL levels were monitored before (Day -1) and after ARO-ANG3 administration
- Deep (> 1 log) ANGPTL3 KD was obtained in both Western diet and standard chow-fed LDLr$^{-/-}$ mice
- Significant decreases in lipid parameters were observed following ARO-ANG3
- Note that LDL is reduced in the absence of LDL receptors

Serum lipid parameters in dyslipidemic mouse model



Conclusions

ARO-ANG3, a new subcutaneous therapeutic candidate, provides deep and durable KD in wild type mice and NHPs. In addition, ARO-ANG3 achieved deep ANGPTL3 protein KD in a dyslipidemic mouse model. Consistent with ANGPTL3's role, reductions in TGs and LDL-C were also observed. ARO-ANG3 holds promise for treatment of patients with certain dyslipidemias and is expected to be in active clinical development in 2019.

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