Abstract

Background: Coronary artery disease (CAD) remains the top cause of mortality in most high-income countries, and is a major contributor to all-cause mortality. It is prevalent and is associated with high mortality and societal costs. Atherosclerosis, a key pathological hallmark of CAD, is characterized by the formation of plaques in blood vessels that can lead to vessel occlusion, ischemia, and ultimately heart attack or stroke. Although several therapies exist for prevention of atherosclerosis, there is a substantial need for new molecules that can impact this disease at the cellular and molecular level. We describe a novel RNA interference (RNAi) therapeutic strategy for targeting the metabolic disease genes PCSK9, ApoC3 and ANGPTL3.

Methods: We have previously developed a modular, robust, and reliable platform for the delivery of RNAi therapeutics to the liver with a large therapeutic index. We have designed chemically-modified siRNAs using bioinformatic algorithms and screened for potency and durability. We set up preclinical studies with ALN-PCSsc, ALN-ANG and ALN-AC3 to target PCSK9, ANGPTL3 and ApoC3, respectively.

Results: We report the preclinical and safety profile of these three RNAi therapeutics in non-human primates (NHPs). ALN-PCSsc achieves potent and stable PCSK9 knockdown and LDL-C lowering with SC dosing. Up to 99% KD of serum ANGPTL3 was observed with a single SC dose of ALN-ANG. ALN-AC3 achieves >90% lowering of ApoC3 protein after a single dose.

Conclusion: We conclude that these three RNAi therapeutics represent attractive candidates for clinical development in patients with hyperlipidemia.

Keywords: RNAi therapeutics, PCSK9, ANGPTL3, ApoC3, metabolic dyslipidemia, non-human primates (NHPs).

Figure 1: Cell-free siRNA delivery to the liver is potential for the treatment of metabolic diseases. (A) RNA interference (RNAi) is a naturally occurring mechanism that uses small interfering RNAs (siRNAs) to silence specific genes. (B) Inhibitory RNAs enter the cell via endocytosis and are processed by endogenous enzymes to yield active siRNAs. These siRNAs bind to the AGO complex and target homologous mRNA, leading to degradation of the mRNA and a reduction in the expression of the target gene.

Figure 2: In vivo activities of multiple siRNA delivery systems in a Laurin and Moore mouse model of PCSK9. (A) ALN-PCSsc is an RNAi therapeutic that targets PCSK9. (B) ALN-ANG targets ANGPTL3. (C) ALN-AC3 targets ApoC3.

Figure 3: Summary of ALN-PCSsc safety studies. (A) Pharmacokinetic parameters for ALN-PCSsc in NHPs. (B) ED90 achieved with 1 mg/kg dose, ED70 ~0.3 mg/kg.

Figure 4: Summary of ALN-ANG safety studies. (A) Summary of pharmacokinetic parameters for ALN-ANG in NHPs. (B) Summary of safety studies for ALN-ANG in NHPs. (C) Summary of safety studies for ALN-ANG in NHPs.

Figure 5: Summary of ALN-AC3 safety studies. (A) Summary of safety studies for ALN-AC3 in NHPs. (B) Summary of safety studies for ALN-AC3 in NHPs. (C) Summary of safety studies for ALN-AC3 in NHPs.

Figure 6: Summary of ALN-PCSsc pre-clinical efficacy in NHP. (A) Inhibitory RNA activity in human APPTL3 knockout (APPTL3KO) and human APPTL3 wildtype (APPTL3WT) liver explants. (B) Summary of efficacy studies for ALN-PCSsc in NHP.

Figure 7: Summary of ALN-ANG pre-clinical efficacy in NHP. (A) Inhibitory RNA activity in human APPTL3 knockout (APPTL3KO) and human APPTL3 wildtype (APPTL3WT) liver explants. (B) Summary of efficacy studies for ALN-ANG in NHP.

Figure 8: Summary of ALN-AC3 pre-clinical efficacy in NHP. (A) Inhibitory RNA activity in human APPTL3 knockout (APPTL3KO) and human APPTL3 wildtype (APPTL3WT) liver explants. (B) Summary of efficacy studies for ALN-AC3 in NHP.

Figure 9: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 10: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 11: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 12: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 13: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 14: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 15: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 16: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 17: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 18: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 19: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.

Figure 20: Summary of ALN-PCSsc, ALN-ANG and ALN-AC3 in human POC for GalNAc-siRNA conjugates. (A) Summary of safety studies for ALN-PCSsc. (B) Summary of safety studies for ALN-ANG. (C) Summary of safety studies for ALN-AC3.