ALN-CC5, an Investigational RNAi Therapeutic Targeting C5 for Complement Inhibition

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Cobra venom factor (CVF) treated rats receive daily injections of ALN-CC5 (5 mg/kg) for 8 weeks. Complement activity was assessed using the sensitized sheep RBC hemolysis assay in pre-dose samples and serum samples collected on day 6 post-α1-antitrypsin injection. Whole tissue mRNA was isolated and C5 and GAPDH mRNA levels quantified by gene-specific Taqman probesets. Expression levels in the respective tissues were normalized to age and gender-matched controls.

Inflammation

Classical Pathway

Alternative Pathway

Classical Pathway Serum Hemolytic Activity

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Summary

ALN-CC5, an investigational RNAi therapeutic targeting human, primate and rodent C5. C5 silencing and complement activity inhibition were examined in rodents and hemolytic activity was observed. ALN-CC5 was safe and well tolerated in both rat and non-human primate toxicology studies. In addition to wild type animals, ALN-CC5 was tested in several animal models of disease in which complement activation plays a prominent role. Silencing of murine C5 was highly efficacious in a model of anti-collagen.
Abstract

Excessive complement activation plays a pivotal role in a variety of disorders. Complement component C5 is a clinically validated therapeutic target for treatment of both paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic-uremic syndrome.

We developed a robust RNAi therapeutics platform for the delivery of siRNAs to the liver using trivalent GalNAc conjugates, enabling specific silencing of hepatocyte-expressed genes following subcutaneous injection. The liver produces essentially the entirety of C5 and other complement pathway proteins. We are developing ALN-CC5, an investigational RNAi therapeutic targeting human, primate and rodent C5. C5 silencing and complement activity inhibition were examined in rodents and primates. Multi-dose SC ALN-CC5 treatment resulted in sustained lowering of cyno serum C5 with ≤3% residual protein remaining. C5 reduction was associated with >90% and >95% inhibition of classical and alternative complement pathways, respectively, as measured by ELISA-based assays. Additionally, >80% lowering of complement serum hemolytic activity was observed. ALN-CC5 was safe and well tolerated in both rat and non-human primate toxicology studies. In addition to wild type animals, ALN-CC5 was tested in several animal models of disease in which complement activation plays a prominent role. Silencing of murine C5 was highly efficacious in a model of anti-collagen antibody-induced arthritis (CIA) with a disease modifying activity equivalent to that of an anti-C5 antibody. Furthermore, C5 silencing was effective at reducing proteinuria in a rat model of membranous nephropathy. Up-regulation of C5 expression, observed in both models, had no effect on the extent of C5 silencing, suggesting that ALN-CC5 could be efficacious in the context of inflammation. These data demonstrate a prominent role for circulating, liver-derived C5 in mediating pathology at extrahepatic sites and the potential utility of an RNAi therapeutic targeting C5.

In summary, RNAi-mediated silencing of liver-derived C5 is a promising novel therapeutic approach for inhibiting systemic complement activity, with the potential to enable, low-volume, subcutaneous treatment for patients with PNH and other disorders where complement activation plays a role in disease progression.

Figure 1. GalNAc-siRNA Conjugates as Investigational RNAi Therapeutics

Asialoglycoprotein Receptor (ASGPR)
• Highly expressed in hepatocytes
• High rate of uptake
• Recycling time ~15 minutes
• Conserved across species

GalNAc-siRNA Conjugates (revisiran, ALN-AT3, ALN-CC5, ALN-PCSc, other programs)
• siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand
• Efficient delivery to hepatocytes following subcutaneous administration
• “Enhanced stabilization chemistry” (ESC) used with ALN-AT3, ALN-CC5, ALN-PCSc, and other programs
  – Significantly improved potency and durability compared with revisiran

Figure 2. Sustained C5 Knockdown and Potent Complement Activity Inhibition in NHP

Robust knockdown of serum C5 with SC dosing in NHP for >7 months
• Q2W Regimen: Every other week dosing (5 mg/kg, qw x 8, q2w thereafter)
• GM Regimen: Every month dosing (5 mg/kg, qd x 5, qw x 8/10 mg/kg qm thereafter)
– 2xW Regimen: Twice a week dosing (5 mg/kg for 8 weeks)
Up to 99.2% knockdown of serum C5
• 56±4% knockdown as group average
• Low inter-animal variation
• Q2W Regimen provides optimal C5 knockdown results in NHP
  – Expect GM dosing regimen in humans based on translation of ESC-GalNAc-siRNA conjugates

Both classical and alternative complement pathways greatly reduced in serums of NHPs treated with ALN-CC5
• Up to 96.9% inhibition of alternative pathway (CAP) activity (mean 95.1±0.93%); and up to 99.2% inhibition of hemolyis (mean 88.8±6.1%)
• Q2W Regimen provides optimal inhibition of complement activity in NHP but monthly likely in humans based on translation of ESC-GalNAc conjugates
• In line with results observed in published reports on ezulinzumab

NHP Efficacy Conclusions

• Knockdown of cyno C5 with long-term SC treatment with ALN-CC5 results in up to 99.2% knockdown of serum C5
  – Stable knockdown maintained for greater than 7 months with a Q2W regimen
  – Full recovery to baseline following 5 mg/kg 2xw dosing for 8 weeks
• C5 knockdown associated with up to 96.9% inhibition of serum complement activity
  – Knockdown of liver C5 results in robust inhibition of both classical and alternative complement pathways
The Complement Pathway

C3 Convertase

C3

C3a

C3b

C5a

C5b-9

Alternative Pathway

Classical Pathway

C1

C4bC2a

C5aR

C1q

C4

C3

Membrane attack complex (MAC)

Terminal Pathway

The complement pathway is illustrated with the following components:
- C3 Convertase
- C3
- C3a
- C3b
- C5a
- C5b-9
- Alternative Pathway
- Classical Pathway
- C1
- C4bC2a
- C5aR
- C1q
- C4
- C3
- Membrane attack complex (MAC)
- Terminal Pathway

Figure 3. C5 Silencing Reduces Proteinuria and Glomerular MAC Deposition

Rat Membranous Nephropathy Model

- ~90% reduction in urinary albumin levels with C5 knockdown in rat model of Passive Heymann Nephritis (PHN)
- Nephritis induced by injection of sheep anti-rat kidney fraction antiserum (antifX1a)
- Cobra venom factor (CVF) treated rats receive daily injections
- Similar reduction in urinary albumin excretion to CVF complement depletion

- No effect on kidney C5 mRNA expression with C5 siRNA treatment
- Kidney expresses 10x lower level of C5 mRNA than liver

- Robust and specific silencing of liver C5 mRNA

- No effect on kidney C5 mRNA expression with C5 siRNA treatment
- Kidney expresses 10x lower level of C5 mRNA than liver

Glomerular MAC Deposition Is Prevented With C5 Silencing

- No glomerular C5b-9 deposition with C5 siRNA or CVF treatment
- Equivalent glomerular sheep IgG deposition regardless of treatment, as expected

Figure 4. C5 Silencing Equivalent to Anti-C5 Antibody in a Mouse CAIA Model

- ALN-CC5 CTA filed; Initial clinical data expected in mid '15
- Wide therapeutic index
- Low inter-animal variation
- No effect on kidney C5 mRNA expression with C5 siRNA treatment
- Kidney expresses 10x lower level of C5 mRNA than liver
ALN-CC5 is an innovative therapeutic strategy for complement-mediated diseases

- GalNAc-conjugate RNAi platform is promising approach for knockdown of liver-expressed target genes
- ALN-CC5, an ESC-GalNAc conjugate targeting C5, characterized in pre-clinical models
  - Sustained lowering of plasma C5 and complement pathway activity in NHPs with q2w and qm subcutaneous dosing regimens for >7 months
  - Lower dose/less frequent dosing expected in humans with ESC-GalNAc conjugates based on ALN-AT3 human translation
- RNAi knockdown of C5 protective in rodent models of disease
  - Membranous nephropathy
  - Urinary albumin reduction equivalent to complement depletion with cobra venom factor
  - Rapid liver uptake of GalNAc siRNA has potential advantages in indications with increased kidney clearance
- Arthritis model
  - RNAi knockdown of liver-derived C5 shows equivalent efficacy to anti-C5 antibody
  - No modulation of C5 silencing in an inflammatory context
- GLP toxicity studies confirm wide therapeutic index; >100 mg/kg NOAEL in NHP, >50 mg/kg in rat
- ALN-CC5 CTA filed; Initial clinical data expected in mid ’15

Summary

ALN-CC5 CTA-enabling studies completed

- Wide therapeutic index
  - NOAEL >100 mg/kg in NHP 4-week GLP toxicology/Tk study
  - NOAEL >50 mg/kg in rat 4-week GLP toxicology/Tk study
  - Highest dose tested for both, weekly dosing
  - NOEL >100 mg/kg in NHP CV/safety pharmacology study
  - 13-week GLP toxicology/Tk studies in rat and NHP ongoing
    - No in-life findings
    - No findings in genetic toxicity studies at ICH limit doses

Phase 1 study expected to start in early ’15; Initial data in mid ’15

- ALN-CC5 with SC dosing
  - Parts A/B: SAD/MAD in up to 60 normal healthy volunteers
    - Randomized, double-blind, placebo-controlled study
    - Assess safety, tolerability, PK/PD, and clinical activity
  - Part C: Multi-dose in up to 8 PNH patients
    - Open-label study
    - Assess safety, tolerability, PK/PD, clinical activity, and LDH reduction

Conflicts of Interest Disclosure