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

Anna Borodovsky1; Kristina Yucius1; Andrew Sprague1; Nirmal K. Banda2; V. Michael Holers2; James Butler1; Shannon Fishman1; Akshay Vaishnaw1; Martin Maier1; Rajeev Kallanthottathil1; Klaus Charisse1; Satya Kuchimanchi1; Muthiah Manoharan1; Kevin Fitzgerald1; Benny Sorensen1; Rachel Meyers1

1Alnylam Pharmaceuticals Inc., 300 Third Street, Cambridge, MA, USA; 2Division of Rheumatology, University of Colorado School of Medicine, Aurora, CO, USA
**Abstract**

The complement system is a pivotal player in multiple diseases. Antibody blockade of the C5 component of complement has been approved as a treatment for both paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic-uremic syndrome (aHUS), validating C5 as an important therapeutic target.

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 (SC) injection. The liver produces essentially the entirety of C5 and other complement pathway proteins.

To examine the utility of the siRNA approach for targeting complement pathway components we developed ALN-CC5, a GalNAc conjugated siRNA targeting rodent, primate and human C5. C5 silencing and serum hemolytic activity inhibition were evaluated in rodents using single and multi-dose SC treatment regimens. In rodent models, ALN-CC5 demonstrated >90% C5 silencing with >80% inhibition of classical pathway serum hemolytic activity with a mouse single dose ED50 of 0.25 mg/kg. In cynomolgus macaques, >97% C5 silencing and >80% lowering of complement serum hemolytic activity were observed with a multi-dose schedule. ALN-CC5 was found to be safe and well tolerated in a preliminary rat toxicology study. Silencing of murine C5 was highly efficacious in a mouse model of anti-collagen antibody-induced arthritis, demonstrating a prominent role for liver derived C5 in joint destruction. Evaluation of C5 silencing in additional animal disease models is ongoing to further validate the utility of an RNAi therapeutic approach to C5 inhibition.

**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 (ALN-TTRrc, ALN-AT3, ALN-CC5, ALN-PCSsc, other programs)**
  - siRNA conjugated to N-acetylglactosamine (GalNAc) ligand
  - Efficient delivery to hepatocytes following subcutaneous administration
  - “Enhanced stabilization chemistry” (ESC) used with ALN-AT3, ALN-CC5, ALN-PCSsc, and other programs
  - Significantly improved potency and durability compared with ALN-TTRsc

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**Figure 2. Sustained C5 knockdown and potent complement activity inhibition in NHP**

- Up to 96.8% inhibition of alternative pathway (CAP) activity (mean 94.6 ± 1.8%), and up to 91.3% inhibition of hemolysis (mean 84.9 ± 7.1%)

**Figure 3. C5 level and anti-C5 complement inhibition**

- **Figure 4. C5 silencing reduces proteinuria in the rat membranous nephropathy model**

- **Figure 5. C5 level and anti-C5 antibody**

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We would like to thank Dr. David Salant of Boston University for the anti-FX1a antiserum and Elizabeth Chlipala of Premier Laboratory (Boulder, CO) for arthritis histology scoring.

**Summary**

- Nephritis induced by injection of sheep anti-rat kidney fraction antiserum (anti-Fx1A)

- C5 levels for all animals were quantified

- Proteinuria was quantified using a turbidity assay on overnight urine collection samples from rats single-housed in metabolic cages. Baseline urine samples were collected on day -2

- Relationship between C5 and anti-C5 antibody

- Consistent C5 knockdown during inflammation

- Demonstrates potential for anti-C5 mAb sparing approach

- Equivalent to anti-C5 antibody

- Rapid liver uptake of GalNAc conjugates has potential advantages in indications with increased kidney clearance
Robust knockdown of serum C5 with SC dosing in NHP; Ongoing study
- Two dose regimens evaluated
  - Q2W Regimen: Every other week dosing (5 mg/kg, qw x 6, q2w thereafter)
  - QM Regimen: Every month dosing (5 mg/kg, qd x 5, qw x 6/10 mg/kg qm thereafter)

In line with results observed in published reports on eculizumab
- Both classical and alternative pathways of complement activity greatly reduced in serum of NHPs treated with ALN-CC5
- Up to 96.8% inhibition of alternative pathway (CAP) activity (mean 94.6 ± 1.8%), and up to 91.3% inhibition of hemolysis (mean 84.9 ± 7.1%)
- In rodent models, ALN-CC5 demonstrated >90% knockdown associated with up to 96.8 % inhibition of serum complement activity
- Robust knockdown in C5 siRNA treated animals
- Consistent C5 knockdown during inflammation
- Efficacy on both disease activity and joint histology
- Conserved across species
- Highly expressed in hepatocytes
- RNAi knockdown of liver-derived C5 sufficient for therapeutic efficacy in mouse arthritis model
- Defines innovative therapeutic strategy for complement-mediated diseases
- Wide therapeutic index; up to 250 mg/kg per week well tolerated in initial safety studies in rodents and NHP

Evaluation of C5 silencing and serum hemolytic activity inhibition were evaluated in rodents using single and multi-dose SC treatment regimens. In rodent models, ALN-CC5 demonstrated >90% silencing of C5 following subcutaneous (SC) injection. The liver produces essentially the entirety of C5 and other complement pathway proteins.

Both classical and alternative pathways of complement activity greatly reduced in serum of NHPs treated with ALN-CC5
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- In line with results observed in published reports on eculizumab

Figure 2. Evaluation of C5 Silencing and Complement Activity inhibition with ALN-CC5 in Non-Human Primates. Groups of 3 cynomolgus monkeys were treated with two regimens of ALN-CC5. Q2W Regimen: every other week maintenance (5 mg/kg, qw x 6, q2w thereafter); QM Regimen: every month maintenance (5 mg/kg, qd x 5, qw x 6/10 mg/kg qm thereafter). Serum samples were collected throughout the study. (A) Serum C5 levels quantified by ELISA, values plotted as % knockdown of serum C5 relative to the 3 bleeds collected prior to the initial dose. Insert graph shows levels of C5 in individual animals (blue Q2W regimen, purple QM regimen). (B) Complement activity assays (QM Regimen), classical pathway activity was assessed using the sensitized sheep RBC hemolysis assay, values are normalized to the “maximal lysis control” for the assay. Alternative pathway activity (CAP) was quantified using the Westlab ELISA-based assay by detecting the formation of the C5b-9 neo-antigen with LPS as an activator. Values were normalized to the level of CAP activity in the 3 pre-dose samples. Error bars are SEM. (C) Relationship between C5 silencing and complement activity assays. R² value derived using a modified Hill equation curve-fit.

NHP Efficacy Conclusions
- Knockdown of cyno C5 with long-term SC treatment with ALN-CC5 results in up to ~99% silencing of serum C5
  - Both Q2W and QM regimens maintain C5 silencing
  - Ongoing study
- C5 knockdown associated with up to 96.8 % inhibition of serum complement activity
  - Complement pathway ELISA assays and sensitized sheep RBC hemolysis assay demonstrate robust inhibition of both classical and alternative pathway activity with knockdown of liver derived C5
Figure 3. C5 Silencing equivalent to anti-C5 antibody in a mouse CAIA model

**A** Disease Activity

Circulating liver-derived C5 key driver of pathology in mouse CAIA
- Little or no role for locally produced C5
- Up-regulation of serum C5 observed during CAIA study

**B** Serum C5

- Robust C5 knockdown in C5 siRNA treated animals
- No modulation of C5 silencing by LPS (injected on day 3)

**C** Histology

C5 silencing preserves joint histology and prevents C3 deposition equivalent to anti-C5 antibody

**D** C3 Deposition

Figure 3. C5 Silencing in a mouse collagen antibody-induced arthritis model. Groups of 6-7 mice were treated SC with 5 mg/kg "siRNA-C5" or "siRNA-Ctr" on day -5, 0 and 5 or IV with 50 mg/kg anti-C5 Mab (BB5.1) on day 0. Arthrogen (Col Ab) was injected IP on day 0 followed by LPS injection on day 3. (A) Clinical disease activity was scored daily by a blinded observer, data shown represent a meansSEM. 85.2% decrease in CDA for siRNA-C5 and 86.4% decrease for anti-C5 Ab relative to PBS. (B) C5 levels for all animals were quantified by ELISA. Values are normalized to the day -5 levels for each animal, meansSEM are plotted. (C) Histopathological scoring (scored 0-5, 5=normal, 5-severe) for inflammation, pannus formation, and cartilage and bone damage for 7 joints from each animal was done following tissue processing and H&E and Toluidine Blue staining. Average Mean Joint (AJM) for each animal was determined and meansSEM for each group are plotted. AJM values show 99% correlation with CDA score. (D) C3 deposition scoring performed on sections from 7 joints per animal following IHC with anti-mouse C3 antibody (score 0-3, 0-normal, 3-severe). AJM for C3 deposition in the synovium and on the surface of cartilage as well as the total C3 deposition score are plotted as meansSEM for the treatment groups. 90% reduction in C3 deposition with C5 silencing or anti-C5 antibody treatment.
Circulating liver-derived C5 key driver of pathology in mouse CAIA

- Knockdown of cyno C5 with long-term SC treatment with ALN-CC5 results in up to ~99% silencing of serum C5

Relationship between C5 silencing and complement activity assays. Dose samples. Error bars are SEM. (C using the Weislab ELISA-based assay by detecting the formation of the C5b-9 neo-antigen with LPS as an activator. Values were normalized to the level of CAP activity in the 3 pre-

Figure 2. Robust knockdown of serum C5 with SC dosing in NHP; Ongoing study

- Highly expressed in hepatocytes

Evaluation of C5 silencing in additional animal disease models is ongoing to further validate the utility of an RNAi therapeutic approach to C5 inhibition.

C5. C5 silencing and serum hemolytic activity inhibition were evaluated in rodents using single and multi-dose SC treatment regimens. In rodent models, ALN-CC5 demonstrated >90% C5 knockdown associated with up to 96.8% inhibition of serum complement activity

Figure 3. C5 Silencing equivalent to anti-C5 antibody in a mouse CAIA model

- Low inter-animal variation
- Up to 98.7% silencing of C5
- Significant improvement in potency and durability compared with ALN-TTRsc

Figure 5. C5 level and anti-C5 complement inhibition

- Linear relationship between C5 serum level and concentration of anti-C5 antibody needed for 80% hemolysis suppression

Figure 6. C5 vs anti-C5 mAb Inhibition

- Nephritis induced by injection of sheep anti-rat kidney fraction antiserum (anti-Fx1A)
- 70.5% reduction in proteinuria with C5 silencing in rat model of Passive Heymann Nephritis
  - Control rats receive no siRNA or anti-Fx1A treatment

Figure 4. C5 silencing reduces proteinuria in the rat membranous nephropathy model

- Proteinuria was quantified using a turbidity assay on overnight urine collection samples from rats single-housed in metabolic cages. Baseline urine samples were collected on day -2 prior to anti-FX1a injection and had levels of proteinuria equivalent to that of the control group. Mean ± SEM for the 4 animals per group plotted. C5 silencing was confirmed by anti-C5 Western and quantification of C5 mRNA levels in the liver (not shown).
- Linear relationship between C5 serum level and concentration of anti-C5 antibody needed for 80% hemolysis suppression
- 95% silencing of C5 allows for use of ~20x lower levels of antibody to achieve equivalent amount of hemolysis inhibition
  - Demonstrates potential for anti-C5 mAb sparing approach

**Figure 5. C5 level and anti-C5 complement inhibition**

- **A** C5 vs anti-C5 mAb Inhibition
- **B** C5 vs 80% Hemolysis Inhibition

Figure 5. C5 Level and Hemolysis inhibition in presence of anti-C5 antibody. (A) Human C5 depleted serum was reconstituted with different amounts of purified human C5 protein and hemolytic activity in the sensitized sheep RBC hemolysis assay was assessed in presence of different amounts of anti-C5 antibody. (B) Relationship between C5 and anti-C5 antibody concentration needed to achieve 80% inhibition of hemolysis.
Summary

ALN-CC5 enables robust knockdown of serum C5 in NHP with SC dosing
- GalNAc-conjugate RNAi platform is promising approach for knockdown of liver-expressed target genes
  - ALN-CC5, an ESC-GalNAc conjugate targeting C5, identified
    - Sustained lowering of plasma C5 and complement pathway activity in NHPs with q2w and qm dosing regimens
  - Lower dose/less frequent dosing expected in humans with ESC-GalNAc conjugates
- RNAi knockdown of liver-derived C5 sufficient for therapeutic efficacy in mouse arthritis model
  - Efficacy on both disease activity and joint histology
  - Equivalent to anti-C5 antibody
  - Consistent C5 knockdown during inflammation
- RNAi knockdown of C5 protective in rat model of membranous nephropathy
  - Rapid liver uptake of GalNAc conjugates has potential advantages in indications with increased kidney clearance
- Wide therapeutic index; up to 250 mg/kg per week well tolerated in initial safety studies in rodents and NHP
- Defines innovative therapeutic strategy for complement-mediated diseases
- ALN-CC5 IND/IND equivalent filing in late 2014; Initial clinical data in mid 2015

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