Reduction of Hepatic Factor XII Expression in Mice by ALN-F12 Inhibits Thrombosis without Increasing Bleeding Risk

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Investigational RNAi Therapeutics
A Potential New Class of Innovative Medicines

Harness natural pathway of RNAi
- RNAi = RNA interference
- Catalytic mechanism
- Mediated by small interfering RNA or “siRNA”

Therapeutic gene silencing
- Any gene in genome
- Distinct mechanism of action vs. other drug classes
- Unique opportunities for innovative medicines

Clinically validated platform
- Human POC across multiple targets in healthy subjects and multiple clinical indications*

* Zimmermann TS et al. Mol Ther. 2017; 25(1): 71-78
ALN-F12: An Investigational RNAi Therapeutic Targeting FXII

- siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand
- Efficient delivery to hepatocytes following subcutaneous (SC) administration
- Targets the F12 gene encoding for FXII
- Robust activity in NHPs
  - ED50 < 1 mg/kg; ED80 < 3 mg/kg
  - >50% reduction for up to 3 months at 3 mg/kg
FXII: A Potential Target for the Treatment of Thrombosis

- Serine protease, auto-activated by contact with negatively charged surfaces
  - PolyP, protein aggregates, DNA, RNA, etc.
- FXIIa activates FXI and triggers fibrin formation (intrinsic coagulation)
- FXII deficiency (Hageman trait) is not associated with disease
  - Increased aPTT with no bleeding disorder
- FXII inhibition prevents thrombosis in various venous and arterial thrombosis models in mouse, rat, rabbit and baboon
  - Antisense oligonucleotides, mAb, Corn Trypsin Inhibitor (CTI), Infestin-4
- FXII primarily expressed in hepatocytes, amenable to GalNAc-siRNA


Preclinical Evaluation of ALN-F12 for Thromboprophylaxis
Overview of Mouse Thrombosis and Hemostasis Models

Thrombosis Models

• Venous Electrolytic Injury
  ◦ Electrolytic “shock” injury allows precise control of thrombosis initiation
  ◦ Fluorescently labeled platelets & fibrin enable real time imaging of platelet & fibrin deposition

• Arterial Ferric Chloride Injury (10%)
  ◦ Redox-induced endothelia cell injury
  ◦ Measure time to occlusion

Hemostasis Models

• Saphenous Vein Bleeding
  ◦ Calculate average hemostatic time during 30 minute observation period

• Tail Tip Transection
  ◦ Measure time to occlusion following injury
ALN-F12 Inhibits Venous Thrombosis in Mice
Venous Electrolytic Injury Model

- Single SC Dose
- 10 days post dosing
- N=8 per group
F11-siRNA Inhibits Venous Thrombosis in Mice
Venous Electrolytic Injury Model

- Single SC Dose
- 10 days post dosing
- N=8 per group
ALN-F12, F11-siRNA Inhibit Venous Thrombosis in Mice
Real Time Images of Fibrin and Platelet Deposition in Electrolytic Injury Model

Control

ALN-F12

F11-siRNA

Red: Fibrin
Green: Platelet
ALN-F12 Inhibits Arterial Thrombosis in Mice
FeCl₃-induced Arterial Thrombosis Model

- Single SC Dose
- 10 days post dosing
- N=8 per group
ALN-F12 Does NOT Impair Hemostasis
No Bleeding Phenotype at > 95% FXII or FXI Reduction

• Single SC Dose
• 10 days post dosing
• N=8 per group

One-way ANOVA: n.s., not significant; ***, p<0.001
Summary

Reduction of FXII by ALN-F12 Prevented Thrombosis Without Increased Bleeding Risk in Rodent Models of Thrombosis and Hemostasis

- ALN-F12 reduced liver F12 mRNA and plasma FXII in a dose dependent manner in rodents and NHPs
- ALN-F12 mediated reduction of FXII prevented platelet and fibrin accumulation in the Venous Electric Injury thrombosis model
  - Dose-dependent effect
  - Reduction of FXII >95% led to ~10 fold reduction in fibrin deposition
  - Reduction of FXI (>95%) also reduced platelet and fibrin accumulation (~5 fold reduction in fibrin deposition)
- ALN-F12 mediated reduction of FXII inhibited FeCl3 induced arterial thrombosis
- > 95% reduction of F12 or F11 had no impact on bleeding time or blood loss (Saphenous Vein Bleeding, Tail Tip Transection),
- Reduction of plasma FXII by ALN-F12 represents a promising approach for the prophylactic treatment of thrombosis