



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

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Alnylam Pharmaceuticals

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Investigational RNAi Therapeutics

A Potential New Class of Innovative Medicines

Harness natural pathway of RNAi

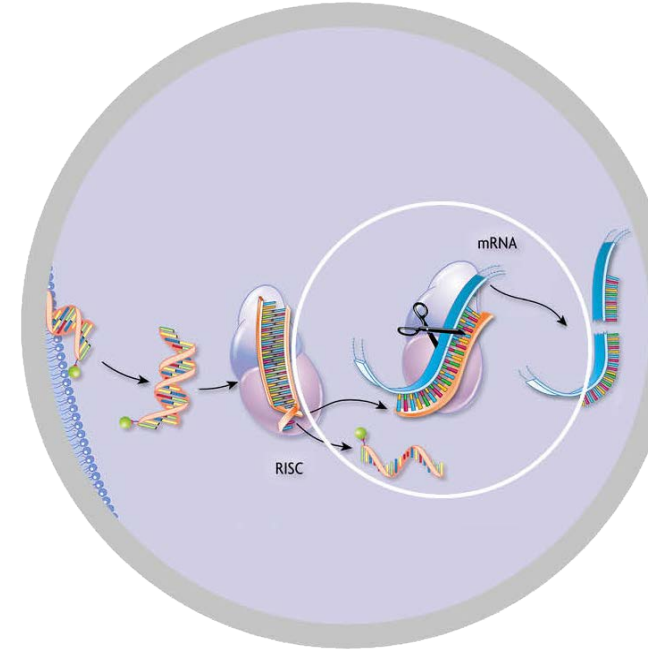
- RNAi = RNA interference
- Catalytic mechanism
- Mediated by small interfering RNA or “siRNA”

Therapeutic gene silencing

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

Clinically validated platform

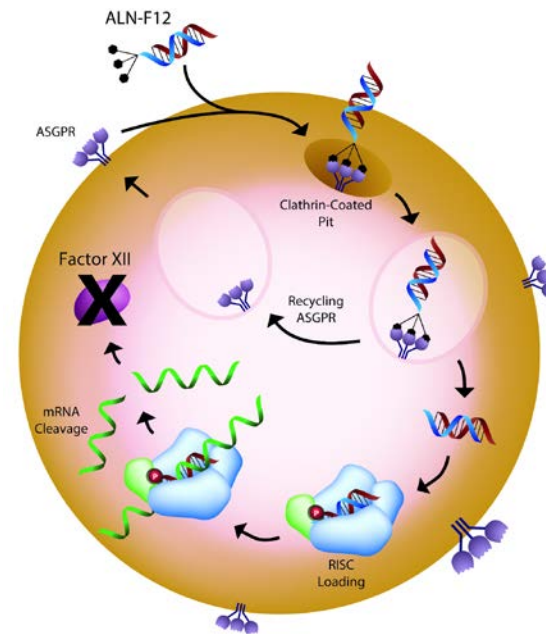
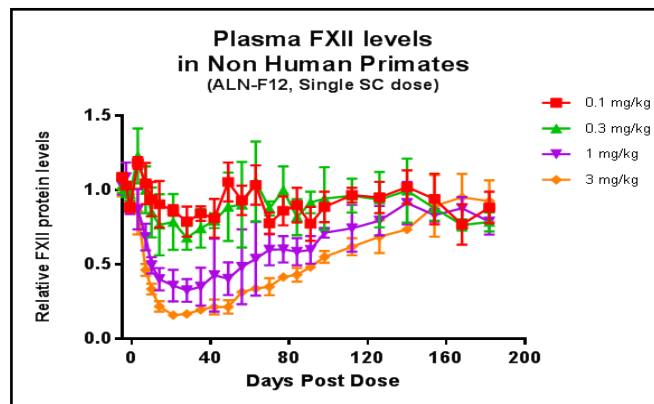
- Human POC across multiple targets in healthy subjects and multiple clinical indications*



* Zimmermann TS et al. *Mol Ther.* 2017; 25(1): 71-78
Fitzgarald K et al. *N Engl J Med.* 2017; 376(18): e38

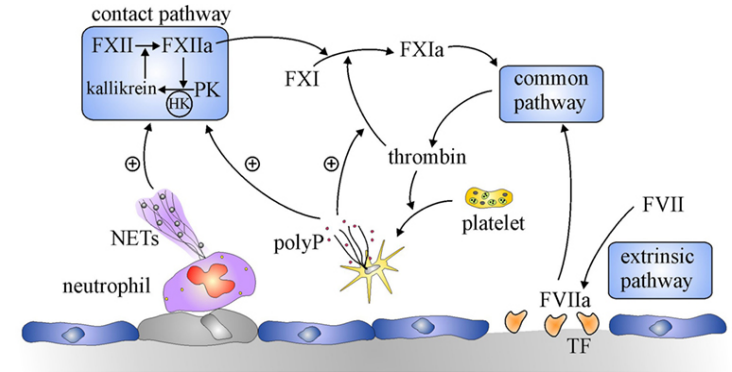
ALN-F12: An Investigational RNAi Therapeutic Targeting FXII

- 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



Weitz & Fredenburgh, *Front Med*, 2017, 4:19

Nickel KF et al. *Art Thromb Vasc Biol*. 2017; 37(1): 13-20
Kenne E et al. *J Intern Med*. 2015; 278(6): 571-85
Yau JW et al. *Blood* 2014; 123(13): 2102-7
Matafonov A et al. *Blood* 2014; 123(11): 1739-46

Preclinical Evaluation of ALN-F12 for Thromboprophylaxis

Overview of Mouse Thrombosis and Hemostasis Models

Thrombosis Models

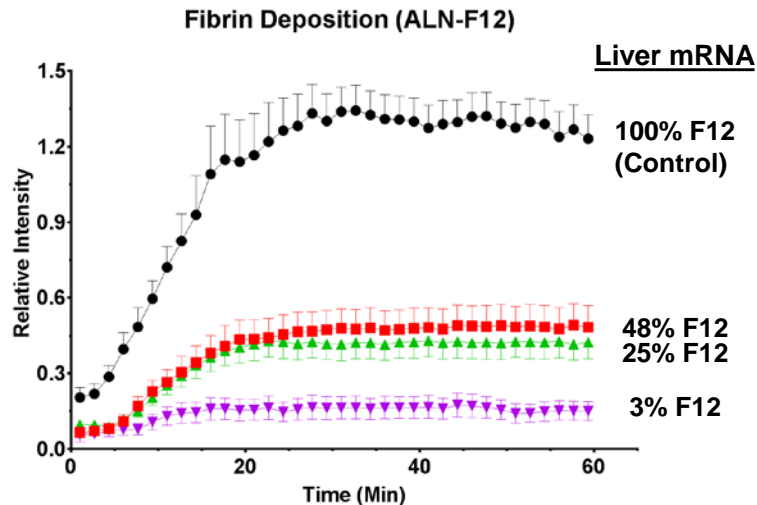
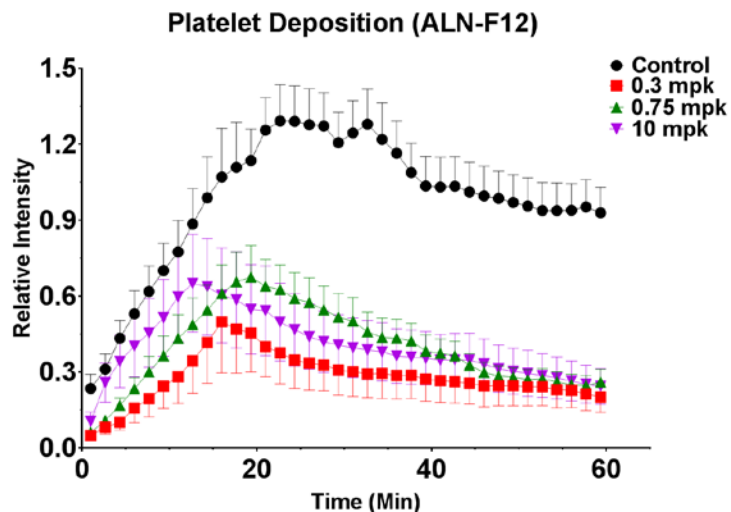
- Venous Electrolytic Injury
 - Electrolytic “shock” injury allows precise control of thrombosis initiation
 - Fluorescently labeled platelets & fibrin enable real time imaging of platelet & fibrin deposition
- Arterial Ferric Chloride Injury (10%)
 - Redox-induced endothelial cell injury
 - Measure time to occlusion

Hemostasis Models

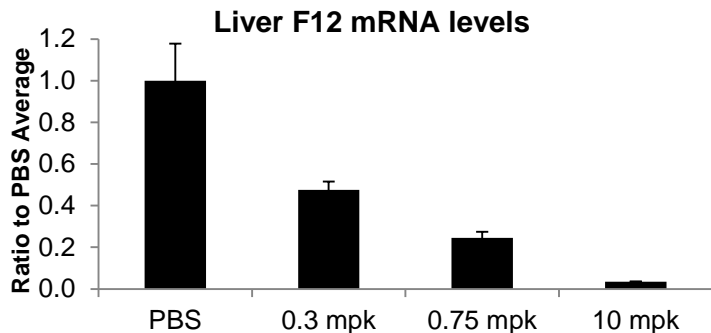
- Saphenous Vein Bleeding
 - Calculate average hemostatic time during 30 minute observation period
- Tail Tip Transection
 - Measure time to occlusion following injury

ALN-F12 Inhibits Venous Thrombosis in Mice

Venous Electrolytic Injury Model

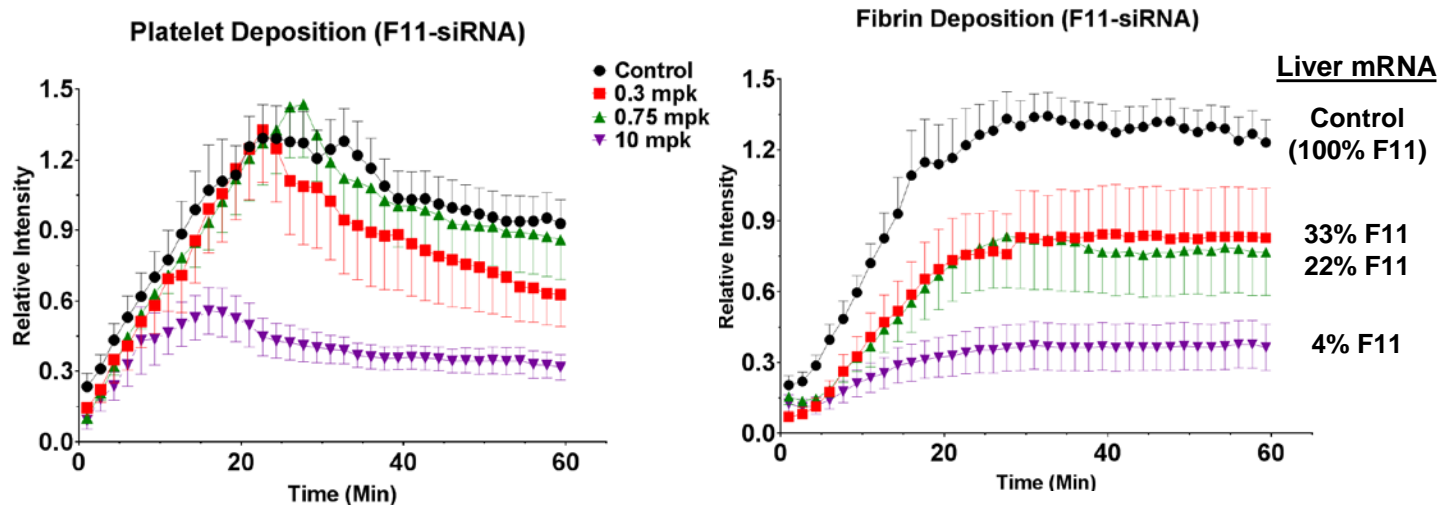


- Single SC Dose
- 10 days post dosing
- N=8 per group

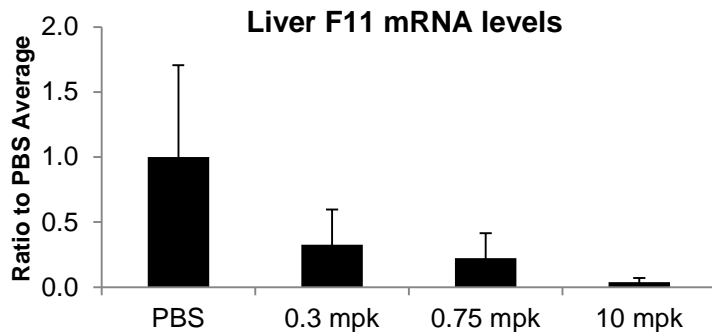


F11-siRNA Inhibits Venous Thrombosis in Mice

Venous Electrolytic Injury Model



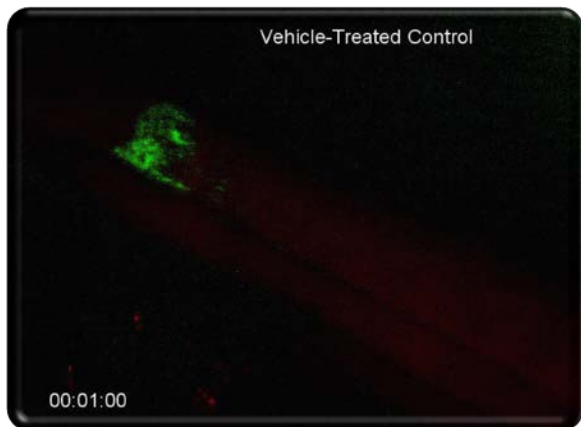
- Single SC Dose
- 10 days post dosing
- N=8 per group



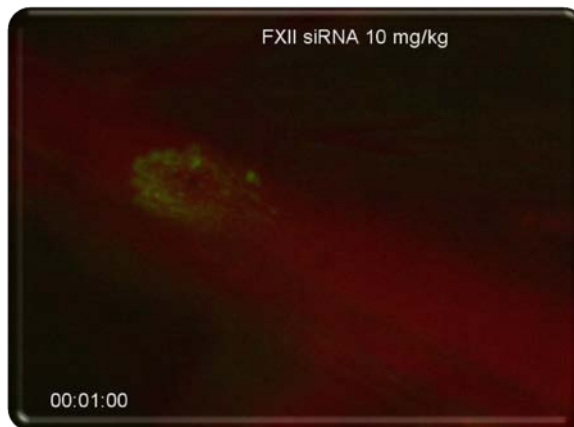
ALN-F12, F11-siRNA Inhibit Venous Thrombosis in Mice

Real Time Images of Fibrin and Platelet Deposition in Electrolytic Injury Model

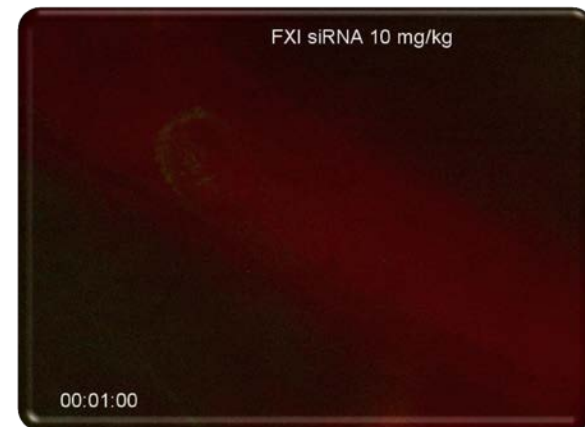
Control



ALN-F12



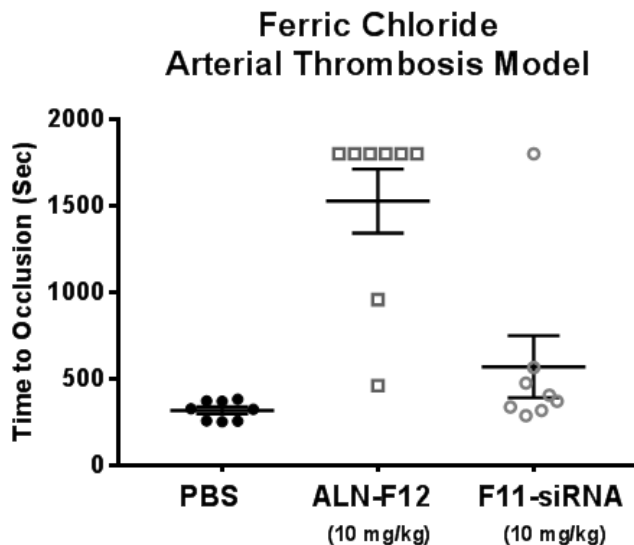
F11-siRNA



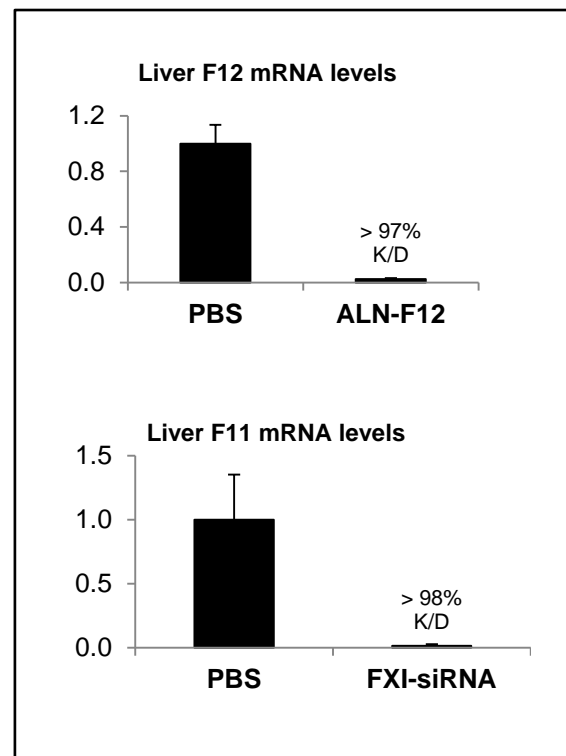
Red: Fibrin
Green: Platelet

ALN-F12 Inhibits Arterial Thrombosis in Mice

FeCl₃-induced Arterial Thrombosis Model

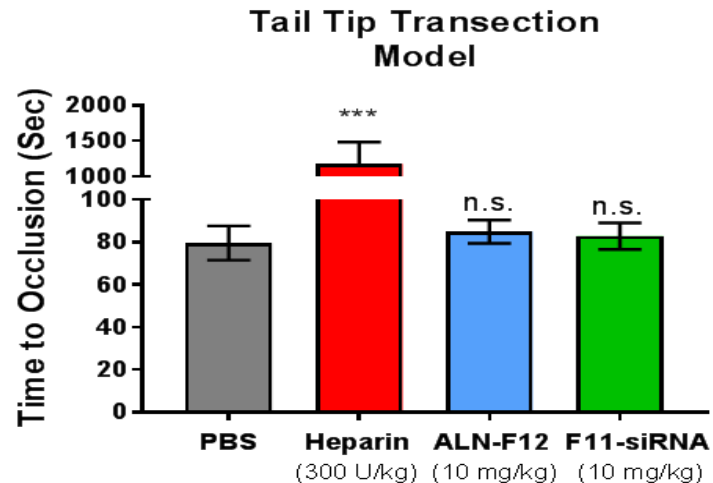
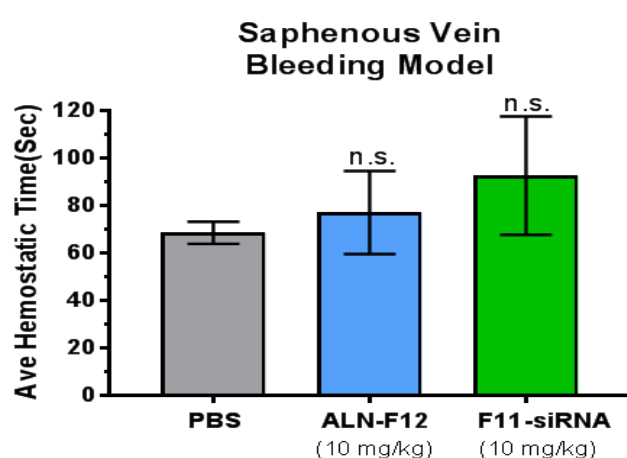


- Single SC Dose
- 10 days post dosing
- N=8 per group



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**