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RNAi-Mediated Inhibition of a Natural Anticoagulant for the Treatment of Hemophilia

OTS 2012—Session VI: RNAi/ASO Preclinical Studies

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Hemophilia

- X-linked, recessive bleeding disorders caused by deficiency of functional clotting factors VIII and IX

	Hemophilia A	Hemophilia B
Percent of Hemophilic Population	80%	20%
Deficient Clotting Factor	Factor VIII (FVIII)	Factor IX (FIX)
Approximate Incidence[1]	1 in 5,000 male births	1 in 20,000 male births
	400 new cases each year	
Reported number of Hemophilia Patients in US/EU[2]	~41,000	~9,600

1. The Haemophilia A Mutation, Structure, Test and Resource Site - http://hadb.org.uk/
2. WFH Global survey 2009

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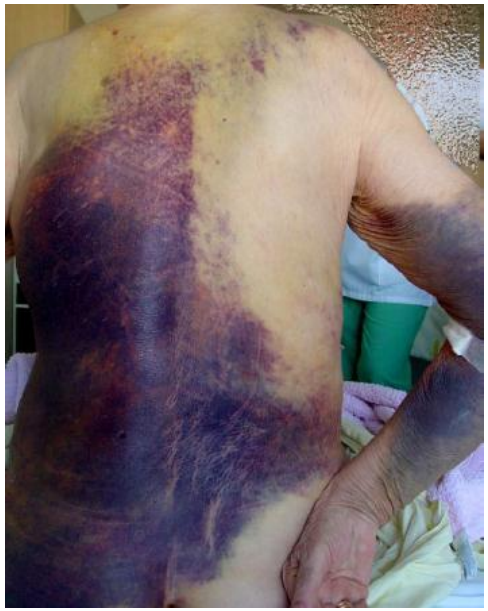
Medical Impact

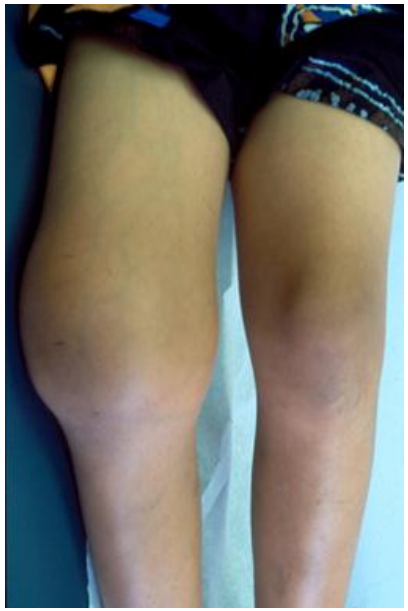
- Typically do not suffer from hemorrhage from minor cuts or abrasions due to normal platelet count and function
- Availability (in developed nations) of safe plasma-derived and recombinant factor has lead to near normal life expectancies; however,
- Recurrent joint and soft-tissue bleeds leads to disabling arthropathy
- Potential life-threatening complications associated with surgery or acute trauma
- Development of inhibitors to replacement factors renders patients more risky and costly to treat

Soft Tissue Bleed



Collins *et al., BMC Research Notes*; **3**: 161 (2010)

Joint Bleed



www.hemophilia.org

Current Management and Unmet Need

Current Management

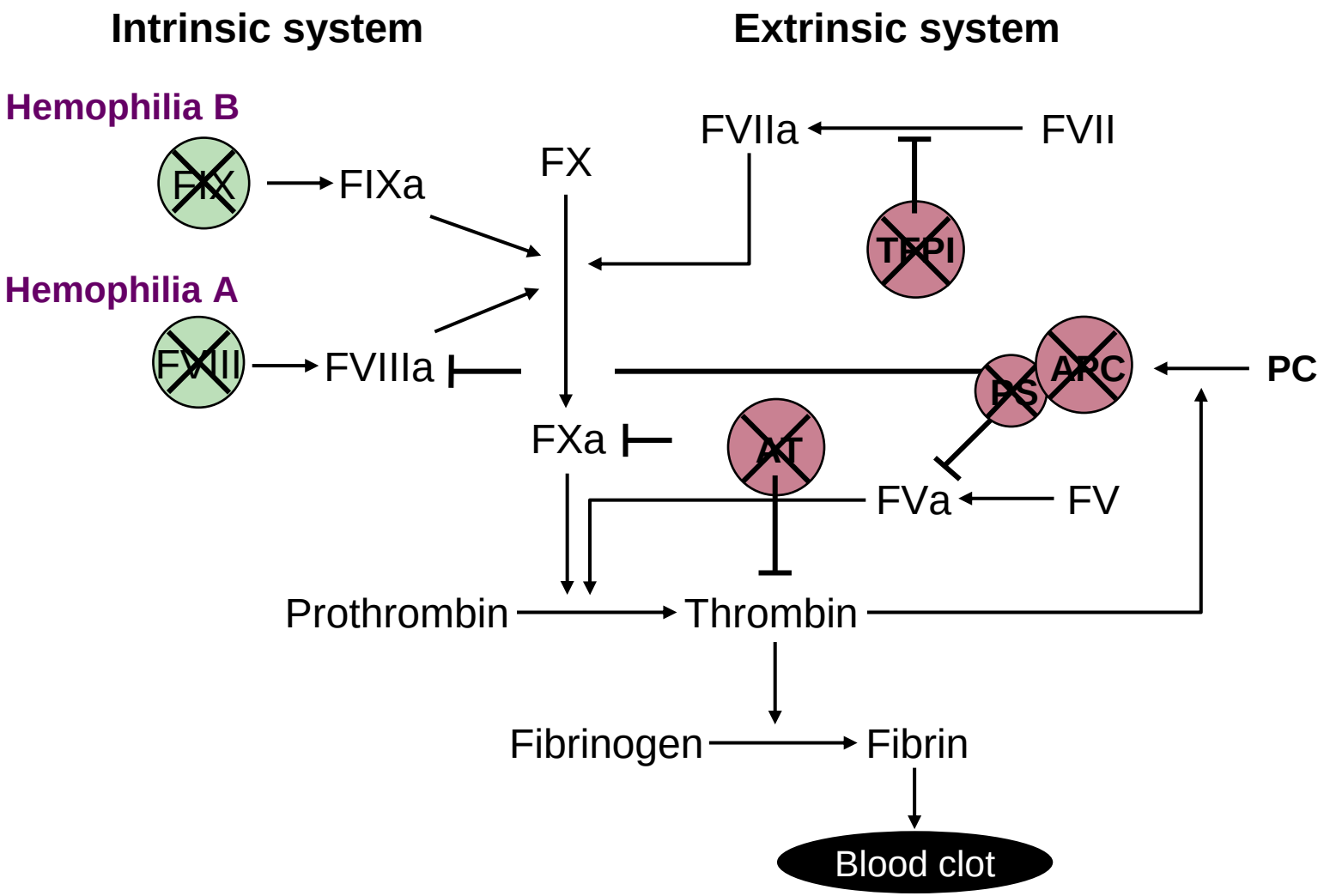
- Treated with replacement factors, either as “on-demand” or prophylaxis
- Prophylaxis is considered standard of care in developed countries for severe hemophilia, particularly for pediatric population
  - » Typically 2-3 times per week IV infusion (w/ target 1% trough level)
  - » Prevention of bleeds protects joint function
- Patients with inhibitors are most challenging to treat
  - » Immune tolerance induction (ITI) therapy may be attempted to clear inhibitor
  - » Generally treated with “bypass agents” (rFVIIa, APCCs)
    - – Prophylaxis not feasible due to short half-life and cost of bypass agents

Unmet Need

- Treatment of inhibitor patients is area of greatest unmet need
  - » Feasible prophylaxis option is needed to prevent arthropathy
  - » ITI very demanding, costly protocol and not always successful
  - » Bypass agents very costly and have short half-life
- Prophylaxis is costly and inconvenient due to need for frequent IV infusion
  - » Frequent IV access can require ports, particularly in children, sometimes resulting in complications (e.g. infection)
  - » Target factor trough levels of 1% are not sufficient to prevent all bleeds
  - » Cost and frequent IV infusion protocol is an impediment for adoption of prophylaxis by some adults
- Effective therapies for the developing world

Therapeutic Hypothesis

Rebalancing the Hemostatic System

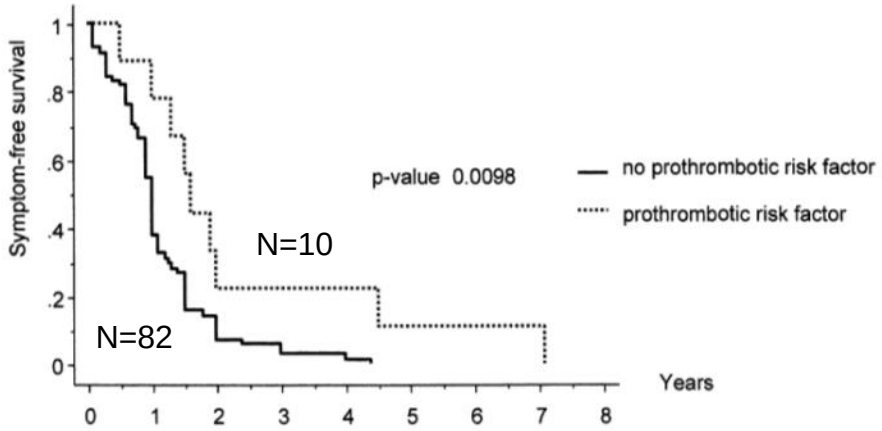


Prothrombotic Factors in Hemophilia

Co-inheritance of Prothrombotic factors and hemophilia

- Prothrombotic factors include Factor V_{Leiden} (FVL), Prothrombin G20210A, Protein C deficiency, Protein S deficiency
- Number of reports of hemophilia patients with co-inheritance of prothrombotic factors with milder disease, e.g. reduced bleeding episodes, arthropathy and FVIII requirements

Symptom-free survival in children with severe hemophilia A (HA) with and without additional prothrombotic risk factors

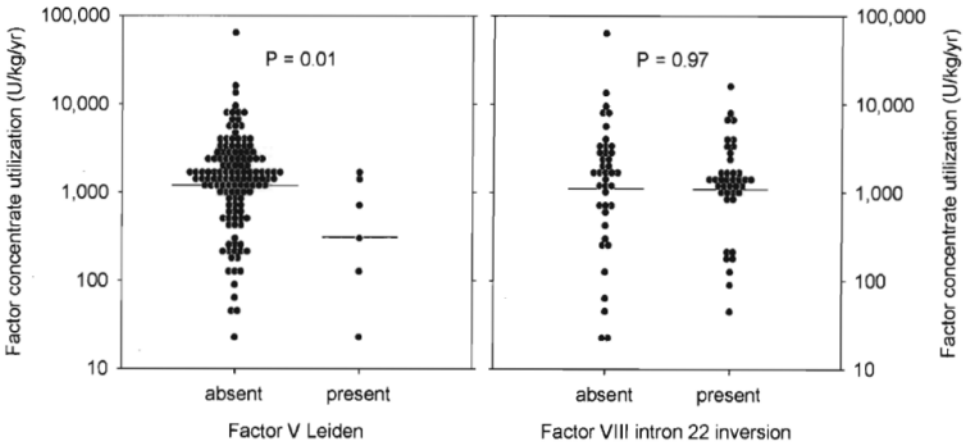


Median age of first symptomatic bleed in severe HA:

- 1.6 years in children with prothrombotic factors:
  - » FVL (6)
  - » Prothrombin (3)
  - » Protein C (type I) deficiency (1)
- 0.9 years in non-carriers

Escuriola-Ettingshausen *et al., Thromb Hemost*; 85: 218-220 (2001)

Impact of FVL
On Severe Hemophilia A Phenotype



Maximum bleeding frequency	Factor V Leiden non-carriers (n = 131)	Factor V Leiden carriers (n = 6)
0 - 10/yr	14	3
> 10/yr	117	3

Lee *et al., Thromb Haemost*; 83: 387-391 (2000)

Table 2. Patient characteristics.

	No thrombophilia	*With thrombophilia*	*p value**
Year of birth	1990 [1991-1999]	1991 [1982-1999]	0.54
Age at first bleeding: years [median/range]	0.9 [0.1-4.0]	1.5 [0.5-7.1]	0.009
Therapy given: number [%]			
on demand	58 [63.0]	8 [53.3]	0.67
prophylaxis	34 [37.0]	7 [46.7]	
Start of prophylactic regimen: Median/range values (years)	1.3 [0.1-6.7]	1.9 [0.8-7.0]	0.44
Factor concentrates used [%]			
pdFVIII	27.3	33.3	0.33
rFVIII	48.5	55.5	
vWFVIII	24.2	11.2	
Annual bleeding frequency	6 [0-30]	1.8 [0-7]	0.012

Kurnik *et al., Hematologica*; 92: 982-985 (2007)

Natural Anticoagulant Factors

Anticoagulant	Mechanism	Genetics	Expression
Protein C	• Inactivates FVa and FVIIIa • Cytoprotective functions; gene expression, anti-inflammatory effects, antiapoptotic effects and protecting endothelial barrier	• Heterozygous PC deficiency associated with increased risk for venous thromboembolism (VTE)	Hepatocytes
Antithrombin	• Direct stoichiometric inhibitor of thrombin and FXa	• Heterozygous AT deficiency associated with increased risk for VTE	Hepatocytes
Protein S	• Cofactor of APC for inactivation of FVa and FVIIIa	• Heterozygous PS deficiency associated with increased risk for VTE	Mainly hepatocytes and vascular endothelium
TFPI	• Binds to FXa, and forms inhibitory quaternary complex with FVIIa-TF	• None described	Mainly vascular endothelium

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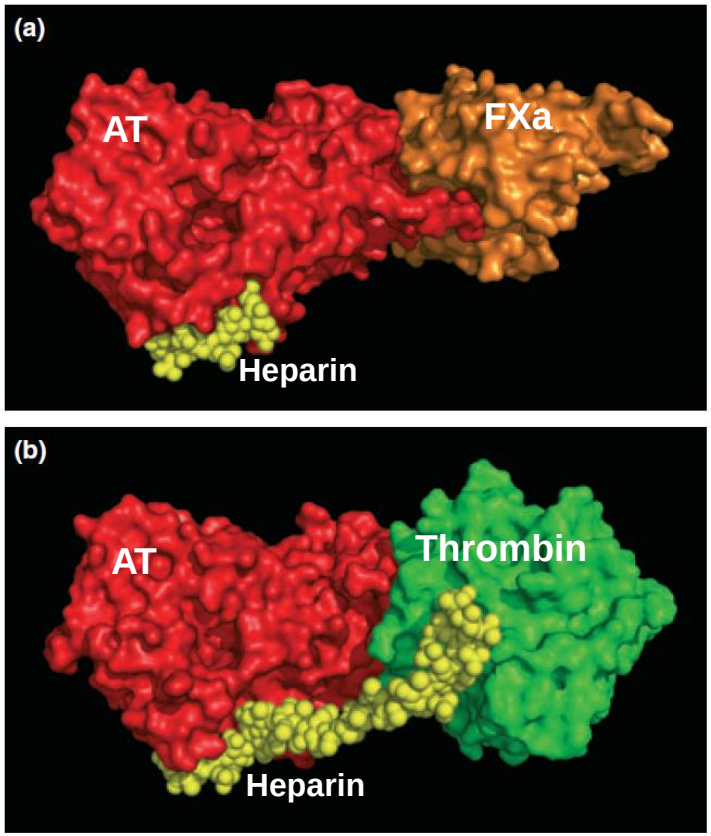
Antithrombin
Inhibitor of Thrombin and FXa

Antithrombin is hepatocyte-expressed serpin

- Encoded by SERPINC1 on Ch1
- Abundant plasma glycoprotein (58 kDa)

Anticoagulant function

- Major role to inhibit thrombin and FXa
- Inhibits other activated factors VIIa, IXa, XIa, and XIIa to lesser degree
- Forms inhibitory stochiometric complex with serine protease
- Activity greatly enhanced by heparin cofactor
  - » Results in ternary complex with thrombin, enhances thrombin inhibition rate 2000-fold
  - » Induces conformational change, enhances FXa inhibition rate 500-1000-fold



Paitnak and Moll, *Haemophilia*; 14, 1229-1239 (2008)

Human Genetics of Antithrombin Deficiency

Incidence

- Estimated between 1 in 500 and 1 in 5000
- AT deficiency classified as Type I (quantitative) or Type II (qualitative) defects
  - » Type I and Type II defects make up 12% and 88% of cases of AT deficiency, respectively
  - » However, Type I defects represent up to 80% of symptomatic cases of AT deficiency

Phenotype

- Homozygous deficiency is almost always fatal *in utero*
- Heterozygous deficiency is associated with 40-60% of normal AT activity levels
- AT deficiency is associated with the highest VTE risk among inherited thrombophilias
- Risk of VTE depends on heavily on personal history, family history, and subtype of AT deficiency
  - » Approximately 40-60% of incidence attributable to other transient risk factors (e.g. surgery, pregnancy, puerperium, immobilization, oral contraceptives)
  - » Asymptomatic patients have ~1.7%/year risk of VTE
  - » Patients with incidence of VTE not on long-term anticoagulation have 10-17%/year risk of recurrence
  - » Patients with incidence of VTE on long-term anticoagulation have 2.7%/year risk of recurrence

Treatment

- Asymptomatic patients are not on long-term thromboprophylaxis
- Patients with incidence of VTE are considered for long-term thromboprophylaxis
- Standard thromboprophylaxis for surgery and immobility

Paitnak and Moll, *Haemophilia*; 14, 1229-1239 (2008)
Bucciarelli *et al*., *Arterioscler Thromb Vasc Biol*; 19: 1026-1033 (1999)

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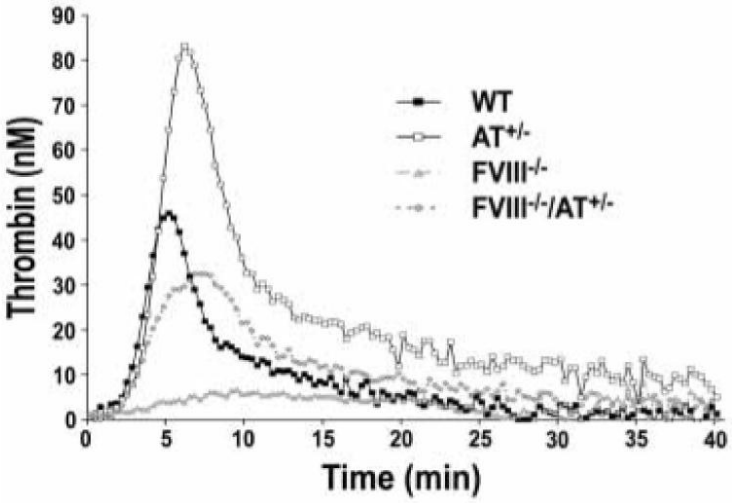
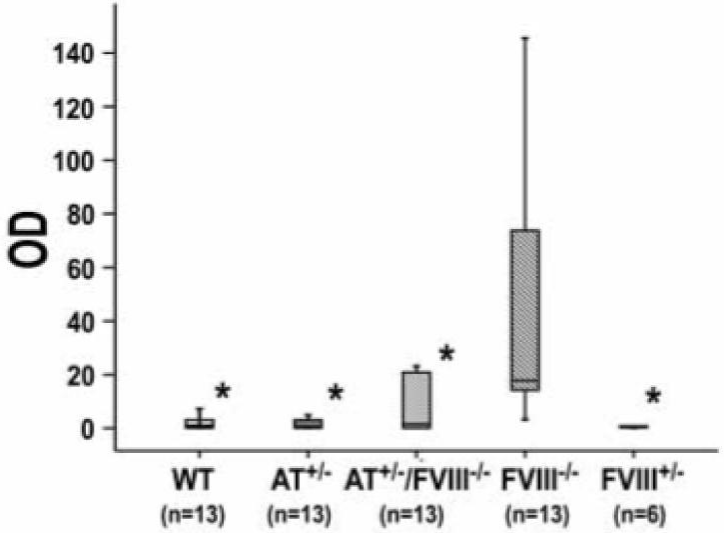
Proof of Concept in Mouse Model of Hemophilia

Increased Thrombin Generation



Reduced Blood Loss



	WT	AT$^{+/-}$	FVIII$^{-/-}$	FVIII$^{-/-}$/AT$^{+/-}$	FVIII$^{+/-}$
	(n=15)	(n=15)	(n=15)	(n=15)	(n=5)
Lag time (min)	2.3 ± 0.9	2.8 ± 1.1	1.9 ± 4.0	1.2 ± 0.7	2.1 ± 2.1
Thrombin peak (nM)	45 ± 22§	58 ± 22§	10 ± 11*	29 ± 17	37 ± 26
ETP (nM)	600 ± 497§	831 ± 291§	94 ± 237*	303 ± 258	390 ± 291
Slope (nM/min)	10.5 ± 6.7§	15.1 ± 4.1§	2.0 ± 2.1*	4.1 ± 3.1*	8.3 ± 6.1

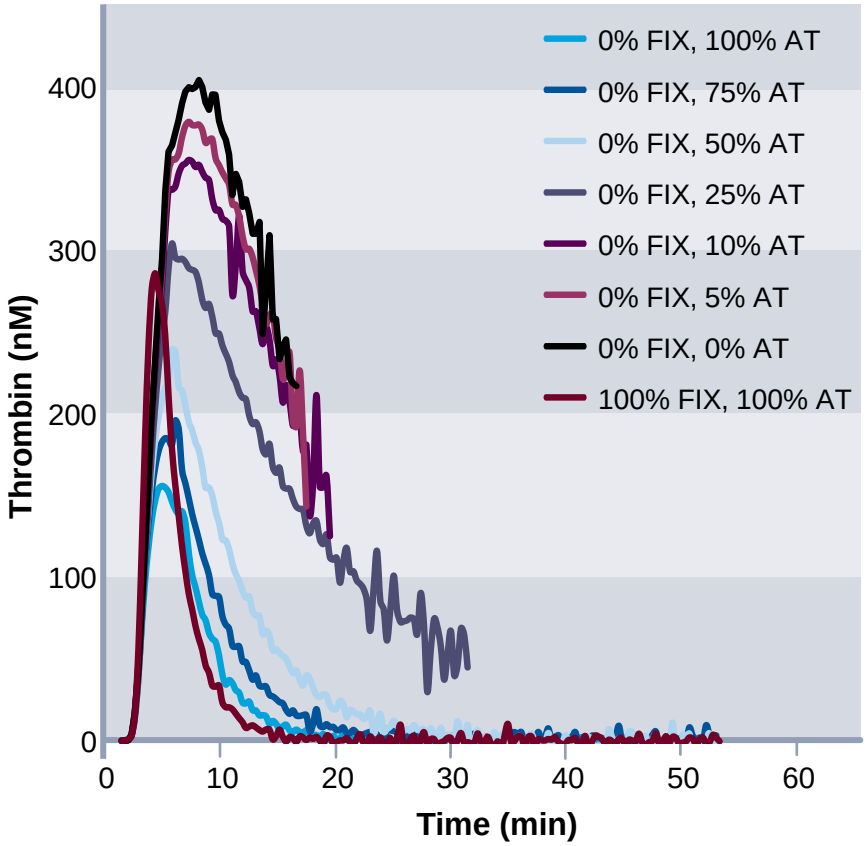
Values are mean ± SD. Intergroup differences were evaluated by ANOVA followed by Bonferroni's posthoc test. * = $p < 0.01$ vs. WT mice. § = $p < 0.01$ vs. FVIII$^{-/-}$ mice.

Bolliger *et al., Thromb Haemost*; 103:1233-1238 (2010)

Proof of Concept in Human Plasma Model of Hemophilia

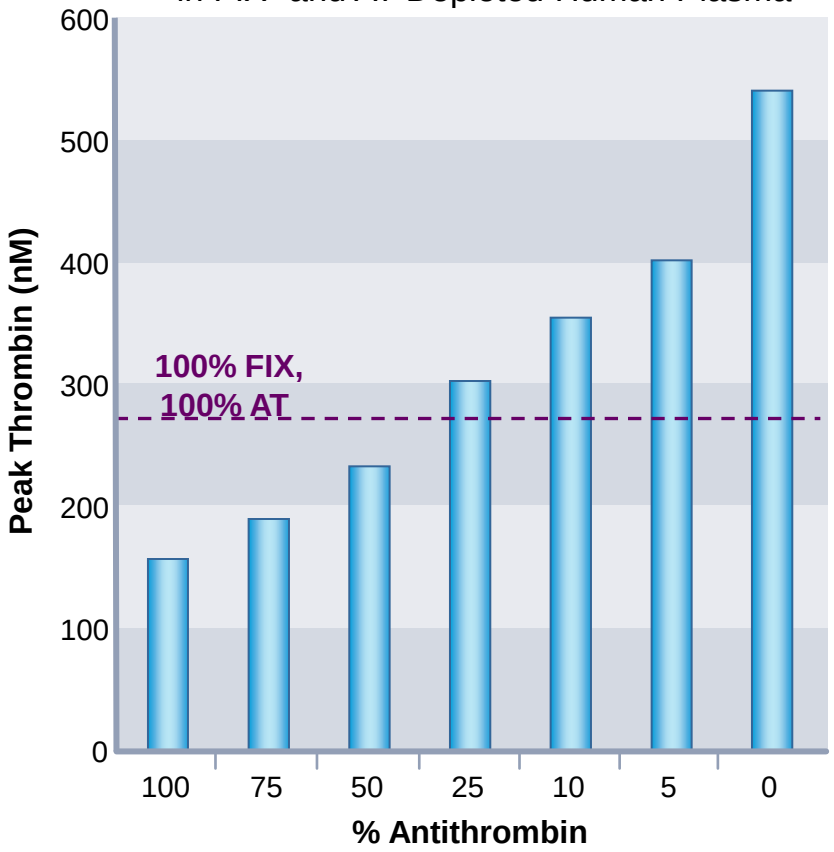
Thrombin Generation

in FIX- and AT-Depleted Human Plasma



Peak Thrombin

in FIX- and AT-Depleted Human Plasma



In collaboration with Haematologic Technologies, Inc.

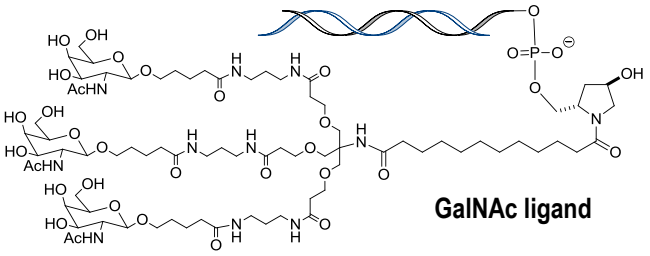
siRNA Conjugate Approach
For Targeted Delivery to Hepatocytes

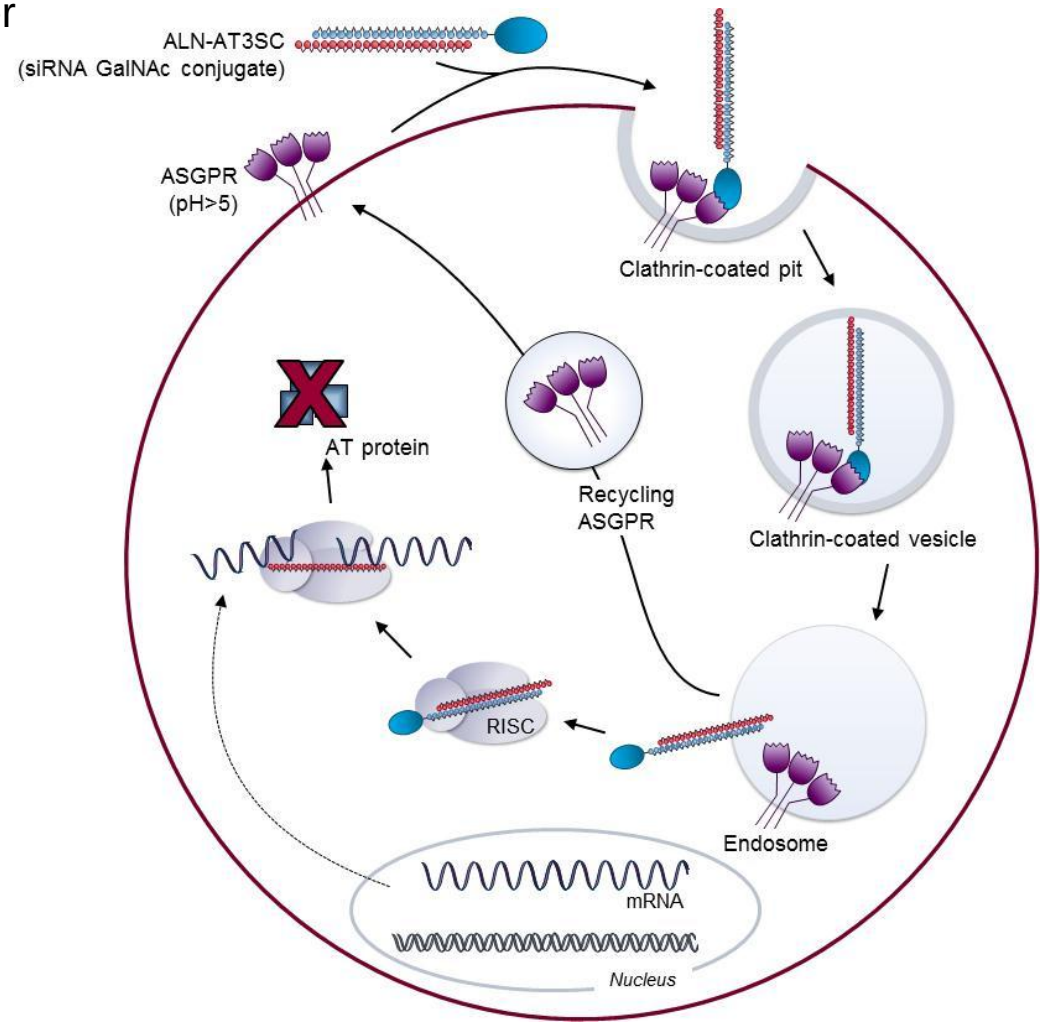
GalNAc-siRNA

- Trivalent GalNAc carbohydrate cluster has high affinity (nM) for ASGPR
- GalNAc ligand conjugated to chemically-modified, AT-targeting siRNA
- Administered subcutaneously (SC)

ASGPR

- Highly expressed in hepatocytes
  - » 0.5-1 million copies/cell
- Clears serum glycoproteins via clathrin-mediated endocytosis
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species



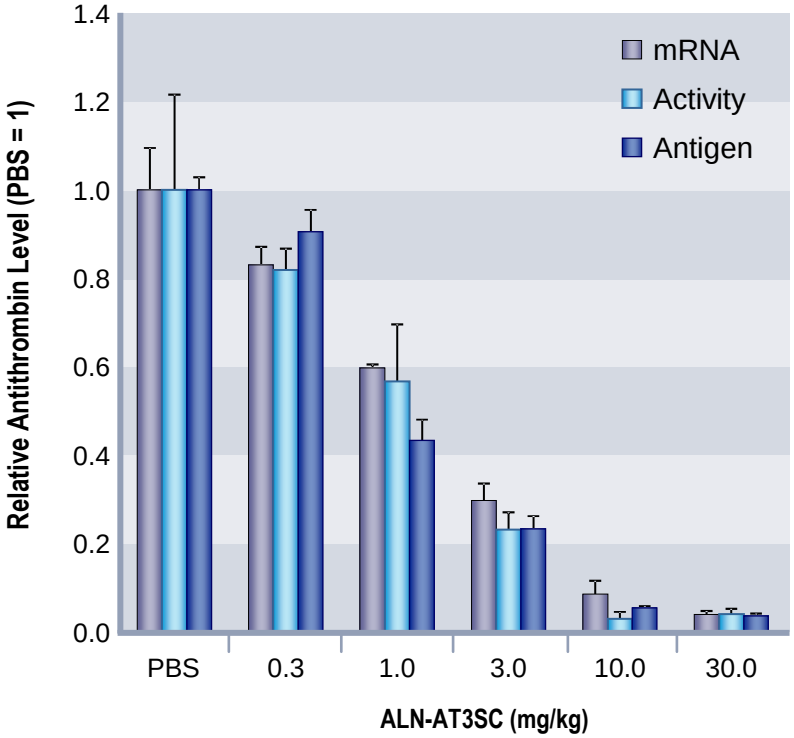


Administration of ALN-AT3

Potent and Durable Suppression of Antithrombin in Mice

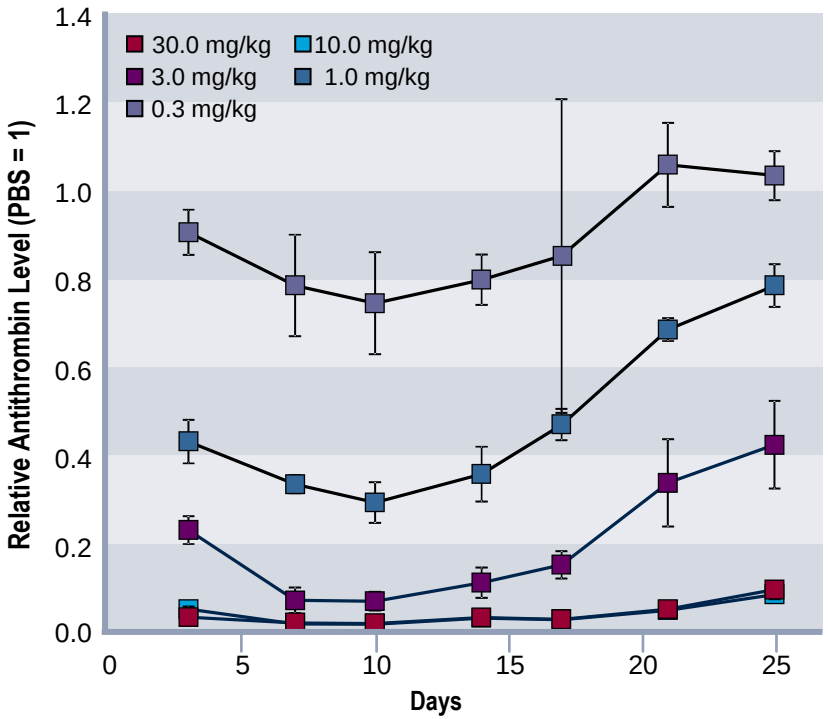
Dose Response

- Single subcutaneous dose
- N = 5, 72 hour time point
- AT protein measured in serum
- AT mRNA measured in liver



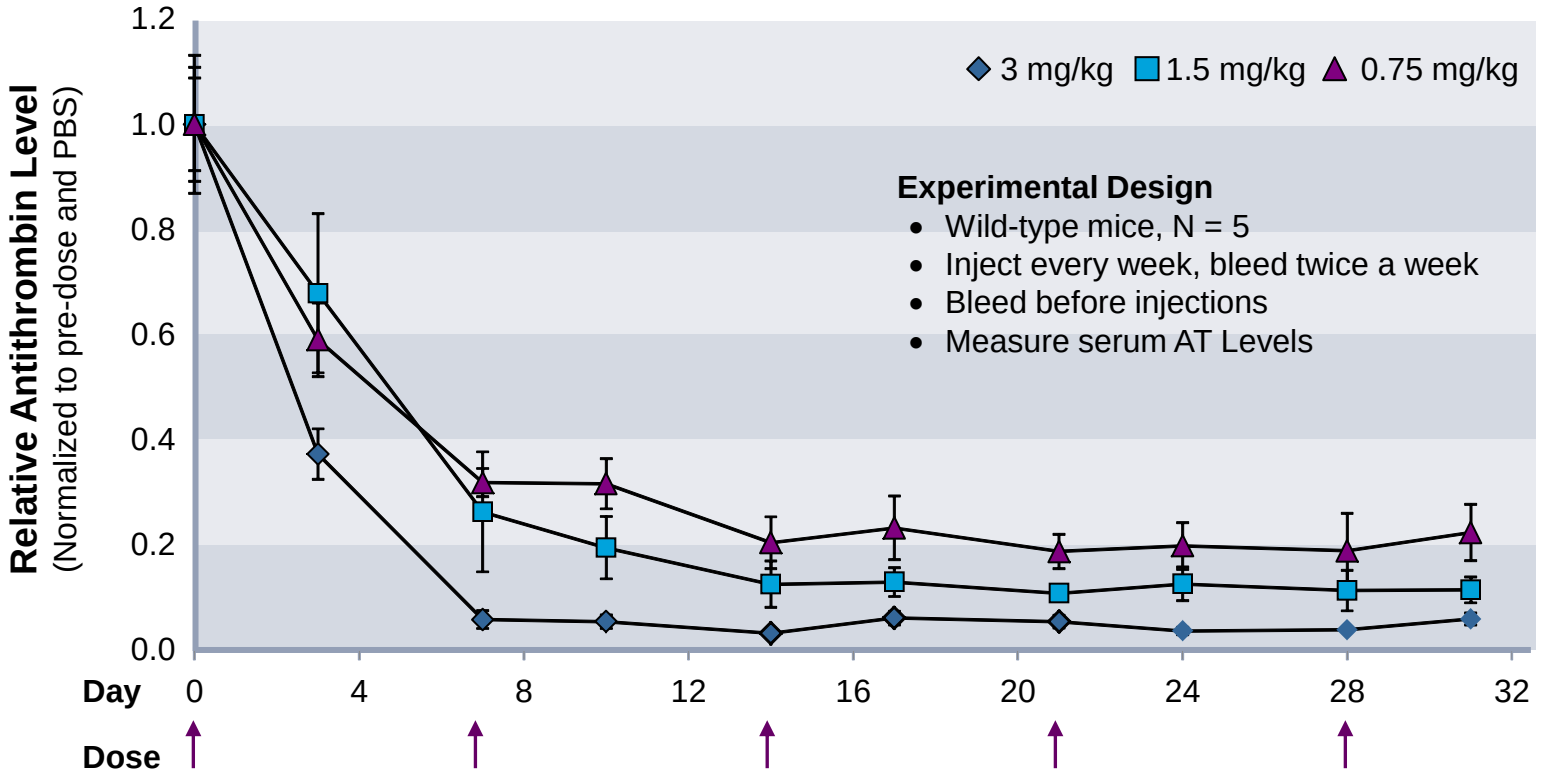
Duration

- Single subcutaneous dose
- N = 5
- AT protein measured in serum



Maintenance of AT Levels

Subcutaneous Weekly Injections

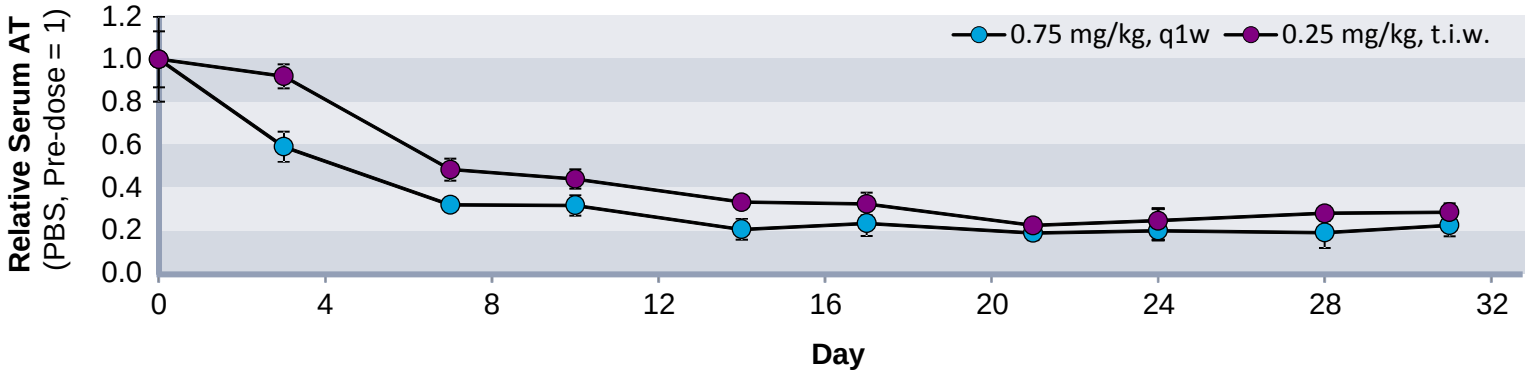


Weekly dosing results in sustained suppression of AT levels, with minimal deviations from steady-state levels

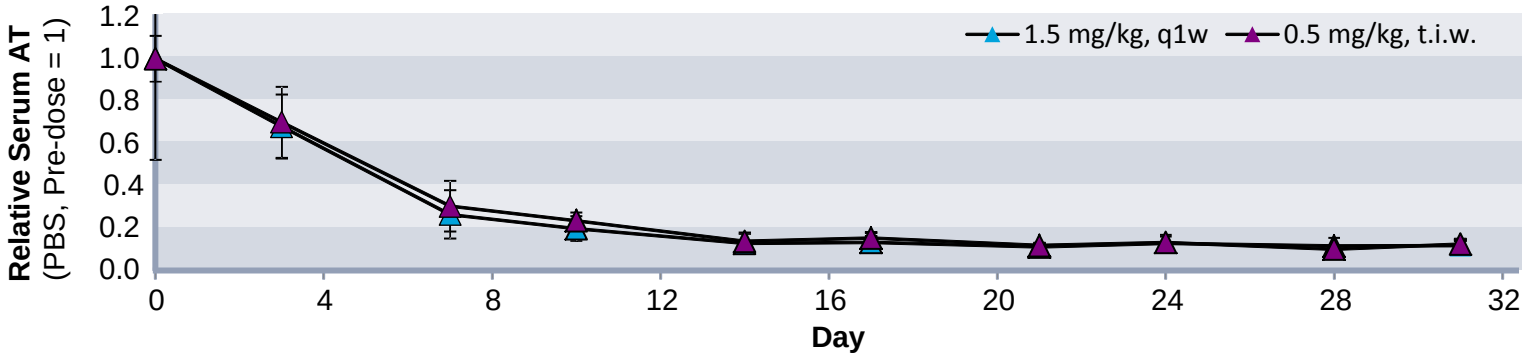
Weekly and 3X Weekly Dosing

Yields Similar Steady-State Silencing

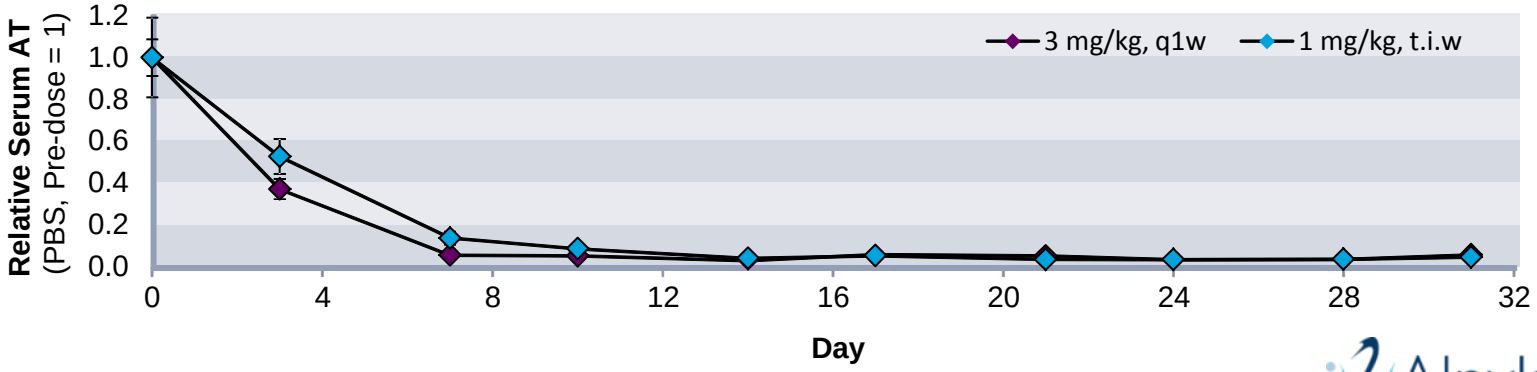
0.75 mg/kg cumulative weekly



1.5 mg/kg cumulative weekly



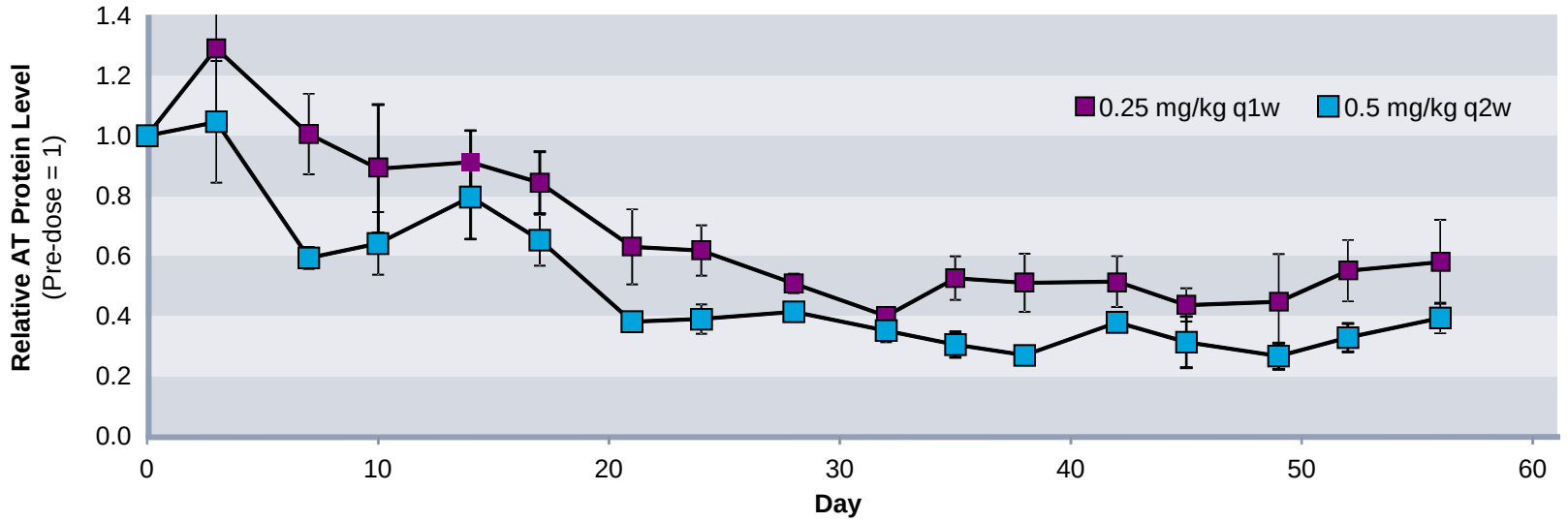
3 mg/kg cumulative weekly



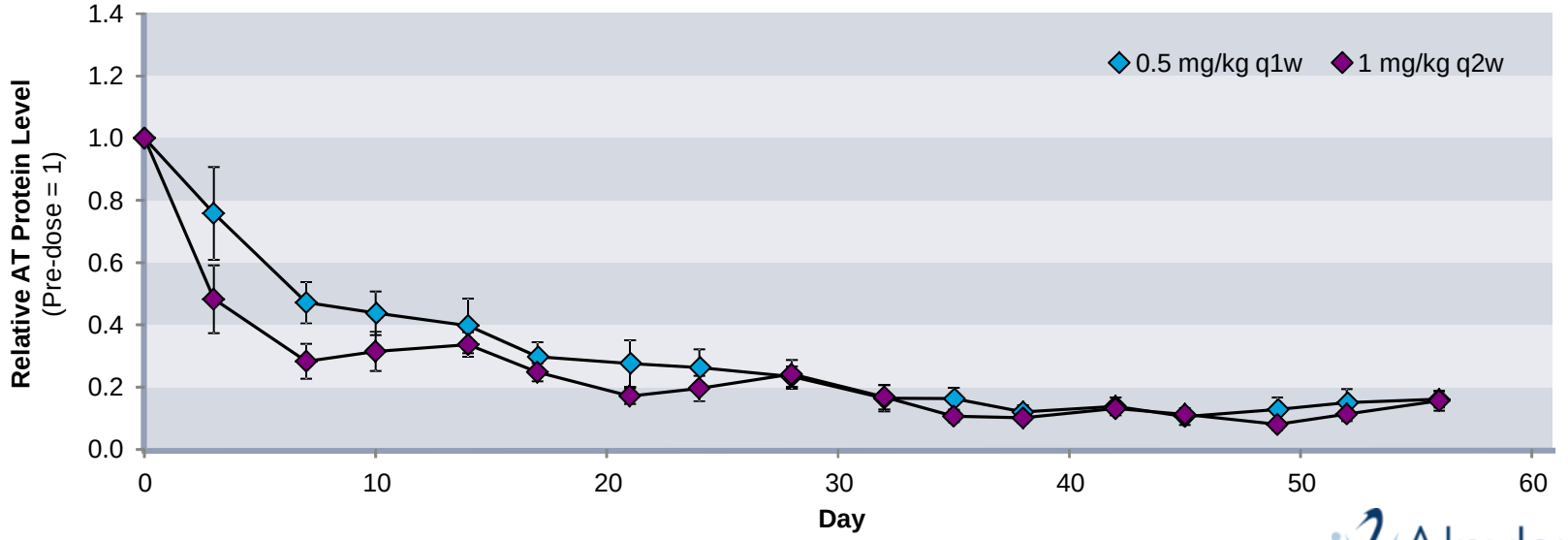
Weekly and Every Other Weekly Dosing

Yields Similar Steady-State Silencing

0.25 mg/kg cumulative weekly



0.5 mg/kg cumulative weekly



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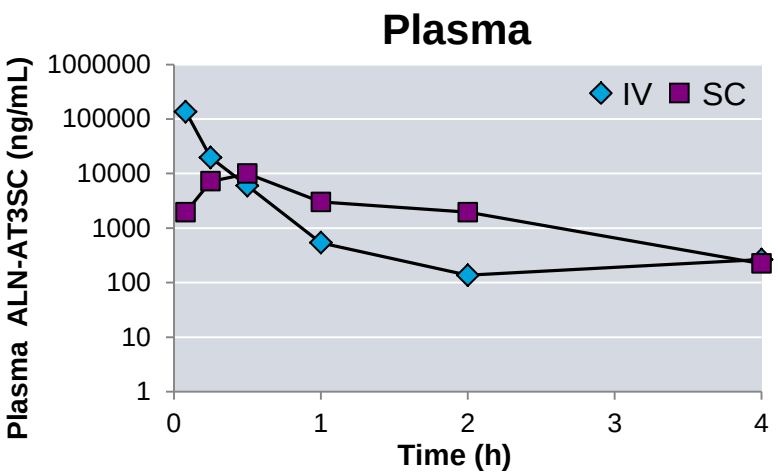
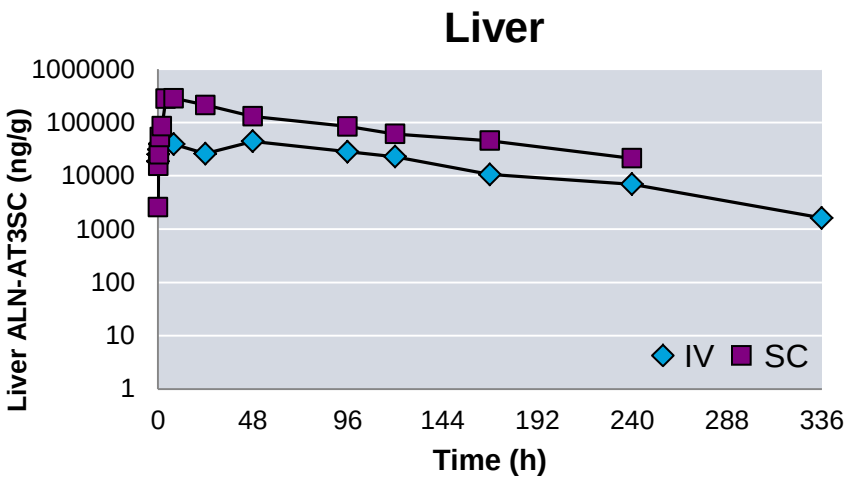
ALN-AT3 Mouse PK Data



Dose (mg/kg)	25	
Route	IV	SC
Tmax (h)	0.083	0.50
Cmax (µg/mL)	137	9.97
AUC_{0-t} (h·µg/mL)	37.9	10.9
MRT_{0-t} (h)	0.14	1.03
F_{sc} (%)	-	28.8



Dose (mg/kg)	25	
Route	IV	SC
Tmax (h)	2	8
Cmax (µg/g)	45.2	285
AUC_{0-t} (h·µg/g)	5881	21546
MRT_{0-t} (h)	95.5	72.0
AUC_{0-24} (h·µg/g)	843	5576
AUC_{0-48} (h·µg/g)	1684	9697
% Dose at Tmax	8.6 %	53 %

- ALN-AT3 persisted in liver to last time-point collected
- Exposure after SC administration was much higher after IV administration
- % of dose in liver after SC administration at Tmax was 53 %

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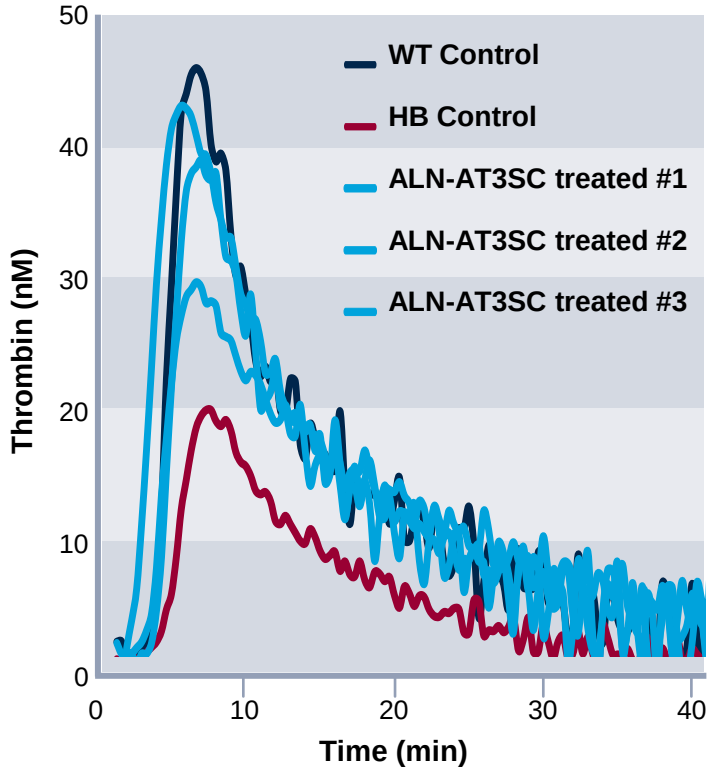
ALN-AT3 Activity in Hemophilia B Mice

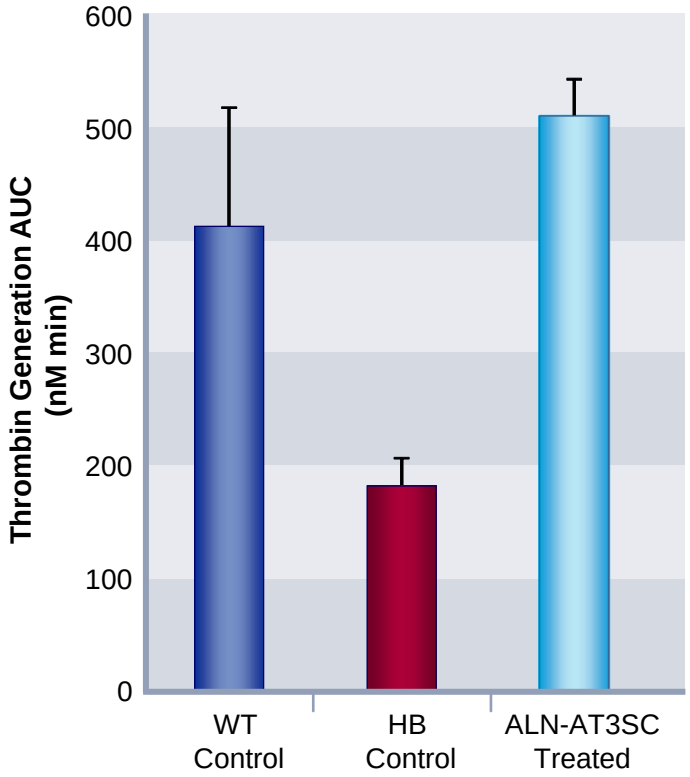
Hypothesis: AT knock-down should increase thrombin generation in hemophilic mice

- N = 3, hemophilia B (FIX $^{-/-}$) mice or wild-type mice
- Single subcutaneous injection at 30 mg/kg
- 72 hour time point
- Blood collected in sodium citrate with CTI
- TGA using Calibrated Automated Thrombinoscope (CAT)

Thrombin Generation in HB Mice



Normalization of ETP in HB Mice

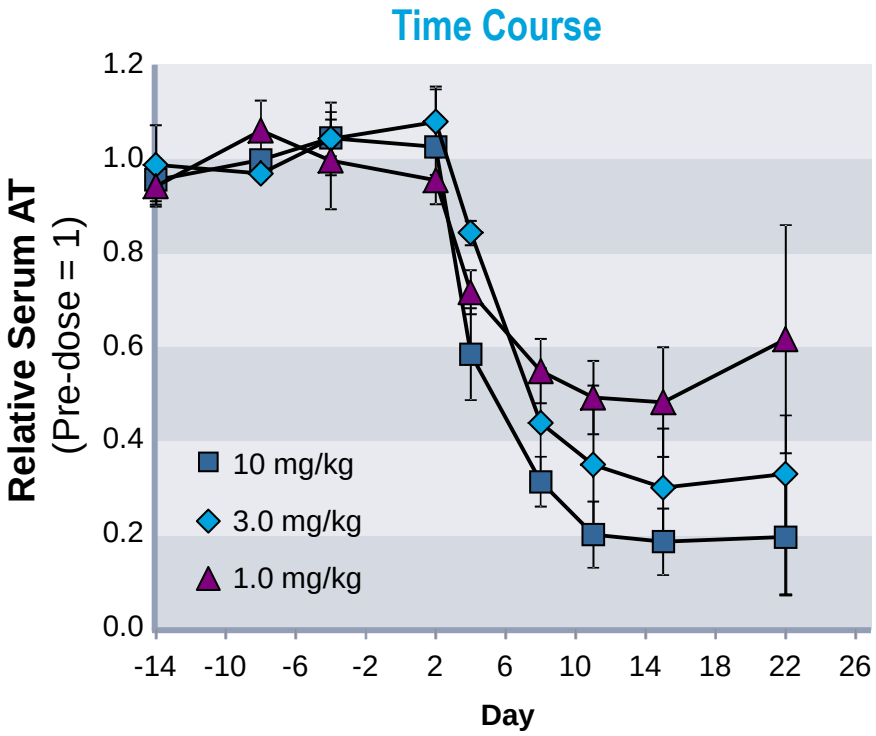


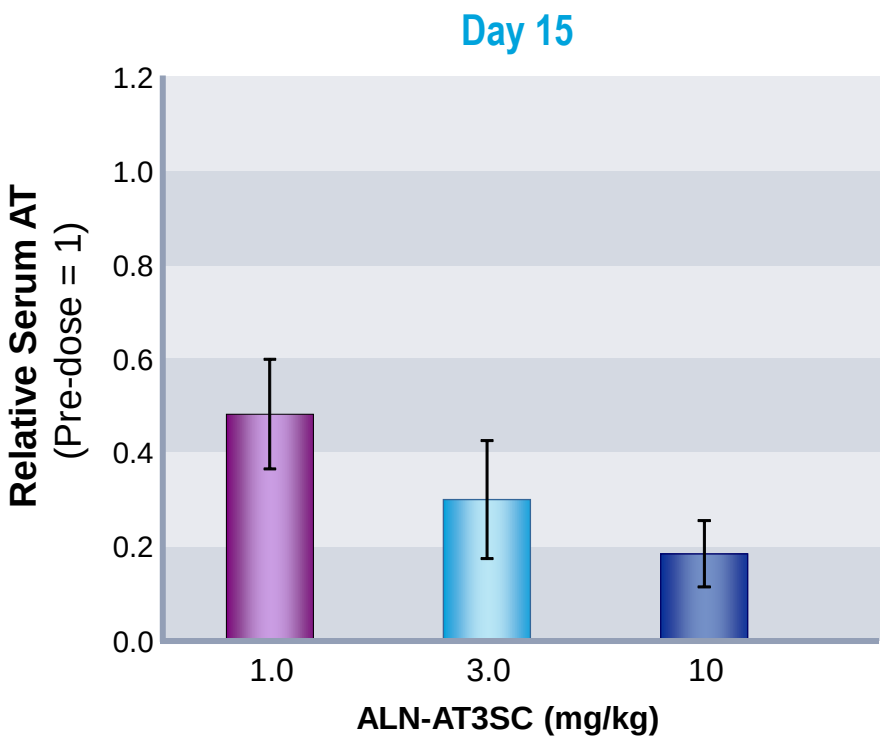
In collaboration with Dr. Claude Negrier

ALN-AT3 Activity in Nonhuman Primates

Experimental Design

- Wild-type cynomolgus monkeys, N = 3
- Single SC injection (1 mL/kg)





Summary

- ALN-AT3 treatment results in potent inhibition of AT in mice and NHPs
- Given long duration of action, twice monthly SC dosing is likely possible
- Proof-of-concept achieved in experimental settings with AT reduction
  - » Increased thrombin generation in human factor-depleted plasma
  - » Normalization of thrombin generation in hemophilia mouse
- Hemostatic rebalancing approach utilizing ALN-AT3 represents a potentially new prophylaxis therapy option in persons with hemophilia and rare bleeding disorders

Acknowledgements

Alnylam Pharmaceuticals

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