In Silico Modeling of the Coagulation Cascade and Thrombin Generation: Simulating Antithrombin (AT) Lowering in Hemophilia and Rare Bleeding Disorders (RBDs)

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Background: Hemophilia and Antithrombin (AT) lowering

- Hemophilia A and B are bleeding disorders characterized by insufficient thrombin generation due to deficiencies in factors VIII and IX, respectively.
- Fitusiran is a once-monthly, subcutaneously administered investigational RNAi therapeutic targeting antithrombin (AT) as a means to improve thrombin generation and promote hemostasis in patients with hemophilia A and hemophilia B with or without inhibitors (Pasi KJ et al. NEJM, 2017[1]).

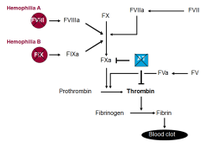

Figure 1: A simplified coagulation model. In hemophilia A and B the lack of factors VIII and IX, respectively, leads to a decrease in thrombin potential, resulting in a bleeding phenotype. Inhibiting antithrombin, a powerful natural anticoagulant in the pathway, has the potential to increase thrombin generation in hemophilia A or B and correct the bleeding phenotype.

- Patients on fitusiran experiencing breakthrough bleeding may require treatment with replacement factor; thus, a deeper understanding of the relationship between AT level, factor dosing, and thrombin generation (TG) is needed.

Methods: In Silico Modeling of Coagulation

- *In silico* Quantitative Systems Pharmacology (QSP) kinetic model describing coagulation cascade (Nayak et al.[2]) was used to simulate the *ex vivo* TG assay.
- Model is described by 66 Species, 66 Reactions, 106 Parameters and the initial plasma factor concentrations were varied to simulate hemophilia and RBD conditions.
- Since plasma factor concentrations vary ± 50% across individuals, we applied Monte Carlo methods to simulate normal range of TG:
  Mean - 140 nM; Range - 50-250 nM

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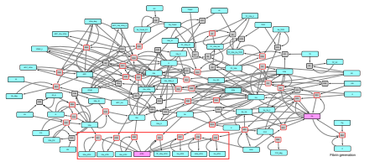

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Factor	Baseline, nM (normal healthy patient)	Condition	Range of Parameter Values, nM	Simulations Tested (% of Baseline)
ATIII	3400	On Fitusiran	34 - 3400	1-100
VIII	0.7 (100 IU/dL)	Hemophilia A	0.0007 - 0.07 (0.1 IU/dL - 10 IU/dL)	0.1, 0.5, 1, 2, 5, 10, 100
IX	90 (100 IU/dL)	Hemophilia B	0.09 - 9 (0.1 IU/dL - 10 IU/dL)	0.1, 0.5, 1, 2, 5, 10, 100
V	20 (100 U/dL)	Factor V Deficiency	0.02 - 2	0.1, 0.5, 1, 2, 5, 10, 100
VII	10 (100 U/dL)	Factor VII Deficiency	0.01 - 1	0.1, 0.5, 1, 2, 5, 10, 100
X	160 (100 U/dL)	Factor X Deficiency	0.16 - 16	0.1, 0.5, 1, 2, 5, 10, 100

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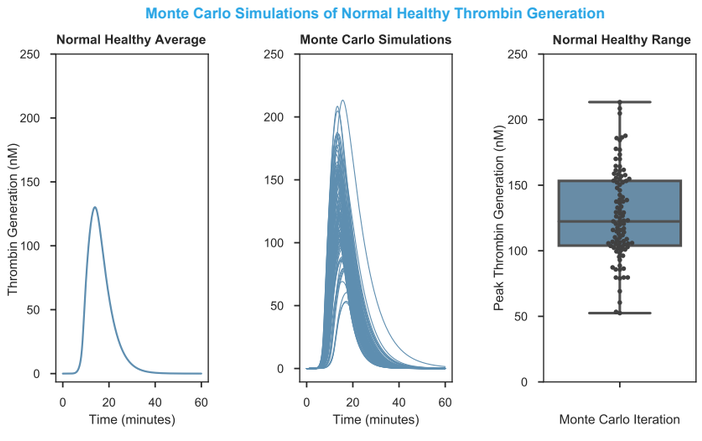

Figure 2: (A) A Cytoscape[3] network visual of the 66 reactions and 66 species in the model, which shows that AT lowering will reduce flux through seven reactions involved in forming a AT-factor complex and promote thrombin generation. **(B)** Table describing the input factor concentrations used for the model under various disease conditions. **(C)** Thrombin generation curve for an average normal healthy individual and range of thrombin generation peaks based on Monte Carlo simulations of factor heterogeneity.

Results: Model Validation Using Clinical Data

- QSP model is predictive of TG with AT lowering.
- Clinical normal range for TG (Figure 3A) aligns with QSP prediction (Figure 2C) and a correlation is observed between clinically measured and model predicted TG for patients with AT lowering.

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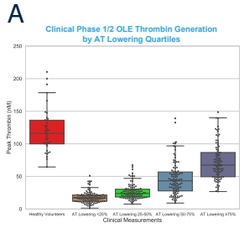

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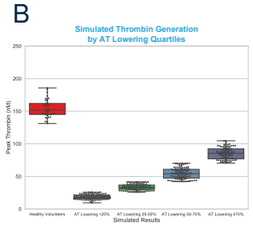

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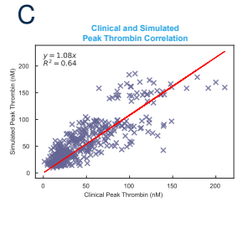

Figure 3: Based on interim Fitusiran Phase 1: (Parts A-C) results, we show a strong correlation between the simulated TG (based on AT knockdown) and the measured TG in the clinic, as binned by AT lowering quartiles **(A,B)** as well as by scatter plot **(C)**

Results: Concomitant Factor Dosing at Different AT Levels

- *In silico* QSP model was applied to quantify nonlinear relationship between TG, AT lowering and factor VIII dose for severe hemophilia A.
- Heatmap representation of surface reveals that between 5-10 IU/kg of factor VIII at AT levels observed with fitusiran (10-25%) may be sufficient to normalize TG.

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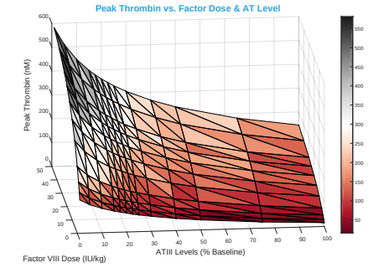

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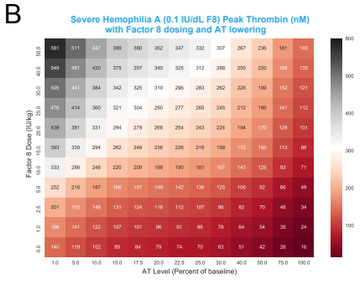

Figure 4: (A) Simulated *in silico* thrombin generation curves for various AT levels and 0.1% Factor FVIII (simulating severe Hemophilia A) **(B)** Heatmap representation of peak thrombin at various Factor VIII doses (single dose) and AT level.

Results: Time-Course Peak Thrombin Potential

- Factor pharmacokinetics (one compartment model) and the QSP model were integrated to simulate peak thrombin potential as a function of time after factor dose.
- AT lowering is predicted to augment thrombin generation at both the peak and trough time points after FVIII administration for breakthough bleeding episodes.

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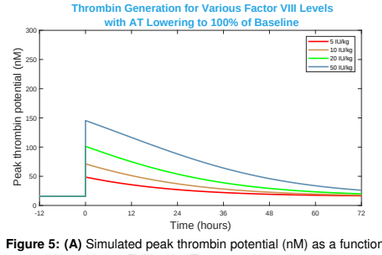

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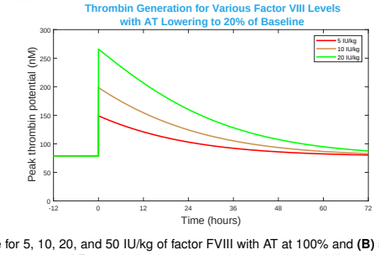

Figure 5: (A) Simulated peak thrombin potential (nM) as a function of time for 5, 10, 20, and 50 IU/kg of factor FVIII with AT at 100% and **(B)** 5, 10, 20 IU/kg of factor FVIII with AT at 20% of baseline to show the time-course impact of Factor dosing in patients with severe hemophilia A.

Results: AT Lowering for Rare Bleeding Disorders (RBDs)

- Rare bleeding disorders (RBDs) arise from deficiencies in various clotting factors. Like hemophilia A or B, some RBDs (e.g. arising from deficiencies in factors V, VII, or X) are also characterized by an inability to generate sufficient thrombin to prevent bleeds.
- Like patients with hemophilia, some RBD patients suffer from frequent and severe bleeding episodes, but often have fewer treatment options.
- Fitusiran may potentially have clinical applicability in RBDs and the *in silico* QSP model may be used to explore the impact of AT lowering in RBD settings.
- We applied the QSP model to predict the impact of AT lowering on TG in plasma with various degrees of deficiencies in factors V, VII, and X.

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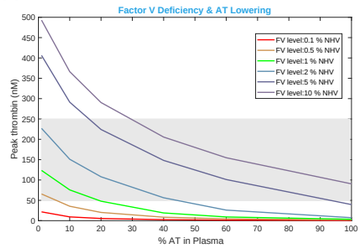

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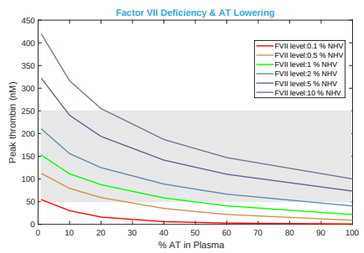

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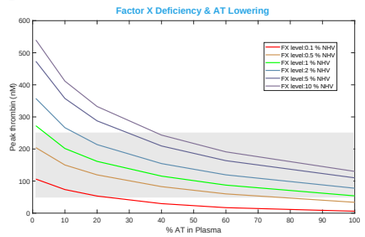

Figure 6: Consistent with published results in hemophilia A and B, the model predicted an increase in thrombin generation as AT was lowered in the setting of factor V, VII, and X deficiencies. AT lowering to 20% resulted in increased thrombin generation by 14, 4, and 3-fold for conditions of 1% factor V **(A)**, VII **(B)**, and X **(C)**, respectively.

Conclusions

- The QSP model was able to describe the clinically observed relationship between AT lowering and thrombin generation.
- The QSP model may be used to support factor dosing guidelines while on an AT lowering agent. Model reveals synergistic relationship between AT levels and FVIII, where TG increases more with combined AT lowering and FVIII dose than would be expected by a linear additive model.
- Based on model predictions, AT lowering may be a therapeutic strategy for certain RBDs, including factor V, VII, or X deficiencies.

Acknowledgements & Disclosures

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References:

[1]Pasi, K. John, et al. "Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy." *New England Journal of Medicine* 377.9 (2017): 819-828. APA

[2]Nayak, S., et al. "Using a systems pharmacology model of the blood coagulation network to predict the effects of various therapies on biomarkers." *CPT: pharmacometrics & systems pharmacology* 4.7 (2015): 396-405.

[3]Shannon, Paul, et al. "Cytoscape: a software environment for integrated models of biomolecular interaction networks." *Genome research* 13.11 (2003): 2498-2504.