Measurement of Thrombin Generation in Conditions of Low Antithrombin

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Measuring Thrombin Generation: A Global Hemostasis Assay

Calibrated Automated Thrombogram (CAT)[1,2]

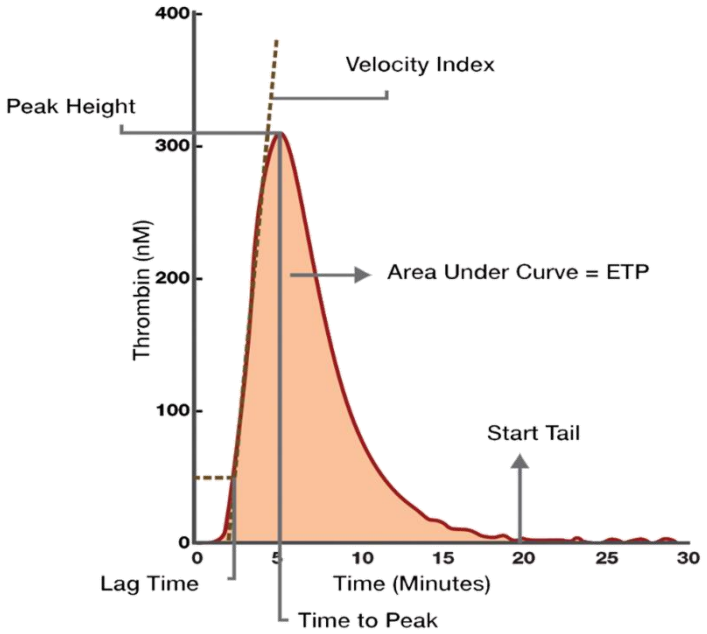
- Citrated platelet poor plasma
- Standardized reagents to initiate thrombin generation
- Thrombin generation measured real-time with thrombin-specific fluorescent substrate
- Thrombin calibrator used to correct signal for substrate consumption and plasma turbidity

Advantages

- Global hemostasis assay; provides information on key parameters of coagulation
- Can be standardized

Limitations

- Closed, static system (reagents can be consumed)
- Lacks important cellular contributions to coagulation
- Not all anticoagulant pathways reflected (e.g. Protein C/S pathway)
- Requires careful sample collection and control of pre-analytical variables



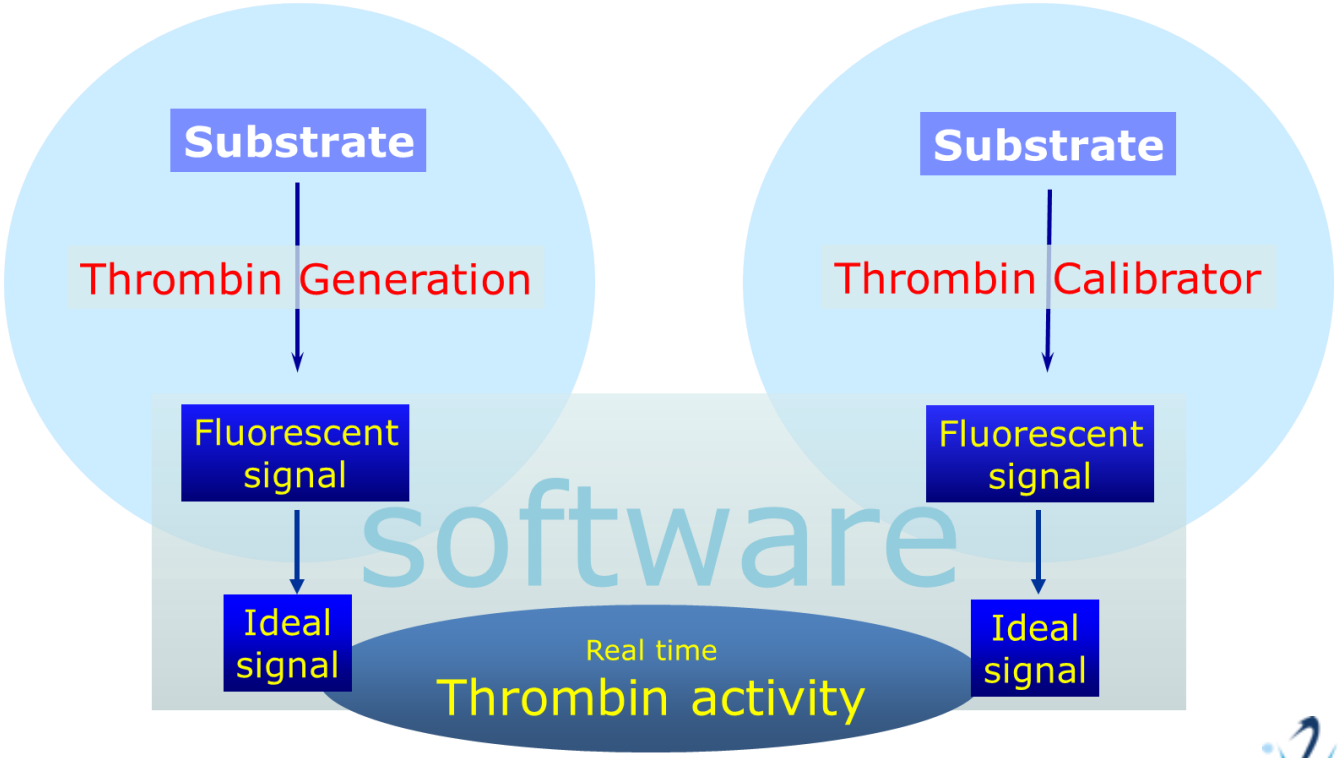
From Diagnostica Stago
http://www.genengnews.com/gen-articles/thrombin-generation-assay-aids-development/4099/

1. PHemker HC, et al. *Pathophysiol Haemost Thromb*. 2003;33(1):4-15. 2. Spronk HM et al, *Thromb Haemost*. 2008 Aug;100(2):362-4.

Alnylam PHARMACEUTICALS

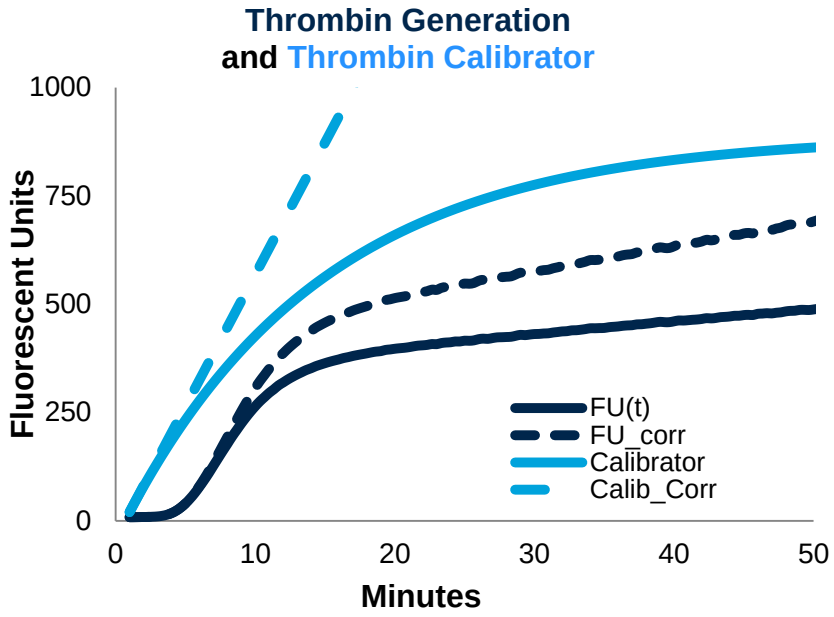
Continuous Calibration

- **Sample divided in two parts:**
  - **Thrombin Generation (TG)**
  - **Thrombin Calibrator (TC)**
- **Software uses both signals to calculate thrombin in time**



Continuous Calibration

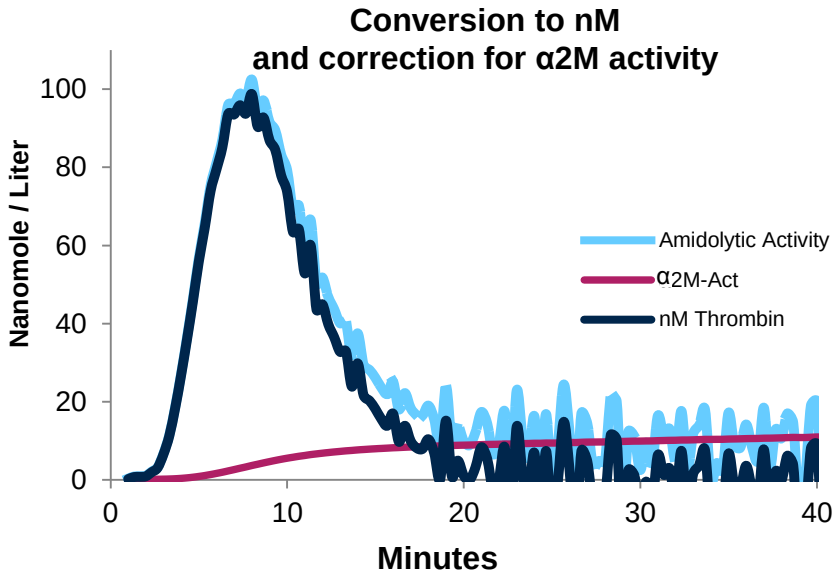
Thrombin Generation and Thrombin Calibrator



- **Simultaneous measurement of TG (dark blue) and TC (light blue)**
- **TC contains fixed amount of thrombin activity**
- **The initial slope of TC is used to translate from FU/min into nM thrombin.**
- **Substrate consumption leads to the bending of the curves**
- **The software corrects the bend**

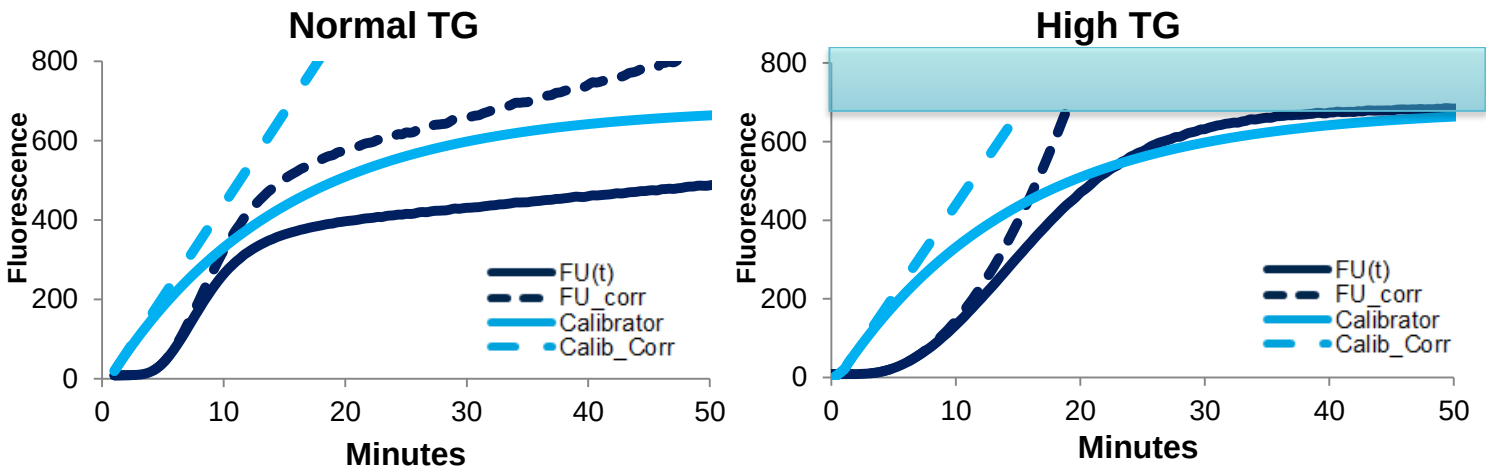
Continuous Calibration

Conversion to nM and Correction for α2M Activity



- **The first derivative of TG is taken and converted into Nanomolar (nM) (dashed light blue)**
- **α2M-thrombin activity is subtracted which results in nM of thrombin in time (solid dark blue).**

Automatic Detection of Reliability in the CAT

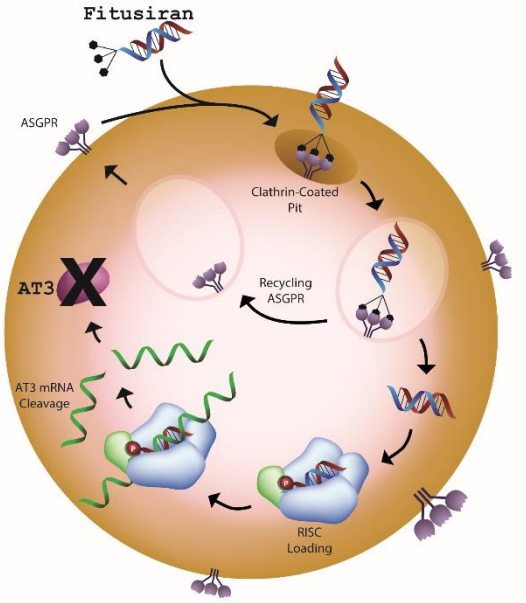


- **Both thrombin generation (dark blue) and thrombin calibrator (light blue) curves bend with substrate consumption. Dashed curves were mathematically corrected.**
- **At TC plateau level (~700 FU), substrate is used up**
  - Left: TG remains lower than the plateau level of TC
  - Right: TG reaches the plateau level of TC, which indicates thrombin generation was not yet finished before substrate was consumed.

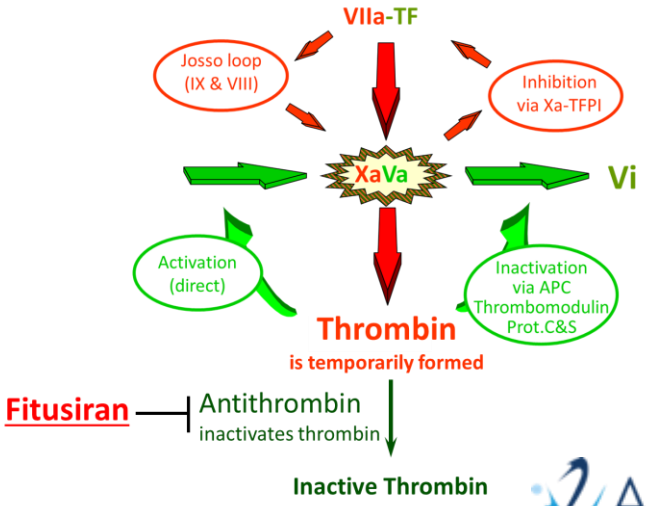
Thrombin Generation Detection by CAT for Fitusiran Treated Plasma

Fitusiran (ALN-AT3) is a SC-administrated GalNAc-conjugated investigational RNA interference (RNAi) targeting Antithrombin (AT)

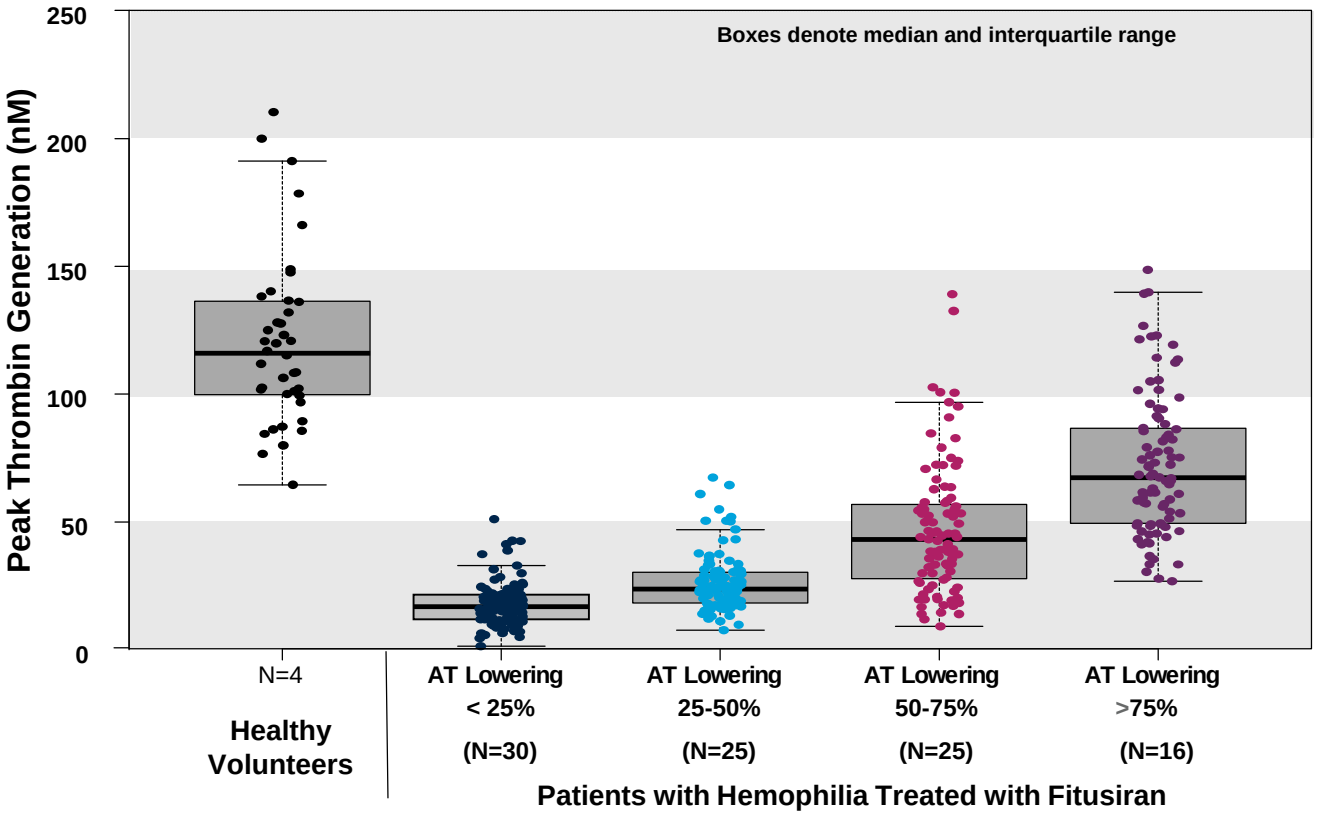
- Non-biologic, chemically-synthesized, with GalNAc ligand to specifically deliver to liver-site of AT synthesis
- Harnesses natural RNAi mechanism for regulation of plasma AT levels



- Thrombin generation is a global parameter that describes hemostasis
- Fitusiran reduces plasma AT levels
- With reduced AT, thrombin is inactivated more slowly and reaches higher concentrations

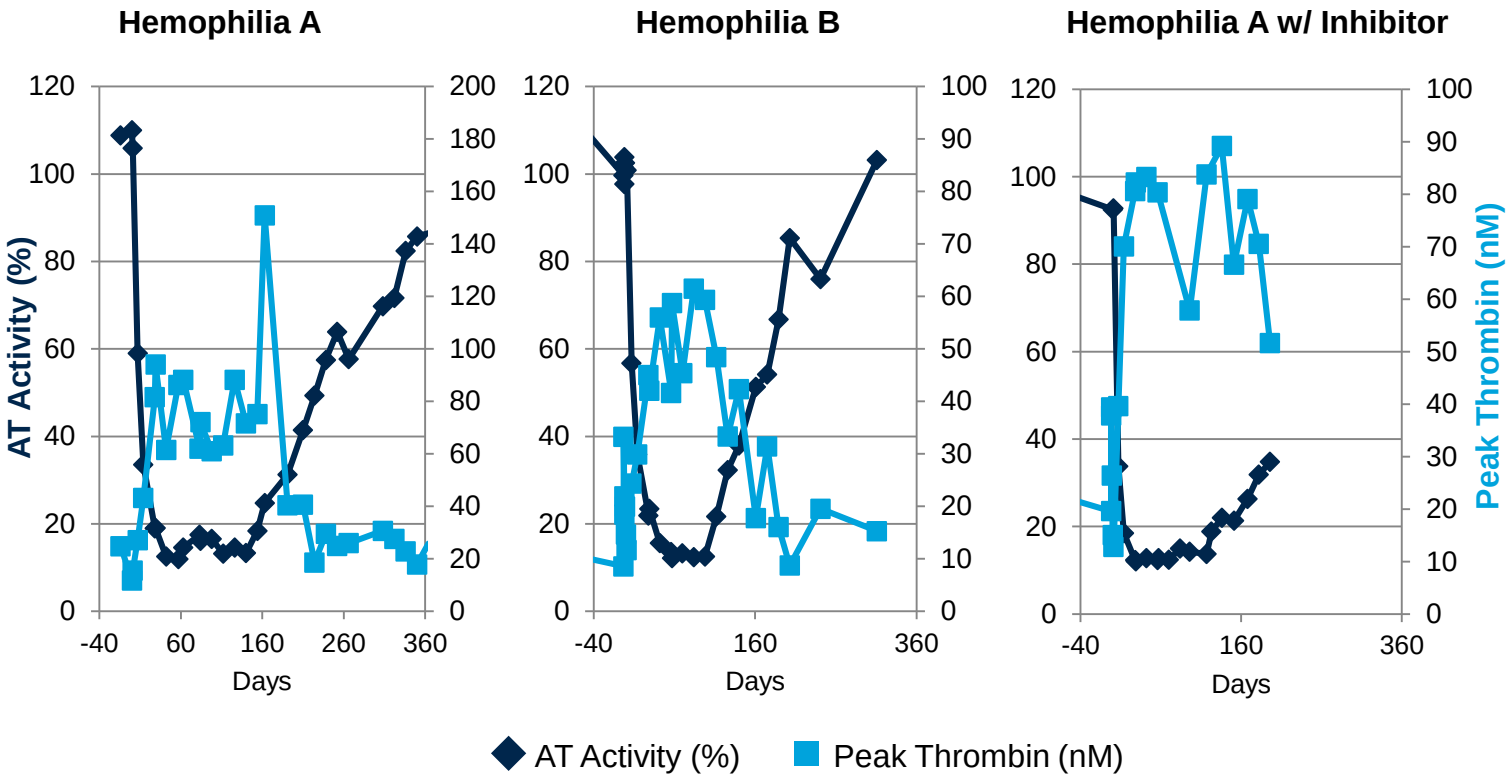


AT Lowering is Correlated with Increased Thrombin Generation



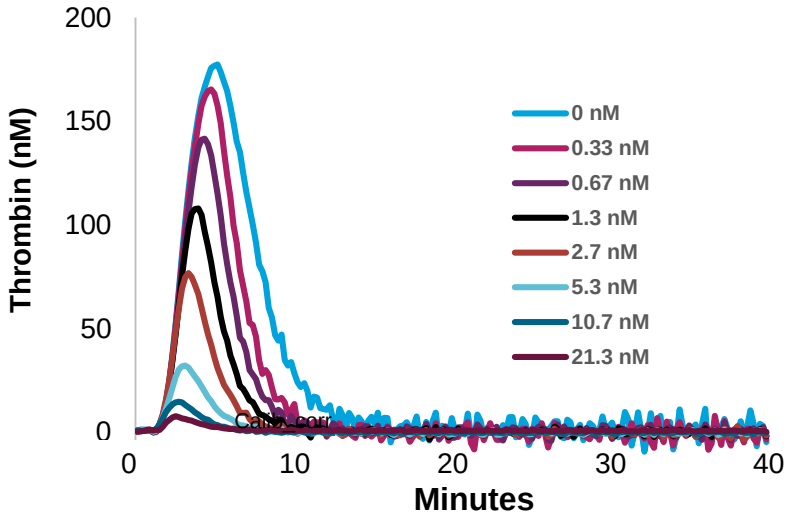
[1]Pasi KJ, et al. *N Engl J Med.* 2017; epub ahead of print.

Fitusiran Patient Plasma with lowered AT displayed increased Peak Thrombin by CAT assay

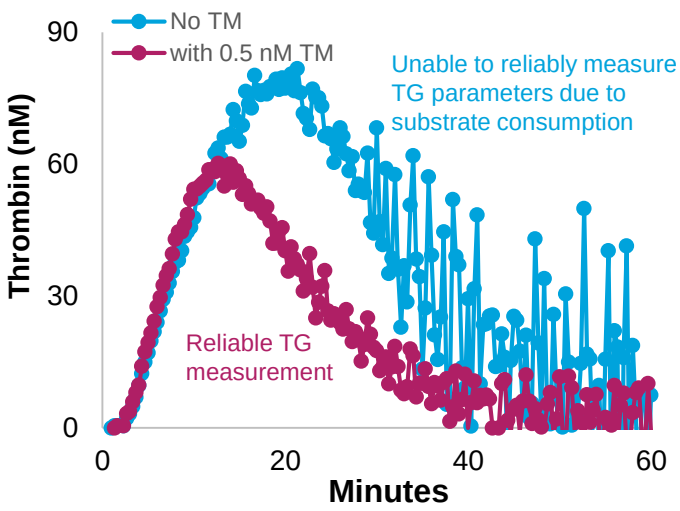


CAT Assay May be Modified by Thrombomodulin (TM) Addition to Activate Protein C Pathway

A. TM Dose-Dependent Influence on Thrombin Generation



B. Influence of TM on Fitusiran Patient Plasma Thrombin Generation



- **Under low AT conditions, thrombin generation assay could be unreliable due to lack of inhibitory machinery**
- **Addition of thrombomodulin activates the anticoagulant protein C pathway which reduces thrombin formation[1,2]**
- **Curves that would otherwise be unreliable could benefit from this addition**

A. Dose-dependent suppression of thrombin generation by TM in normal human plasma

B. Addition of TM (0.5 nM) enables robust measurement of thrombin generation in fitusiran treated hemophilia patient

1. Dielis et al. *Haematologica*. 2008 Sep;93(9):1351-7.; 2. Couturaud et al. *Thromb Haemost*. 2008 Jan;99(1):223-8.

Alnylam PHARMACEUTICALS

Summary

The CAT is a global hemostasis assay that has built-in checks for reliability of the data.

When CAT is unreliable due to conditions of AT and substrate depletion, the assay may be modified by thrombomodulin addition to activate the APC pathway and allow for robust TG measurement