VI Foro Stago Academy

# Role of thrombin generation assays in the management of haemophilia

**Yesim Dargaud MD, PhD**
**University of Lyon**
**Coordinator of the French Hemophilia Reference Centre**

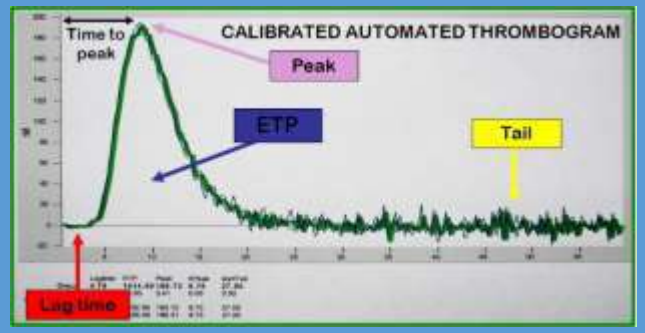
Stago
Diagnostics is in our blood.

# COI Declaration

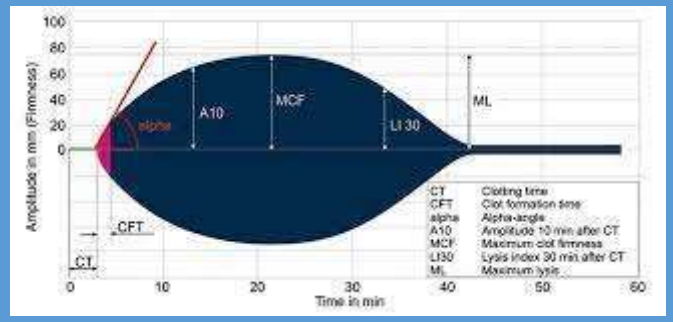
- **Grants/research support from**: Bayer, Baxter, NovoNordisk, CSL Behring, LFB Biomedicaments, Pfizer, Leo Pharma, Octapharma, Baxalta - Shire, Diagnostica Stago
- **Educational grant from**: NovoNordisk, Shire-Takeda, CSL-Behring, LFB, Sobi, LeoPharma
- **Speaker's honoraria from**: Bayer, Baxter, LFB, NovoNordisk, CSL Behring, Sobi and Octapharma, Sanofi, Leo-Pharma, Pfizer
- **Consultancy honoraria from**: Bayer, Baxalta-Shire-Takeda, CSL-Behring, Octapharma, LFB, Biomarin
- **Instructor honoraria from**: NovoNordisk, Sanofi

# GLOBAL HAEMOSTASIS ASSAYS

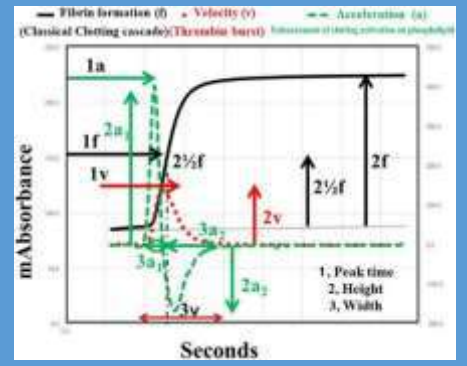
## THROMBIN GENERATION ASSAY




## THROMBOELASTOGRAPHY

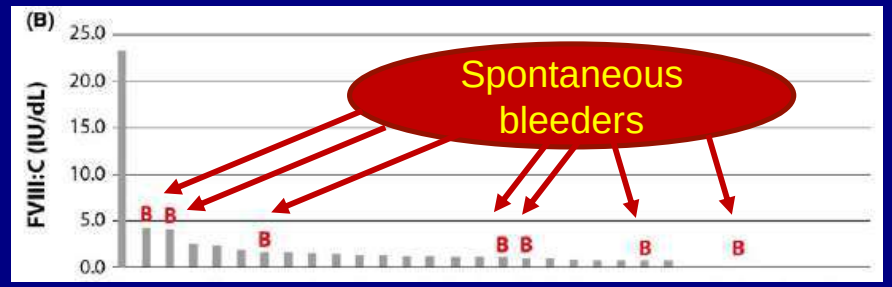



## CLOT WAVEFORM ANALYSIS

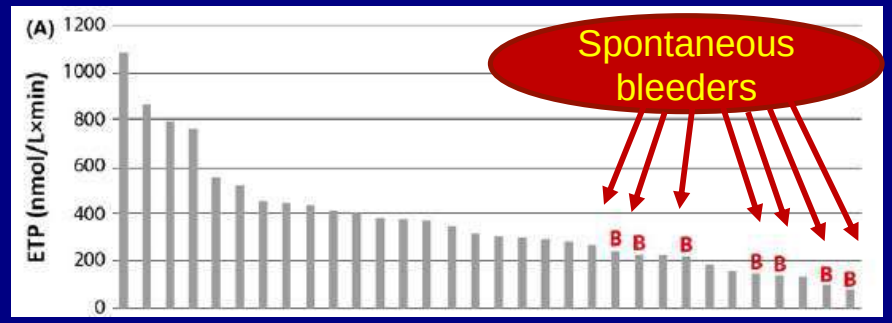



# What is the difference between TGA and routine haemostasis assays ?

# Accurate assessment of Coagulation Capacity is important in haemophilia treatment




Patients ranked according to trough Factor VIII level

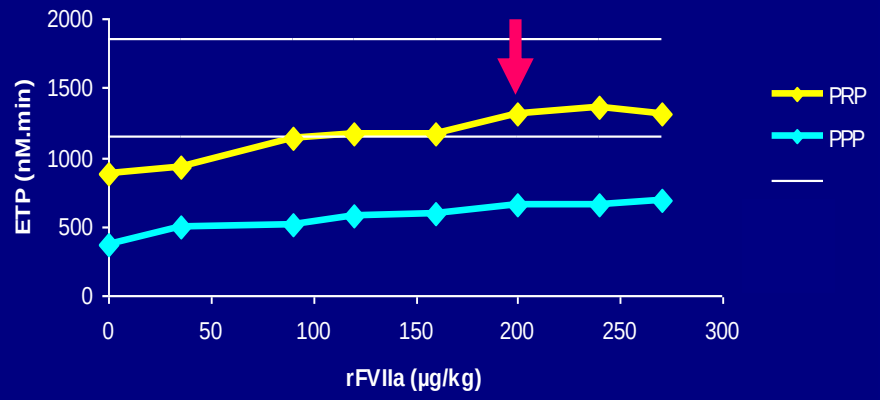



Patients ranked according to trough ETP

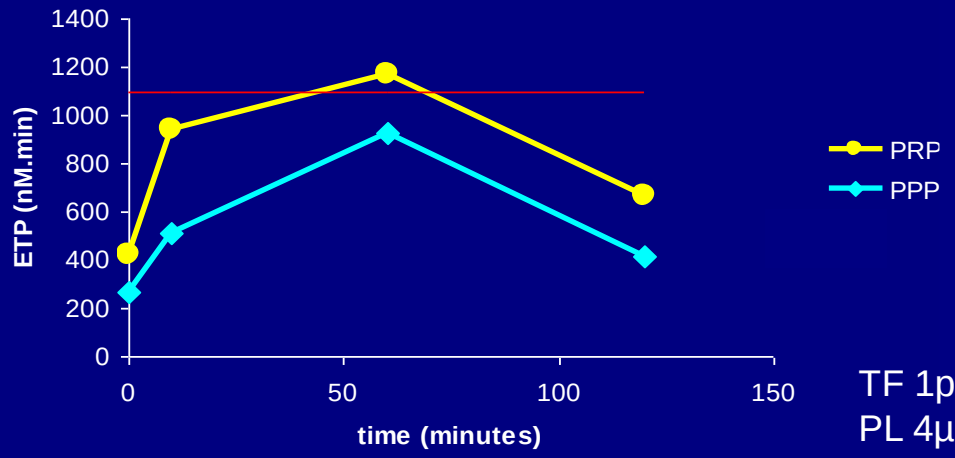
Limit spontaneous bleeding : 200 nM.min i.e. 12.5 % of normal

Dargaud et al, Haemophilia 2018

## In vitro




## Ex vivo
## rFVIIa 200µg/kg




TF 1pM
PL 4µM
CTI 1.45µM
CAT method

FVIII < 1 IU/dl
Ab = 21 BU/ml

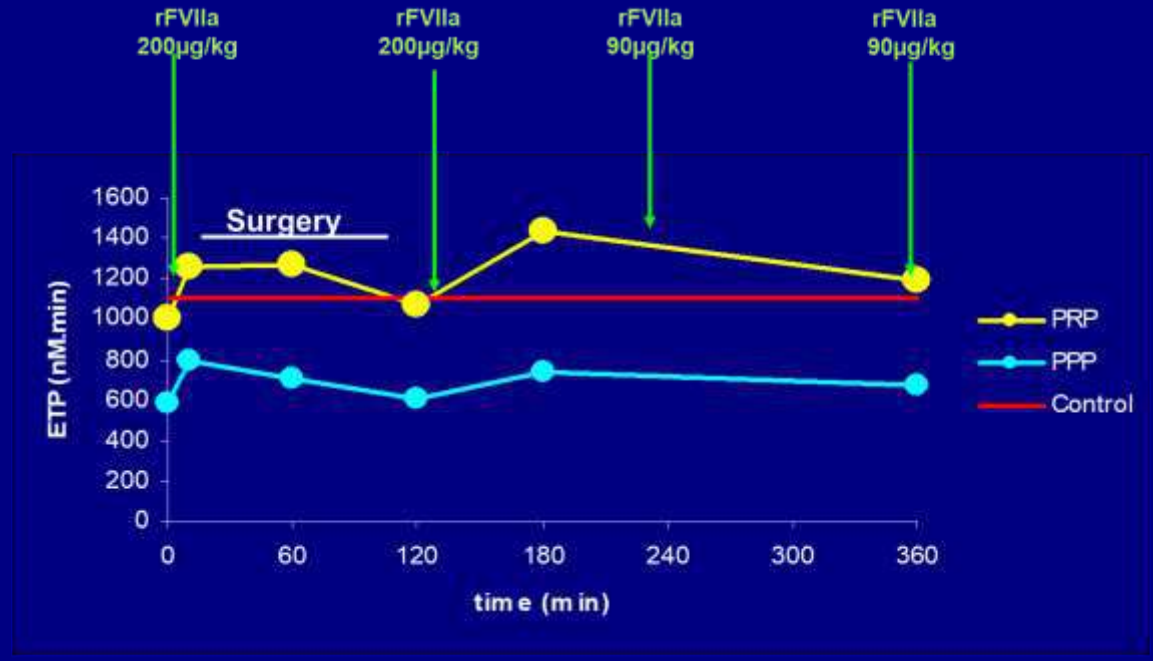
Major orthopaedic surgery

blood

2010 116: 5734-5737
Prepublished online Sep 1, 2010;
doi:10.1182/blood-2010-06-291905

Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery

Yesim Dargaud, Anne Lienhart and Claude Negrier





*Dargaud Y. et al. Haemophilia, 2005;11:552-8*
*Dargaud et al. Blood 2010; 116:5734-37*
*Tran HTT et al. Haemophilia 2015; 275-283*
*Luna Zaizar et al. Haemophilia 2014; e7-14*
*Ay Y et al. Clin Appl Thromb Haemost 2013*
Livnat et al. Blood Clls Mol Dis 2017;66:1-5
Van Veen JJ et al. Int J Lab Haematol 2009

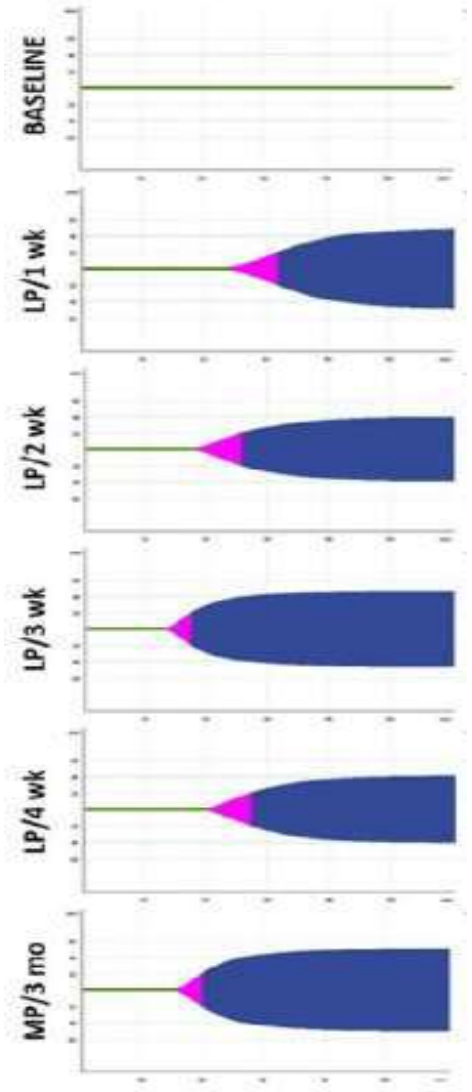
# Can GHA may be helpful to monitor non factor therapies for haemophilia ?

## EMICIZUMAB

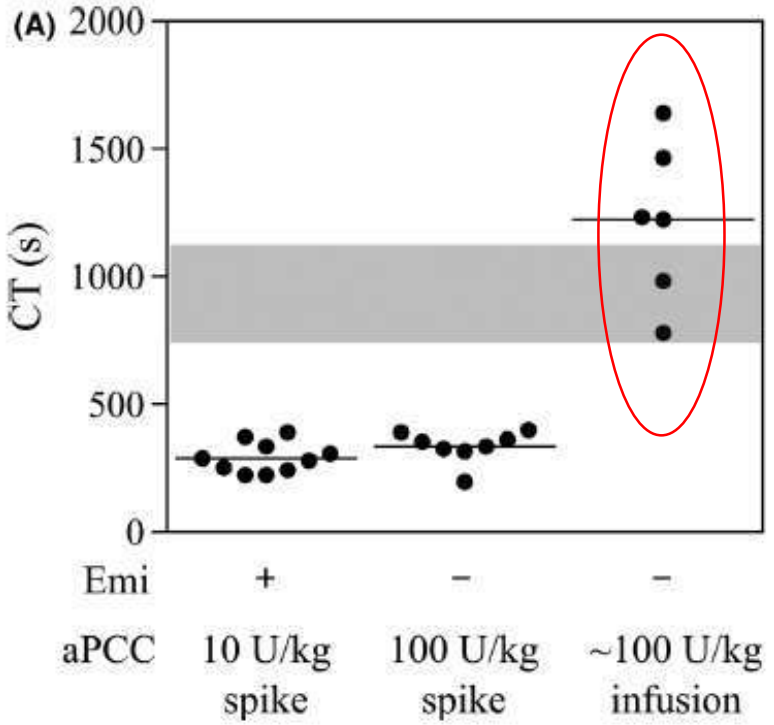
| FVIIIa | ACE910/Emicizumab |
|---|---|
| Multiple sites of interaction | Single sites of interaction |
| High affinity for enzyme & substrate *(low to high nanomolar range)* | Low affinity for enzyme & substrate *(micromolar range)* |
| Specific for FIXa and FX *(no binding to FIX and FXa)* | No distinction between zymogen and enzyme *(FIX vs FIXa and FX vs FXa)* |
| Full cofactor activity<br>- *promotes phospholipid binding*<br>- *stabilizes FIXa active site*<br>- *bridges FIXa to FX* | Partial cofactor activity<br>- *bridges FIXa to FX* |
| Enzyme and substrate are in excess over cofactor | Antibody is in excess over enzyme and substrate |
| FVIIIa has on/off mechanism | Emicizumab has no on/off mechanism |
| High level of self-regulation | Low level of self-regulation |

Lenting P et al. Blood 2017; 130:2463-68

Amrani et al. J Chromatogr A. 2021 Oct 11;1655:462489.
Donners et al. Res Pract Thromb Haemost. 2022 Jun 8;6(4):e12725
Josset L & Dargaud Y et al J Pharm Biomed Anal. 2023 Jan 20;223:115163.

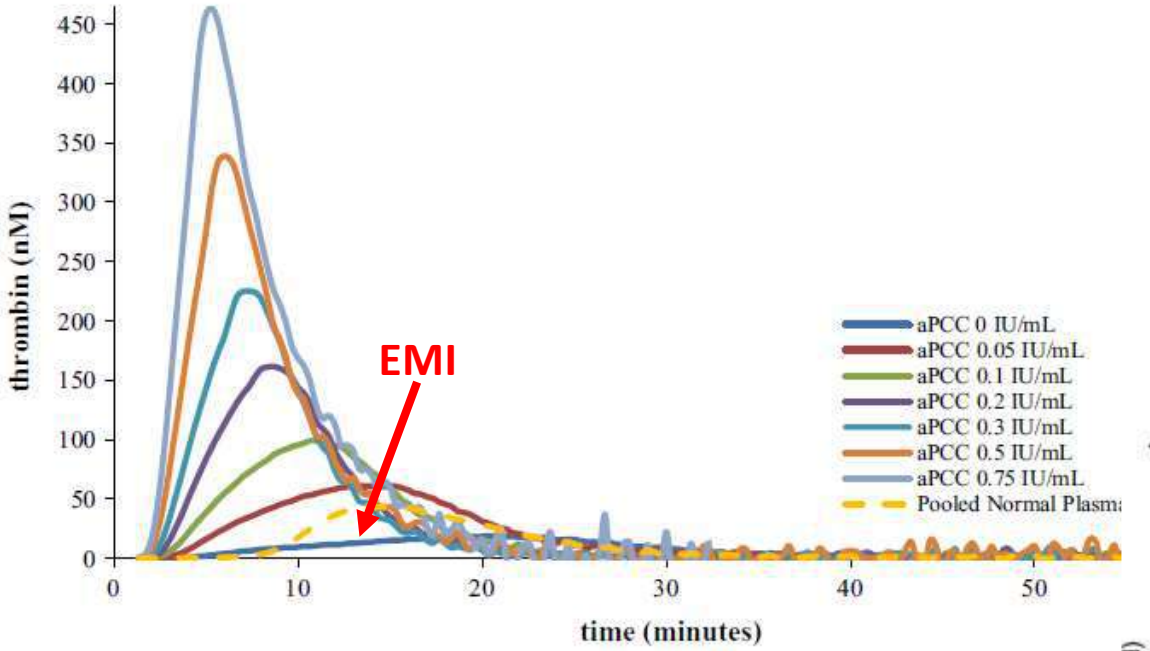



Szanto&Lassila R. Haemophilia 2021

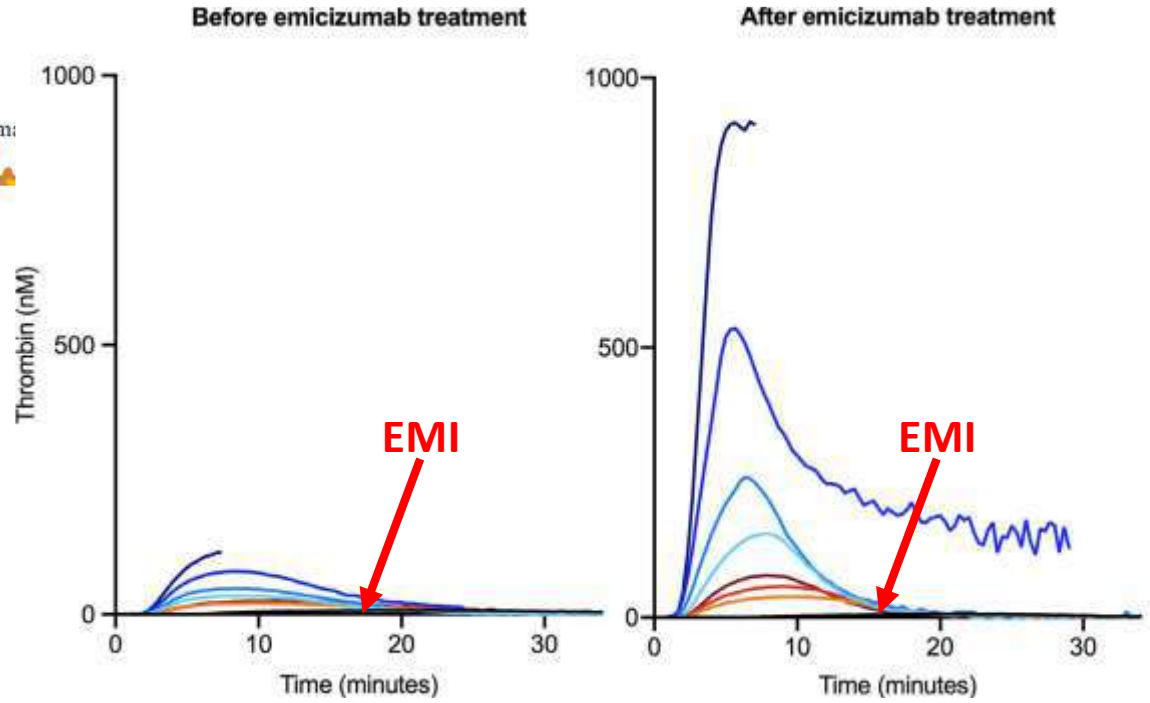



Furukawa &Shima M. Br J Haematol 2020

# Can TGA may be useful to determine individual response of patients to combined EMI+By passing agents?

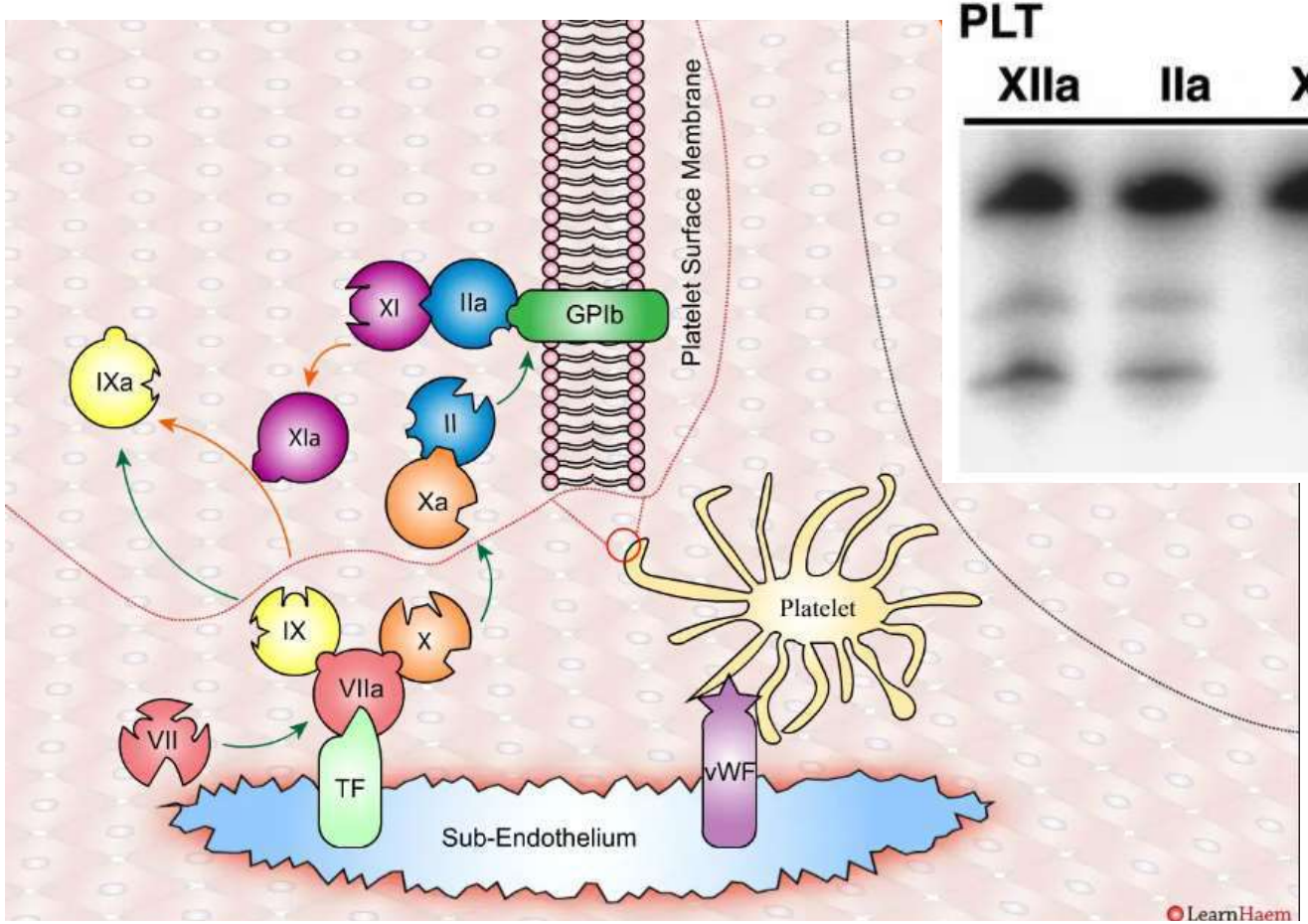



Kizilocak H & Young G et al Haemophilia 2020
Kizilocak H & Young G et al Haemophilia 2021
Kizilocak H & Young G et al Haemophilia 2022




Schultz NH & PA Holme et al RPTH 2020

# GHA have not been designed to monitor « FVIII mimicking Ab »

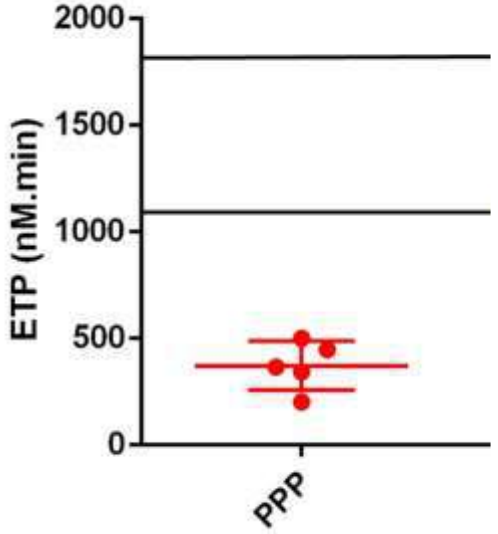






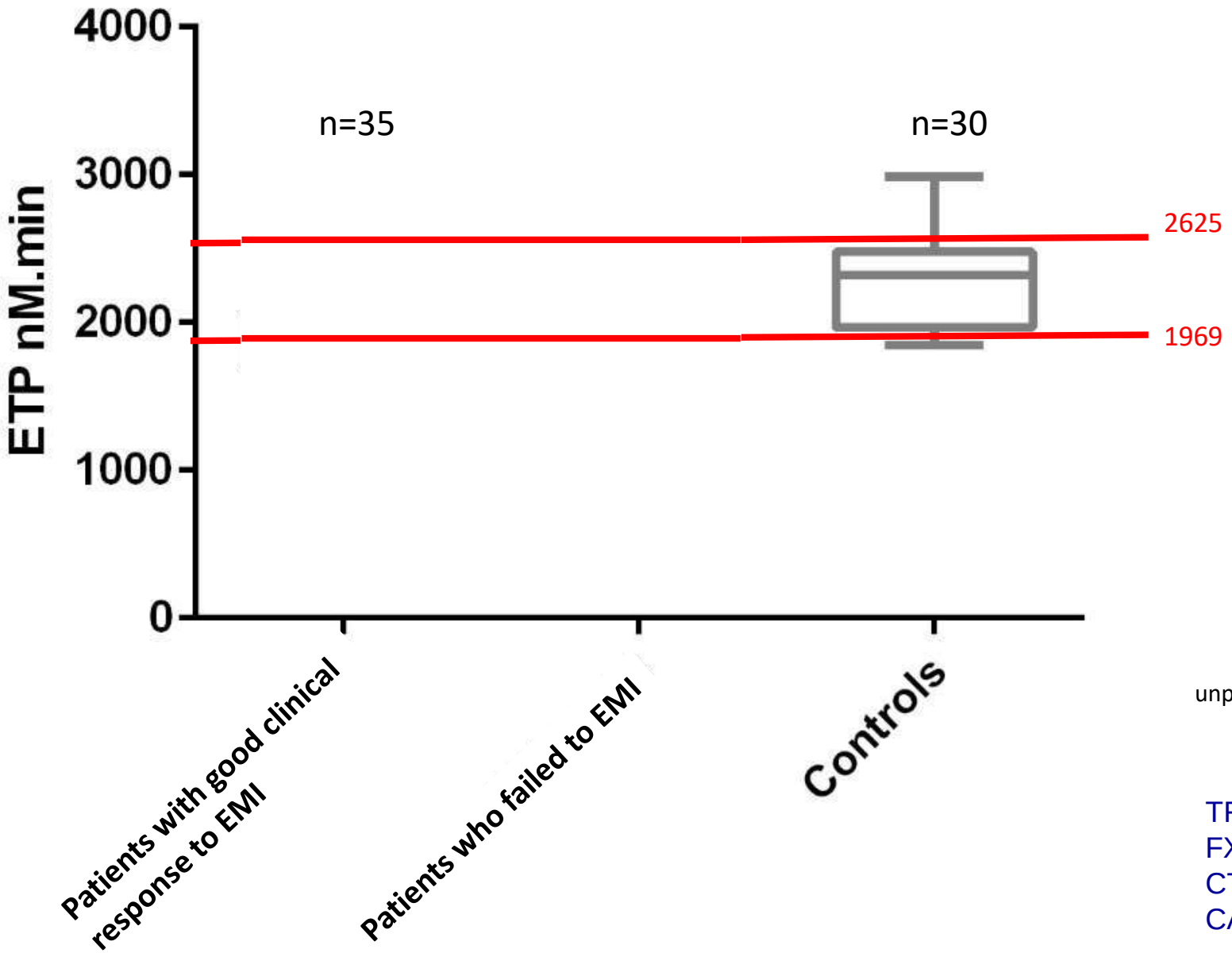
**Thrombin Activates Factor XI on Activated Platelets in the Absence of Factor XII**

Julie A. Oliver, Dougald M. Monroe, Harold R. Roberts, Maureane Hoffman




unpublished personal data

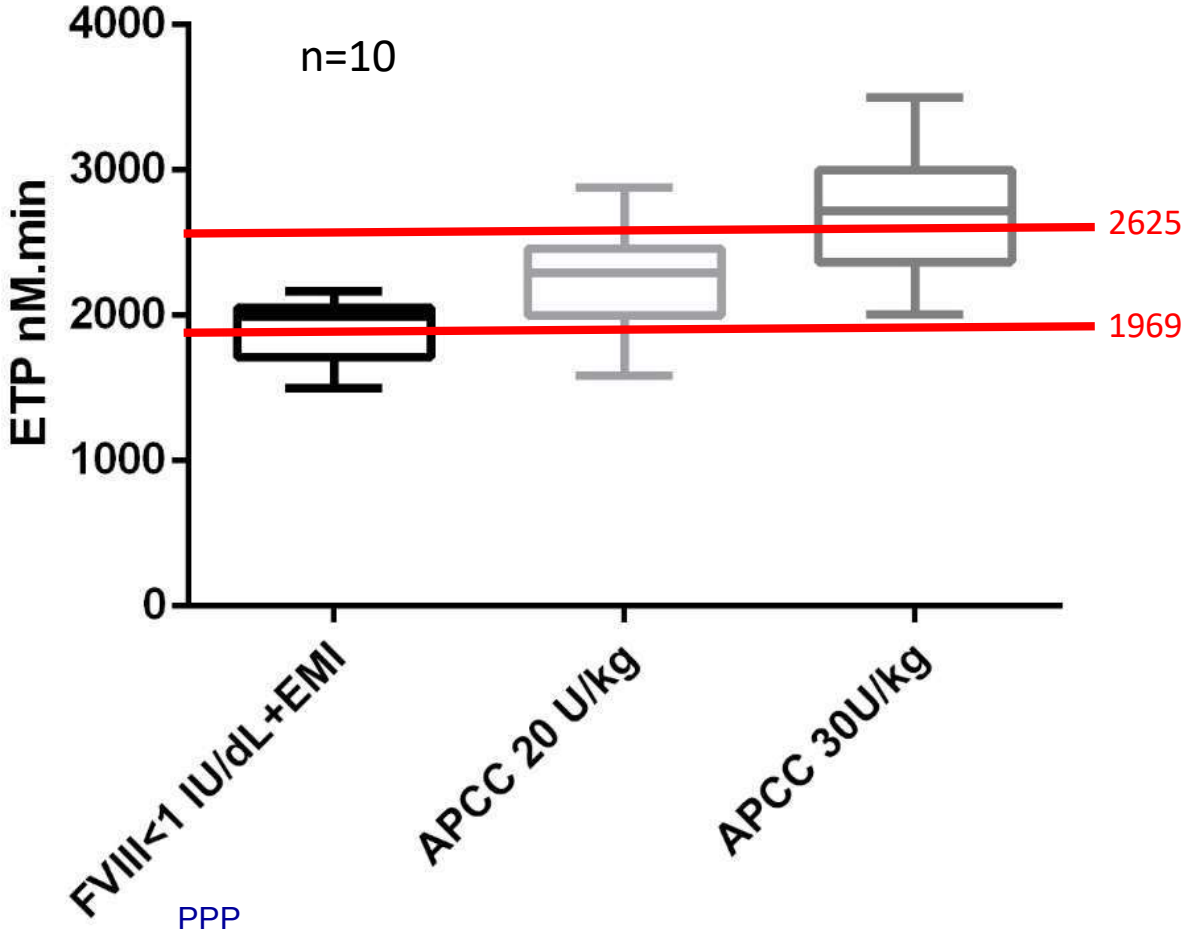
TF 1pM
PPP Low Reagent
CTI 1.45µM
CAT method



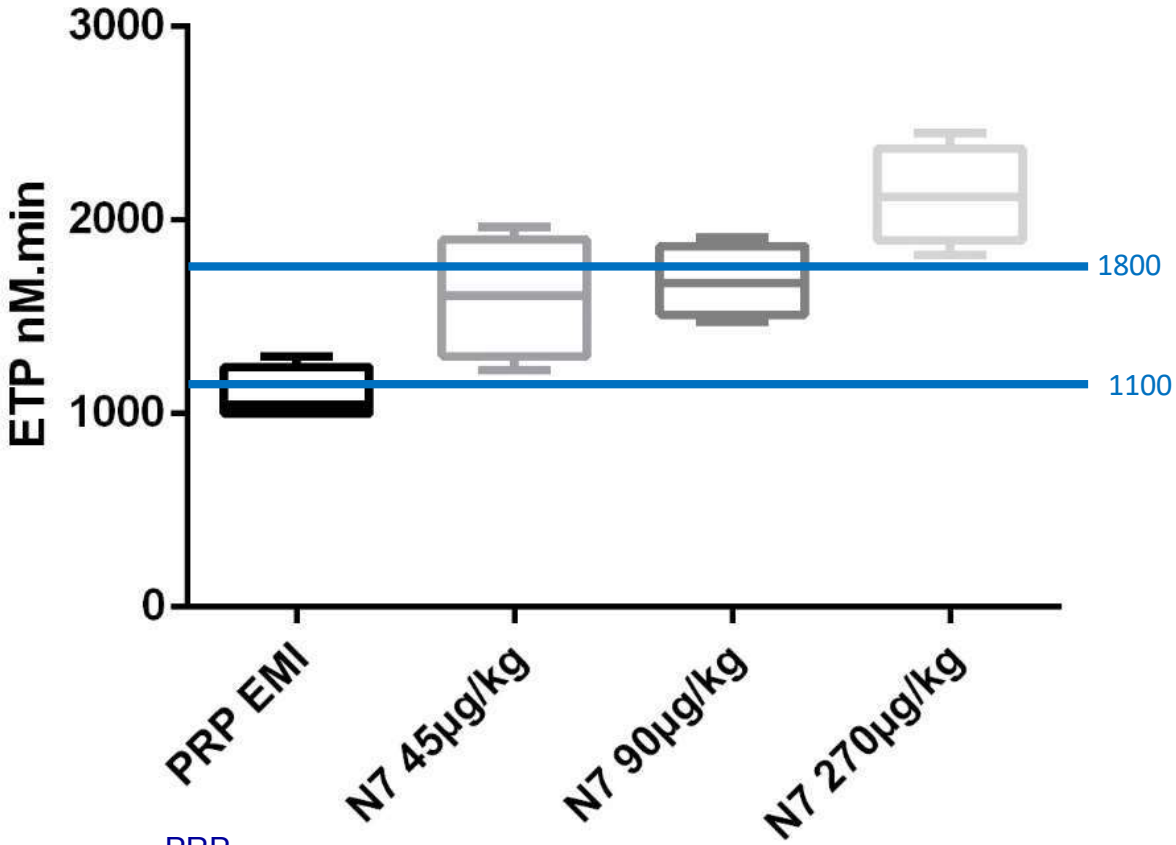

unpublished personal data

TF 1pM (PPP Low Reagent)
FXIa 1.5pM
CTI 1.45µM
CAT method

# Plasma samples from patients on prophylaxis with EMI + *in vitro* spiking with by-passing agents



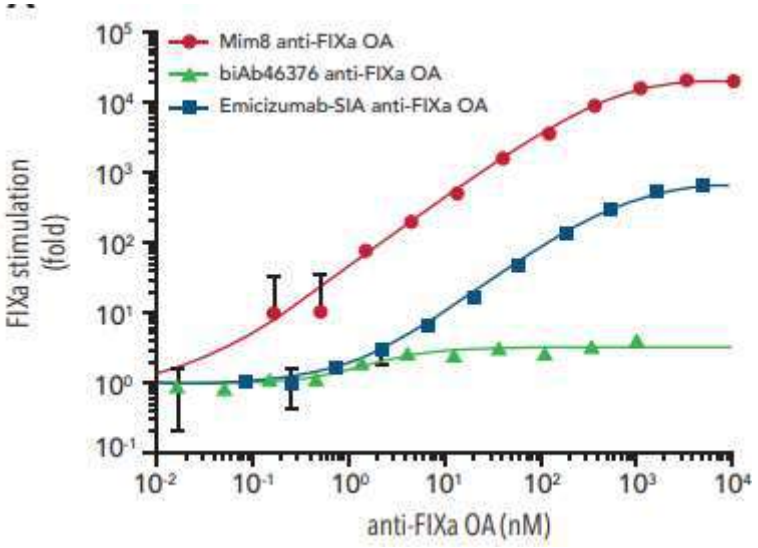

PPP
TF 1pM (PPP Low Reagent)
FXIa 1.5pM
CTI 1.45µM
CAT method



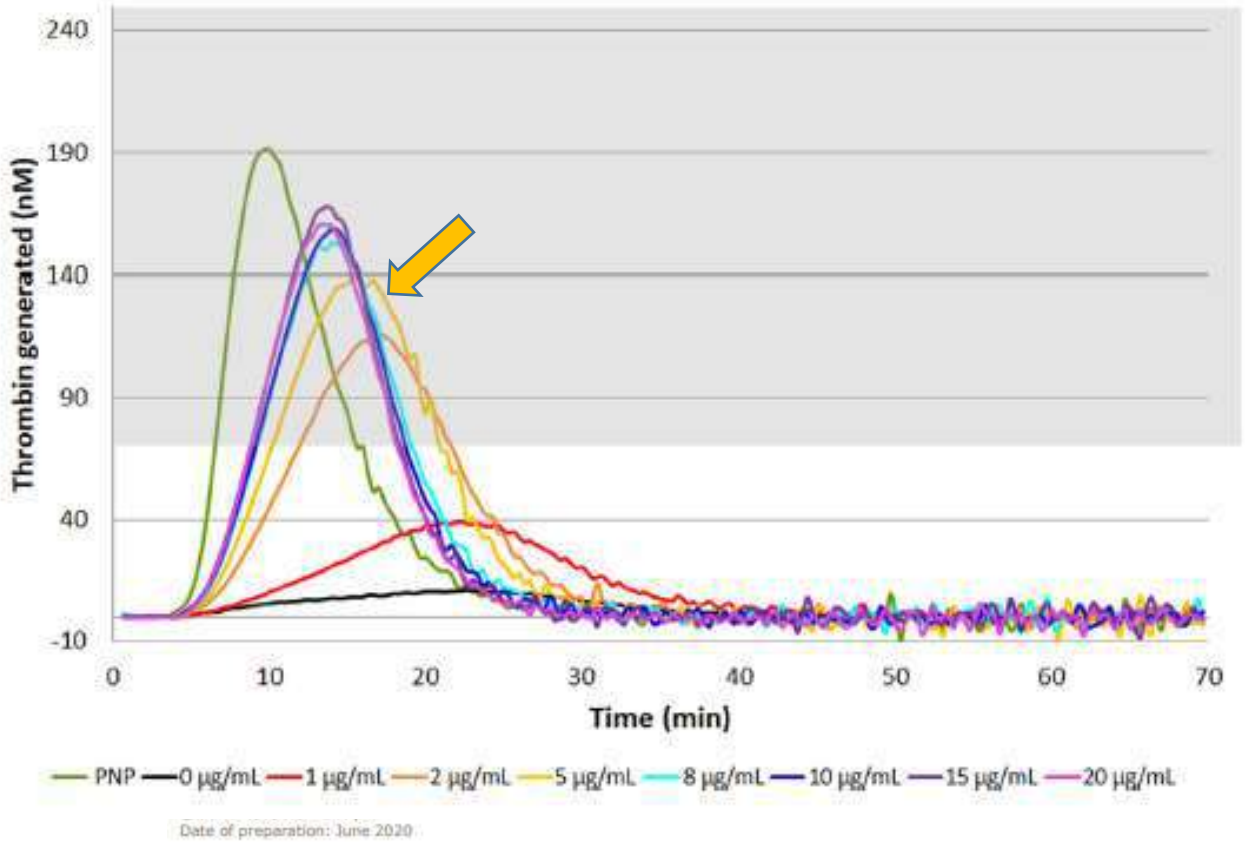

PRP
TF 1pM (PRP Reagent)
FXIa 1.5pM
CTI 1.45µM
CAT method

unpublished personal data

# Mim8: Next Generation FVIII Mimetic Bispecific Ab

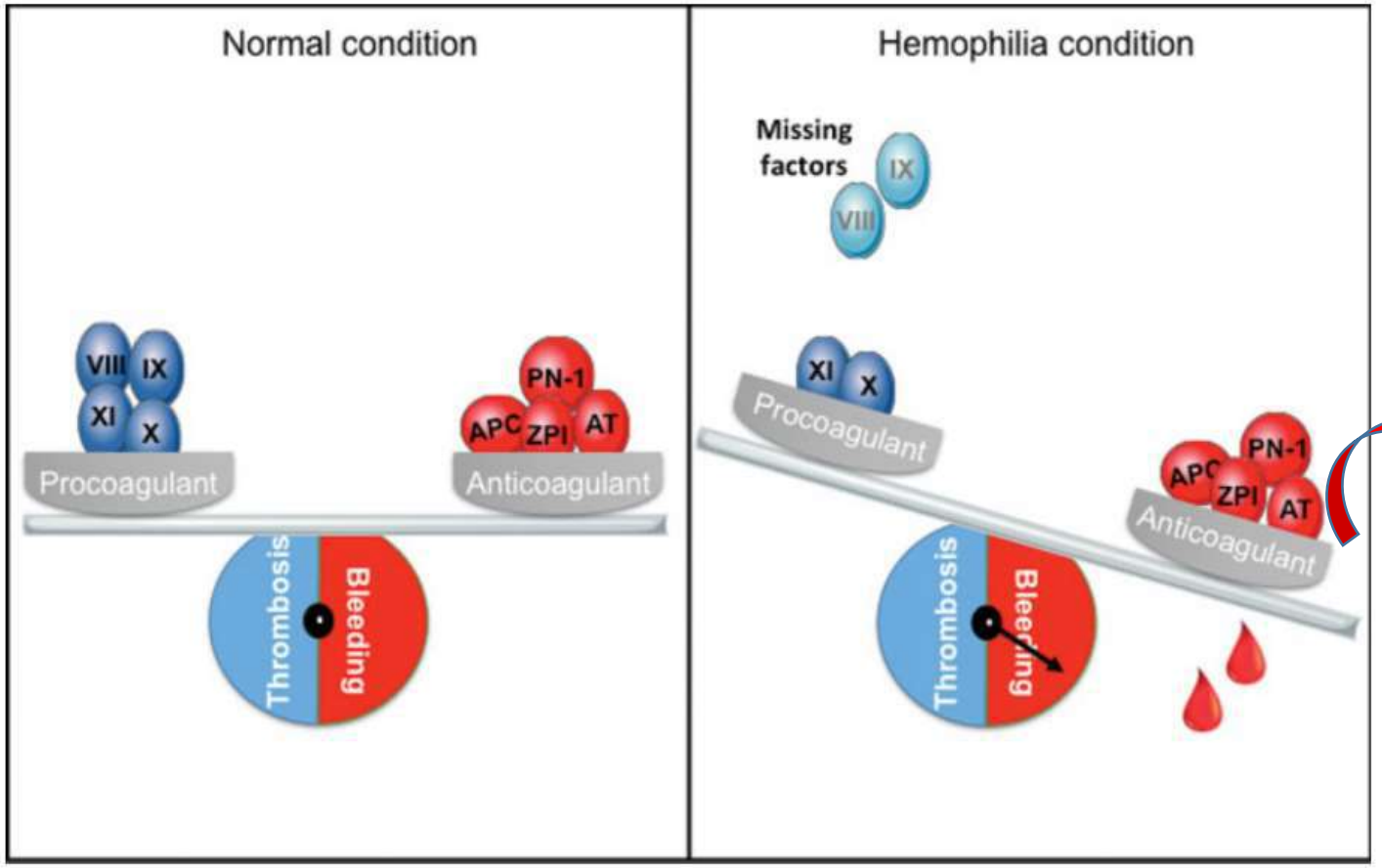



Ostergaard et al . Blood 2021;138:1258-68

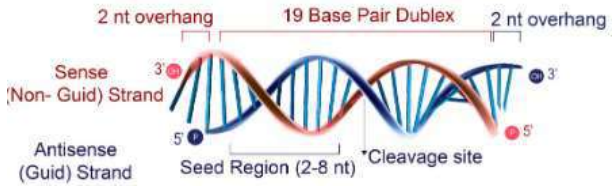


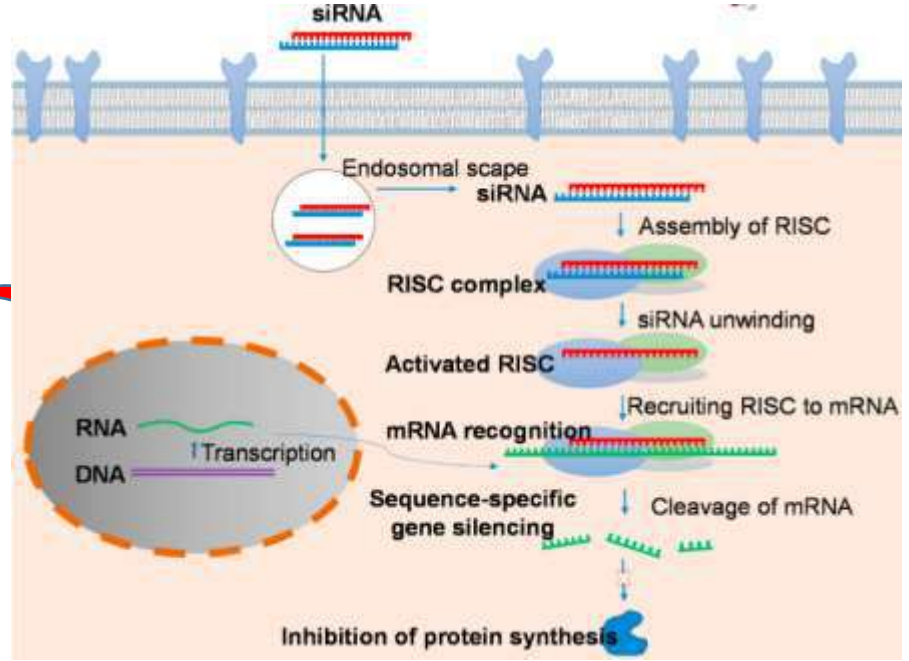

Bowyer A et al . J Thromb Haemost 2023

# Rebalancing Therapies



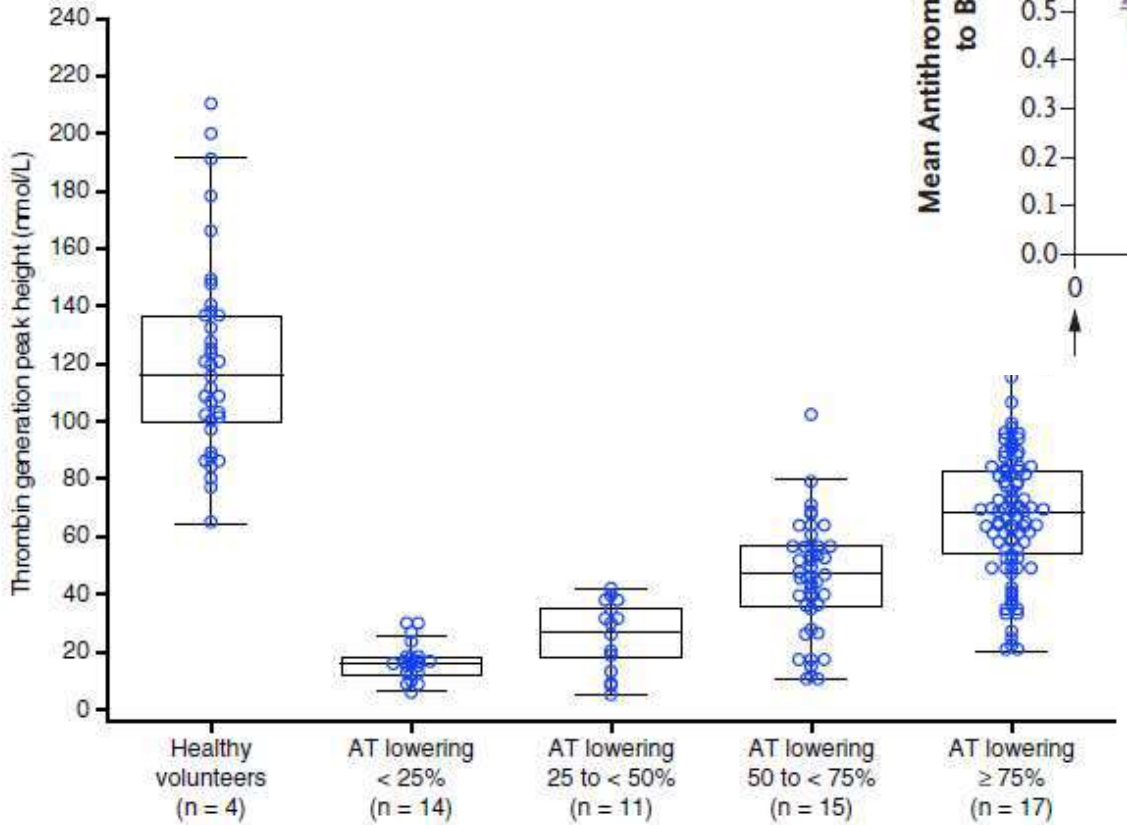

**FITUSIRAN**



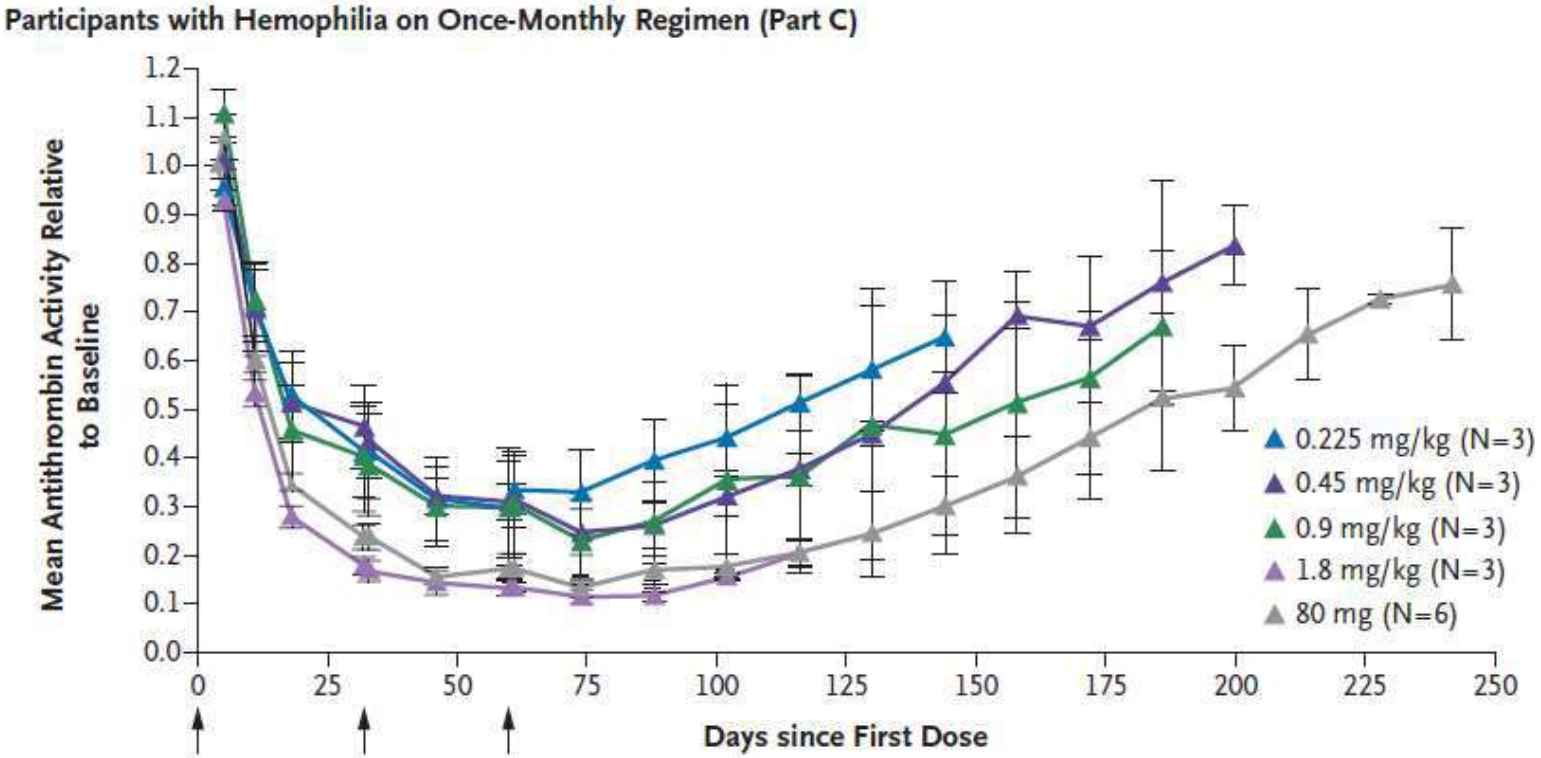




Aymonnier et al. Thromb Haemost 2021;121:261-9

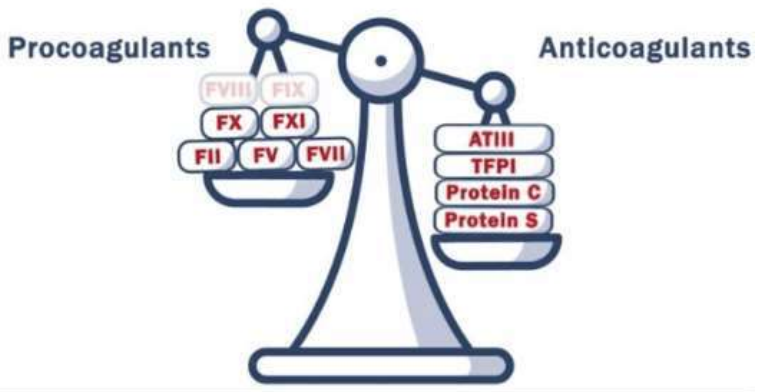
# Fitusiran




Pasi J& Ragni M et al. J Thromb Haemost 2020



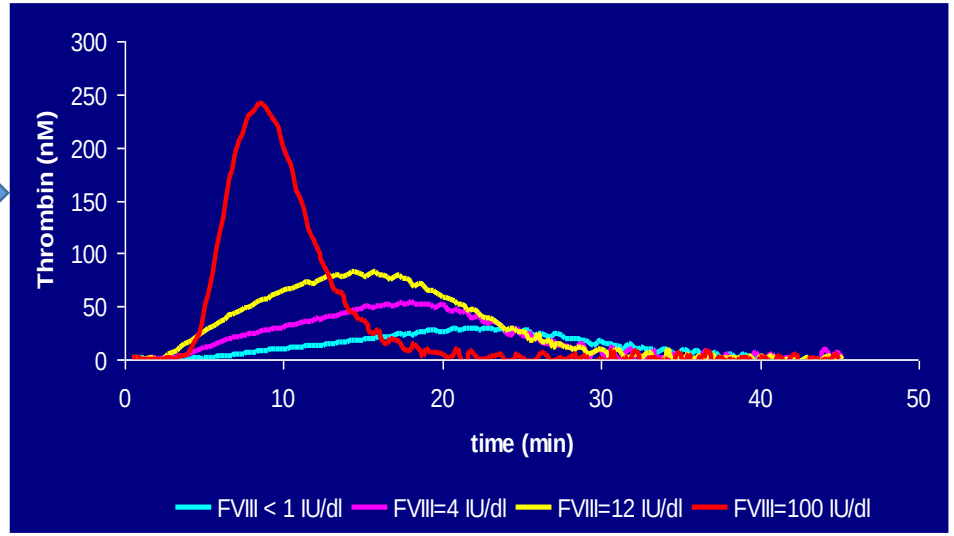

Pasi et al. NEJM 2017



# Reversal of Fitusiran can be guided by TGA

**In case of surgery or major breakthrough bleed**

**Measure AT and TGA**




Dargaud et al. Thromb Haemost 2005

| AT Reagents | Source | Incubation time |
|---|---|---|
| **Enzyme IIa** | | |
| Biophen AT (Hyphen Biomed) | Bovine IIa | 60-240 sec |
| Berichrom AT (Siemens) | Bovine IIa | 180 sec |
| Stachrom AT (Stago) | Bovine IIa | 60 sec |
| AT Cobas (Roche diagnostics) | Bovine IIa | 120-200 sec |
| **Enzyme Xa** | | |
| Biophen AT anti-hXa-LRT (Hyphen Biomed) | Human Xa | 60-240 sec |
| Coamatic LR AT | Bovine Xa | 90 sec |
| HemosIL AT (IL) | Bovine Xa | 100 sec |
| Innovance AT (Siemens) | Human Xa | 185-210 sec |

ECAT FOUNDATION

*External quality Control for Assays and Tests*
*With a focus on Thrombosis and Haemostasis*

Version: 1.0.0

Survey: 2021-M2
Page 5 of 155
23-July-2021
Labcode: 339C

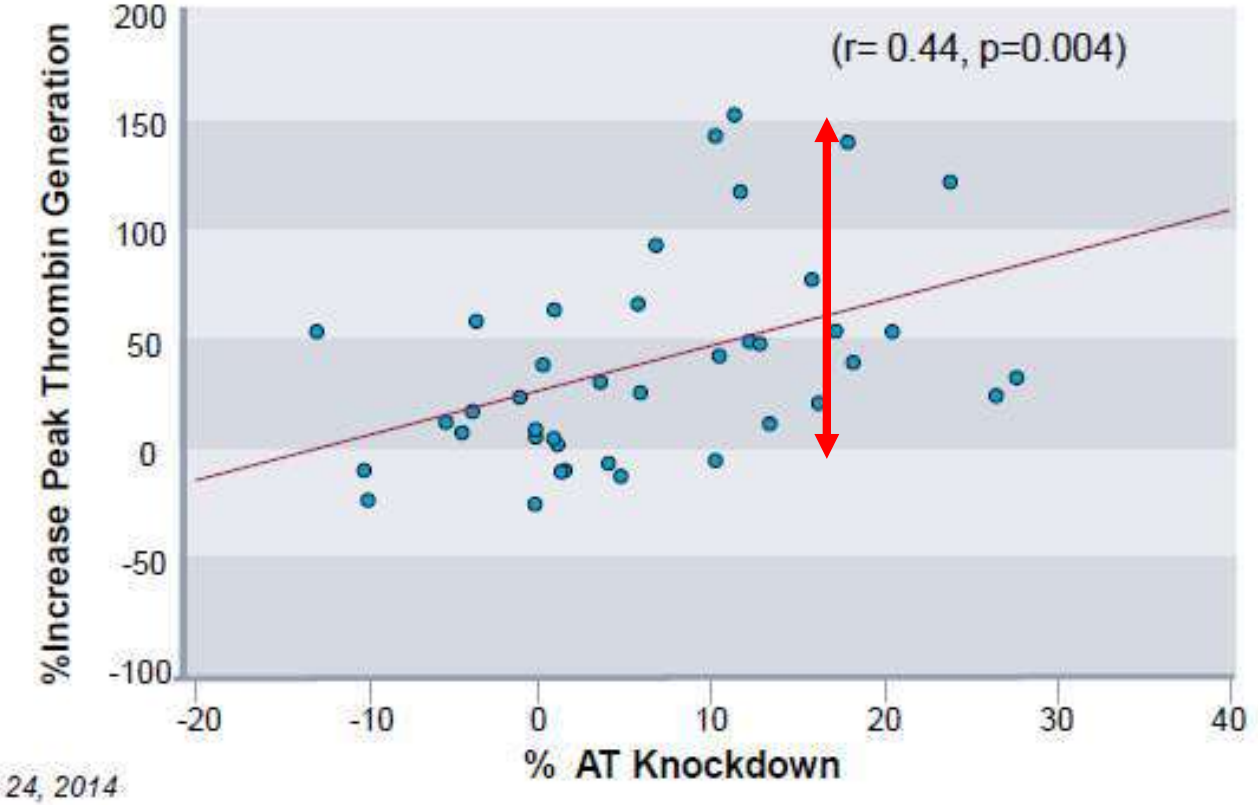
| | n | assigned value | Uncert. | CV (%) | Range |
|---|---|---|---|---|---|
| Total Group | 416 | 33 | 0.47 | 23.2 | 13 - 52 |
| Chromogenic, anti-IIa | 172 | 37 | 0.42 | 11.8 | 24 - 49 |
| Chromogenic, anti-Xa | 244 | 30 | 0.63 | 26.5 | 13 - 52 |

# ALN-AT3 Phase 1 Study Part A (SAD)*

## Pharmacodynamics

### Increase in thrombin generation with AT knockdown

- Significant association between AT knockdown and peak thrombin generation
- Up to 152% increase in peak thrombin generation
- Mean maximum increase of peak thrombin 138% ± 8.9% (mean ± SEM)
  - » Consistent with increased sensitivity for thrombin generation increase with AT knockdown in background of normal levels of Factor VIII or IX



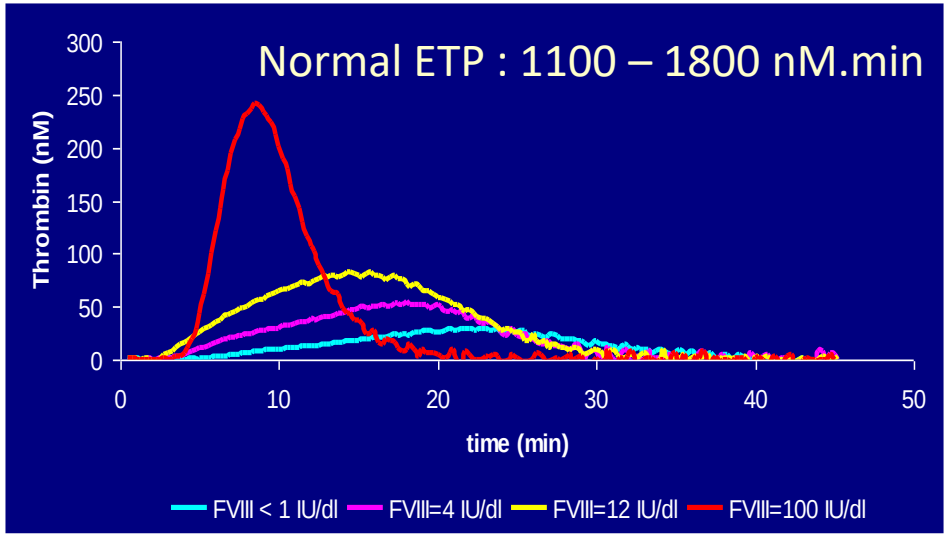

*Data as of Nov. 24, 2014

# Reversal of Fitusiran can be guided by TGA

**In case of surgery or major breakthrough bleed**

## Measure AT and TGA

If TGA is into the normal range, no need to give factor supplementation




**Perioperative Management of Patients with Hemophilia Receiving Fitusiran, an Investigational RNAi Therapeutic Targeting Antithrombin for the Treatment of Hemophilia**

*K.J. Pasi[1,2], C. Négrier[3], M. Ragni[4], P. Georgiev[5,6], T. Lissitchkov[7], S. Kichou[8], B. Mei[8], S. Andersson[8]*

**Abstract Number:** PB1142

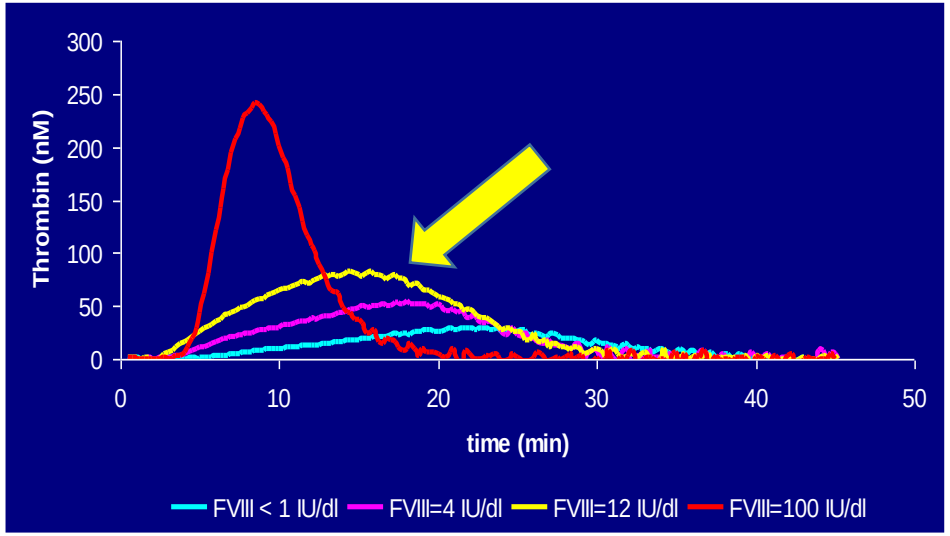
**Meeting:** ISTH 2020 Congress

**Theme:** Hemophilia and Rare Bleeding Disorders » Novel Biotherapeutics in Hemophilia

# Reversal Strategy 1

**If TGA is NOT into the normal range**

Determine the personalized dose of the factor concentrates to correct thrombin generation without inducing hypercoagulability




Dargaud et al. Thromb Haemost 2005

## No Inhibitor

**FVIII or FIX Supplementation**

In vitro spiking with FVIII/FIX concentrate in plasma samples from patients receiving prophylaxis with fitusiran

**FVIII 5 – 10 – 20 - 30 – 50 IU/dL in PPP or PRP**

## Inhibitor +

**By-Passing Therapy**

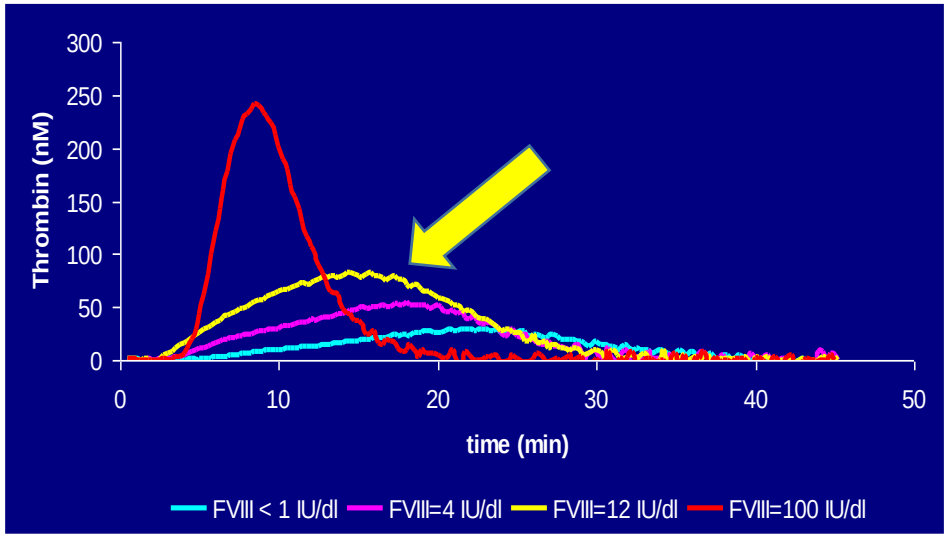
In vitro spiking with rFVIIa and aPCC in blood samples from patients receiving prophylaxis with fitusiran

**rFVIIa 22.5 – 45 – 90 – 270µg/kg in PRP**

**aPCC 5 – 10 – 15 – 20 – 25 – 50 U/kg in PPP or PRP**

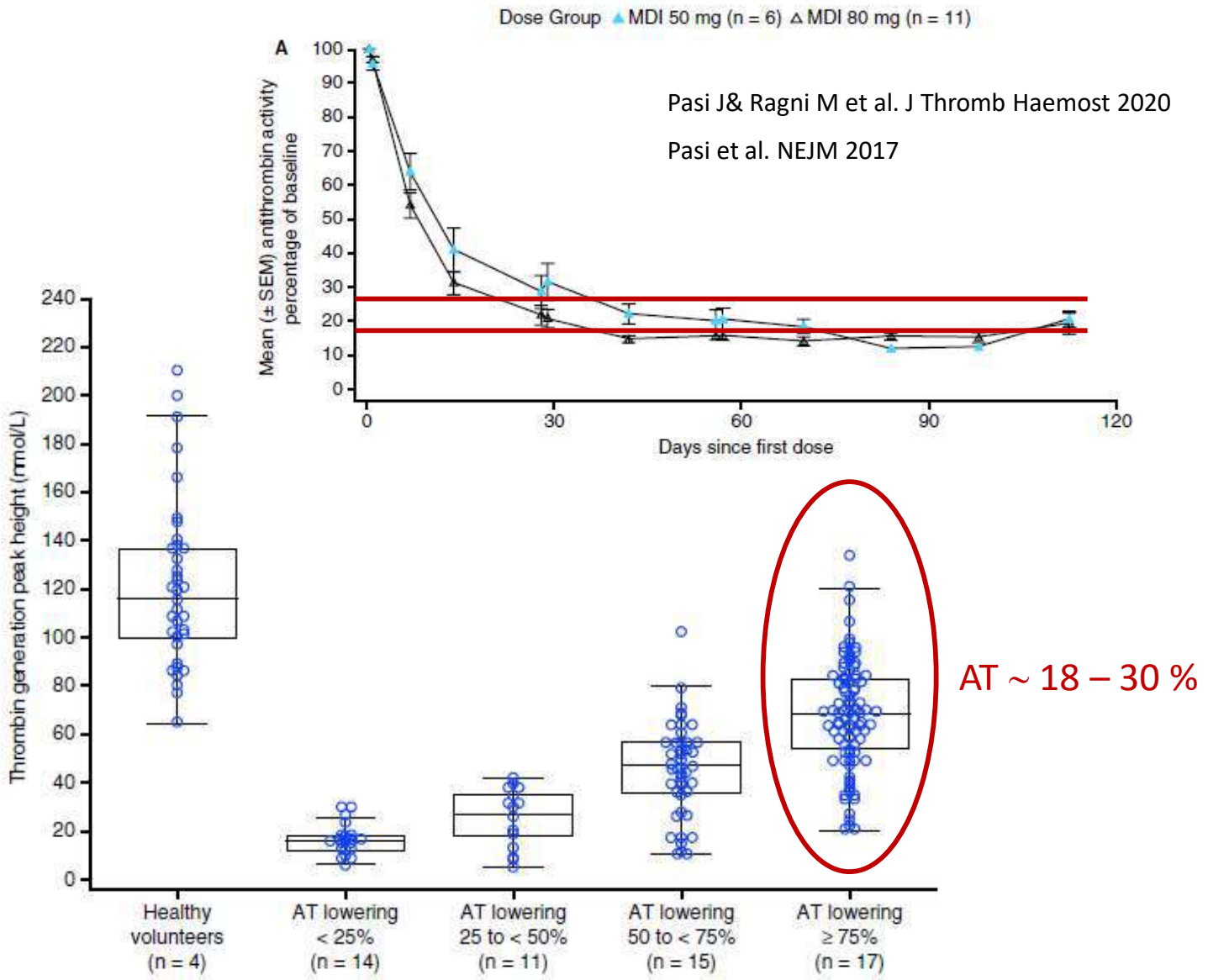
# Reversal Strategy 2

**Correct AT deficiency first and treat patients with usual doses of factors**




Pasi J& Ragni M et al. J Thromb Haemost 2020

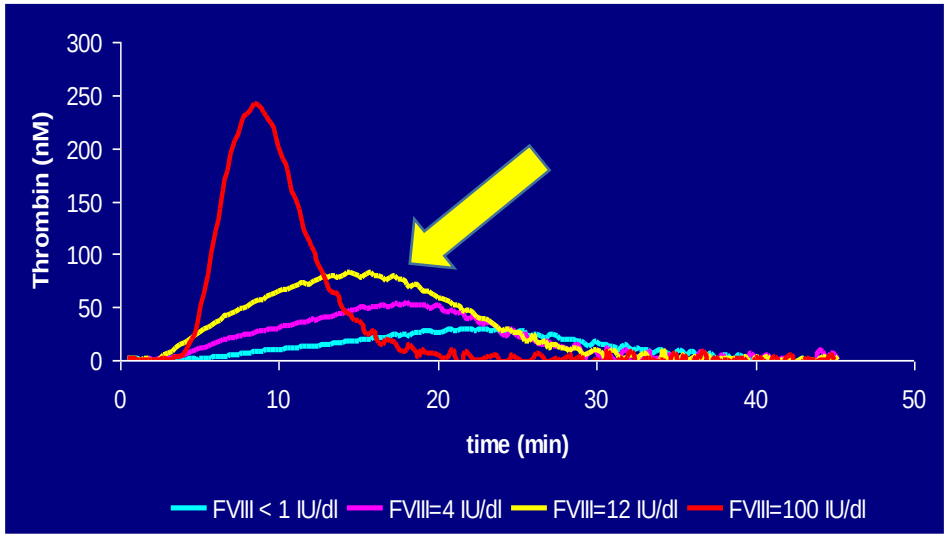
Pasi et al. NEJM 2017




AT ~ 18 – 30 %

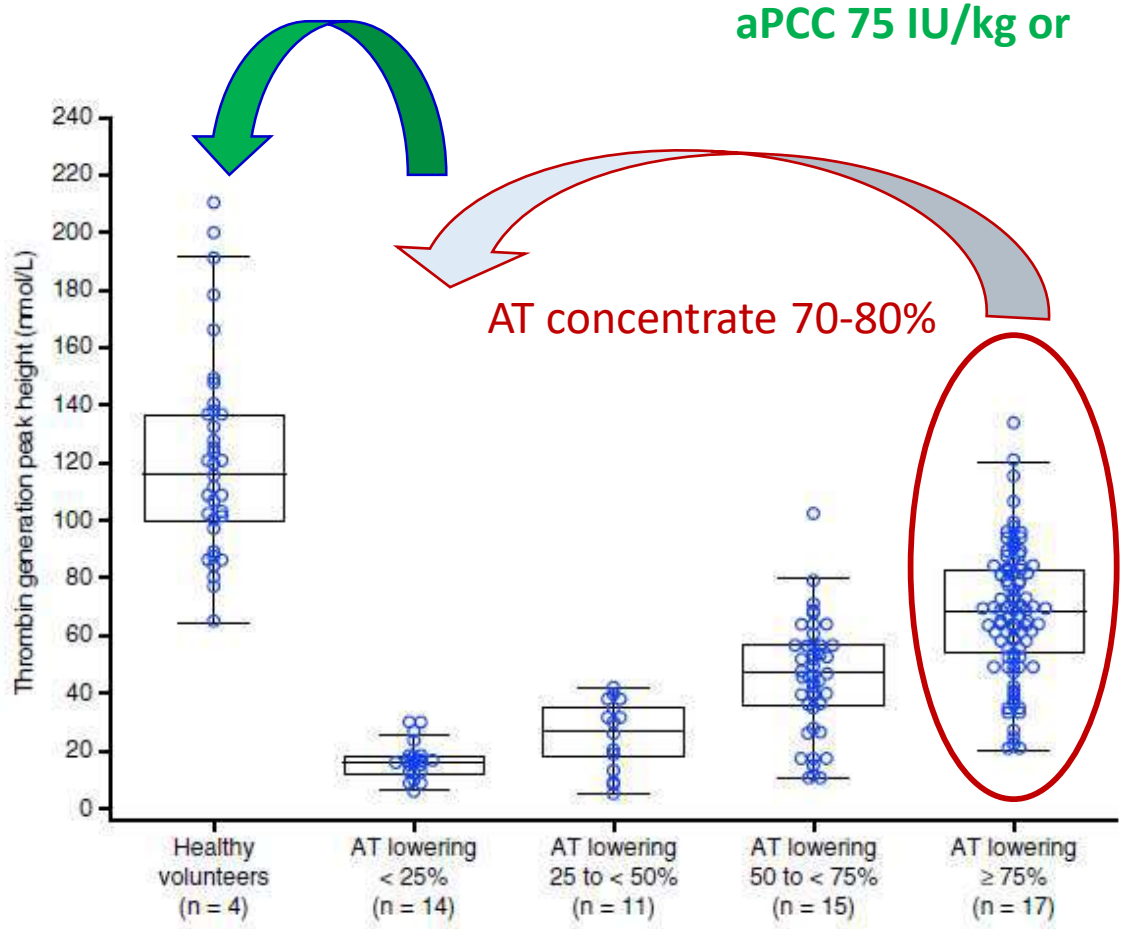
# Reversal Strategy 2

**Correct AT deficiency first and treat patients with usual doses of factors**




**Severe HA no inhibitor FVIII 50 IU/kg**
**Severe HB no inhibitor FIX 100 IU/kg**

**Haemophilia with inhbitors rFVIIa 90 or 270µg/kg**
**aPCC 75 IU/kg or**




AT ~ 18 – 30 %

Received: 15 June 2022 | Revised: 24 October 2022 | Accepted: 9 November 2022

https://doi.org/10.1016/j.rpth.2022.100018

REVIEW

# Thrombin generation and implications for hemophilia therapies: A narrative review

Robert F. Sidonio Jr. MD, MSc[1,2] | Maureane Hoffman MD, PhD[3] | Gili Kenet MD[4,5] | Yesim Dargaud MD, PhD[6]

| Therapy, route of administration, indication | Mechanism of action | Thrombin generation parameter[a]: Lag time (time to start) | Thrombin peak (Velocity of thrombin generation – reflects "thrombin bursting") | ETP (total amount of thrombin generated – area under the curve) | Factor level | Hemostasis status |
|---|---|---|---|---|---|---|
| **Factor therapy** | | | | | | |
| **FVIII concentrate, intravenous dosing, hemophilia A**[47,50,53,56] | Replaces the missing procoagulant factor | ↓ | ↑ | ↑ | | |
| **FIX concentrate, intravenous dosing, hemophilia B**[47,54,56] | Replaces the missing procoagulant factor | ↓ | ↑ | ↑ | | |

| Assay | Pros | Cons |
|---|---|---|
| **Non-factor therapy** | | |
| **Emicizumab, subcutaneous dosing, hemophilia A with and without inhibitors**[44,47,53,55,56] | Bispecific antibody that mimics the function of FVIII by enabling the generation of FXa, the key factor for optimal thrombin generation and maintaining hemostasis | ↓ ↑ ↑ |
| **Fitusiran, subcutaneous dosing, hemophilia A or B, with or without inhibitors**[44,47,51,53,55,57] | siRNA therapeutic that targets and interferes with the expression of the antithrombin protein, decreasing its production and increasing the amount of thrombin generation in people with hemophilia, sufficient to rebalance hemostasis | ↓ ↑ ↑ |
| **Anti-TFPI antibodies, subcutaneous dosing, hemophilia A and B, with or without inhibitors**[47,51,55] | Monoclonal antibodies that favor thrombin generation by neutralising TFPI-mediated inhibition of FVIIa and FXa | ↓ ↑ ↑ |
| **Activated protein C (APC) inhibitor**[4,58] | Modified plasma serine protease inhibiting APC, prolonging the life-span of the prothrombinase complex to directly increase thrombin generation | ↓ ↑ ↑ |

# Standardization of GHA

ISTH 2023
JUNE 24-28 CONGRESS
#ISTH2023 ISTH2023.ORG
montréal

**SSC THROMBIN GENERATION AND GHA**
June 27th 16h30-18h30

*Journal of Thrombosis and Haemostasis*, **15**: 1704–1707 DOI: 10.1111/jth.13743

**RECOMMENDATIONS AND GUIDELINES**

## Proposal for standardized preanalytical and analytical conditions for measuring thrombin generation in hemophilia: communication from the SSC of the ISTH

Y. DARGAUD,* A. S. WOLBERG,† E. GRAY,‡ C. NEGRIER,* H. C. HEMKER,§ FOR THE SUBCOMMITTEE ON FACTOR VIII, FACTOR IX, AND RARE COAGULATION DISORDERS
*Hospices Civils de Lyon, Clinical Haemostasis Unit, Hopital Cardiologique Louis Pradel - Universite Lyon 1, Lyon, France; †Department of Pathology and Laboratory Medicine, UNC, Chapel Hill, NC, USA; ‡Ha Standards and Control, Potters Bar, Hertfordshire, UK; and §Cardiova Maastricht, the Netherlands

*Journal of Thrombosis and Haemostasis*, **12**: 103–106 DOI: 10.1111/jth.12458

**RECOMMENDATIONS AND GUIDELINES**

## Recommendations for performing thromboelastography/ thromboelastometry in hemophilia: communication from the SSC of the ISTH

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# Conclusion

1- Routine laboratory assays are efficient to monitor SHL FVIII and FIX molecules
Need improvements for EHL products (specific calibration)

2- Haemophilia therapies have been tremendously and very rapidly improved during the last decade but laboratory assays did not

3- We have laboratory assays able to detect the **concentration** of non-factor therapies (ELISA, LC-MS or chrFVIII:C based measurements, AT….) but :
- these assays do not correlate with the clinical efficacy of these drugs
- and they can not indicate the haemostatic efficacy of combined therapies (EMI+BPA or Fitusiran+FVIII/FIX or Mim8+rFVIIa…..)

4- Global haemostasis assays are good candidates to monitor new therapies
Need for a working group of the ISTH FVIII-FIX SSC to work on this topic

# GRACIAS!!!!