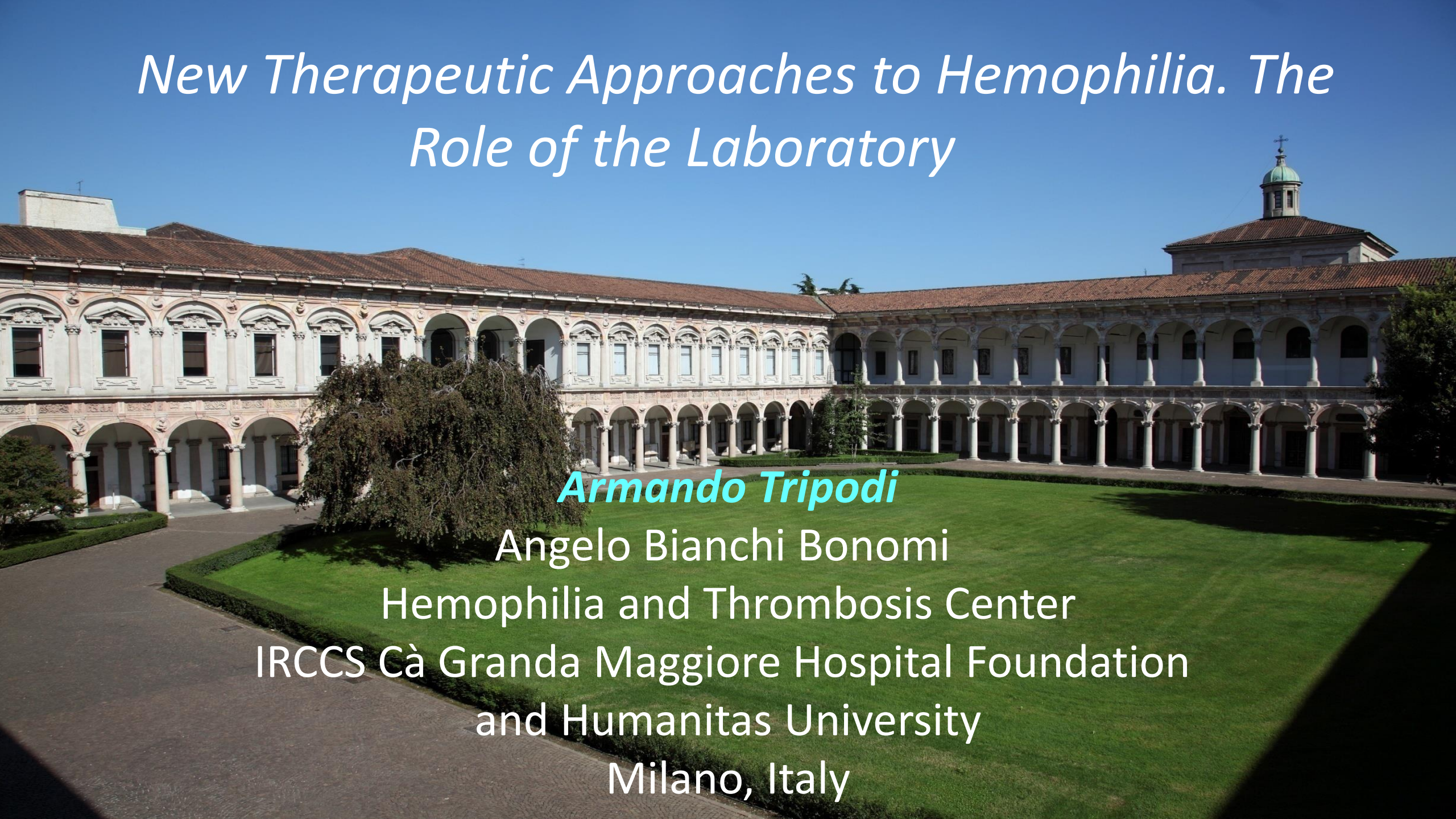
New Therapeutic Approaches to Hemophilia. The Role of the Laboratory



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Coagulation Laboratory & Hemophilia
Current Challenges

- *Monitoring conventional bypassing agents (aPCC, rFVIIa)*
- Monitoring *long-acting* FVIII/IX concentrates
- Monitoring *innovative drugs*, not based on replacement therapy

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Need for Bypassing Agents in Hemophilia

- ***20-30%*** of hemophilia patients develop inhibitors to FVIII
- Because of these inhibitors, treatment with FVIII or IX concentrates is ineffective
- Hence, agents ***bypassing FVIII*** are needed

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Current Bypassing Agents to Treat Hemophilia

- (Activated) prothrombin complex concentrates (aPCC)
  - *FII, VII(a), IX and X*
- Activated recombinant FVII
  - *rFVIIa*

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Laboratory monitoring of conventional bypassing agents

- aPCC achieves hemostatic efficacy without modifying the plasma levels of FVIII/IX
- Measuring post-infusion FVIII/IX is unsuitable to monitor aPCC efficacy
- Unknown if measuring post-infusion FVII is useful to monitor rFVIIa
- Global coagulation assays should be the candidates
  - *Thromboelastometry (whole blood)*
  - *Thrombin generation (platelet-poor or platelet-rich plasma)*

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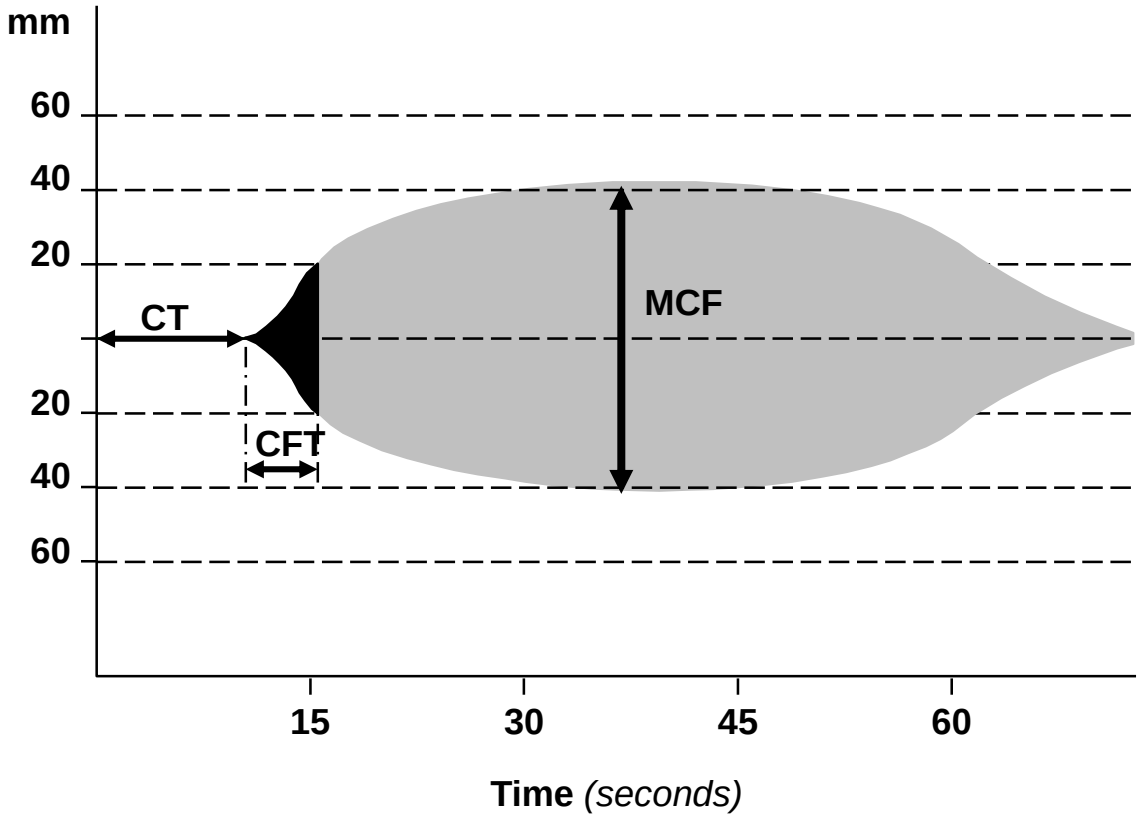
Thromboelastometry Parameters



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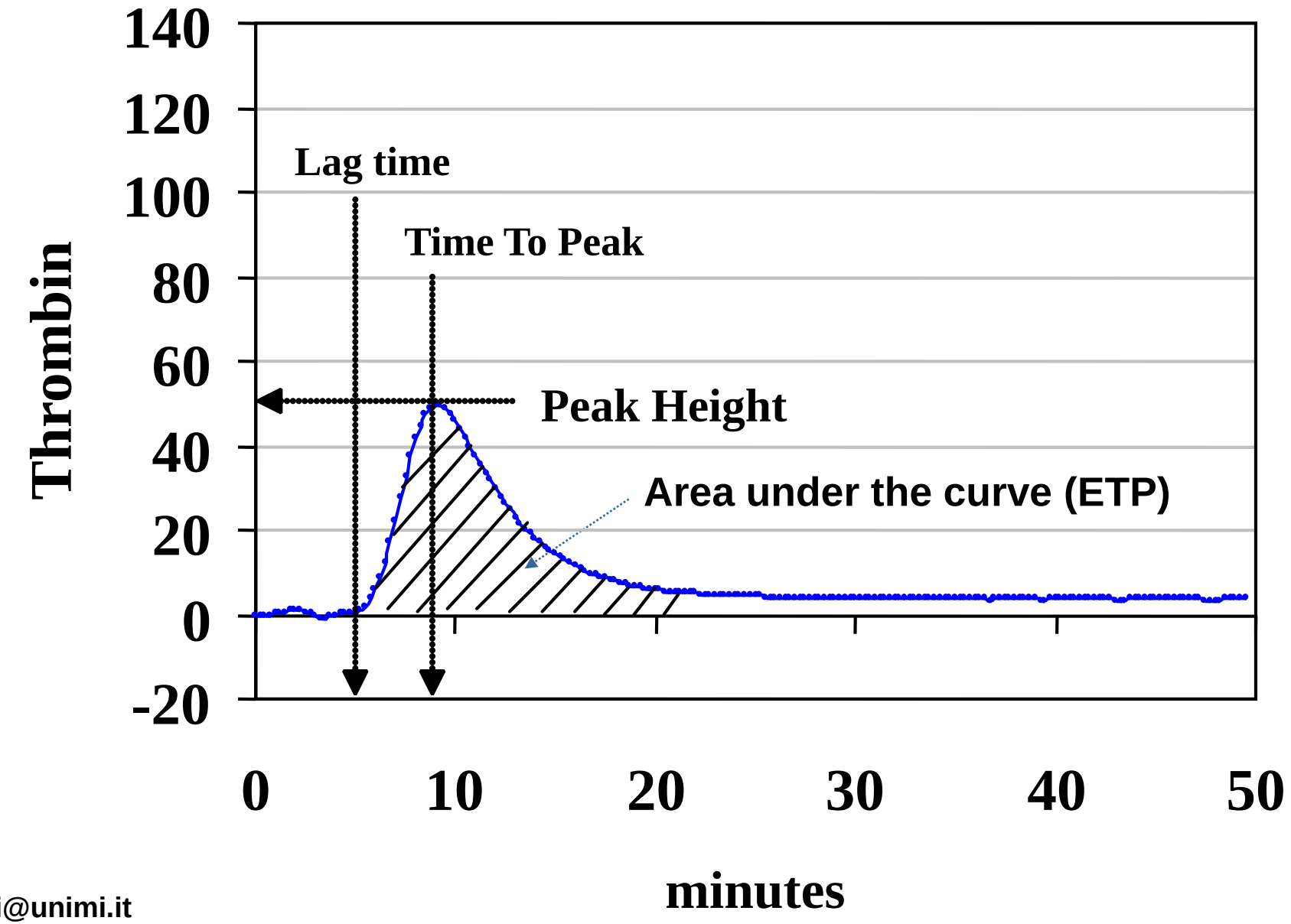
Thrombin Generation Parameters



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Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and
the Hemostasis & Thrombosis Research Society

Haemophilia (2015), **21**, 275–283 DOI: 10.1111/hae.125

ORIGINAL ARTICLE *Laboratory science*

Monitoring bypassing agent therapy – a prospective crossover study comparing thromboelastometry and thrombin generation assay

H. T. T. TRAN,*†‡ B. SØRENSEN,§ S. BJØRNSEN,* A. H. PRIPP,*¶ G. E. TJØNNFJORD†‡ and P. ANDRE HOLME*†‡

Laboratory endpoint only

Positive Study

Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and
the Hemostasis & Thrombosis Research Society

Haemophilia (2016), **22**, e292–e300 DOI: 10.1111/hae.12939

ORIGINAL ARTICLE *Laboratory science*

Low thrombin generation during major orthopaedic surgery fails to predict the bleeding risk in inhibitor patients treated with bypassing agents

M. E. MANCUSO,* V. CHANTARANGKUL,* M. CLERICI,* M. R. FASULO,* L. PADOVAN,* E. SCALAMBRINO,* F. PEYVANDI,*† A. TRIPODI*‡ and E. SANTAGOSTINO*

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Clinical endpoint!

Negative Study

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Dargaud Y et al, Blood 2010

THROMBOSIS AND HEMOSTASIS

Brief report

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

Yesim Dargaud,[1,2] Anne Lienhart,[1] and Claude Negrier[1,2]

Clinical endpoint!

Positive Study

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Summary on Monitoring Bypassing Agents

(according to the literature)

- Thrombin generation or thromboelastometry are responsive to aPCC or rFVIIa
- Contrasting results on the clinical value of the two assays
- None of the two is licensed by regulatory authorities
- Thrombin generation not yet standardized
- Thromboelastometry relatively simple as bedside device

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Coagulation Laboratory & Hemophilia
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- *Monitoring long-acting FVIII/IX concentrates*
- Monitoring *innovative drugs*, not based on replacement therapy

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Need for Long-acting FVIII/IX

- *Native FVIII & IX have relatively short half-life (few hours)*
  - Patients on prophylaxis need repeated infusions over short period
- *Extended half life achieved by conjugation/fusion of FVIII/FIX with:*
  - Polyethylene glycol (PEG)
  - Fc fraction of Ig
  - Albumin
- *Extension of half-life is greater for FIX than FVIII*

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Types of Assays to measure FVIII/IX

- One-stage clotting
  - *APTT reagent(s) & factor-deficient plasma(s)*
- Chromogenic
  - *Activation of coagulation and FXa generation*
  - *FXa measurement by synthetic chromogenic substrates*
- Both need standard(s) (pooled normal plasma) run in parallel to calculate factor activity

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Summary on Monitoring Long-acting Factors

- Discrepant results depending on whether one-stage-clotting or chromogenic assays are used for post-infusion measurement
- One-stage clotting assays may give different post-infusion results according to the APTT reagent
  - *Activators (silica or ellagic acid)*
  - *Phospholipids*

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Approaches to minimize discrepancy of post-infusion results (one-stage clotting vs chromogenic)

- Switch to chromogenic assays for all products
- Use product-specific standards *(like-vs-like)*
- Use the same method employed for potency assignment
- Use product-specific methods based on data from literature and info provided by manufacturers
- Whatever the choice, tight collaboration clinicians/lab operators is essential

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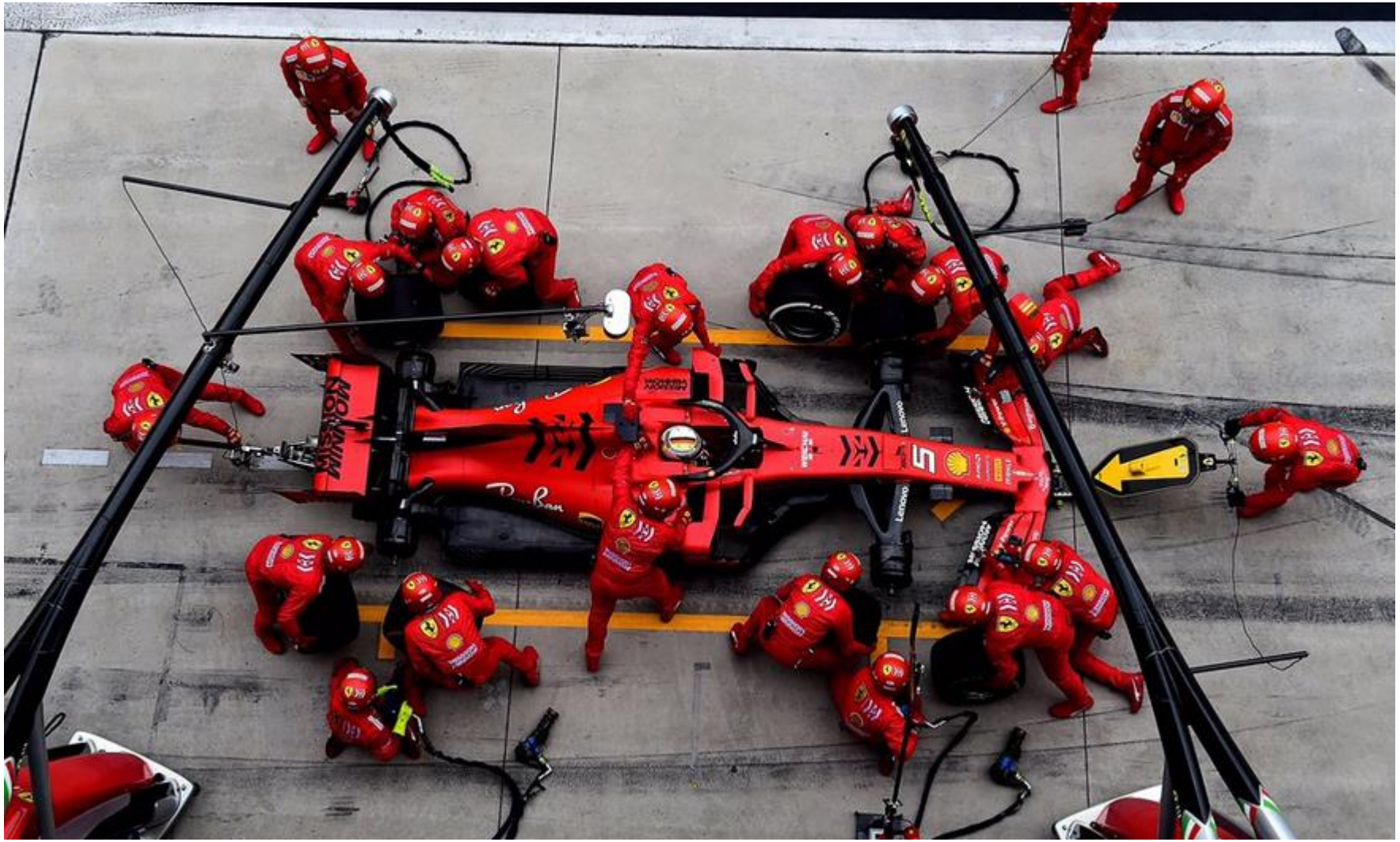

Tight collaboration between operators is essential

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Coagulation Laboratory & Hemophilia

Current Challenges

- *Monitoring conventional bypassing agents (aPCC, rFVIIa)*
- *Monitoring long-acting FVIII/IX concentrates*
- *Monitoring innovative drugs, not based on replacement therapy*

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Drugs not based on replacement therapy

- ***Emicizumab***
  - Recombinant, humanized **bi-specific antibody** binding FIXa to FX, thus bypassing FVIII in the activation of FX
- ***Fitusiran***
  - RNA interference molecule **reducing antithrombin expression**, thus enhancing thrombin generation
- ***Concizumab***
  - Humanized monoclonal **anti-TFPI** antibody, enhancing thrombin generation

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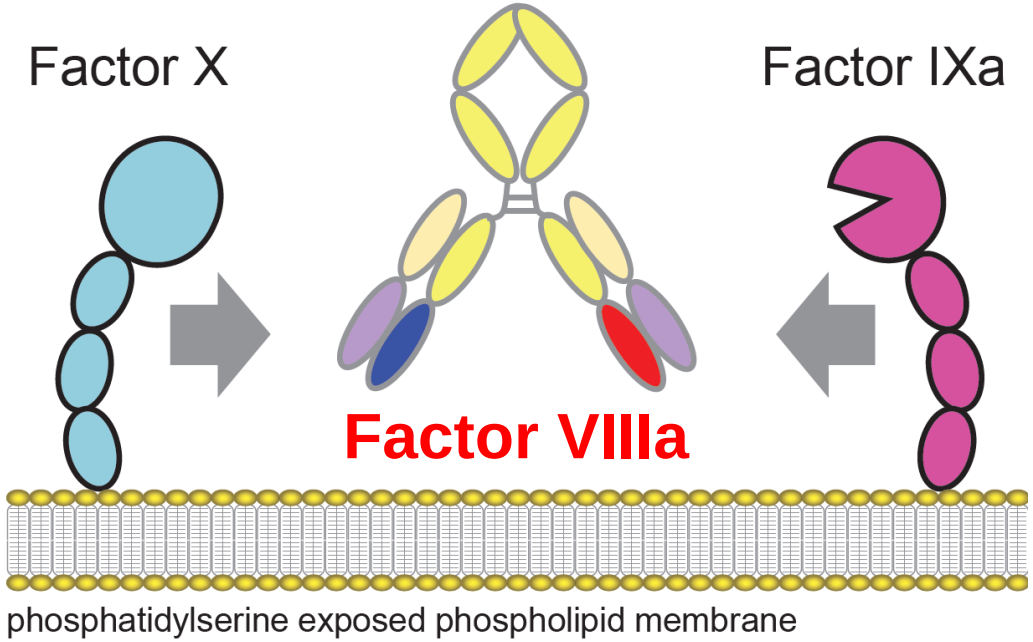
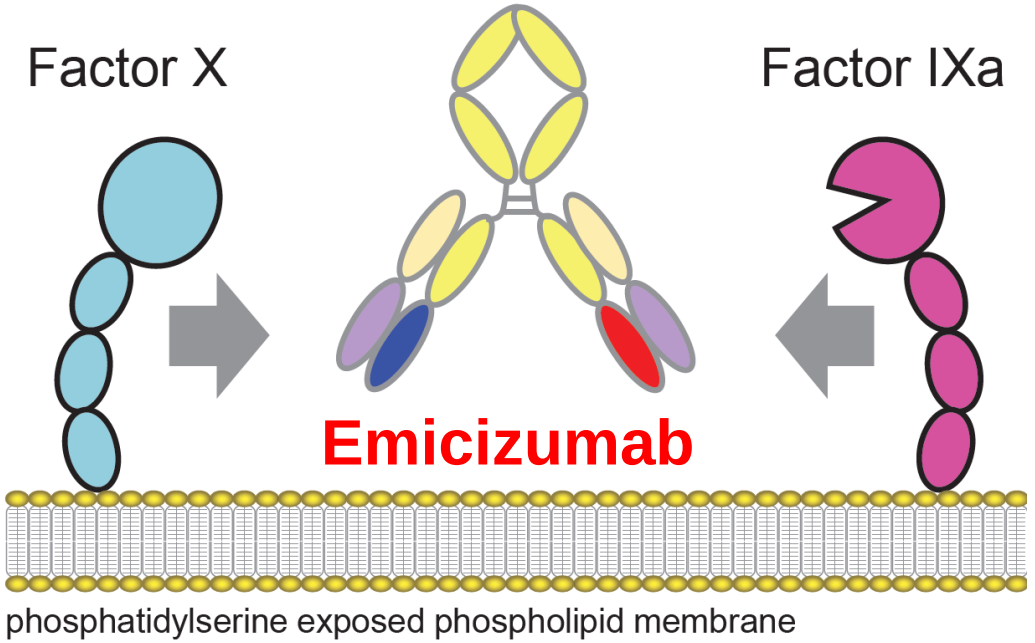
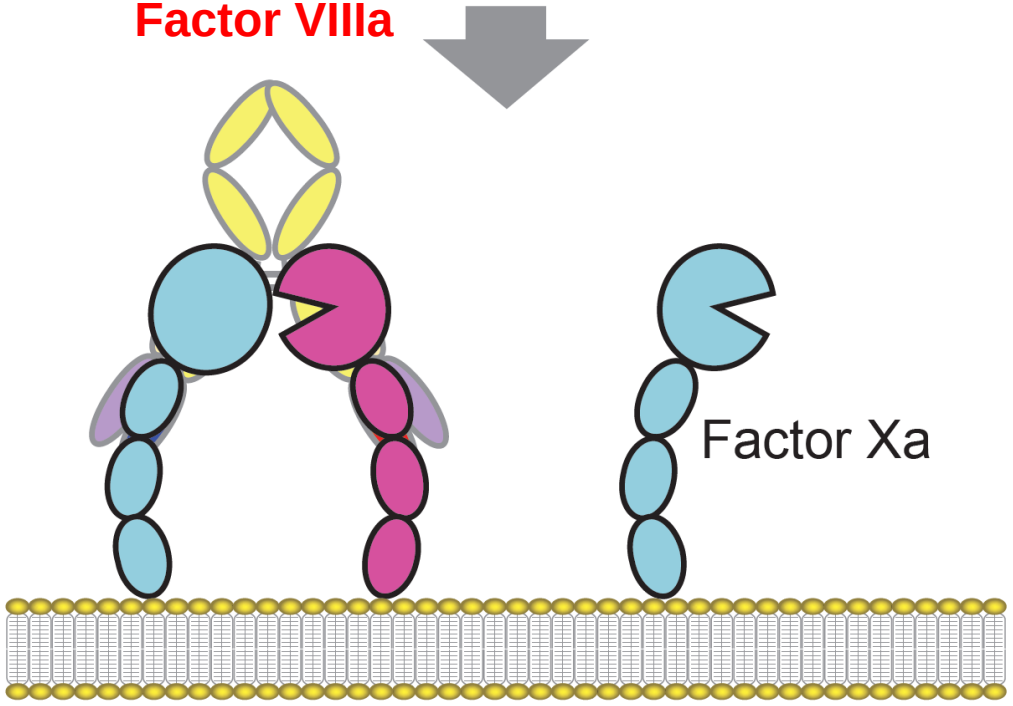
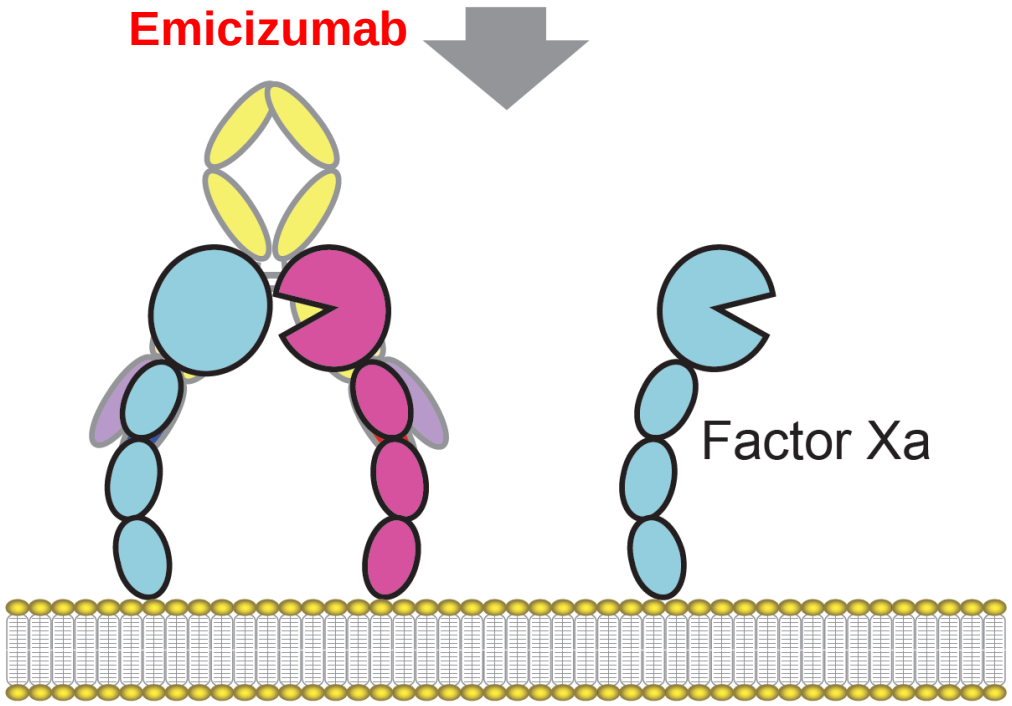

Factor X
Factor IXa
Factor VIIIa
phosphatidylserine exposed phospholipid membrane


Factor X
Factor IXa
Emicizumab
phosphatidylserine exposed phospholipid membrane


Factor VIIIa
Factor Xa


Emicizumab
Factor Xa

Laboratory Monitoring of Emicizumab

- Apparently, lab monitoring **(i.e., dose-adjustment)** is not needed for this drug
- However, assessment of its activity **(concentration)** may be useful in special circumstances

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Measuring the Effect of Emicizumab

- APTT
- Measurement of FVIII-surrogate activity based on:
  - *One-stage clotting assays*
- Measurement of emicizumab concentration based on:
  - *Modified one-stage clotting or chromogenic assays & plasma calibrators*

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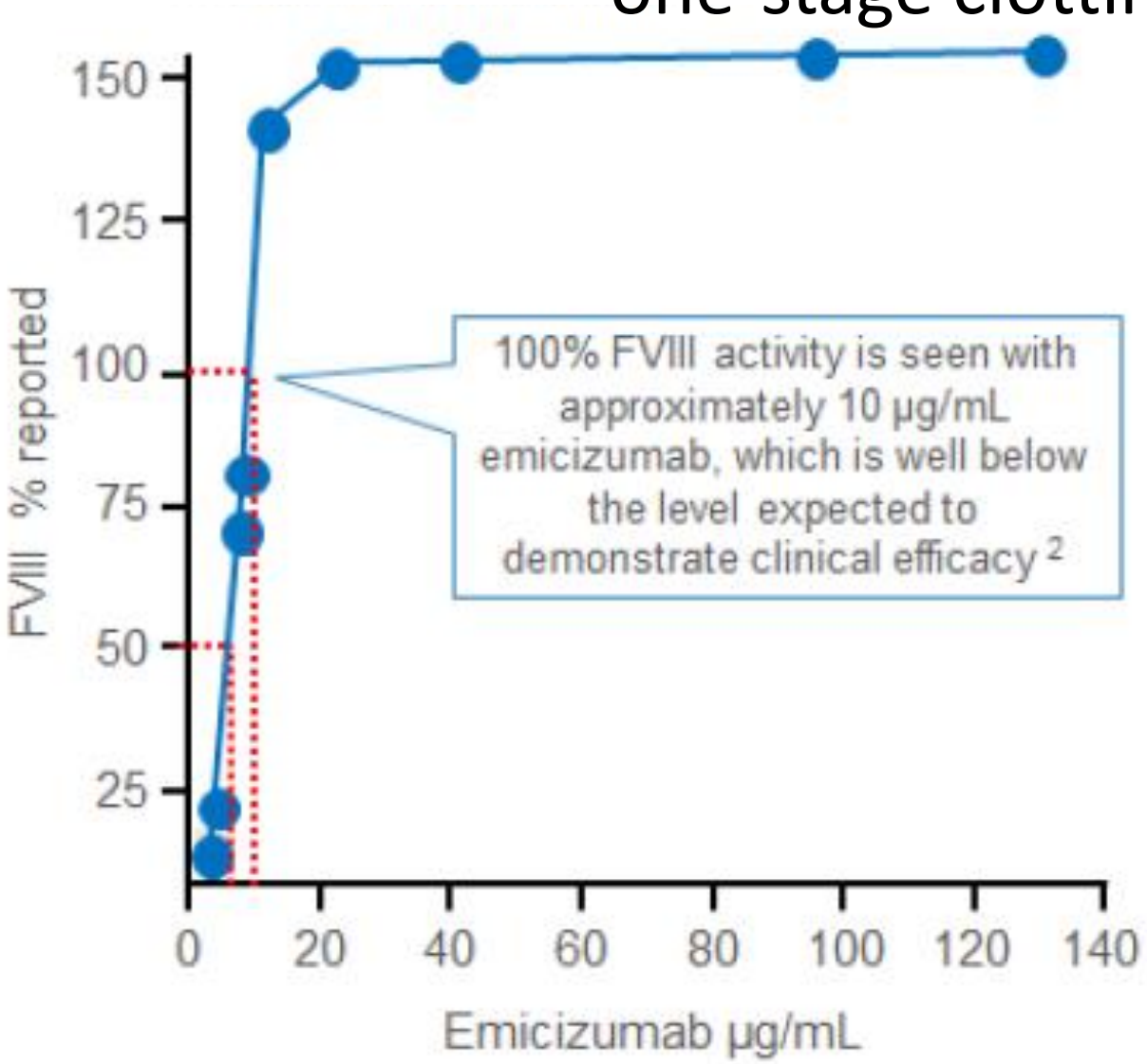
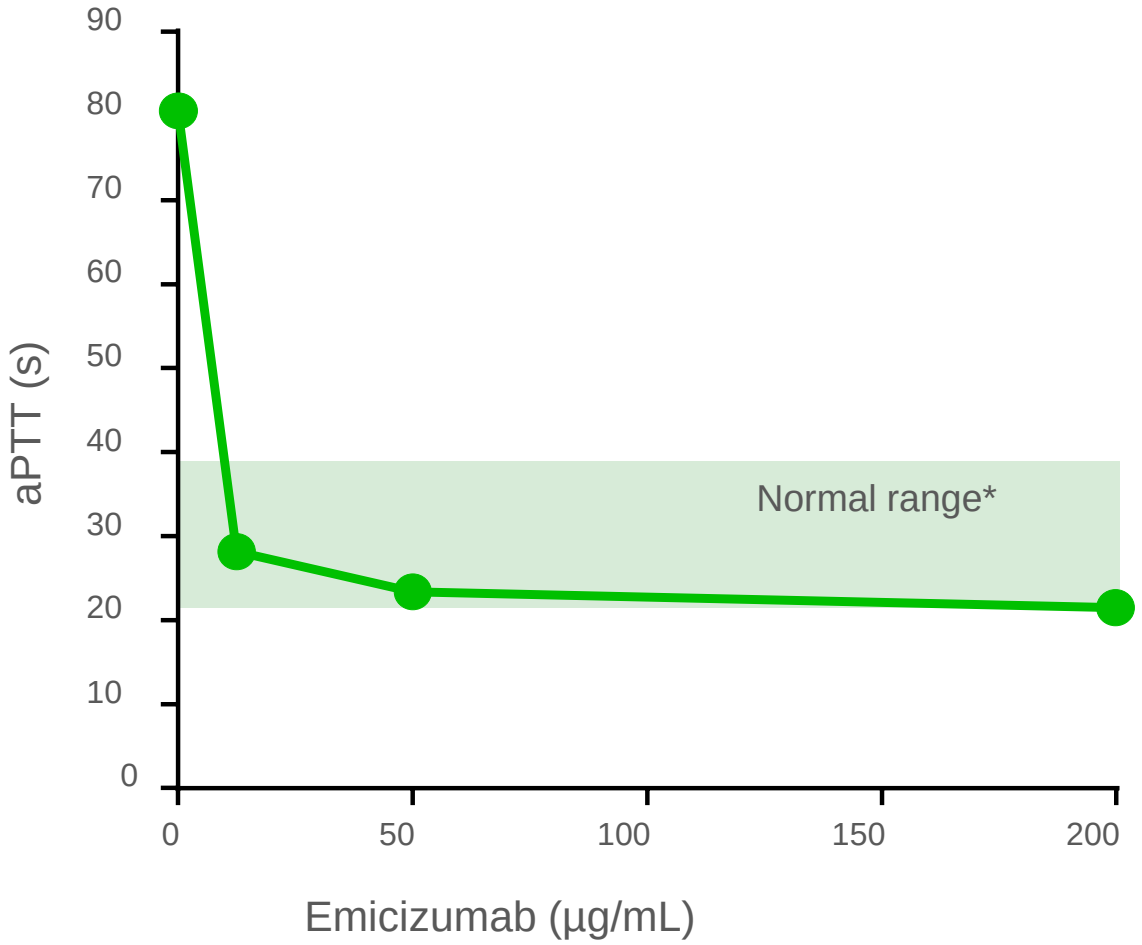
FVIII-surrogate activity measured with the regular one-stage clotting assay



Calatzis A, ECTH, 2016

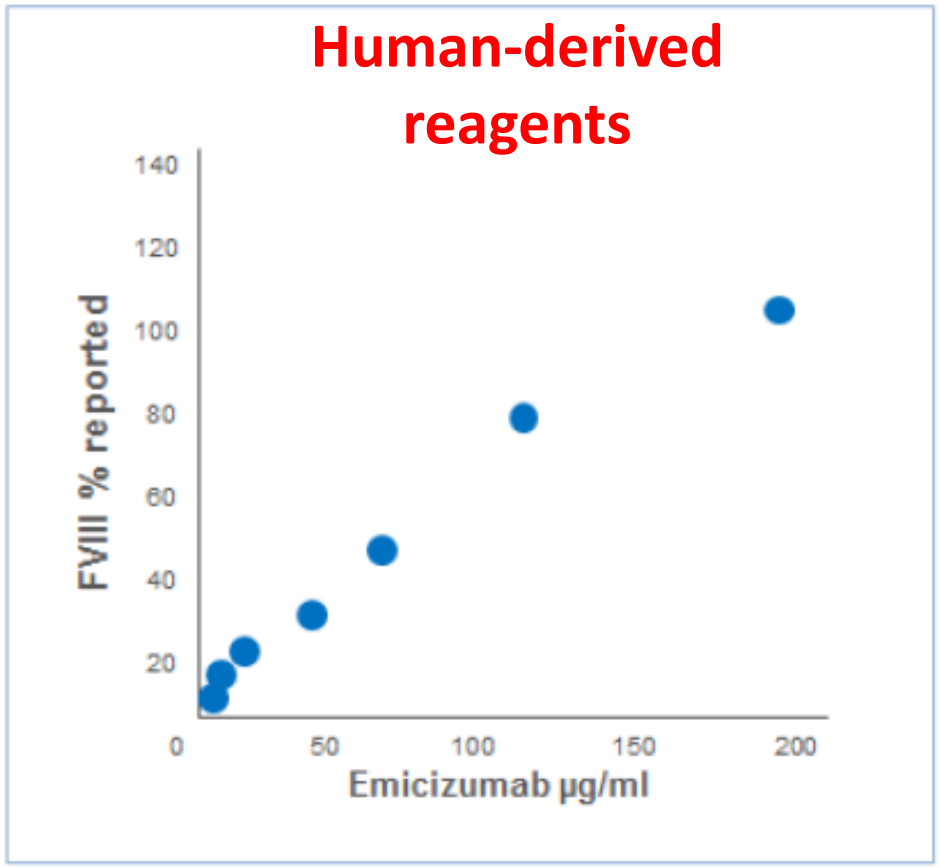
Emicizumab & APTT



APTT of hemophilic plasma is normalized at very low emicizumab concentrations

Calatzis et al. ISLH 2017

Chromogenic assay to measure emicizumab



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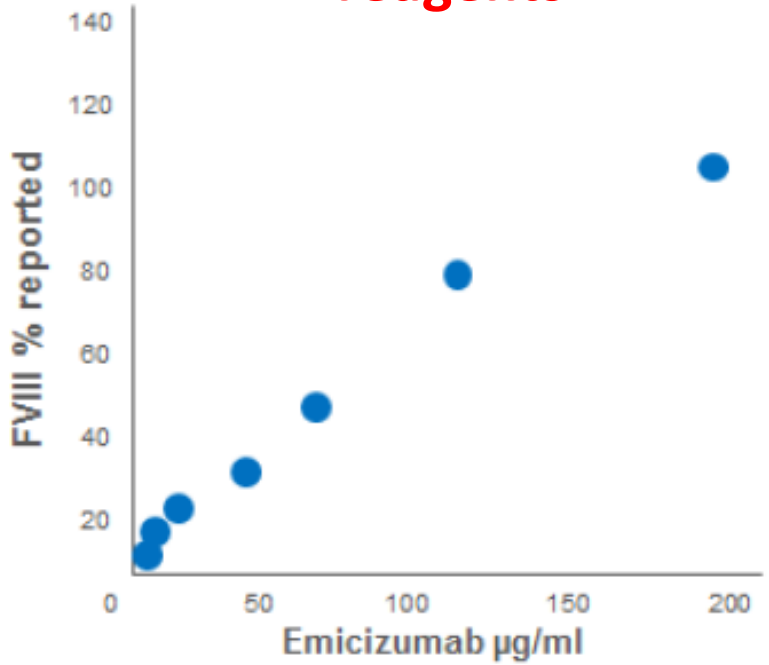
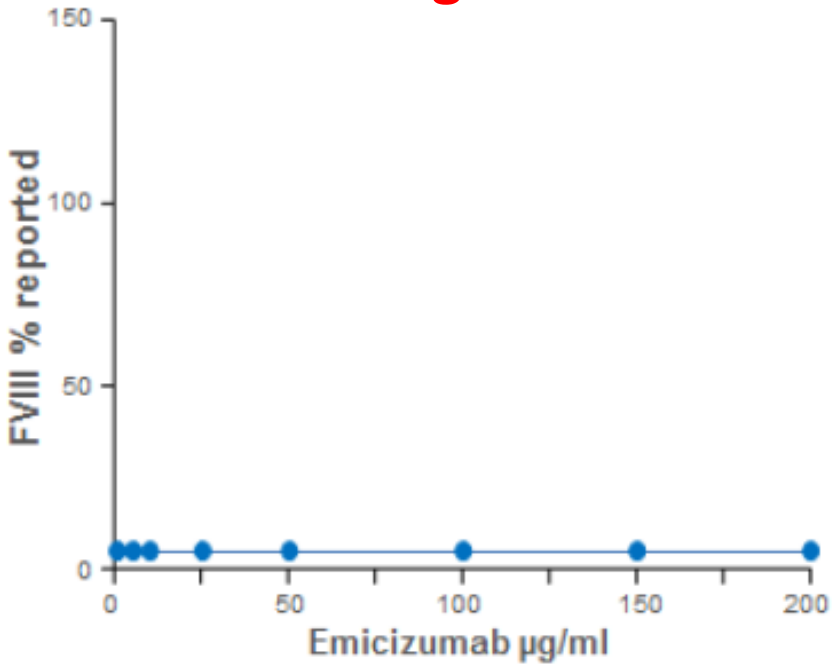
Calatzis A, ECTH, 2016

Chromogenic assay to measure emicizumab

Human-derived reagents



Bovine-derived reagents



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Calatzis A, ECTH, 2016

Summary on Emicizumab and Lab Tests

Assay	Results for patients on emicizumab
APTT	Over-responsive

Summary on Emicizumab and Lab Tests

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FVIII-surrogate activity (unmodified one-stage clotting) assay)	Over-responsive

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Drug concentration (modified one-stage clotting assay)	Responsive (dose-dependent)

Summary on Emicizumab and Lab Tests

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Drug concentration (Chromogenic assay, ***human***)	Responsive (dose-dependent)

Summary on Emicizumab and Lab Tests

Assay	Results for patients on emicizumab
APTT	Over-responsive
FVIII-surrogate activity (**unmodified** one-stage clotting) assay)	Over-responsive
Drug concentration (**modified** one-stage clotting assay)	Responsive (dose-dependent)
Drug concentration (Chromogenic assay, ***human***)	Responsive (dose-dependent)
Chromogenic assay, ***bovine***	Completely insensitive (useful to assess inhibitors to FVIII or FVIII replacement)

Emicizumab Plasma Calibrators & Controls are Available

r^2 Diagnostics Inc.
South Bend, IN

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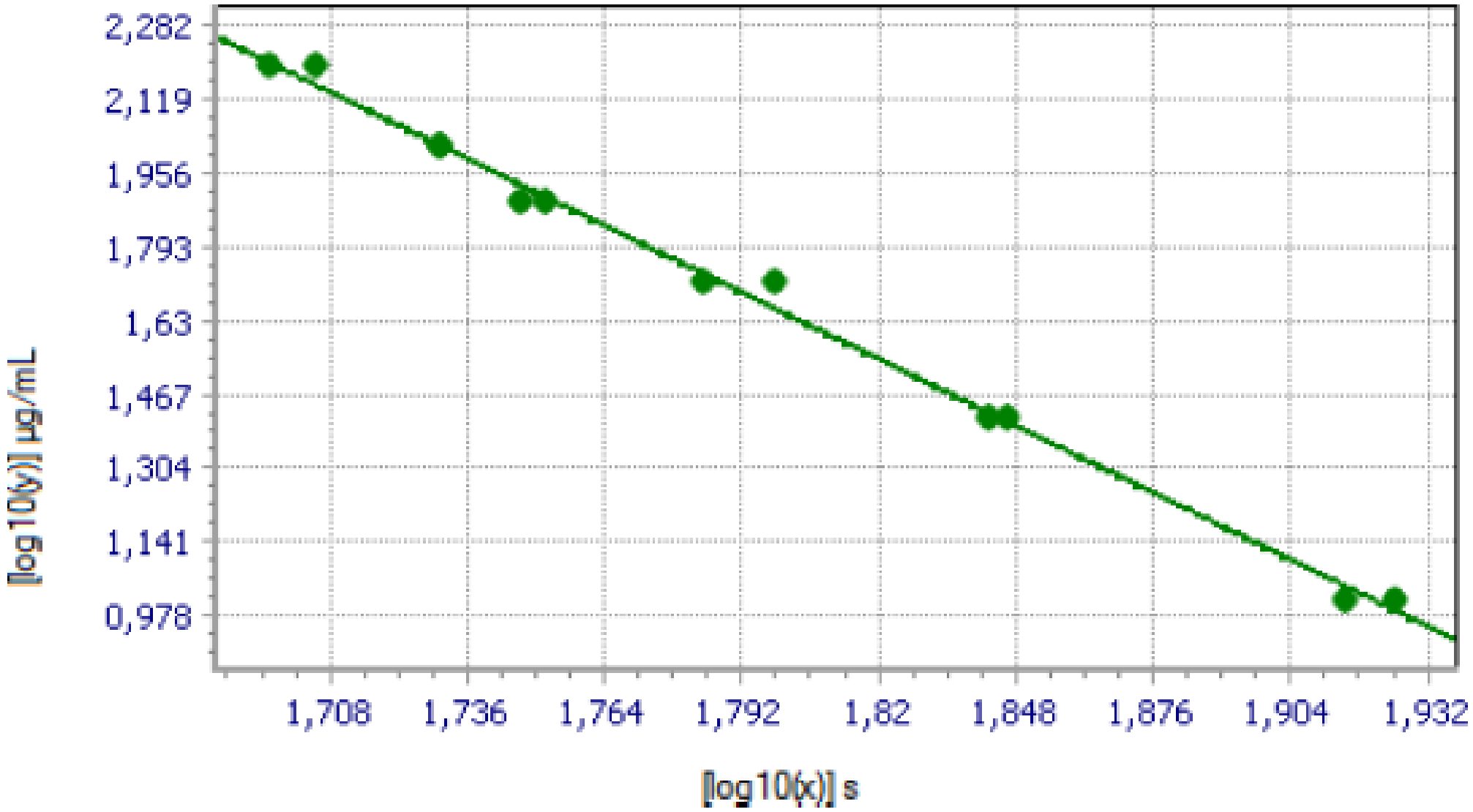
Emicizumab Calibration Curve (modified one-stage clotting assay Werfen)



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Conclusions (1)

- Monitoring *conventional bypassing agents* is still a challenge. Global coagulation assays are the candidate assays. Clinical experience is needed
- Monitoring *long acting factors* requires careful consideration on the product and lab methods. Switching to chromogenic assays might be the pragmatic solution

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Conclusions (2)

- Emicizumab plasma concentration can be measured by modified one-stage clotting or chromogenic assays *(human reagents)* combined with emicizumab calibrators
- FVIII inhibitors in patients on emicizumab can be measured by the modified chromogenic assay with *bovine reagents*

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Emicizumab may interfere with some of the most common hemostatic parameters

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THIEME OPEN ACCESS

Coagulation and Fibrinolysis

Effects and Interferences of Emicizumab, a Humanised Bispecific Antibody Mimicking Activated Factor VIII Cofactor Function, on Coagulation Assays

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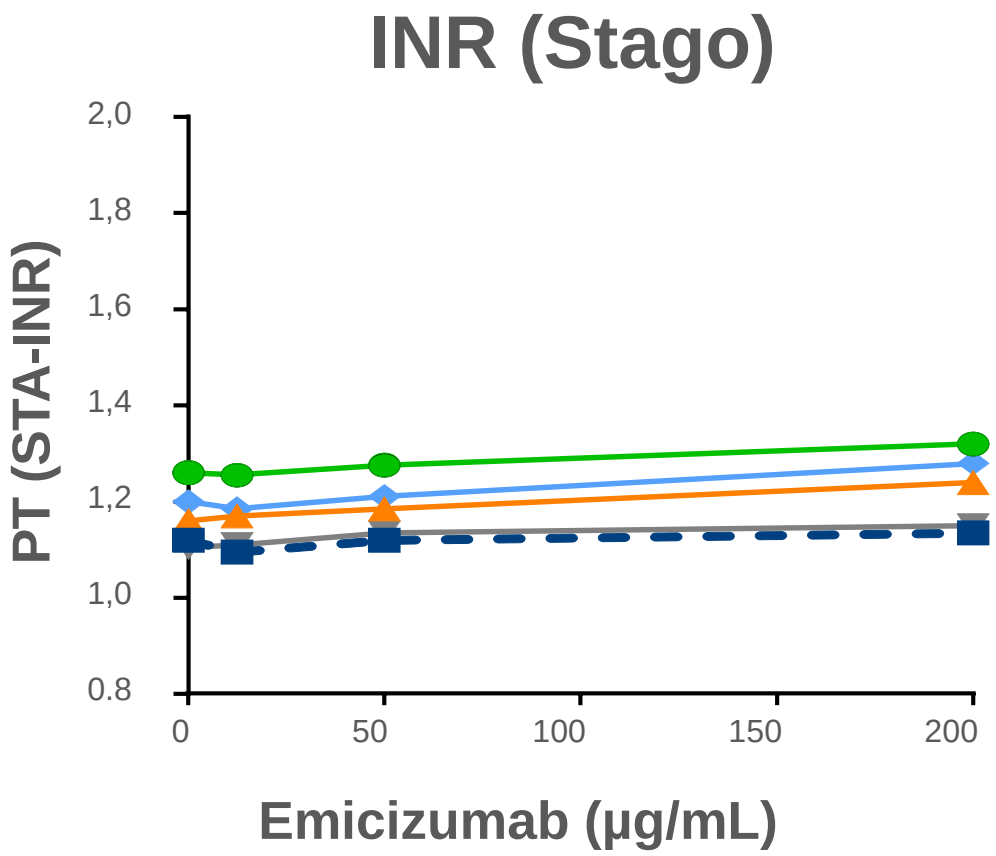
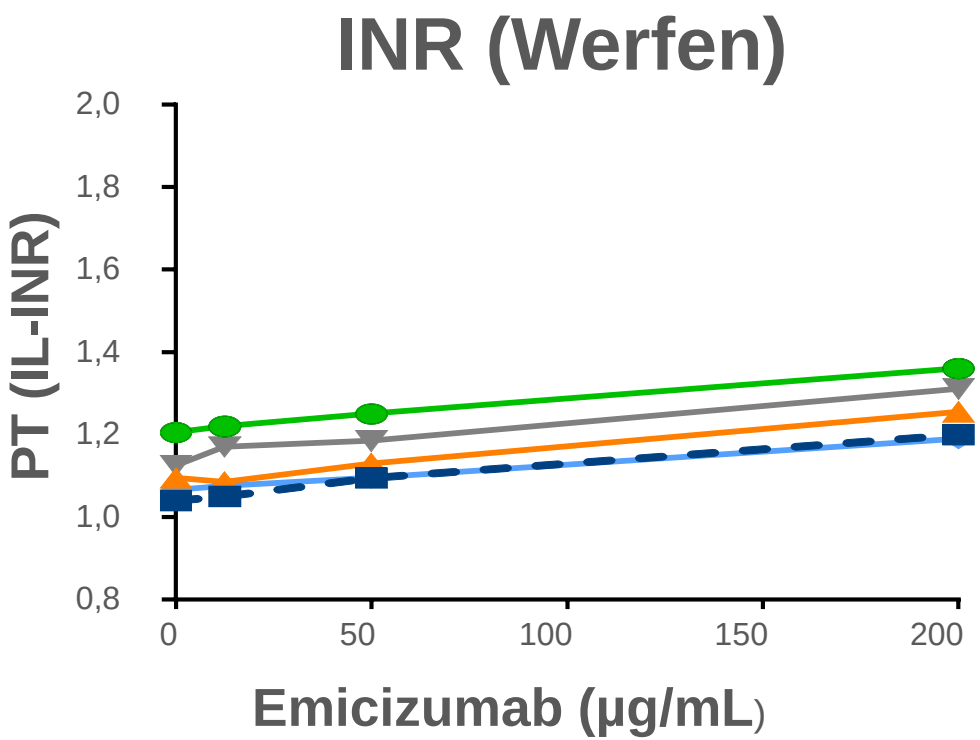
[1] Genentech, Inc., South San Francisco, California, United States

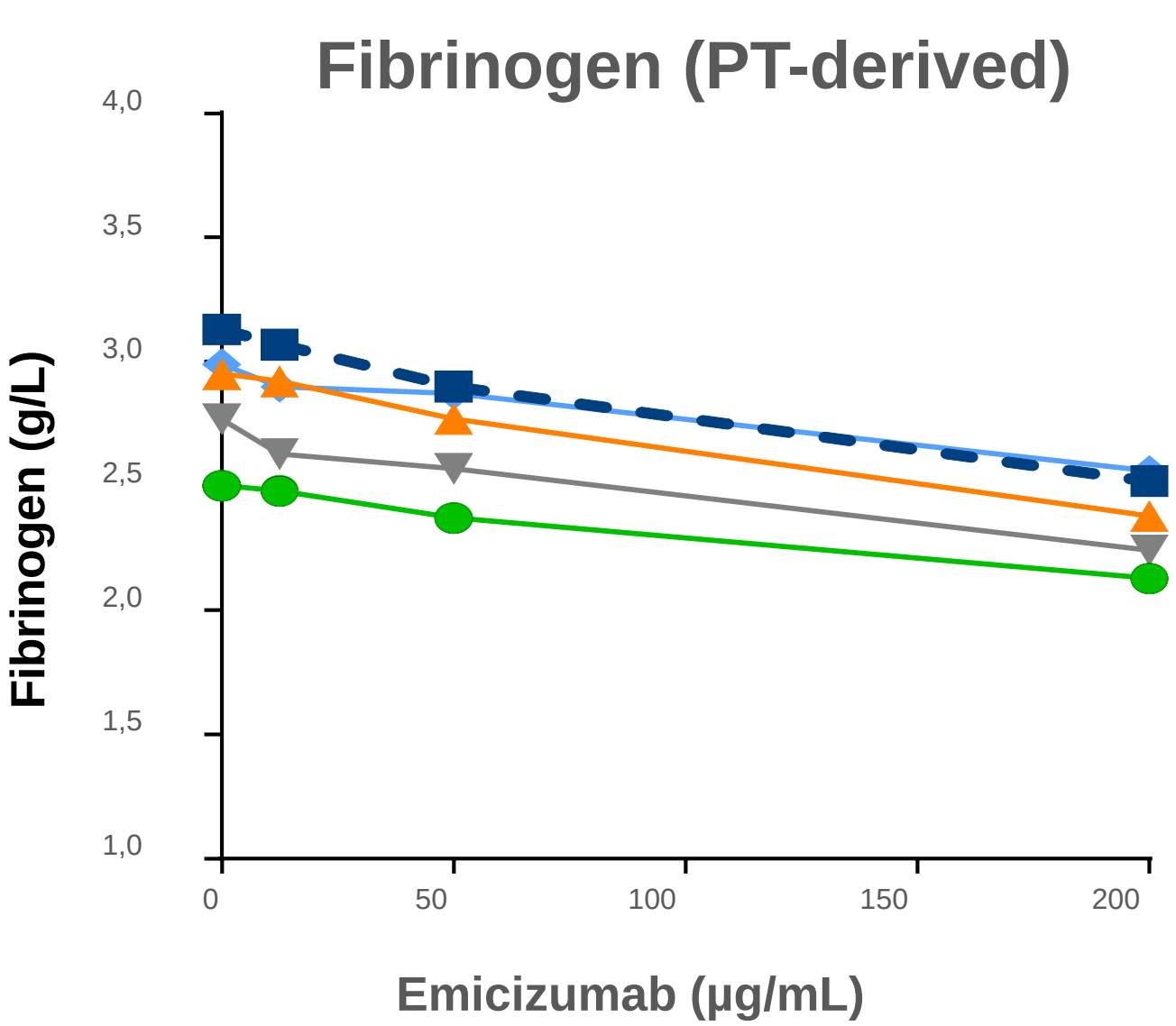
Thromb Haemost

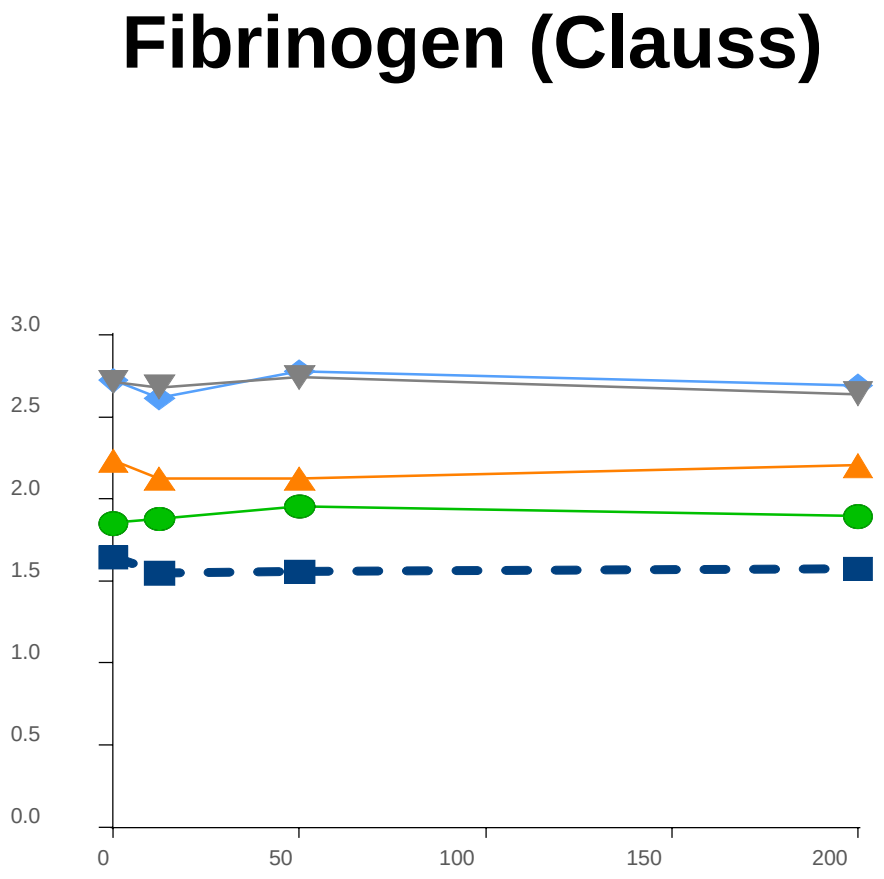
Address for correspondence Joanne I. Adamkewicz, PhD, Genentech, Inc., 1 DNA Way, MS 422a, South San Francisco, CA 94080, United States (e-mail: adamkewicz.joanne@gene.com).





Calatzis A et al, 2017





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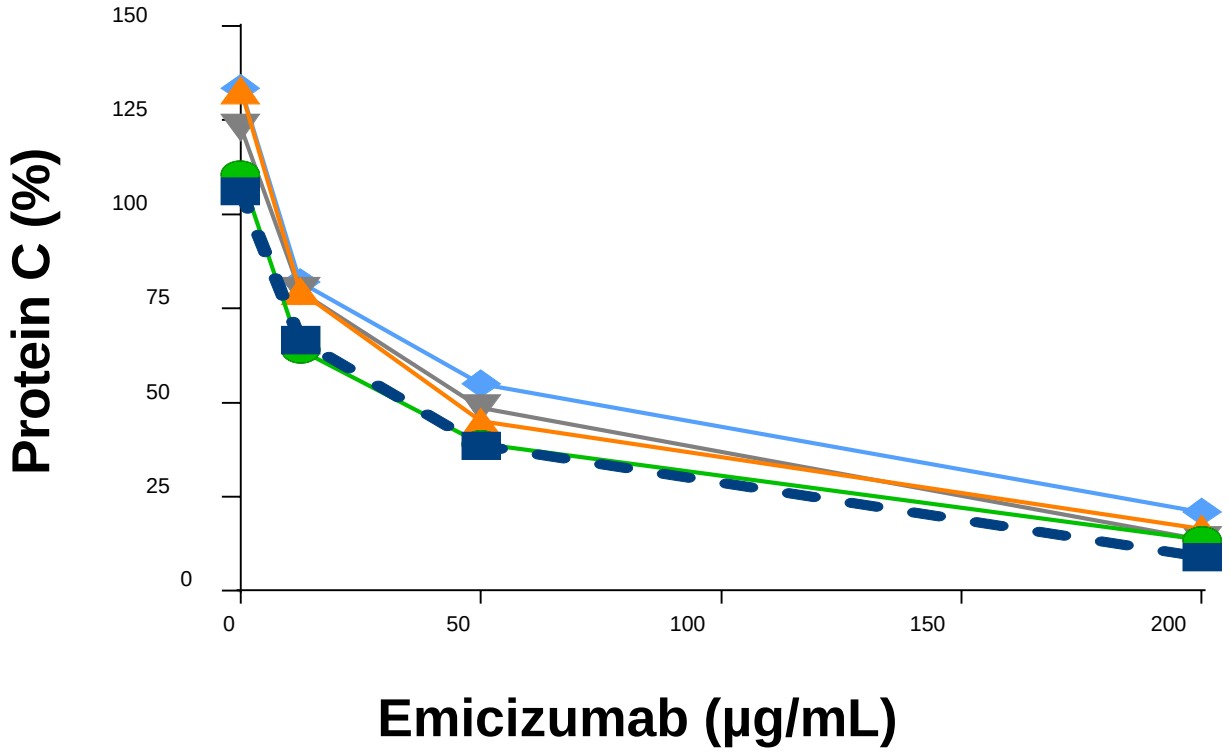
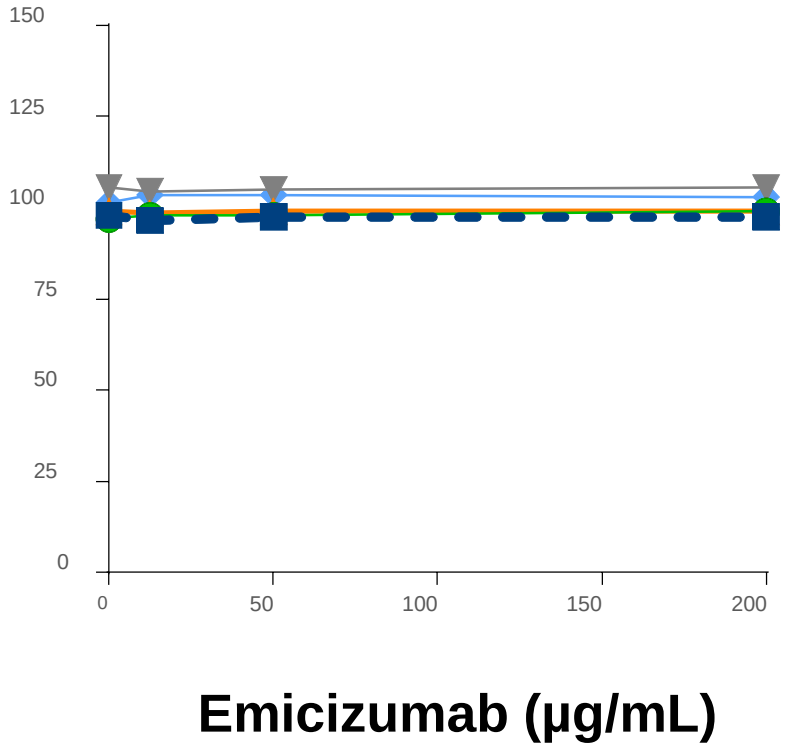
Protein C (Anticoagulant activity)



Protein C (Chromogenic activity)



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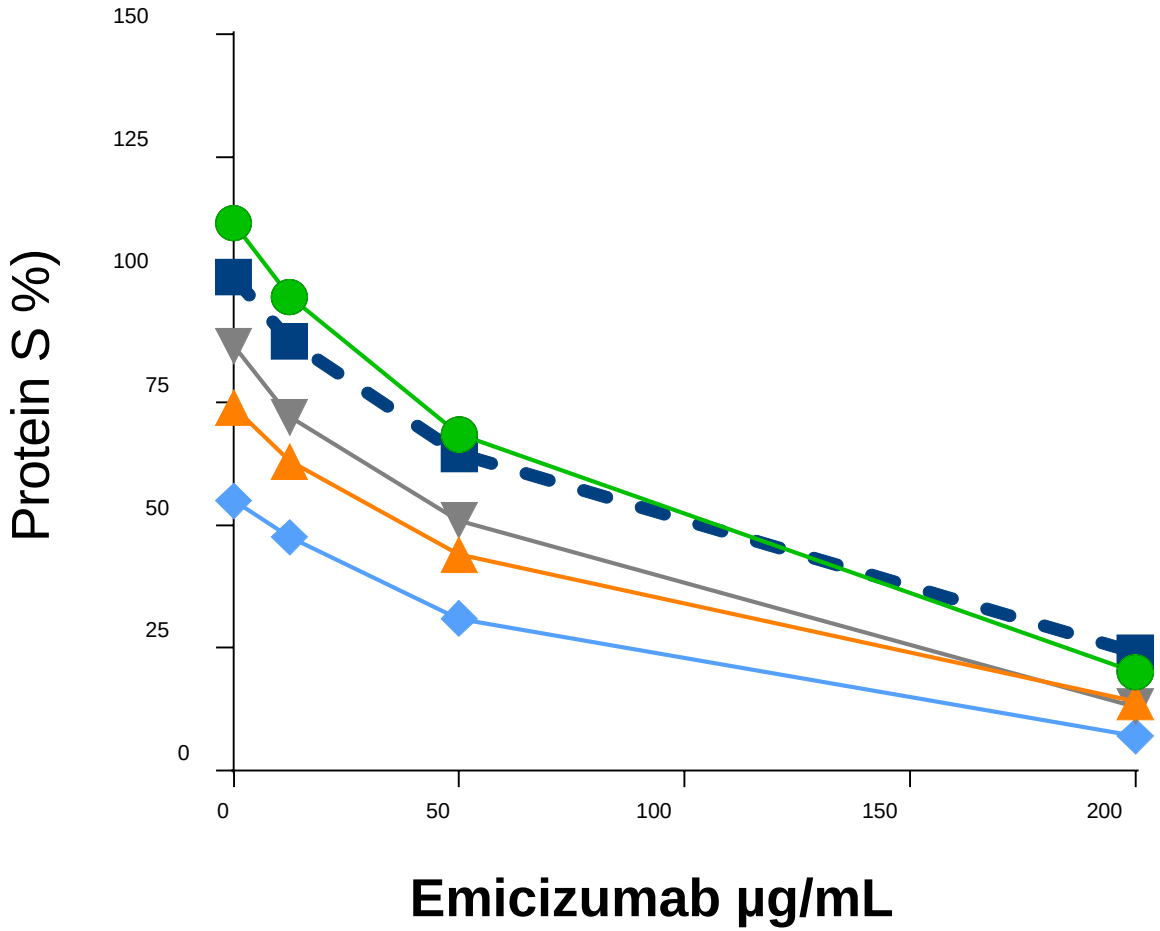
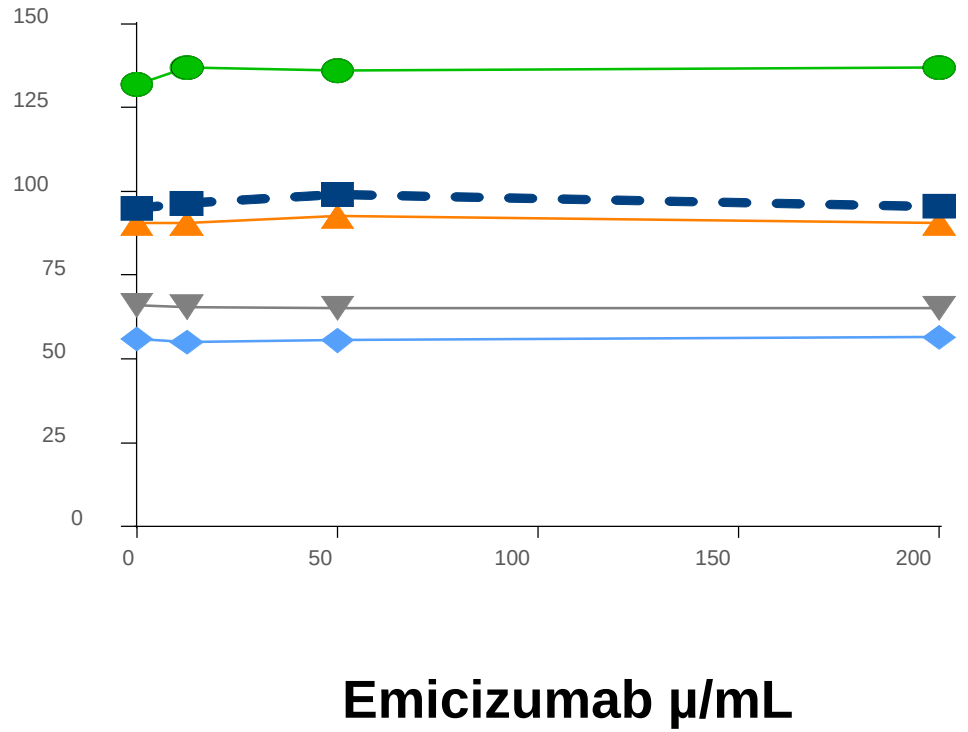
Protein S activity



Protein S antigen



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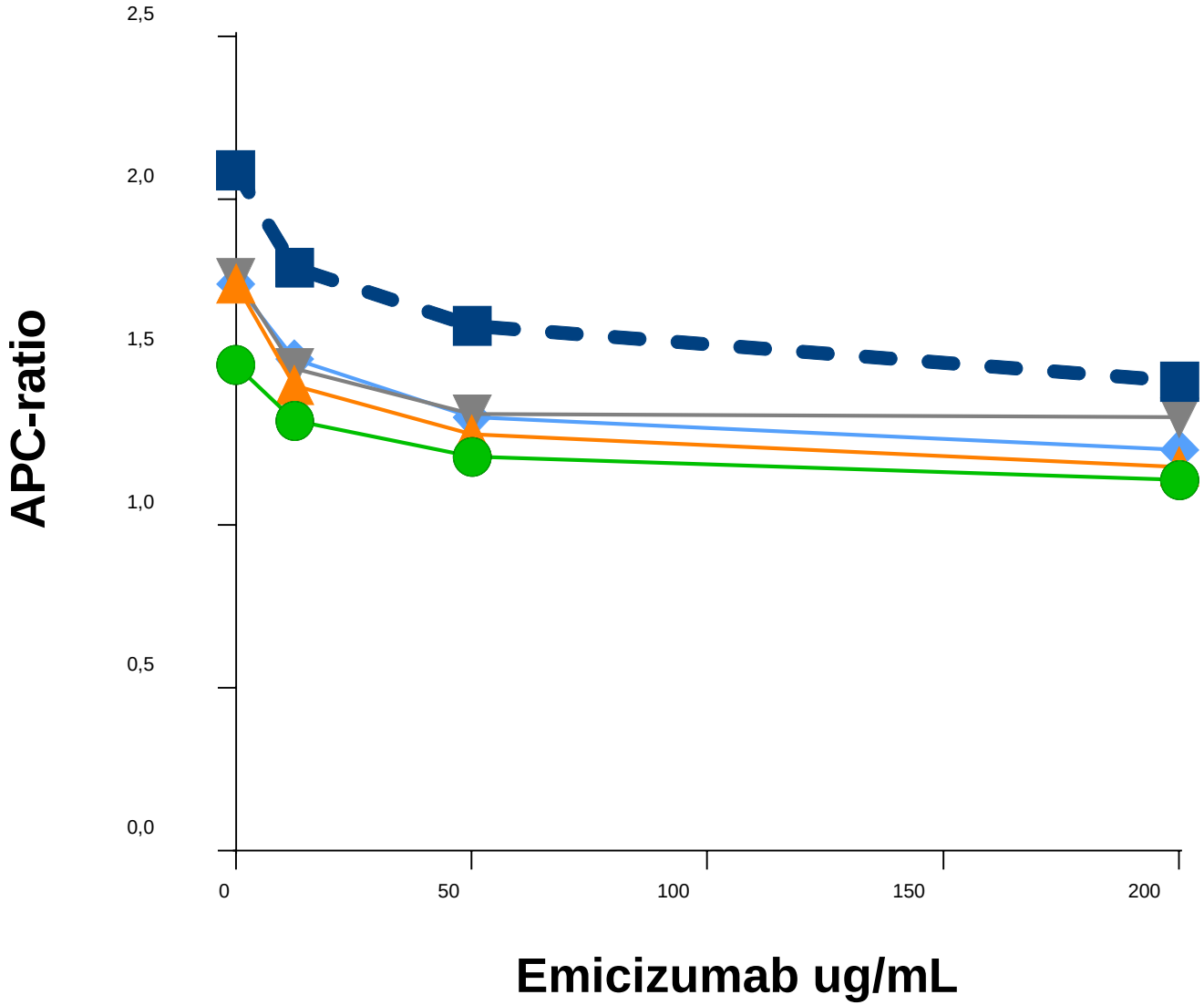
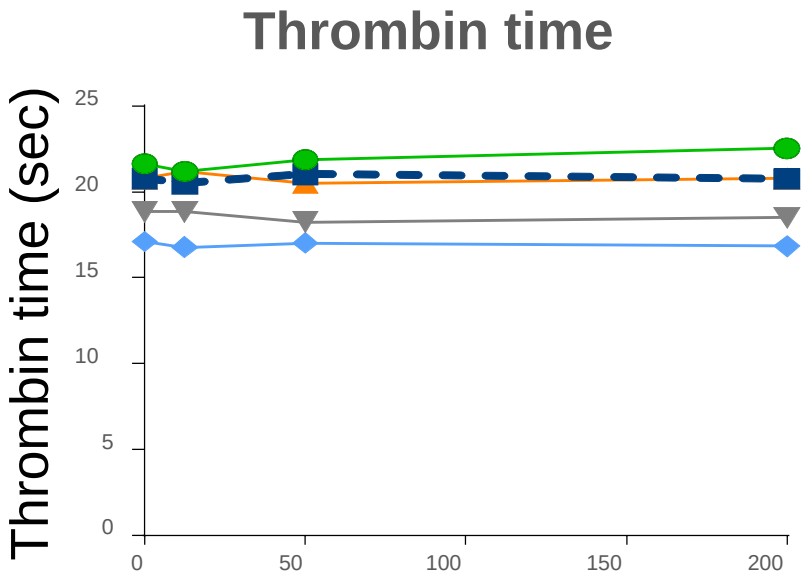
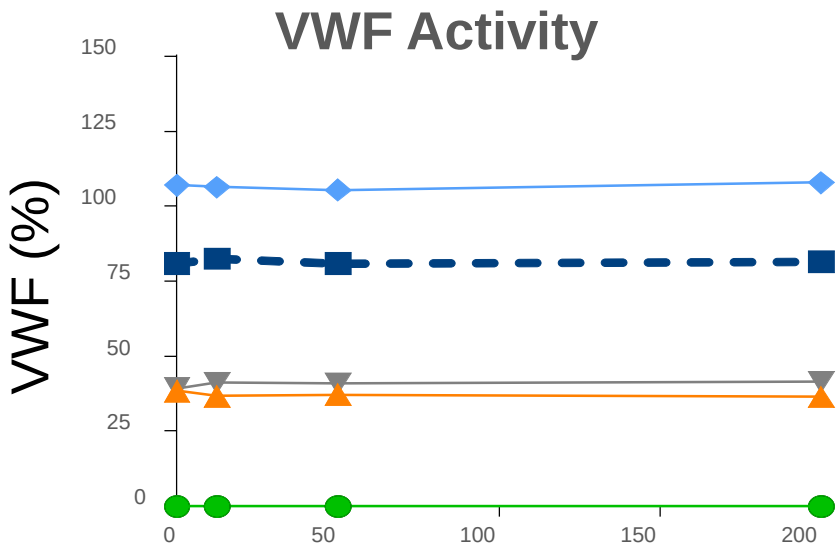
APC-resistance

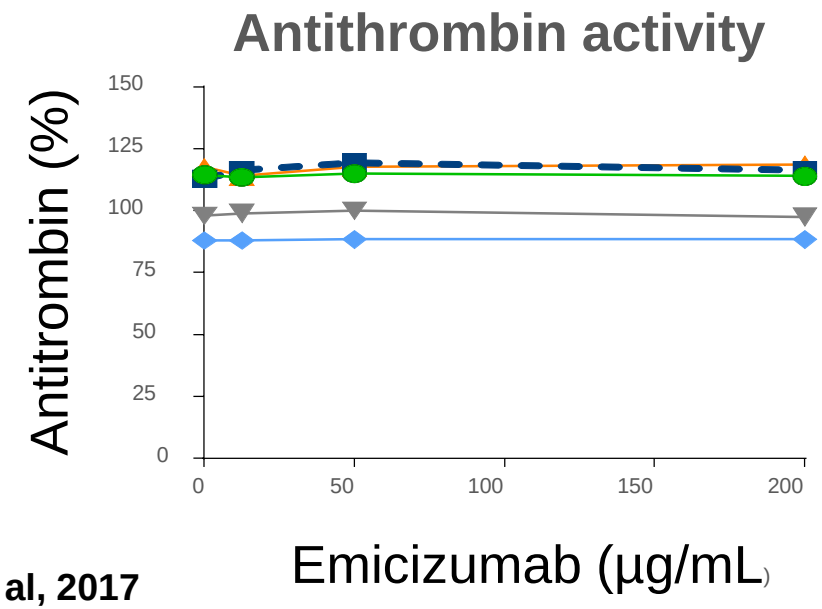


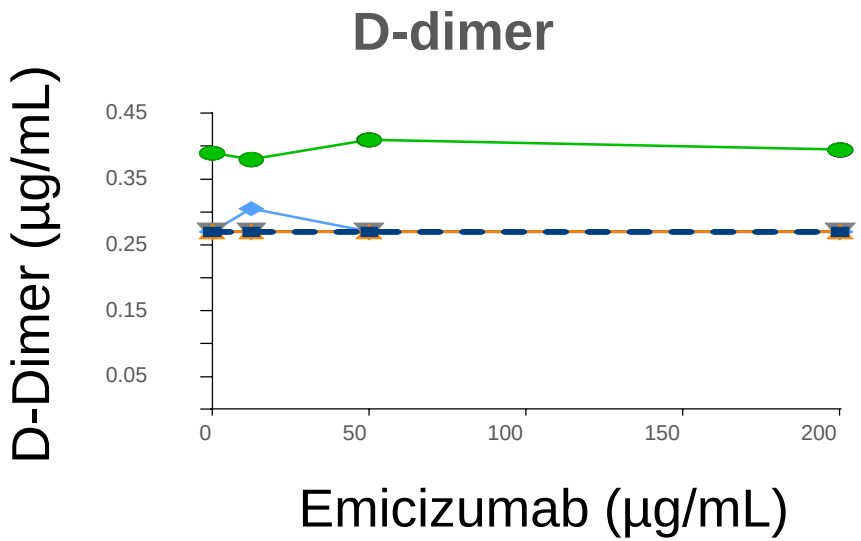
Calatzis et al, 2017

No emicizumab effect on the following parameters









Calatzis A et al, 2017

Possible Options for Lab Monitoring of Fitusiran or Concizumab

Thrombin generation or thromboelastography are (presumably) suitable lab tools

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Additional Lab Monitoring for Fitusiran, Concizumab or Emicizumab

- Antithrombin activity could be monitored in patients on fitusiran
- TFPI activity could be monitored in patients on concizumab
- Detection of antibodies against emicizumab, fitusiran or concizumab may be required when they occur

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Clinical Chemistry 65:2
000–000 (2019)

Reviews

Advances in the Treatment of Hemophilia: Implications for Laboratory Testing

Armando Tripodi,[1,4*] Veena Chantarangkul,[1,4] Cristina Novembrino,[1,2,4] and Flora Peyvandi[1,3,4]

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