UMC Utrecht

Microlyse:
Busting Clots by Targeting VWF

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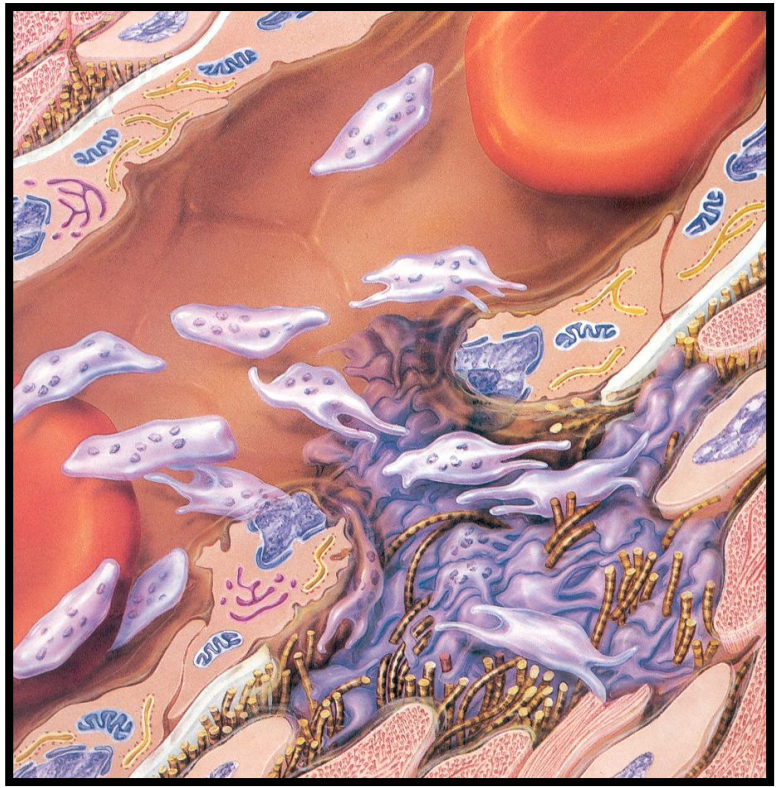
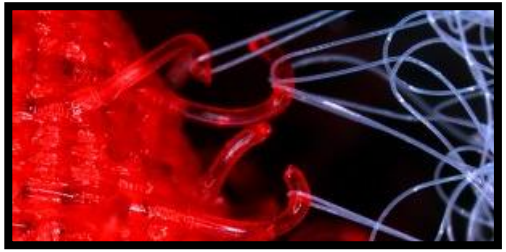
Conflicts of interest

TargED Biopharmaceuticals

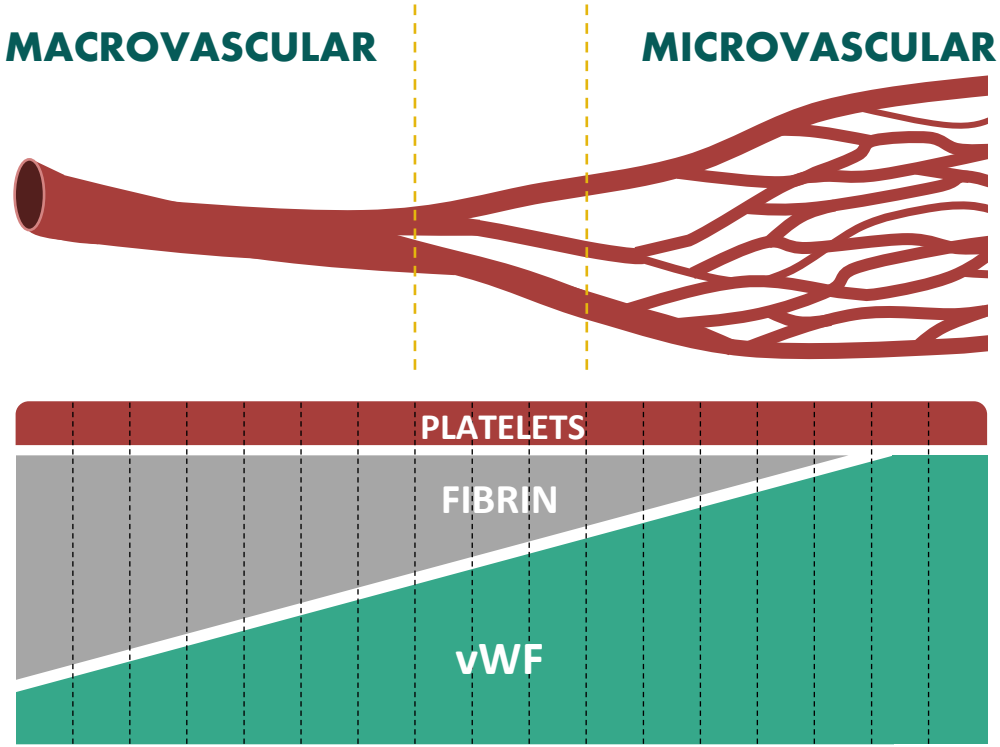
- Academic cofounder / inventor
- Shareholder (minor)

Von Willebrand Factor: molecular velcro



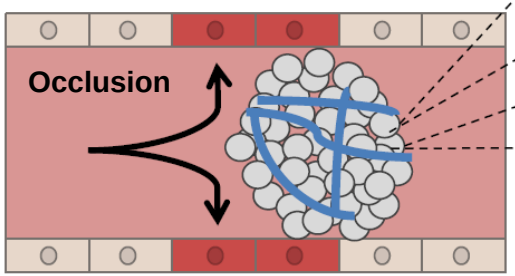


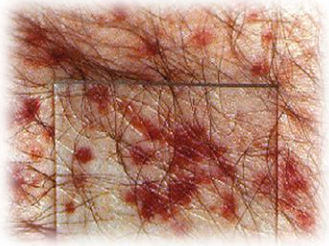
Thrombus composition in the vasculature

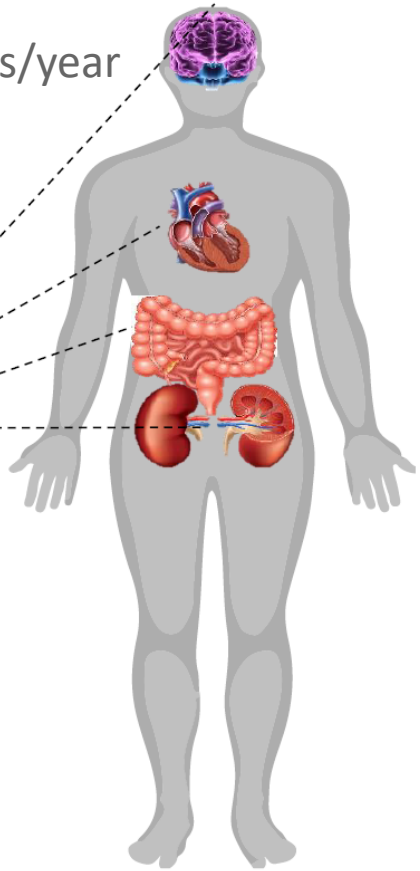


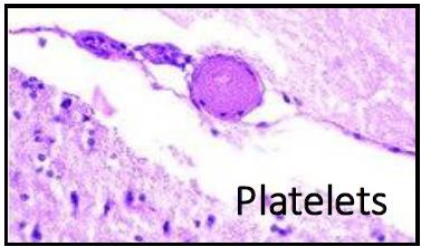
Thrombotic Thrombocytopenic Purpura (TTP)

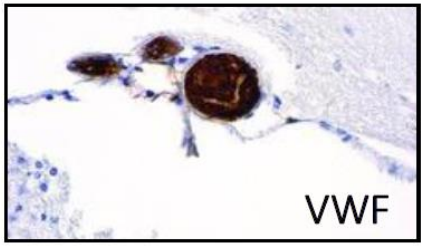
- **Incidence 7MM:** 7.500 episodes/year
- **Mortality without SoC:** 90%
- **Recurrence rate:** 80%

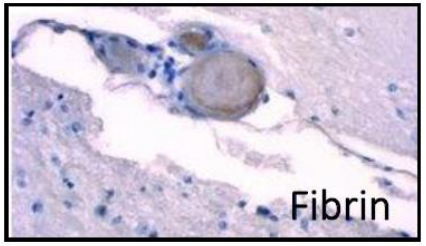




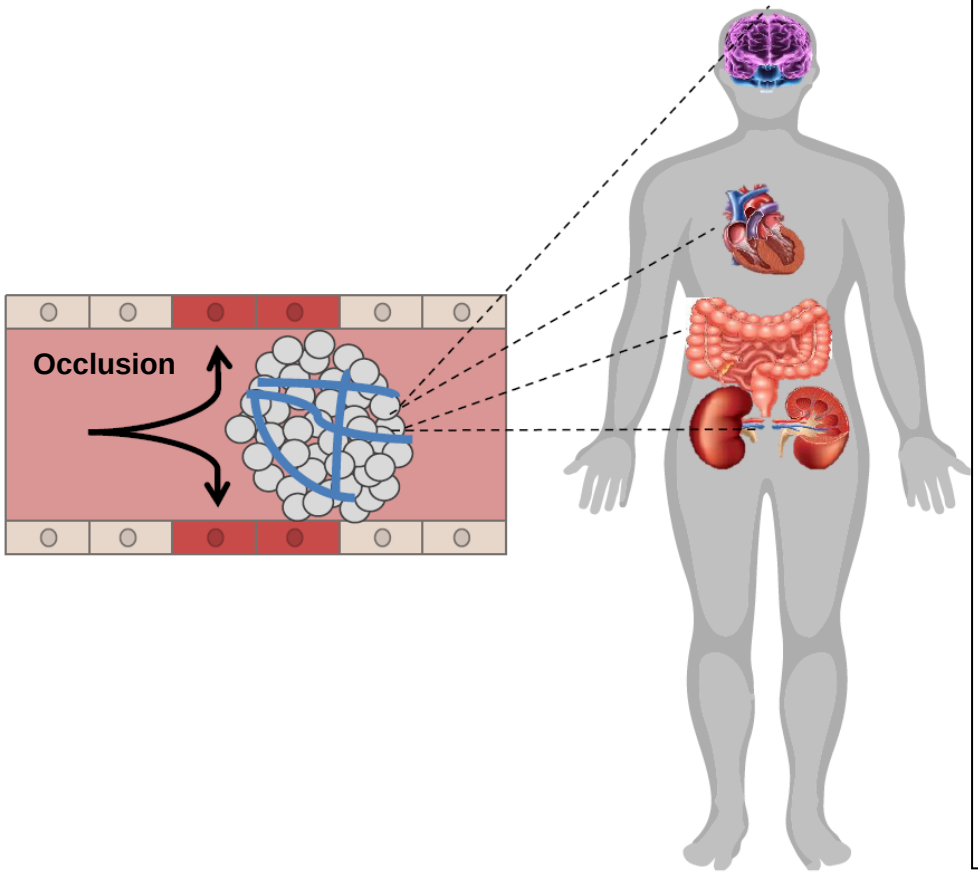








TTP: Normal D-dimer



American Journal of Hematology AJH

Original Article

Fibrinogenolysis in thrombotic thrombocytopenic purpura

Dr. Eizo Kakishita, Tetsuji Koyama, Mitsuhiro Higuchi, Osamu Kunitomi, Yoshio Oura, Kiyoyasu Nagai

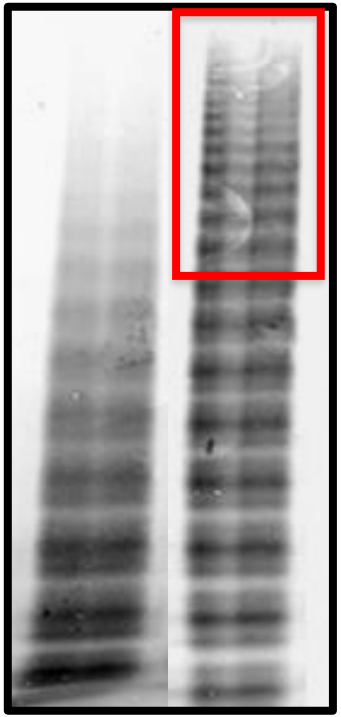
First published: September 1989 | https://doi.org/10.1002/ajh.2830320104 | Citations: 10

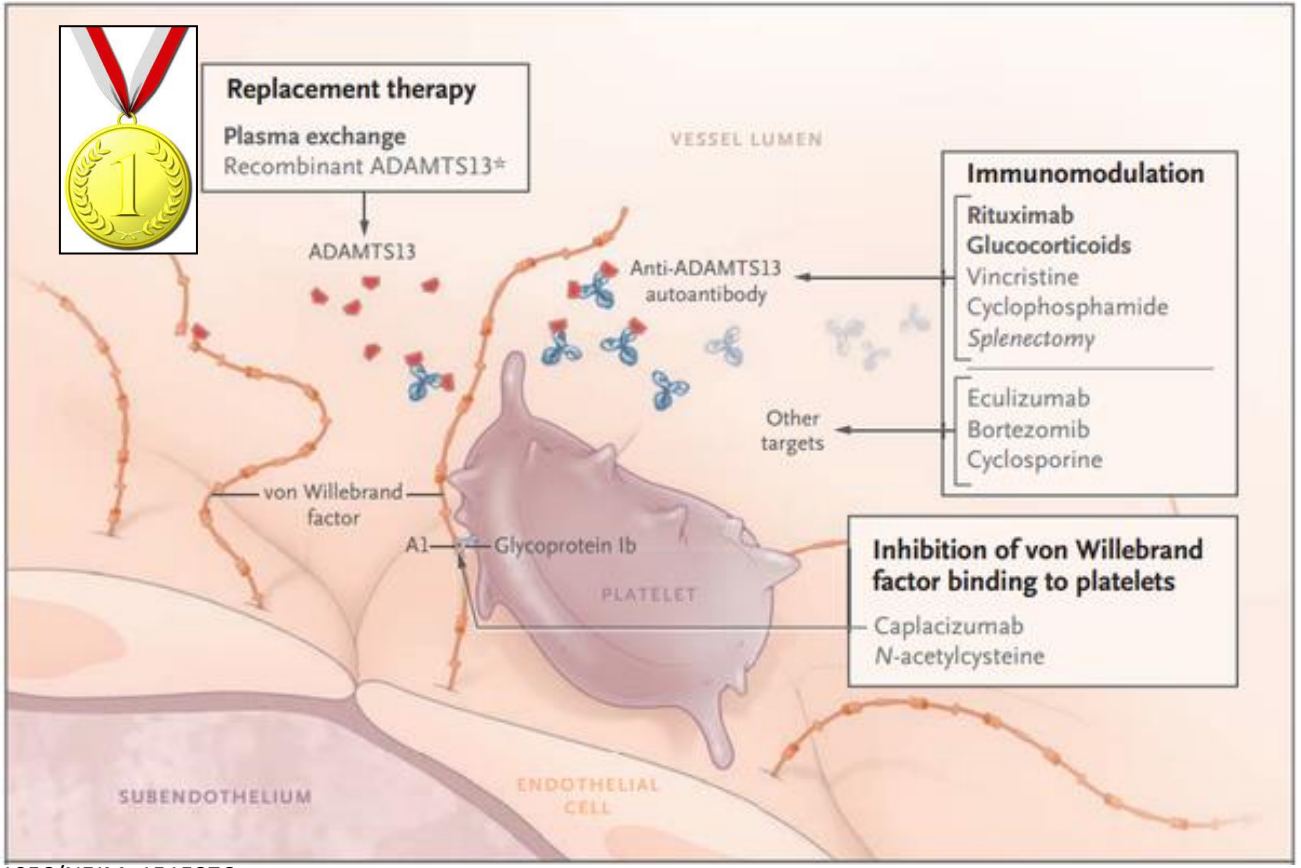
Abstract

Coagulo-fibrinolytic factors were studied in five patients suffering from thrombotic thrombocytopenic purpura (TTP). The change in coagulation factors in the acute stage was mild compared with that found in disseminated intravascular coagulation (DIC). We observed a slight increase of fibrin-fibrinogen degradation products (FDP) in the plasma of four patients during the acute stage of TTP, but the level of the D-dimer remained within normal variation and was extremely low compared with that in 27 samples from patients with DIC showing the same level of FDP. At the same time, both antigen levels of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) were elevated in three of the four patients tested. Although a similar change was recognized in DIC patients' plasma, the elevation of PAI- far higher than in overt DIC. The antigen levels of t-PA ar remission, and a mild elevation of PAI-1 was detected in the early stage of TTP relapse. Enzymography revealed t an increase of a substance with a 110 kD molecule, assu complex, in TTP plasma in the acute stage, but the findir

Cause and therapeutic options

Normal **ADAMTS13 deficiency**





Veyradier. N Engl J Med 2016; 374:583-585 DOI: 10.1056/NEJMe1515876;

Goal: Short attacks

= less tissue damage

	PEX + IS	+ Caplacizumab
Mortality	~20%	~15%
Attack days	5	3-4
Plasma	40L (€100K)	30L

THROMBOSIS AND HEMOSTASIS

Cost effectiveness of caplacizumab in acquired thrombotic thrombocytopenic purpura

George Goshua,[1] Pranay Sinha,[2] Jeanne E. Hendrickson,[3] Christopher Tormey,[3] Pavan K. Bendapudi,[4-6] and Alfred Ian Lee[1]

[1]Division of Hematology, Yale University School of Medicine, New Haven, CT; [2]Division of Infectious Diseases, Boston Medical Center, Boston, MA; [3]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT; [4]Division of Hematology and Blood Transfusion Service, Massachusetts General Hospital, Boston, MA; [5]Division of Hemostasis and Thrombosis, Beth Israel Deaconess Medical Center, Boston, MA; and [6]Harvard Medical School, Boston, MA

"The addition of caplacizumab to standard of care treatment in acquired thrombotic thrombocytopenic purpura is **not cost effective** compared with standard of care alone."

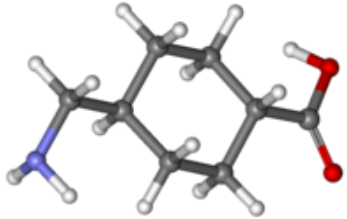
Goshua et al *Blood* . 2021 Feb 18;137(7):969-976.

A mysterious bleeding case

Male, 43

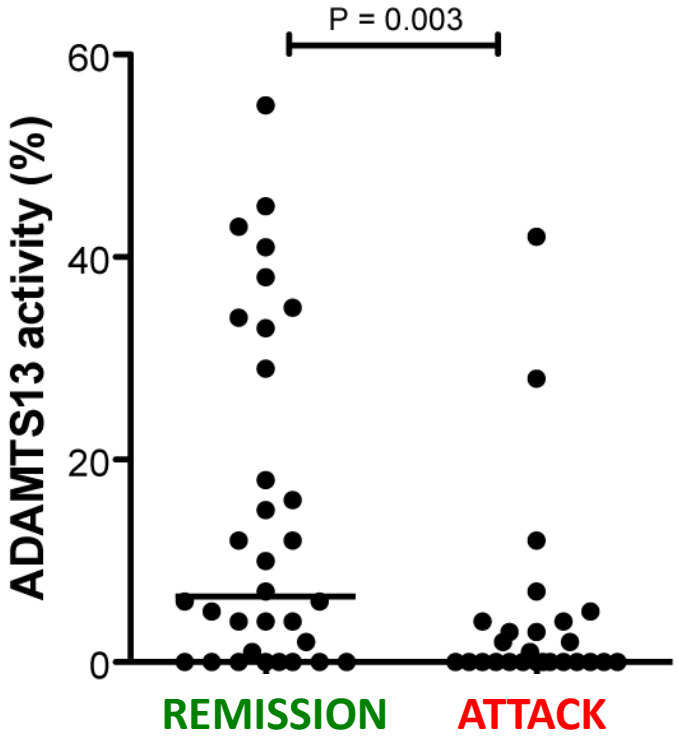
Day 1
- Stent placement

Day 4
- TTP (0% ADAMTS13)
- Admission for PEX
- IC for respiratory and hemodynamic support



Day 7
- Major GI bleed
- Treated with **cyclokapron** (plasmin blocker)
- Bleeding stopped
- Death within 50 minutes (heart)
- Obduction: widespread microthrombosis

How do you get a TTP attack?

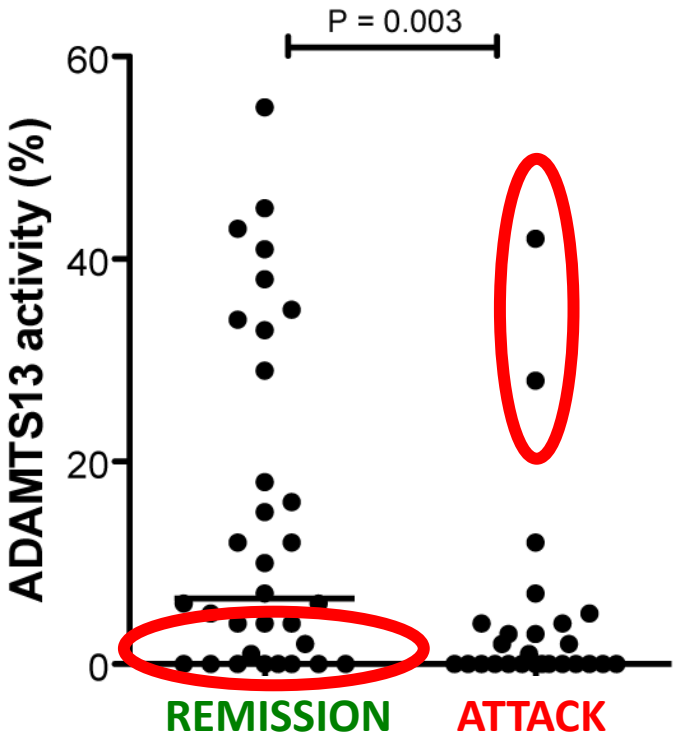


Autoantibodies

ADAMTS13 Activity

Tersteeg et al. *Circulation* 2014 Mar 25;129(12):1320-31.
Ergic et al. *J Biol Chem.* 2021 Oct;297(4):101132.

TTP paradox



Complete ADAMTS13 deficiency

- Congenital (Upshaw-Schulman)
- Knockout mice
- Persistent inhibitory antibodies

No continuous disease

Unexpected rescue

More than one VWF-cleaving enzyme?

Dr. Claudia Tersteeg

ADAMTS13

- Since 2001

Plasmin

- Since 1987

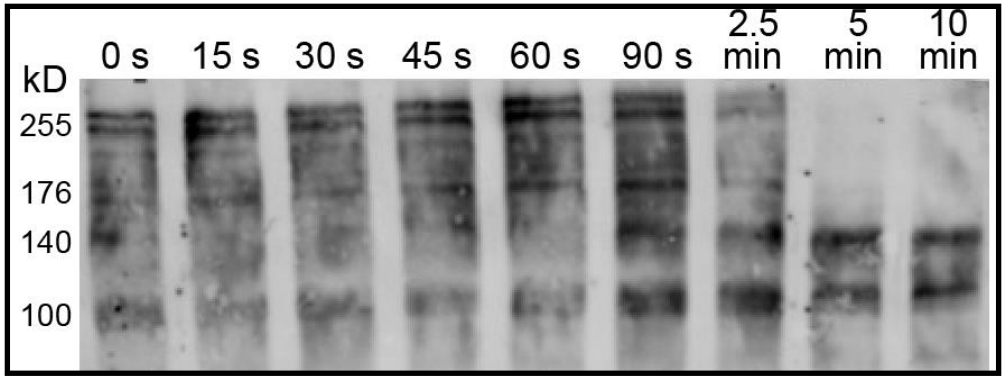
Berkowitz et al. *J Clin Invest* 1987;
Wohner et al. *Thromb Res*. 2012

REVIEW IN TRANSLATIONAL HEMATOLOGY | JULY 1, 2005

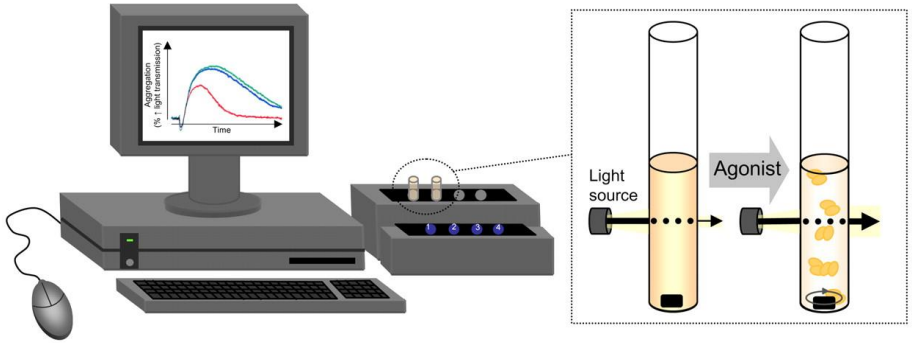
ADAMTS13 turns 3

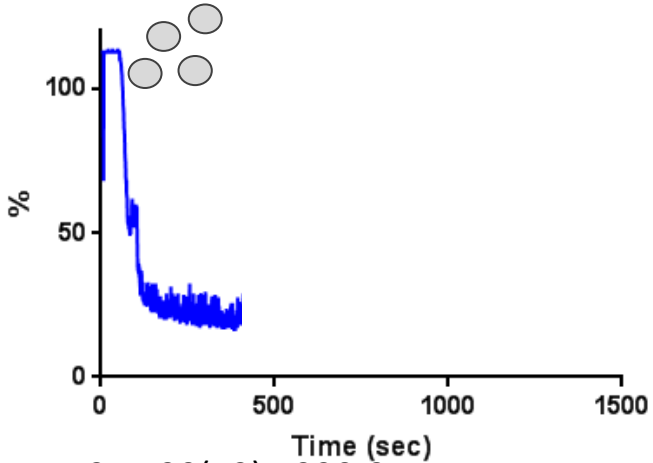
Clinical Trials & Observations

Gallia G. Levy, David G. Motto, David Ginsburg



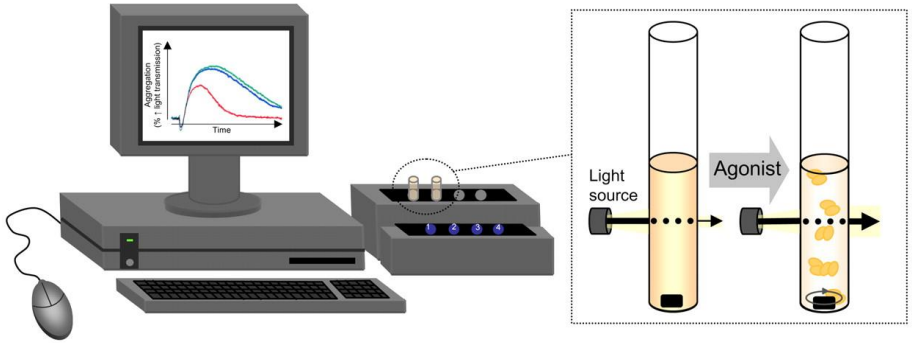
Microthrombolysis

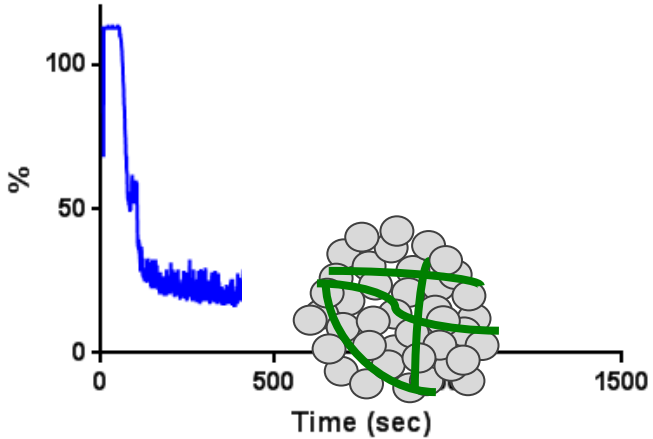




Tersteeg et al. *Circulation* 2014 Mar 25;129(12):1320-31.

Microthrombolysis





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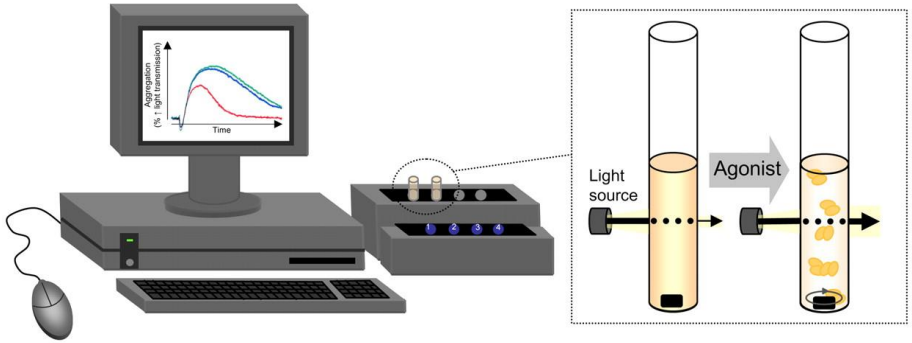
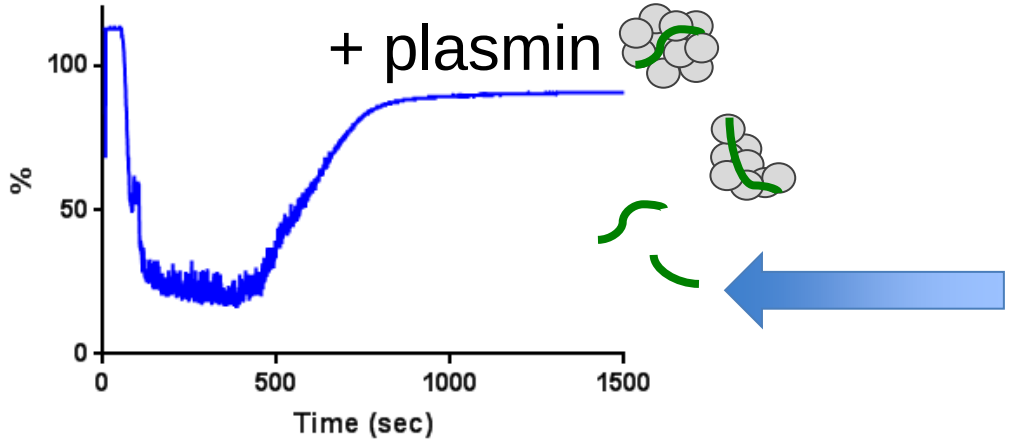
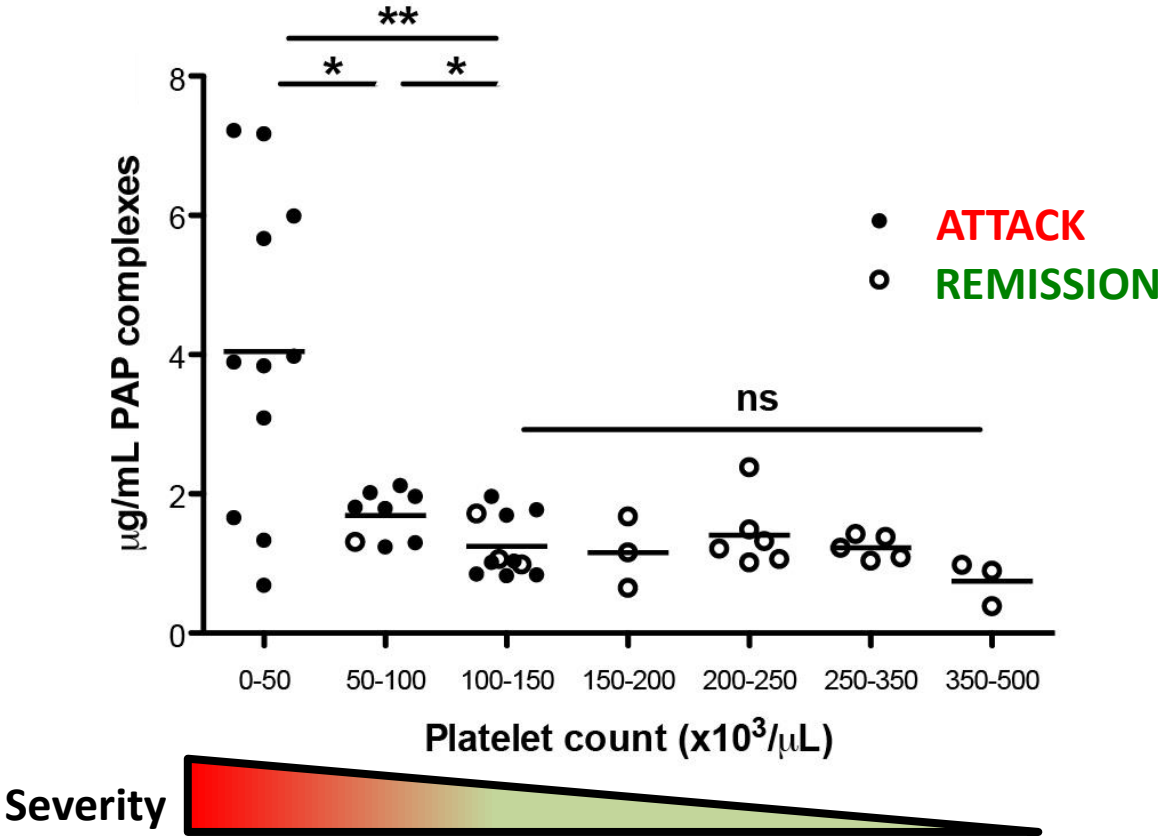




Biomarker?

Hinde El Otmani

Plasminogen activation in TTP patients



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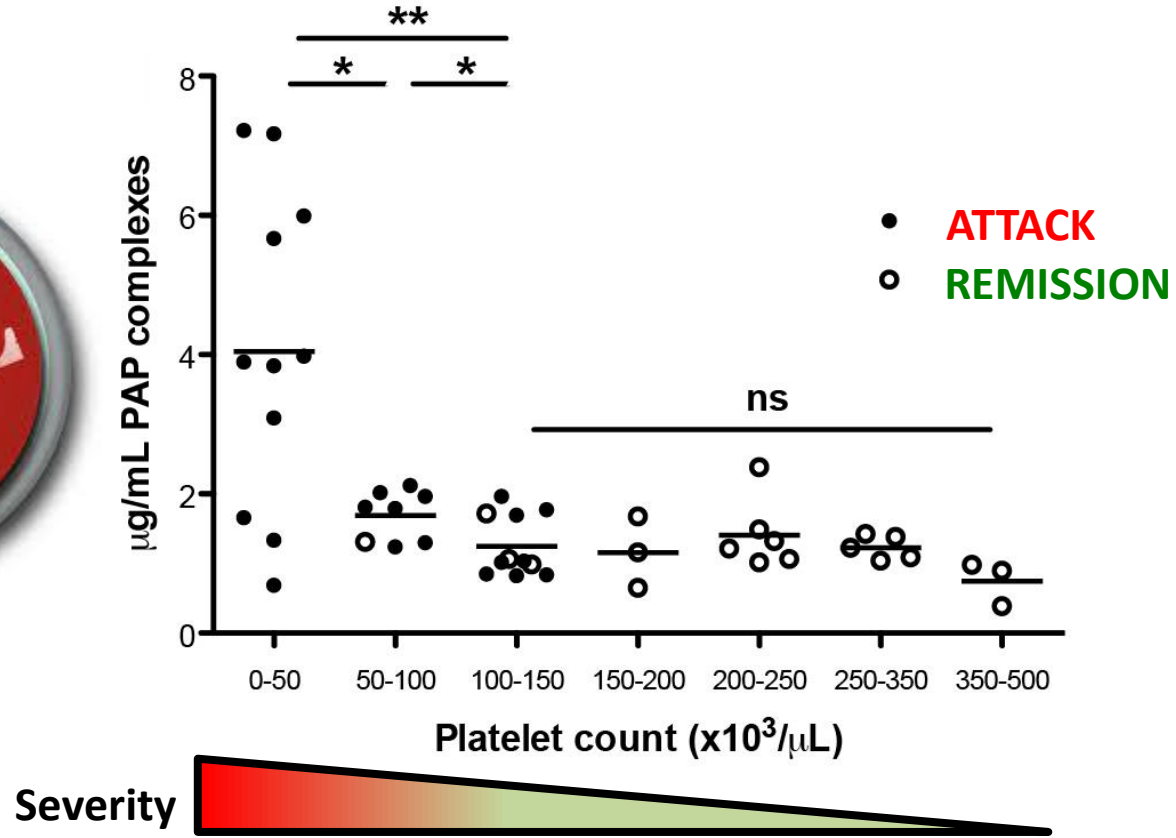
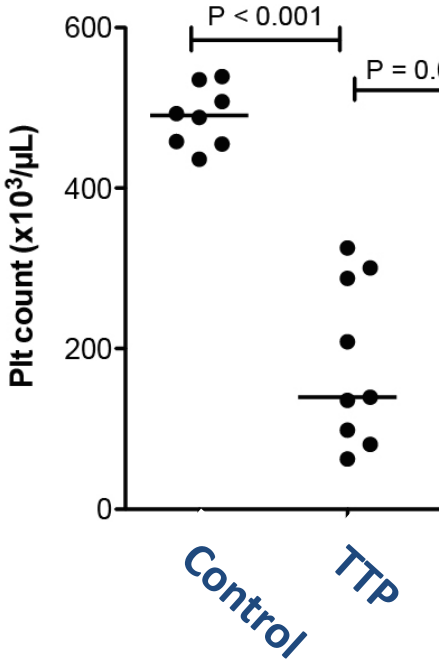
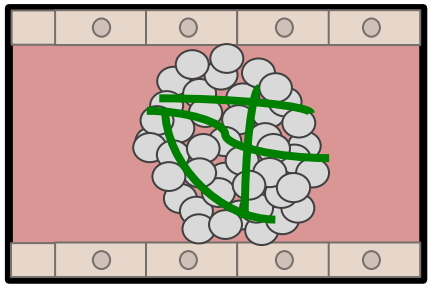


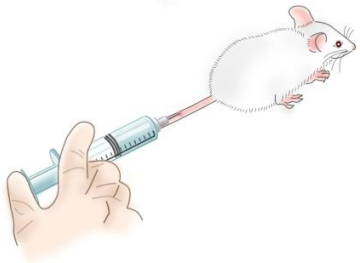
Thrombolysis for TTP *in vivo*





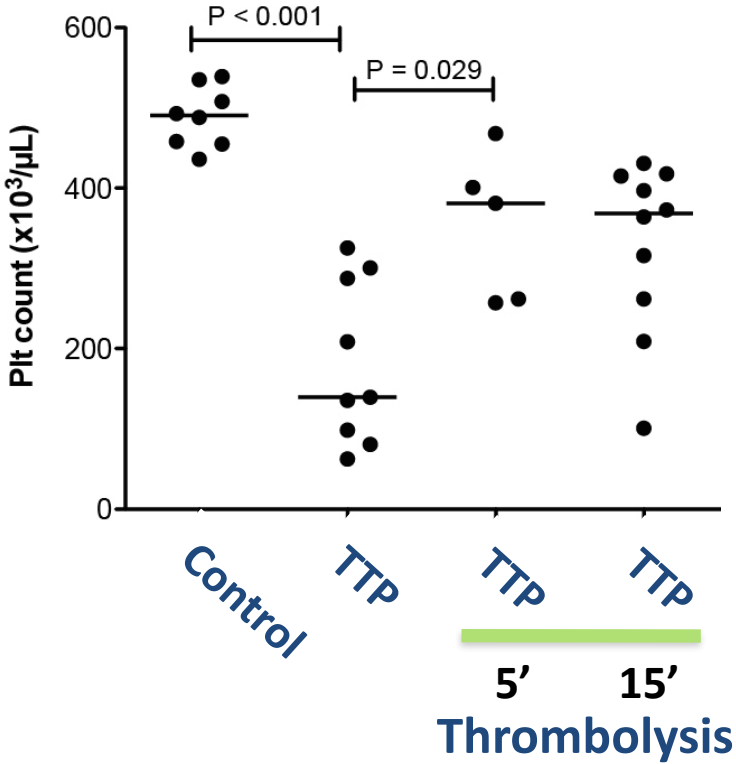
ADAMTS13$^{-/-}$
VWF "overdose"

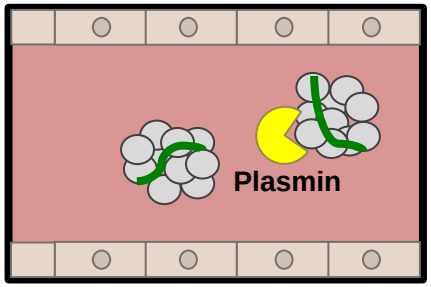
- **"ER" thrombolysis**
- **analyses after 24h**



Tersteeg et al. *Circulation* 2014 Mar 25;129(12):1320-31.

Thrombolysis for TTP *in vivo*





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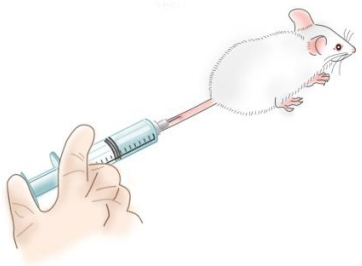
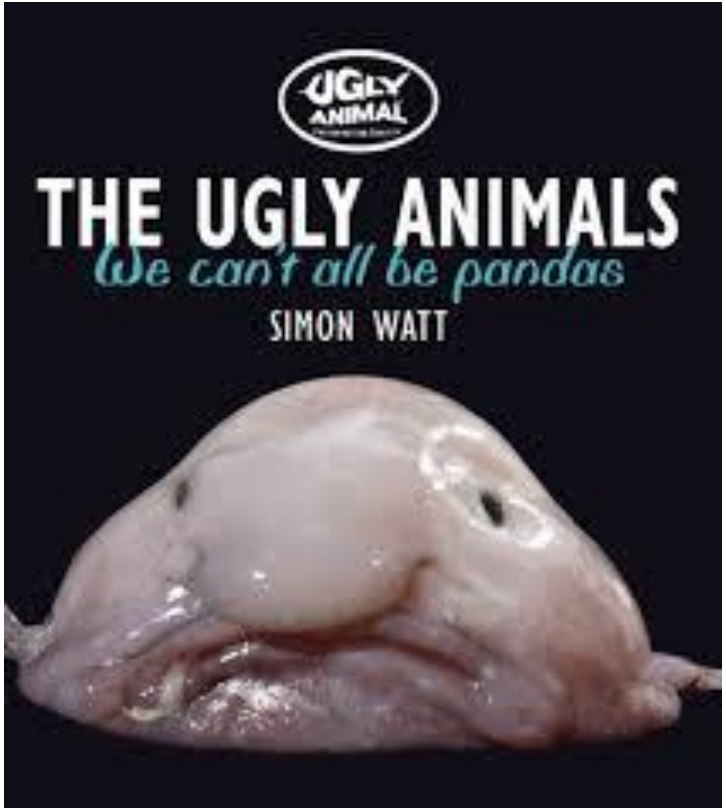


Proof of concept - but no beauty prize

Streptokinase

- Bacterial
- Systemic
- Insusceptible to inhibition
- Requires **human** plasminogen



Proof of concept - but no beauty prize

Streptokinase

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- Systemic
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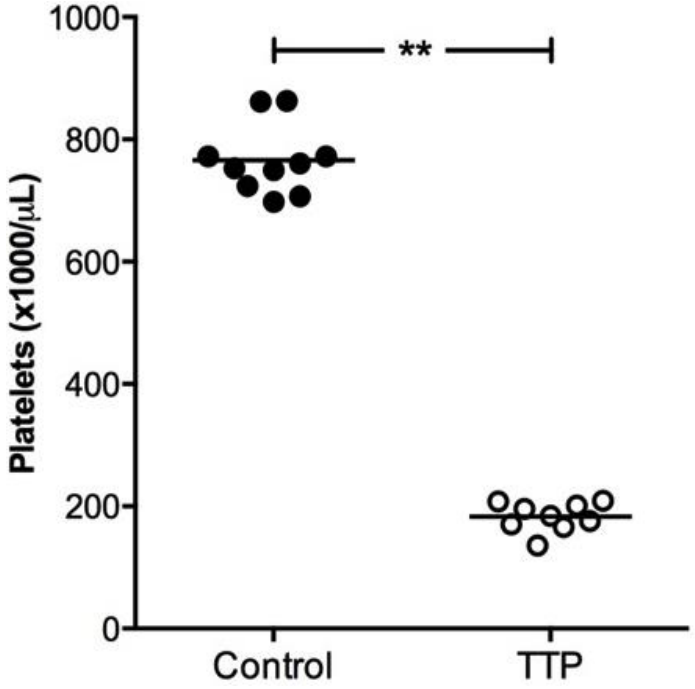
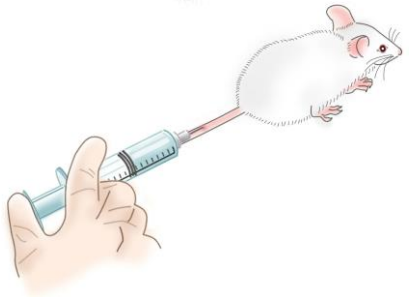
In the mean time….. Acquired TTP mice (aTTP)

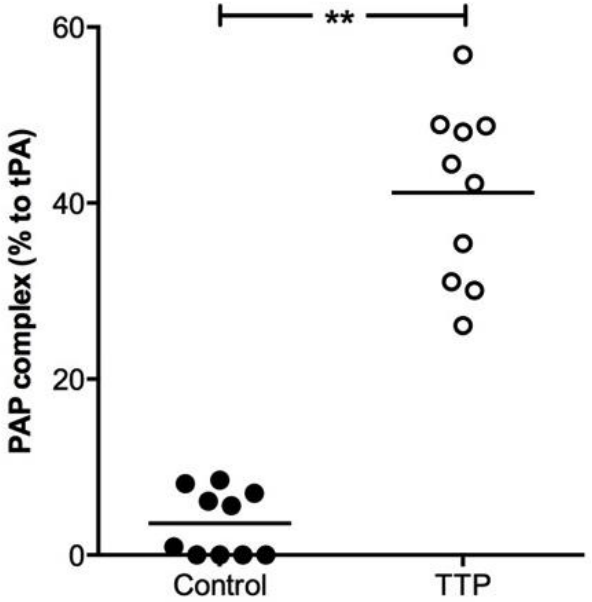


anti-ADAMTS13
VWF “overdose”

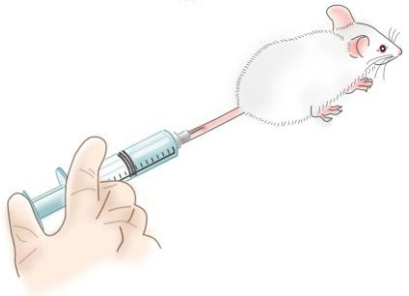


Tersteeg et al. *J Thromb Haemost.* 2017 Dec;15(12):2432-2442.

Plasmin formation aTTP mice

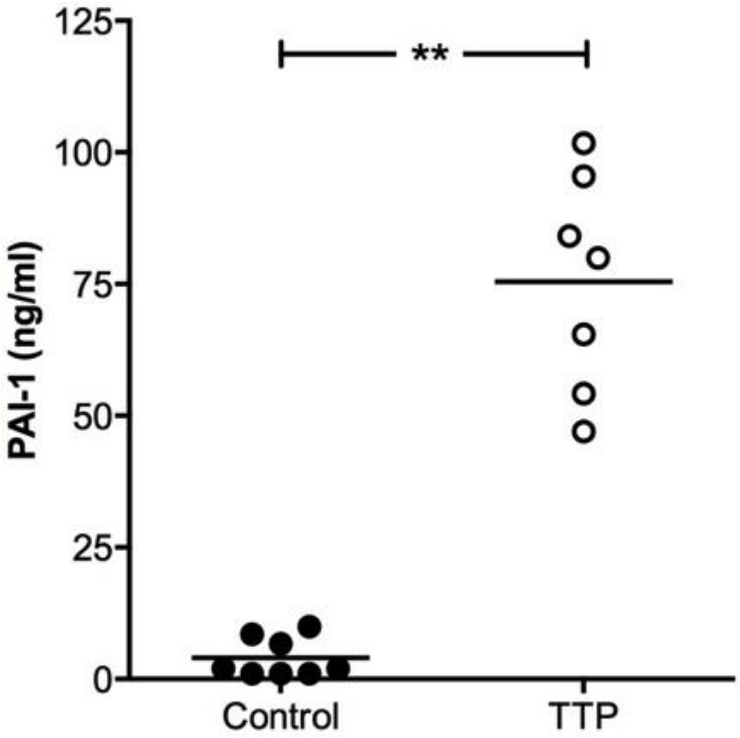


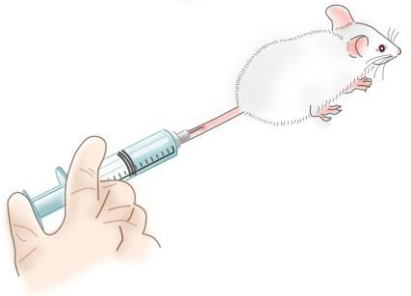
anti-ADAMTS13
+VWF “overdose”



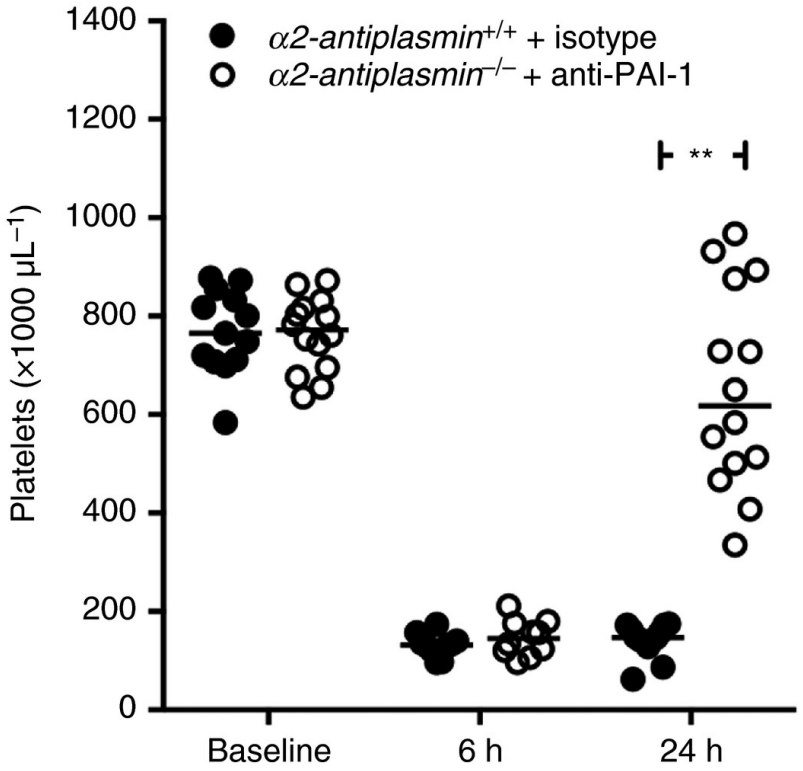
Tersteeg et al. *J Thromb Haemost.* 2017 Dec;15(12):2432-2442.

PAI-1 release in aTTP mice (=endothelial injury)





Amplified endogenous plasminogen activation



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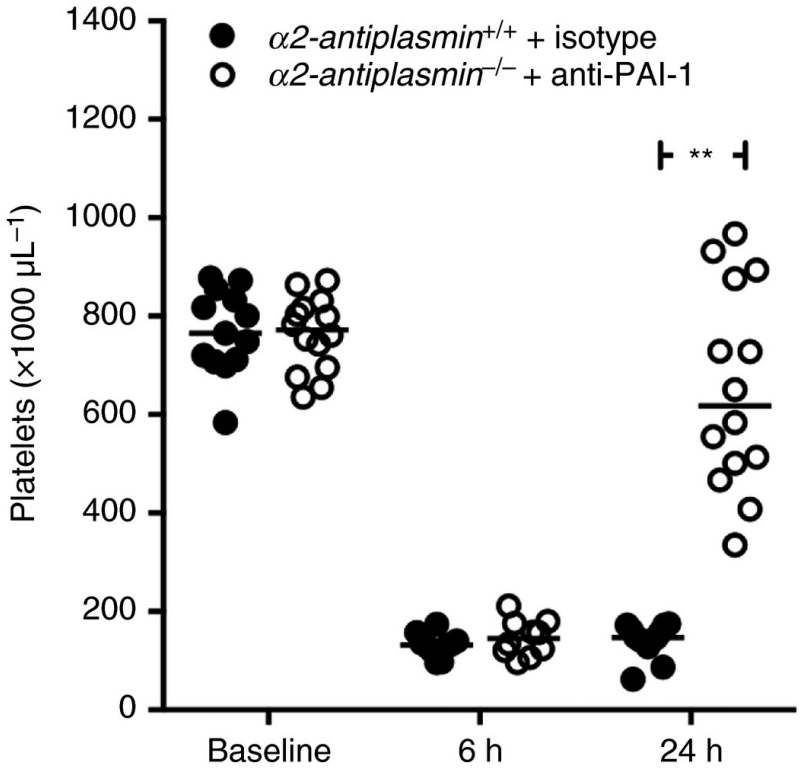
Amplified endogenous plasminogen activation

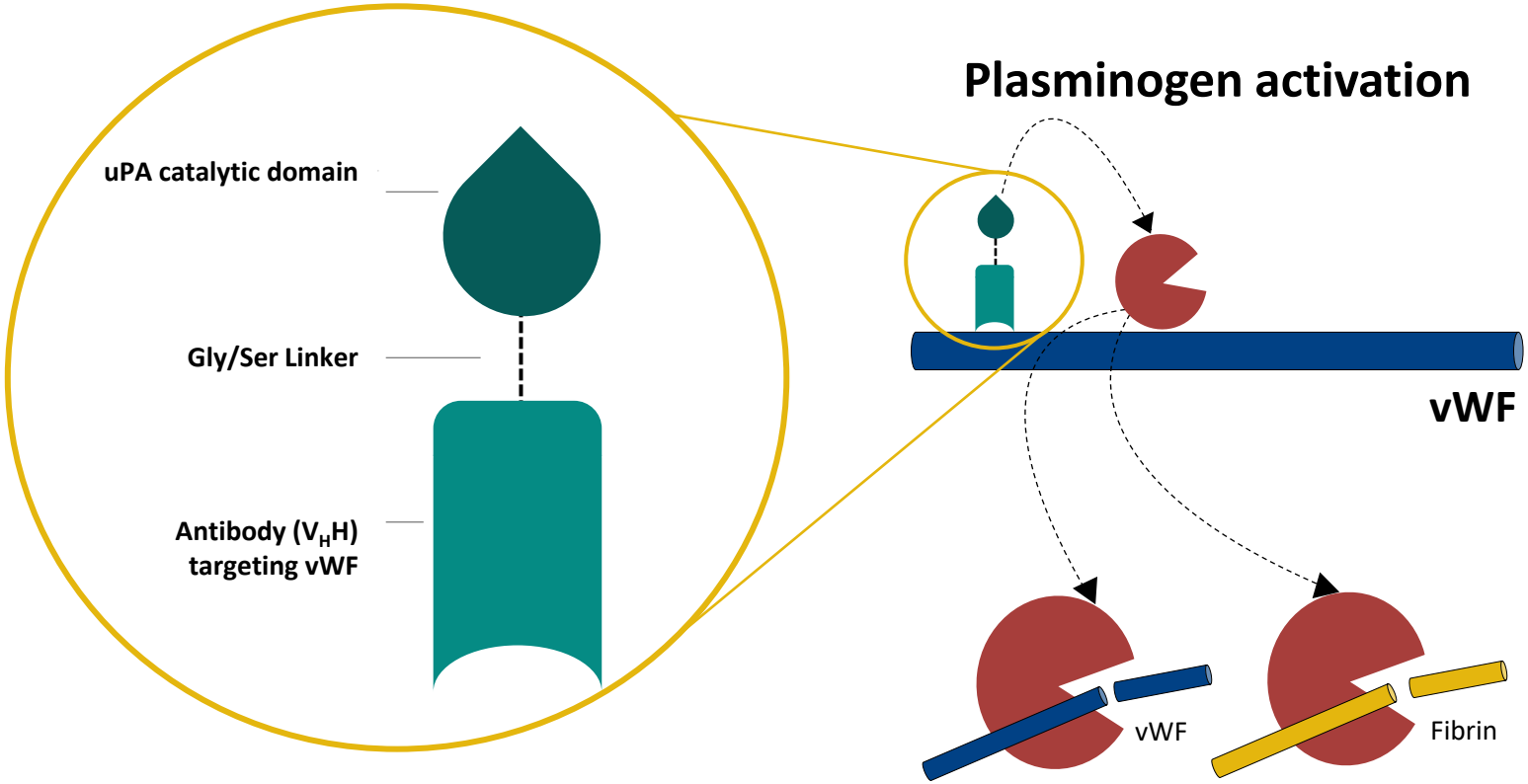


- Plasmin tries (but fails)
- SERPINs interfere

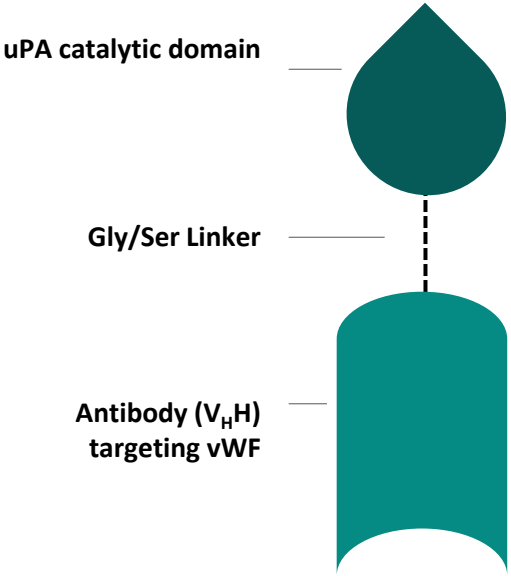
How can we do better?

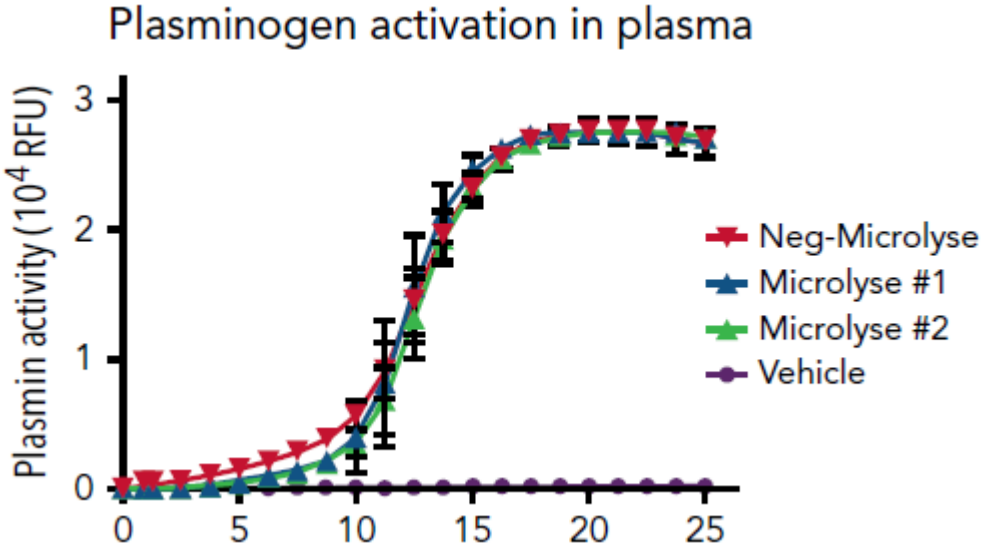
Tersteeg et al. *J Thromb Haemost.* 2017 Dec;15(12):2432-2442.

Microlyse design

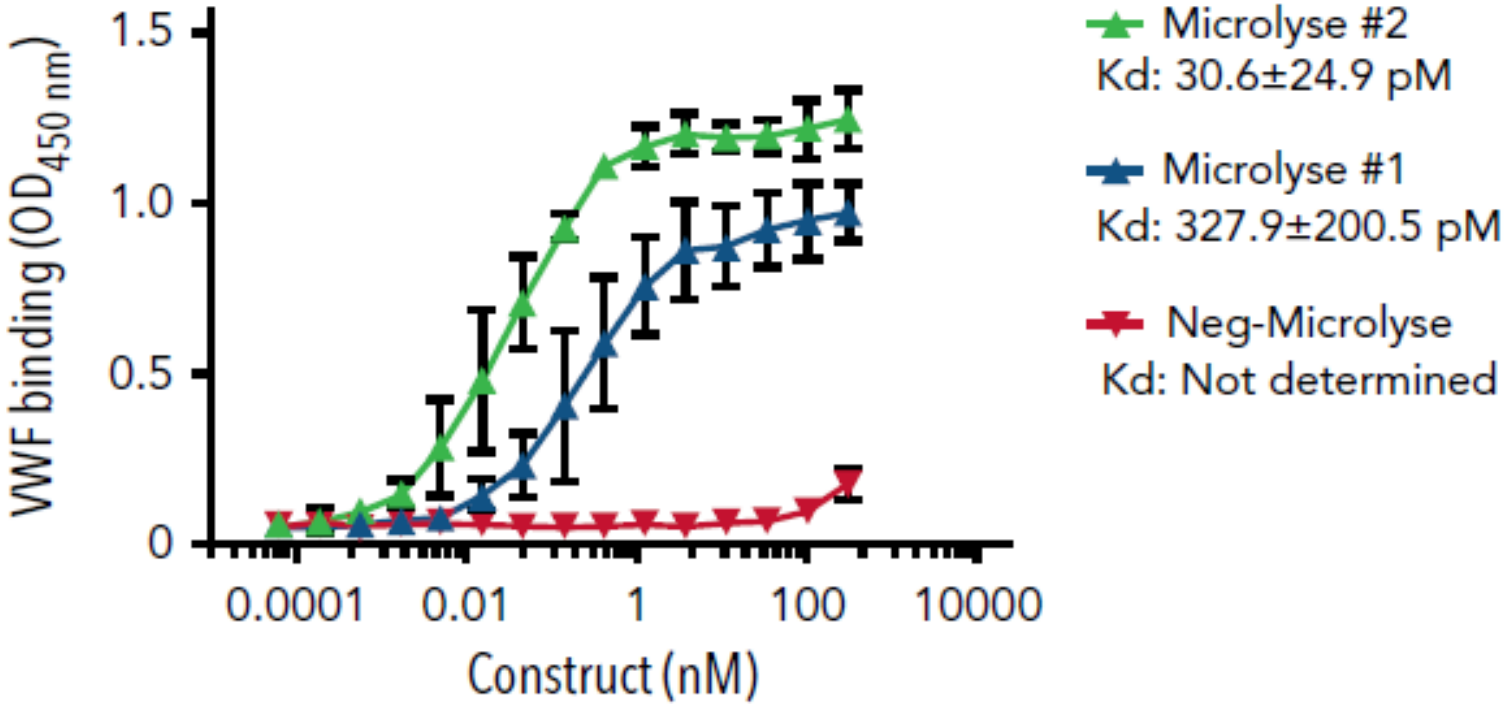


Microlyse active site



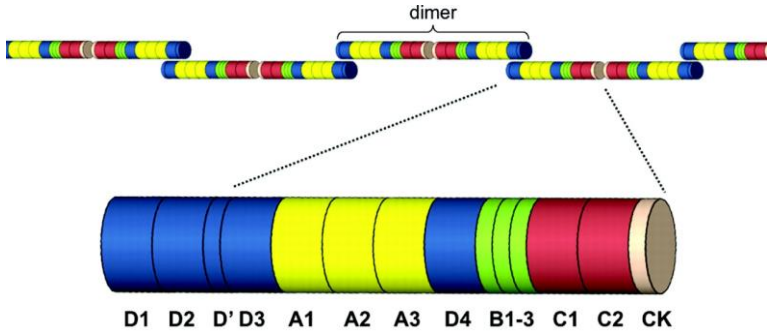


VWF Binding

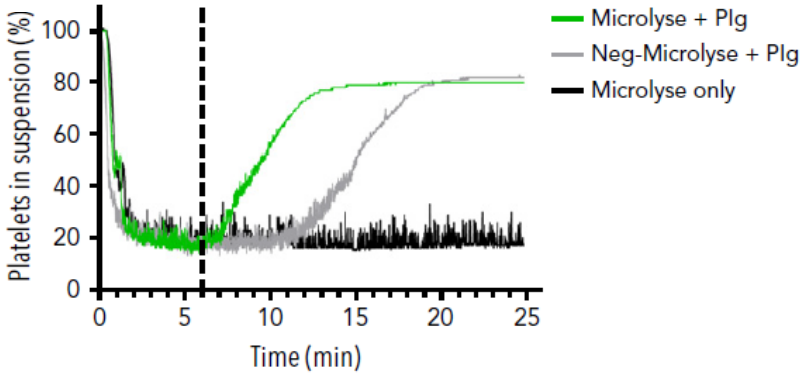

VWF binding ($OD_{450\ nm}$)
1.5
1.0
0.5
0
0.0001
0.01
1
100
10000
Construct (nM)
Microlyse #2
Kd: 30.6±24.9 pM
Microlyse #1
Kd: 327.9±200.5 pM
Neg-Microlyse
Kd: Not determined

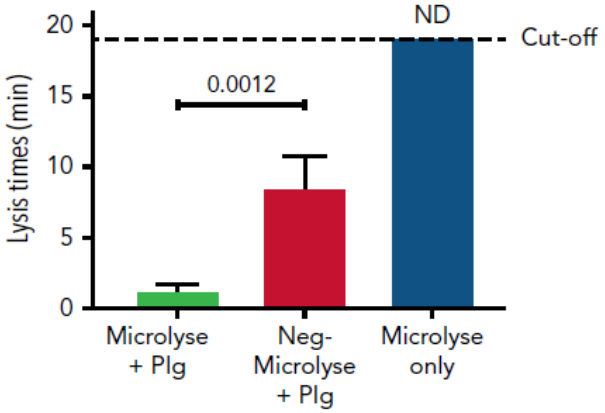
Binding site

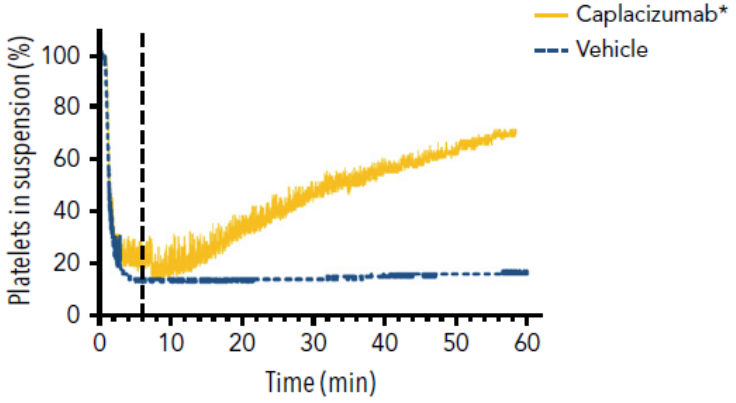
Variant	Domain	Blocks	Continue?
Microlyse #1	D'D3	FVIII binding	NO
Microlyse # 2	CTCK	-	YES
Neg-Microlyse	-	-	-
Caplacizumab	A1	Platelet binding	-

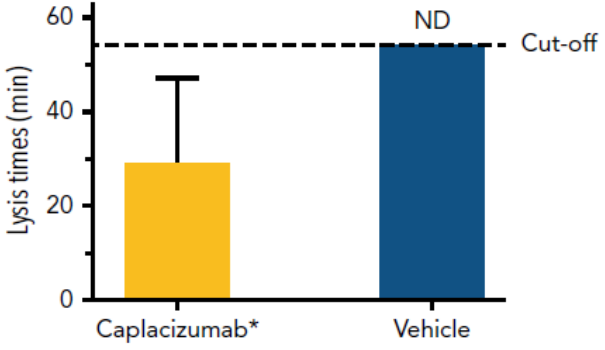


In vitro: Microlyse vs Caplacizumab*

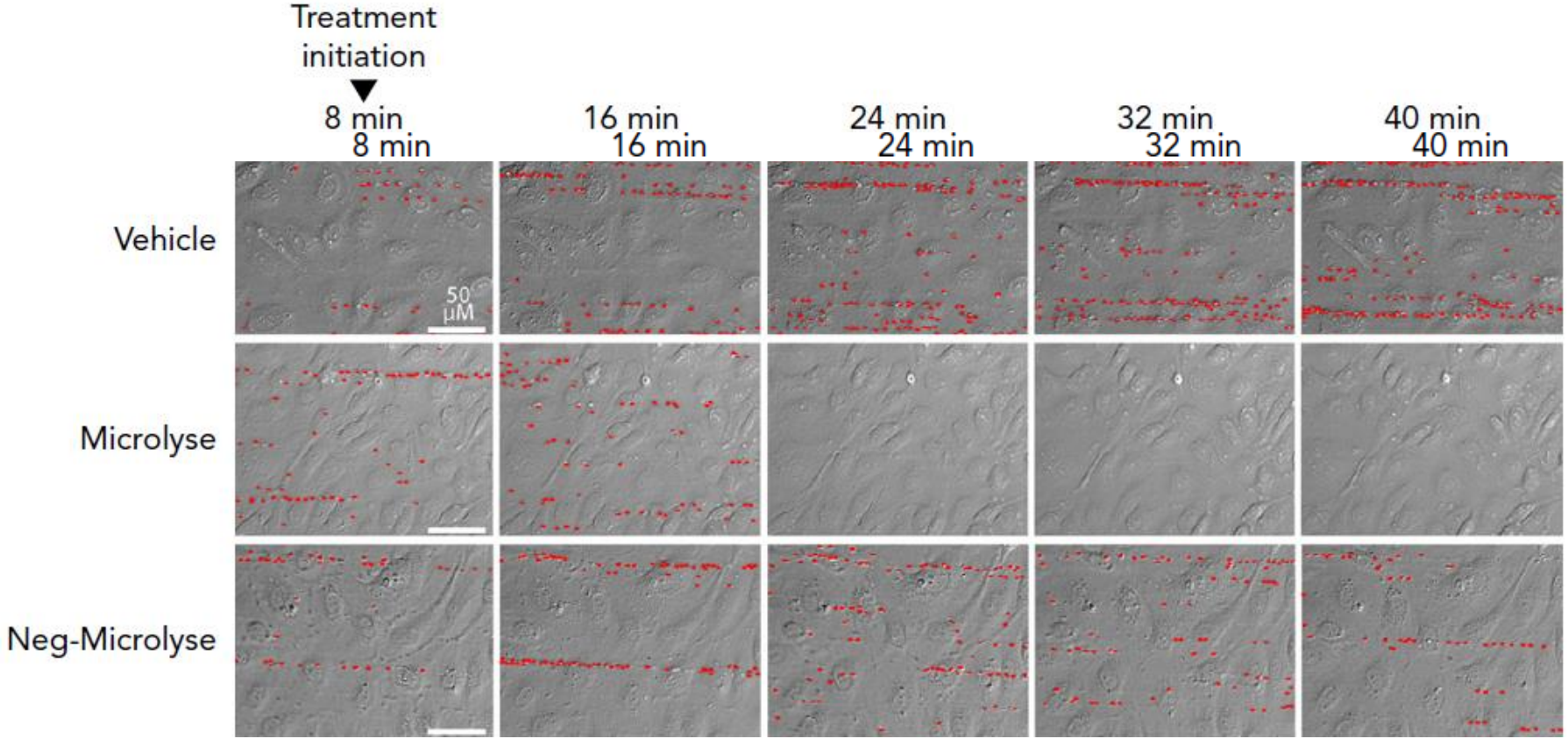




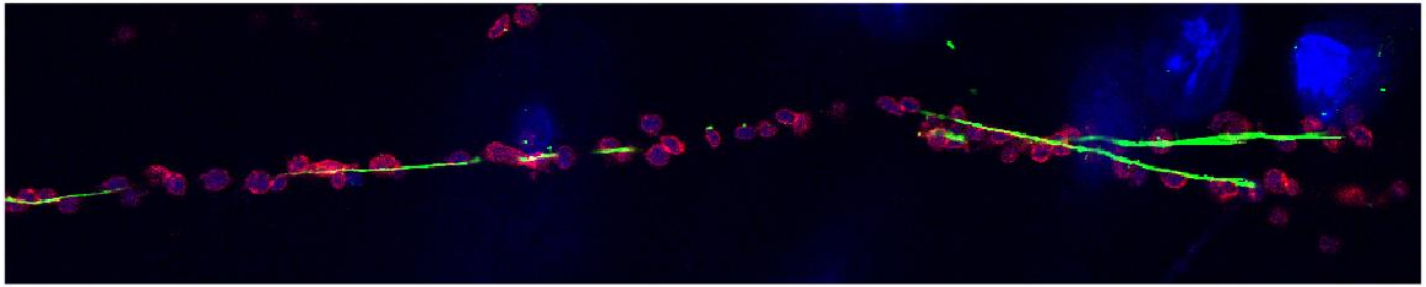




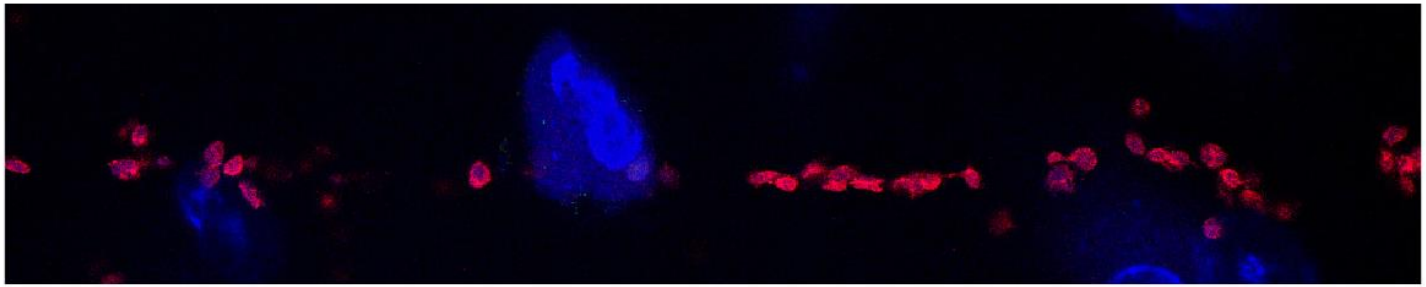
Microthrombolysis under flow (adamts13-/-plasma)



Binding under flow

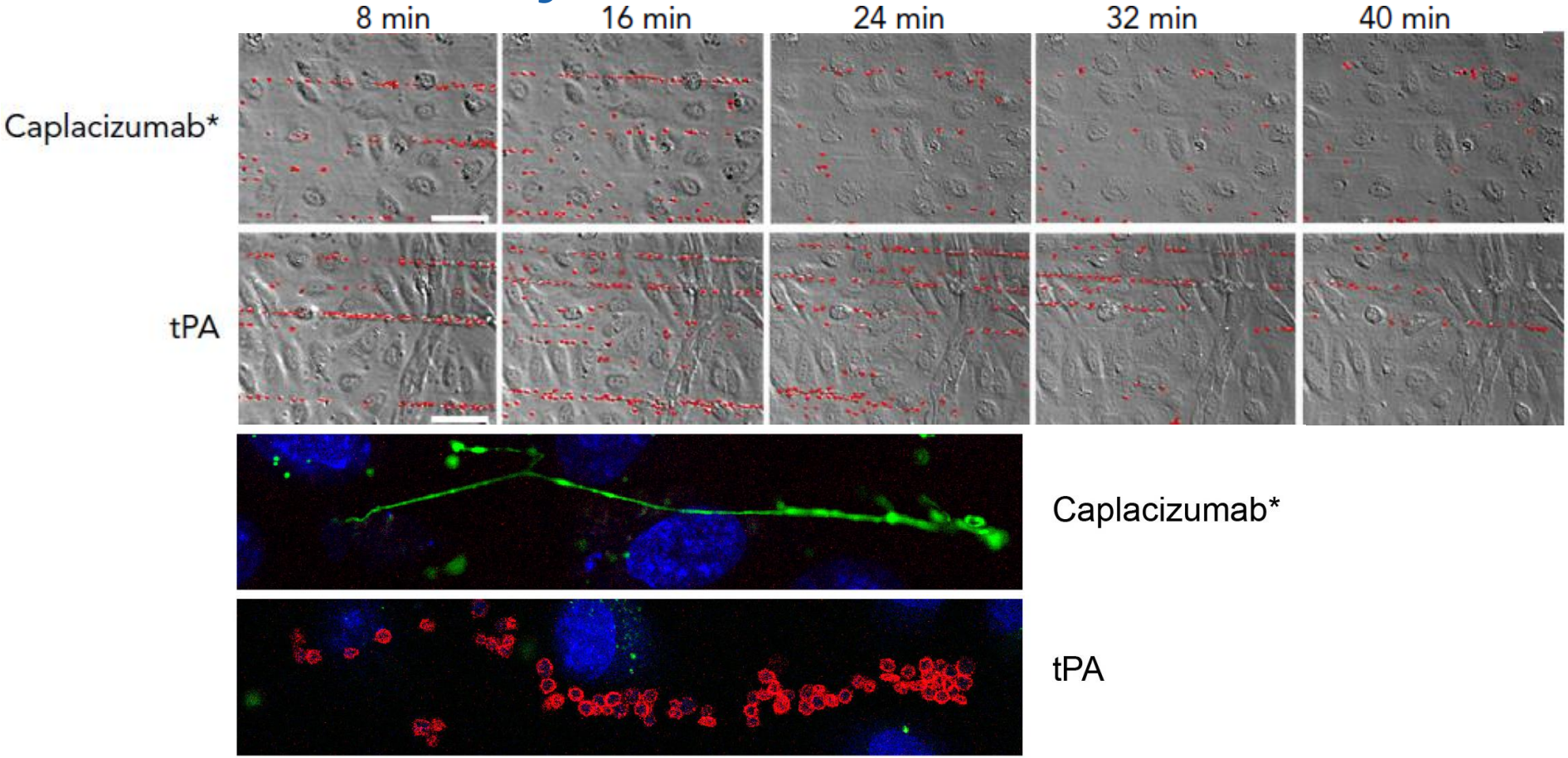


Microlyse

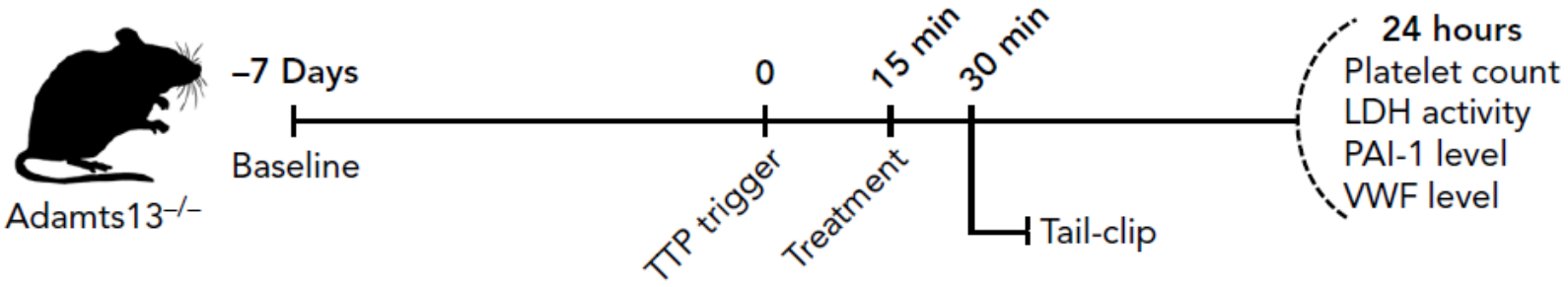


Neg-Microlyse

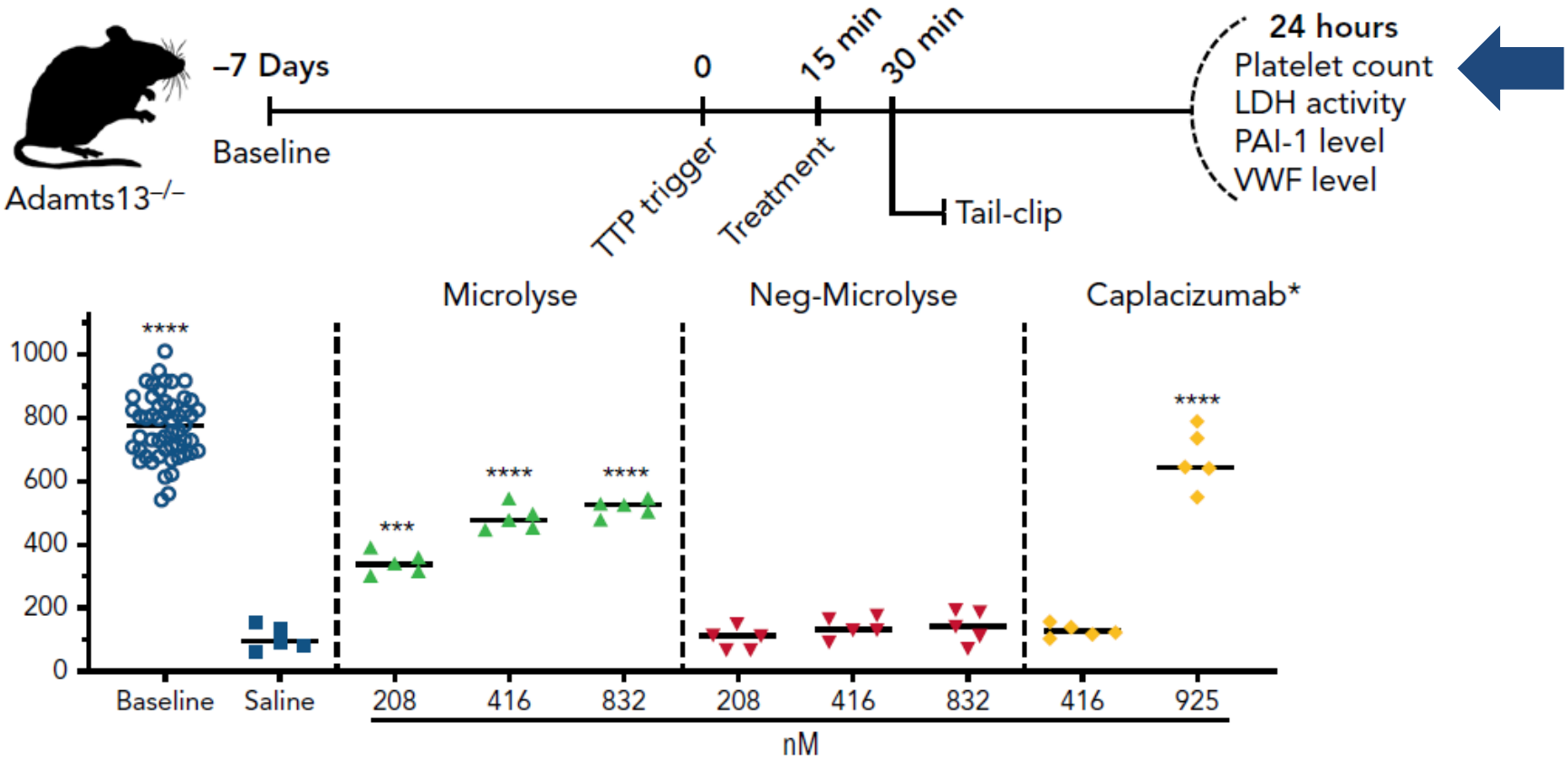
Microthrombolysis under flow (adamts13-/-plasma)



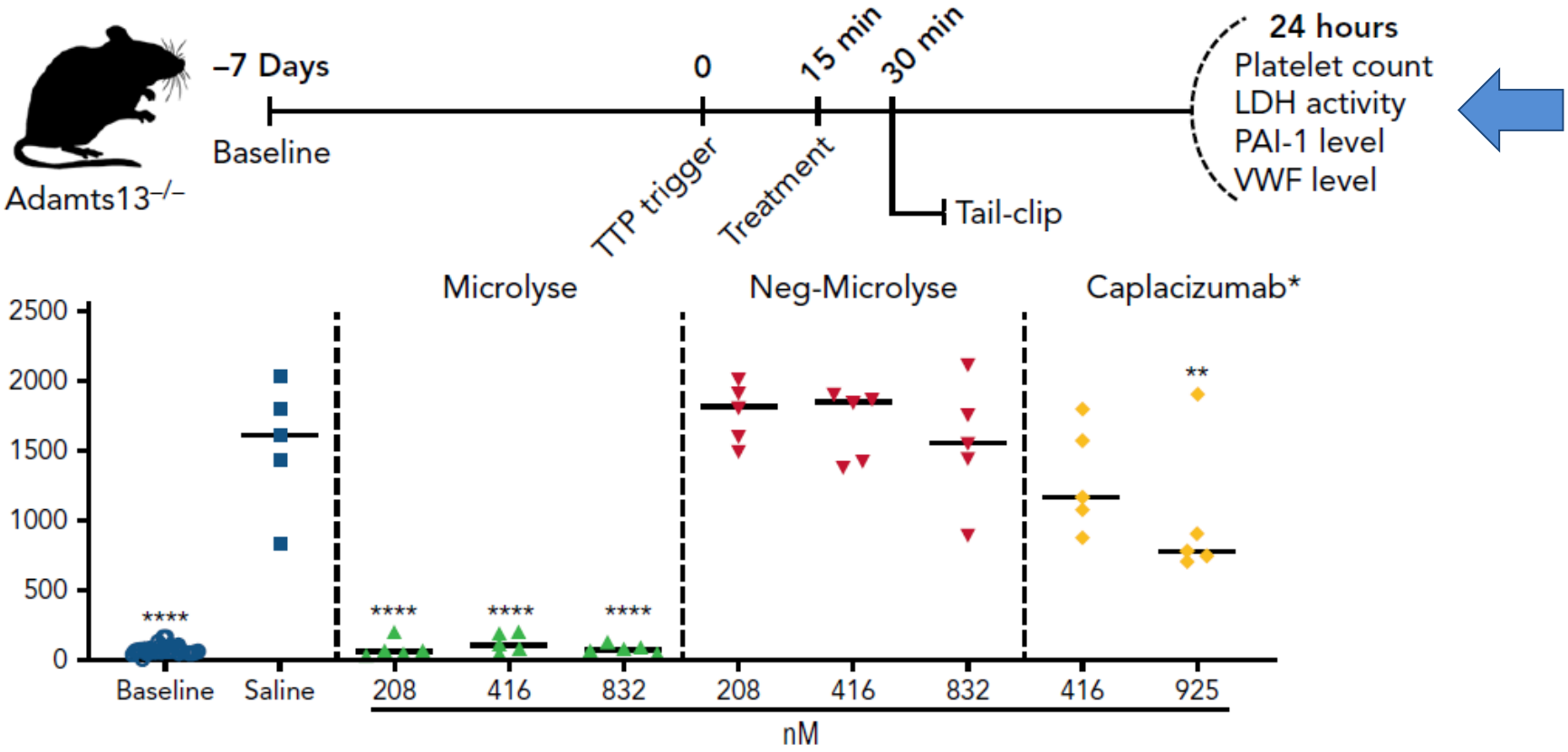
TTP in *vivo*


Adamts13−/−
−7 Days
Baseline
0
TTP trigger
15 min
Treatment
30 min
Tail-clip
24 hours
Platelet count
LDH activity
PAI-1 level
VWF level

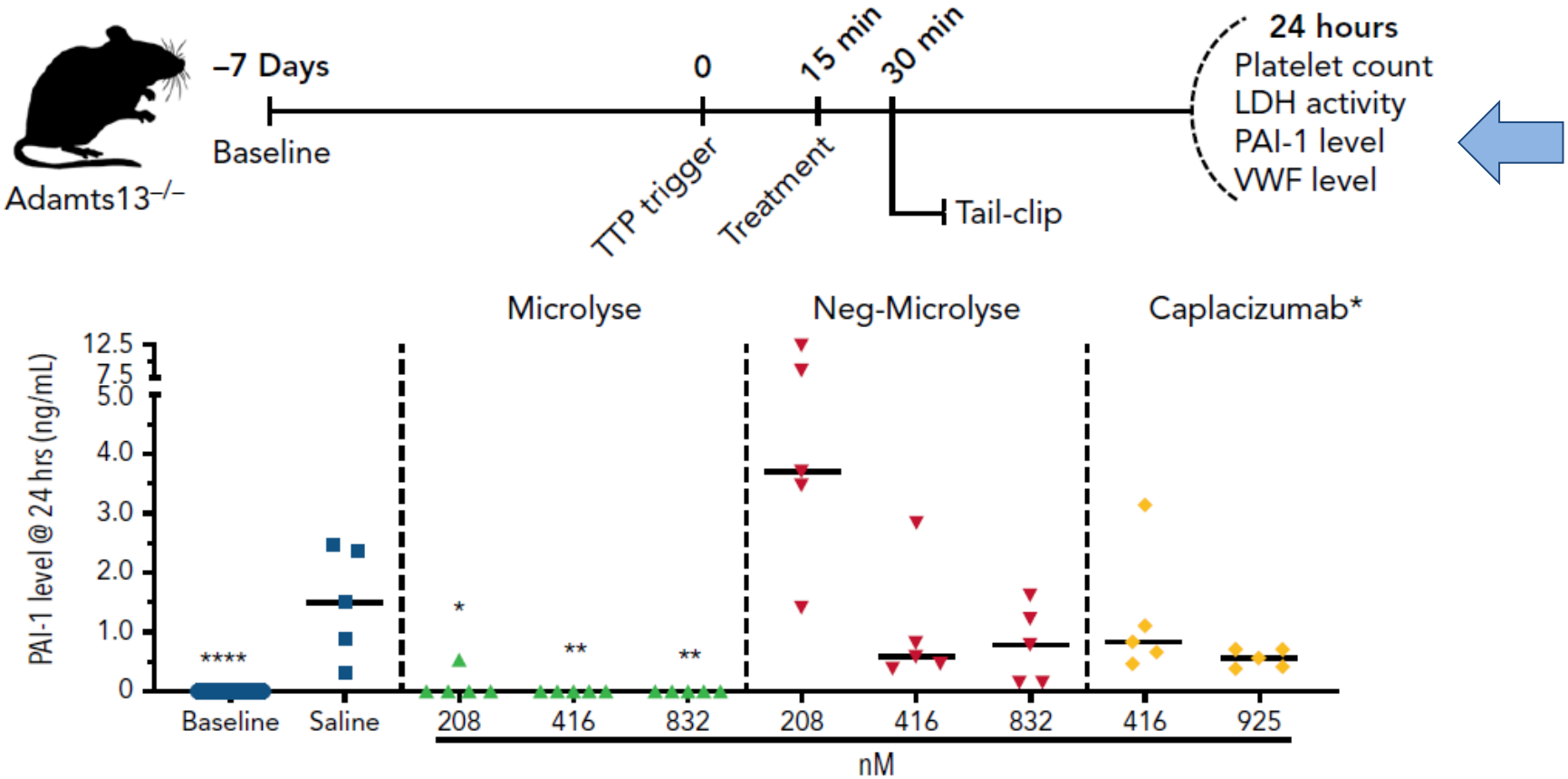
In *vivo:* thrombocytopenia

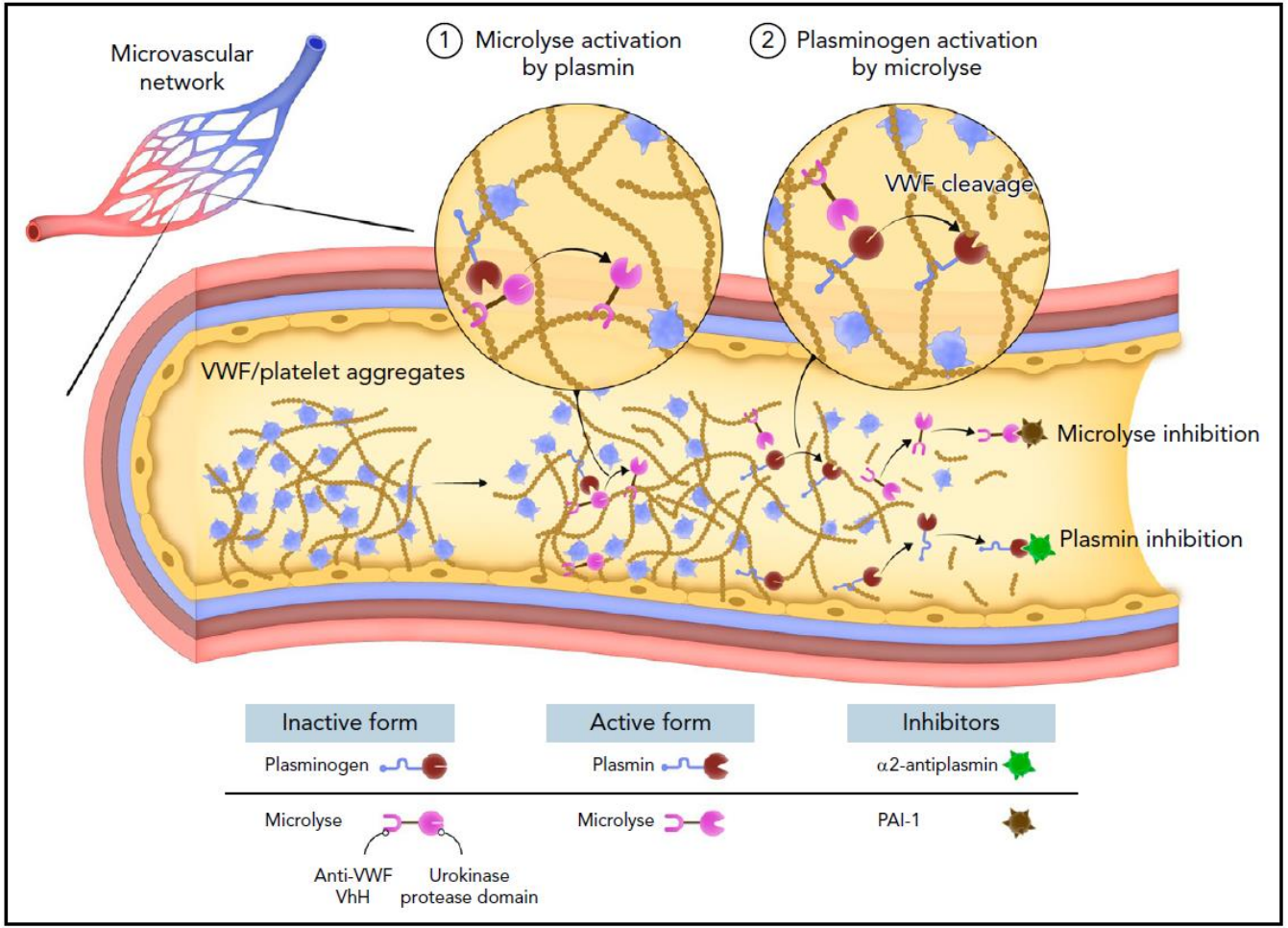


In *vivo:* tissue damage



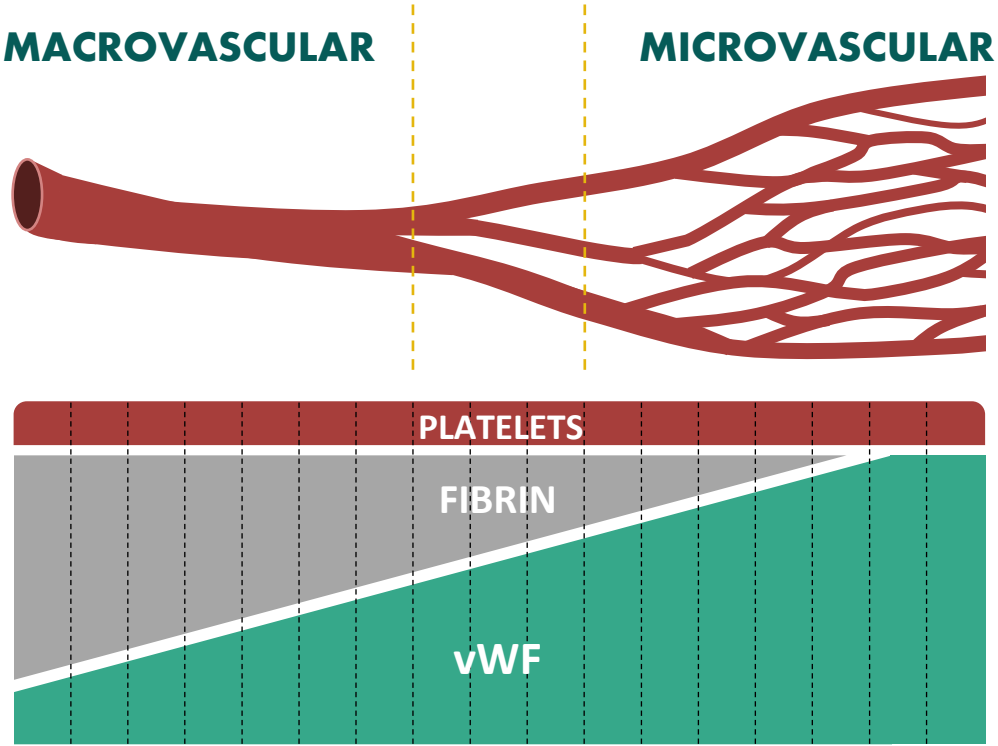
In *vivo:* PAI-1 release (endothelial injury)





Kretz et al. *Blood*. 2022 Jan 27;139(4):477-479 de Maat et al. *Blood*. 2022 Jan 27;139(4):477-479

VWF: always there



Acute ischemic stroke

EPISODE

SYMPTOMS

- One side paralysis
- Sudden confusion
- Speech impairment
- Loss of vision / coordination
- Sudden, severe headache

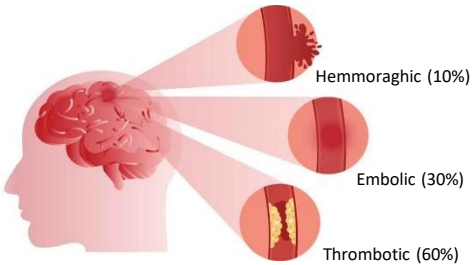
EPIDEMIOLOGY

- **Incidence:** 15 million/year
- **Mortality:** 5 million/year
- **Permanent disability:** 5 million/year

DIAGNOSIS

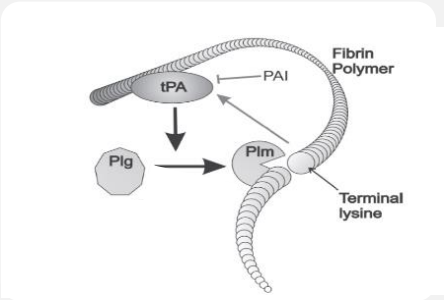
ER METHODOLOGY

- CT to differentiate occlusion from bleeding
- CT to determine location of blood clot



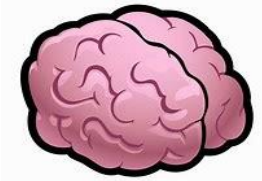
THERAPY

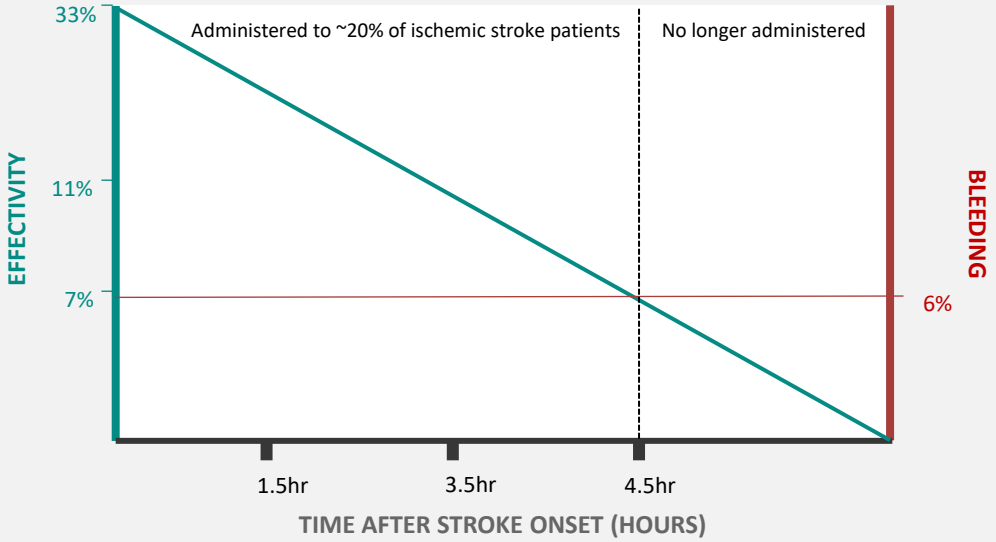
FIRST-IN-LINE: thrombolysis with rh-tPA (~20%)



ADDITIONAL: thrombectomy (~5%)

Problem: tPA-resistance





UNMET CLINICAL NEEDS

LIMITATIONS OF tPA

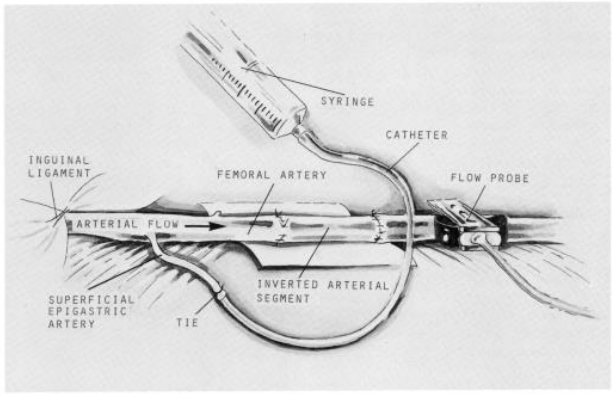
Time after onset	Therapeutic response
0 - 1.5 hr	33%
1.5 - 3 hr	11%
3 – 4.5 hr	7%
>4.5 hr	Outweighed by bleeding risk

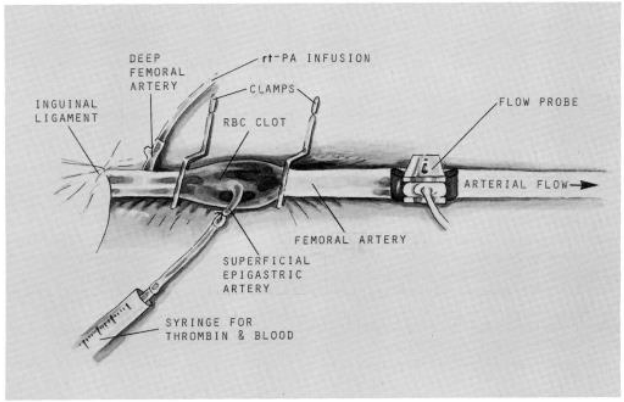
UNMET NEEDS

- Therapy for untreated AIS patients
- Faster clot lysis
- Reduced bleeding risk

Thrombus composition as possible cause

Circulation *Vol 79, No 4, April 1989*

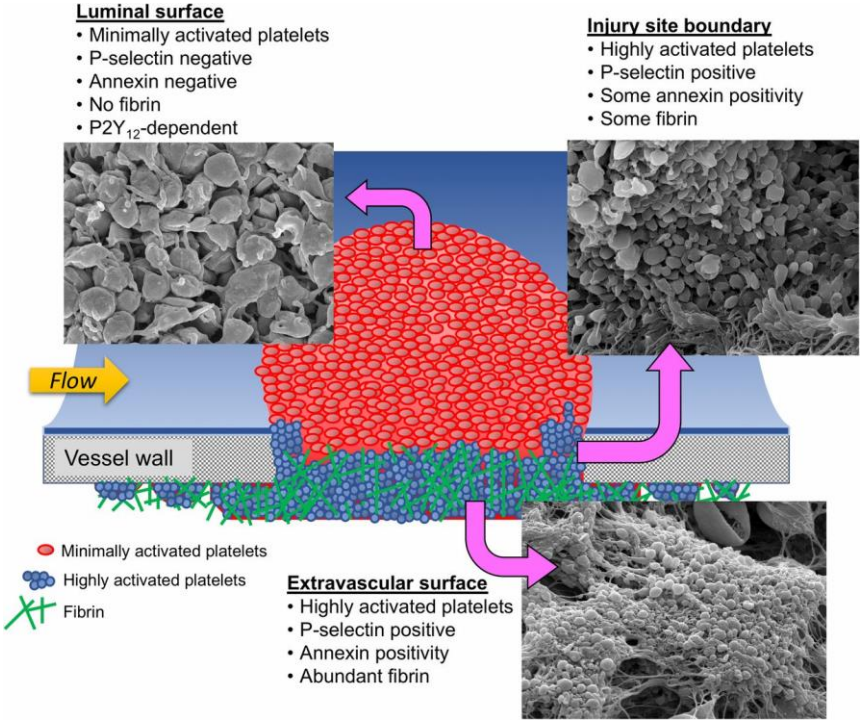




Acute myocardial infarction is triggered by coronary artery occlusion that may be recanalized by thrombolytic therapy with a success rate of up to 75% only. The resistance of coronary artery occlusion to thrombolysis may either be due to obstruction of the lumen by a nonthrombotic mechanism or by intrinsic resistance of thrombus to dissolution. Coronary arterial thrombi are composed of platelet-rich and erythrocyte-rich material in variable proportions. To evaluate the relative sensitivity of these thrombus components to thrombolysis, we have used two femoral arterial thrombosis models in the rabbit, consisting of erythrocyte-rich clot produced by injecting whole blood and thrombin in an isolated segment and of platelet-rich thrombus spontaneously formed on an everted (inside out) femoral arterial segment. Intravenous infusion of recombinant tissue-type plasminogen activator (rt-PA) at a rate of 30 μg/kg/min consistently reperfused arteries occluded with erythrocyte-rich clot (six of six animals compared with zero of six placebo-treated animals, $p=0.002$), whereas infusion of 30 or 100 μg/kg/min was significantly less efficient for reperfusion of everted segments occluded with platelet-rich material (only four of 12 animals, $p=0.01$). Intra-arterial infusion proximal to the occlusion, at a rate of 20 μg/kg/min reperfused six of seven rabbits with erythrocyte-rich clots but only one of seven rabbits with occluded everted segments ($p=0.03$). A dose of 100 μg/kg/min was necessary to reperfuse platelet-rich occlusions in five of six rabbits. We conclude that platelet-rich arterial thrombus is much more resistant to thrombolysis with rt-PA than erythrocyte-rich clot. This differential sensitivity to lysis may explain the failure of thrombolytic therapy in a significant percentage of patients with acute myocardial infarction who may have a predominantly platelet-rich occlusion. The rabbit femoral arterial eversion graft model may represent a useful tool for developing strategies directed at the dissolution of platelet-rich thrombus. (*Circulation* 1989;79:920–928)

Jang et al. *Circulation* 1989;79:920-928

Core-shell architecture x Thrombectomy studies



- 1-2% contain unusual components

Aspegen et al. *Front Neurol.* 2022 May 17;13:846293.

- VWF drives formation of platelet-rich thrombi

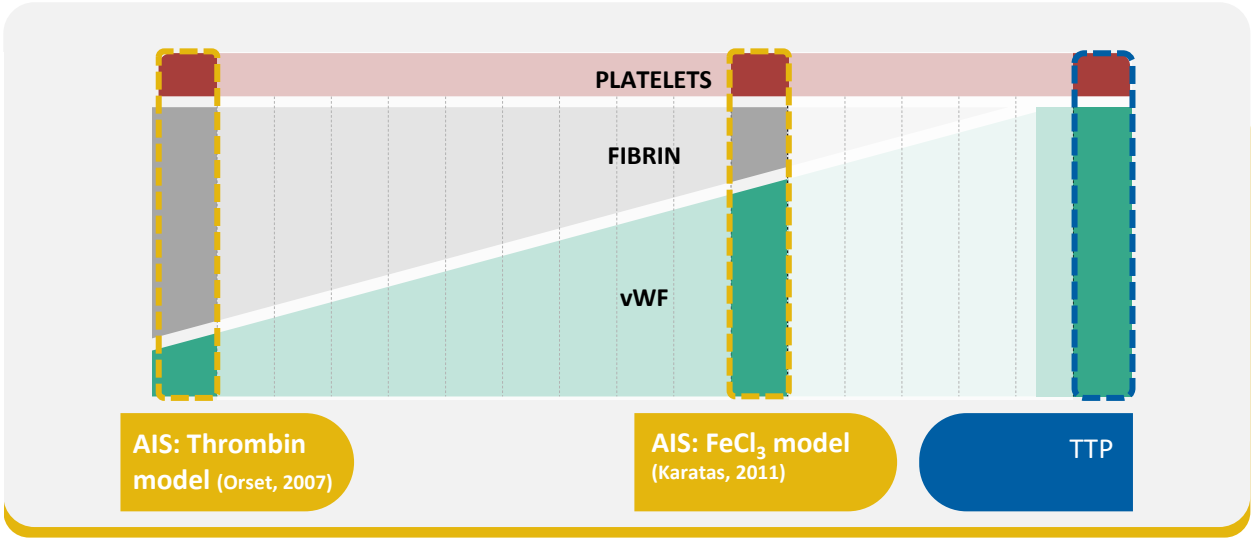
Prochazka et al. *Med Sci Monit*. 2018 Jun 11;24:3929-3945.

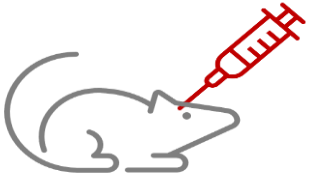
- Soft core, hard shell
  - contains VWF and fibrin
  - tPA resistant

Di Meglio et al. *Neurology*. 2019 Oct 29; 93(18): e1686–e1698.

Tomaiuolo et al. *Proc Natl Acad Sci USA*. 2019 Feb 5;116(6):2243-2252.

tPA response in ischemic stroke models

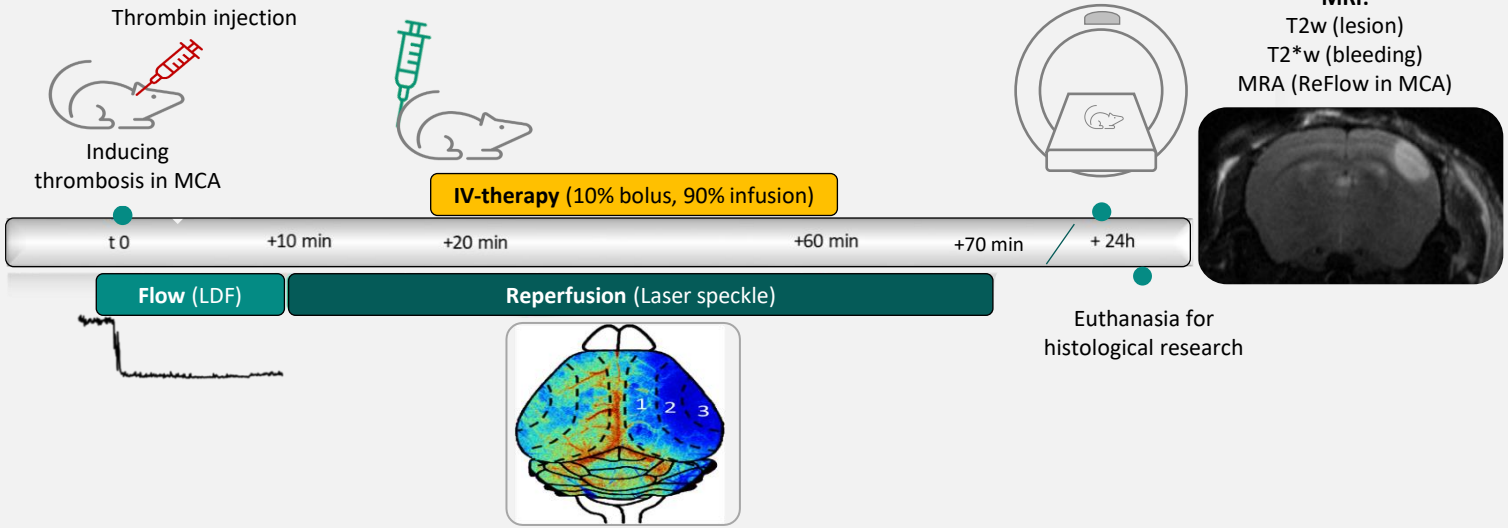




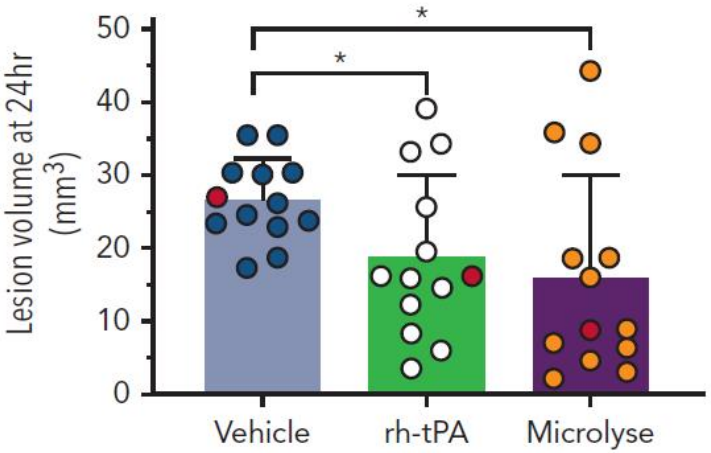
- Fibrin-rich
- tPA-responsive

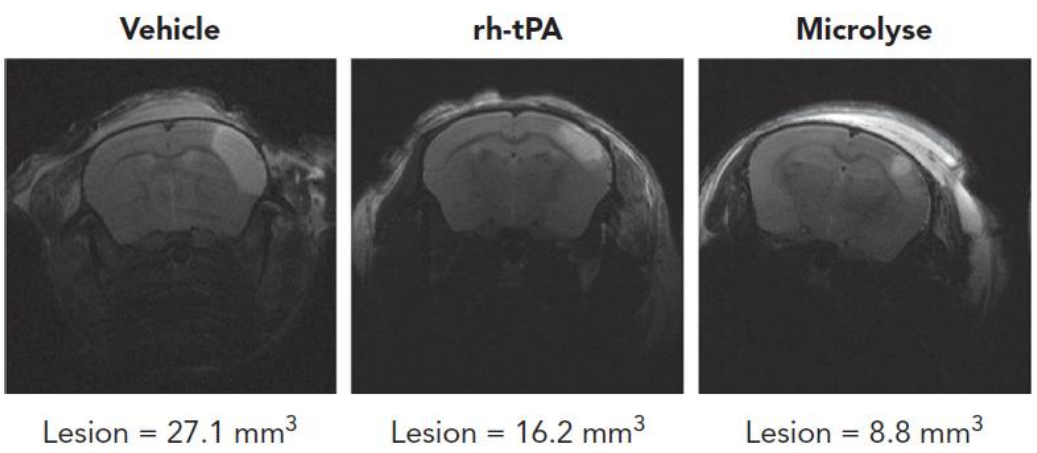
- Platelet-rich
- tPA-resistant

tPA-responsive stroke model



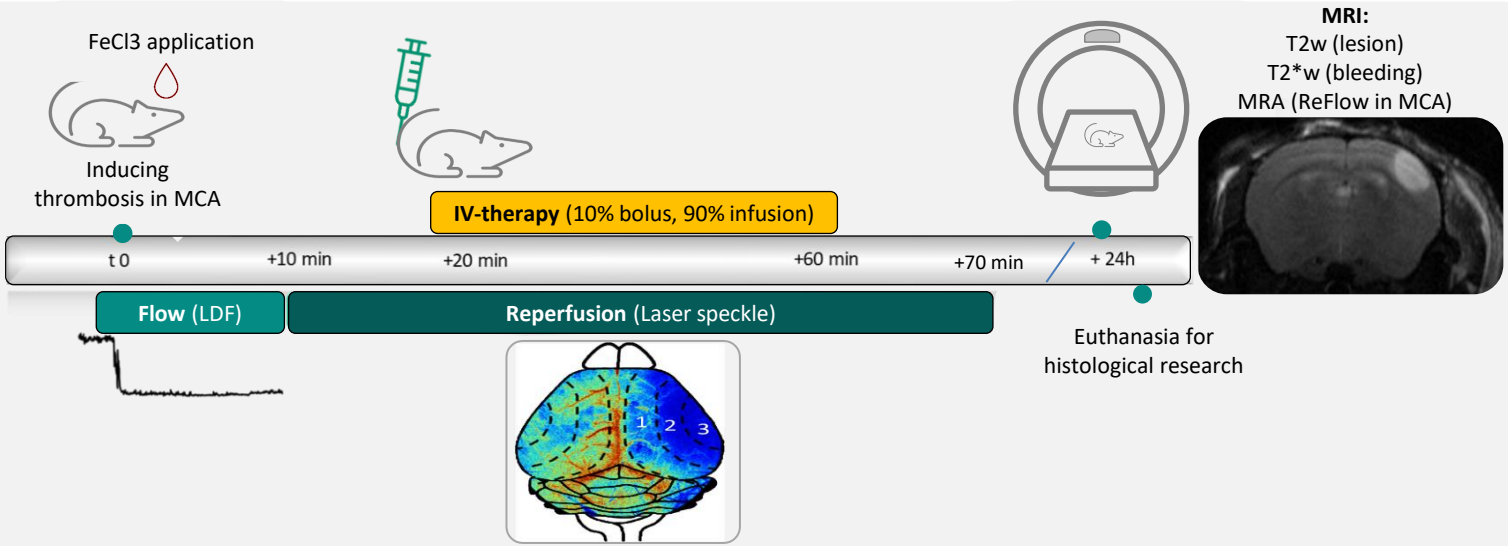
Microlyse non-inferior to rh-tPA in fibrin-rich AIS



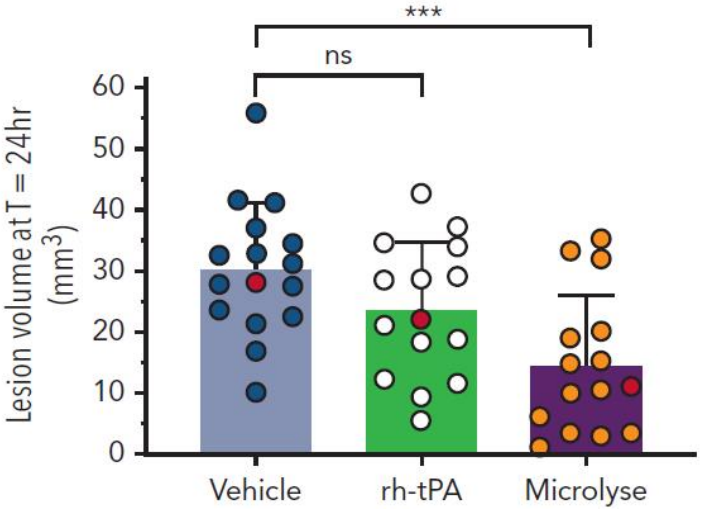


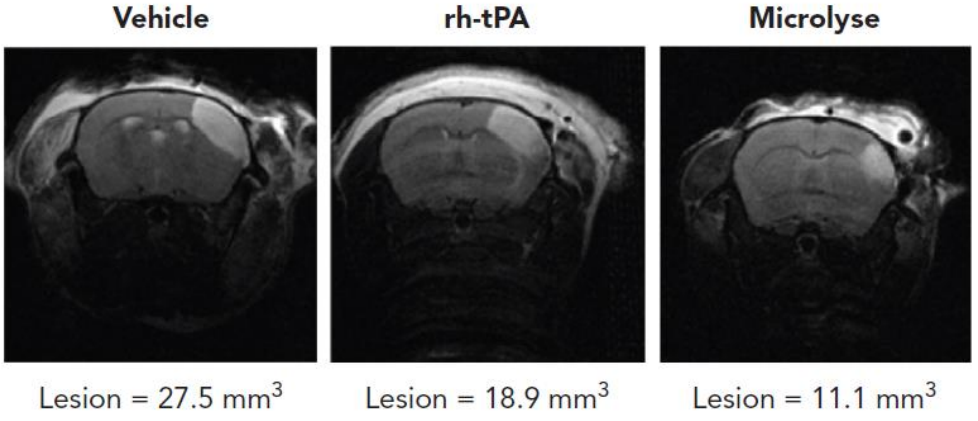
Van Moorsel et al. *Blood*. 2022 Dec 29;140(26):2844-2848.

tPA-resistant stroke model



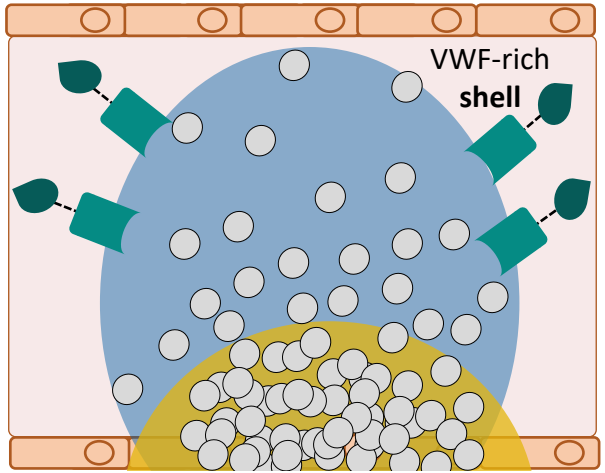
Microlyse superior to rh-tPA in platelet-rich AIS



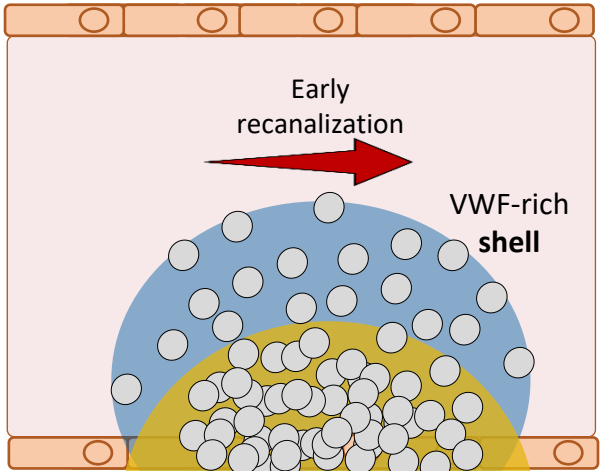


Van Moorsel et al. *Blood*. 2022 Dec 29;140(26):2844-2848.

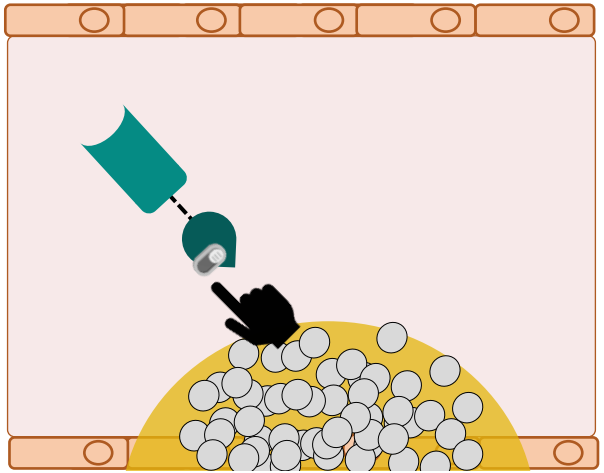
Targeting "shell-VWF”



fibrin-rich **core**



fibrin-rich **core**



fibrin-rich **core**

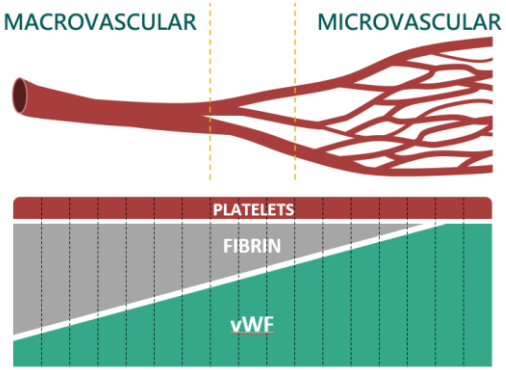
Summary

Plasmin targets and degrades VWF

- Natural emergency backup for ADAMTS13

VWF-targeted plasminogen activation

- Attenuates TTP in preclinical model
  - Reverses thrombocytopenia = prohemostatic
  - Superior to blockade of GPIbα with a V_HH
- Effective in preclinical models for AIS
  - Non-inferior to rh-tPA in fibrin-rich model
  - Superior to rh-tPA in platelet-rich model



Many thanks!!!

UMC Utrecht
Steven de Maat
Hinde El Otmani
Rowan Frunt
Wariya Sanrattana
Chantal Clark
Arjan Barendrecht
Marc van Moorsel

KU Leuven Campus Kulak Kortrijk
Karen Vanhoorelbeeke
Claudia Tersteeg
INSERM U1176 Paris
Peter Lenting
Thibaud Sefiane
Amsterdam UMC
Joost Meijers
UMC Groningen
Ton Lisman
Oregon Health and Science University
Owen McCarty
Karolinska Institutet and University Hospital
Thomas Renné

Michigan State University
James Luyendyk
Luigi Sacco Hospital Milan
Marco Cicardi
Semmelweis University Budapest
Henriette Farkas, Lilian Varga
Justus Liebig University
Malgorzata Wygrecka
Medical University of South Carolina
Allen Kaplan
Charité - Universitätsmedizin Berlin
Karoline Krause, Marcus Maurer

LSBR Landsteiner Foundation for Blood Transfusion Research

TROMBOSESTICHTING NEDERLAND

NWO Netherlands Organisation for Scientific Research

HAEi

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