Réunion du Collège d'Hématologie des Hôpitaux
Lundi 4 décembre 2023, Paris

Actualités sur le diagnostic du Purpura Thrombotique Thrombocytopénique

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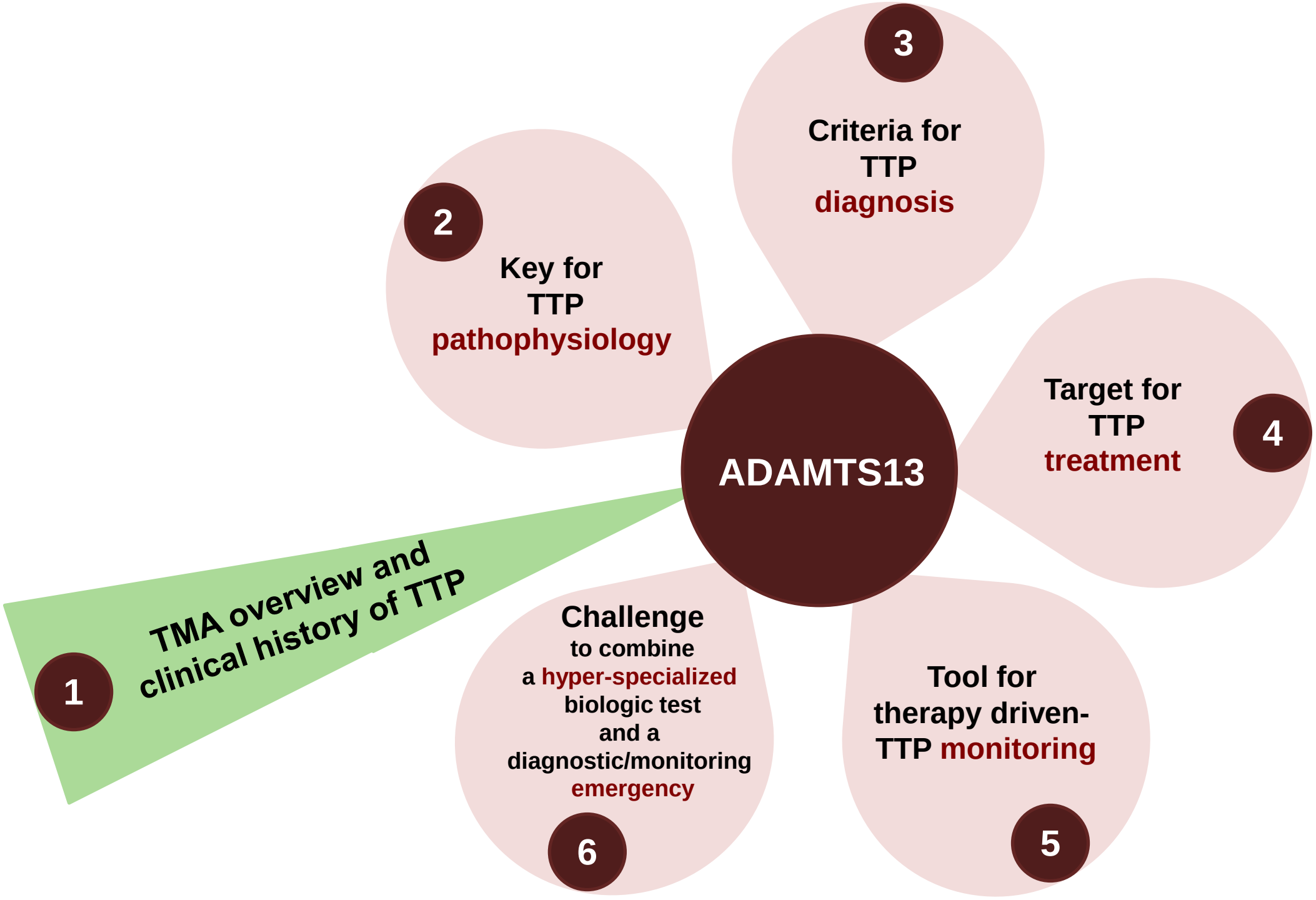
Plateforme biologique ADAMTS13 du CRMR des MAT


ADAMTS13
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TMA overview and clinical history of TTP
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Key for TTP pathophysiology
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Criteria for TTP diagnosis
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Target for TTP treatment
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Tool for therapy driven-TTP monitoring
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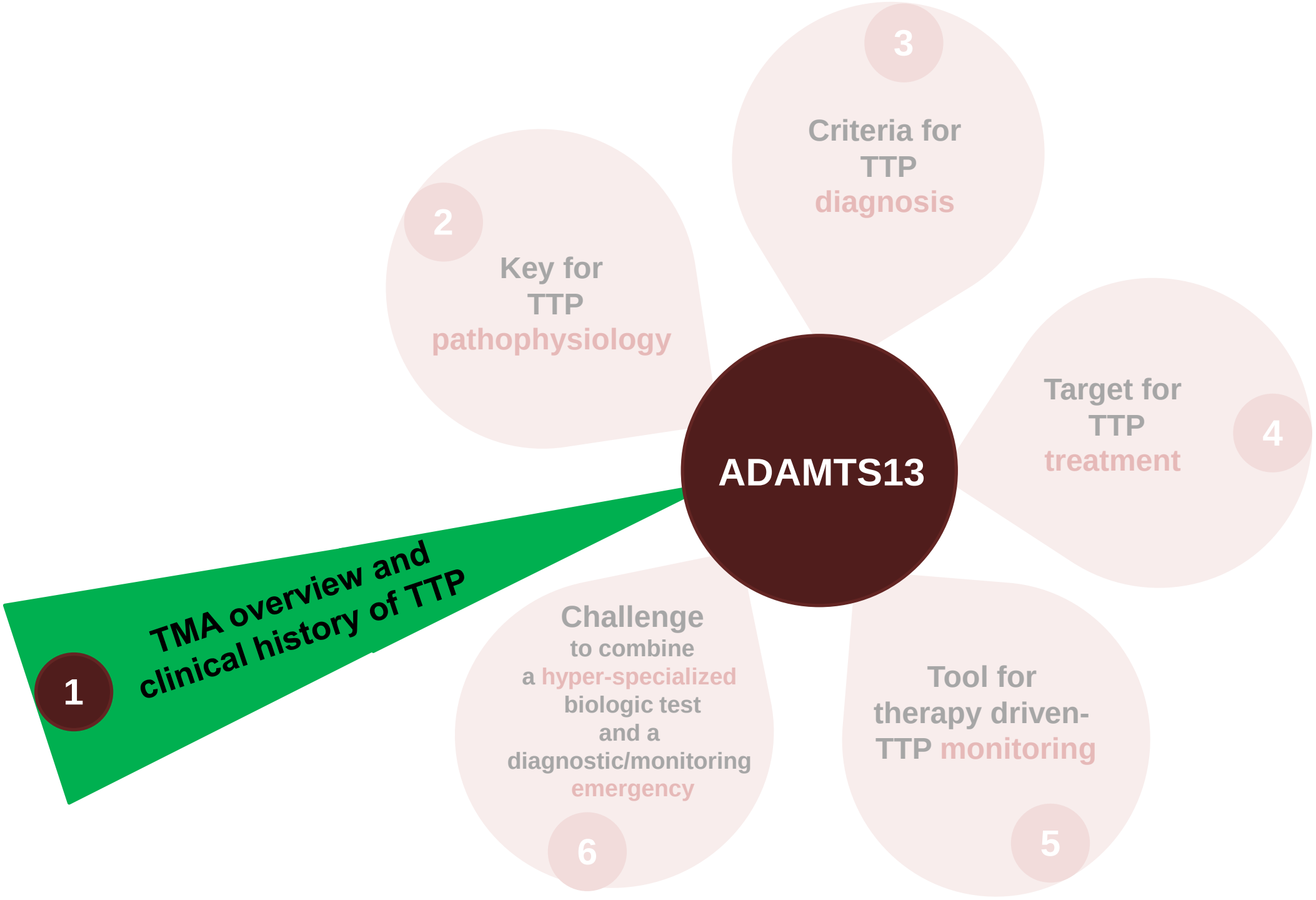
Challenge to combine a hyper-specialized biologic test and a diagnostic/monitoring emergency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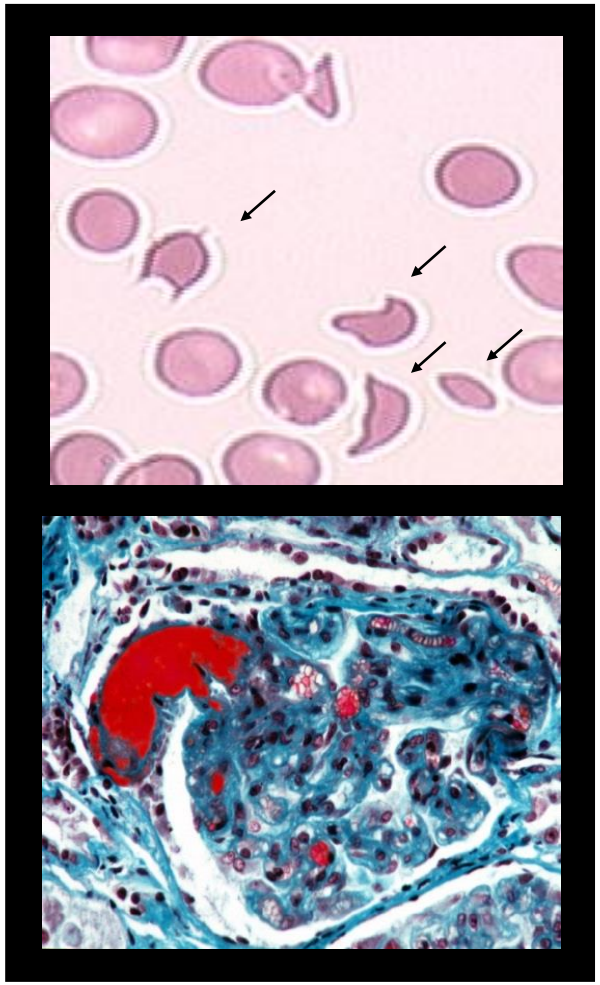
Thrombotic microangiopathies (TMAs)

Global prevalence of TMA worldwide : ~50-100 cases / million people / year

The TMA syndrome associates:

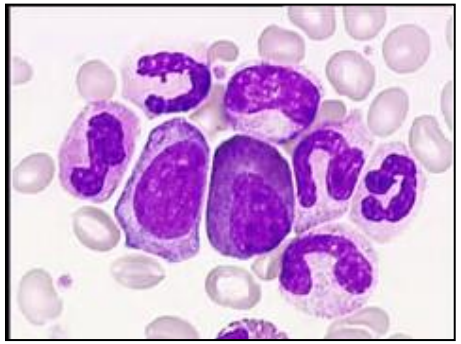
- **Microangiopathic hemolytic anemia**
- **Peripheral thrombocytopenia**
- **Organ failure of variable severity related to thrombosis of the microvessels**

Examples: TTP, HUS, others…



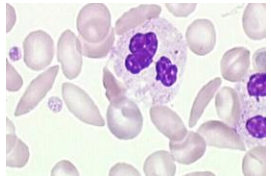
TMAs in the global picture of hematology

Malignant hematology



Benign hematology

Cellular hematology



Anemia
Thrombocytopenia

Hemostasis

Bleeding and thrombosis of the microvessels

TMAs are at the border between cellular hematology and hemostasis

Princeps clinical descriptions of TTP

E. Moschcowitz, 1924

HYALINE THROMBOSIS OF THE TERMINAL ARTERIOLES AND CAPILLARIES: A HITHERTO UNDESCRIBED DISEASE *

ELI MOSCHCOWITZ, M.D.

The history of this case is as follows:

A girl aged sixteen with an uneventful previous history and in a state of perfect health was suddenly attacked with a high fever (103° to 104° F.). The only complaint was pain in the arms. Even in the first days of her illness her physician noted an extreme pallor. She was admitted to Beth Israel Hospital a few days after the onset of the illness, where she remained one

* Presented January 10, 1924.

AN ACUTE FEBRILE PLEIOCHROMIC ANEMIA WITH HYALINE THROMBOSIS OF THE TERMINAL ARTERIOLES AND CAPILLARIES

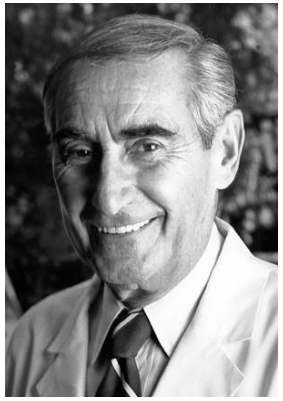
AN UNDESCRIBED DISEASE *

ELI MOSCHCOWITZ, M.D.
NEW YORK

This case is remarkable, clinically and anatomically.

- **16 year-old girl**
- **Fever, <u>cerebral manifestations</u>**
- **Anemia, <u>haemorrhagia</u>**
- **<u>Renal</u> failure**
- **<u>Heart</u> failure**
- **Death within 2 weeks**

<u>Thrombi</u> in arterioles and capillaries of most organs at autopsy



I Schulman, 1960

BLOOD *The Journal of Hematology*

JULY, 1960 VOL. XVI, NO. 1

Studies on Thrombopoiesis. I. A Factor in Normal Human Plasma Required for Platelet Production; Chronic Thrombocytopenia Due to its Deficiency

By IRVING SCHULMAN, MILA PIERCE, ABBY LUKENS AND ZINET CURRIMBHOY

Congenital Deficiency of a Factor in Normal Plasma That Reverses Microangiopathic Hemolysis and Thrombocytopenia

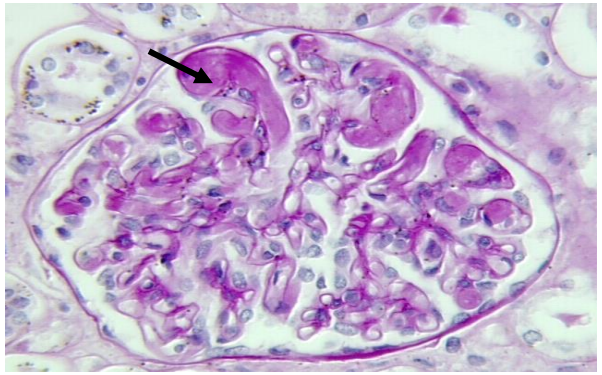
Jefferson D. Upshaw, Jr., M.D.

***New England Journal of Medicine 1978**. 298 (24): 1350–2*

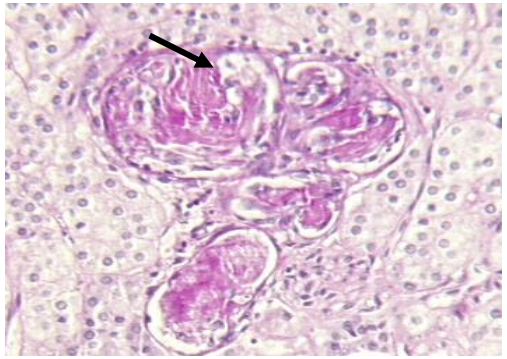
JD Upshaw Jr, 1978

TTP is a systemic disease

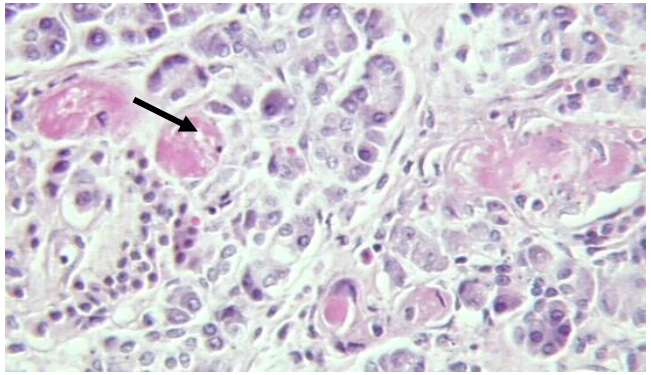
kidney



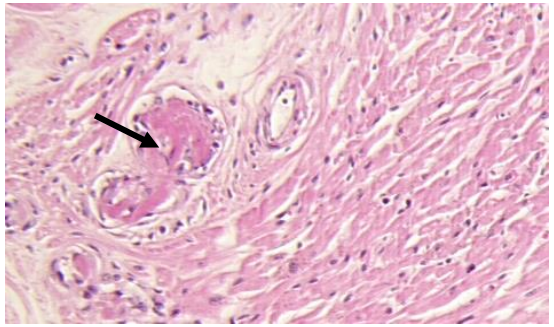
surrenal



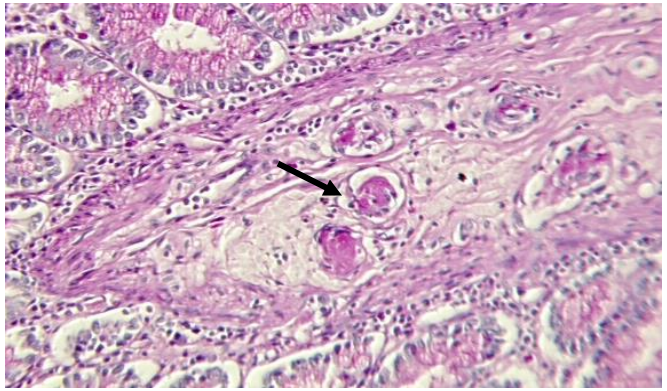
pancreas



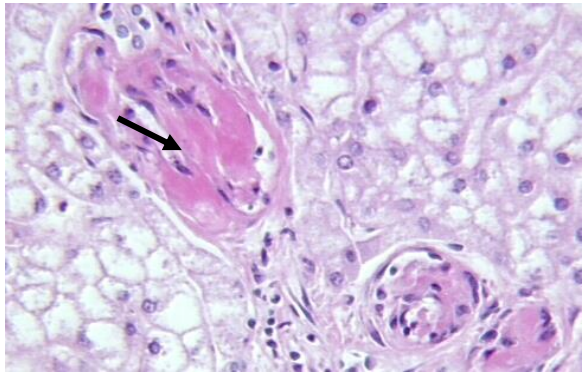
heart



colon



liver



Empiric treatment of TTP with plasmatherapy

Vol. 325 No. 6 PLASMA EXCHANGE VS. PLASMA INFUSION FOR TTP — ROCK ET AL. 393

COMPARISON OF PLASMA EXCHANGE WITH PLASMA INFUSION IN THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA

Gail A. Rock, Ph.D., M.D., Kenneth H. Shumak, M.D., Noel A. Buskard, M.D., Victor S. Blanchette, M.D., John G. Kelton, M.D., Rama C. Nair, Ph.D., Robert A. Spasoff, M.D., and the Canadian Apheresis Study Group*

398 THE NEW ENGLAND JOURNAL OF MEDICINE Aug. 8, 1991

IMPROVED SURVIVAL IN THROMBOTIC THROMBOCYTOPENIC PURPURA–HEMOLYTIC UREMIC SYNDROME

Clinical Experience in 108 Patients

William R. Bell, M.D., Hayden G. Braine, M.D., Paul M. Ness, M.D., and Thomas S. Kickler, M.D.

Since the 1990's,

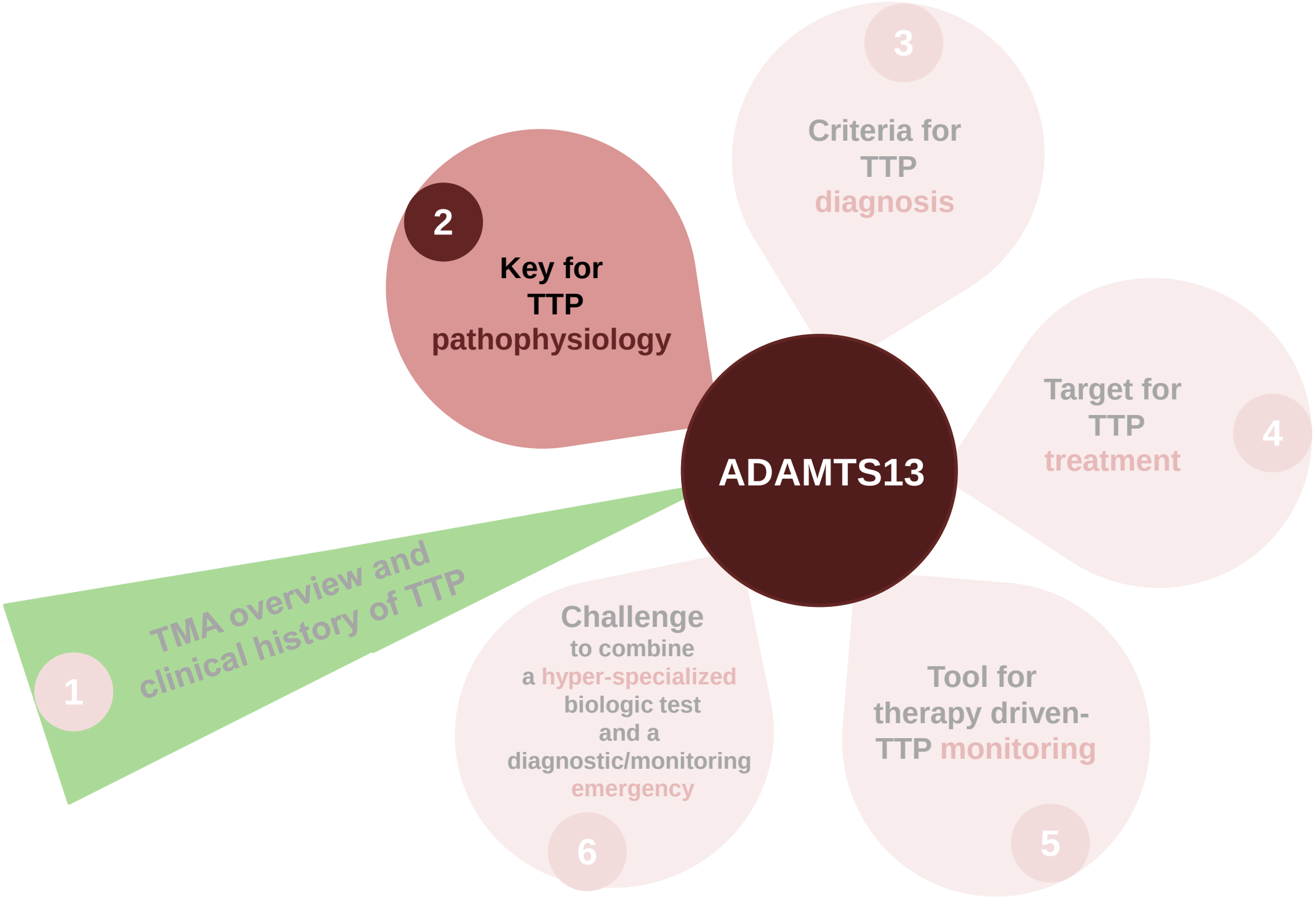
empiric plasmatherapy by daily plasma exchange initiated in emergency

until clinical/hematologic remission

has dramatically been improving TTP prognosis allowing a 85% survival

***versus* an almost systematic death before.**

Rock GA et al, NEJM. 1991; 325(6): 393-7
Bell WR et al; NEJM 1991; 325(6): 397-403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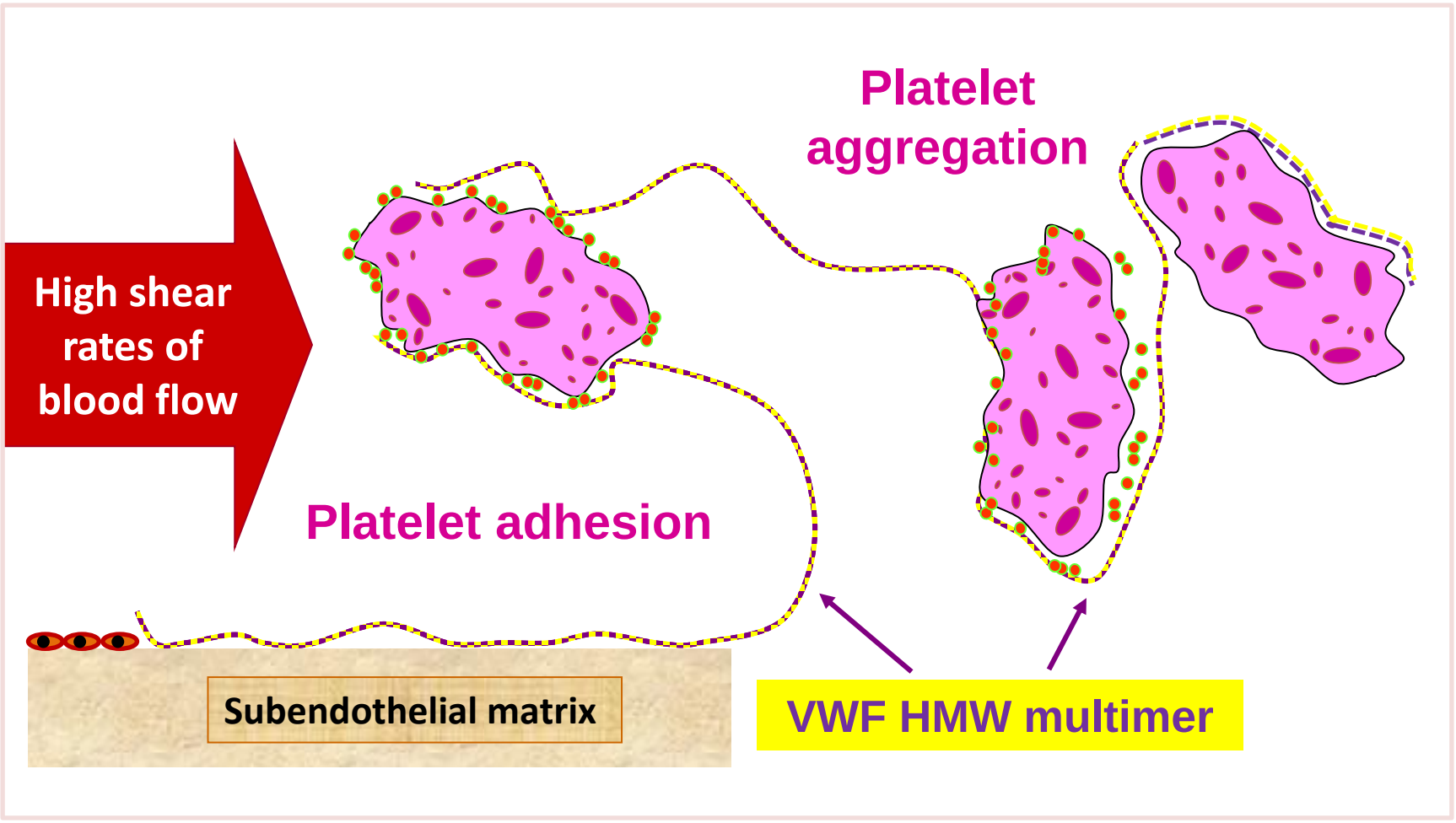
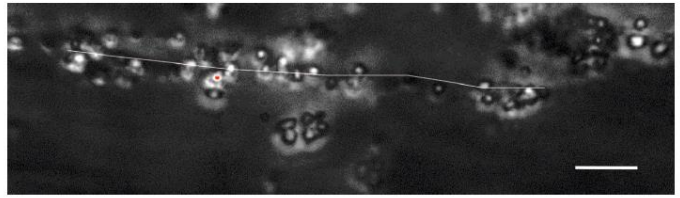
VWF is the key protein for platelet clumping within the microvessels



High shear rates of blood flow
In the microvessels

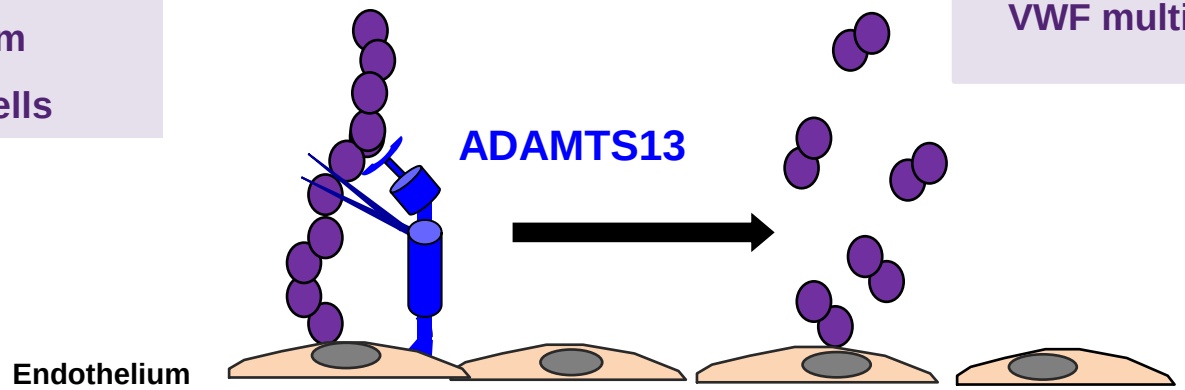


Videomicroscopy with fluorescence

Schneider et al, PNAS 2007; 104:7899-03

VWF-dependent platelet adhesion and aggregation is regulated by ADAMTS13 (1)

Ultralarge VWF multimers secreted from endothelial cells

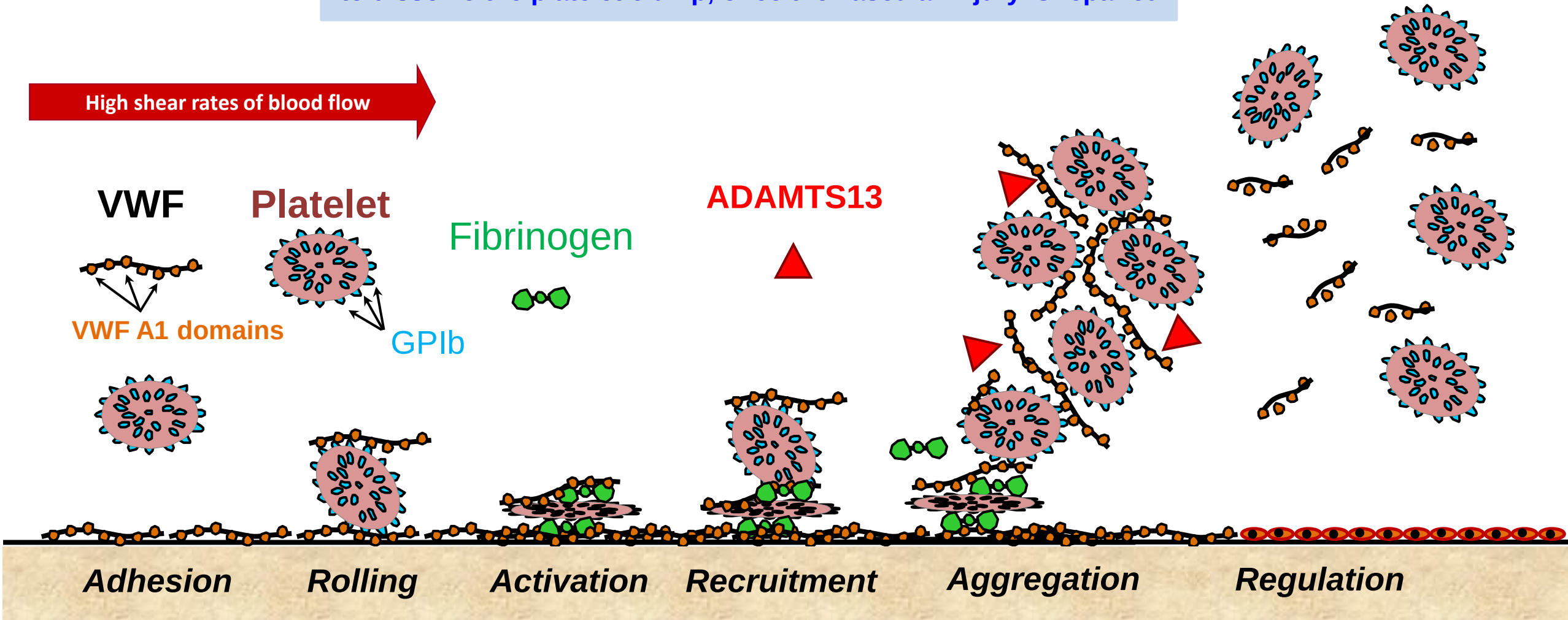


Circulating normal VWF multimers

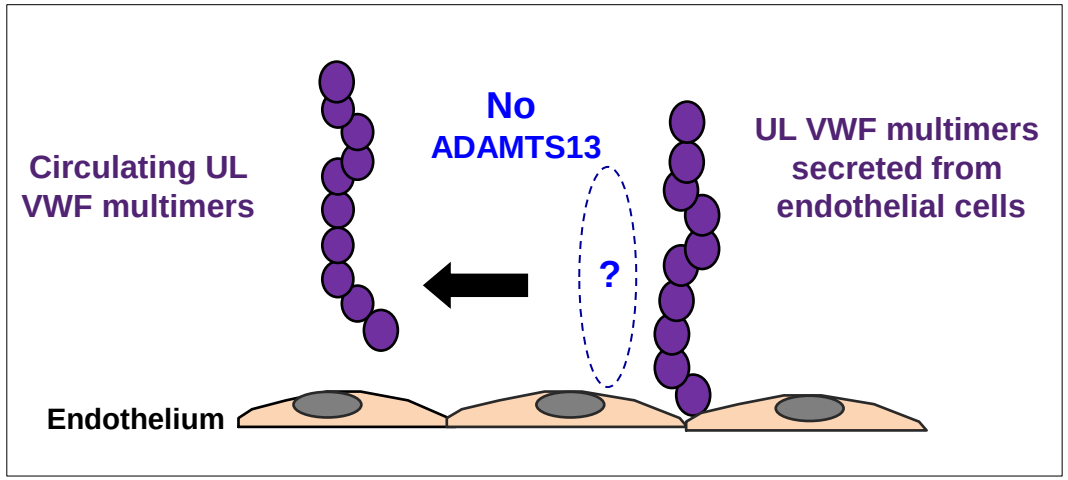
ADAMTS13 cleaves VWF UL multimers released from endothelial cells

VWF-dependent platelet adhesion and aggregation is regulated by ADAMTS13 (2)

ADAMTS13 is able to cleave VWF multimers bound to platelets to dissolve the platelet clump, once the vascular injury is repaired



Pathophysiology for TTP



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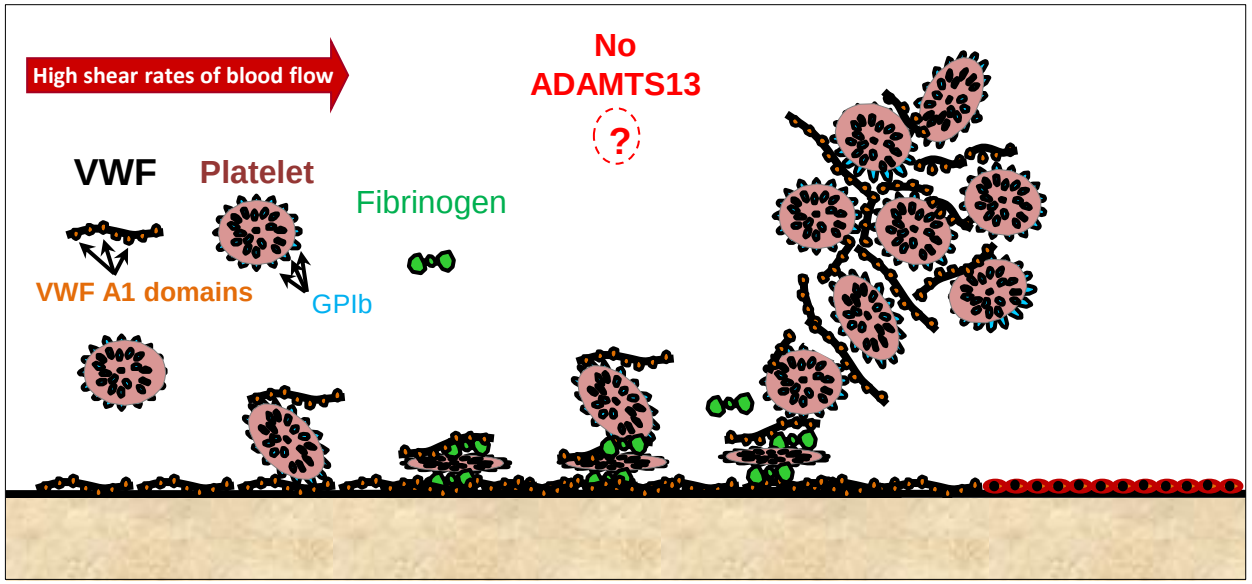
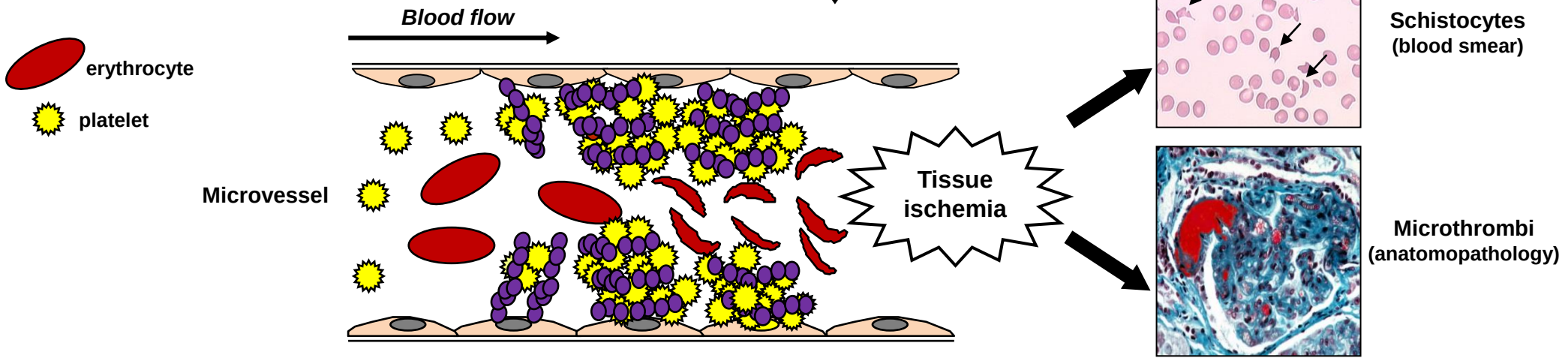




Joly BS et al, Blood 2017.

Pathophysiology for TTP

Severe functional deficiency of ADAMTS13
(ADAMTS13 activity < 10%)

↓

Accumulation of hyperadhesive UL-VWF multimers
circulating in plasma and mediating platelet clumps in small vessels

↓

Spontaneous formation of platelet microthrombi in the microcirculation
and absence of physiologic dissolution of platelet clumps

↙ ↘

Mechanical hemolytic anemia
Consumption thrombocytopenia

Multivisceral ischemia
-> organ dysfunction

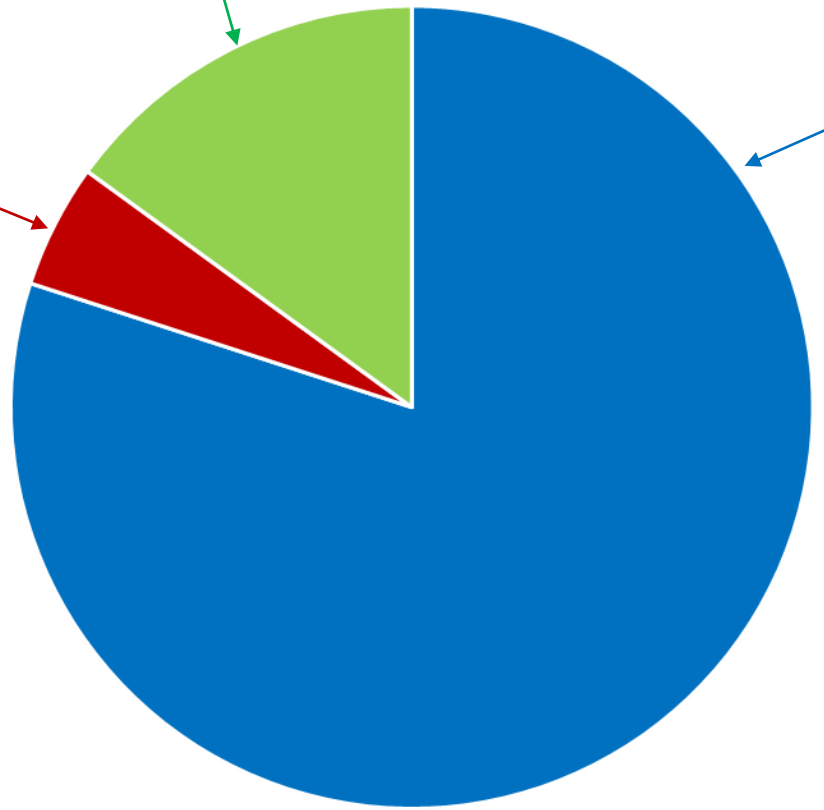
Causes of ADAMTS13 severe deficiency

Non immune acquired TTP

Deficient synthesis, excessive degradation, non immune inhibition… of ADAMTS13 (~15%)

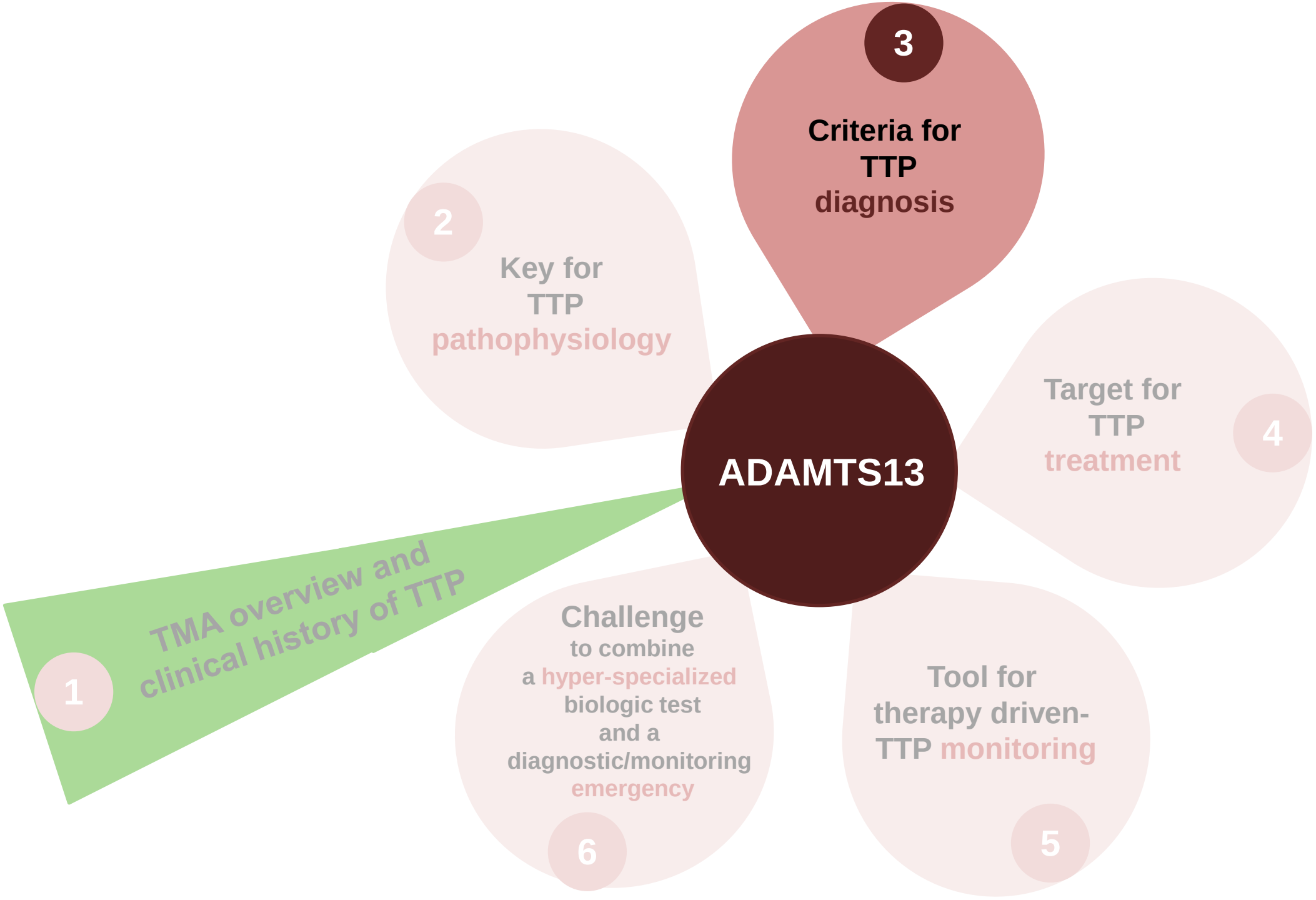
Bi-allelic recessive mutations of ADAMTS13 gene (~5%)

Congenital TTP (cTTP) Upshaw-Schulman syndrome



Anti-ADAMTS13 auto-antibodies (IgG) (~80%)

Immune TTP (iTTP)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TTP definition

Life-threatening Thrombotic Microangiopathy (TMA) and therapeutic emergency characterized by:

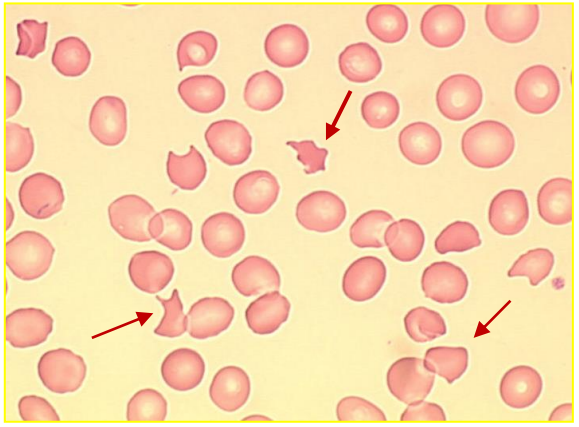
. Mechanical hemolytic anemia

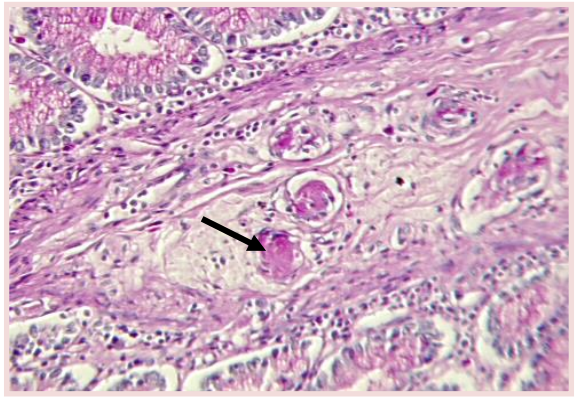
. Severe thrombocytopenia

. +/- multivisceral ischemia (brain, heart, kidney…)

. Severe functional deficiency of ADAMTS13 (activity < 10%), the specific VWF-cleaving protease

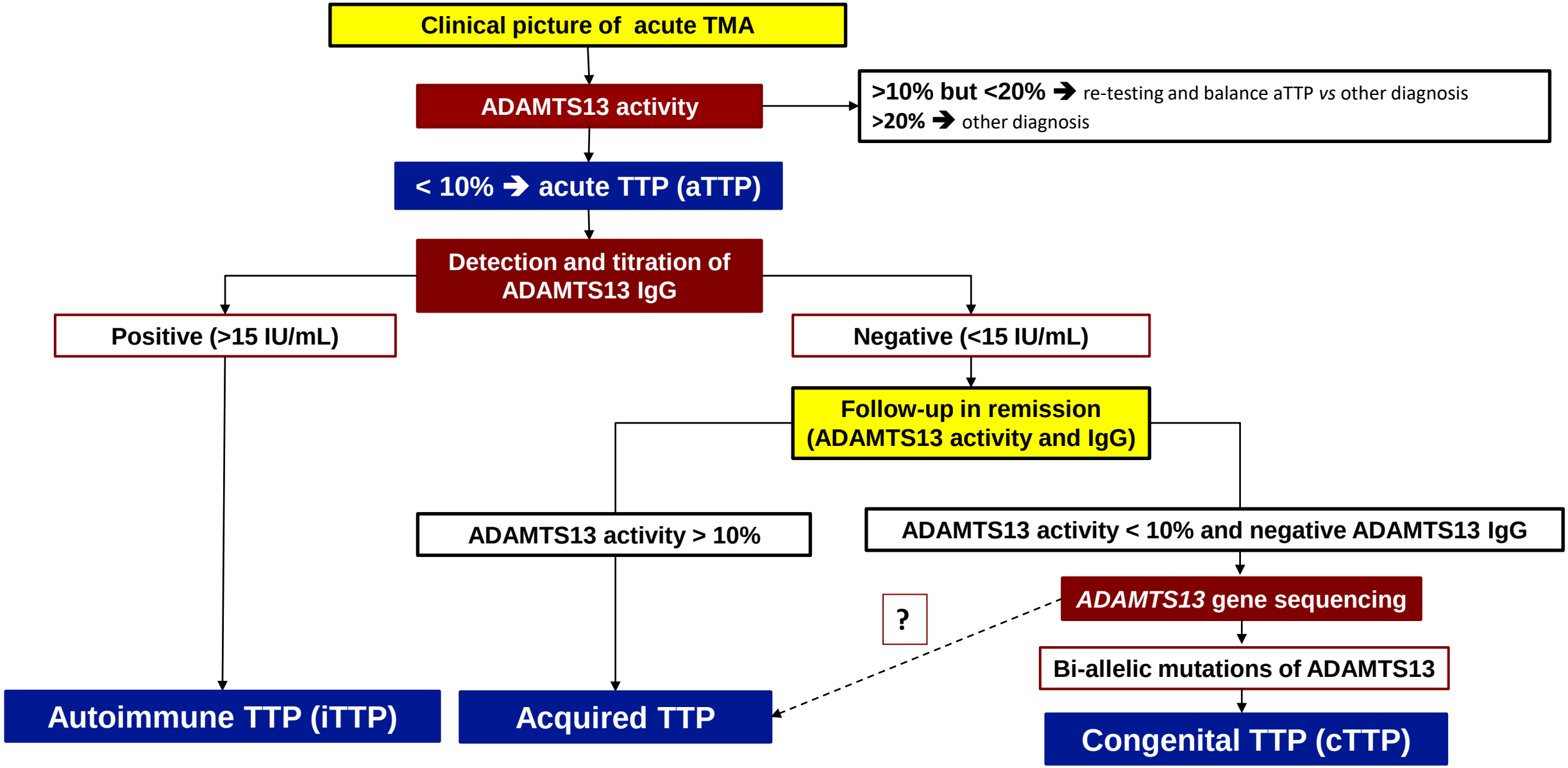
unique sensitive and specific biological marker able and mandatory to distinguish TTP from other TMAs and other diseases





Sadler JE. Hematology (Am Soc Educ Hematol Program) 2015 - Joly BS et al. Blood 2017; 129: 2836-46 - Zheng XL et al. J Thromb Haemost. 2020; 18:2486-95 - Mackie I et al. Int J Lab Hematol. 2020; 00:1-12.

Simplified flow-chart to identify TTP forms



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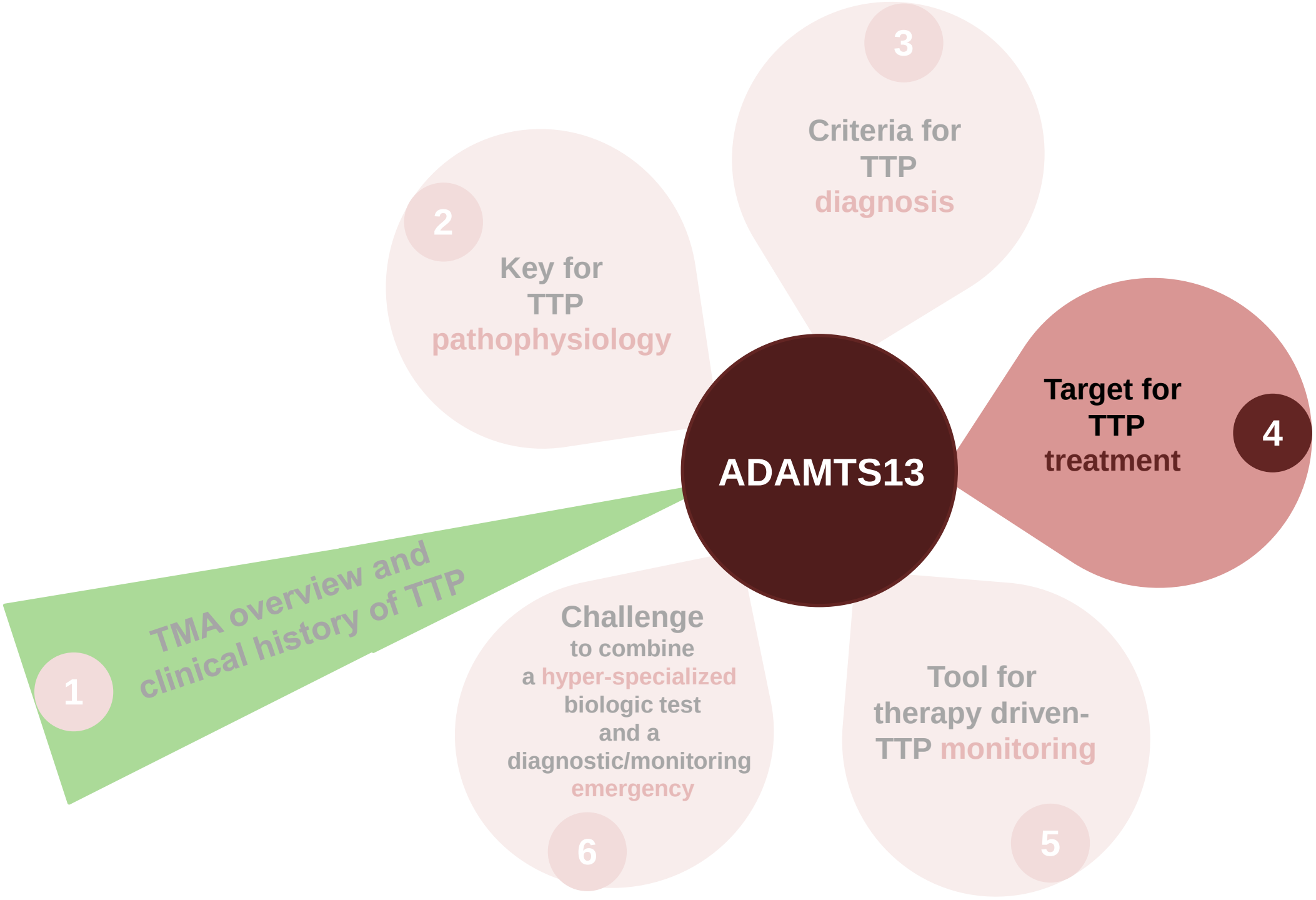
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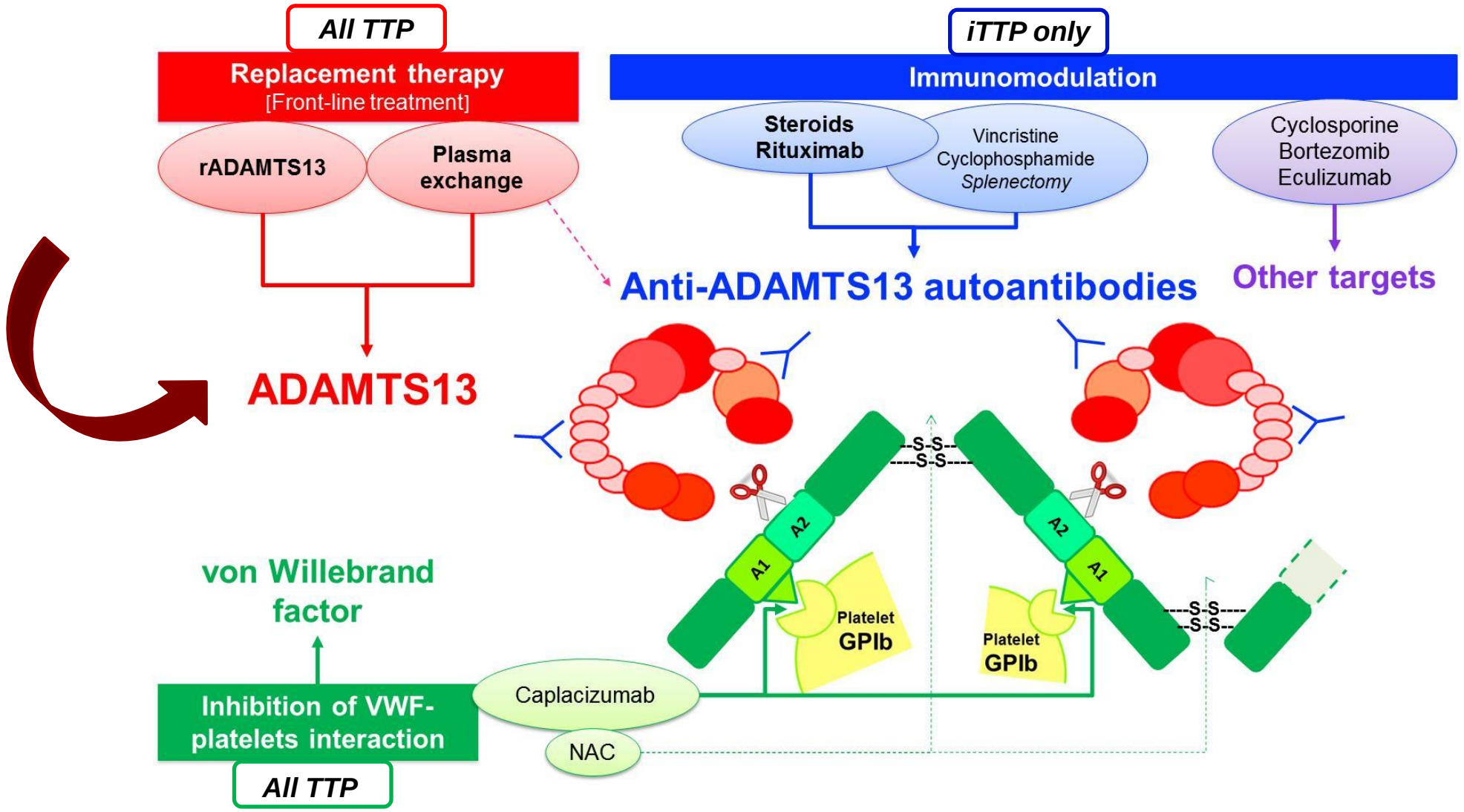
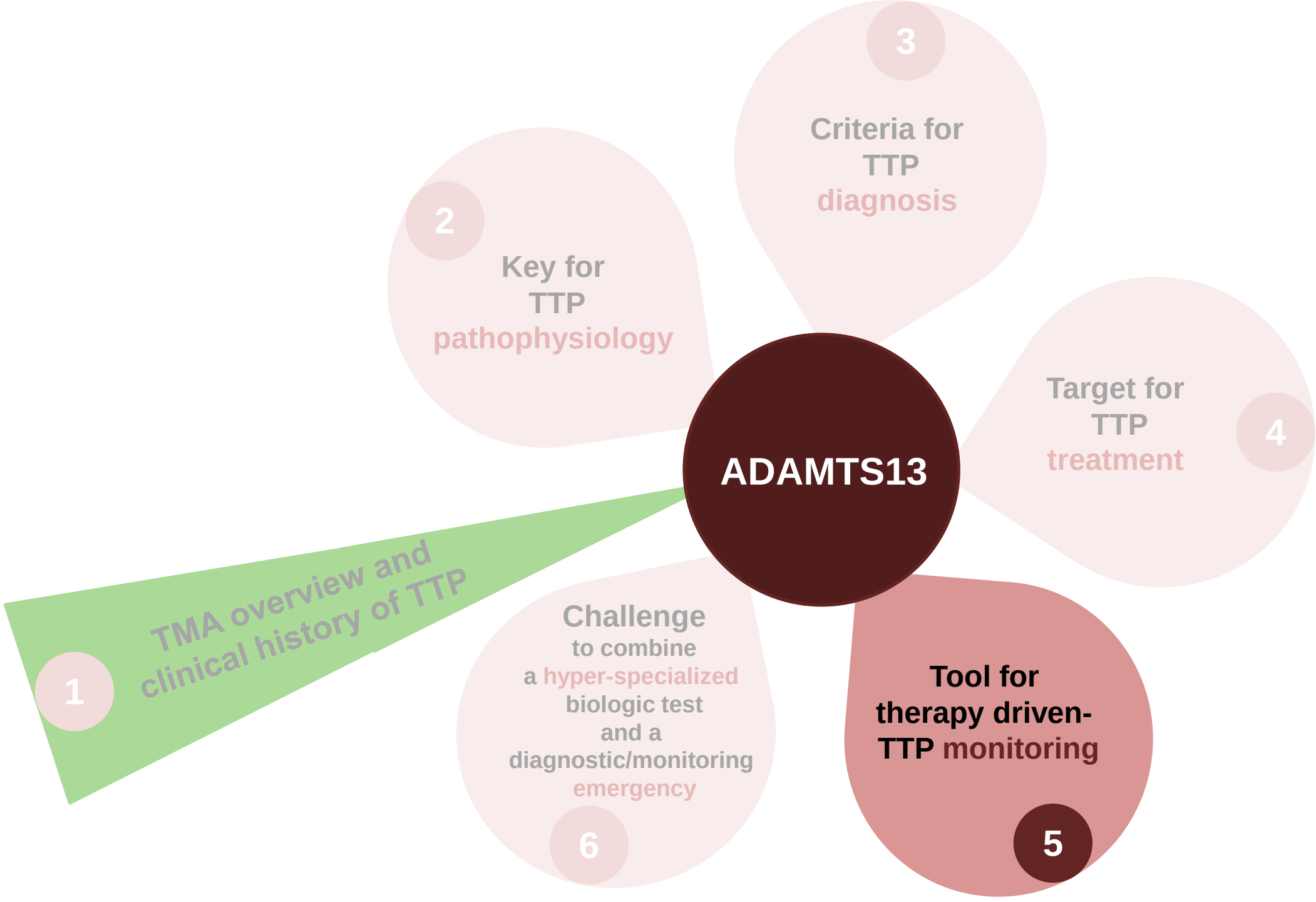
Therapeutic targets in TTP



Joly B et al, Int Care Med 2017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ADAMTS13 to optimize TTP management

Acute episode

Diagnosis

Before TPE, 1 blood sample collected at day 0

↓

ADAMTS13 <10%
➔ Confirmation of acute TTP diagnosis

Caplacizumab Monitoring [a]

One week after stopping TPE, 1 blood sample collected once a week

↓

ADAMTS13 >20%
➔ Stop caplacizumab ???

Clinical remission

Prediction of relapse

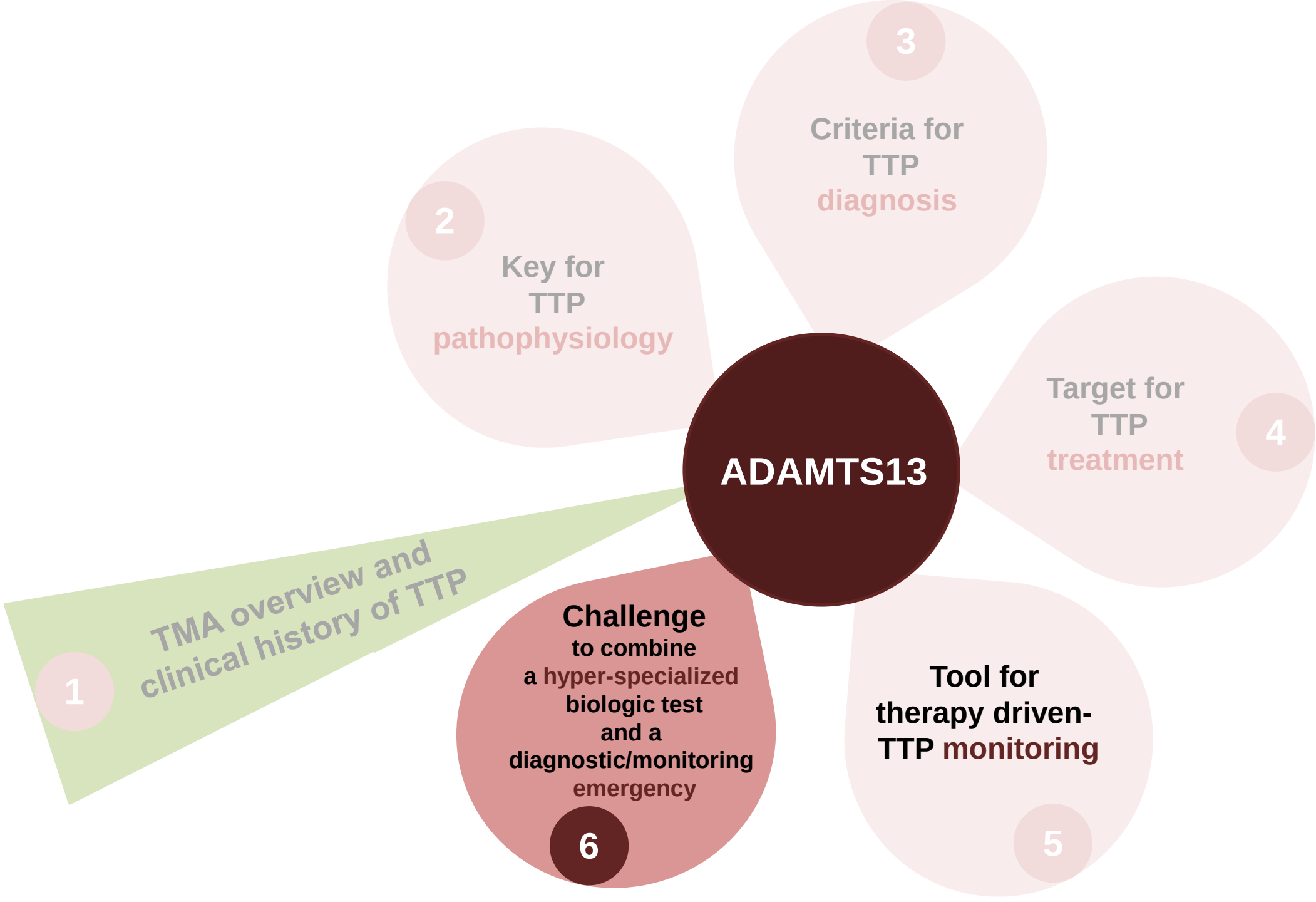
1 blood sample collected / trimester for 1 to 2 years

↓

ADAMTS13 <10-20%
➔ Preemptive rituximab

[a]There are no controlled data available to tailor treatment according to ADAMTS13 monitoring. According to the EU SmPC[4] and US PI5, caplacizumab should be administered once daily during PEX (first dose given as IV bolus prior to PEX followed by a daily SC dose after PEX) and once daily for 30 days after the last PEX. For patients with evidence of unresolved immunological disease, the US PI recommends extending treatment for a maximum of 28 days. The EU SmPC recommends optimizing the immunosuppression regimen and continuing daily caplacizumab administration until signs of underlying immunological disease are resolved.
ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; EU, European Union; PI, product information; SmPC, summary of product characteristics; TPE, therapeutic plasma exchange; TTP, thrombotic thrombocytopenic purpura; US, United States.

Zheng XL et al. ISTH guidelines for the diagnosis of TTP. J Thromb Haemost. 2020; 18:2486-95.
Mackie I et al. ICSH recommendations for laboratory measurement of ADAMTS13. Int J Lab Hematol. 2020; 00:1-12.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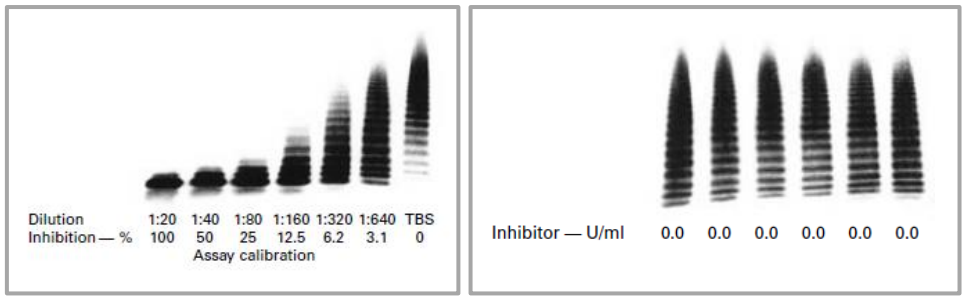
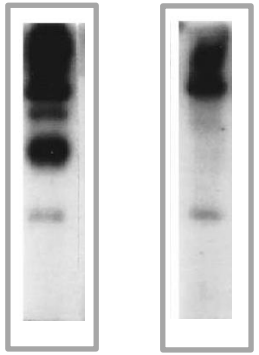
First-generation ADAMTS13 Activity assays: 1998–2005

- **Principle[1–5]:**
  - **Degradation of a full-length VWF substrate by ADAMTS13 present in the tested sample**
  - **Quantification of residual VWF by electrophoresis, IRMA, CBA ELISA**

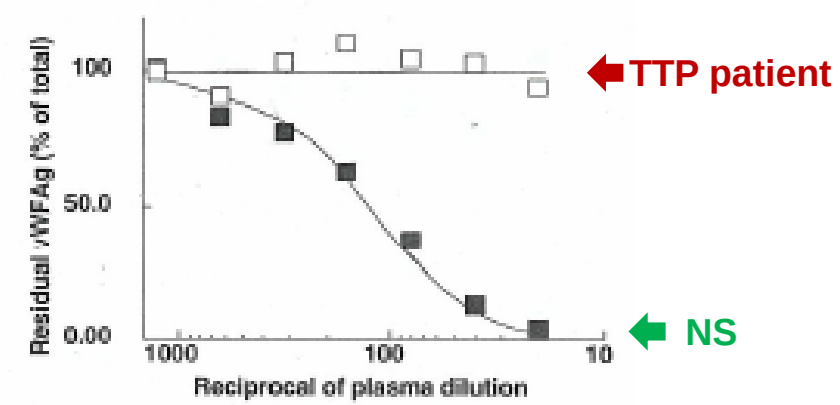
Activity of VWF-cleaving protease, calibrated against dilutions of normal plasma[1]



VWF-cleaving protease activity[2]



Hydrolysis of recombinant VWF[3]



- **Homemade assays developed and available only in research/highly specialized hospital labs**
- **Time-consuming, requires high technical skills, poor precision and very low turn-over**
  - ***Not adapted for clinical diagnosis***
- **Still available in TTP reference labs (~1/country) to further document some rare complex TTP cases**

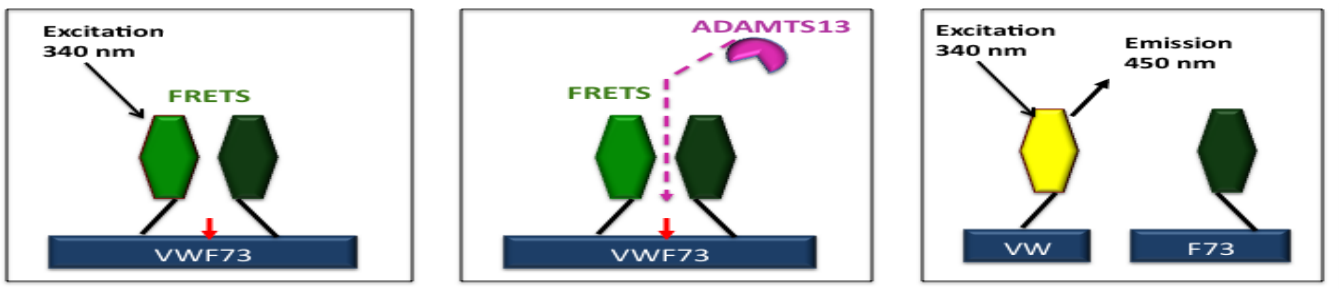
ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CBA ELISA, collagen binding enzyme-linked immunosorbent assay; IRMA, immunoradiometric assay; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

1. Furlan M, Robles R, Galbusera M, *et al.* von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998;339:1578-84.
2. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585-94.
3. Obert B, Tout H, Veyradier A, *et al*. Estimation of the von Willebrand factor-cleaving protease in plasma using monoclonal antibodies to vWF. *Thromb Haemost*. 1999;82:1382-85.
4. Gerritsen HE, Turecek PL, Schwarz HP, *et al.* Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding affinity of degraded vWF: a tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP). *Thromb Haemost*. 1999;82:1386-89.
5. Mackie I, Mancini I, Muia J, *et al.* International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of ADAMTS13. *Int J Lab Hematol*. 2020;00:1-12

Second-generation ADAMTS13 Activity assays: since 2005

- **Principle:**
  - **Degradation of a small VWF peptide substrate by ADAMTS13 present in the tested sample[1–3]**
  - **Quantification of residual VWF by FRET assay or chromogenic ELISA methods[1–4]**

- **Original and still *gold standard reference method since 2005 : FRETS-VWF73 assay*[1]**
  - **Homemade and requires technical skills -> performed only in TMA-expert accredited labs**
  - **Results available in half-a-day (usually 5 days/week and 8 hours/day)**



- **Commercial chromogenic ELISA kits, since ~2010[5,6,7]**
  - **Sensitivity for TTP diagnosis of ~90% *versus* FRETS-VWF73 reference [7]**
  - **No requirement for technical skill but need for biologic expertise +++ [7]**
  - **Results available in one day (usually 5 days/week and 8 hours/day) [7]**

Image courtesy of Agnes Veyradier.

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; FRET, fluorescence resonance energy transfer; ELISA, collagen binding enzyme-linked immunosorbent assay; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

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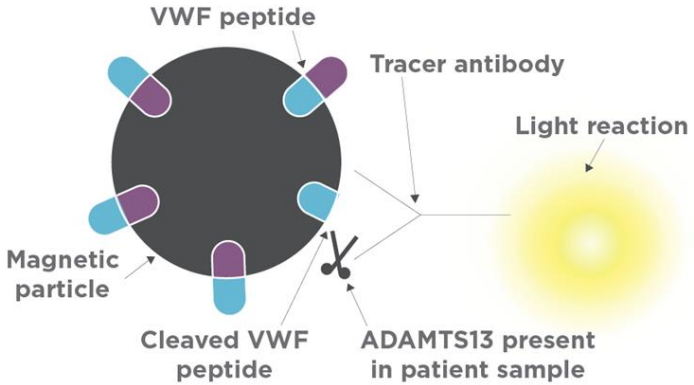
Third-generation ADAMTS13 Activity assays: since 2019

Principle[1]

- **Degradation of a small VWF peptide substrate by ADAMTS13 present in the tested sample**
- **Automated chemiluminescence assay (CLIA) [specific couple automate/reagent]**



. HemosIL® Acustar ADAMTS13 Activity Assay* (Werfen) run on ACL AcuStar® Hemostasis testing system
.Two-step immunoassay
. Magnetic particles coated with a GST-VWF73 peptide substrate
. Chemiluninescent detection based on an isoluminol-labelled monoclonal antibody that reacts with the cleaved peptide

- **First automated rapid turnover assay, very recently available in selected markets**
- **Sensitivity for TTP diagnosis of ~90-95% *vs* FRETS-VWF73 or commercial reference *(6 published studies, 1,851 miscellaneous samples)*[1–6]**
- **No requirement for technical skill but need for biologic expertise +++ [1-6]**
- **Results available in 35 min after control check/calibration[1-6]**
- **Results potentially available 24 hours/day and 7 days/week, depending on local hospital [1-6]**

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; FRET, fluorescence resonance energy transfer; ELISA, collagen binding enzyme-linked immunosorbent assay; GST, glutathione S-transferase; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

*Note: Assay not FDA marketing authorized or Canadian licensed. Not saleable in the US or Canada.

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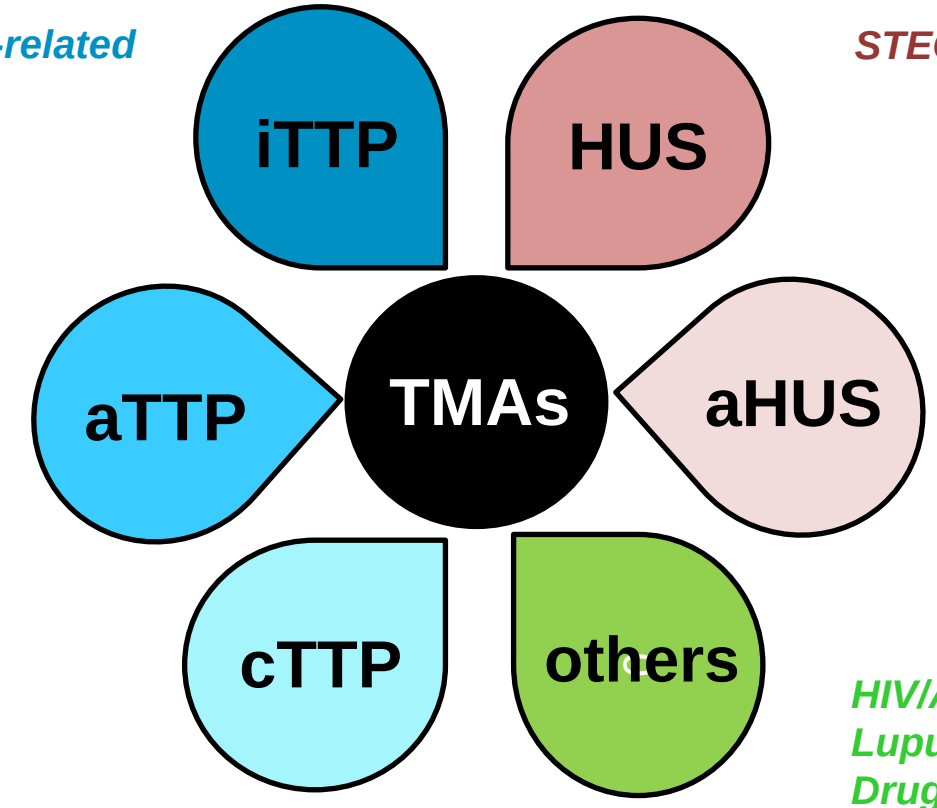
Pathophysiology-based classification of TMA syndromes

ADAMTS13 autoantibodies-related
Immune TTP

Non immune ADAMTS13 deficiency-related
Acquired TTP

ADAMTS13 mutations related-
Congenital TTP



STEC+ related
HUS

Complement-related
Atypical HUS

HIV/AIDS
Lupus, other CTD
Drugs
Cancers, HELLP Sd
Malignant AHT, CAPS

TTP: Thrombotic Thrombocytopenic Purpura; HUS: Hemolytic Uremic Syndrome

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