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Roma 23 - 25 Settembre 2010

Attualità nel trattamento delle Microangiopatie Trombotiche

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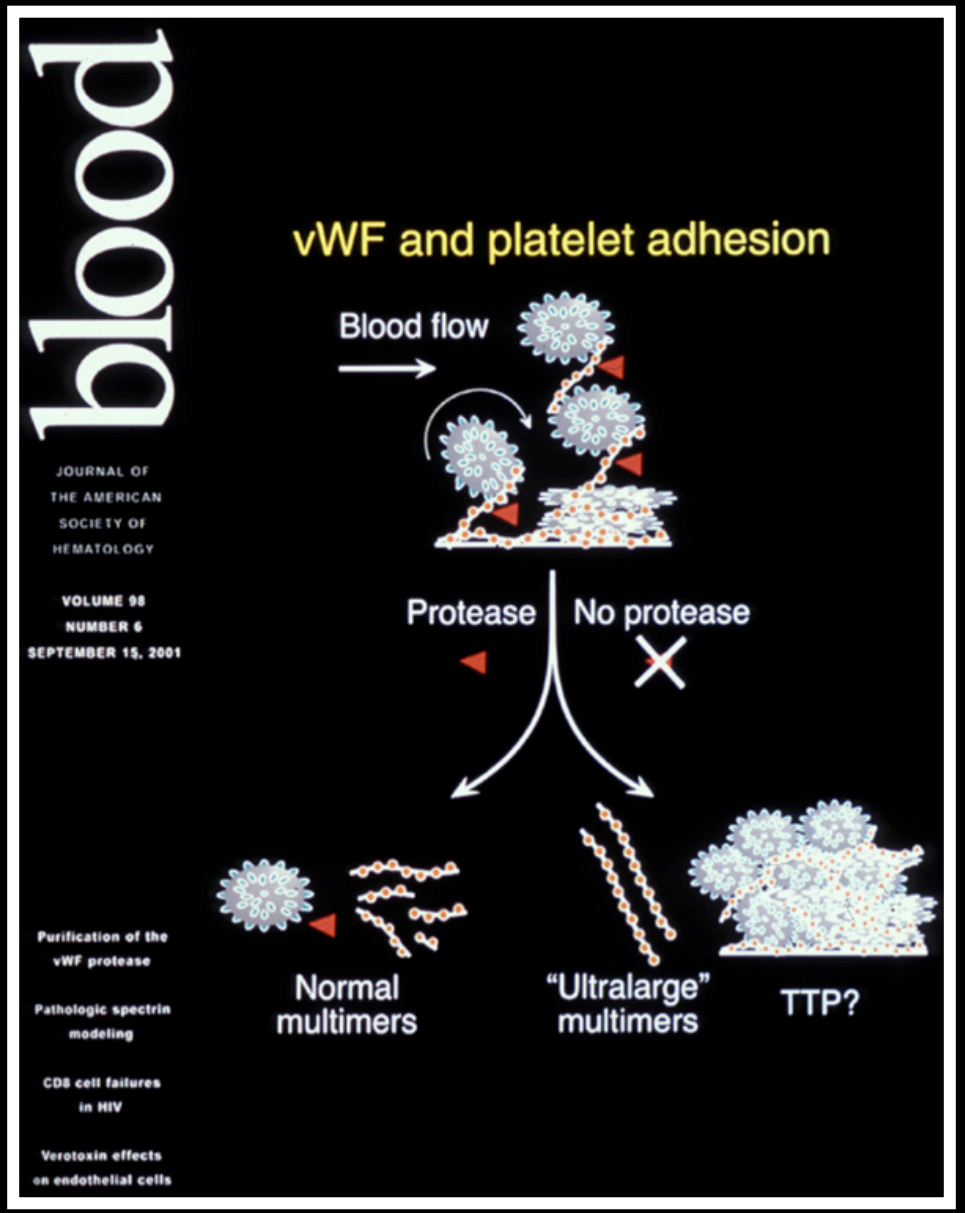
blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY
VOLUME 98
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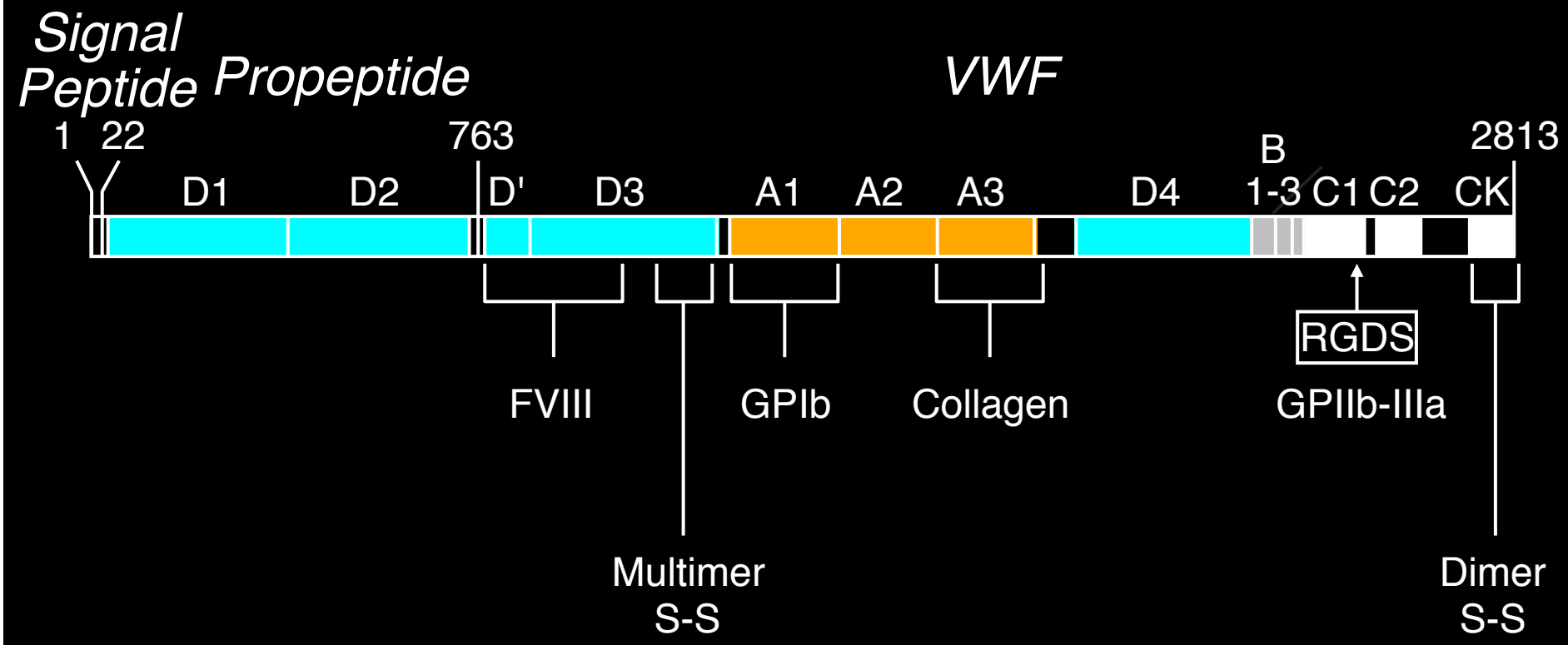
SEPTEMBER 15, 2001
Purification of the vWF protease
Pathologic spectrin modeling
CD8 cell failures in HIV
Verotoxin effects on endothelial cells
vWF and platelet adhesion
Blood flow
Protease
No protease
Normal multimers
"Ultralarge" multimers
TTP?

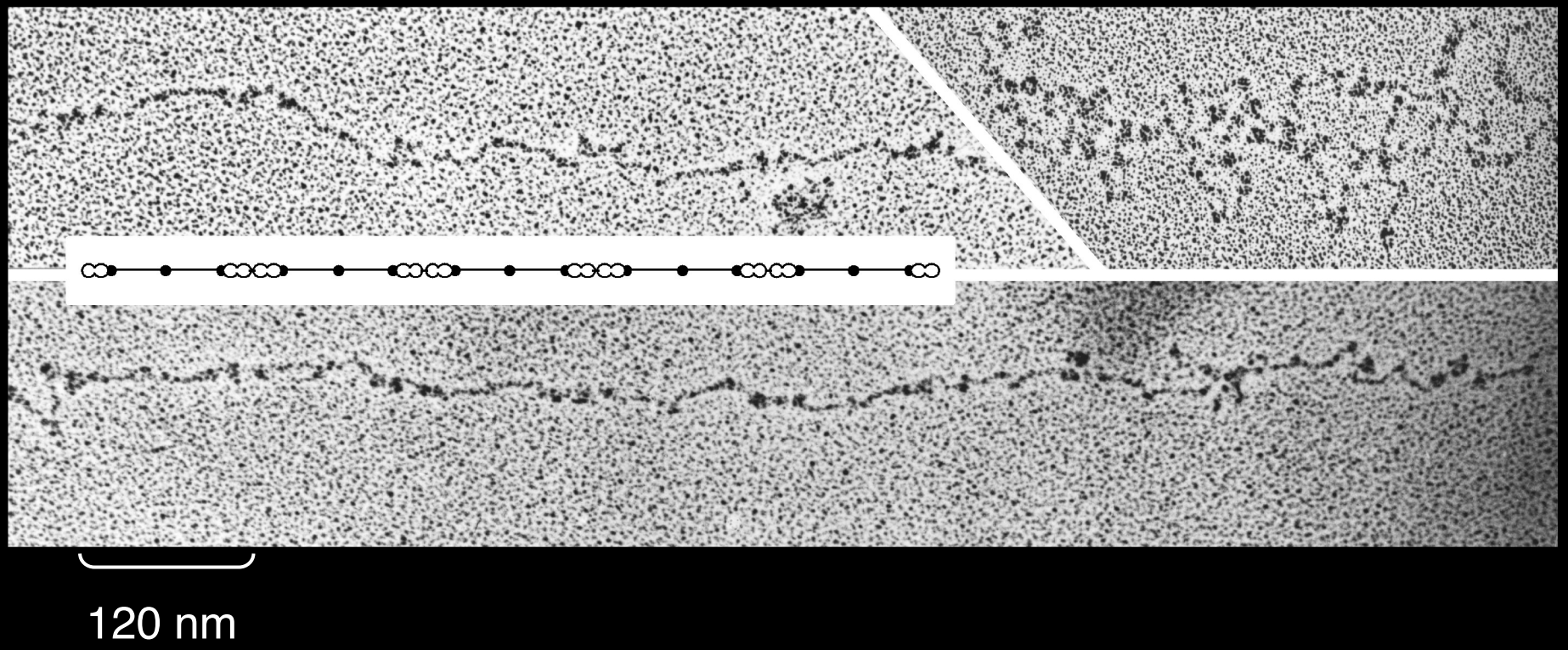
VWF and TTP

- VWF, ADAMTS13, platelet adhesion
- Pathophysiology, diagnosis, treatment of thrombotic microangiopathy
- Clinical Research Questions

The Von Willebrand Factor Precursor



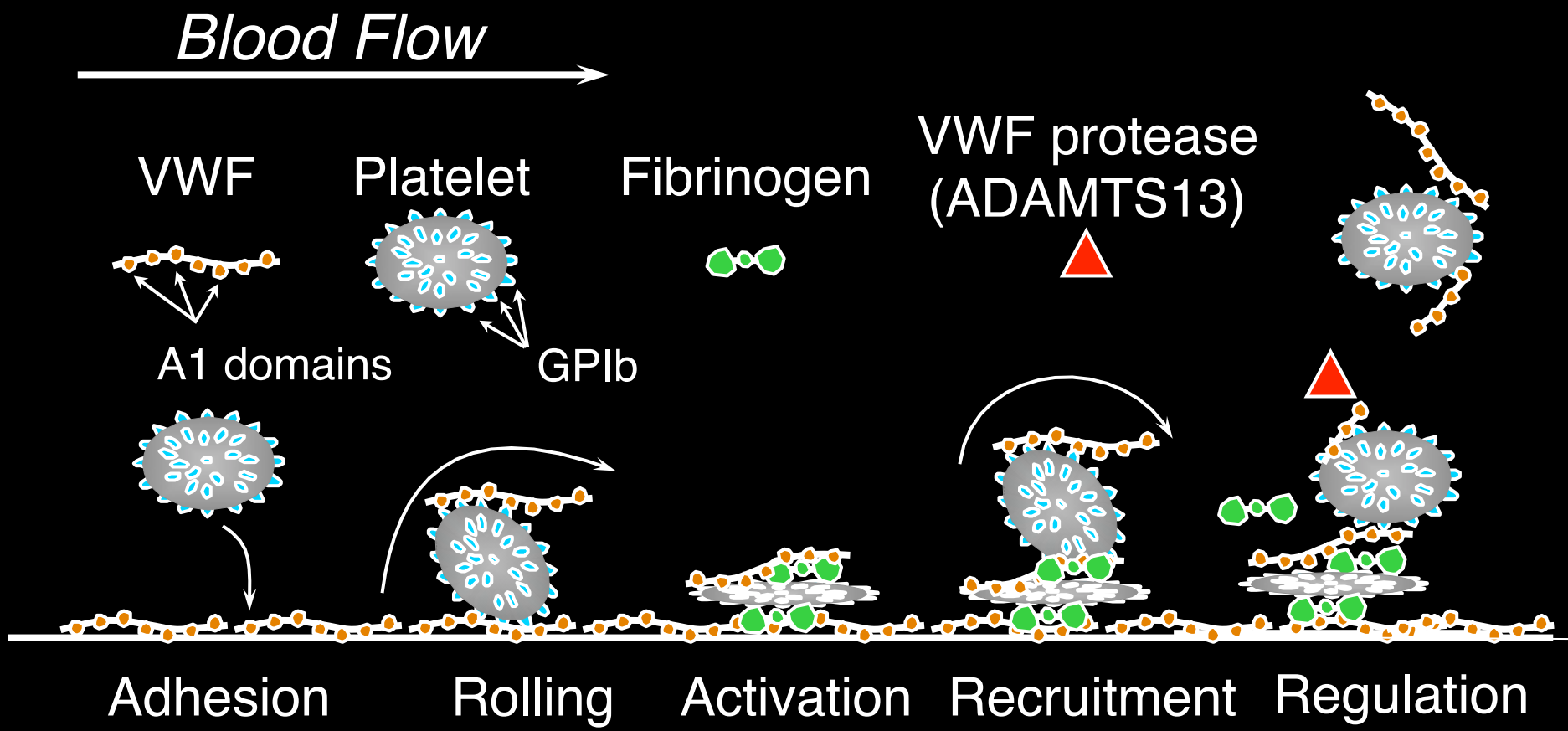
von Willebrand Factor Multimers



Adapted from Fowler et al, *J Clin Invest* **76:**1491-1500, 1985

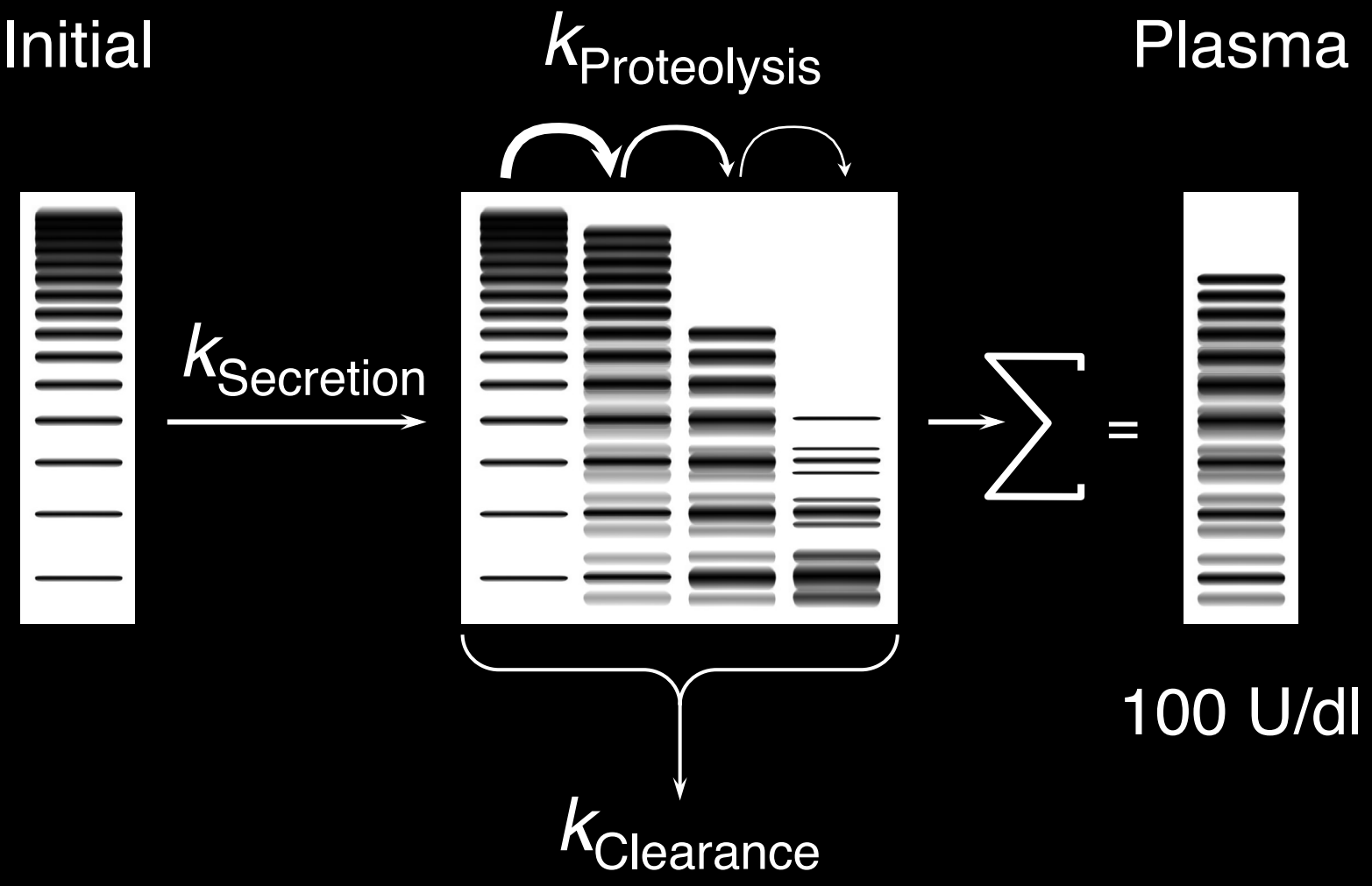
VWF and Factor VIII Survival

VWF, ADAMTS13 and Platelet Adhesion



Assembly and Catabolism of VWF

Normal Steady-State



Thrombotic Thrombocytopenic Purpura

History:

Dr. Eli Moschcowitz – 1924, NYC
(*Arch Intern Med* 31:89, 1925)

- 16 yo girl with weakness, joint pain, 40°C fever, hemolytic anemia, WBC, no platelet count; 4 days paresis (L), coma, death
- "Acute febrile pleiochromic anemia associated with widespread microvascular occlusive thrombi"

History

1923 Eli Moschcowitz described "acute febrile pleiochromic anemia"
– autopsy showed hyaline microthrombi

1936 Baehr found microthrombi composed of fibrin and platelets, the pathognomonic lesion

1947 Singer named "TTP" to emphasize these lesions

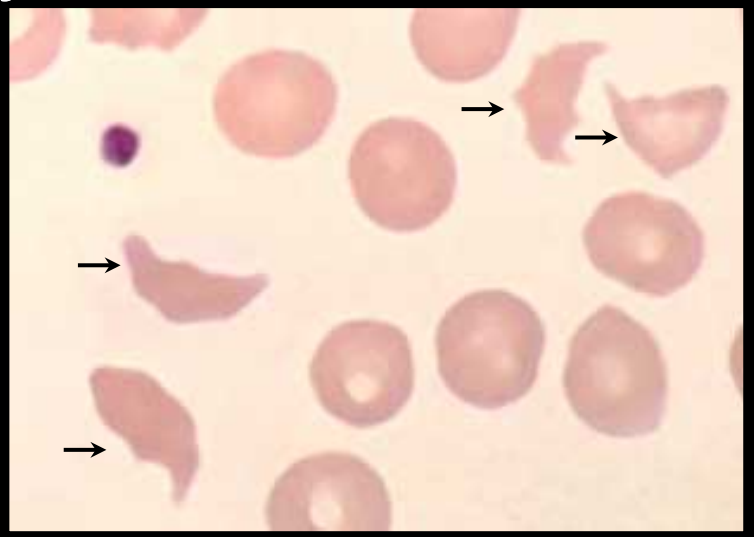
1957 Named "Moschcowitz's disease" by Bernheim

Thrombotic Thrombocytopenic Purpura

A Disorder of VWF Proteolysis?

A classic pentad of signs:

- Microangiopathic hemolytic anemia
- Thrombocytopenia
- Neurologic dysfunction
- Renal failure
- Fever



Incidence 4/million/year

Often strikes young adults, mainly females

Untreated, mortality >90%

Treated with plasmapheresis, mortality <20%

Definitions

Thrombotic microangiopathy:

- Microangiopathic hemolytic anemia (Coombs' negative)
- Thrombocytopenia
- Variable pattern of tissue injury from microvascular thrombosis

Definitions

Idiopathic TTP:

- Thrombotic microangiopathy
- Without a predisposing condition
- Without oliguric renal insufficiency at presentation

Definitions

Secondary TTP:

- Thrombotic microangiopathy
- With a predisposing condition (e.g., cancer, sepsis, malignant hypertension, marrow or organ transplantation, HELLP, eclampsia, cyclosporine, tacrolimus, other drugs)

Definitions

Hemolytic Uremic Syndrome (HUS):

- Thrombotic microangiopathy
- With acute oliguric or anuric renal failure
- Relative sparing of other organ systems

Definitions

Shiga toxin-associated HUS:

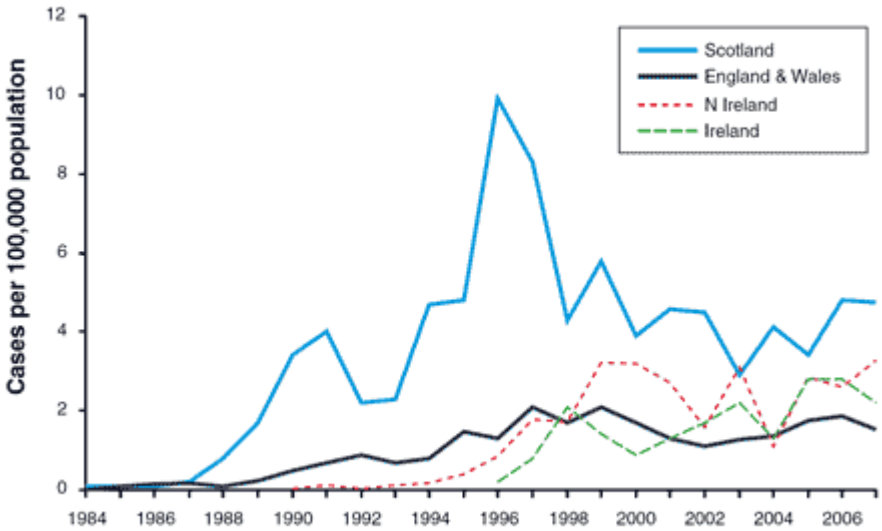
- Shiga toxin-producing *E. coli*
- Preceded by painful bloody diarrhea
- Usually a childhood disease

Atypical HUS:

- No diarrheal prodrome
- Complement regulatory defects in some

Enhanced surveillance of thrombotic microangiopathies in Scotland, 2003-2008

FIGURE 1: *E. coli* O157 cases: rates per 100,000 population, 1984-2007



Data from outwith Scotland courtesy of Health Protection Agency Belfast & London and Health Protection Surveillance Centre Dublin (2007 figures are provisional)

TABLE 1: Predisposing factors in development of haemolytic uraemic syndrome (HUS) or thrombotic thrombocytopaenic purpura (TTP) – χ^2 analyses performed on predisposing factors and development of thrombotic microangiopathy

Predisposing factor	HUS (cases)	TTP (cases)
Infection with verocytotoxin-producing O157 *E. coli*	133	1
Infection with verocytotoxin-producing non-O157 *E. coli*	4	0
Diarrhoea and vomiting (no pathogen identified)	8	0
Pre-existing renal disease	4	7
Relapsing disease	2	3
Immuno-suppression	2	7
Pregnancy	0	2
Severe sepsis	0	12
Collagen/vascular disorders	2	12
Malignancy	1	2
Other	9	11
Total number of cases	**165**	**57**

Idiopathic TTP – Demographics

Gender (Female/Male)[a,c]	≈2/1
Age (peak decade)[a]	35-45
African ancestry (Odds Ratio)[b]	≈2
BMI ≥ 30 kg/m^2 (Odds Ratio)[b]	≈4
Annual incidence[c]	≈4.5 per million

[a] Rock et al, *New Eng J Med* 1991; **325:** 393-397

[b] George et al, *Semin Hematol* 2004; **41:** 60-67

[c] Terrell et al, *J Thromb Haemost* 2005; **3:** 1432-1436

Idiopathic TTP – Evolution

Feature	1925-1964[a]	1964-1980[b]	1982-1989[c]
		Percent	
Microangiopathic hemolytic anemia	96	98	100
Low Platelets	96	96	100
Neurological Signs	92	84	63
Renal Abnormalities	88	76	59
Fever	98	59	24

[a] Amorosi & Ultmann, 1966 (271 patients)

[b] Ridolfi & Bell, 1981 (258 patients)

[c] Rock et al, 1991 (102 patients)

Idiopathic TTP – Laboratory

Variable	Mean ± SD	Normal Range
Platelet count (x 10^9/L)	22 ± 13	150-400
Hemoglobin (g/dL)	8.8 ± 1.9	12-17.5
Creatinine (μM)	124 ± 103	40-110
LDH (U/L)	1400 ± 900	100-225
PT (sec)	11.6 ± 1.1	10.5-12.5
aPTT (sec)	30.3 ± 10.3	20-35

Rock et al, *New Eng J Med* 1991; **325:** 393-397

Idiopathic TTP – Clinical Course

20% dead within with 5 weeks

80% complete response in average of 16 days (range, 3-36)

40% of responders have exacerbations within one week

30% of responders relapse within 2 years

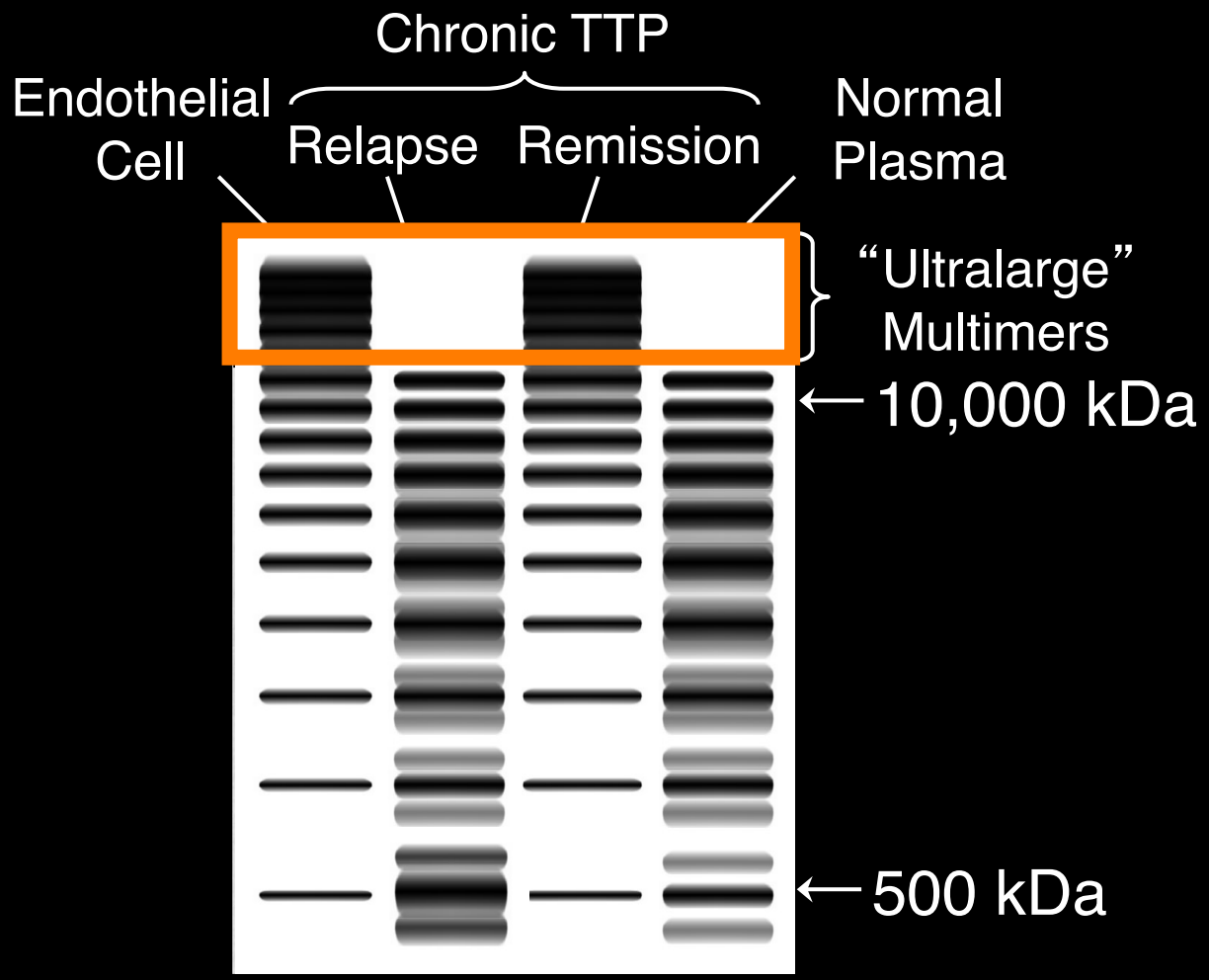
Rock et al, *New Eng J Med* 1991; **325:** 393-397

Idiopathic TTP – Pathophysiology

Why does plasma exchange work at all?

- Removes a toxin or antibody?
- Replenishes a missing factor?

The VWF Hypothesis



Failure to cleave ultralarge VWF causes TTP?

Moake et al, *N Engl J Med* 1982; **307:** 1432-1435

VWF Cleaving Protease in Plasma

1982: Predicted by Moake

1996: Discovered by Tsai and Furlan

1997: Absent in familial TTP

1998: Absent in most idiopathic TTP (because of acquired autoantibody inhibitors)

2001: Cloned, mutated in familial TTP

What's the deal with ADAMTS 13?

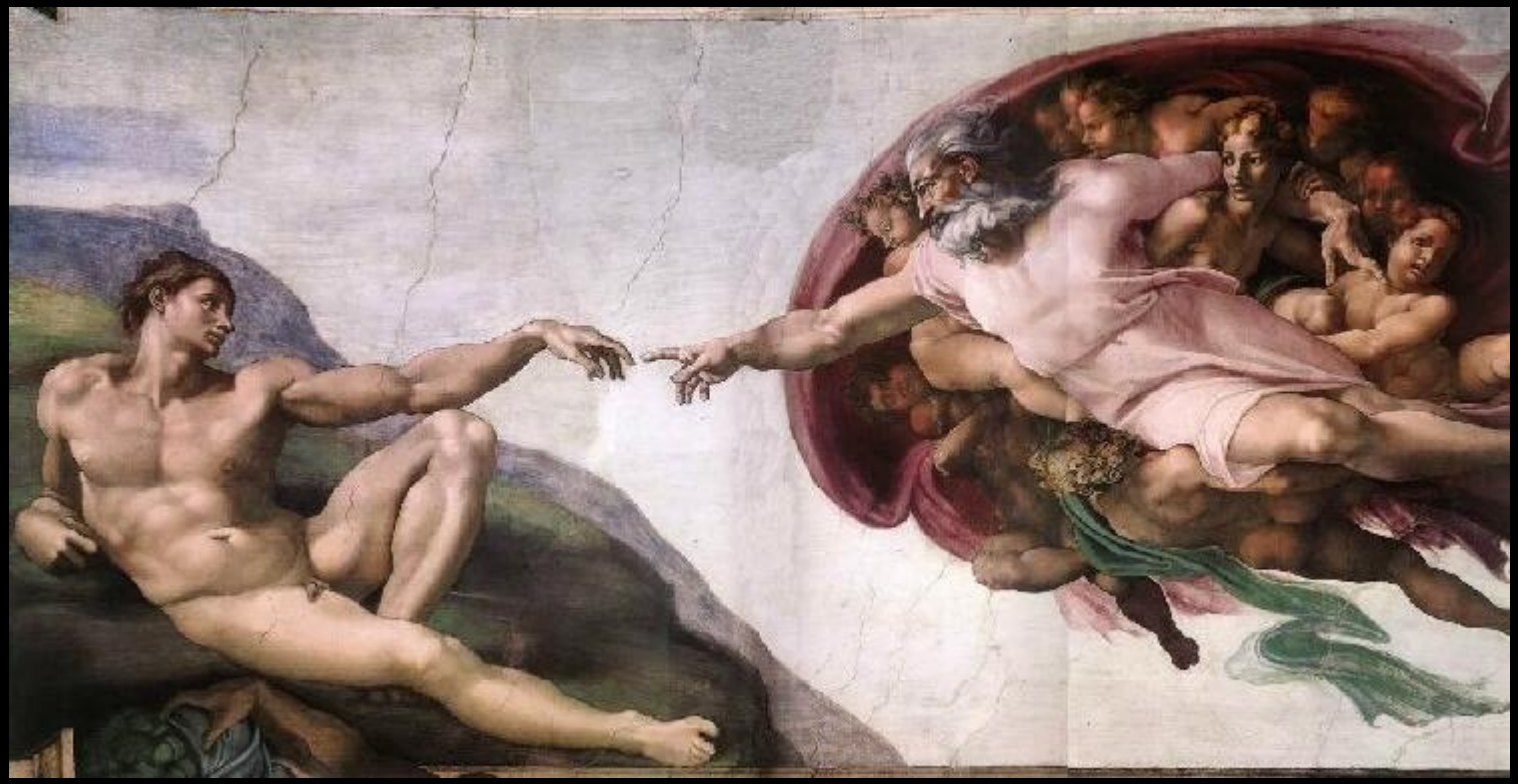
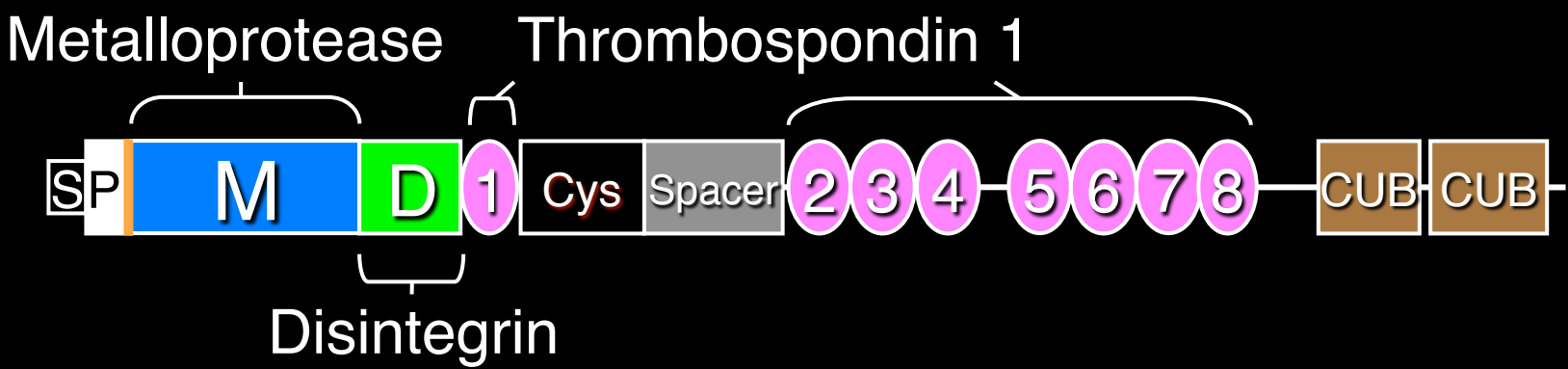


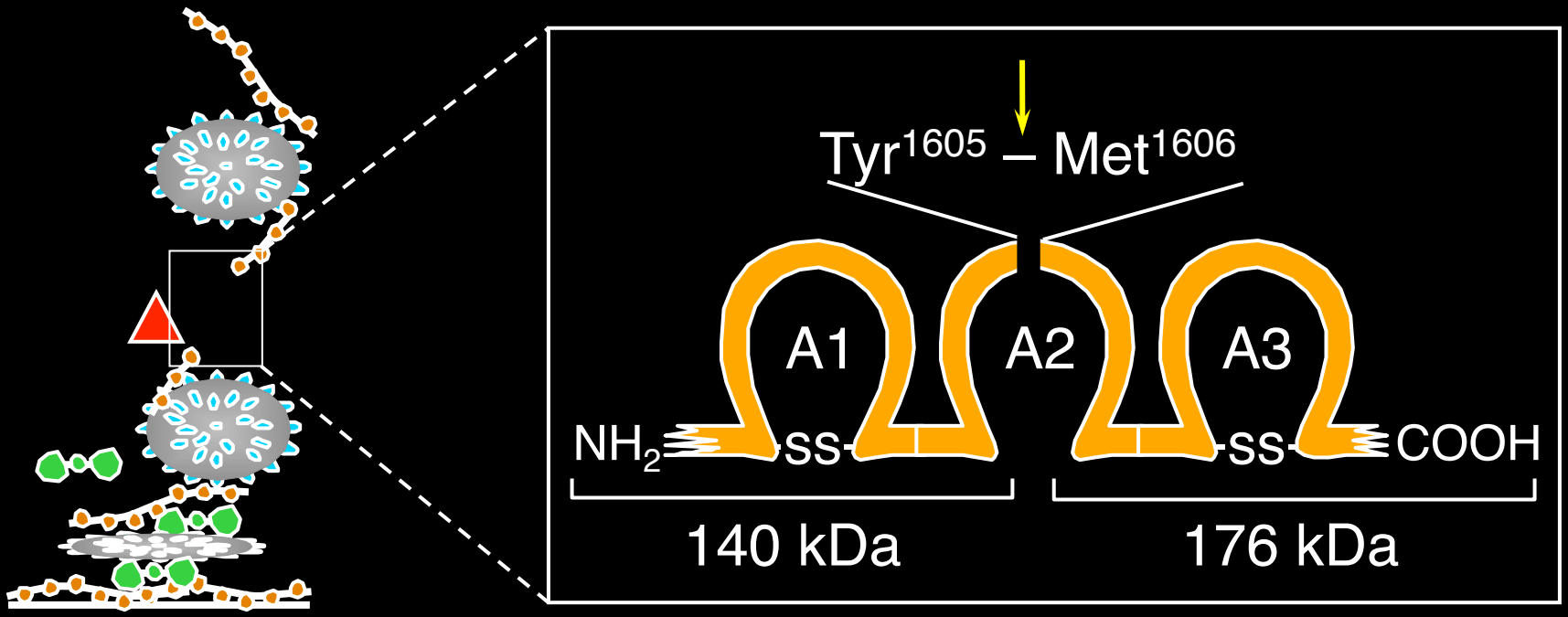
Image of Adam (looking fairly inactive at the moment): Michelangelo, Sistine Chapel.

VWF Cleaving Protease (ADAMTS13)



A *D*isintegrin-like *A*nd *M*etalloprotease
with *T*hrombo*S*pondin-1 repeats

Shear and VWF Proteolysis

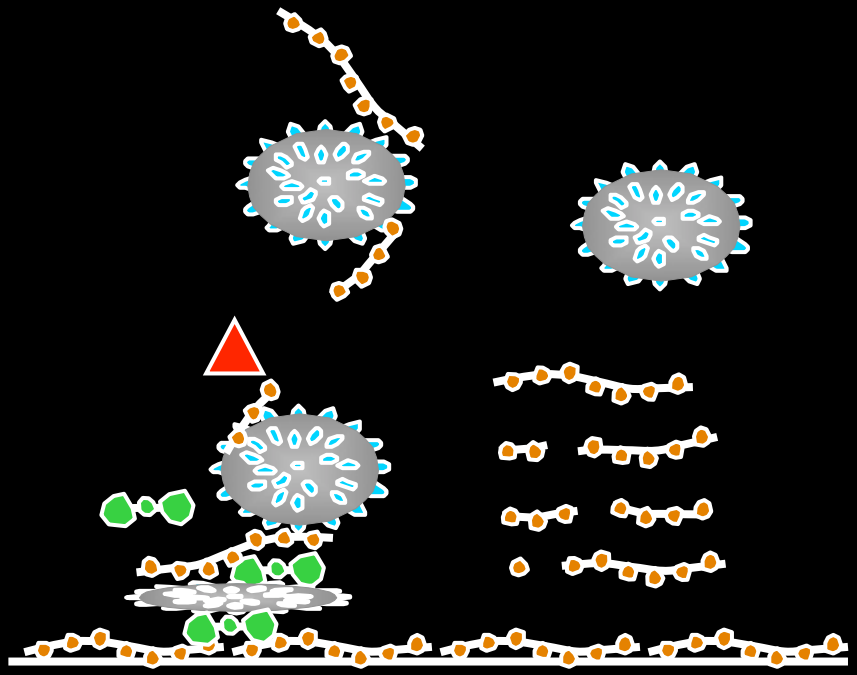


Proteolysis increased by:

- Shear stress (aortic stenosis)
- VWD type 2A mutations
- Denaturants (urea, guanidine)

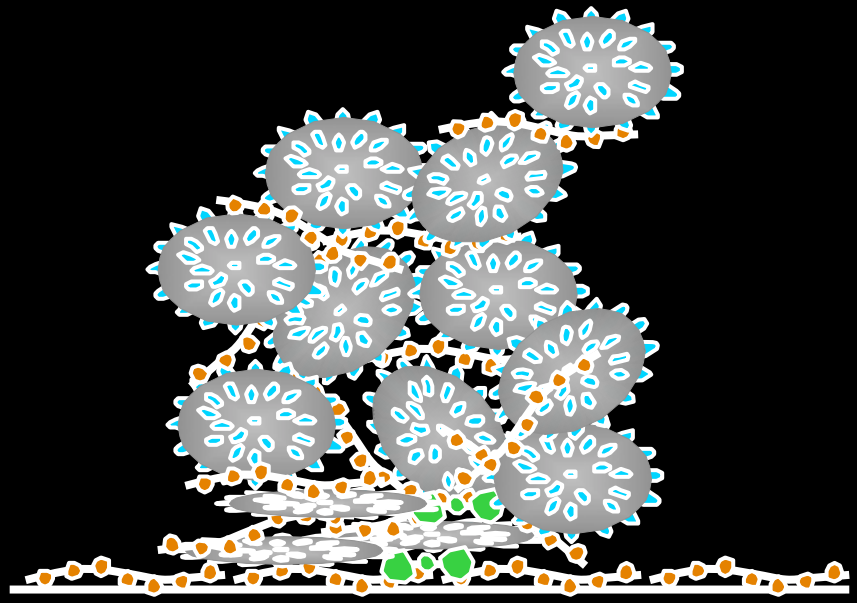
VWF, ADAMTS13 and Platelet Adhesion

With ADAMTS13



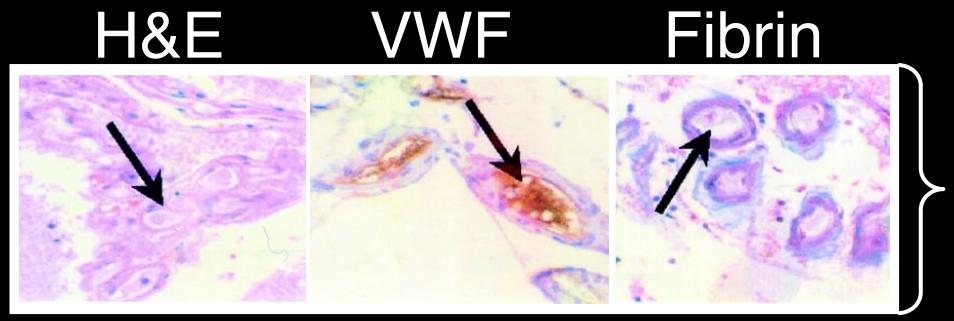
Normal VWF Multimers
Normal Hemostasis

Without ADAMTS13



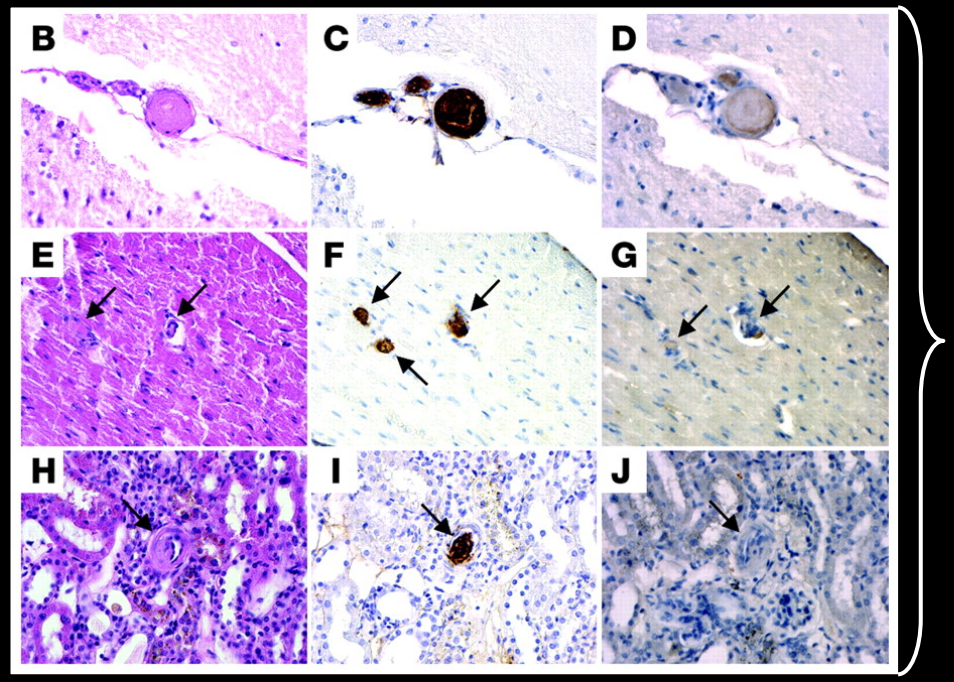
Ultralarge VWF Multimers
Microvascular Thrombosis
(TTP)

TTP Histopathology



Human TTP

Tsai et al, *Pediatr Res* 2001; **49:** 653-659



ADAMTS13 Knockout Mouse

Motto et al, *J Clin Invest* 2005; **115:** 2752-2761

Familial TTP

Upshaw-Schulman Syndrome

Clinical features:

- Autosomal recessive, rare
- Neonatal jaundice
- Some have a relapsing course from infancy
- Others present as young adults
- Precipitated by infection, pregnancy or stress
- Chronic renal failure is uncommon

Familial TTP
Upshaw-Schulman Syndrome

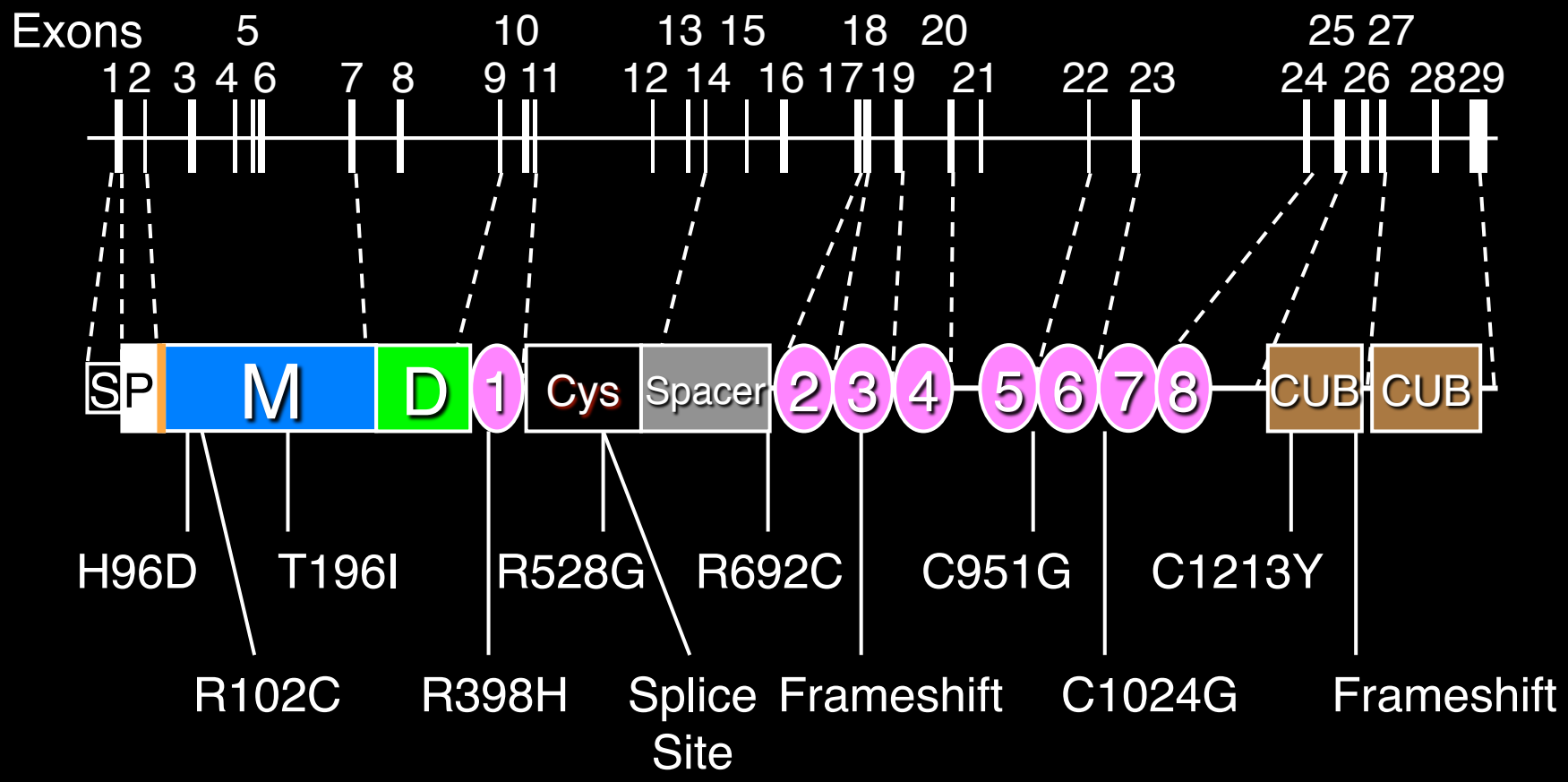
Laboratory Studies:

- Microangiopathic hemolytic anemia
- Thrombocytopenia
- Mild renal insufficiency, microhematuria

Treatment and Prognosis:

- Plasma 20-40 ml/kg every 2-4 wks, or less often
- ADAMTS13 plasma half life is 2-3 days
- 5% of normal ADAMTS13 level prevents disease

ADAMTS13 Mutations in Familial TTP

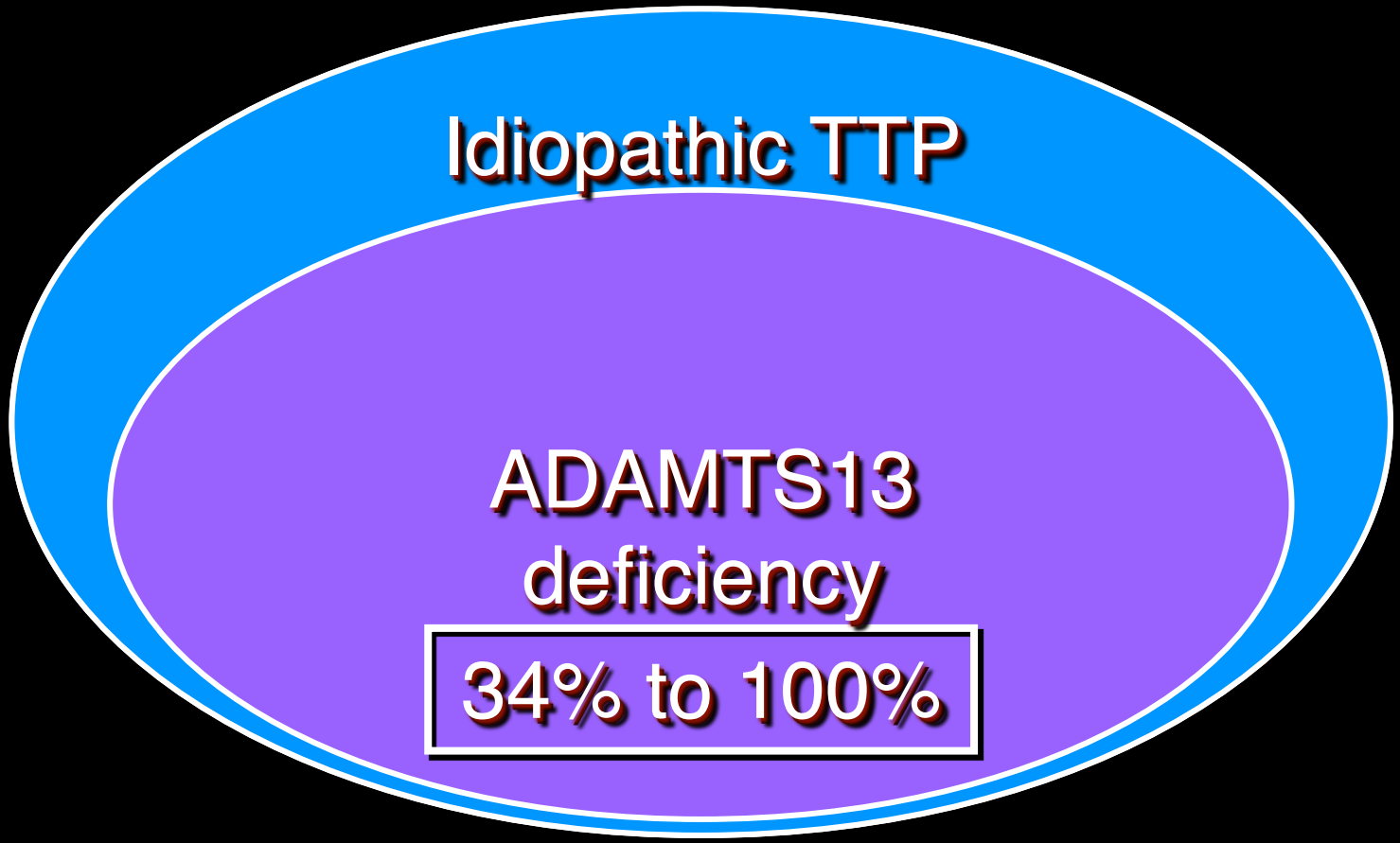


7 families, 12 mutations

Levy et al, *Nature* 2001; **413:** 488-494

Autoimmune Idiopathic TTP

Acquired ADAMTS13 Deficiency



Autoimmune Idiopathic TTP

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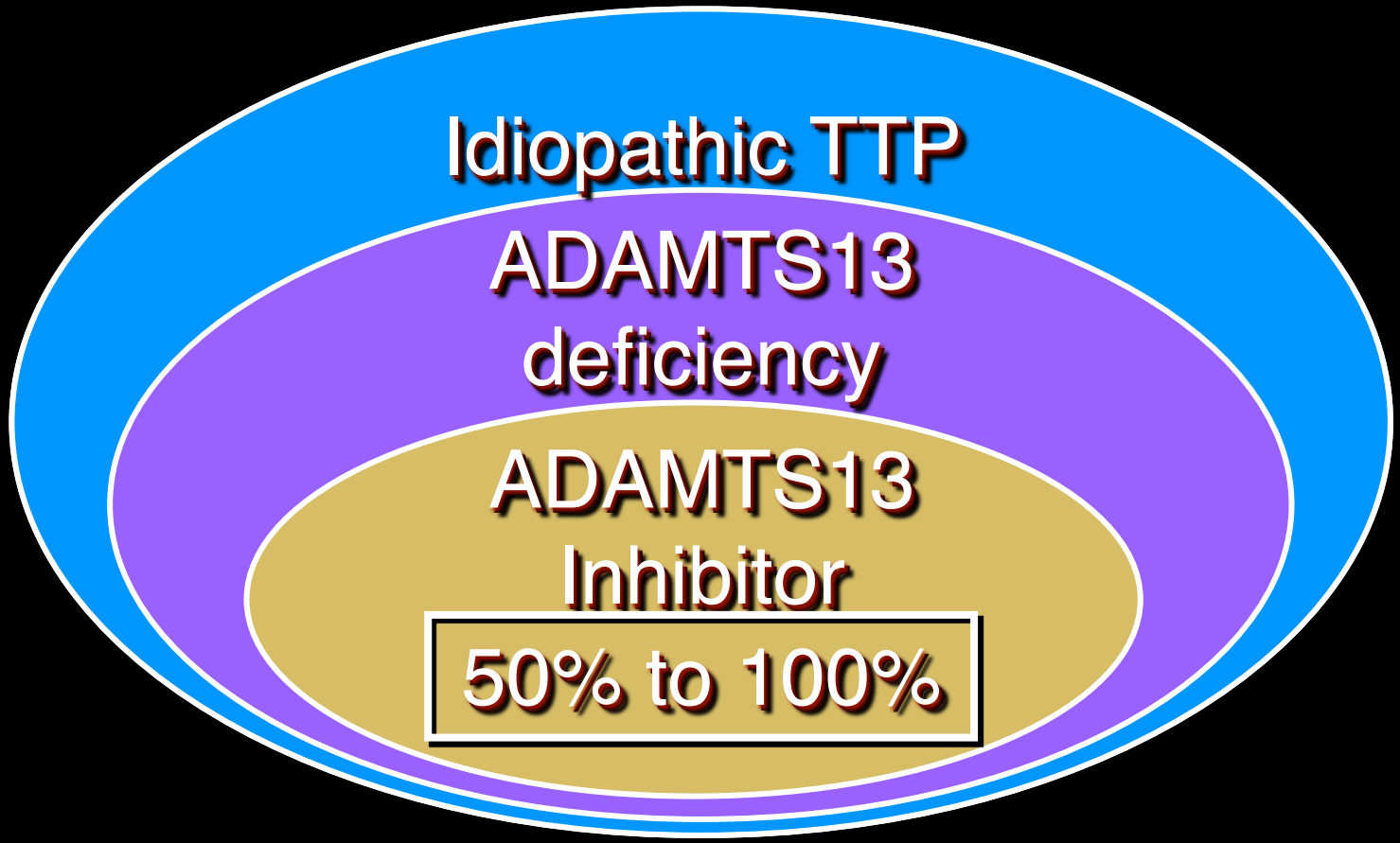
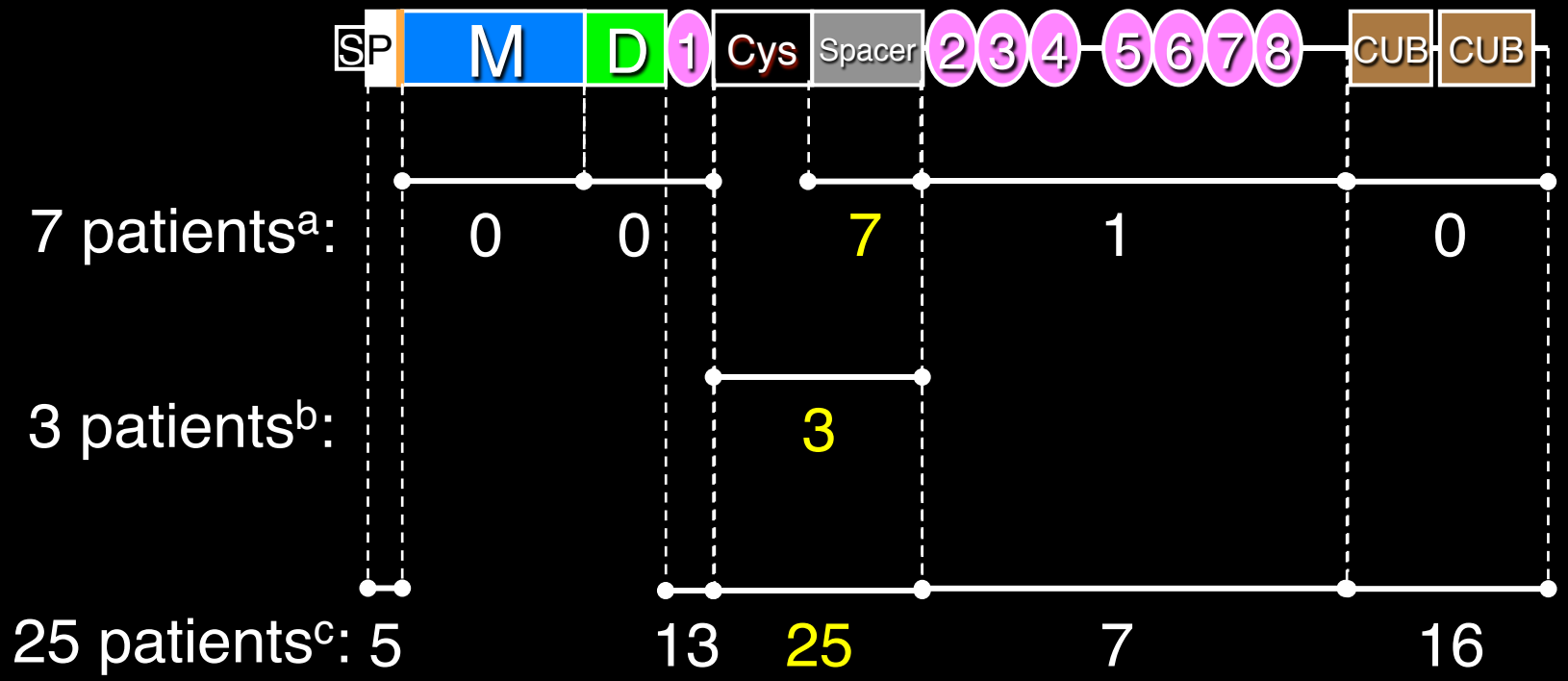


ADAMTS13 Inhibitors in TTP

Epitopes Map to Cys-Rich + Spacer



[a] Luken et al, *Thromb Haemost* 2005; **93:** 267-274

[b] Soejima et al, *Blood* 2003; **102:** 3232-3237

[c] Klaus et al, *Blood* 2004; **103:** 4514-4519

ADAMTS13 Deficient, With Inhibitor

Response to Plasma Exchange

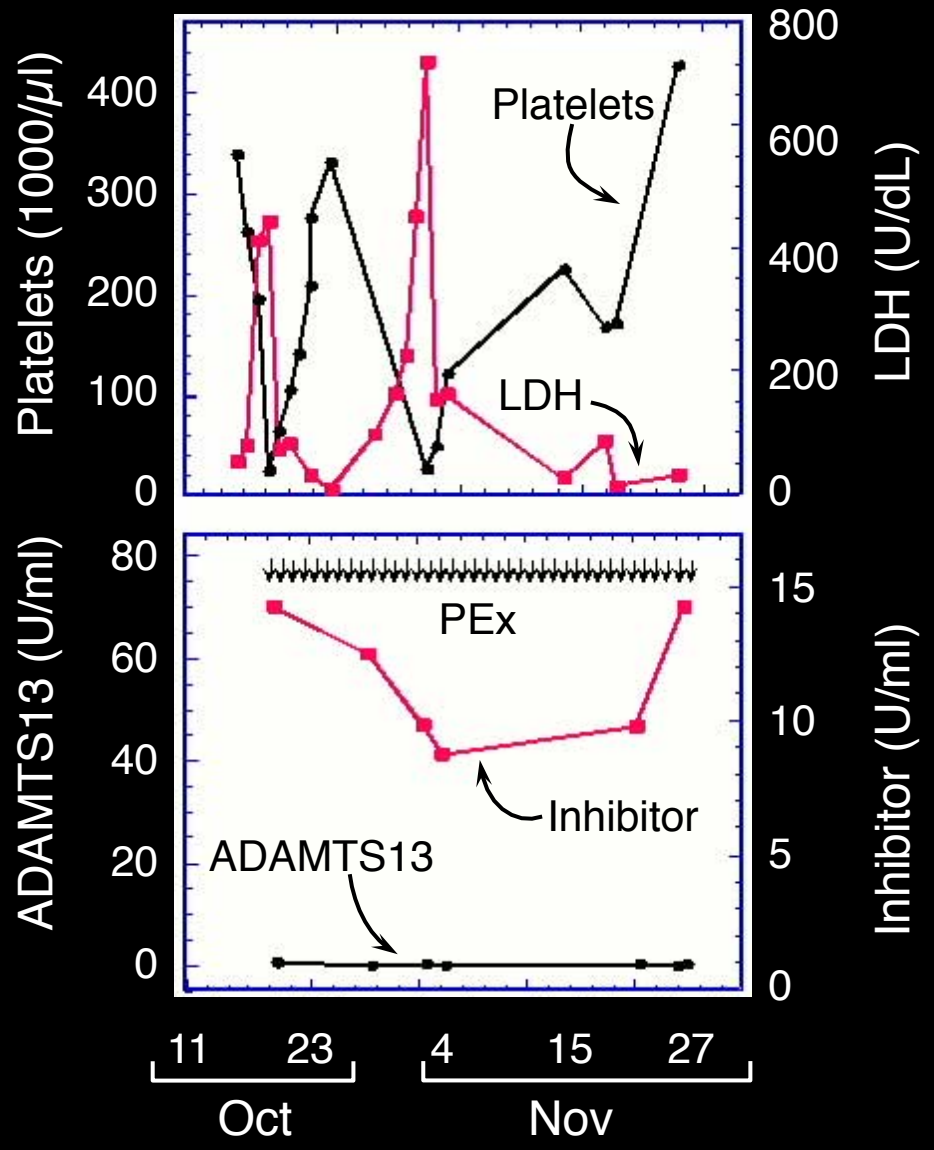
7 patients with:

- ADAMTS13 <5%
- Inhibitor present
- Multiple relapses in 4 and 2 deaths

Plasma exchange:

- Good response
- Unchanged ADAMTS13
- Persistent inhibitor

Why is PE effective?



Zheng et al, *Blood* 2004; **103:** 4043-4049

TTP and Persistent ADAMTS13 Inhibitors

Why Does Plasma Exchange Work?

Stress precipitates TTP in familial ADAMTS13 deficiency

- Childhood triggers: vaginal delivery, upper respiratory infection, pneumonia, otitis media
- Adult triggers: infection, alcohol abuse, pregnancy

Resolution of stress may end an attack of familial (or acquired idiopathic) TTP

ADAMTS13 – Clinical Correlations

ADAMTS13 deficiency (<5%) predicts:

- Idiopathic TTP
- Complete response to plasma exchange
- Survival

ADAMTS13 inhibitor predicts:

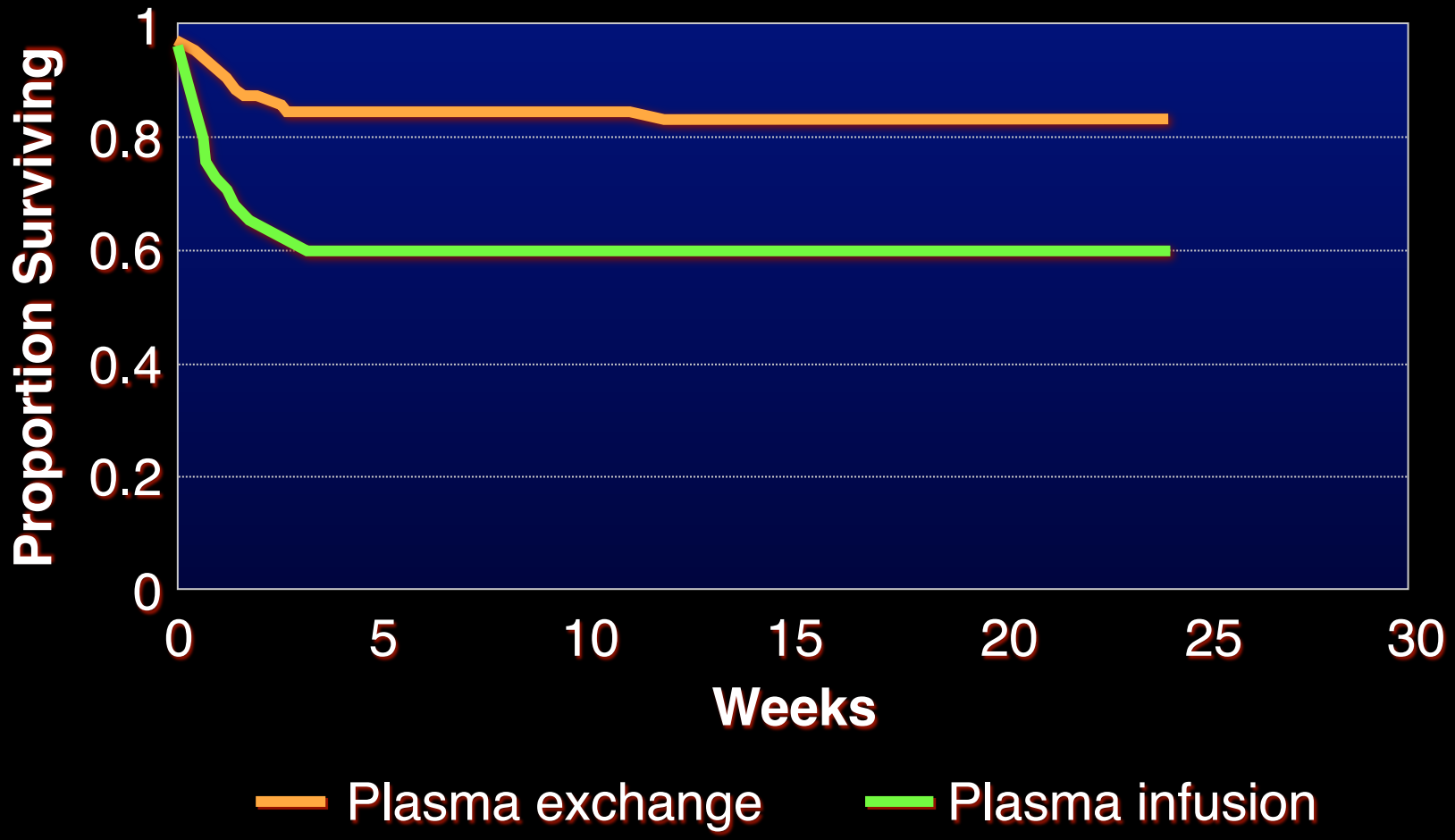
- Prolonged time to complete response
- Death
- Relapse

Reviewed in Coppo et al, *Br J Haematol* 2005; **132:** 66-74

Therapy

1930s-40s	Splenectomy initially; @ 50% response rate
1959	Rubinstein, fresh blood exchange transfusions
1977	Bukowski, plasmapheresis
1991	Rock, plasma exchange superior to infusion
1996	Rock, "cryopoor" plasma more effective than FFP as initial therapy
1997	One PE daily and antiplatelet drugs until complete remission; otherwise not standardized; ***do not transfuse platelets***

Plasma Infusion vs. Plasma Exchange



Rock et al. *NEJM* 325(6): 396

Management

Plasma Infusion vs. Exchange

- Rock et al. *NEJM*, Aug 1991
- Randomized trial 102 pts, PE vs. PI
- All pts received ADA/dipyridamole and no steroids
- 6m mortality PE: 22%
  PI: 37%

Plasma Infusion vs. Exchange

PE superior to infusion

- Canadian Apheresis Trial

But, unequal volumes of plasma infused

- Rock GA, Shumak KH, Buskard NA, et al: Comparison of plasma exchange with plasma infusion. *N Eng J Med* 1991;325:393-7

No prospective trials

Idiopathic TTP – Initial Therapy

Initiate treatment:

- Plasma exchange 60 ml/kg daily
- Plasma infusion if >12 hour delay
- Prednisone 2 mg/kg/day

Cardiac monitoring

Avoid:

- Platelet transfusions
- ?Antiplatelet drugs (e.g., aspirin, dipyridamole, clopidogrel)

Idiopathic TTP – Initial Therapy

Continue PE to complete response:

- Platelets >150K/μl for 3 days
- and LDH normal
- and Neurologically stable

After complete response:

- PE every other day for 4 days and stop
- Taper prednisone
- Resume treatment for exacerbation (<30 days) or relapse (>30 days)

Idiopathic TTP – Initial Therapy

Laboratory monitoring daily:

- Hemoglobin, white blood cell and platelet count, LDH, electrolytes, calcium, creatinine

Evaluate for causes of secondary TTP

Complications of plasma exchange:

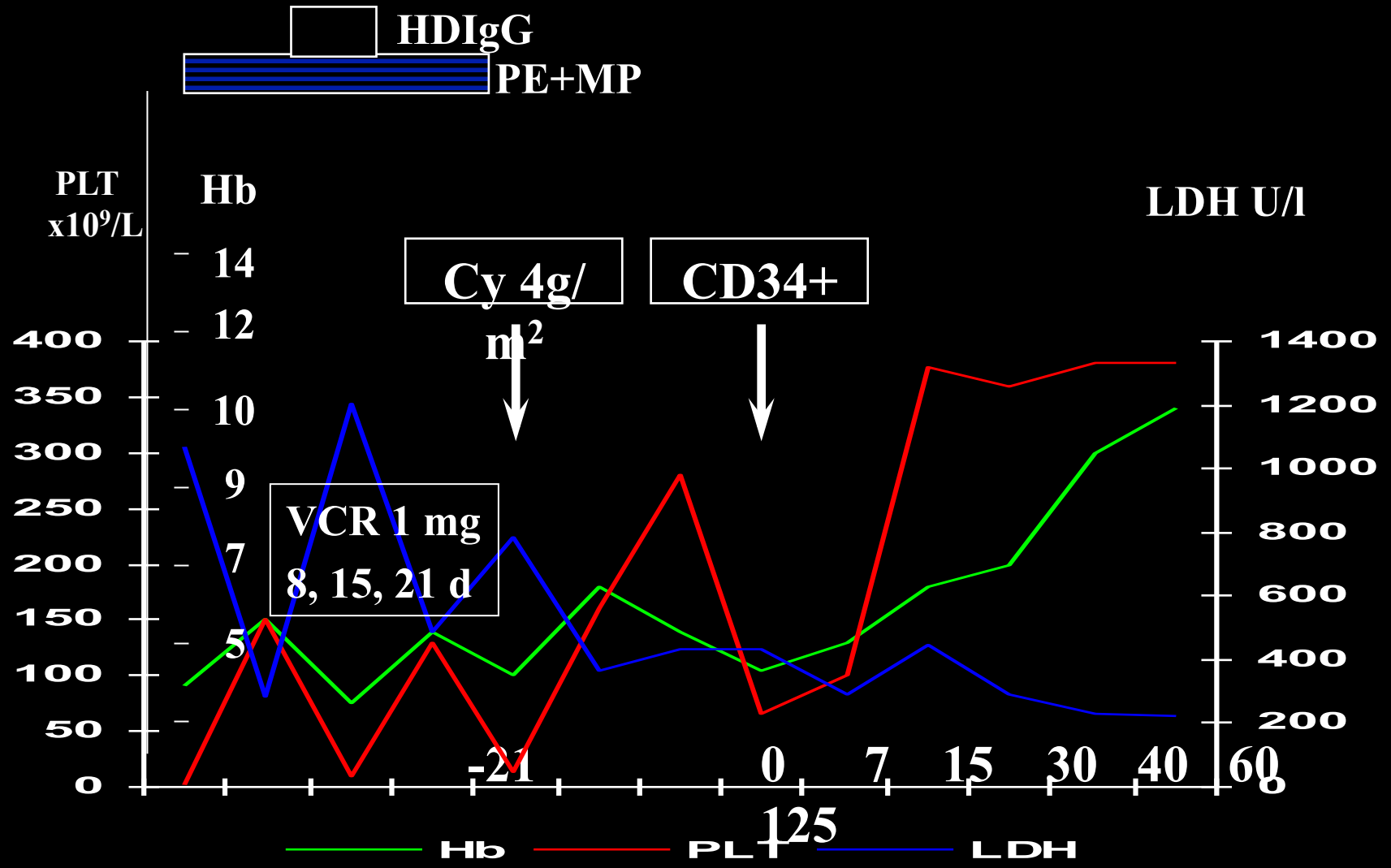
- Catheter (28%) – Sepsis, thrombosis, pneumothorax, hemorrhage
- Plasma (8%) – Allergic reaction, alkalosis, blood-borne infection, hypocalcemia

Idiopathic TTP – Salvage Therapy

Refractory or relapsing disease may benefit from immunosuppression:

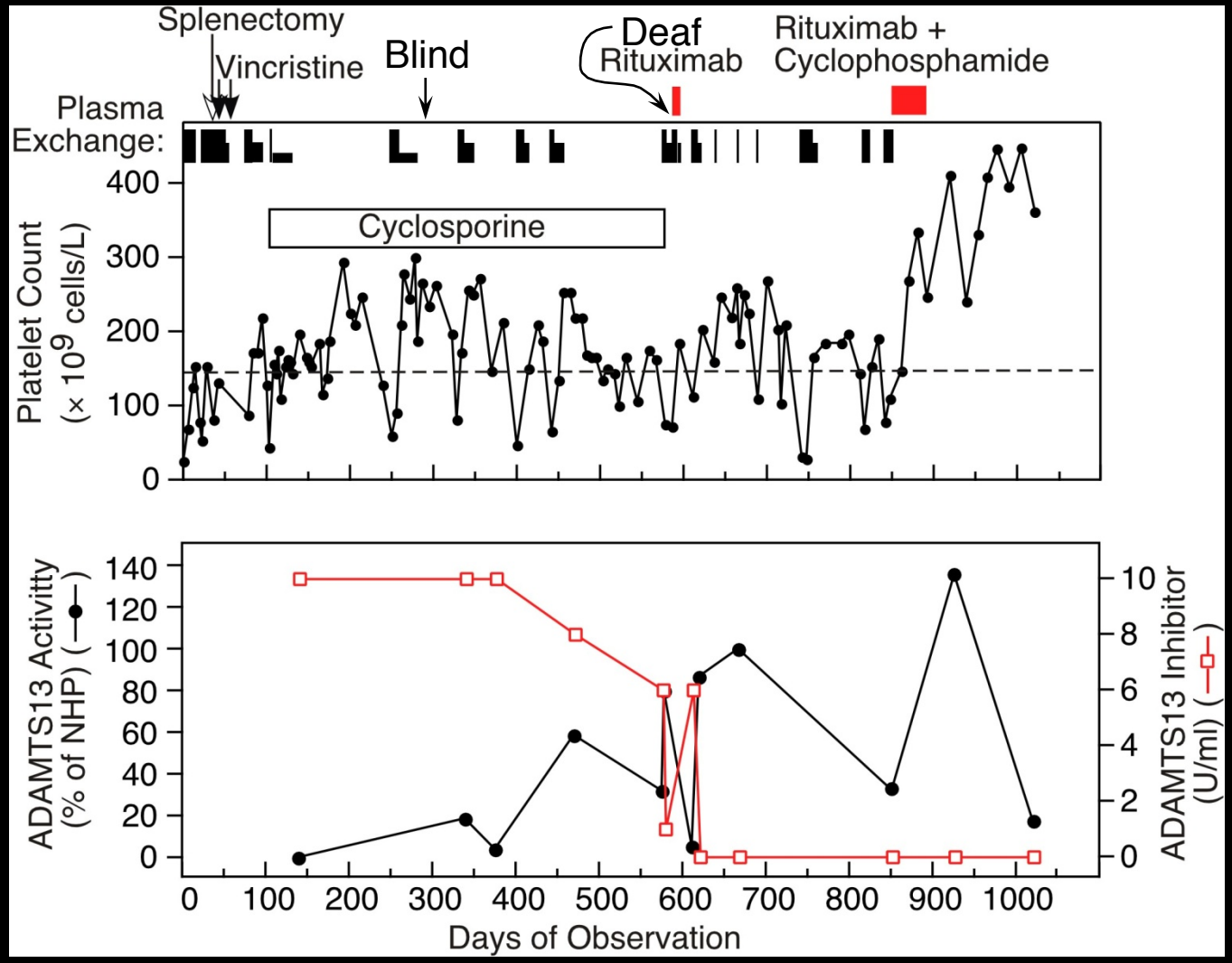
- Vincristine
- Splenectomy
- Rituximab (monoclonal anti-CD20)

M.G. 20, M, CD34+ ASCT for TTP



Musso M Bone Marrow Transplant. 1999 Jul;24(2):207-9

Immunosuppressive Therapy in TTP



Zheng et al, *Ann Intern Med* 2003; **138:** 105-108

Rituximab for Refractory TTP

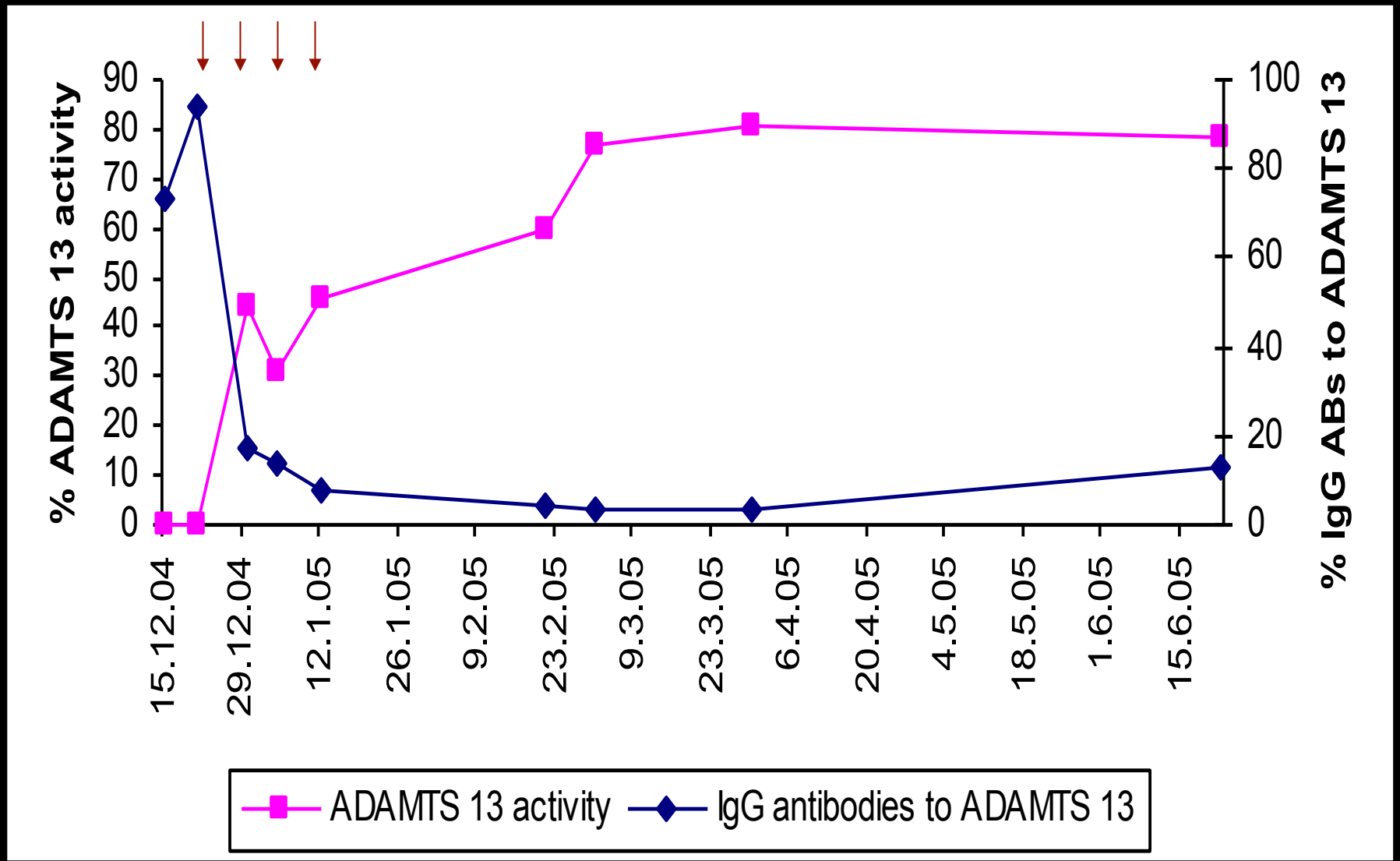
At least 18 reports, 43 patients failed PE, many failed steroids, vincristine, splenectomy

Rituximab 375 mg/m^2 weekly, up to 8 doses

Responses in 2-5 weeks:

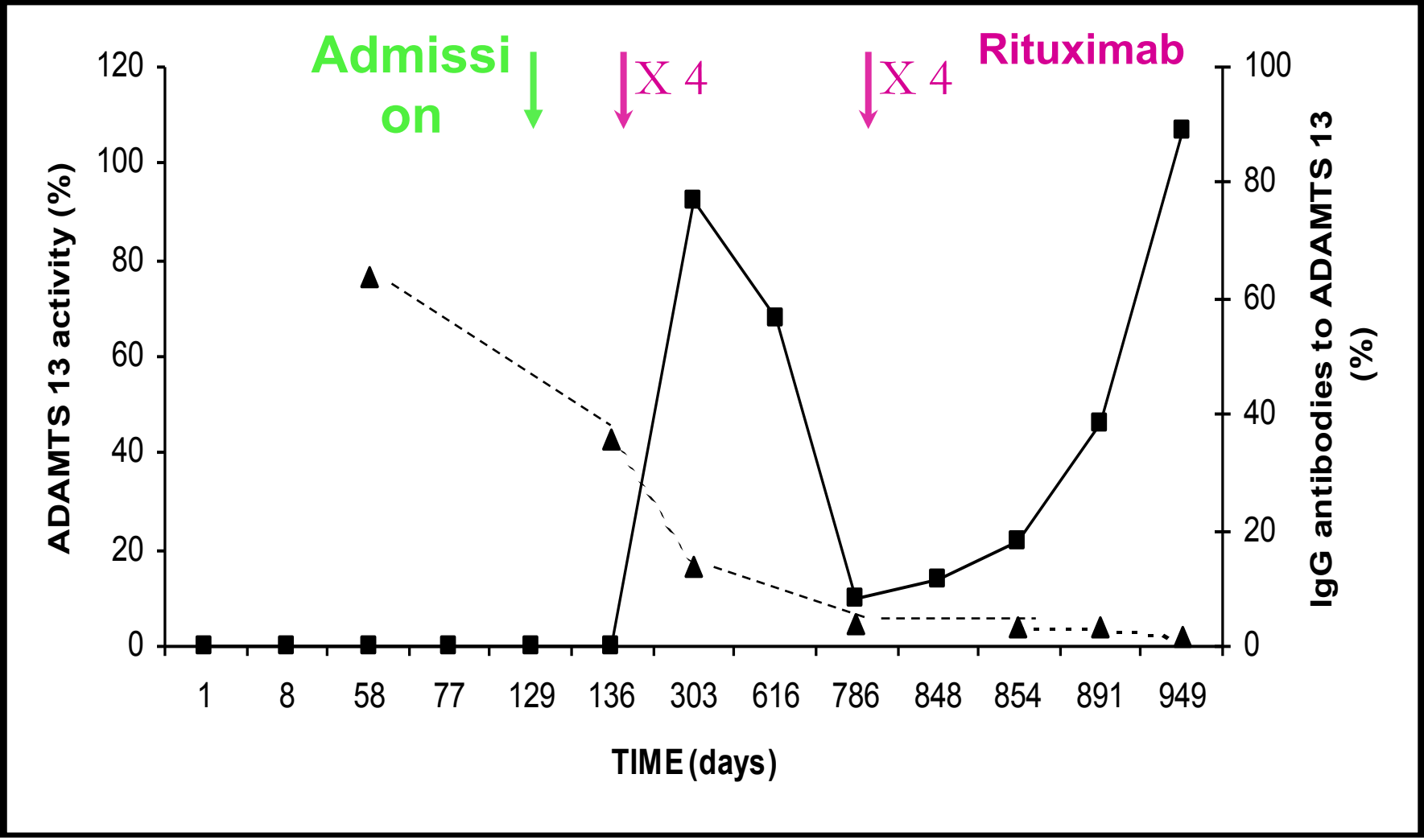
- 38 durable complete responses
- 2 late relapses, 1 achieved CR upon retreatment
- 1 partial response
- 2 no response (one with lung cancer)

Effect of Rituximab in acute refractory TTP

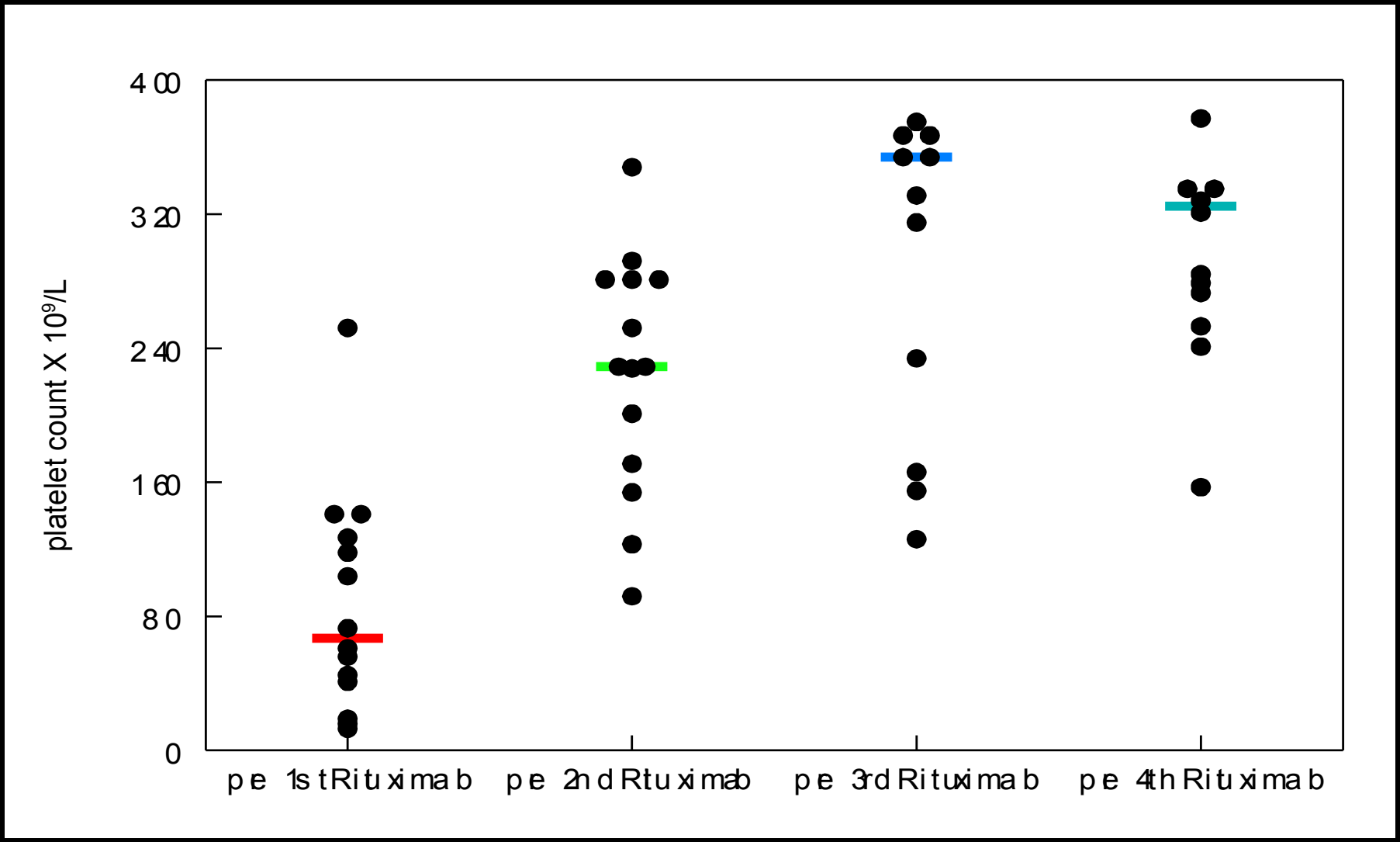


Acute and elective use of Rituximab

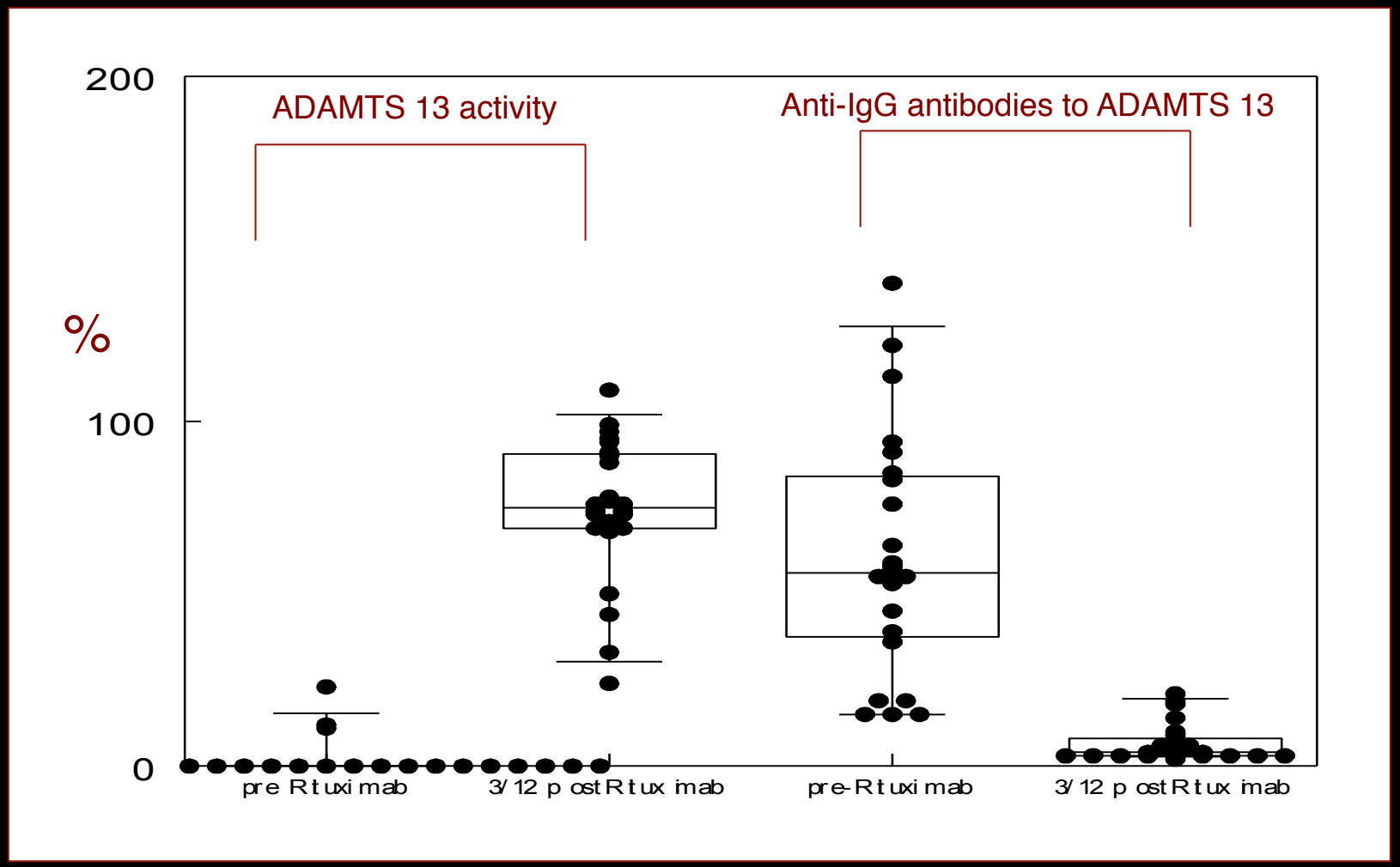
ADAMTS 13 activity: ■ **IgG antibodies to ADAMTS 13:** ▲



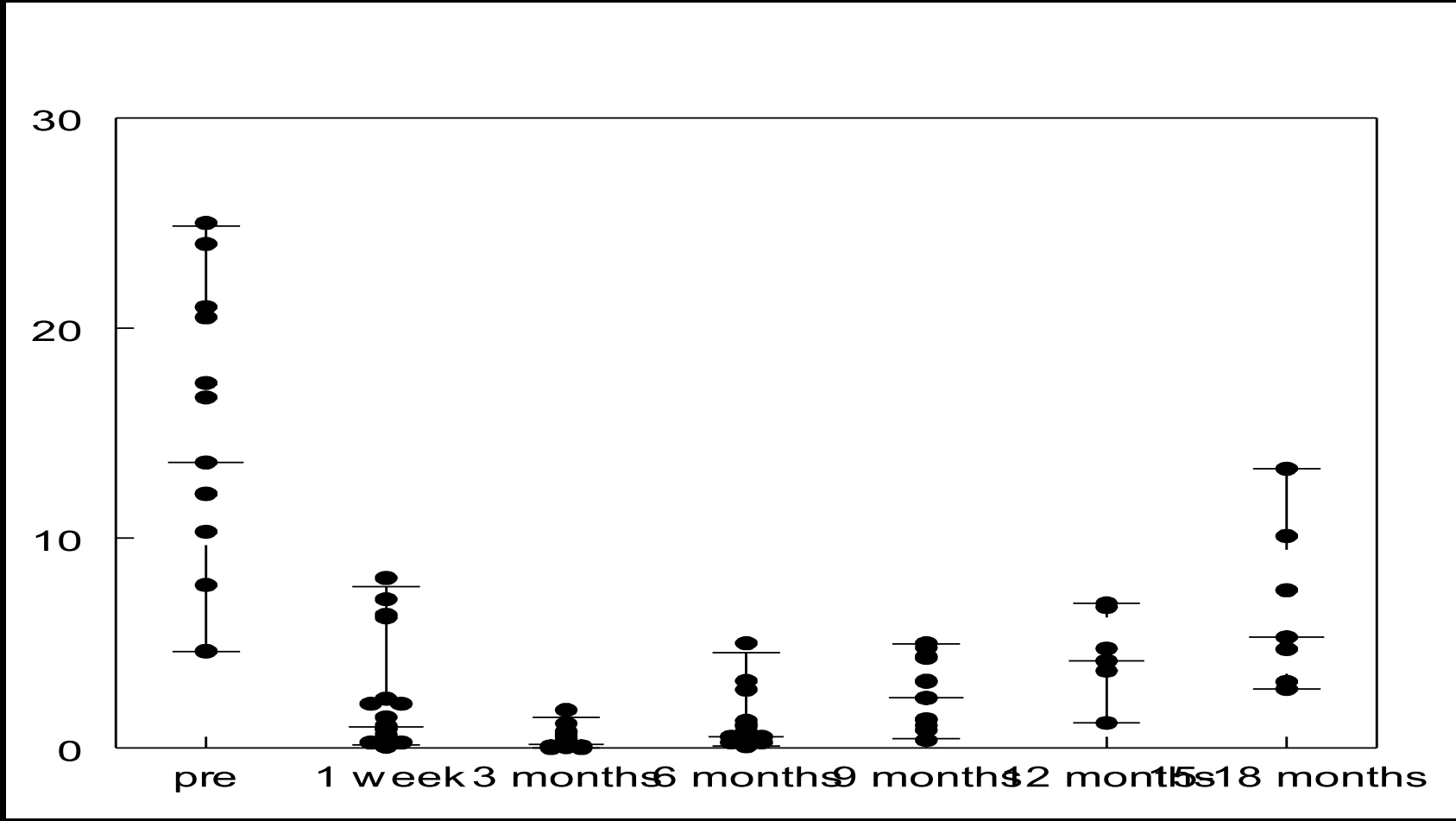
Platelet count Pre Rituximab Therapy



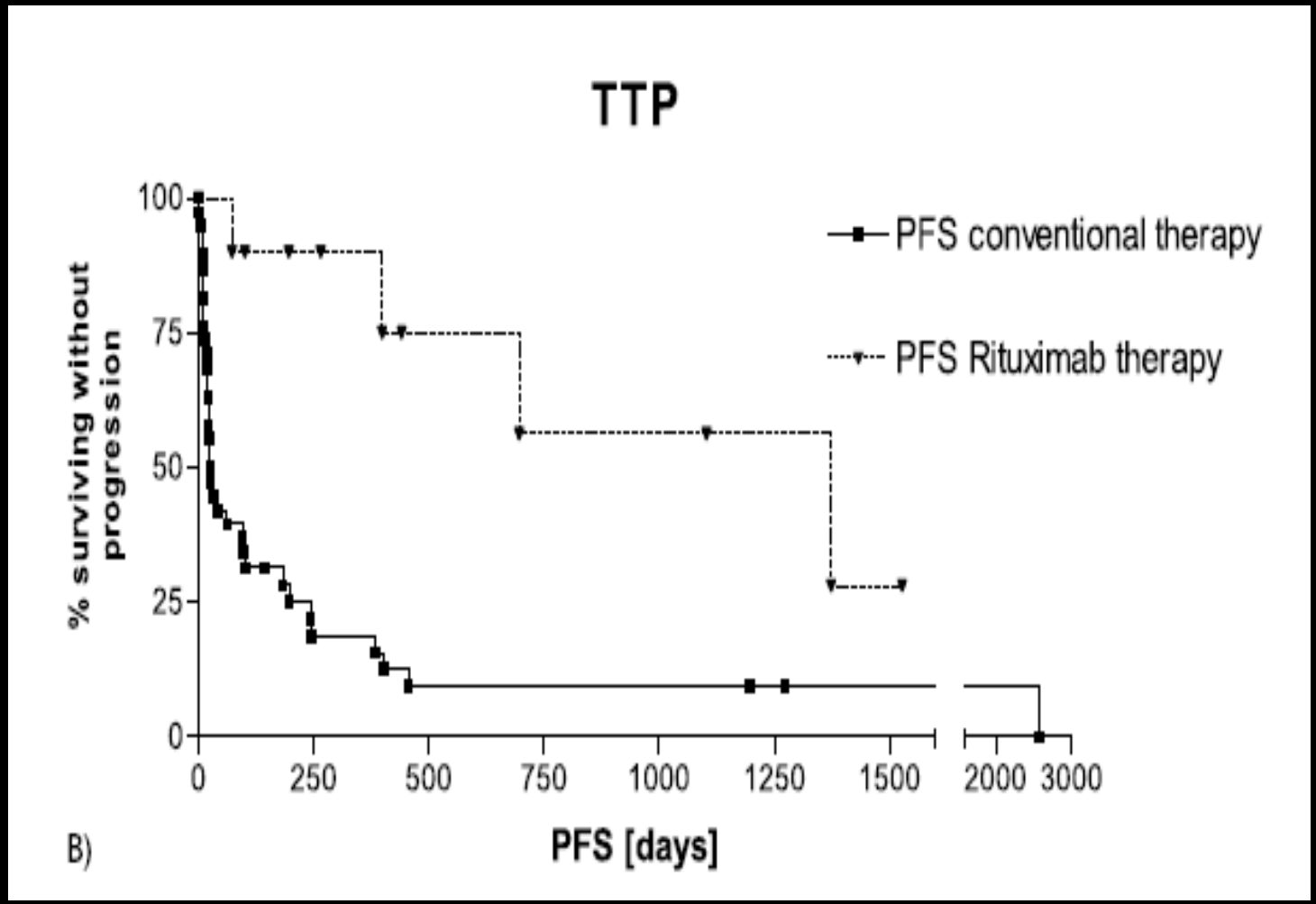
ADAMTS 13 activity and IgG antibody to ADAMTS 13 before and 3 months following Rituximab



CD 19 levels before and following Rituximab in acute TTP



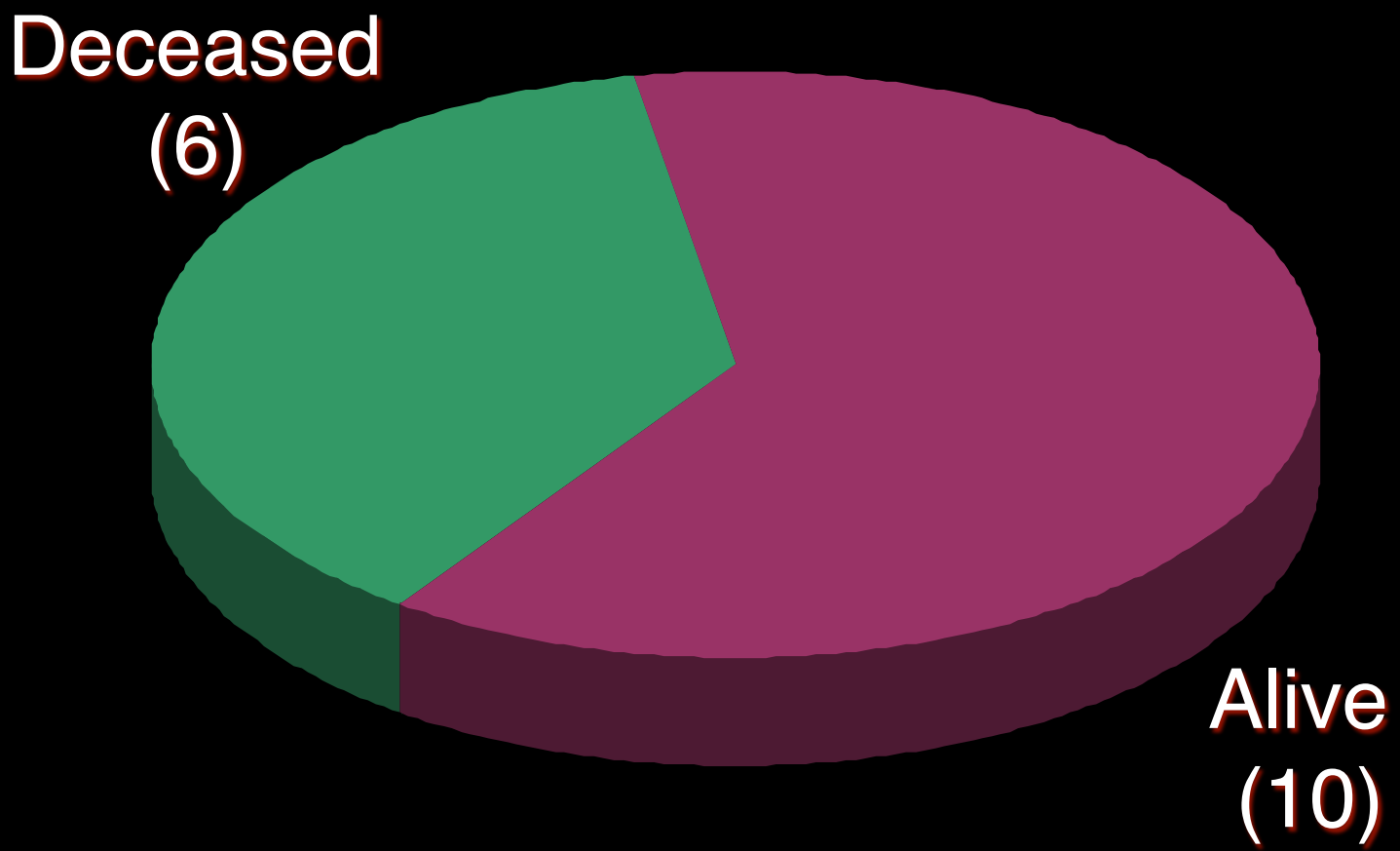
Rituximab improves Response Rate and PFS in Relapsed/refractory TTP



Heidel, TH 2007

Should Splenectomy Still Have a Role in the Treatment of Patients with Refractory TTP?

TTP Patients Who Had Splenectomy



VWF and TTP

- Look for hemolysis and schistocytes in all patients with anemia and thrombocytopenia
- ADAMTS13 deficiency correlates with response to PE and survival
- ADAMTS13 inhibitors correlate with relapses and early death
- Refractory patients may respond to immunosuppression

TTP: Clinical Research Questions

Many causes of microangiopathic hemolytic anemia, thrombocytopenia

Are ADAMTS13 data useful:

- To diagnose autoimmune idiopathic TTP?
- To stratify patients by risk?
- As biomarkers of disease activity?

TTP: Clinical Research Questions

Should we stratify patients by ADAMTS13 activity and inhibitor level?

- Does plasma exchange benefit patients with normal ADAMTS13?
- Inhibitors predict relapse, death
- Evaluate PE + rituximab as initial therapy for high-risk disease

TTP: Clinical Research Questions

Should asymptomatic patients with ADAMTS13 deficiency be treated?

- Relapses occur unpredictably
- Evaluate preemptive immunosuppression
- Evaluate ADAMTS13 activity and inhibitor as biomarkers of response

TTP: Clinical Research Questions

What is the best way to replace ADAMTS13 during active TTP?

- Fresh frozen plasma or cryosupernatant
- Virucidally-treated plasma fractions
- Recombinant ADAMTS13

"Prediction is very difficult, especially about the future."

Niels Bohr

"Those who cannot remember the past are condemned to repeat it."

George Santayana,
Reason in Common Sense
(New York: Scribner's, 1905)