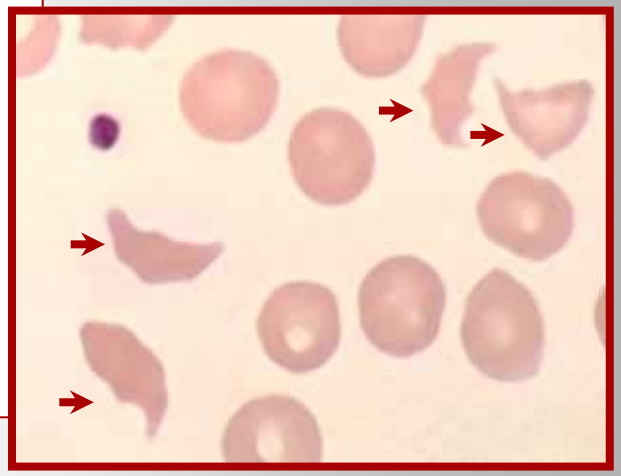
Porpora Trombotica Trombocitopenica (TTP)
Una diagnosi difficile in oncologia

Flora Peyvandi
A. Bianchi Bonomi Haemophilia and Thrombosis Center
IRCCS Policlinico, Mangiagalli and Regina Elena Foundation
University of Milan

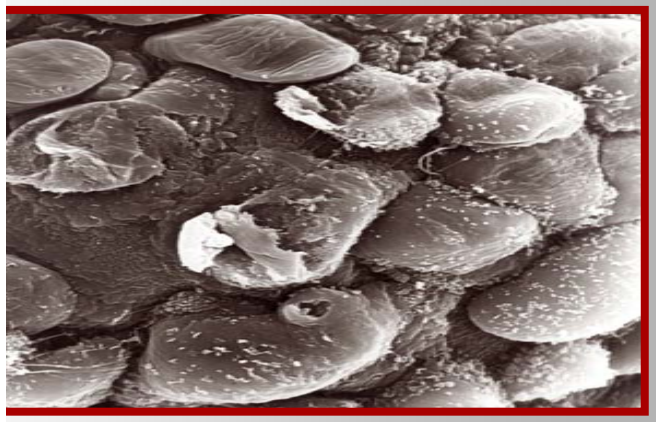
THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

Eli Moschowitz, 1924

- Disseminated form of thrombotic microangiopathy characterized by:
- Microangiopathic haemolytic anemia
- Thrombocytopenia
- Microvascular thrombosis → degree of tissue ischemia and infarction



- Incidence ≈4/million/year
- Often it strikes young adults, mainly females (2/3 of the cases)
- Untreated, mortality >90%
- Treated with plasmapheresis, mortality <20%



TTP Diagnosis

OBJECTIVE EXAMINATION

CLINICAL DIAGNOSIS

P

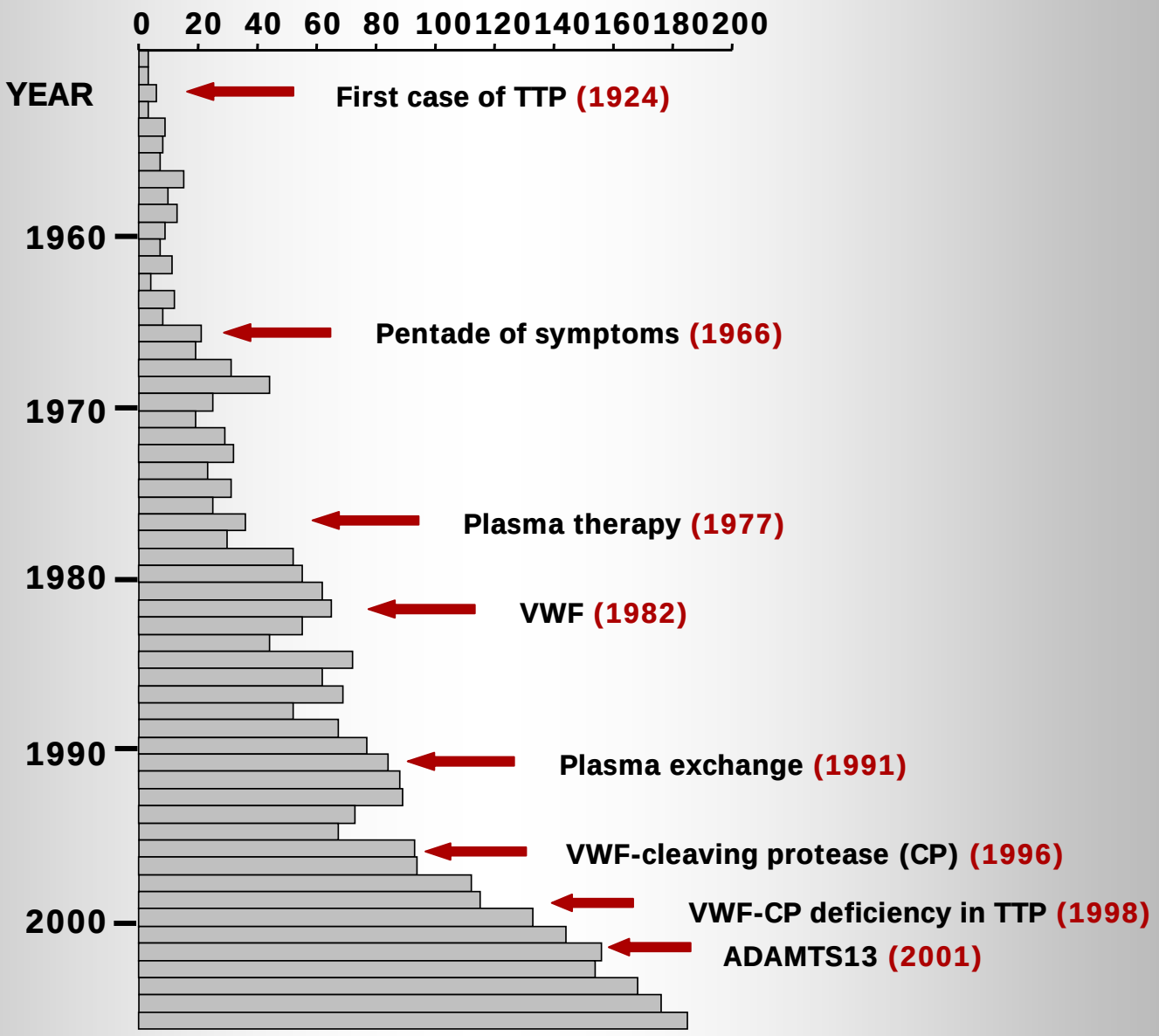
LABORATORY DIAGNOSIS

- Vomiting
- Abdominal pain

- **Trombocytopenia:** Platelet count < 50.000/mm^3

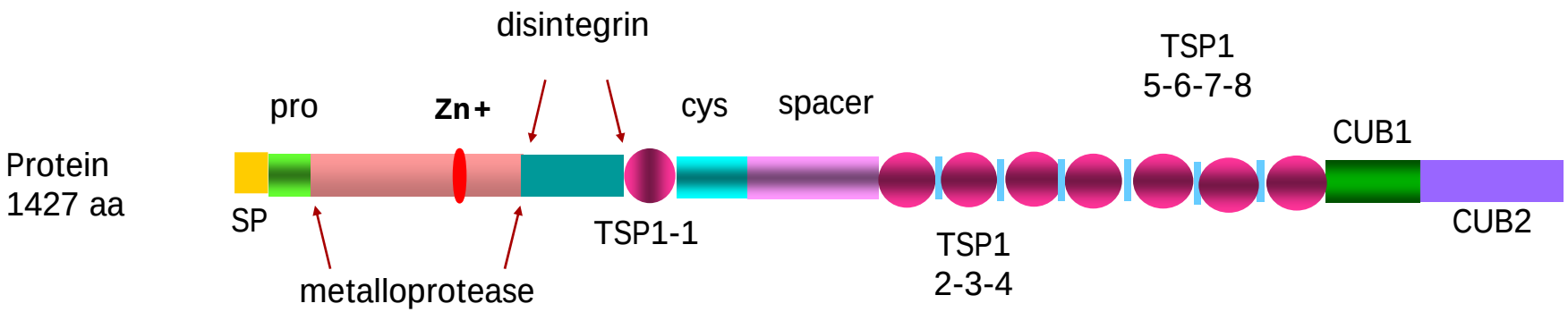
- **Microangiopathic hemolytic anemia:**
  - Hematocrits usually < 20%
  - Hb < 10 g/dl
  - Increased indirect bilirubin
  - Presence of schistocytes on peripehral blood smear
  - Reticulocytosis
  - Low or unmeasurable haptoglobin level
  - High serum lactate dehydrogenase
  - Negative Coombs test

From TTP to ADAMTS13



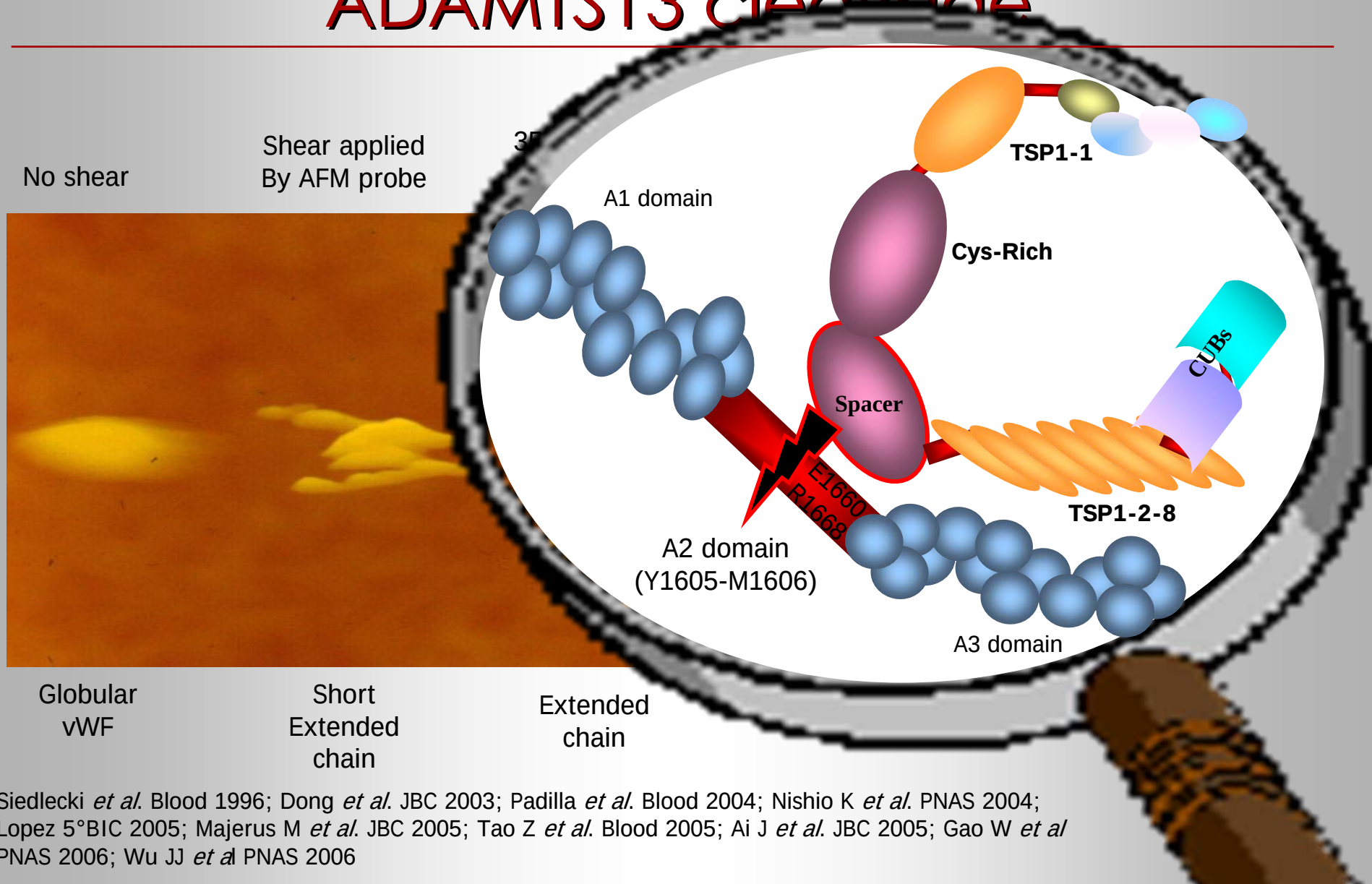
ADAMTS13

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



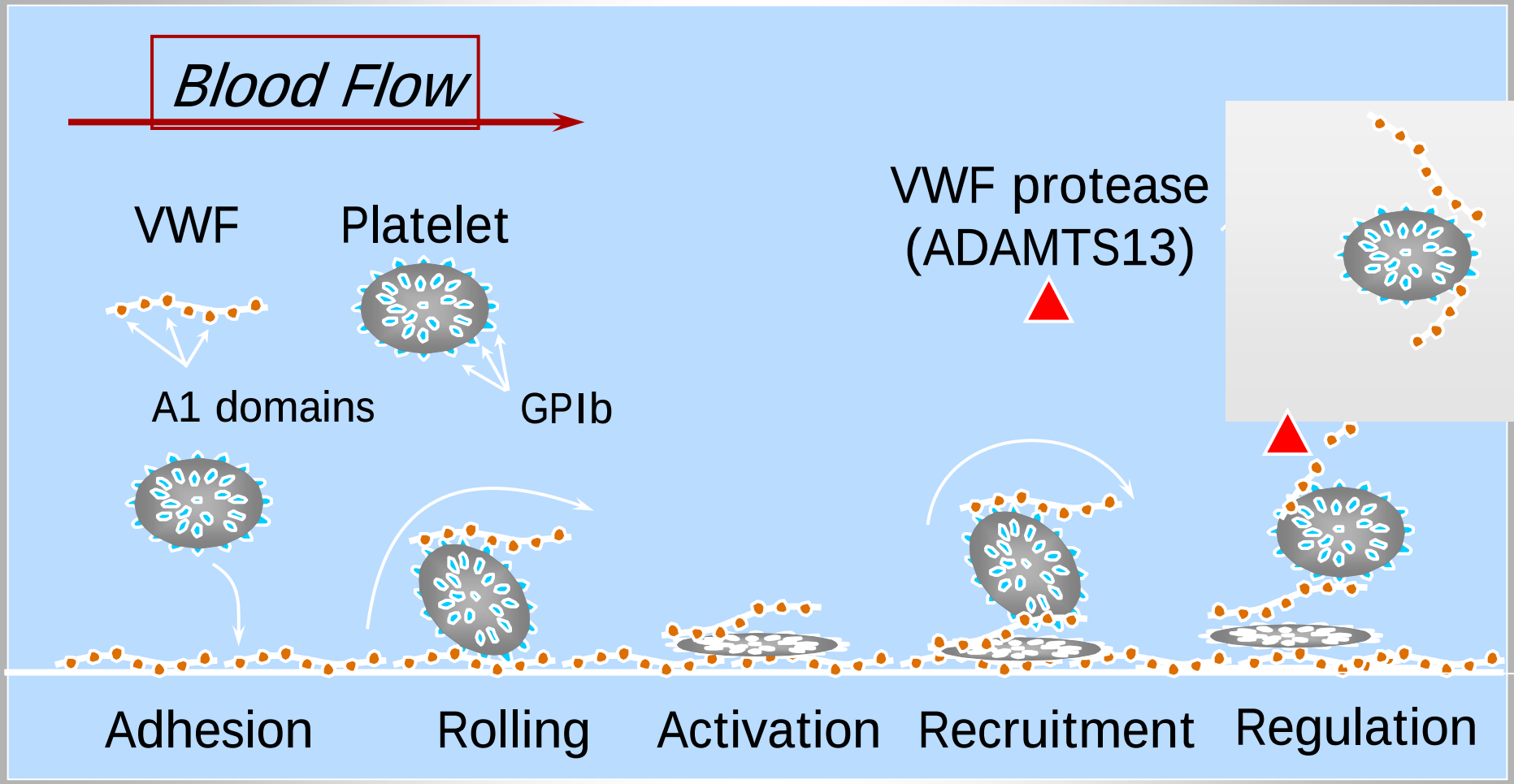
(Zheng XE *et al.* JBC 2001; 276:41059-63)

Shear dependent VWF-changes and ADAMTS13 cleavage

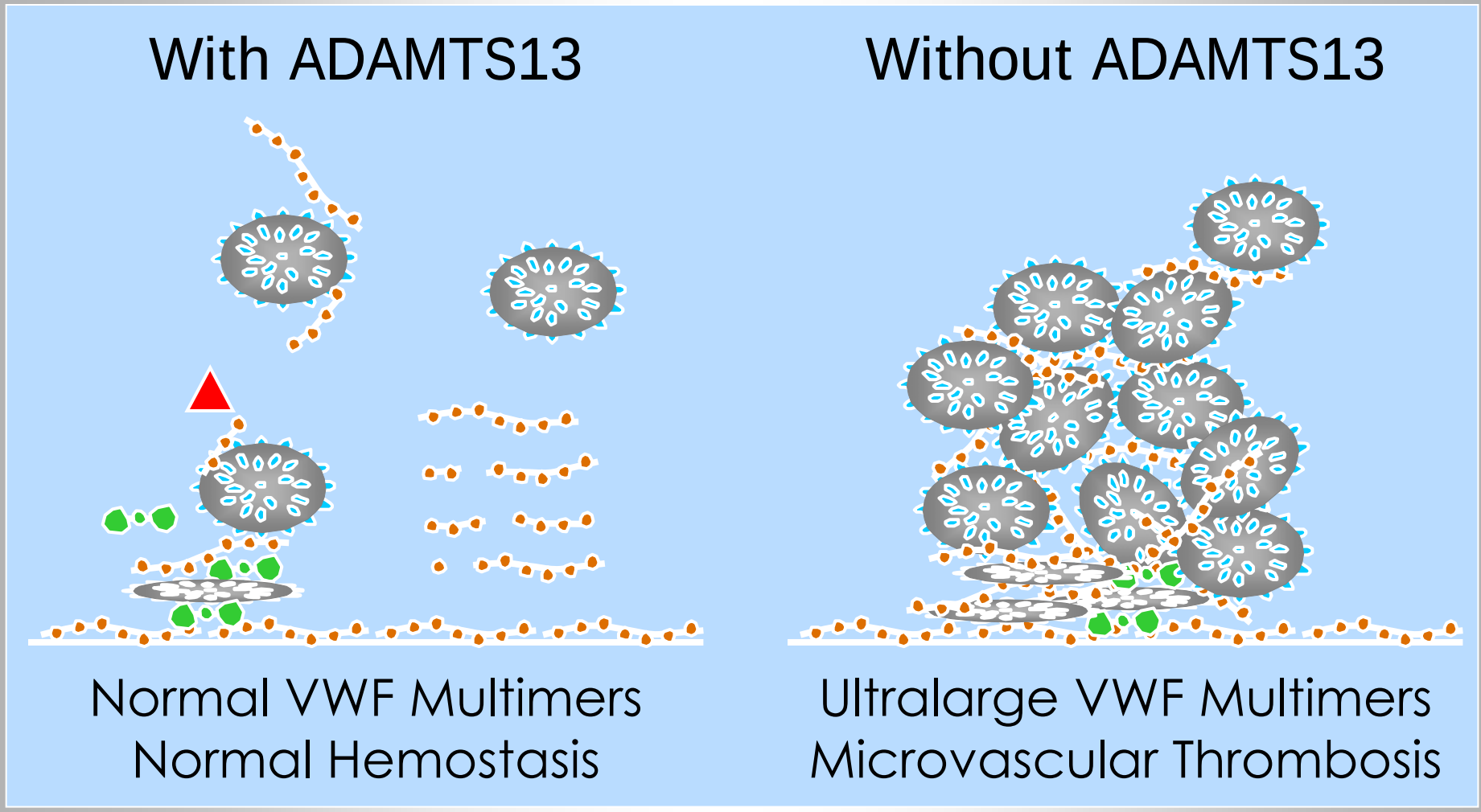


Siedlecki *et al*. Blood 1996; Dong *et al*. JBC 2003; Padilla *et al*. Blood 2004; Nishio K *et al*. PNAS 2004; Lopez 5°BIC 2005; Majerus M *et al*. JBC 2005; Tao Z *et al*. Blood 2005; Ai J *et al*. JBC 2005; Gao W *et al* PNAS 2006; Wu JJ *et al* PNAS 2006

VWF, ADAMTS13 and Platelet adhesion



VWF, ADAMTS13 and Platelet adhesion



TTP

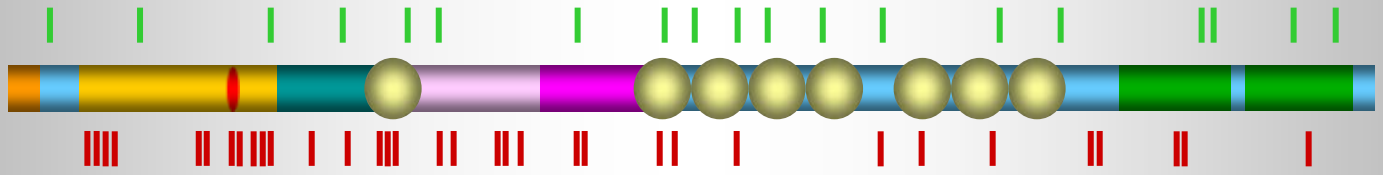
IDIOPATHIC

SECONDARY

- **Autoimmune**
- **Drug induced**
  - Acute, immunomediated drug toxicity
  - Cumulative, dose-dependent drug toxicity
- **Hematopoietic stem-cell transplantation**
- **Cancer associated**
  - Cancer related
  - Chemotherapy related

Congenital TTP

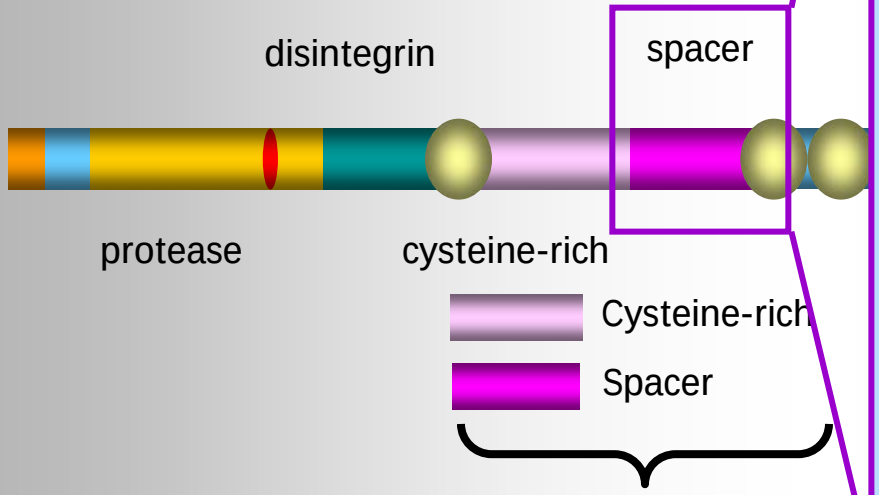
- Greater than 80 mutations identified to date:
  - ~40% nonsense, frameshift, or splice site
  - ~60% missense



- No apparent clustering or mutation "hot-spots"
  - ~75% of missense mutations in first half of ADAMTS13
- No clear genotype-phenotype correlations observed
  - complete deficiency originally thought to be lethal
- About 50% of patients presented within the first 5 years of life, but a second group remained without symptoms until 20 to 40 years of age.

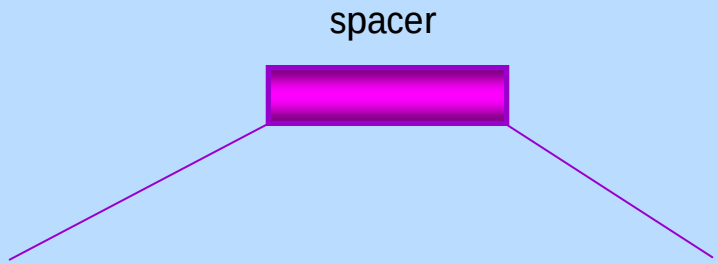
Acquired TTP

ADAMTS13 autoantibodies
of patients diagnosed



100% of cases
(critical epitopes)

Characterization of a human Mab directed against the spacer domain of ADAMTS13: a prototype of antibodies present in patients with acquired TTP



Aminoacid regions 572-579 and 657-666 are required for binding of human Mab

Luken BM *et al* TH 2006

Combination in majority of cases

(Soejima K *et al*. Blood 2003; Klaus K, *et al*. Blood 2004)

Secondary TTP

SECONDARY:

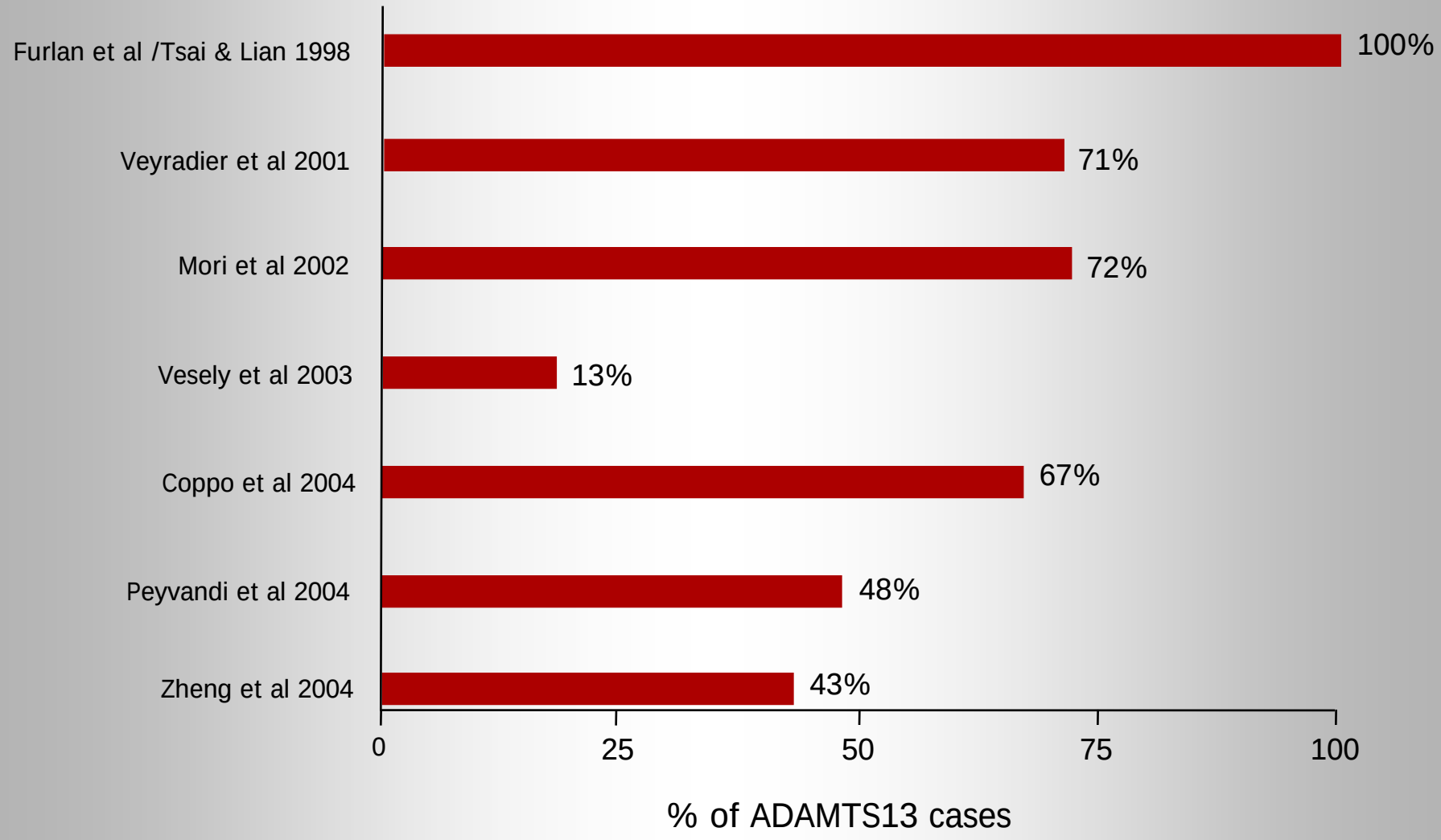
due to an alteration in the balance between VWF as substrate and its cleaving protease activity,reflected by lower levels of ADAMTS13 enzymatic activity compared with the baseline condition

ADAMTS13 measurement

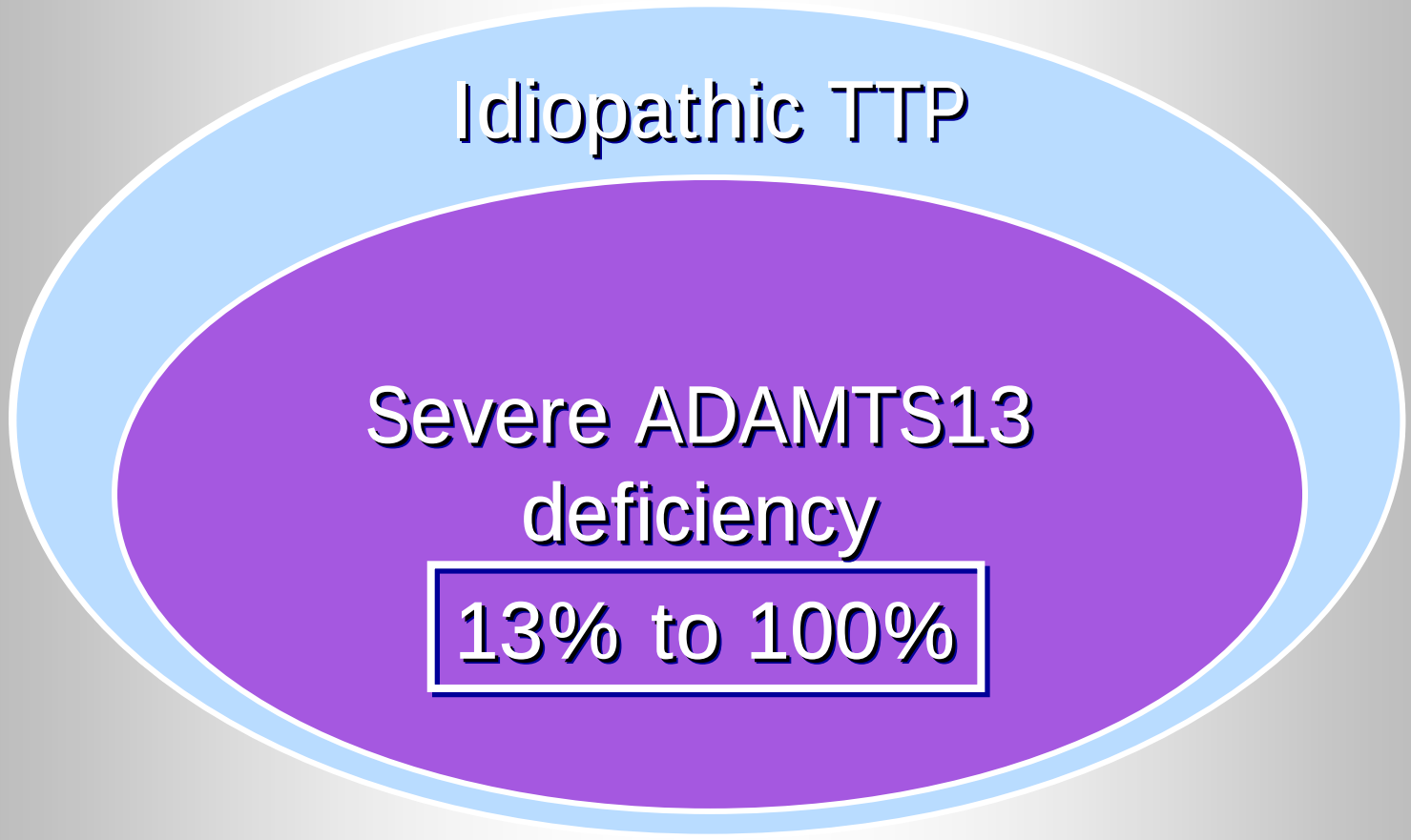
- Functional assay:
  - Direct (Furlan M NEJM '98; Tsai HM NEJM '98; Kokame K Blood '03; Ai J JBC '05; Kokame K. BJH '05; Kato S Transfusion '06; American Diagnostic, Stamford, USA; TECHNOCLONE GmbH, Vienna, Austria; GTI Diagnostic, Waukesha, USA)
  - Indirect (Gerritsen HE TH '99; Bohm M Ann Hematol '02; Dong JF Blood '02 ; Whitelock JL JTH 04; Zhou W TH '04; Shenkman B BJH '03)
- Antigen level:
  - ELISA (American Diagnostic, Stamford, USA; TECHNOCLONE GmbH, Vienna, Austria)
- Inhibitory anti-ADAMTS13 autoantibody:
  - mixing studies
- Anti-ADAMTS13 autoantibody:
  - ELISA (TECHNOCLONE GmbH, Vienna, Austria; American Diagnostic, Stamford, USA)
  - WB

Is ADAMTS13 deficiency sufficient to cause TTP?

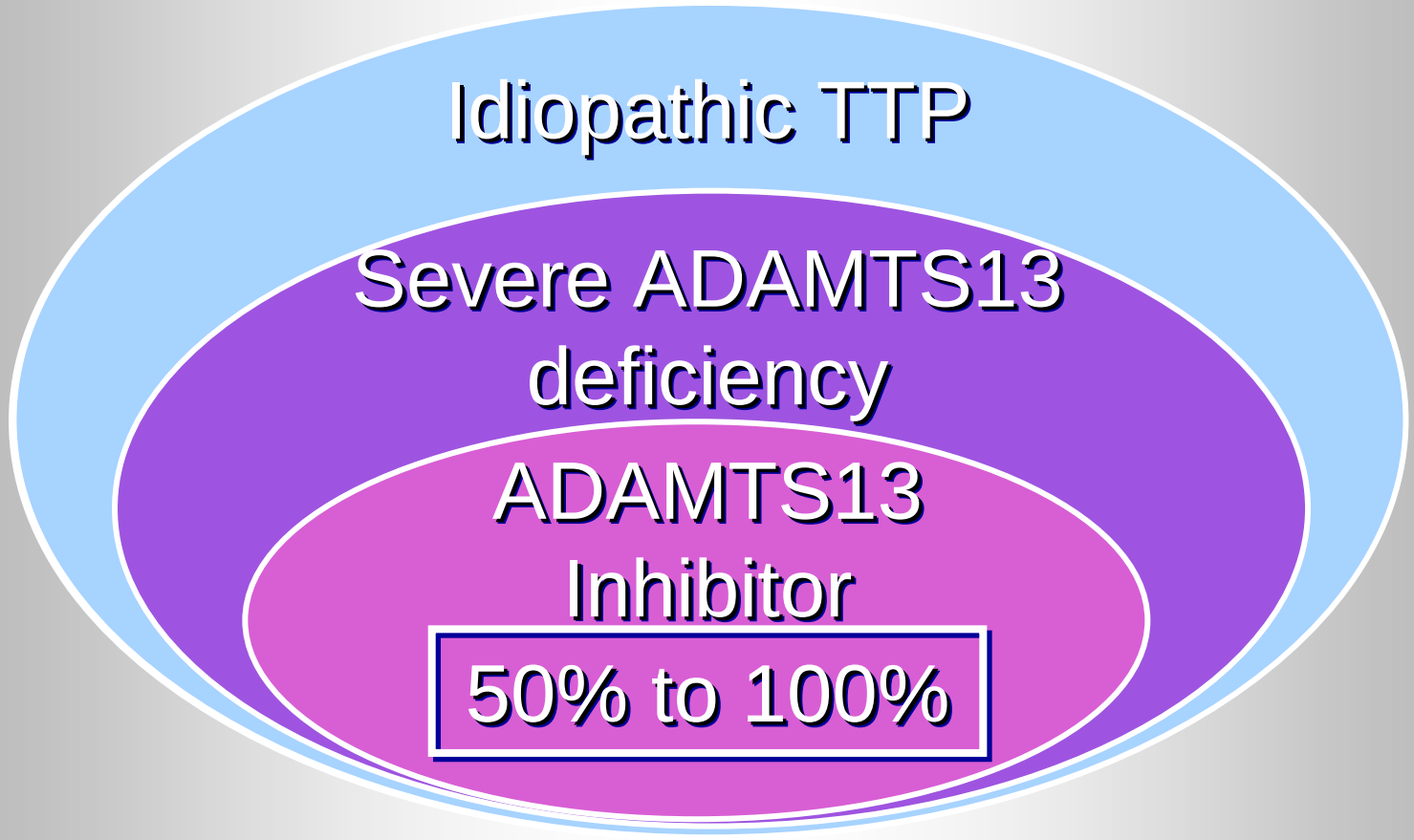
Rates (%) of severe ADAMTS13 deficiency in clinically-diagnosed acute TTP



Acquired Idiopathic TTP



Acquired Idiopathic TTP



ADAMTS13 and other TMA

Disease	Number of patients studied	Patients with severe ADAMTS13 deficiency (<10%)	Patients with reduced ADAMTS13 level (10-40%)
Typical HUS (Veyradier et al, Blood 2001)	46	13%	4%
Atypical HUS (Remuzzi et al, Blood 2002)	29	34%	7%
LES (Mannucci et al, Haematologica 2003)	36	0	14%
HELLP syndrome (Lattuada et al, Haematologica 2003)	17	0	70%
APS (Rieger et al, Blood 2005)	55	0	ND
Sepsis-induced DIC (Ono et al, Blood 2006)	109	16%	51%

A severe ADAMTS13 deficiency is reported in 13-71% of patients clinically diagnosed with TTP

ADAMTS13 was as severely deficient as in TTP also in some patients diagnosed as typical (13%) or atypical HUS (34%) and sepsi induced DIC (16%)

Real time ADAMTS13 testing is not warranted in acute TTP but could confirm this diagnosis

The diagnosis of TTP is still based upon clinical manifestations and laboratory features and the treatment should start early when the diagnostic criteria are met

ADAMTS13 analysis may provide useful indication for treatment and valuable insights to the disease status during the course of therapy

TTP and cancer

CANCER RELATED

CHEMOTHERAPY RELATED

Cancer related TTP

- Few studies in the literature
- Prevalence of 3%
- Clinically indistinguishable from idiopathic TTP
- TTP can be the presenting feature of cancer → disseminated malignancy can be misdiagnosed as idiopathic TTP
- Poor response to plasma exchange
- Usually NOT associated with severe (<10%) ADAMTS13 deficiency

ADAMTS13 and cancer

Oleksowicz *et al.* (Cancer Res 1999) compared:

- 20 adults with **disseminated tumors**
- 15 adults with **localized tumors**

→ Very low or undetectable (<15%) ADAMTS13 levels in patients with disseminated tumors NOT associated with microanigiopathic hemolytic anemia

→ Normal (>88%) ADAMTS13 levels in localized tumors

→ ADAMTS13 deficiency was accompained by a concomitant increase of plasma VWF levels and the presence of ultralarge VWF multimers

Abnormalities of plasma VWF and its cleaving metalloprotease might play a key role in the adhesive interactions between tomur cells, circulating platelets and the vascular endothelium that lead to the formation of metastasis

ADAMTS13 and cancer

Mannucci *et al.* (Haematologica 2003) compared:

- 20 pediatric and adult patients with **disseminated tumors**
- 29 pediatric and adult patients with **localized tumors**
- both groups with 49 healthy matched individuals as a control

→ Patients with both disseminated and localized tumors had significantly lower plasma levels of ADAMTS13 compared to controls

→ ADAMTS13 were lower in patients with disseminated tumors than in localized, but the difference did not reach statistical significance

→ There was a significant inverse correlation beetween VWF:Ag and ADAMTS13

→ None of patients with low ADAMTS13 levels had clinical or laboratory signs of tumor-associated thrombotic microangiopathy

Chemotherapy related TTP

1. Rare but severe complication of chemotherapy
2. Many different drugs involved (gemcitabine, doxorubicin, fluorouracil, tamoxifen…)
3. Chemotherapy-induced endothelial damage is the postulated mechanism
4. Rarely associated with ADAMTS13 severe deficiency

TMA and Bone Marrow Transplantation

- TMA is a known complication of BMT
- The pathogenesis of allo-TMA is poorly understood
- Reported incidence in the literature: 1-6 to 76%

Different factors are involved in **endothelial damage** pathogenesis:

- Cyclosporine A
- Graft-versus-host disease (GVHD)
- Total body irradiation (TBI)
- Intensive conditioning chemotherapy
- Infections

Role of ADAMTS13 in TMA post BMT pathogenesis is not clear

Few retrospective studies have not evidenced a reduction of ADAMTS13 levels in this situation

(George JN *et al*. Transfusion 2004)

TMA and Bone Marrow Transplantation

Peyvandi *et al.* (BJH 2006):

- 27 cases of auto BMT
- 19 cases of allo BMT

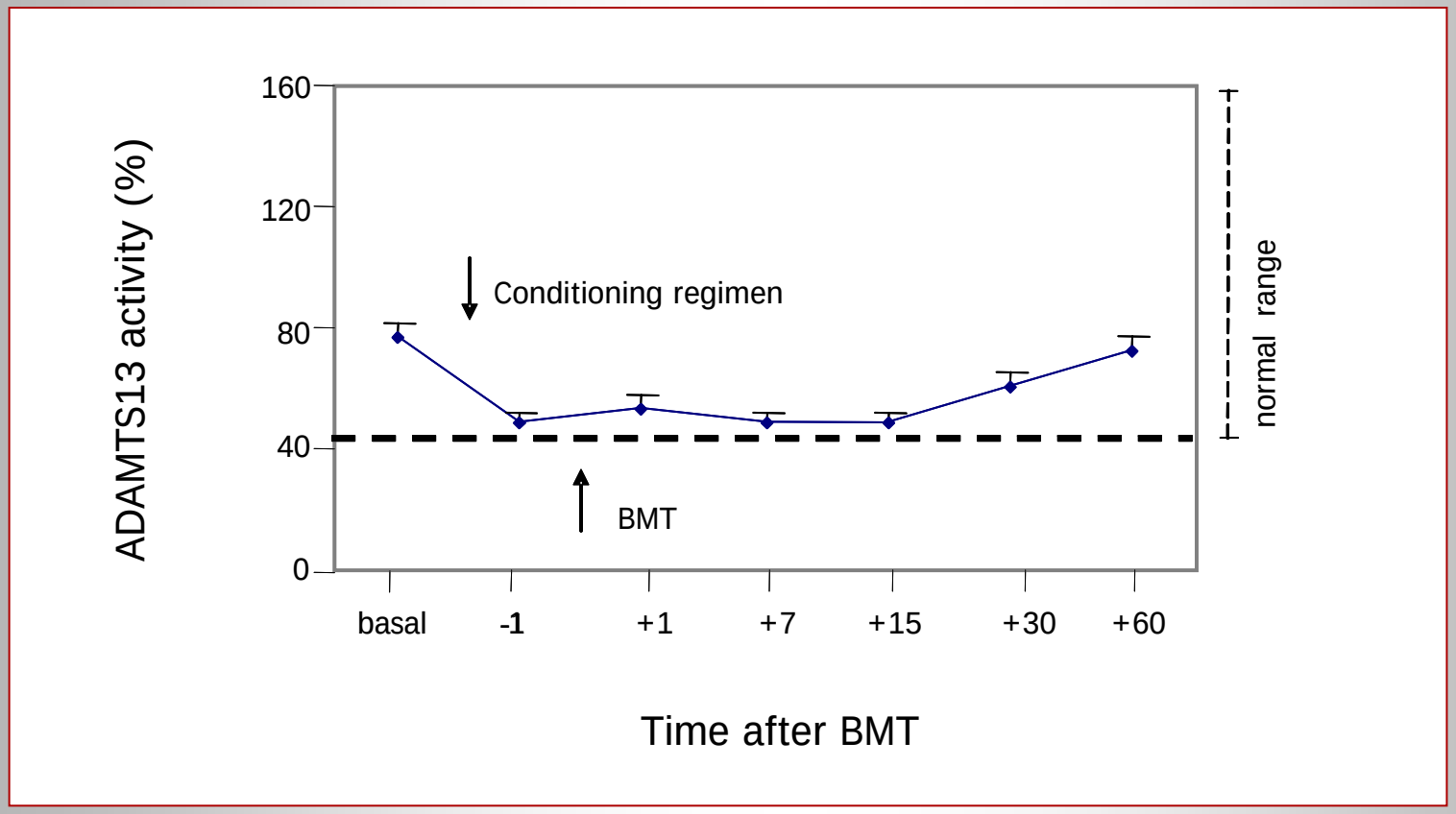
Objectives

1. To evaluate the incidence of TMA after BMT
2. To explore the behaviour of ADAMTS13, VWF antigen and VWF multimer size

RESULTS

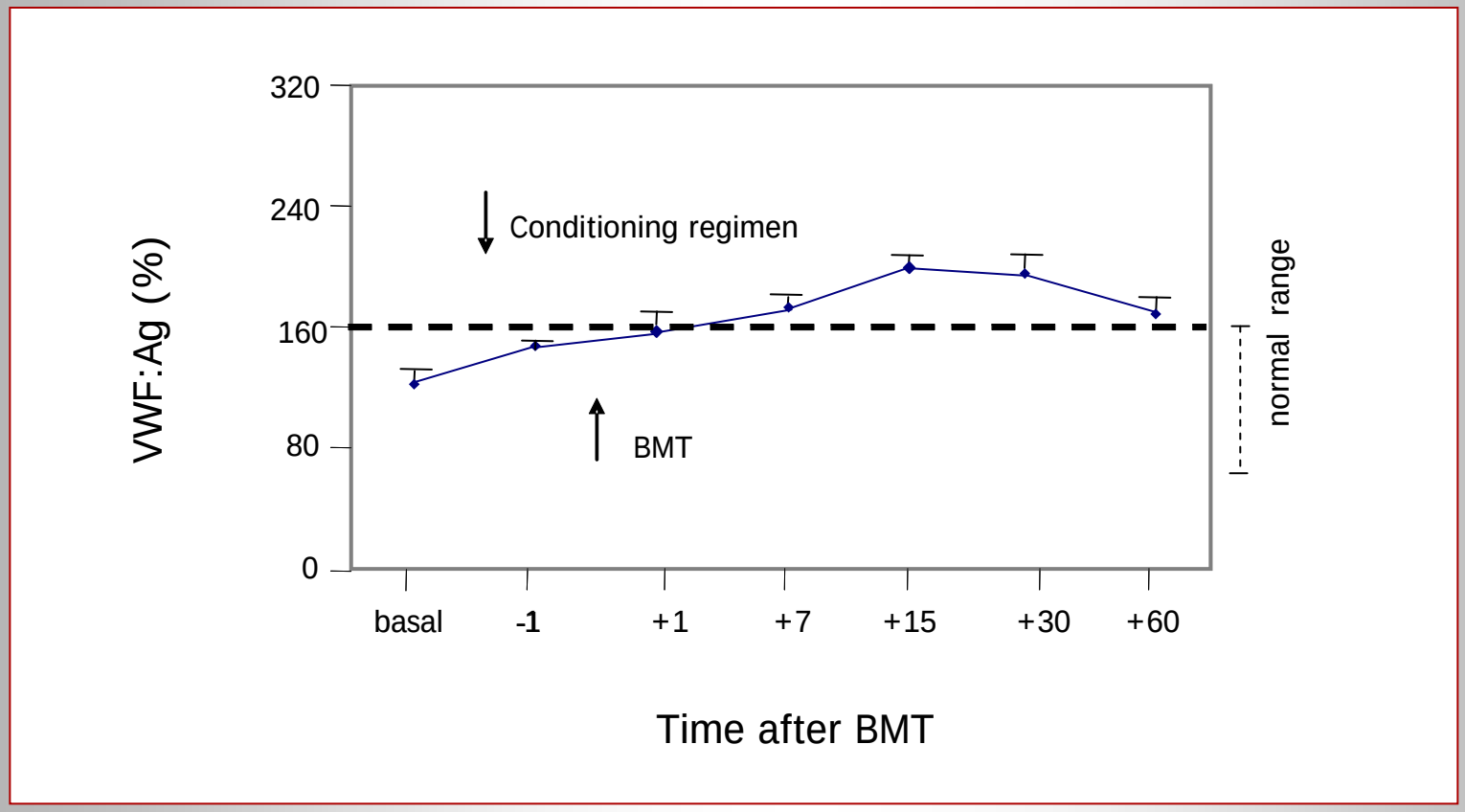
Death rate:	17% (8/46)
GVHD incidence:	16% (3/19 patients who underwent allo BMT)
Incidence of TMA after BMT:	6.5% (3/46)

ADAMTS13 at 7 different times after BMT



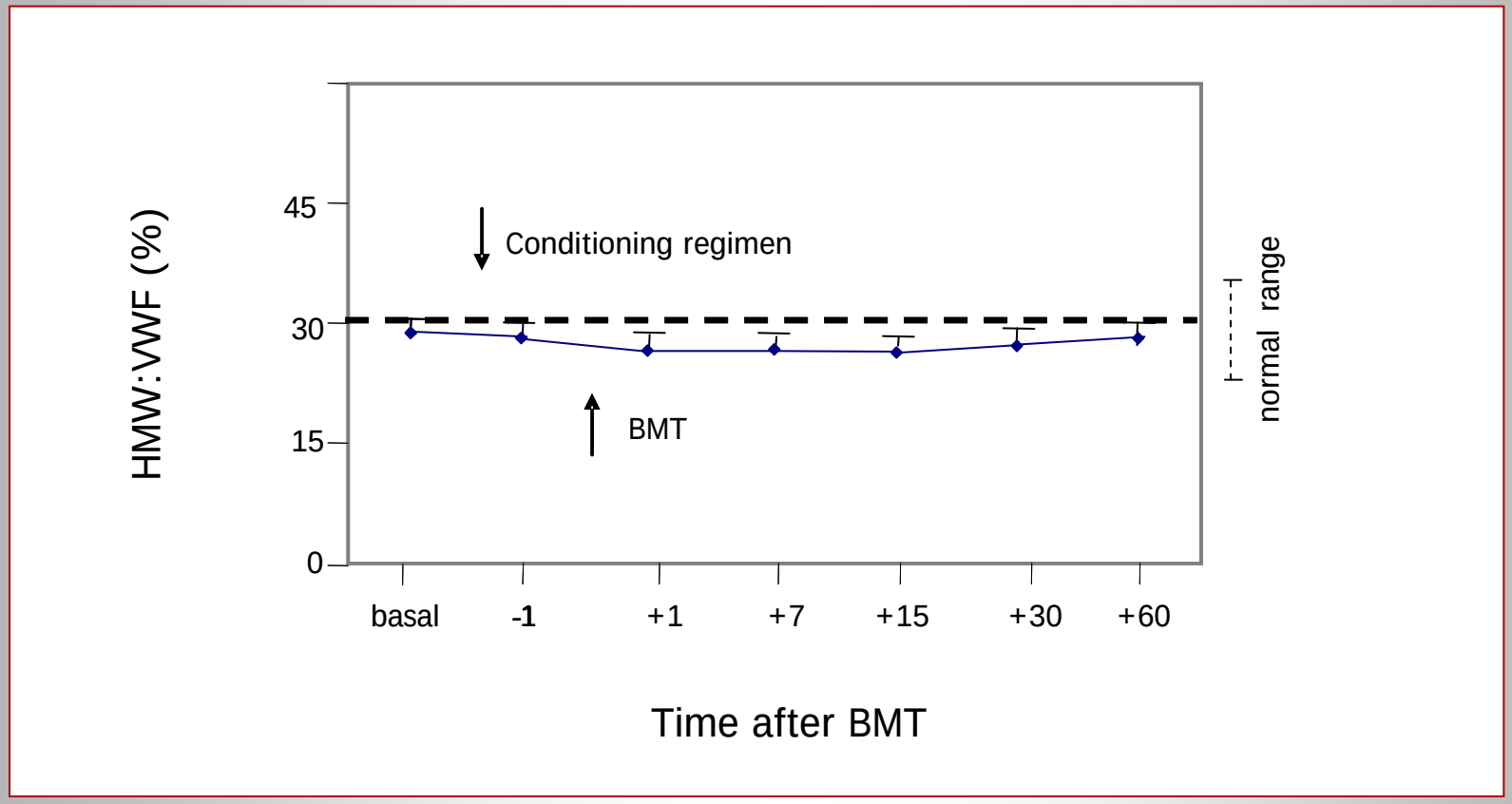
ADAMTS13 activity had already decreased after conditioning chemotherapy and remained lower than before conditioning until +30 day after BMT

VWF:Ag at 7 different times after BMT



There was a significant increase of VWF:Ag after conditioning chemotherapy on day -1 before BMT. High levels were sustained until day +60 with a peak on day +15

HMW:VWF at 7 different times after BMT



The mean percentage of HMW:VWF remained unchanged throughout the BMT period

Conclusions

- This and previous studies have found that **plasma levels of ADAMTAS13 were not dramatically reduced after BMT**

TREATMENT

Management:

- Discontinuation of the responsible drug
- Plasma exchange
- Corticosteroids

Poor prognosis

Conclusions

- ADAMTS13 levels in cancer patients may be decreased because of impaired protein synthesis as a result of:
  - direct tumor involvement of the liver and/or
  - the catabolic action of tumor related cytokines

- The mechanism of negative relationship between ADAMTS13 plasma levels and VWF antigen and its pathophysiologic significance remain to be established

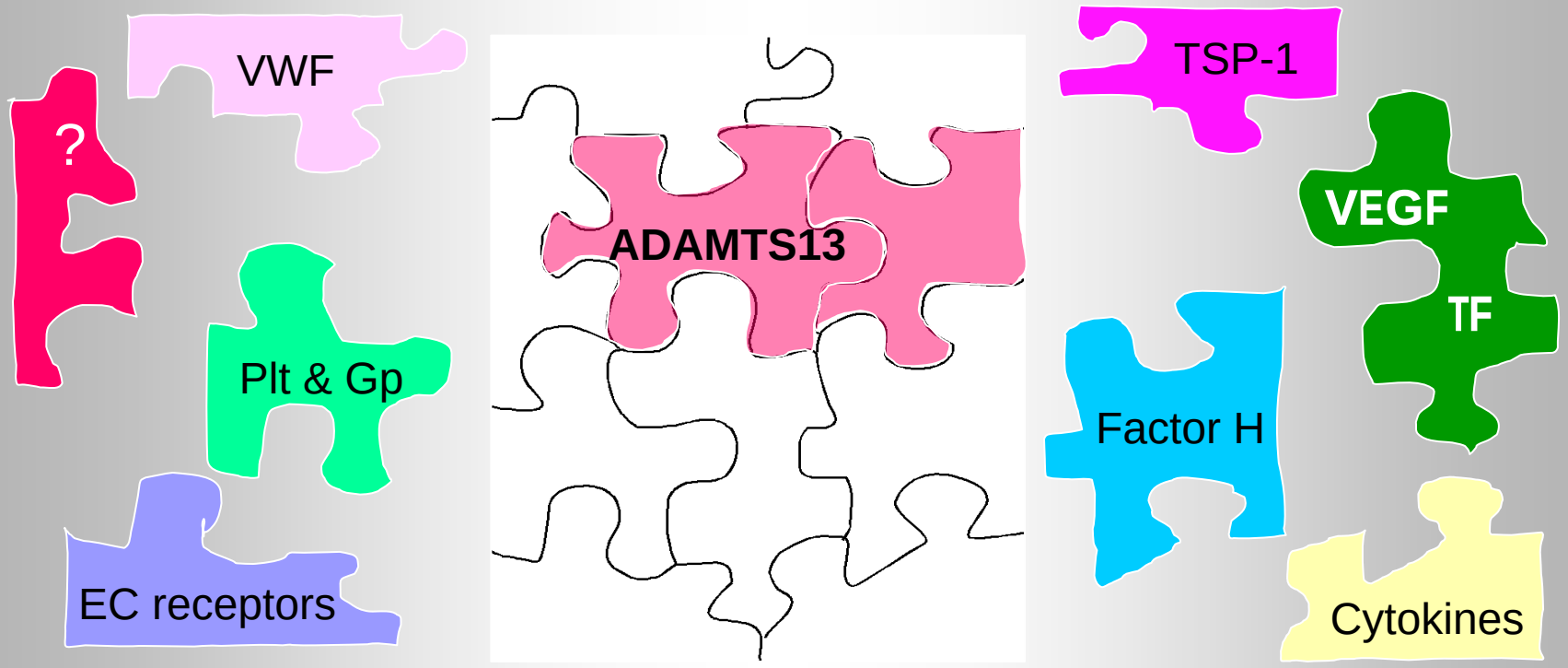
- The low levels of the protease affect the interactions between VWF, tumors cells, platelets and endothelials cells in the process of metastasis formation remains to be determined by animal experiments

Other factors involved in cancer related TMA

Pathol Res Pract. 2008 Oct 1, Chinen K, Fujino T, Horita A, Sakamoto A, Fujioka Y.
Pulmonary tumor thrombotic microangiopathy caused by an ovarian cancer expressing tissue factor and vascular endothelial growth factor.

Detailed molecular mechanisms underlying TMA cancer related remain unclear, but some studies have suggested that tissue factor (TF) and vascular endothelial growth factor (VEGF) expressed by tumor cells may be involved in the pathogenesis for cases of gastric cancer

Other factors



ADAMTS13 deficiency alone is not sufficient to explain the pathogenesis
of an acute TTP, particularly a cancer related TTP and other modulatory factors
should be considered

www.ttpdatabase.org