Von Willebrand Factor

both Friend and Foe

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The Fritsma Factor, Your Interactive Hemostasis Resource

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Thrombotic Microangiopathies (TMAs)

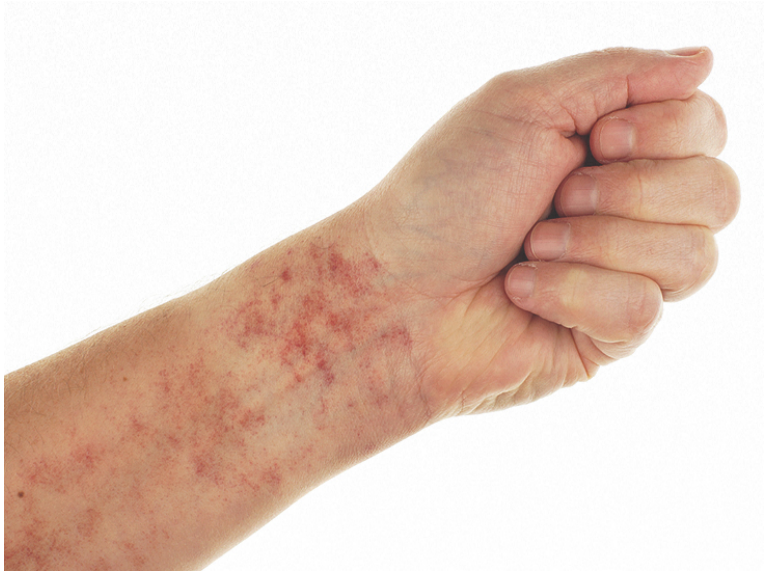
- Thrombotic thrombocytopenic purpura (TTP)
- Shiga-toxin producing Escherichia coli hemolytic-uremic syndrome (STEC-HUS)
- Atypical hemolytic-uremic syndrome (aHUS)
- Hemolysis, elevated liver enzymes and low platelets syndrome (HELLP)
- Von Willebrand factor in inflammation

Please silence your phone.

2-YO Boy with a TMA

- Recurrent bouts of bloody diarrhea, elevated WBC
  - No thrombocytopenia, no anemia
- Appendectomy on a Friday, released Sunday AM
- Grew sicker, some neurological changes
- Readmitted Sunday PM—no urine output, mild MAHA
- Cultured E. coli, strain determined later to be O157:H7
- Renal dialysis 2 weeks, every third day
- Released after 1 month

Nineteen-YO Woman with TMA

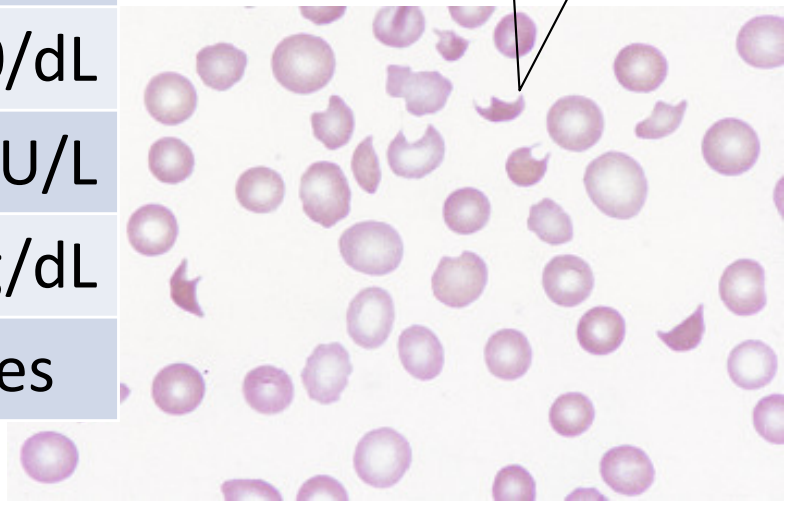


A nineteen-year-old woman came to the ED experiencing rapid onset fever, headache, confusion and weakness. The ED nurse recorded petechiae on her extremities.

19-YO ♀ TMA Relevant Labs

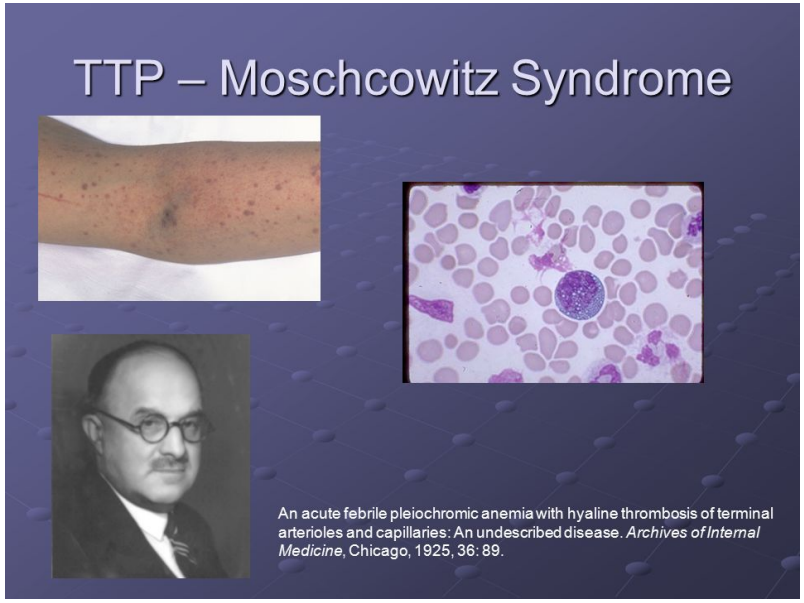
Test	Result	RI
HGB	9.8 g/dL	12.0–15.0 g/dL
HCT	31%	35–49%
MCV	78 fL	80–100 fL
PLT	21,000/dL	150–450,000/dL
LD	420 U/L	140–280 U/L
Creatinine	1.1 mg/dL	0.6–1.2 mg/dL
RBC morphology		2+ schistocytes

Microangiopathic hemolytic anemia (MAHA), schistocytes



19 YO ♀ Presumptive Diagnosis

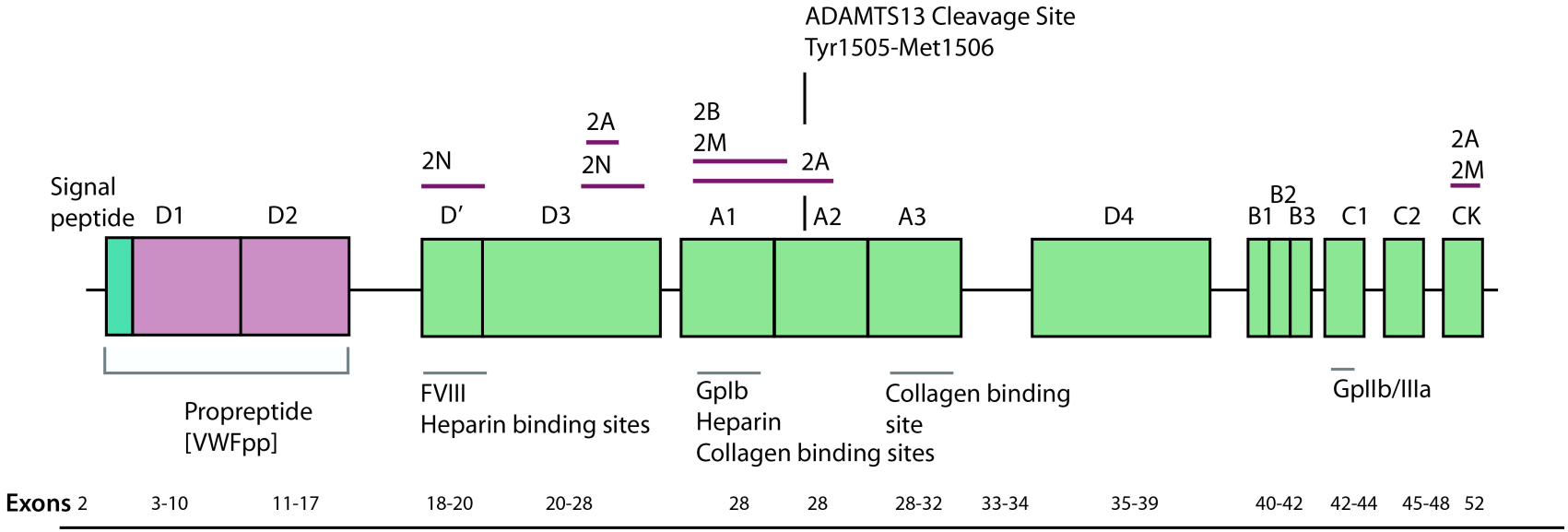
- Thrombotic thrombocytopenic purpura (TTP)
- Moschcowitz, 1924 described a 16-YO ♀ with petechiae, thrombocytopenia, hemiparesis and pulmonary edema
- 70/30 ♀, typical of autoimmune disorders
- "Classic pentad:" marked thrombocytopenia, MAHA, neurologic changes, fever, renal insufficiency
- Now defined by MAHA with elevated lactate dehydrogenase (LD) and thrombocytopenia
- Once rare, now dramatically increasing incidence
- Was 90% fatal, now 20% fatal



Lopez JA, Chen J, Ozpolat HT, Moake JL, Chung DW. Ch 34: Thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. In Kitchens CS, Kessler CM, Konkle BA, Strieff MB, Garcia DA. Consultative Hemostasis and Thrombosis, 4th Edition. Elsevier 2019.

The TMA Culprit: VWF

- Chromosome 12p13;31, 178 kb, 52 exons
- mRNA specifies polypeptide of 2813 amino acids made of…
- 22-aa signal peptide, 742-aa propeptide, and 2050-aa monomer



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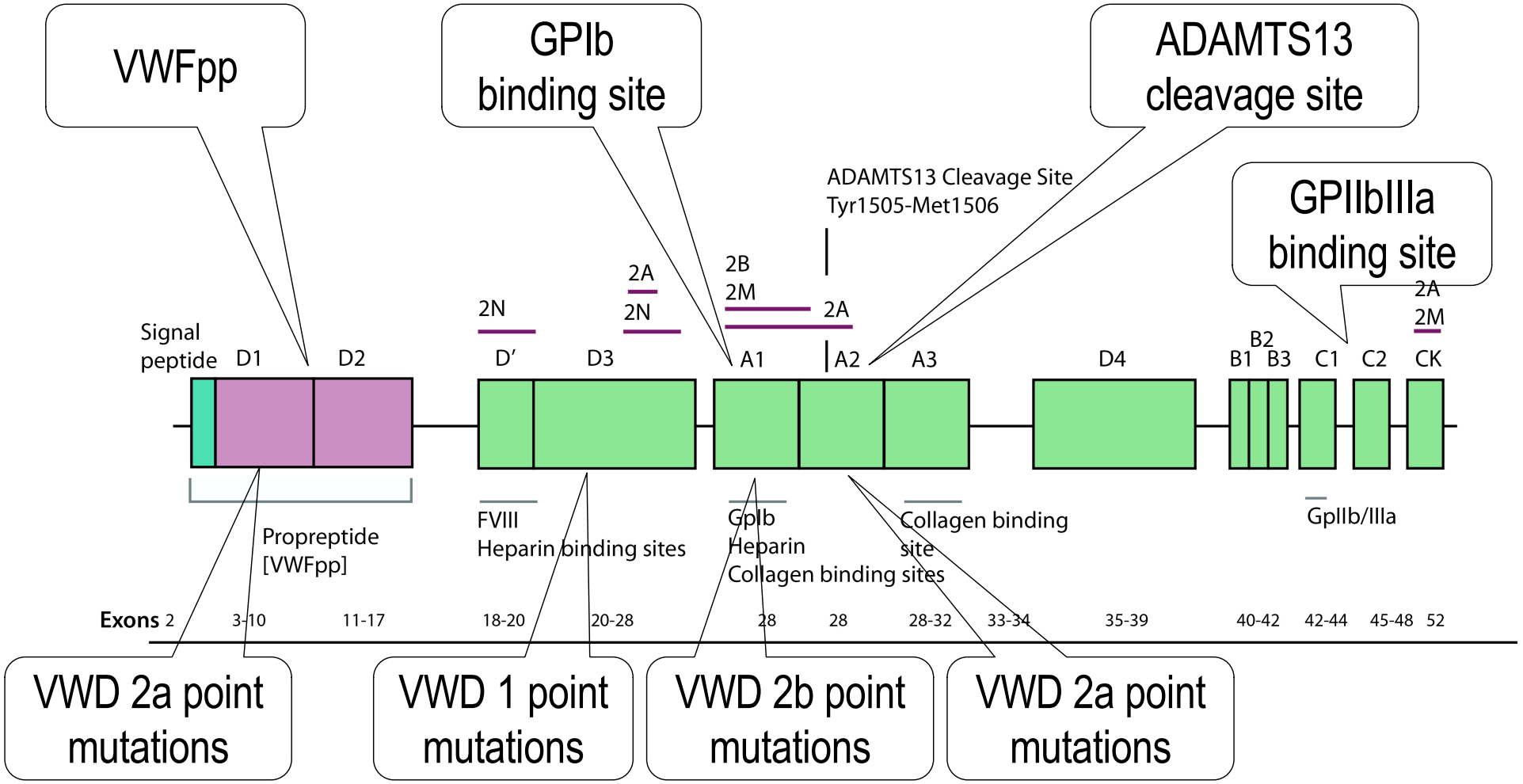
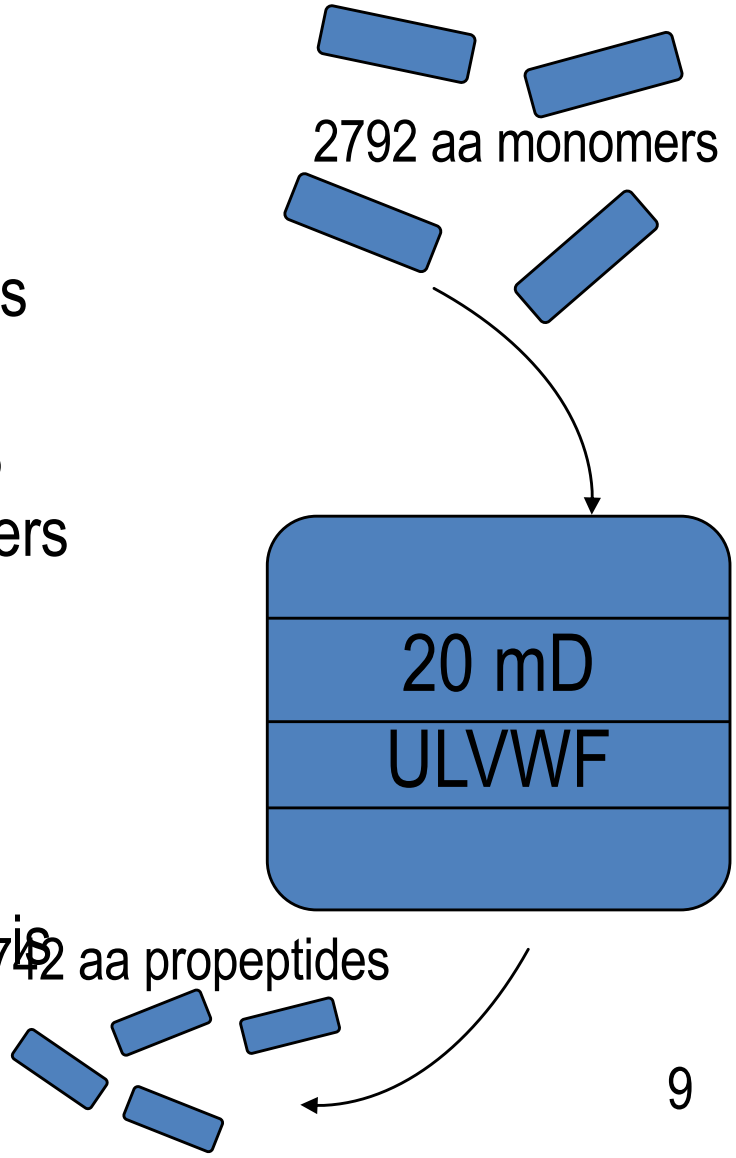
VWF Monomer



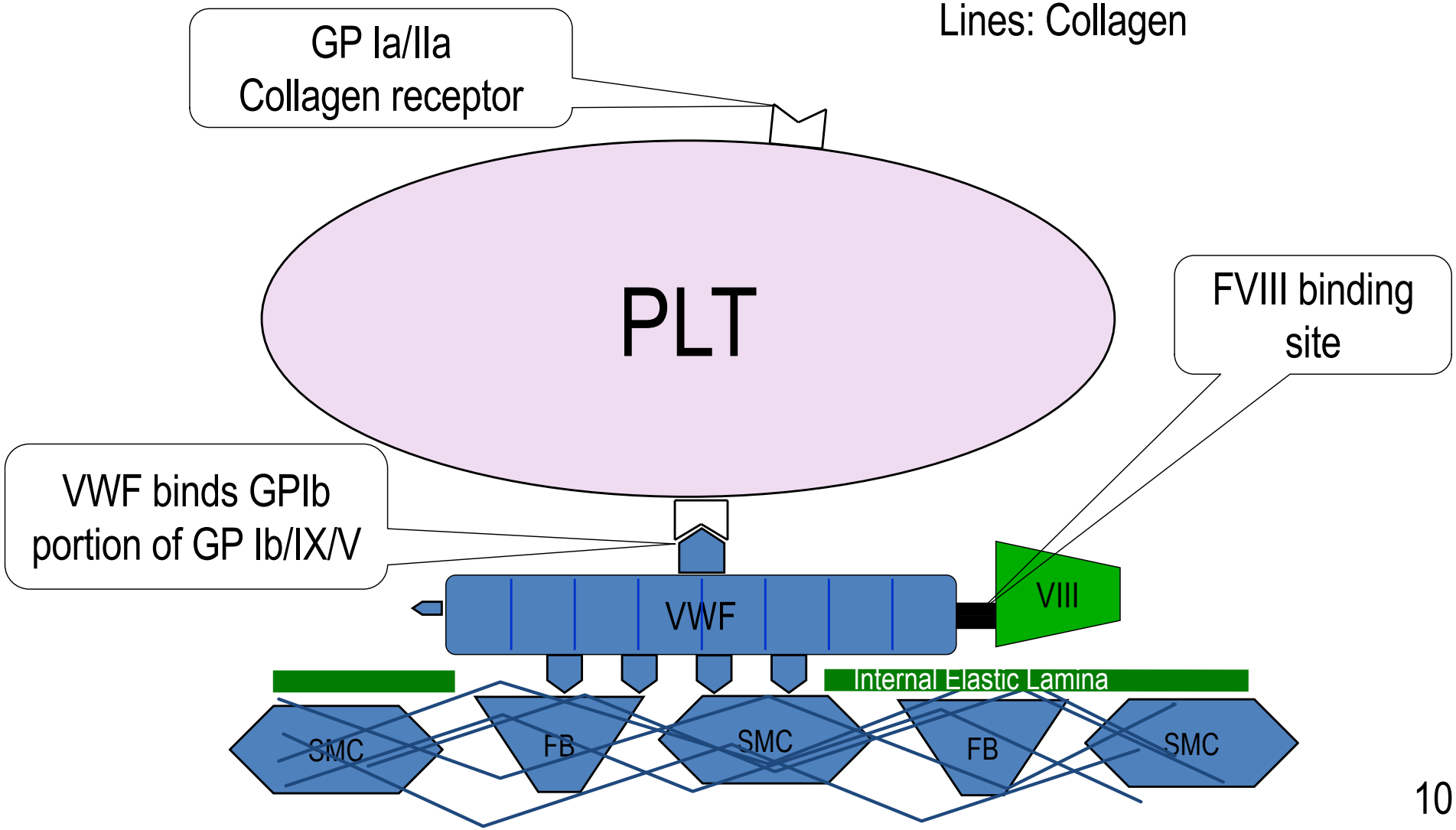
Monomer Assembly in Endothelial Cells

- 22-aa signal peptide is cleaved in endoplasmic reticulum after translation
- 742-aa propeptide-containing monomers dimerize via disulfide linkage of Cys residues near the C-terminus
- In Golgi apparatus, propeptides catalyze D3 region disulfide bonds, creating long multimers composed of dimers arranged head-to head
- The propeptide is then cleaved and the resultant ultra-large VWD multimers are package in Weibel-Palade bodies
- Propeptide may be measured (VWFpp) and is not affected by blood group



VWF-Platelet Interaction

SMC: Smooth muscle cell
FB: Fibroblast
Lines: Collagen



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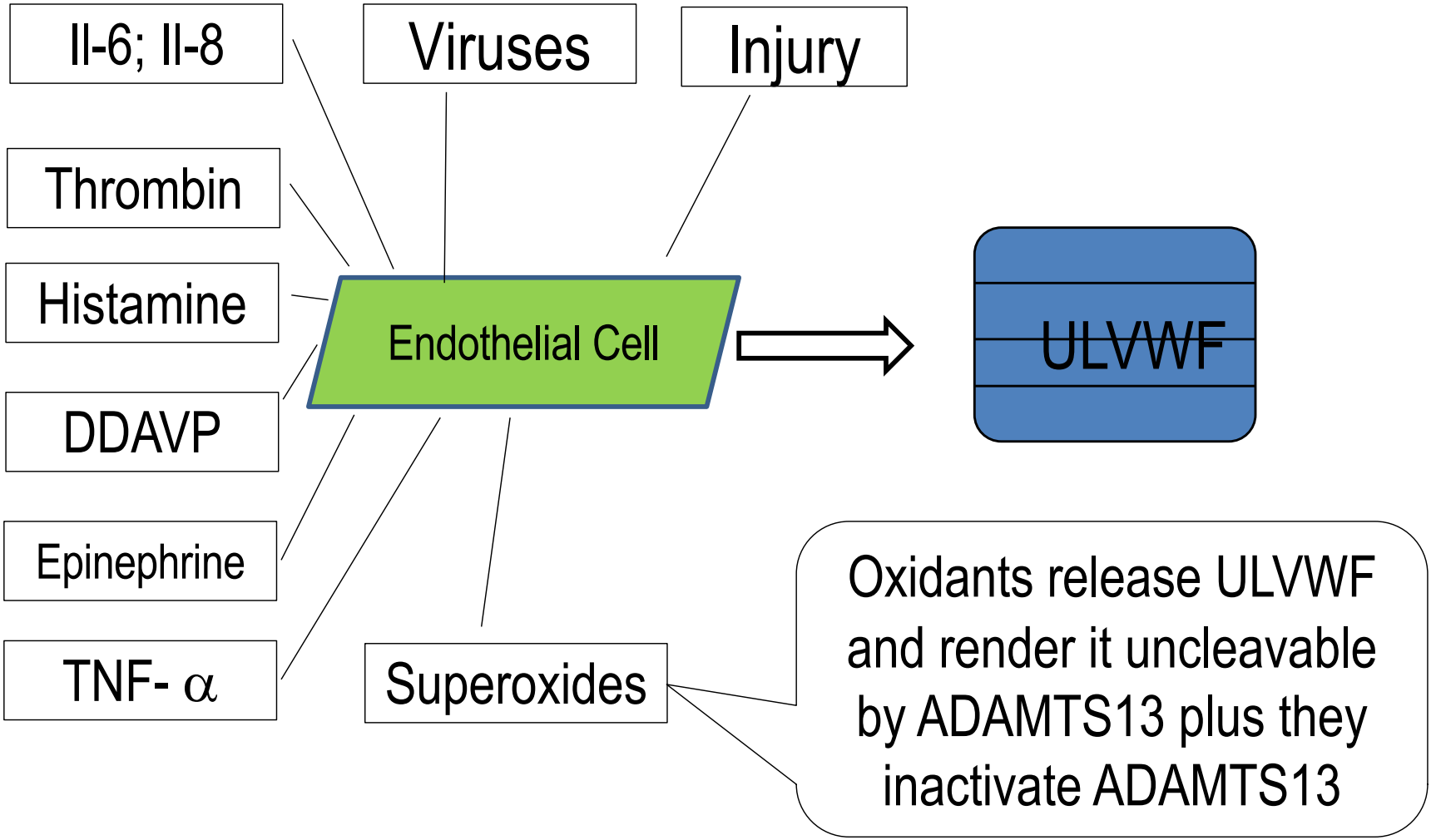
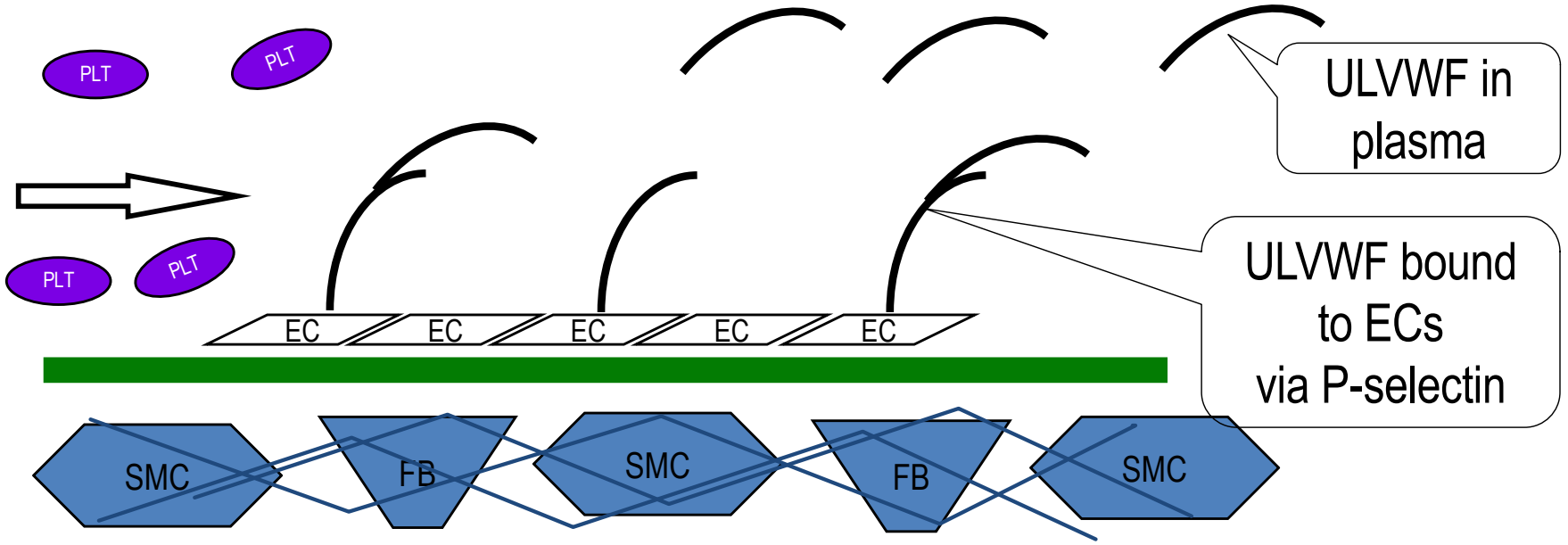
Endothelial Cell Secretes ULVWF

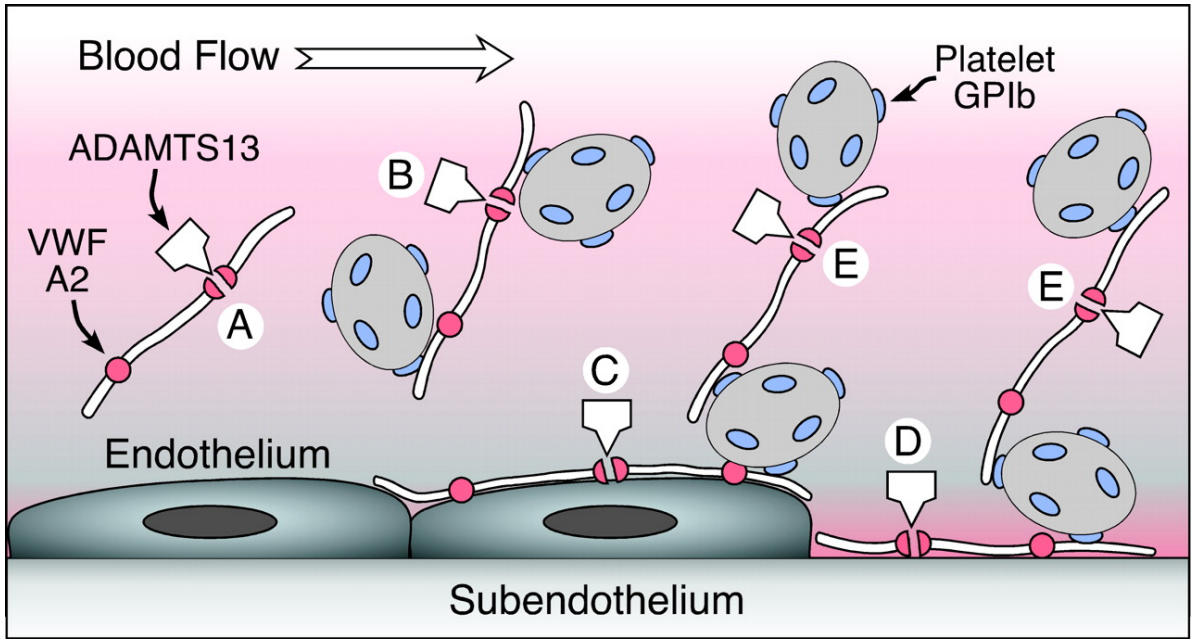


ULVWF Secreted from Endothelium



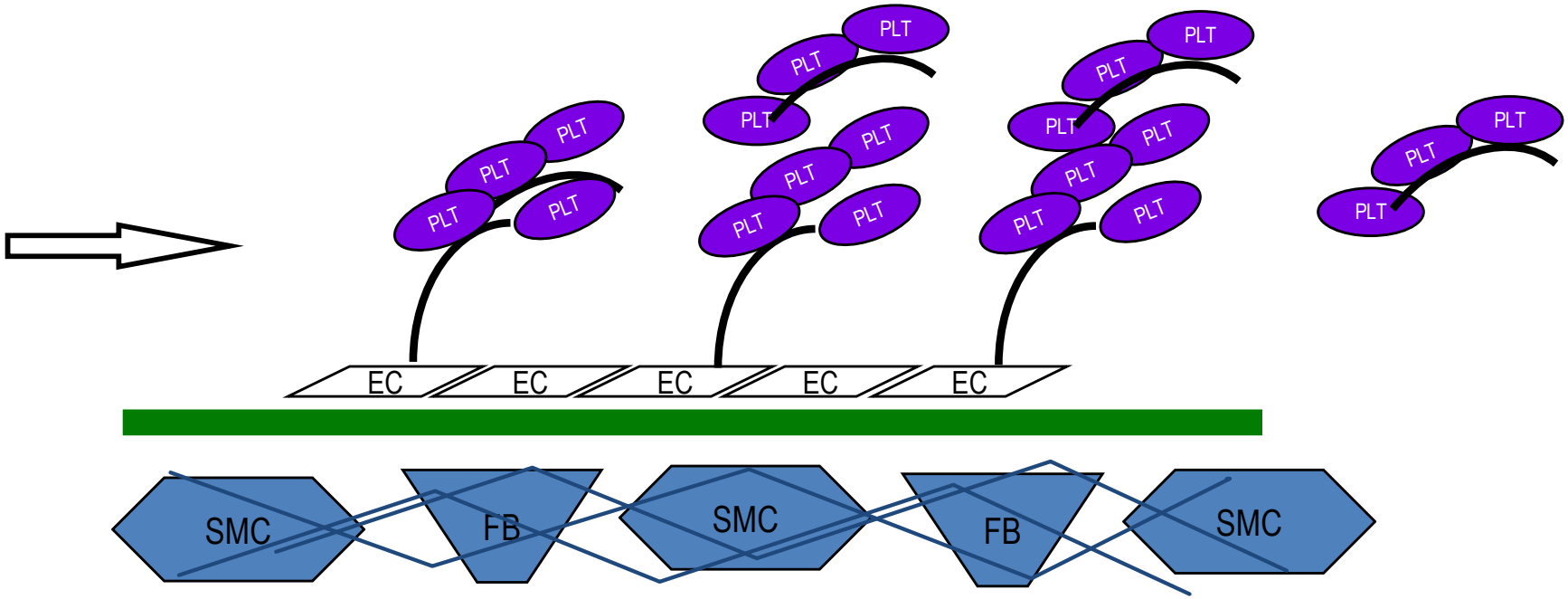
In high shear-stress arterioles ULVWF strands are released to the plasma or remain attached to ECs via P-selectin and other adhesion molecules. Whether in plasma or bound, they bind platelets via GP Ibα, a segment of GP Ib/IX/V with little platelet energy expended.

Platelet Strings



ULVWF is released from ECs and diffuses into the circulation (A and B) or adheres to the EC (C). ULVWF also binds connective tissue exposed at sites of vascular injury (D). Under high shear stress, platelets adhere to VWF in solution (B) or on surfaces (C and D) through their GPIbα receptor. ULVWF also binds to previously adhering platelets (E). ADAMTS13 cleaves a Tyr–Met bond in the A2 domain of the ULVWF subunit. This reaction is slow for VWF in solution (A) but occurs rapidly owing to conformational changes induced by tensile force on the ULVWF multimer.

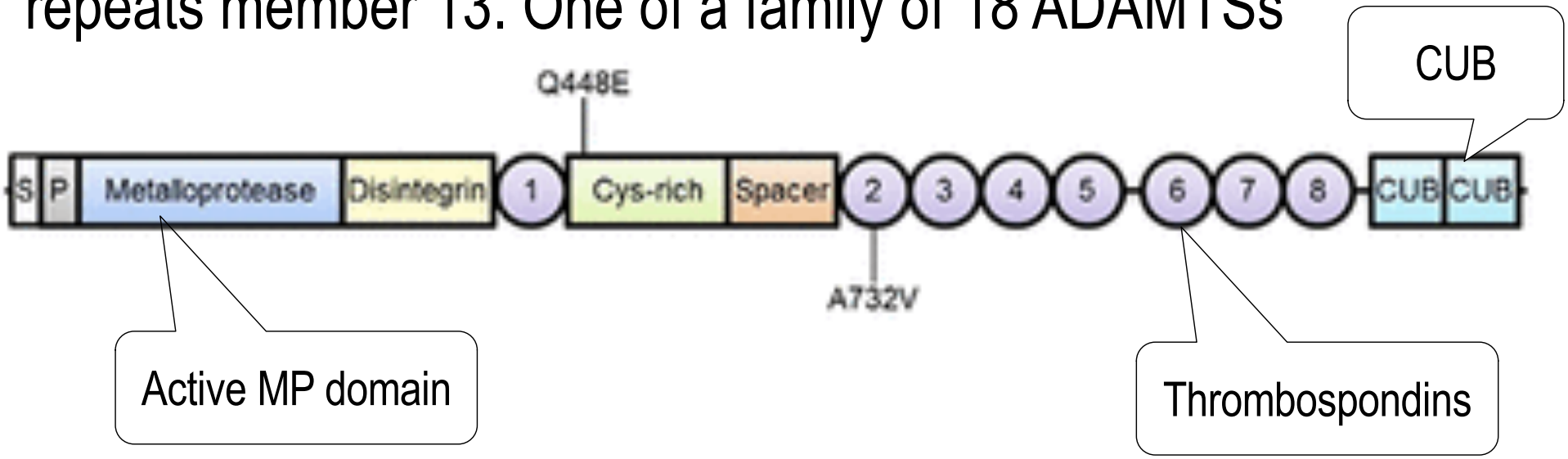
ULVWF Bind Platelets in High Shear Stress Areas



Platelets bind ULVWF forming beaded strings. The strings stack and associate with collagen through platelet collagen receptors and may occlude the vessels. String length is attenuated by HDL.

What Prevents Occlusion? ADAMTS13

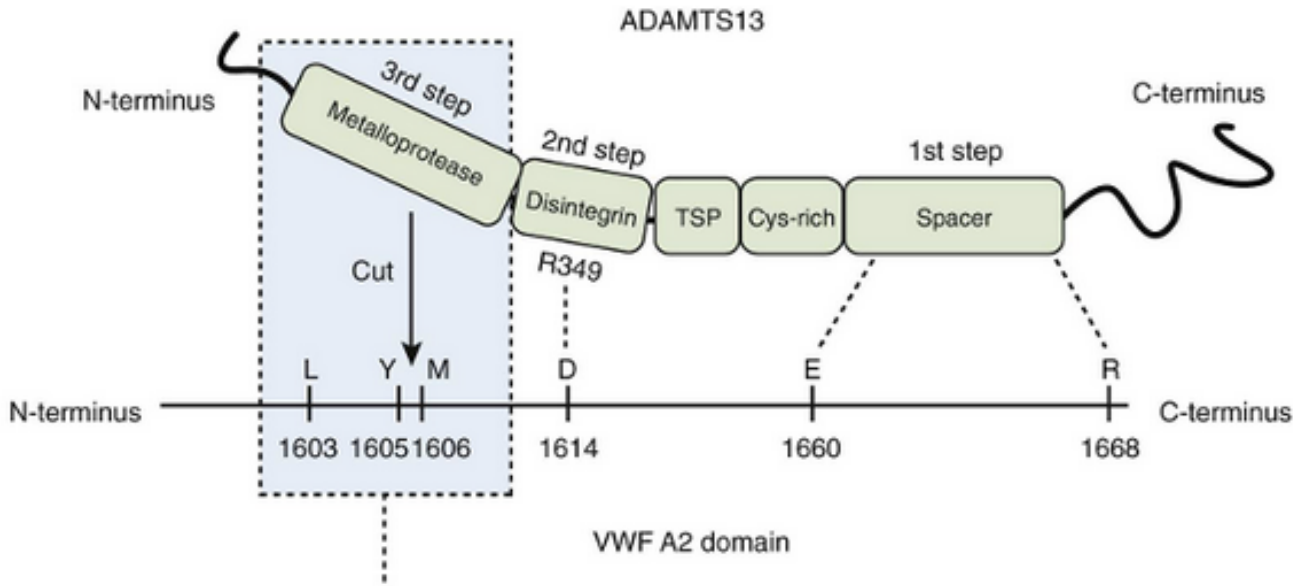
A **d**isintegrin **a**nd **m**etalloprotease with **t**hrombo**s**pondin-1 repeats member 13. One of a family of 18 ADAMTSs



CUB = "**c**omplement components C1r/C1s, embryonic sea **u**rchin protein endothelial growth factor, and **b**one morphogenic protein"

ADAMTS13

- Synthesized from chromosome 9q34 in liver, 190,000 Daltons
- ULVWF A2 domain stress exposes Tyr 1605–Met 1606.
- ADAMTS13 attaches to ULVWF in “zipper” fashion
  - Digests ULVWF
  - Incidentally, A2 domain mutations enhance ADAMTS13 digestion, causing VWD subtype 2A



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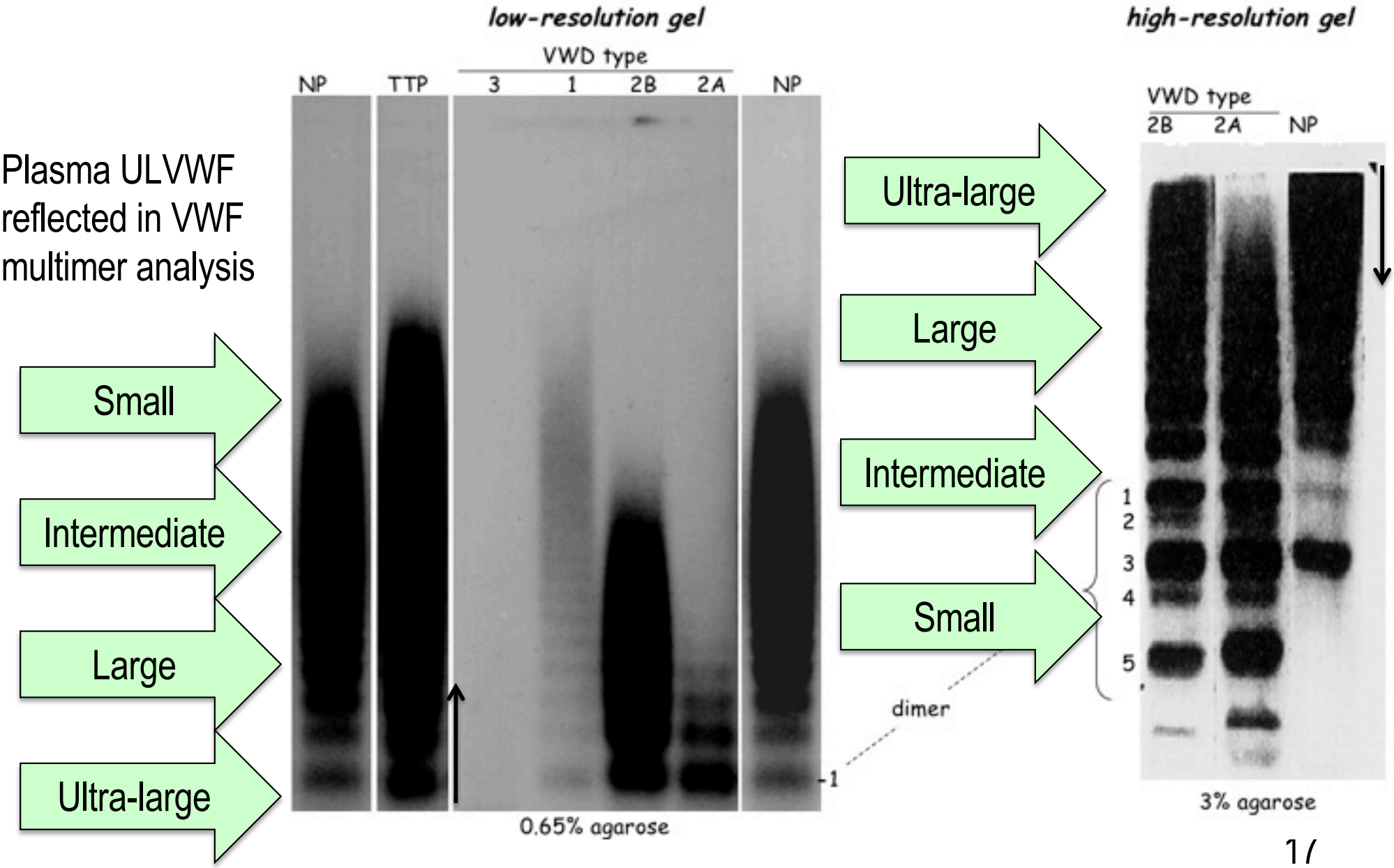
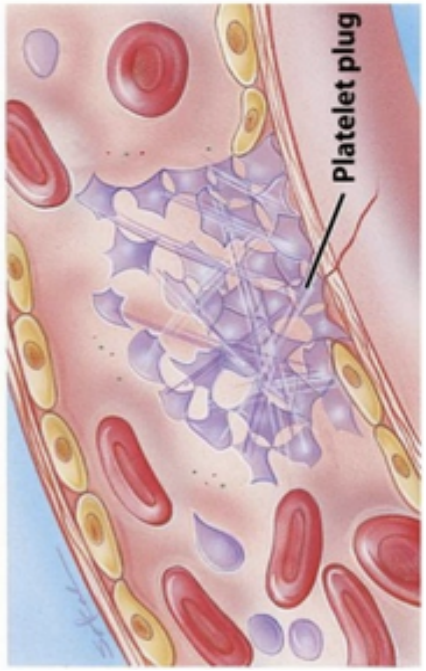
TTP: Reduced ADAMTS13

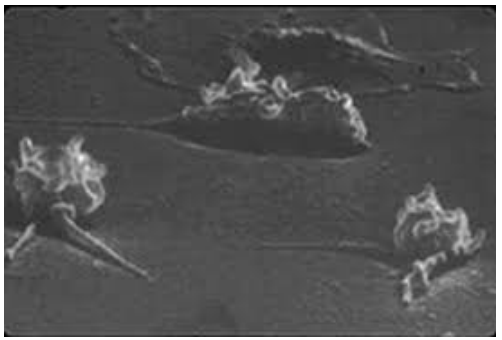
Plasma ULVWF reflected in VWF multimer analysis



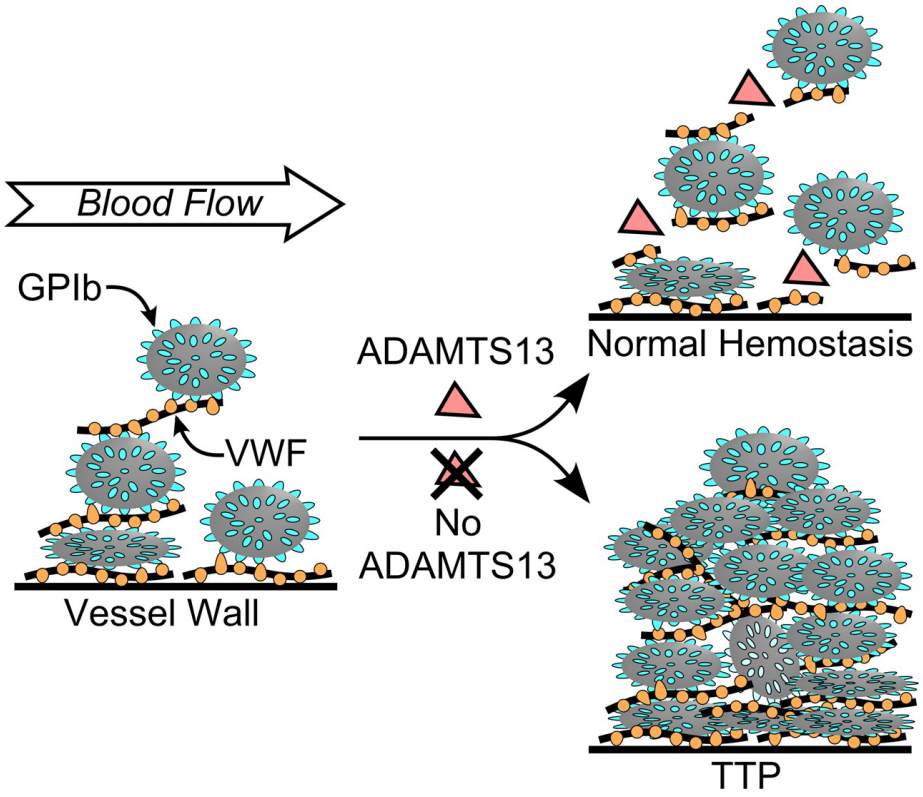
TTP: Reduced ADAMTS13

- Platelets do not bind ADAMTS13-digested plasma VWF (mechanism under study)
- Familial recurrent “rTTP”—10%: congenital ADAMTS13 abnormality or deficiency, <5% activity detected in assay. ULVWF consistent in plasma, childhood symptoms, remission is temporary
- Autoimmune (idiopathic) “iTTP”—90%: episodic deficiency of ADAMTS13 and rise of ULVWF, remission usually sustained
- Accumulated ULVWF/PLT complex occludes vessels, consumes and activates PLTs and splits RBCs, accounting for thrombocytopenia and MAHA



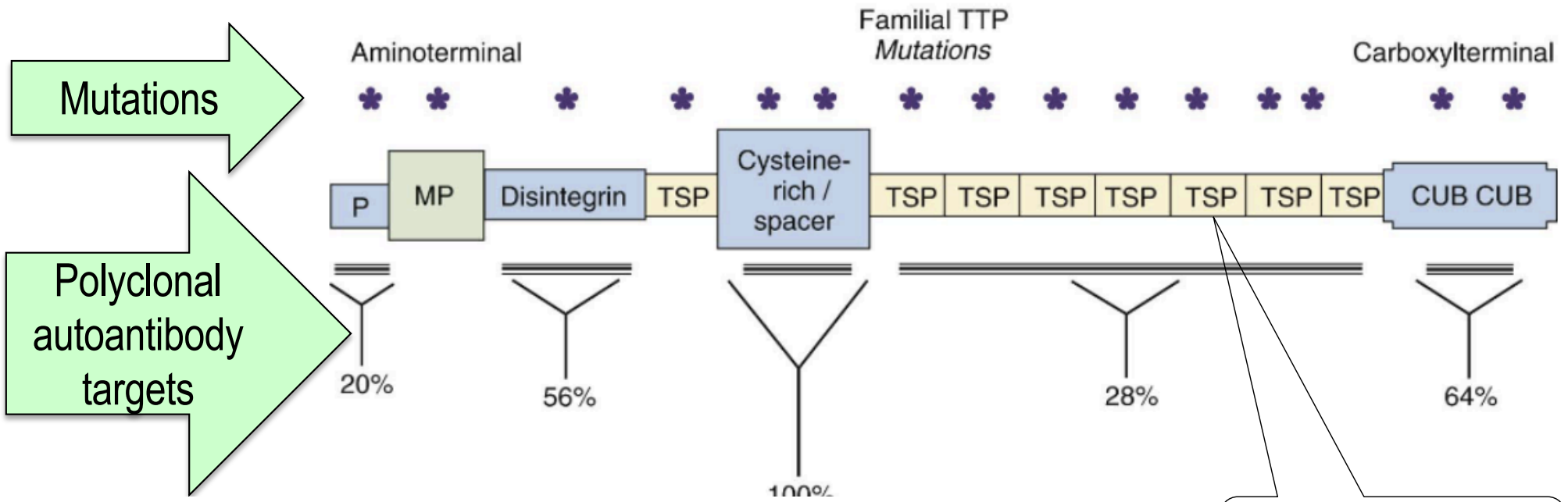


Familial TTP in Pregnancy



- Deficient ADAMTS13 but remains adequate: asymptomatic
- Pregnancy (estrogen) enhances ULVWF secretion
- ULVWF exceeds capacity of ADAMTS13, symptoms appear
- Infants with TTP: renal failure
  - "Upshaw-Schulman syndrome"

Congenital Vs. Acquired (Idiopathic) TTP



IgG 4 autoantibodies, possible familial distribution, all bind the cysteine-rich spacer residue among other portions of the molecule

19-YO ♀, Presumptive TTP
Differential Diagnosis: Various TMAs

- Atypical hemolytic-uremic syndrome (aHUS)
- Shiga toxin E. coli-triggered hemolytic-uremic syndrome (STEC-HUS)
- Hemolysis, elevated liver enzymes, low platelets (HELLP)
- Disseminated intravascular coagulation (DIC)
- Familial recurrent thrombotic thrombocytopenic purpura (rTTP)
- Acquired autoimmune (idiopathic) thrombotic thrombocytopenic purpura (iTTP)

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19-YO ♀, Presumptive TTP "PLASMIC" Score

Criteria	Patient	Points
Platelet count <30,00/uL	15,000.uL	1
Hemo**l**ysis (MAHA, LD)	LD 420 U/L	1
No **a**ctive cancer in previous year	✅	1
No **s**olid organ or stem cell transplant	✅	1
MCV <90 fL	78 fL	1
INR <1.5	1.2	1
Creatinine <2.0 mg/dL	1.1 mg/dL	1
Maximum 7 points, 6–7 indicates TTP, <6 indicates HUS or HELLP		

Li A, Khalighi PR, Wu Q, et al. External validation of the PLASMIC score: a clinical prediction tool for TTP diagnosis and treatment. J Thromb Haemost. 2018;16:169–9.

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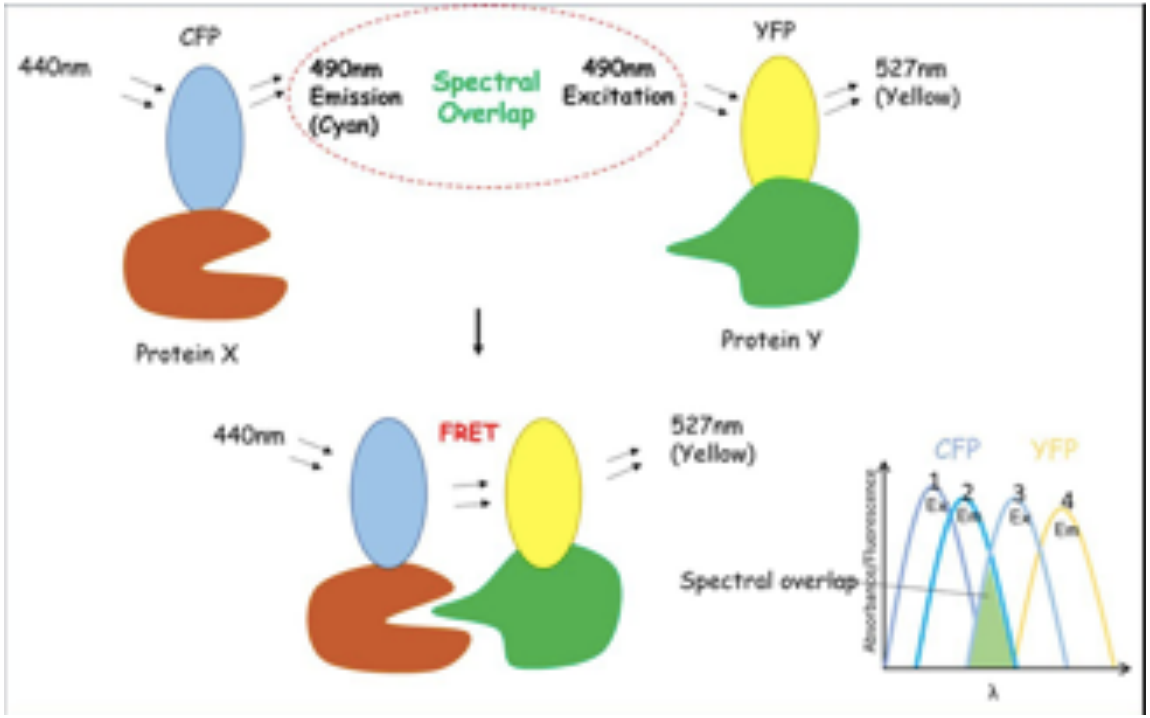
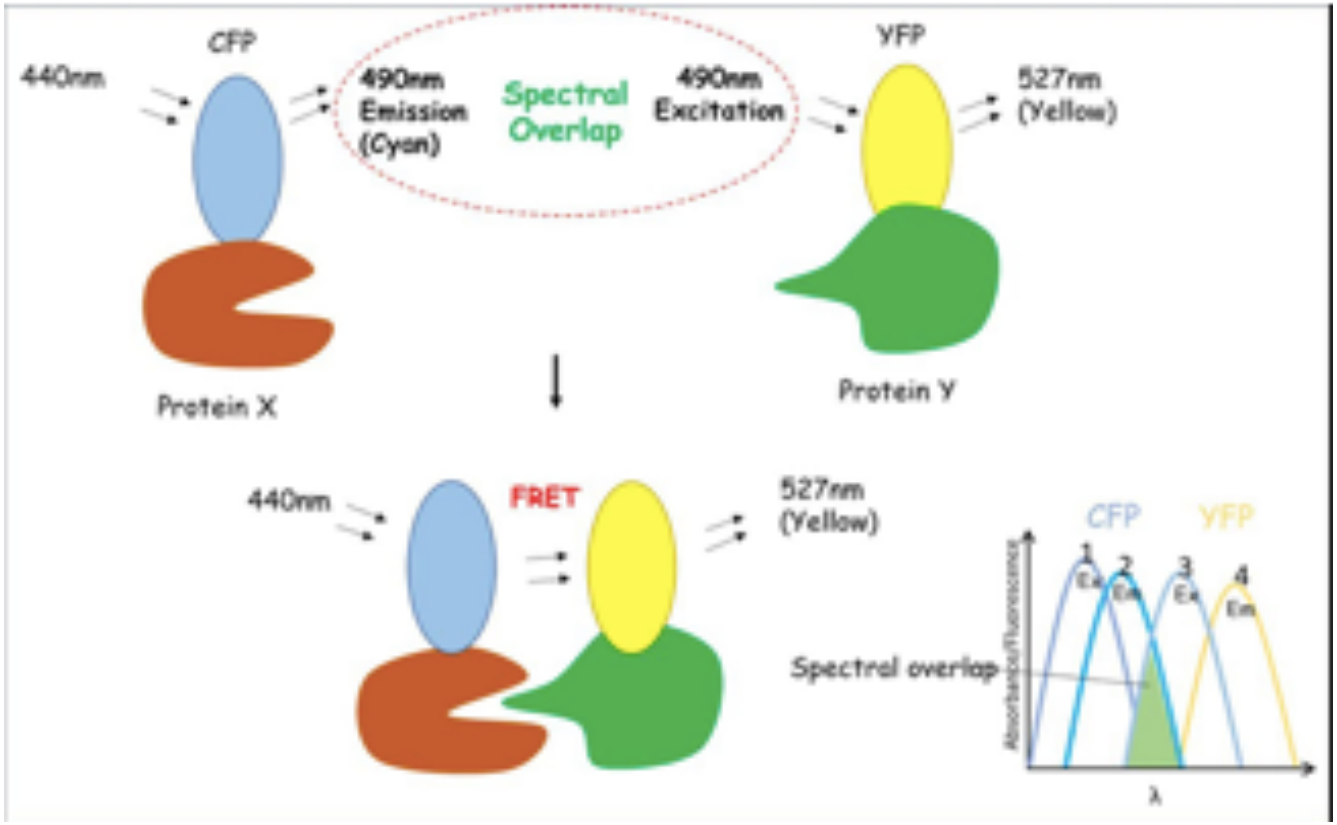
ADAMTS13 Activity Assay FRET-rVWF71

- Fluorescence resonance energy transfer
- Synthetic VWF peptide: 71 aa from the A2 domain
- Serum, heparinized or citrated plasma, not EDTA
- No bilirubin, HGB, or plasma VWF multimer interference
- Does not measure shear force effects



Jones GA, Bradshaw DS. Resonance energy transfer: From fundamental theory to recent applications. Frontiers in Physics. 2019;7:100. doi:10.3389/fphy.2019.00100.

Fluorescence Resonance Energy Transfer



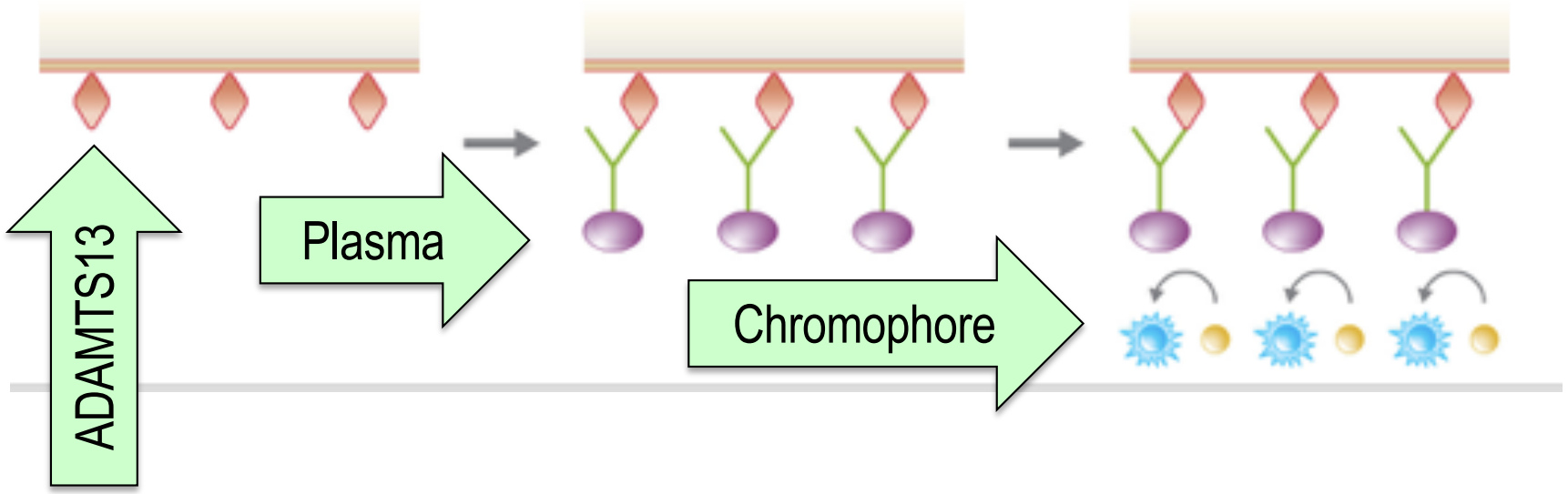
1. Fluorophore CFP excites at wavelength 440nm, emits at 490nm (cyan)
2. Fluorophore YFP excites at 490nm, emits at 527nm (yellow)
3. Emission is a function of distance between the fluorophores
4. Photon (non-radioactive) exchange between fluorophores when adjacent
5. ADAMTS13 (Protein X) binds target VWF71 (Protein Y)
6. Emission intensity is linear with ADAMTS13 activity

ADAMTS13 Antigen Immunoassays

- Seven solid-phase polyclonal or monoclonal enzyme immunoassays available
- WHO international ADAMTS13 standard 12/252, 2015 surveys
  1. Mean ADAMTS13 activity 0.91 U/mL, CV 12.4%
  2. Mean ADAMTS13 antigen 0.92 U/mL, CV 16.3%
- Large CVs reflect antigen epitope recognition and avidity differences

Auto-anti-ADAMTS13 Immunoassay

- Heat-inactivated patient plasma
- Non-antibody ADAMTS13 inhibitors
  - HGB, IL-6, thrombospondin 1, all block the A2 domain
  - Not detected in the auto-anti-ADAMTS13 assay

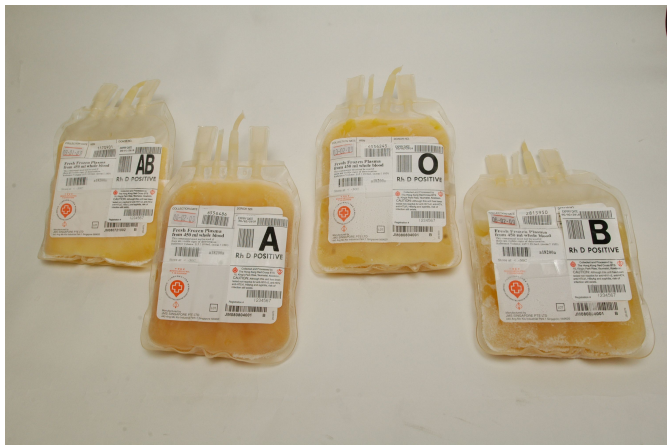


19-YO ♀ TMA Diagnosis

- ADAMTS13 activity by FRET: <5%
- ADAMTS13 concentration by antigen assay: <5%
- Auto-anti-ADAMTS13 immunoassay: 1:64
- Diagnosis: iTTP

Familial TTP Therapy

- Originally: fresh-frozen plasma (FFP) and cryosupernatant restored normal VWF multimers and reversed symptoms
- Relapse in three weeks, repeated infusions
- Initiated plasma exchange (PLEX)



Byrnes JJ, Khurana M. Treatment of thrombotic thrombocytopenic purpura with plasma. N Engl J Med. 1977;297:1386–89.
Moake JL, Brnes JJ, Troll JH, et al. Effects of fresh-frozen plasma and its cryosupernatant fraction on von Willebrand factor multimeric forms in chronic relapsing thrombotic thrombocytopenic purpura. Blood. 1985;65:1232–6.

Standard iTTP Therapy: PLEX

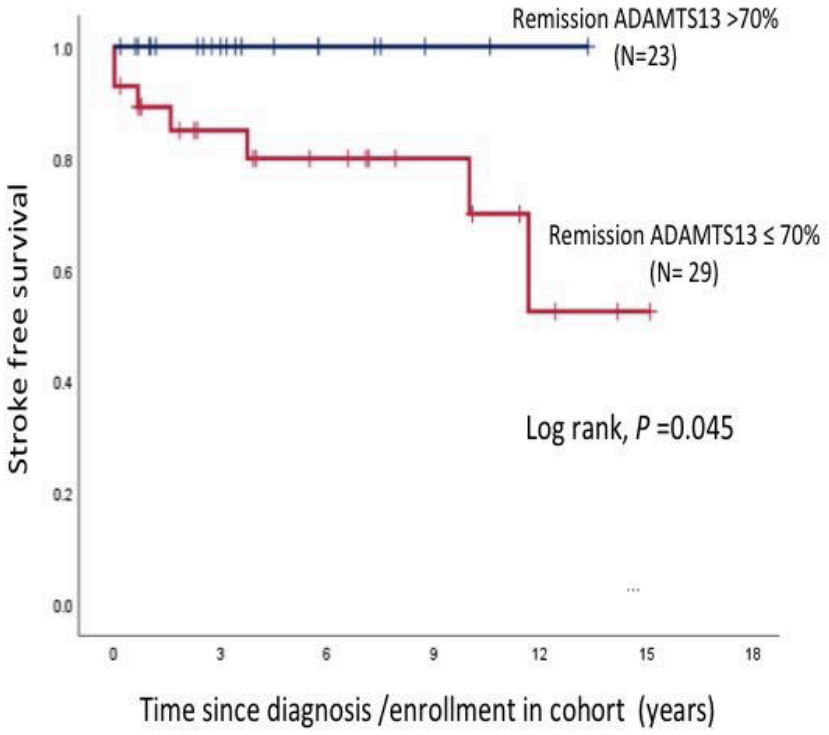
D/C triggers ticlopidine, clopidogrel, quinine	Emergent: FFP at 30 mL/kg/day until PLEX is started	High-dose glucocorticoids or rituximab anti-CD20
PLEX daily, BID if refractory, Rituxan® post-PLEX	Follow efficacy daily w/ PLT counts & LD	Assess remission: symptoms, PLT counts, LD, MAHA, HGB
Continue PLEX 3 days post-remission	PLEX plus steroids or rituximab 80–90% effective	Remeasure ADAMTS13 when in remission

Rituxan

- TTP care with PLEX alone associates with a median 8.2-year reduction in life expectancy and a total cost of $389K direct care and $259K indirect cost including loss of productivity and early mortality.
- Rituxan® (anti-CD20) raises the total cost by $13K but provides a 2.6-year improvement in life expectancy.

iTTP and Stroke

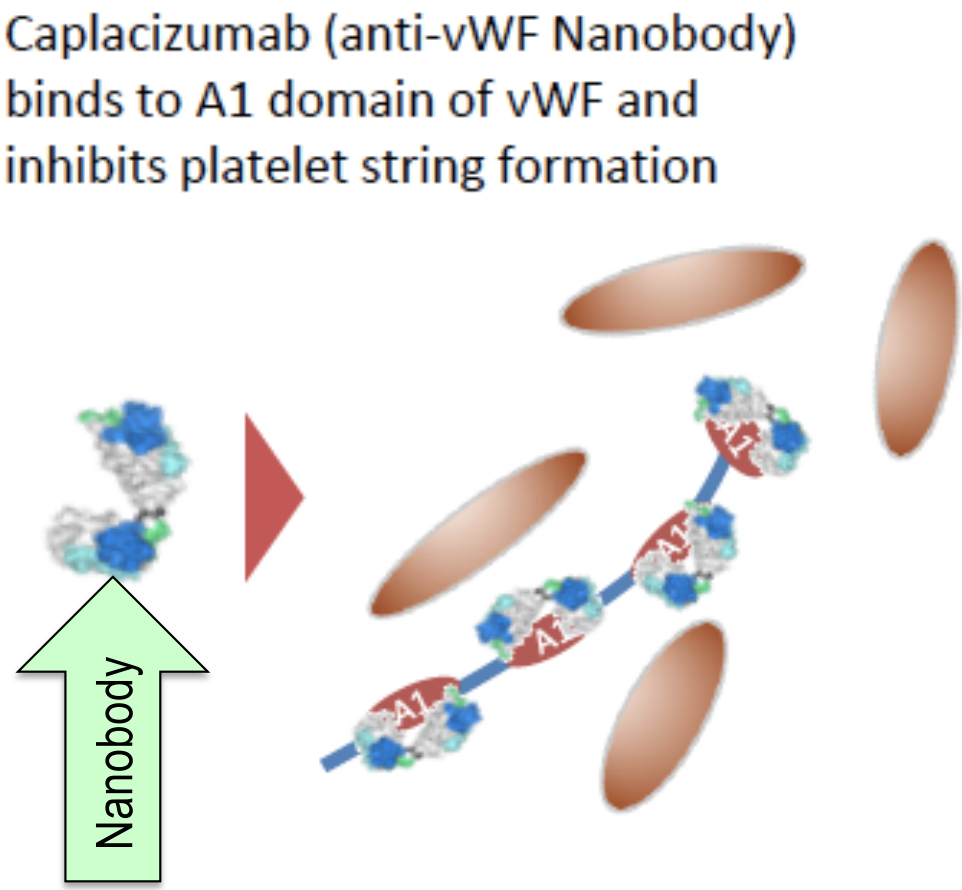
- ADAMTS13 activity (FRET) ≥90 days in 52 PTs after stroke
- ADAMTS13 activity was <10% in 7.7%, 10–39% in 25%, 40–70% in 23.1%, and >70% in 44.2%.
- Stroke rate higher in patients with ≤70% Vs >70% ADAMTS13 activity during remission. 27.6% vs 4.3% (P=0.030)



Chaturvedi S, Upreti H, Dane K, Kasmani J. Persistently low ADAMTS13 activity in remission is associated with stroke in TTP survivors. Abstract

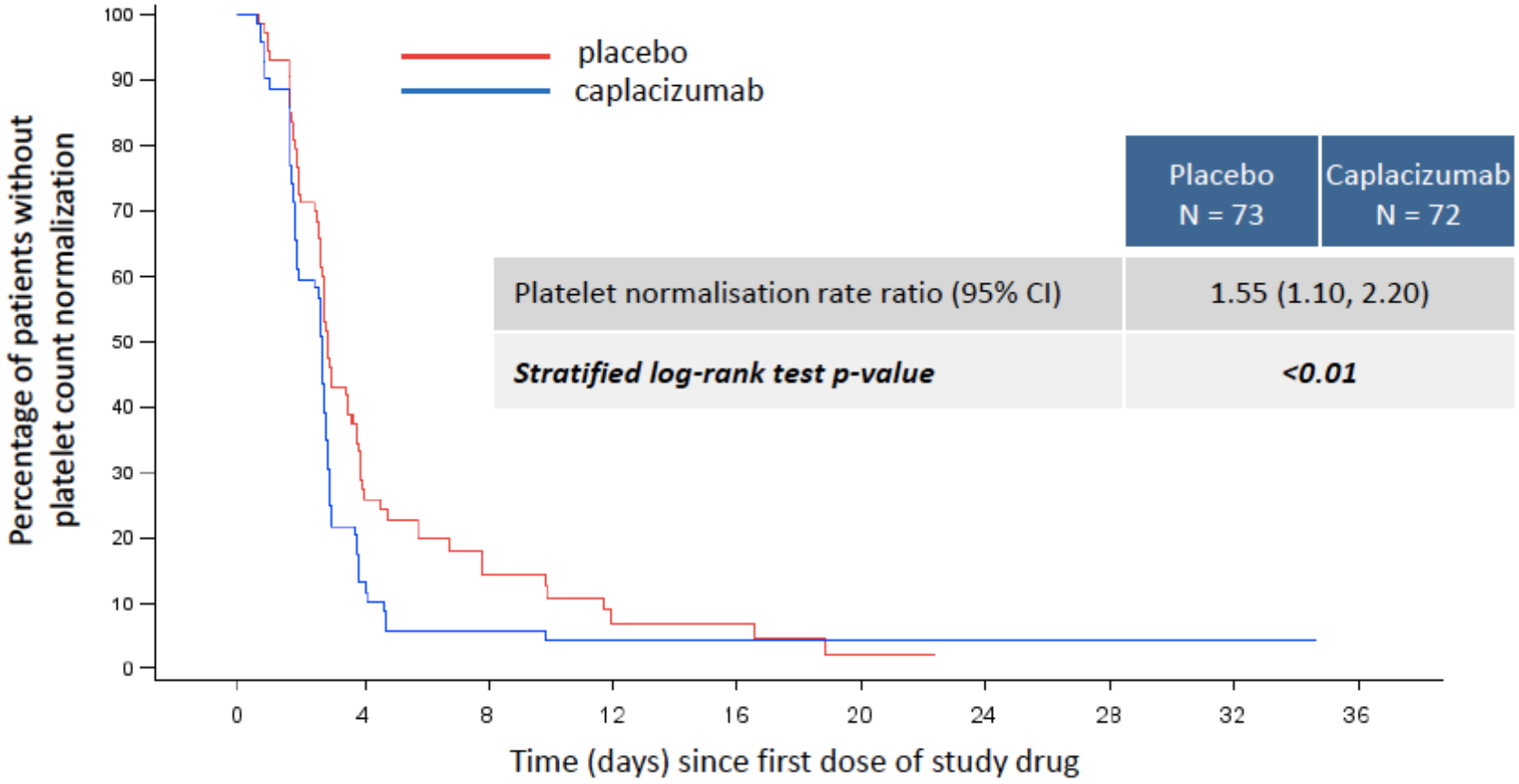
Caplacizumab (Cablivi®) for iTTP

- HERCULES trial, phase III
- FDA-approved Jan, 2019, Sanofi; world's first nanobody Rx
- Binds VWF A1 region, blocks PLT string formation
- Prevents microthrombi



Scully M, Cataland SR, Peyvandi F, et al. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. N Engl J Med. 2019;380:335–46

Cablivi® Primary Endpoint: Time to PLT Response



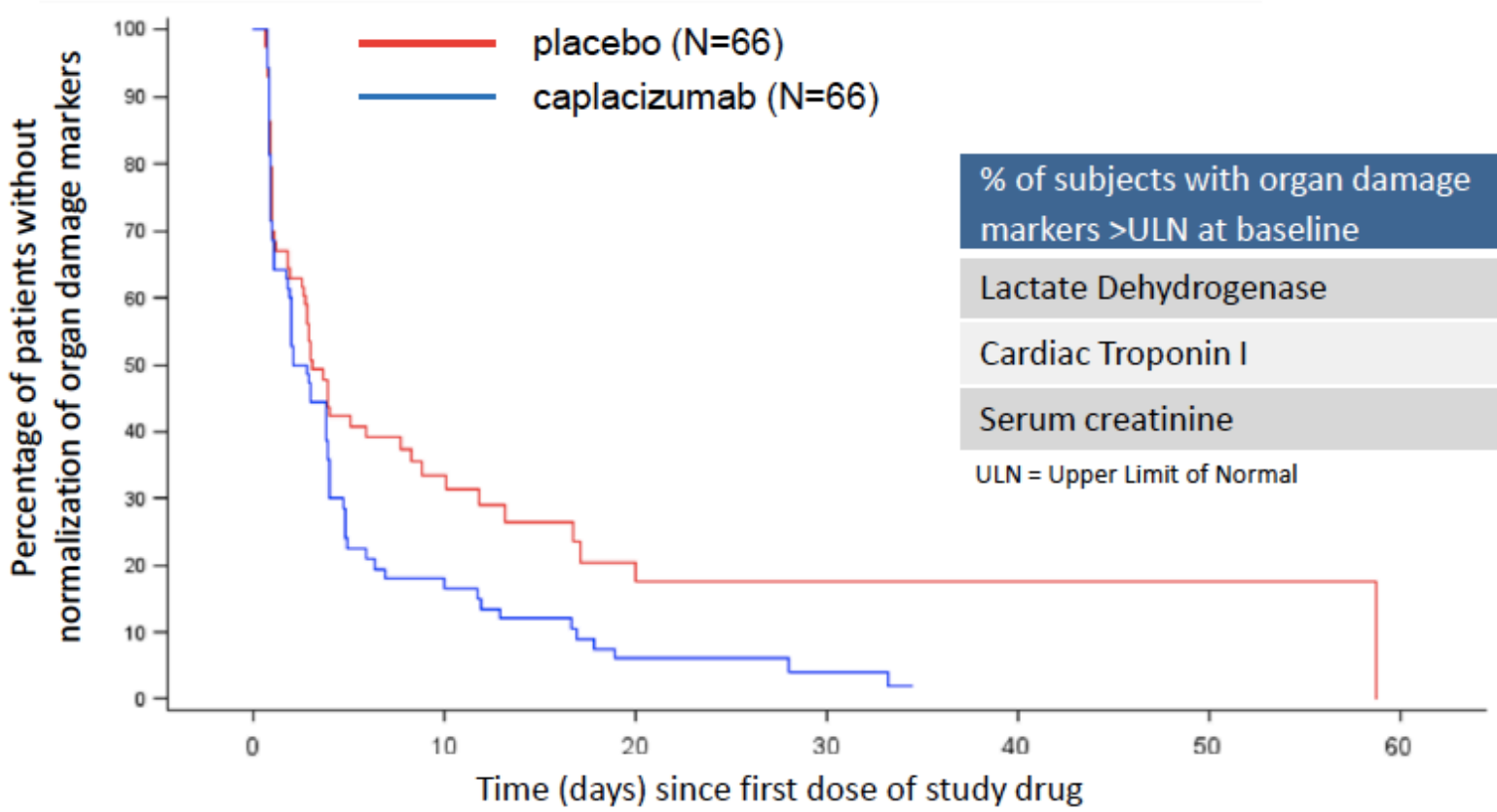
	Placebo N = 73	Caplacizumab N = 72
Platelet normalisation rate ratio (95% CI)	1.55 (1.10, 2.20)	
Stratified log-rank test p-value	***<0.01***	

Defined as initial platelet count ≥150,000/uL, subsequent stop of daily PE within 5 days

HERCULES End Points

Subjects N (%)	Current Rx N = 73	Cablivi N=72
iTTP-related death	3 (4.1%)	0
iTTP recurrence or exacerbation	28 (38.4%)	3 (4.2%)
iTTP recurrence long-term	28 (38.4%)	9 (12.7%)
Refractory iTTP—continued thrombocytopenia	5 (7%)	0
Major thromboembolic event (VTE or arterial)	6 (8.2%)	6 (8.5%)
First four endpoints: P > 0.05		

HERCULES Time to Normalization of Organ Damage Markers



% of subjects with organ damage markers >ULN at baseline	All subjects N=145
Lactate Dehydrogenase	87.1%
Cardiac Troponin I	53.8%
Serum creatinine	22.7%

ULN = Upper Limit of Normal

Camalid Nanobody

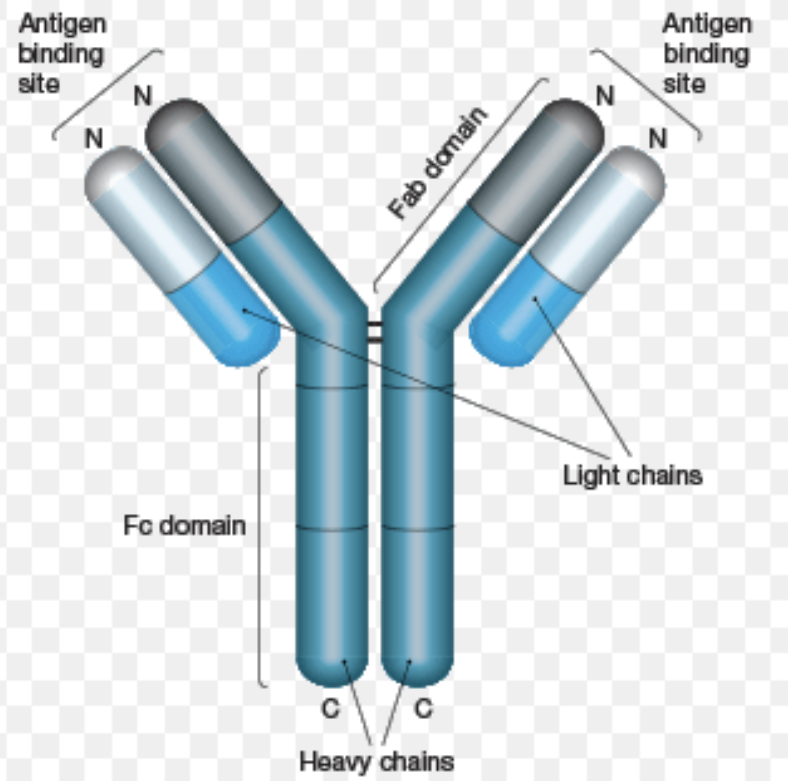
Llama

Alpaca

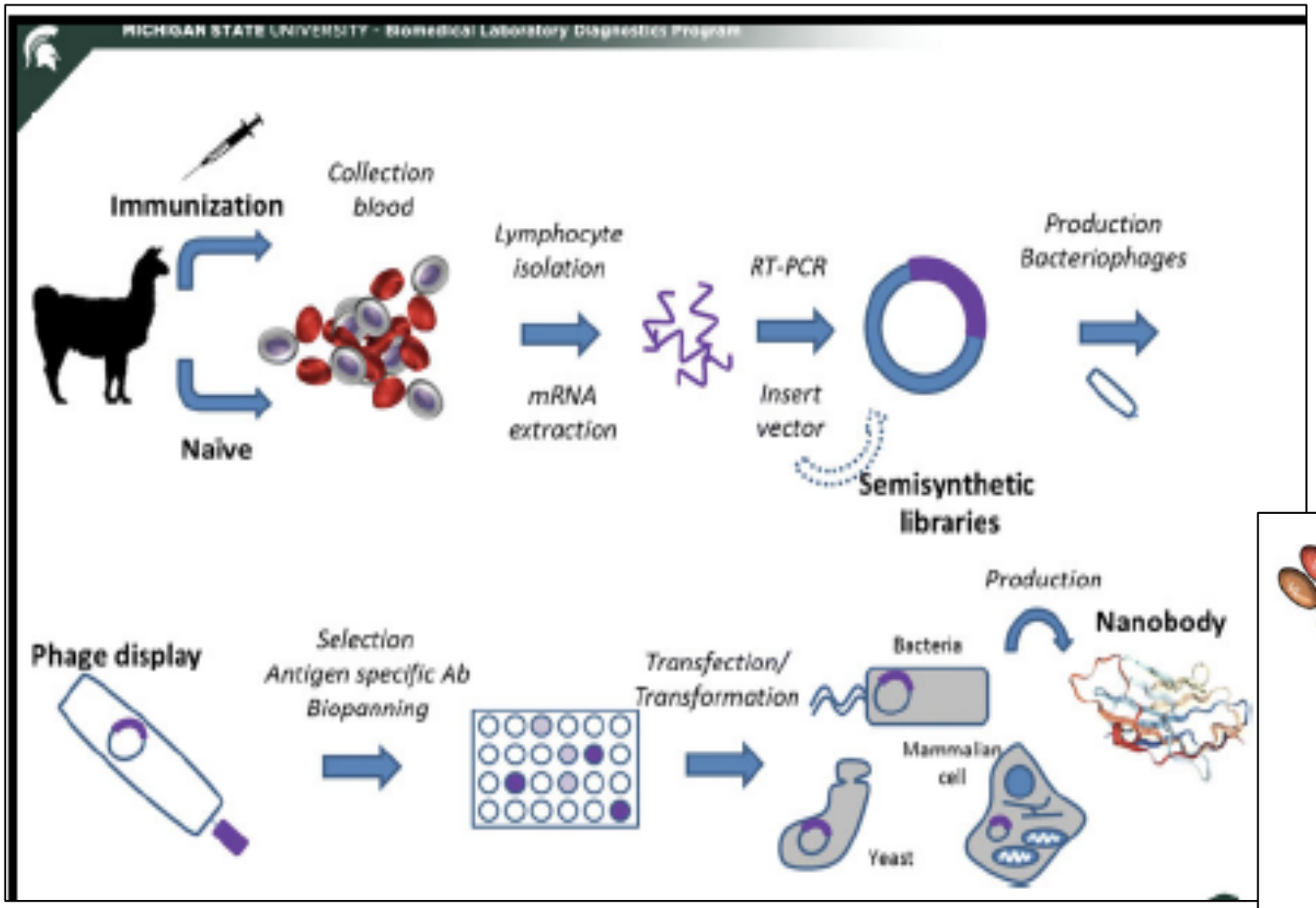
Vicuna

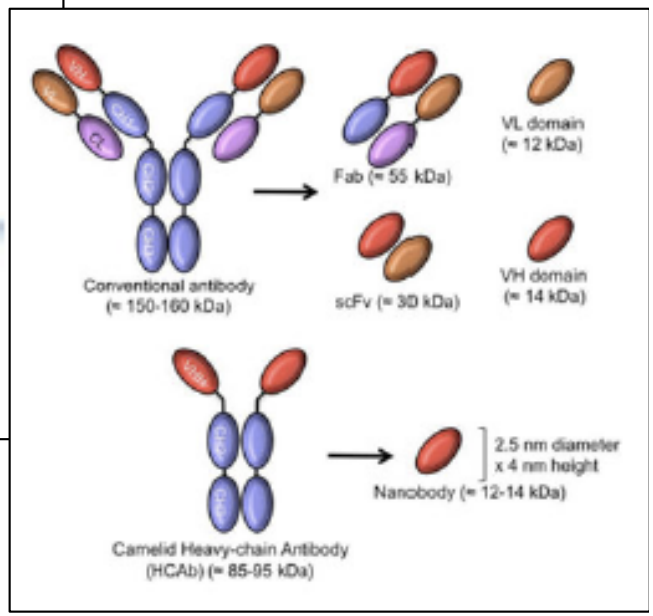
What is a Nanobody?

Camelids: camels, llamas, alpacas, vicunas make heavy chains with no light chains, no VH1 region, direct connection to hinge region



Nanobody Production

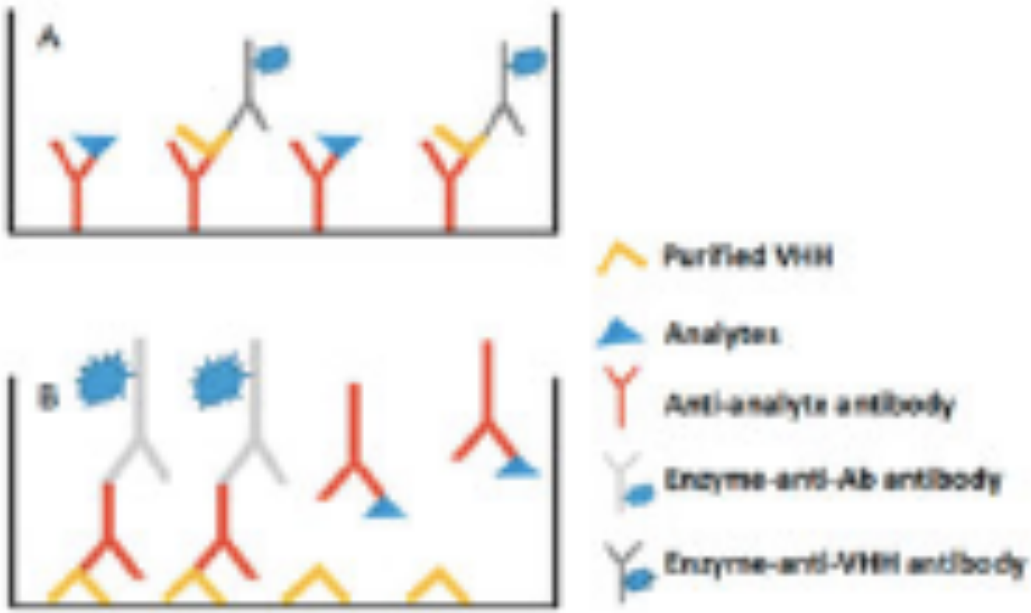




Courtesy of John Gerlach, PhD, Michigan State University

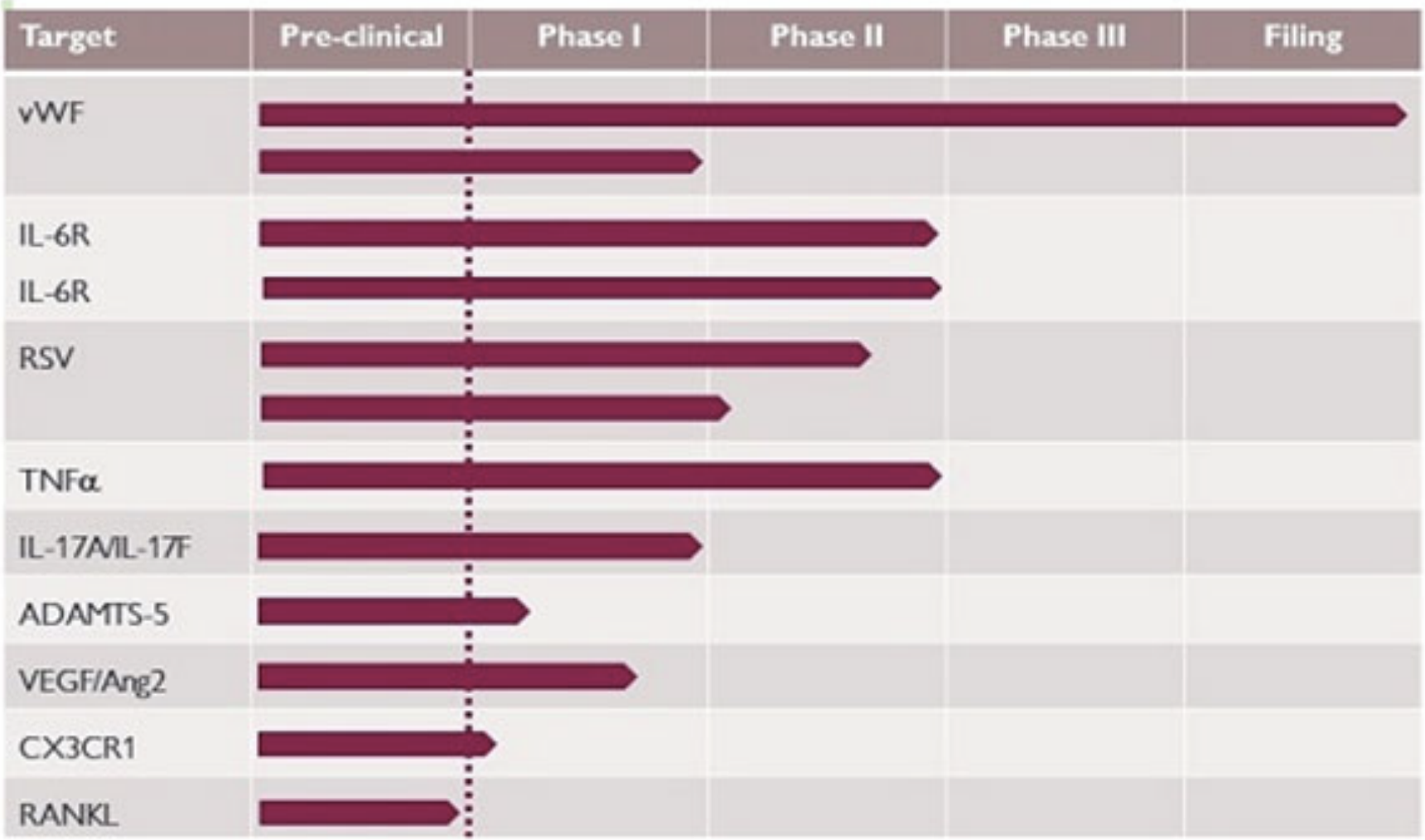
Nanobody Applications

- Antiviral (HIV, HSV, RSV), camel flu
- Anti-allergens
- Antitumor Rx
- Anti-angiogenesis
- Antiinflammatory
- Neutralize venoms
- IL-6-R for rheumatoid arthritis Rx
- Radiolabeled for Her2+ tumor imaging

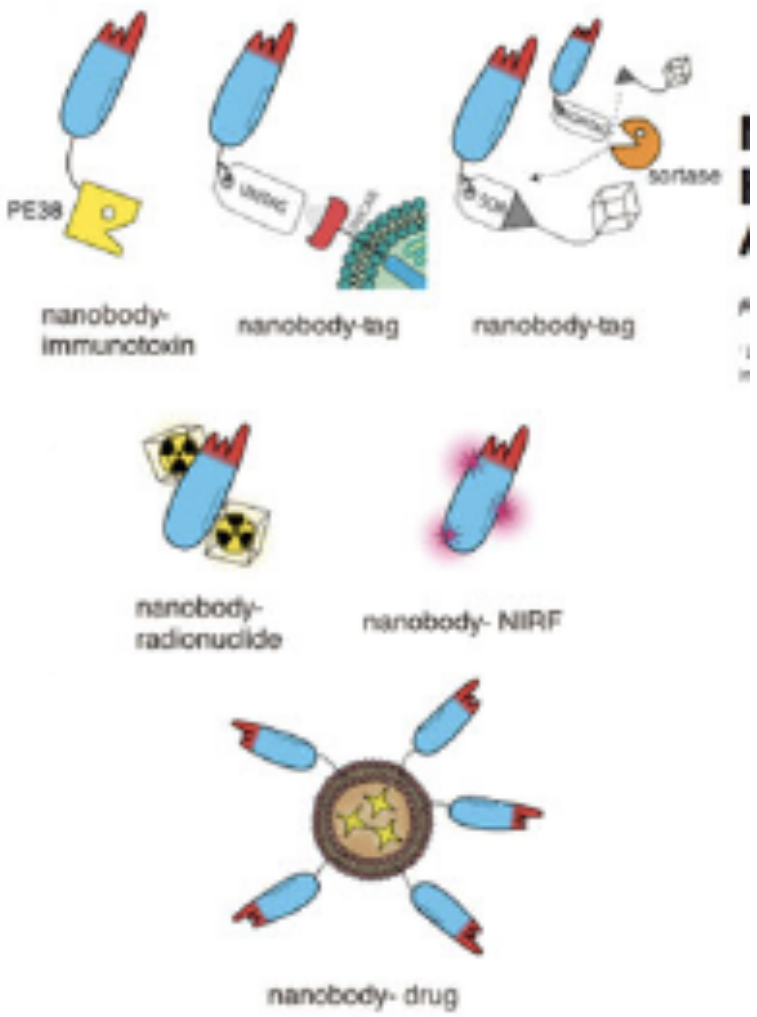


- Diagnostic: viral, fungal, mycotoxins in food, trypanosomes
- Stoke diagnosis and inactivation

Nanobody Applications



Nanobody Features



- High affinity, specificity
- Short half-life, thermostable
- Soluble, penetrate tissues
- Small molecule are non-immunogenic, no heterophile ABs
- Penetrate small molecular pockets
- Cross the blood-brain barrier
- But they don't bind small molecules or carbohydrates

Caleb

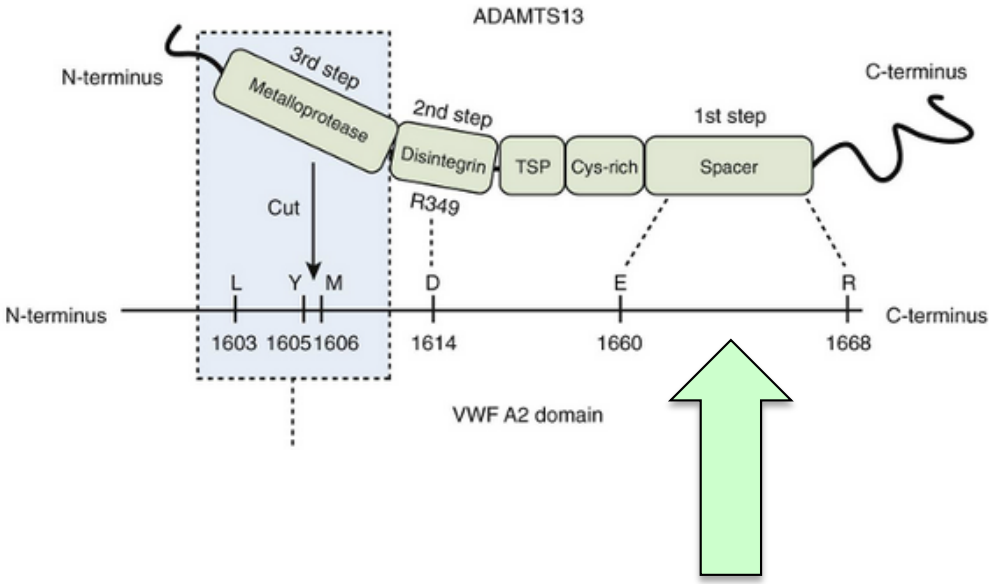
rADAMTS13 Concentrate SHP655

- A phase 2, multicenter, randomized, placebo-controlled, double-blind study in patients with hereditary TTP to evaluate the pharmacokinetics, safety and efficacy of rADAMTS13 (SHP655) administered in addition to standard of care treatment. ClinicalTrials.gov Identifier: NCT03922308
  - Takeda Pharmaceuticals acquired Shire, Dublin, January 2019, Shire was Baxalta, Baxalta was Baxter
- Or a modified ADAMTS13 that evades the autoantibody
- Or ADAMTS13 in platelet concentrate to evade autoantibody

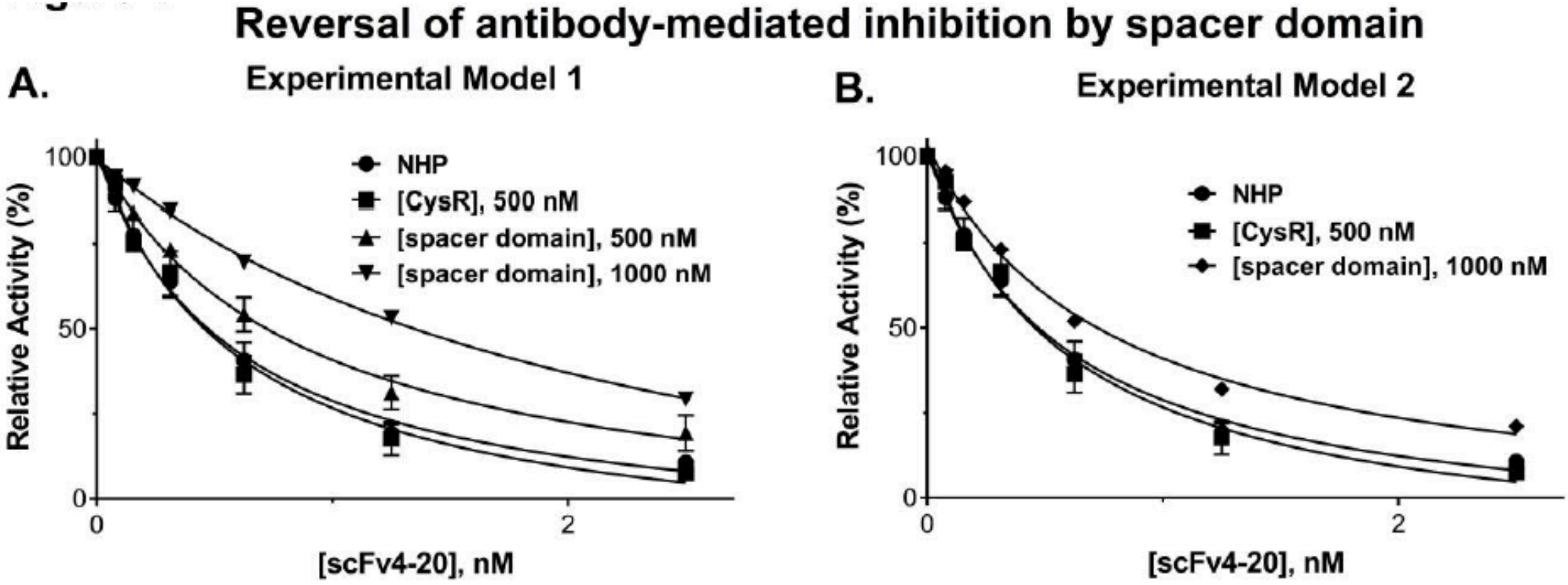
- Jian C, iao J, Gong I, et al. Gain-of-function ADAMTS13 variants that are resistant to autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. Blood 2112; 119: 3836–43.
- Abdelgawwad MS, Cao W, Zheng L, Kocher NK, Williams LA, Zheng XL. Transfusion of platelets loaded with rADAMTS13 is efficacious for inhibiting arterial thrombosis associated with thrombotic thrombocytopenic purpura. Arterioscler Thromb Vasc Biol. 2018;38:2731–43

"Decoy" iTTP Therapy

Spacer domain analogue decreased the in vitro inhibitory activity of synthetic autoantibody against ADAMTS13 in normal human plasma in a concentration-dependent manner. When autoantibody concentration was fixed at 1.25 nM, spacer domain at the final concentration of 1000 nM recovered activity by 2.1-fold.



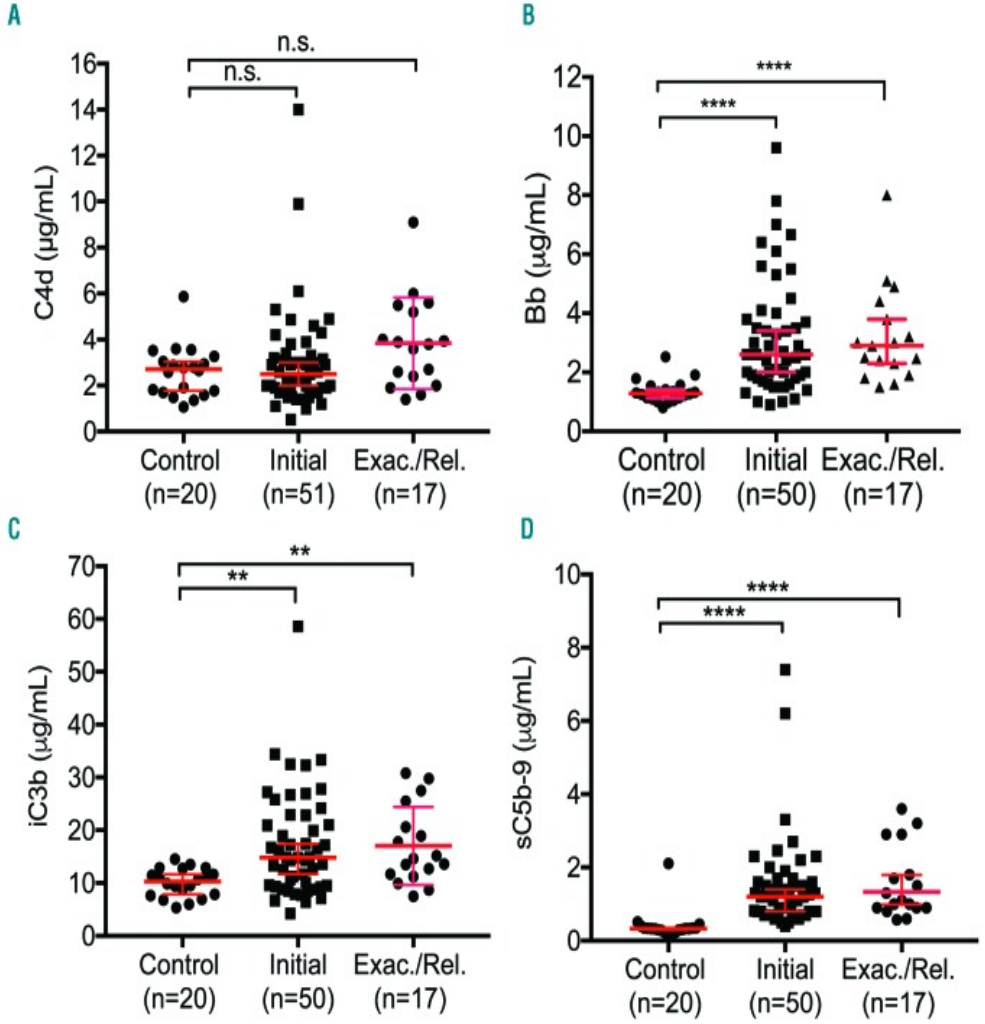
Experimental iTTP Therapy



Halkidis K, Zheng X. Targeting autoantibodies against ADAMTS13 in immune thrombotic thrombocytopenic purpura. Abstract University of Alabama at Birmingham, Birmingham, Alabama, USA

iTTP Outcomes

- ADAMTS13 activity
- VWFag and VWFactivity (collagen-binding assay)
- Platelet count and LD
- Human neutrophil peptides 1–3 (HNP)
- Complement proteins Bb, 5b–9



Staley EM, Can W, Pham HP, et al. Clinical factors and biomarkers predict outcome in patients with immune-mediated thrombotic thrombocytopenic purpura. Haematologica. 2019;104: 166–75.

Childhood Trauma

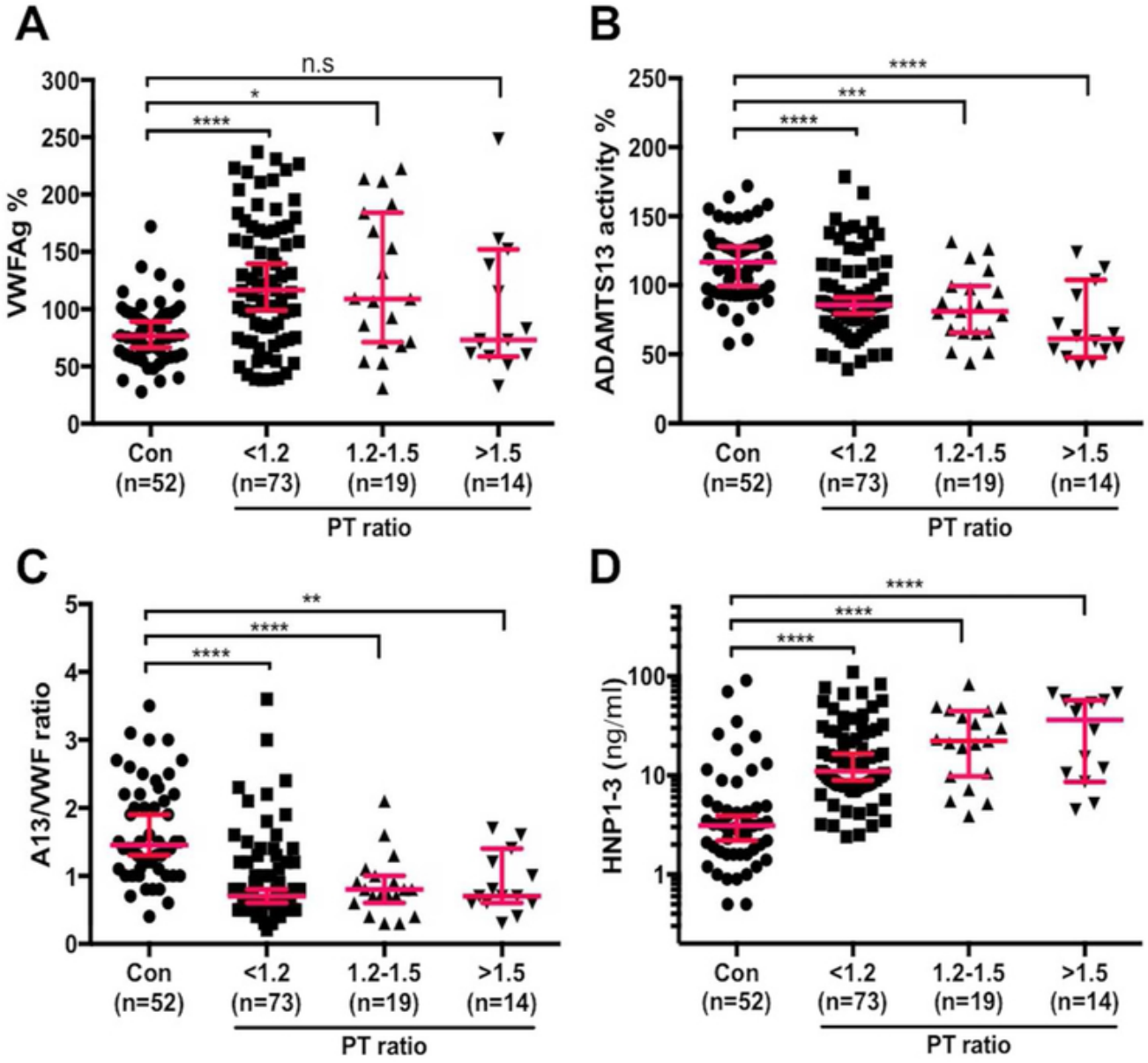
- Children's Hospital of Alabama Level 1 Trauma Center
- 106 Pts 2014–16, median 9 YO, blunt & penetrating trauma
- Injury severity score median 33; $\geq 15{:}\,72;\ \geq 25{:}\,46$
- Measures: PT Ratio vs ADAMTS13, VWFag, VWFac, HNP 1–3
- Endothelial activation occurs in trauma or sepsis and can induce an inflammatory procoagulant state that is associated with microvascular injury and thrombosis.

Russell RT, McDaniel JK, Cao W, Zheng XL, et al. Low plasma ADAMTS13 activity is associated with coagulopathy, endothelial cell damage and mortality after severe pediatric trauma. Thromb Haemost. 2018; 118: 676–87.

The Fritsma Factor
YOUR INTERACTIVE HEMOSTASIS RESOURCE

Childhood Trauma Results by PT Ratio

- Reduced ADAMTS13
- Reduced ADAMTS13 /VWFag ratio
- Elevated HNP 1–3
- So what?

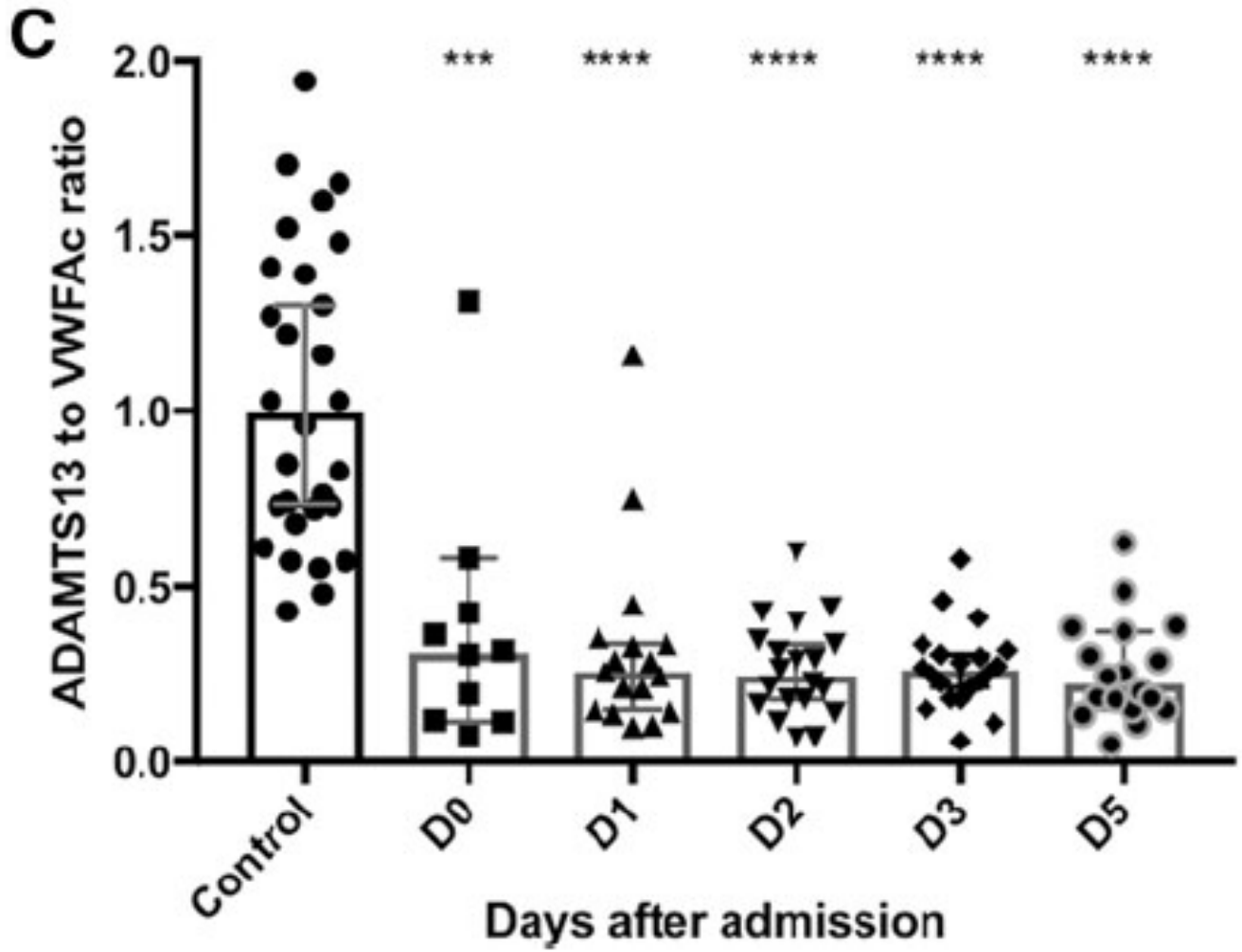


ADAMTS13, VWF, HNP 1–3 in Traumatic Brain Injury

- 33 adult TBI victims 2010–14 Vs 33 controls
- Blood collected at 0, 1, 2, 3, & 5 days
- Traumatic microvascular injury in brain and other organs
- VWFag, VWFac, HNP 1–3 rise over 5 days Vs control
- ADAMTS13 reduces over 5 days compared to controls
- Changes most profound in severe cases

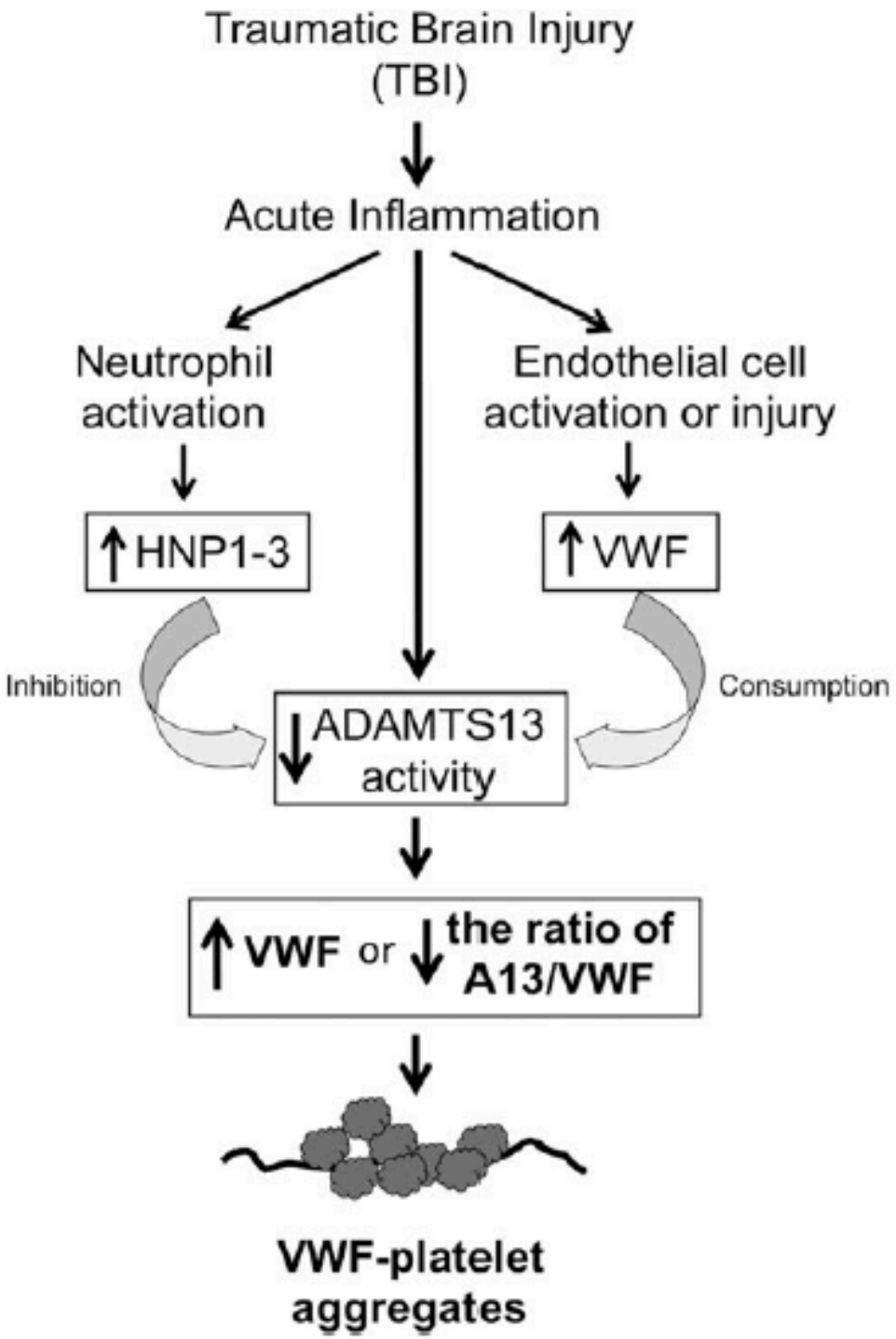
Kumar MA, Can W, Pham HP, Zheng XL, et al. Relative deficiency of plasma ADAMTS13 activity and elevation of human neutrophil peptides in patients with traumatic brain injury. J Neurotrauma 2018; 36. https://doi.org/10.1089/neu.2018.5696

ADAMTS13:VWF Ac



Proposed Mechanism for Traumatic Brain Injury

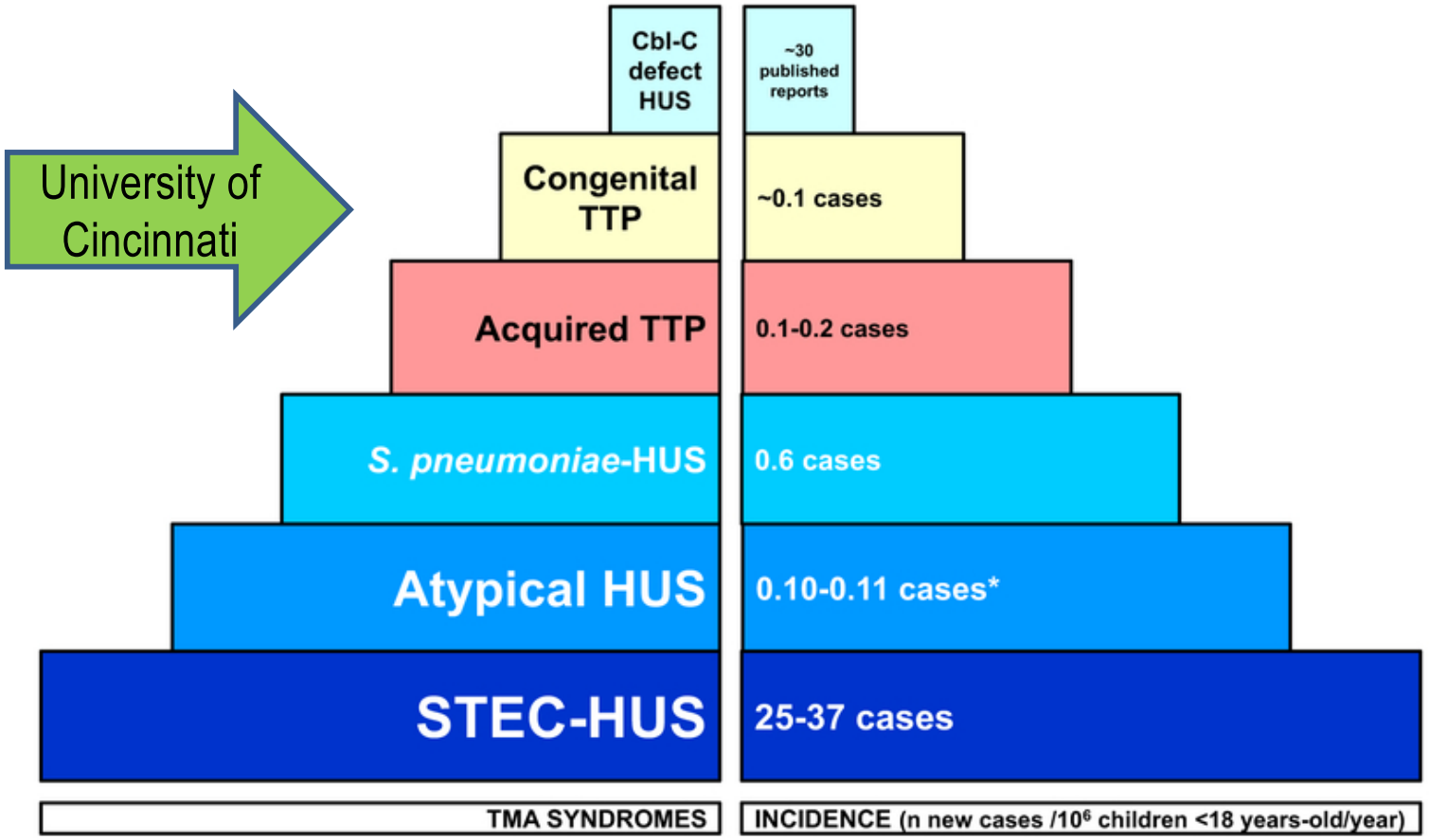
HNP 1-3, the most abundant protein constituents of neutrophil azurophilic granules, consist of 29–30 aa residues that form a tertiary structure by three disulfide bonds, MW ~3.5 kDa. Whether HNP 1-3 is an inflammatory marker or has specific biological activity triggering microvascular thrombosis remains to be determined.



ADAMTS13 Concentrate TBI Therapy ?

"We conclude that the elevated plasma levels of VWF, reduced ADAMTS13 activity, and elevated HNP 1–3 in patients post-TBI may explain the underlying mechanism of microvascular thrombosis found in vessels of brain parenchyma and other organ tissues despite a seeming hypocoagulability revealed by other routine laboratory tests, including low PLT count, prolonged PT and PTT. Our findings may provide a rationale for supporting future clinical trials with recombinant ADAMTS13 as a novel therapy in patients with TBI."

TMA Incidence in Childhood



Berangere SJ, Zheng XL, Veyradier A. Understanding thrombotic microangiopathies in children. Intensive Care Med. 2018; 44:1536–8.

2-YO Boy with STEC-HUS

- Recurrent bouts of bloody diarrhea, elevated WBC
  - No thrombocytopenia, no anemia
- Appendectomy on a Friday, released Sunday AM
- Grew sicker, some neurological changes
- Readmitted Sunday PM—no urine output, mild MAHA
- Cultured E. coli, awaiting strain ID
- Renal dialysis 2 weeks, every third day
- Released after 1 month

STEC-HUS Mechanism

- E. coli 0157:H7, Shigella, S. pneumoniae secrete shigella cytotoxin (STX)
  - Common childhood diarrhea
- STX activates glomerular ECs to secrete ULVWF
  - Platelet strings appear, STX slows ADAMTS13 activity
  - Microvascular ischemia, ADAMTS13 is not consumed
- MAHA: anemia, schistocytosis, elevated LD
- Elevated creatinine, reduced eGFR, anuria
- Self-limiting, offer renal dialysis, maybe PLEX

STEC-HUS Vs. TTP

	HUS	TTP
Patient	Child	All ages
Organ	Renal	Several (CNS)
Episodes	Single	Recurrent
Thrombocytopenia	Moderate	Severe
MAHA	2+	4+
ADAMTS13	Broadly normal	Absent
Clinical and laboratory observations cross boundaries, obscuring diagnosis		

VWF and VWFpp in Diabetes

Elevated VWF levels are found in diabetes and other vasculopathies, and predict cardiovascular mortality. VWF is released from ECs along with equimolar amounts VWDpp.

We measured VWFpp levels as a marker of endothelial secretion in vivo.

VWF but not VWFpp levels are influenced by blood groups, explaining in part the smaller variation in VWFpp levels among normal individuals.

In both controls and insulin-dependent diabetics, we found a modest correlation between VWFpp and VWF:Ag ($r^2 = 0.54$, $p < 0.0001$).

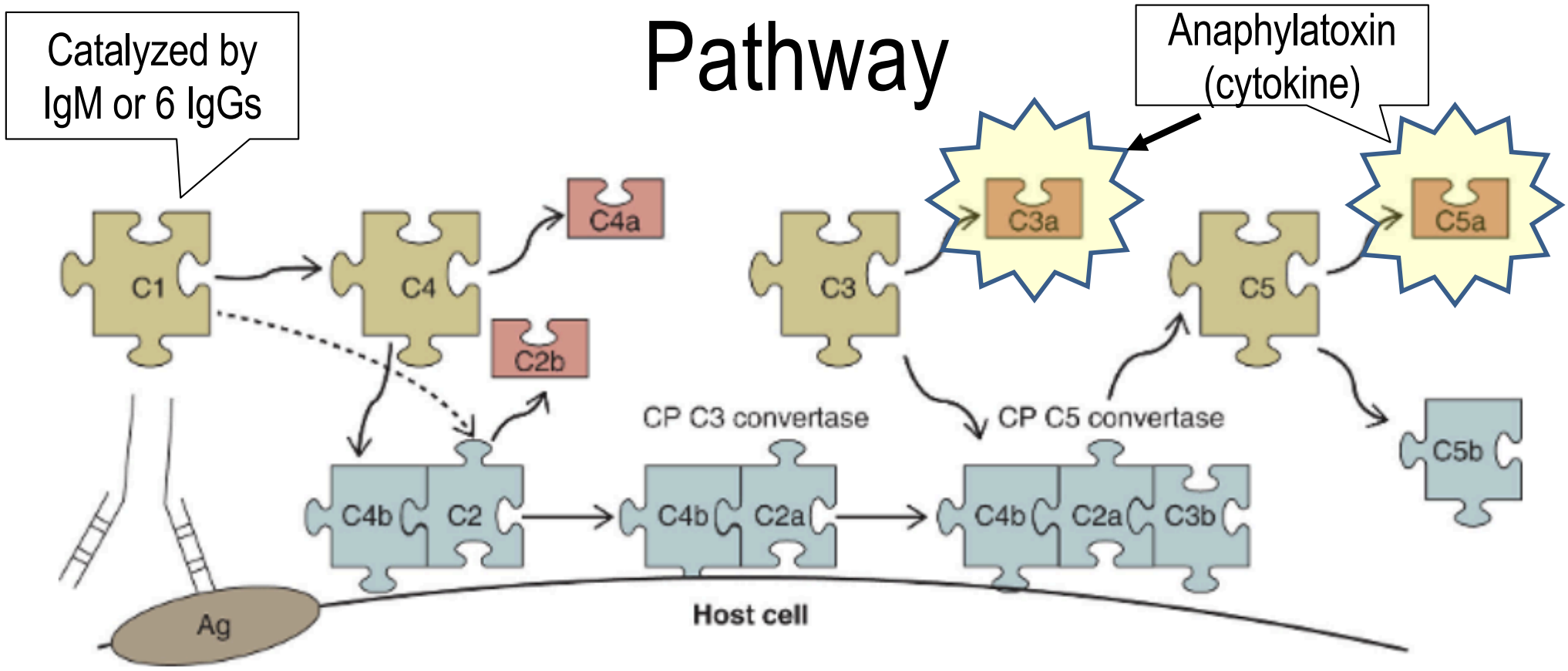
VWf and VWFpp were elevated in patients with microalbuminuria or overt diabetic nephropathy, whereas only VWFpp was significantly elevated in the normoalbuminurics. This observation suggests that in conjunction with VWF, VWFpp measurements may improve the identification of endothelial activation, which occurs frequently even without increased urinary albumin excretion.

In 12 NIDDM patients, a 3-week diet enriched in monounsaturated fat (MUFA) resulted in parallel decreases in VWF (-22%, $p < 0.05$) and VWFpp (-17%, $p < 0.05$) levels, indicating that the experimental diet affected endothelial secretion rather than VWF catabolism. A carbohydrate-enriched control diet did not significantly influence either marker.

aHUS

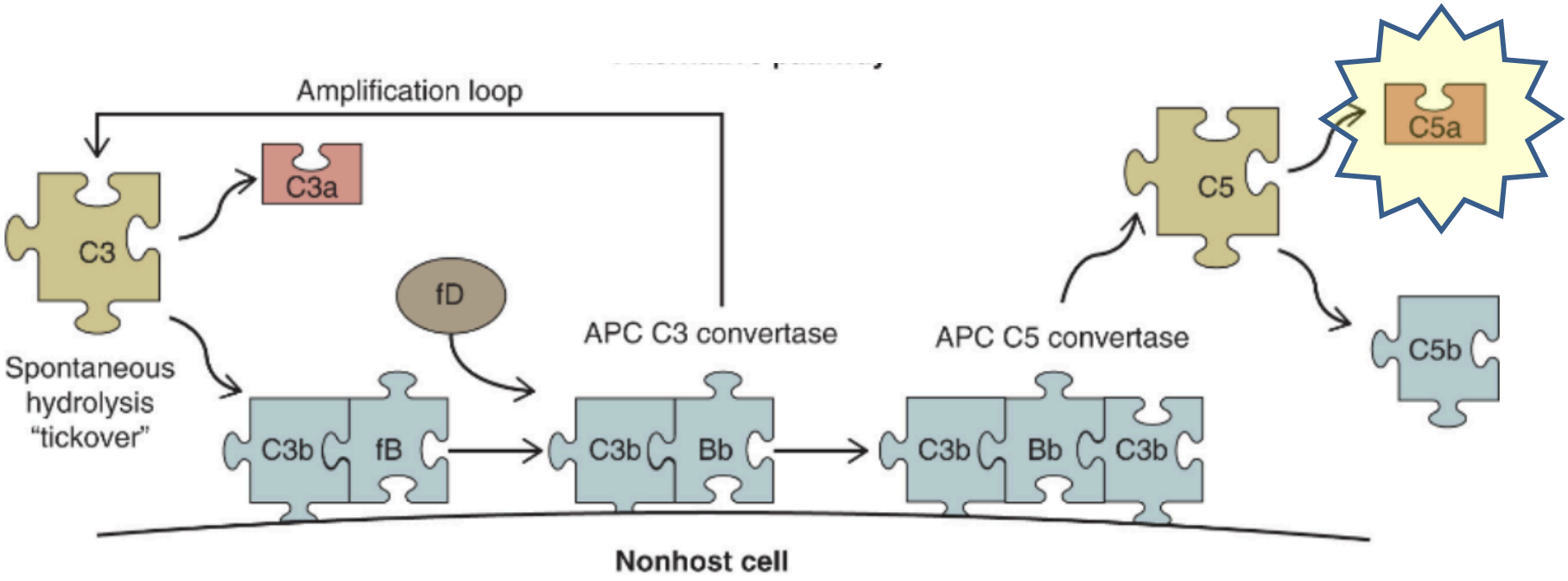
- A recurrent TMA with MAHA, thrombocytopenia, creatinine >2.25 mg/dL, and ADAMTS13 activity >13%
- Pathophysiology: excessive activation of the alternate pathway complement (APC) system
- Related to mutations in APC regulatory proteins genes for H, I, thrombomodulin, and membrane cofactor protein
  - However, these mutations exist in non-aHUS individuals
- Two-hit hypothesis: mutation + pregnancy, inflammation, surgery, or autoimmune disorder
- Could also be acquired aHUS, autoimmune
- Symptoms appear at median 18 YO, severe, recurring

Classical Complement Pathway



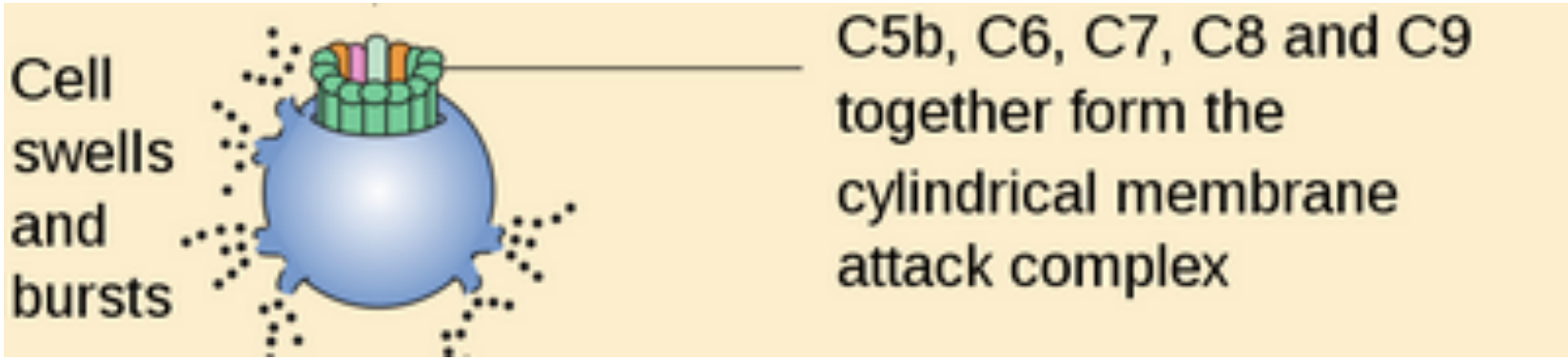
C5a may increase inflammatory cytokines, downregulate ADAMTS-13, generate tissue factor and PAI1, decrease protein S and increase protein C resistance because of increased factor VIII activity, and, most importantly, activate thrombin.

Alternate Complement Pathway (APC)



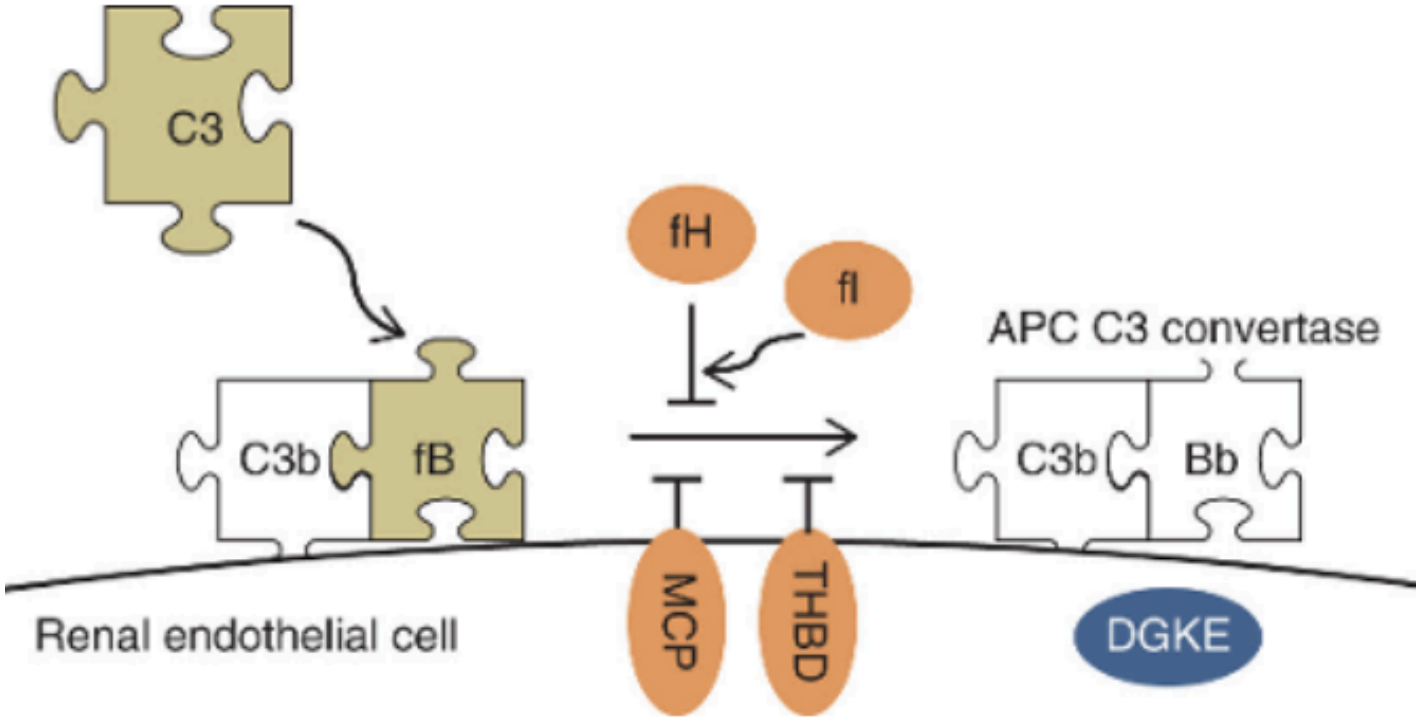
Gavriilaki E, Brodsky RA. Complement-mediated coagulation disorders: PNH and aHUS. In Kitchens CS, Kessler CM, Konkle BA, Streiff MB, Garcia DA. Consultative Hemostasis and Thrombosis, fourth Edition. Elsevier 2019

Membrane Attack Complex (MAC)



Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology Edition 6. Elsevier. 2010:272–88.

APC Dysregulation in aHUS



Activation results from loss-of-function mutations in regulatory factors H/fH, I/fI, membrane cofactor protein (MCP) and thrombomodulin (THBD) shown in orange, gain-of-function mutations of C3 and factor B/fB shown in green. Renal ECs are primary aHUS targets.

How to Test for aHUS

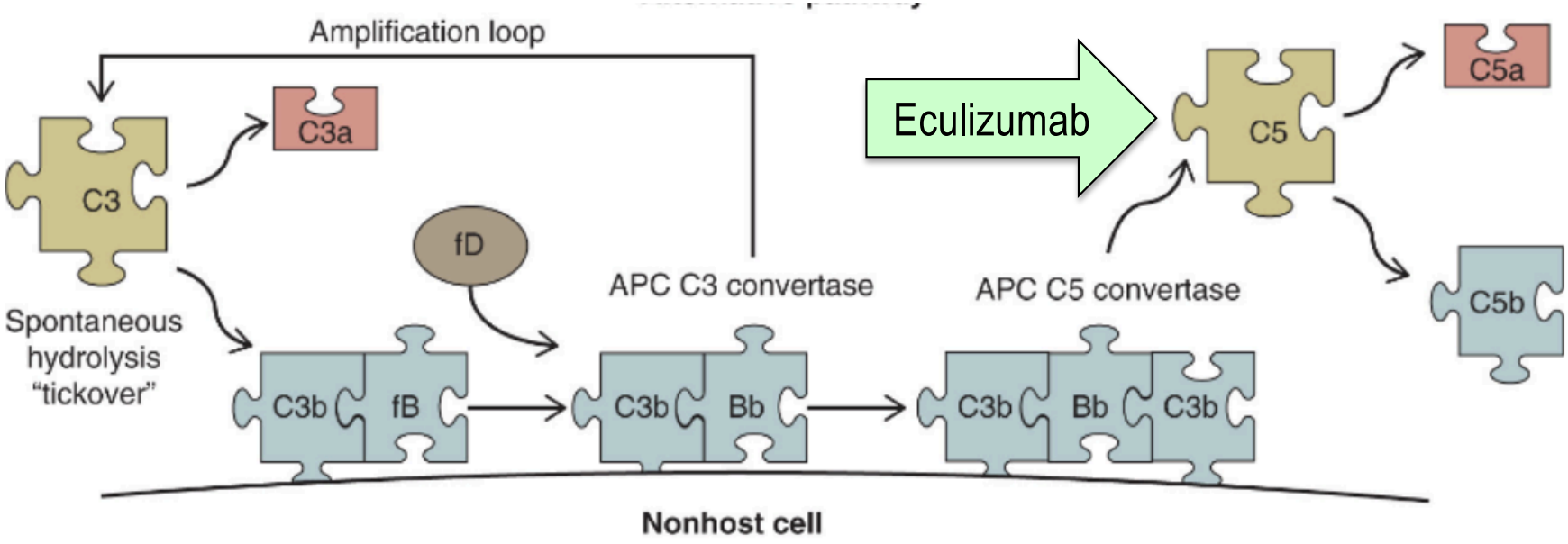
- No reliable complement protein tests
  - Urinary C5b–9?
- Modified Ham test (really!) PNH RBCs incubated with aHUS serum, cells retain dye if complement MAC induces death

aHUS Differential Diagnosis

- DIC: MAHA, thrombocytopenia, PT/INR and PTT prolonged, D-dimer markedly elevated
- Misc. TMAs: malignancy, PNH, organ transplant, drugs
  - Quinine, mitomycin, cyclosporin, chemotherapy
- TTP: ADAMTS13 <10%
- STEC-HUS: Shiga toxin testing

aHUS Treatment

- PLEX: temporarily partially effective
  - 50% progression to end state renal disease
- Complement inhibition, nanobody eculizumab (Soliris®)
  - Cost, relapse?, D/C?, close monitoring

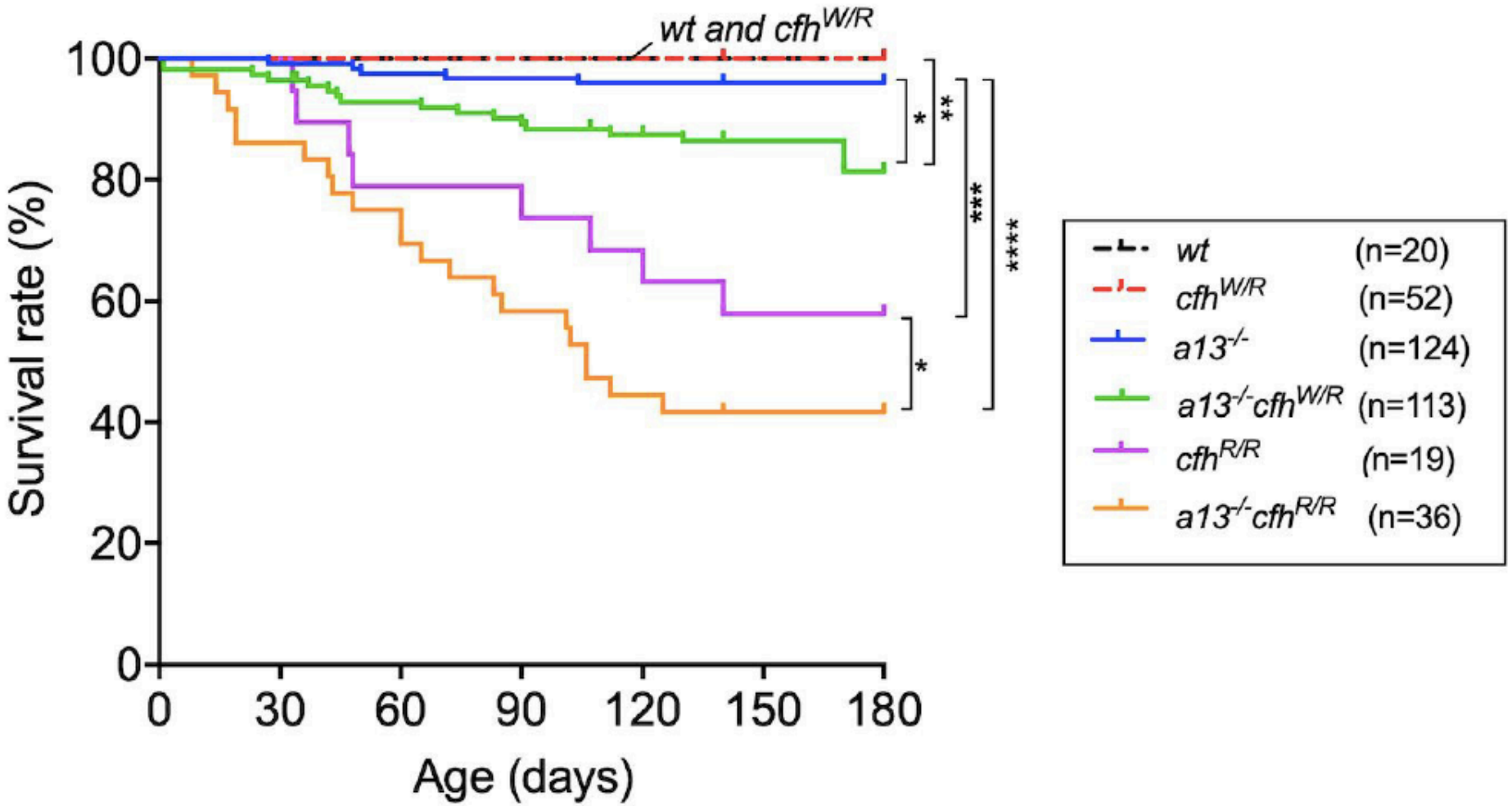


ADAMTS13-Complement Crosstalk

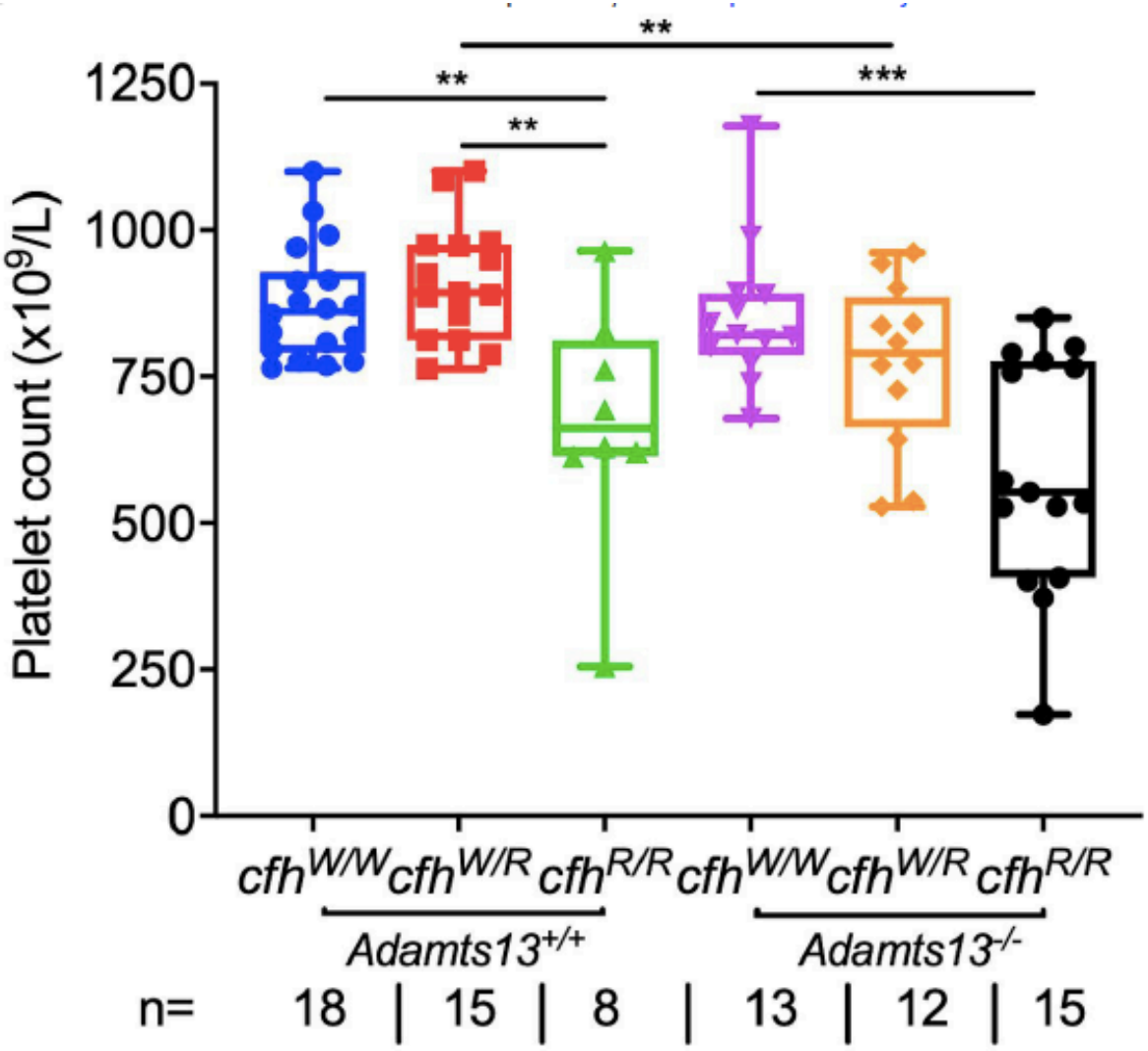
- Mice with *Adamts13*$^{-/-}$ or a heterozygous complement factor H mutation (i.e. *chf*^{W1206R}): no spontaneous TMA.
- However, mice carrying *Adamts13*-/- *cfh*^{W1206R} or *cfh*^{R1206R} develop TMA with a significantly increased mortality rate.

Zheng L, Zhang D, Can W, Song WC, Zheng XL. Synergistic effects of ADAMTS13 deficiency and complement activation in pathogenesis of thrombotic microangiopathy. Blood 2019 pre-pub

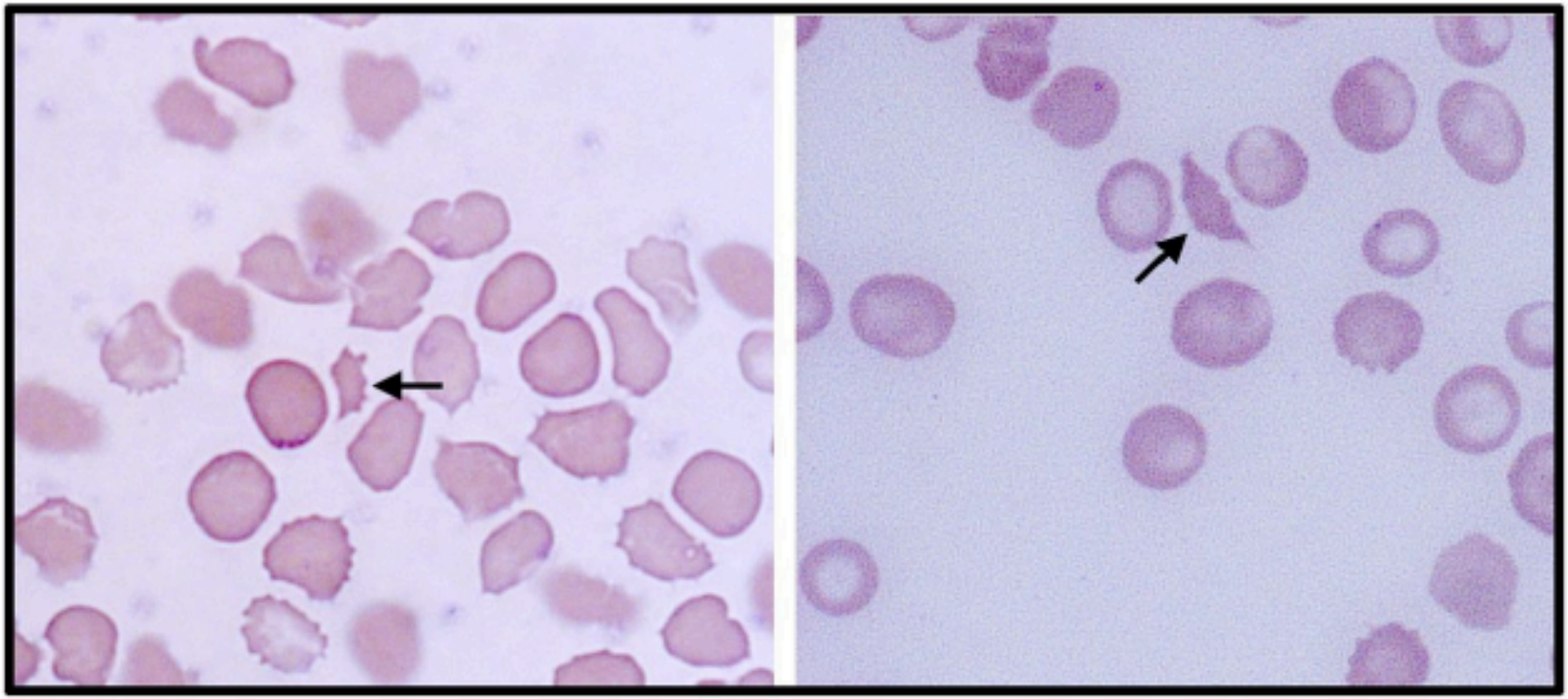
ADAMTS13-Complement Crosstalk



ADAMTS13-Complement Crosstalk



ADAMTS13-Complement Crosstalk



Adamts13$^{-/-}$*cfh*$^{W/R}$
(at 42 days old)

Adamts13$^{-/-}$*cfh*$^{R/R}$
(at 16 days old)

ADAMTS13-Complement Crosstalk

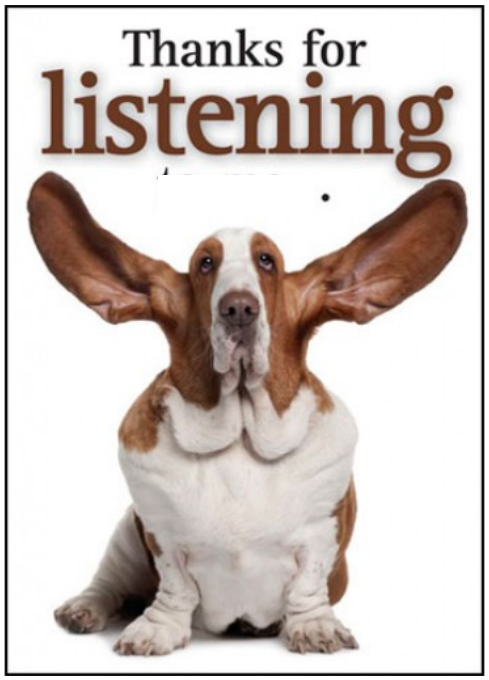
- Turner and Moake showed that C3, CFB, CFD, CFP C5, CFH, and CFI bind newly released ULVWF strings on endothelial surfaces in the amount required for C' alternate pathway components to assemble.
- Small VWF multimers act as a cofactor mediating the CFI-dependent degradation of C3b.
- ULVWF multimers do not inhibit complement, thus promote complement activation.
- ADAMTS13 removes ULVWF multimers. This reduces platelet adhesion and aggregation and eliminates complement component assembly and activation on the injured or activated endothelium.
- When ADAMTS13 is absent, endothelial ULVWF strings may serve as a template for assembling complement components, leading to overactivation of complement in situ, resulting in endothelium damage and thrombus formation.

HELLP Syndrome in Pregnancy

- Hemolysis, elevated liver enzymes, low platelets
- Pre-eclampsia spectrum, severe risk to mother and fetus
  - Hypertension, proteinuria, end organ ischemia
  - MAHA, renal dysfunction, altered mental status, seizures
- Rx: early delivery, Mg for seizures, Rx for hypertension
- APC dysregulation, urine C5b–9 elevation, mutations
- Assay: modified Ham test, Rx eculizumab in trials

HELLP: The Lab Saved My Life

Not this Lab



HELLP

Baby

Mama