Acquired hemolytic, megaloblastic and sideroblastic anemias

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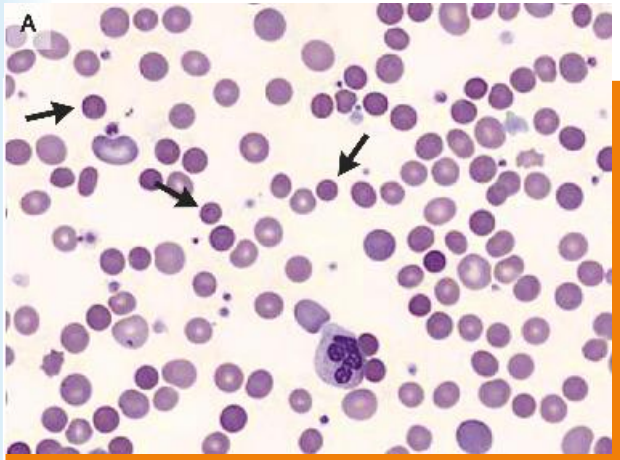
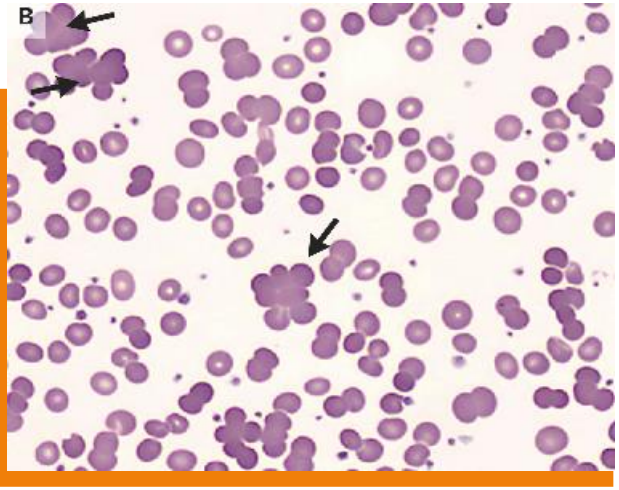
BHS Educational Course on Red Blood Cell Disorders
18th November 2023

Acquired anemias



Hemolytic anemia

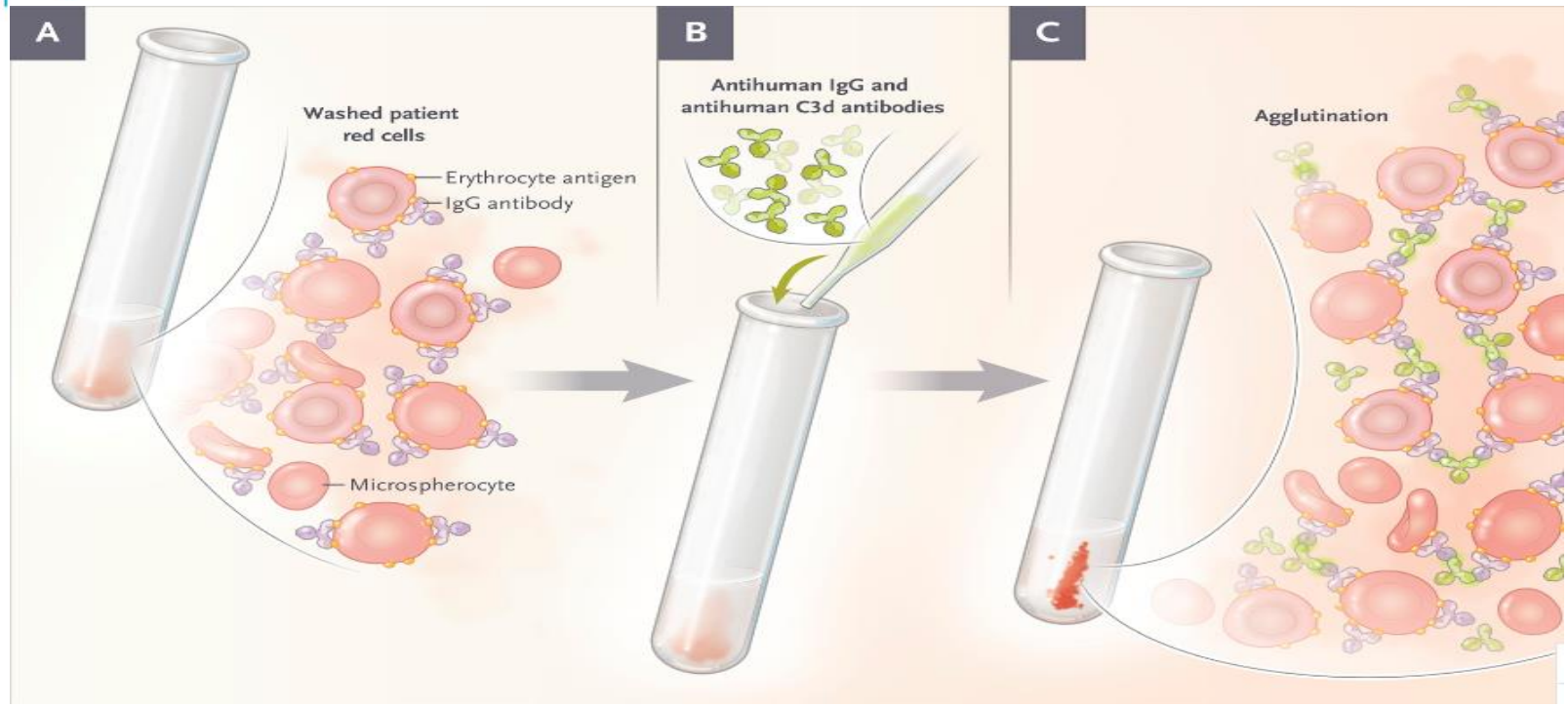
- AIHA
- TMA
- PNH

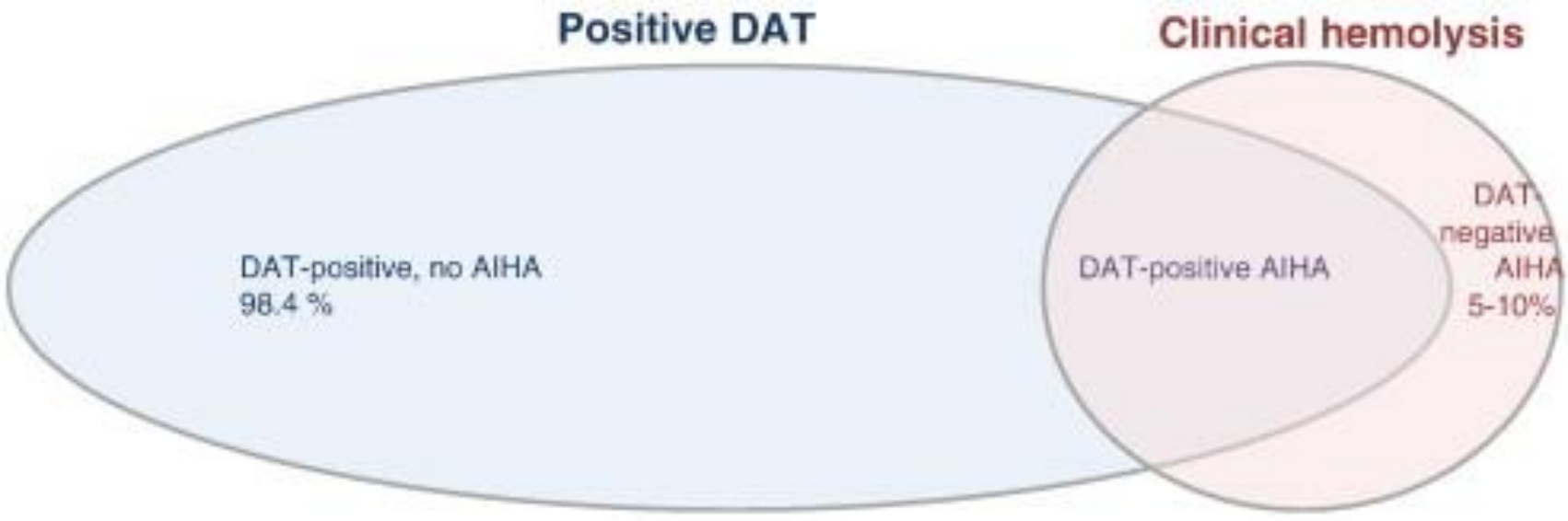


Megaloblastic anemia

Sideroblastic anemia

AIHA - DAT





Meulenbroek EM, et al. Blood Rev 2015;29:369-76
Brodsky RA. N Engl J Med 2019;381:647-54

AIHA - DAT

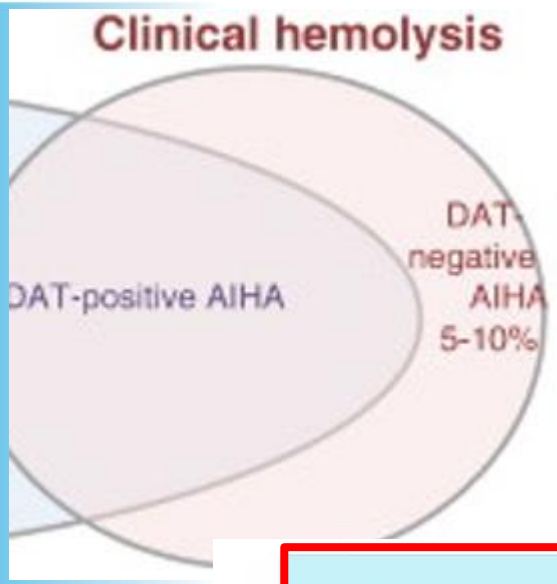
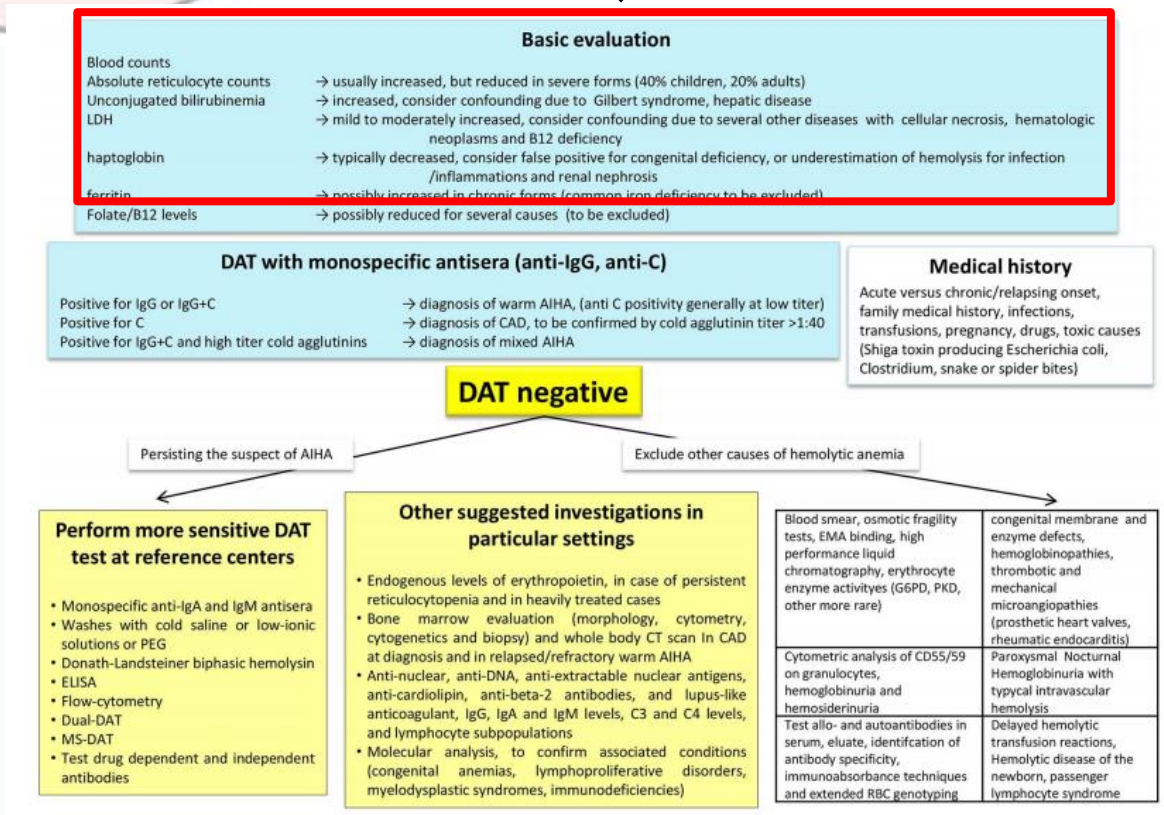


Table 3. Mechanisms involved in DAT-negative WAIHA

1. Erythrocyte-bound antibody below the limit of detection of standard DAT
Erythrocytes from healthy individuals have up to 35 molecules of IgG bound to their surface.
Standard DAT can detect >300-500 bound IgG molecules.
WAIHA can occur with as few as 70-434 bound IgG molecules.
2. Low-affinity IgG antibodies
Loosely bound antibodies are dislodged during the washing of erythrocytes or when samples are left standing at room temperature.
3. IgA antibodies
IgA antibodies may trigger phagocytosis and antibody-dependent cell cytotoxicity, resulting in hemolysis.
Standard anti–human globulin reagents do not have anti-IgA activity, as most polyspecific reagents contain a mixture of monoclonal anti-IgG and anti-C3d.
4. Warm-reacting IgM and monomeric IgM antibodies
IgM antibodies reacting at warm temperatures and monomeric IgM may not fix complement.
Standard anti–human globulin reagents do not detect IgM. However, these antibodies will detect C3d if the IgM antibody fixes complement.

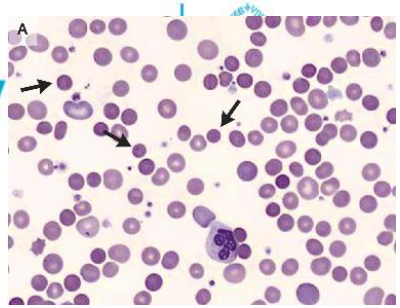


Meulenbroek EM, et al. Blood Rev 2015;29:369-76
Go RS, et al. Blood 2017;129:2971-9
Barcellini W, Fattizzo B. Front Immunol 2020;11:946

AIHA - classification

Based on optimal temperature of antibody binding	Based on presence of underlying disorder
• Warm antibody AIHA • Cold antibody AIHA • Mixed antibody AIHA	• Primary/idiopathic AIHA • Secondary AIHA

AIHA - classification



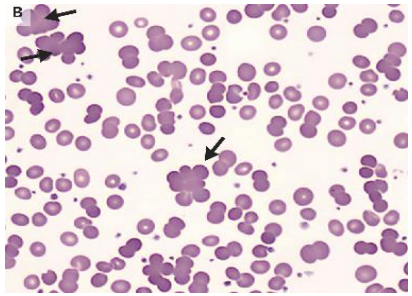


Table 1. Autoimmune Hemolytic Anemias.

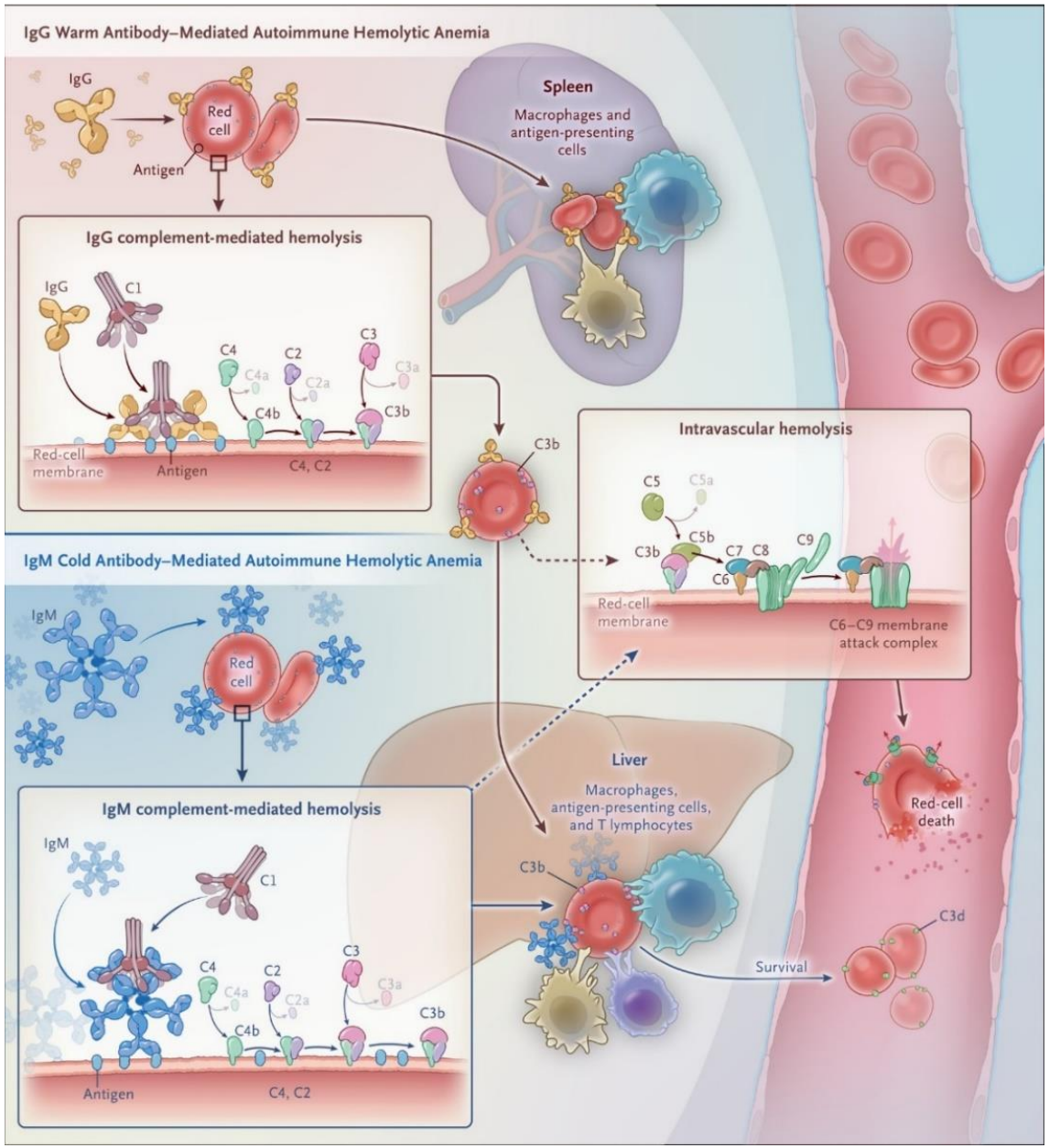
Variable	Warm-Antibody Type	Cold Agglutinin Disease	Secondary Cold Agglutinin Syndrome	Paroxysmal Cold Hemoglobinuria	Mixed Type
Incidence and age at onset	5–10 cases/1 million persons/yr; occurs at any age but frequently in the elderly	0.45–1.9 cases/1 million persons/yr; occurs mainly in the elderly	Rare, any age	Rare in children, ultrarare in adults	Rare, depending on definition
Cause	Unknown in <50% of cases; secondary in ≥50% of cases*	Low-grade lymphoproliferative bone marrow disorder	Secondary*	Postviral (in children); tertiary syphilis, hematologic cancers (in adults)	Unclear
Pathogenesis					
Autoantibody	Warm-reactive, panreactive, polyclonal	Cold agglutinin, anti-I (in rare cases, anti-Pr or anti-IH), monoclonal	Cold agglutinin, anti-I or anti-i, polyclonal or monoclonal	Nonagglutinating, biphasic anti-P, polyclonal	Both warm- and cold-reactive antibodies
Immunoglobulin class	IgG (in rare cases, IgM or IgA)	IgM (in rare cases, IgG)	IgM or IgG	IgG (in rare cases, IgM)	IgG plus IgM
Complement activation†	Frequently none; classical pathway (++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+++)	Present, details not established
Predominant type of hemolysis	Extravascular (mainly in the spleen)	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Intravascular	Not established
Typical findings					
Direct antiglobulin test	IgG positive C3d negative or positive In rare cases, IgA or IgM positive	C3d positive In rare cases, IgG or IgM positive IgA negative	C3d positive IgG positive or negative In rare cases, IgM positive IgA negative	C3d positive In rare cases, IgG or IgM positive IgA negative	IgG and C3d positive In rare cases, IgM positive IgA negative
Cold agglutinin	Absent	High titer	High titer	Absent	High titer

Idiopathic, SLE, CLL, lymphoma, medication

CAD, lymphoma, post-infectious (in particular EBV, mycoplasma)

Brodsky RA. N Engl J Med 2019;381:647-54
Berentsen S, Barcellini W. N Engl J Med 2021;385:1407-19

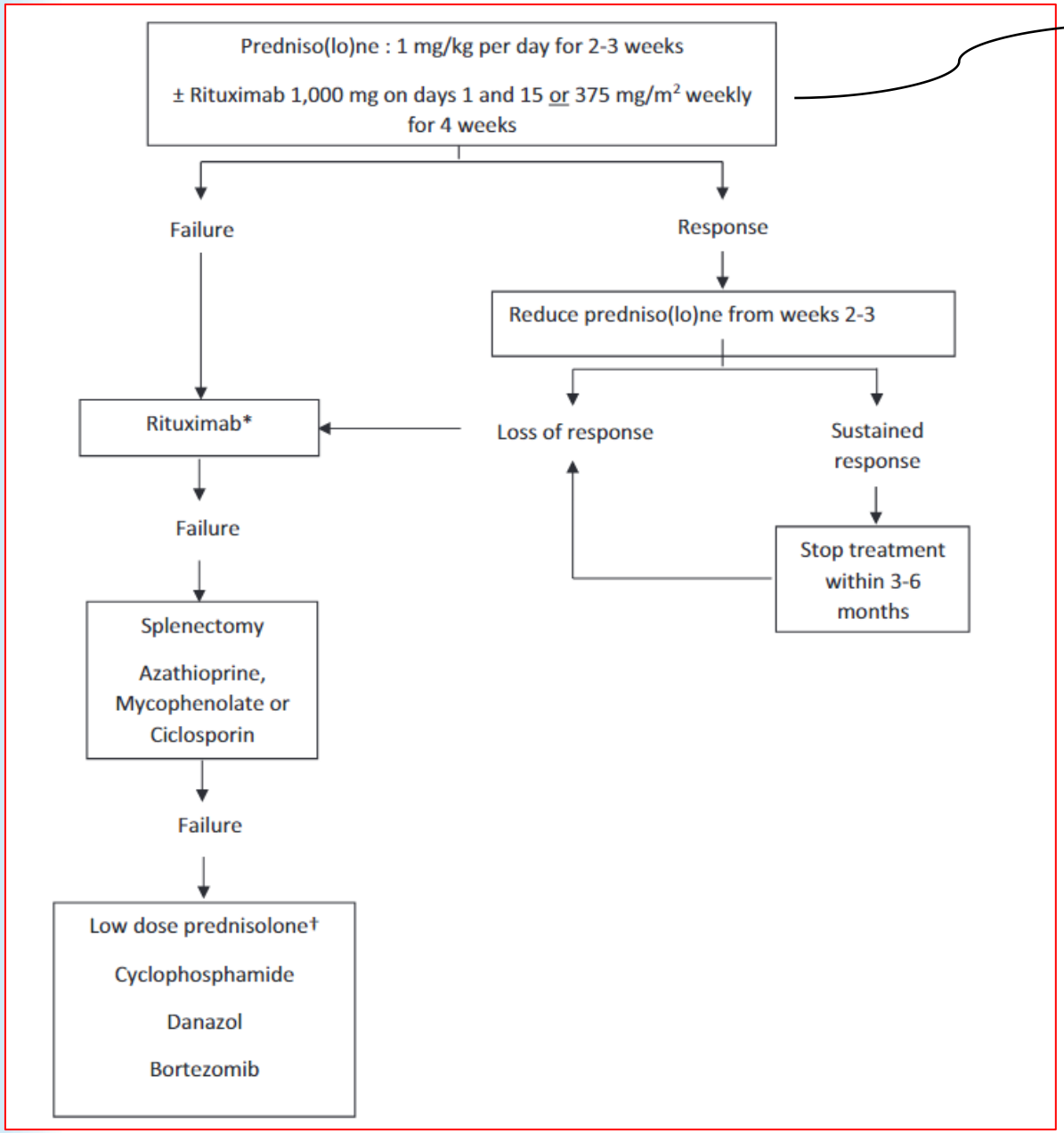
AIHA - classification



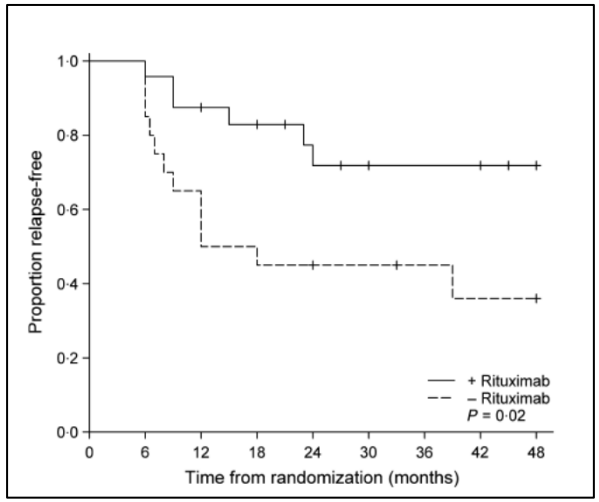
Barcellini W, Fattizzo B. Front Immunol 2020;11:946

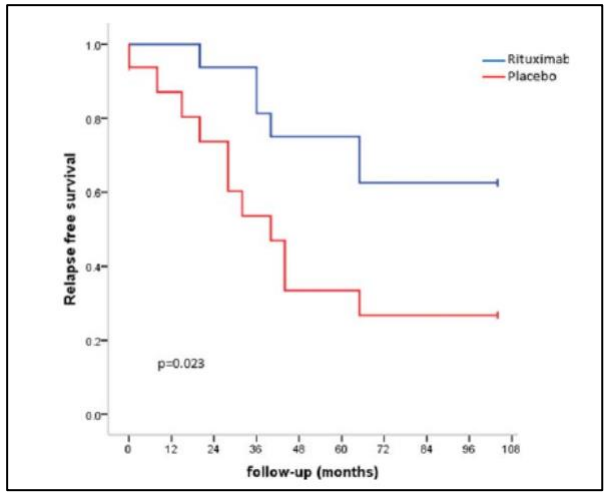
Berentsen S, Barcellini W. N Engl J Med 2021;385:1407-19

Warm AIHA - treatment



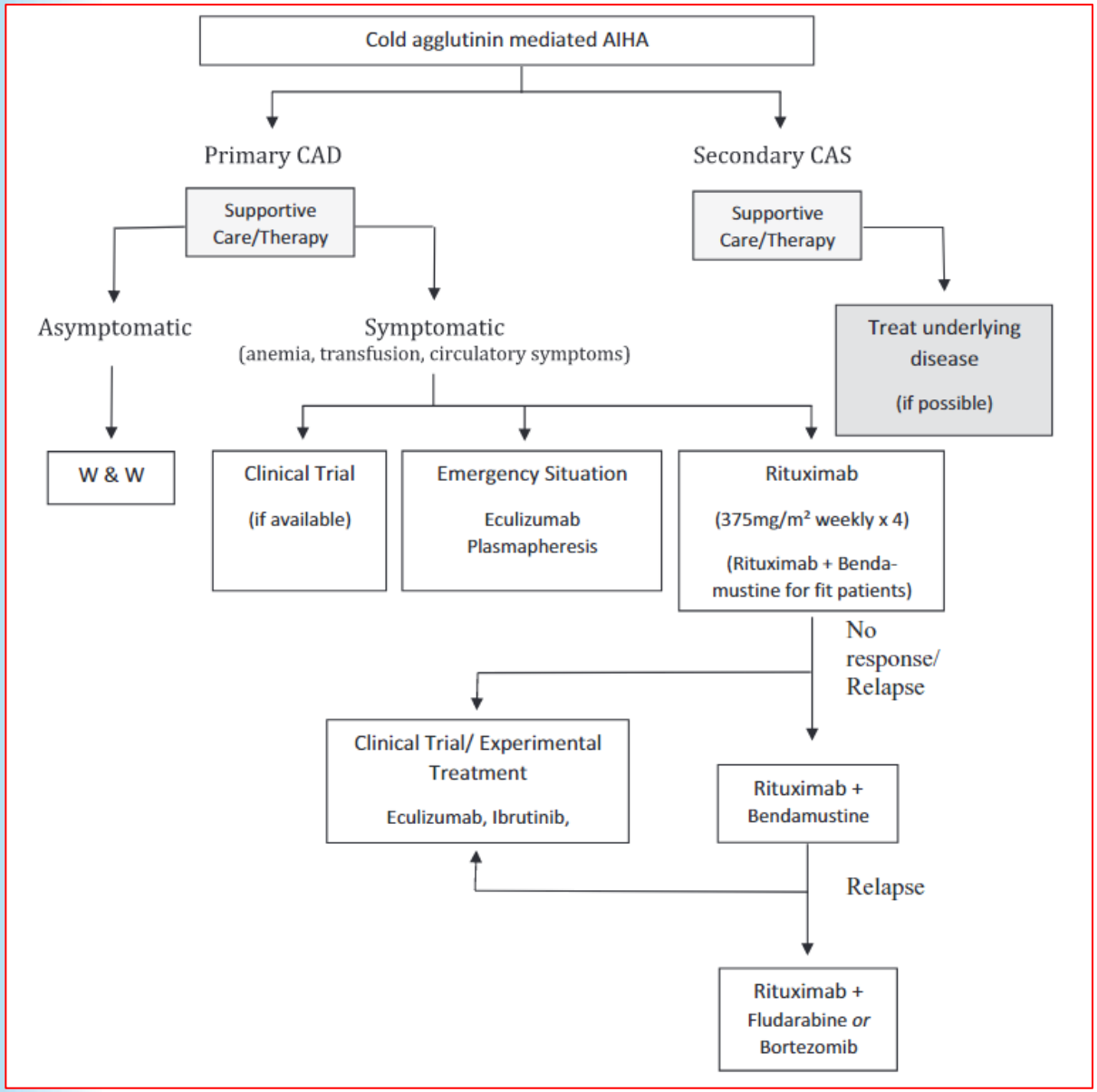
First International Consensus Meeting
Severe disease (Hb < 8 g/dL; atypical AIHA [IgA-mediated, DAT negative]; Evans syndrome)

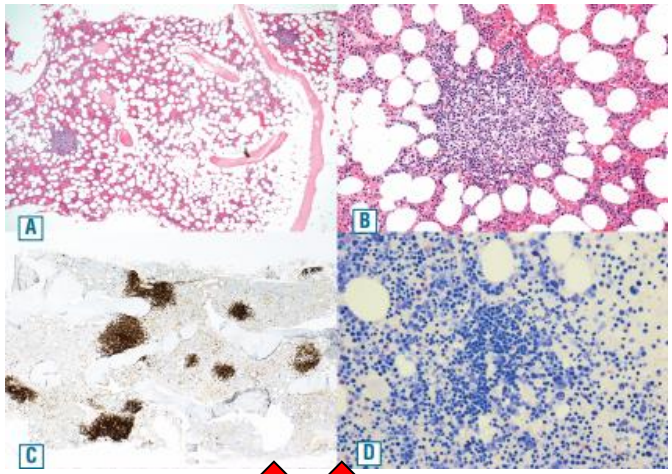




Jäger U, et al. Blood Rev 2020;14:100648
Birgens H, et al. Br J Haematol 2013;163:393-9
Michel M, et al. Am J Hematol 2017;92:23-7

Cold AIHA - treatment

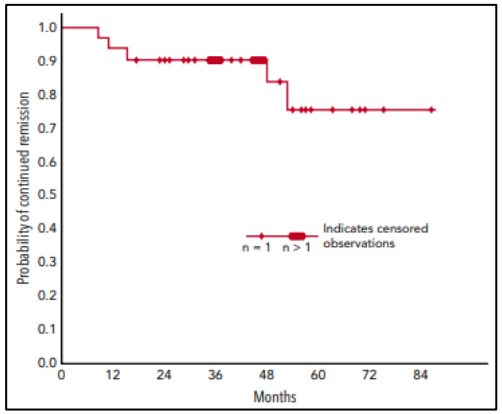




MYD L265P

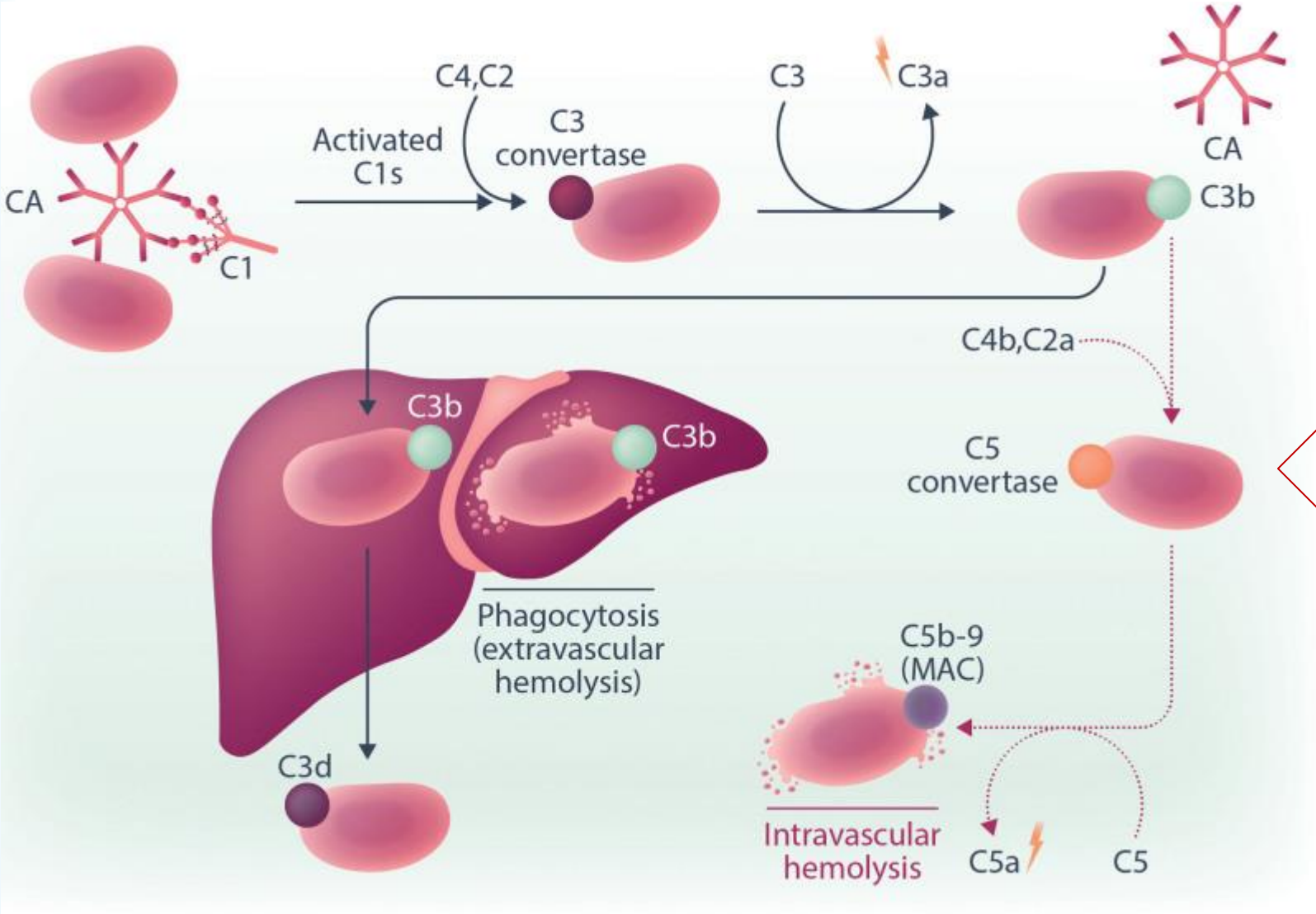
Table 2. Response rates

Response level	n	%
CR	18	40
PR	14	31
NR	13	29
All patients	45	100



Jäger U, et al. Blood Rev 2020;14:100648
Randen U, et al. Haematologica 2014;99:497-504
Berentsen S, et al. Blood 2017;130:537-41
Berentsen S, et al. Blood 2020;136:480-8

CAD – role of complement



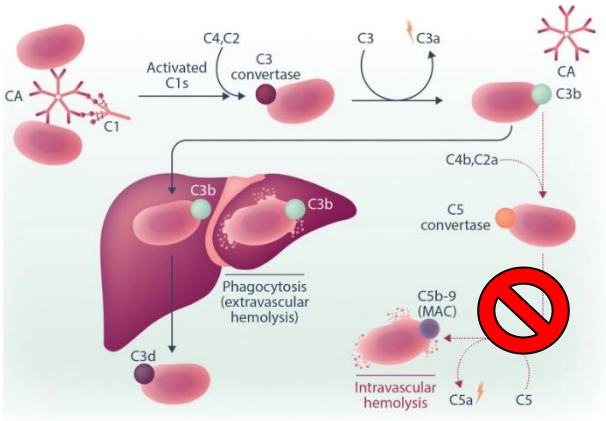
Extra trigger
(infection, surgery, trauma,…)

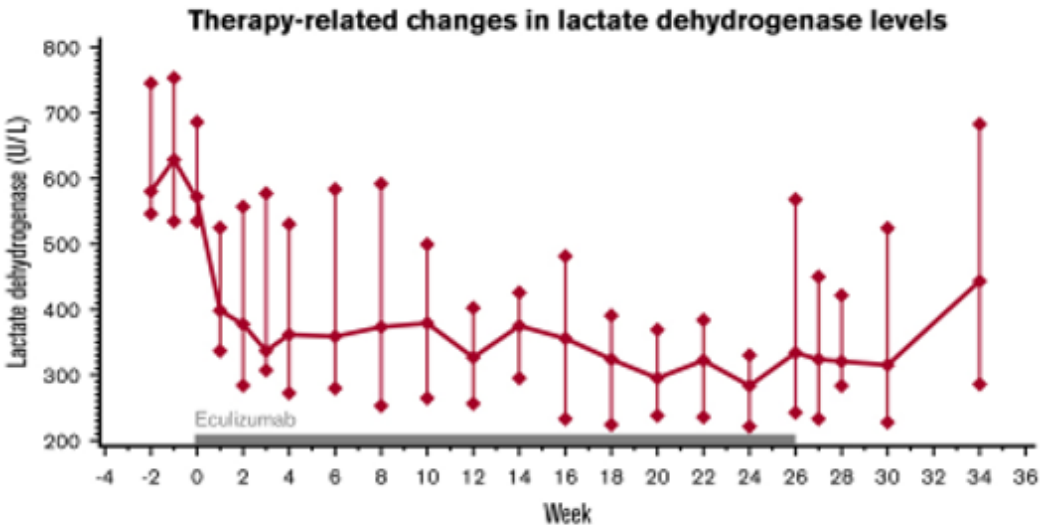
Berentsen S. Hemasphere 2023;2;7:e879

CAD – role of complement

C5

DECADE trial (eculizumab)
n = 13 (open label, phase 2)





Severity/possibly related to treatment	Probably related to treatment
Severe	
Peritonitis*	Pneumonia†
Moderate	
Hemorrhoidal hemorrhage*	---
Fatigue‡	
Muscle cramps‡	
Arterial stenosis‡	
Hypertension	
Pruritus	
Urinary tract infection	
Mild	
Limb pain‡	---
Oral herpes infection	
Fatigue	
Creatinine increase (2 episodes)	

But:

Only limited effect on hemoglobin

No improvement of cold-induced circulatory symptoms

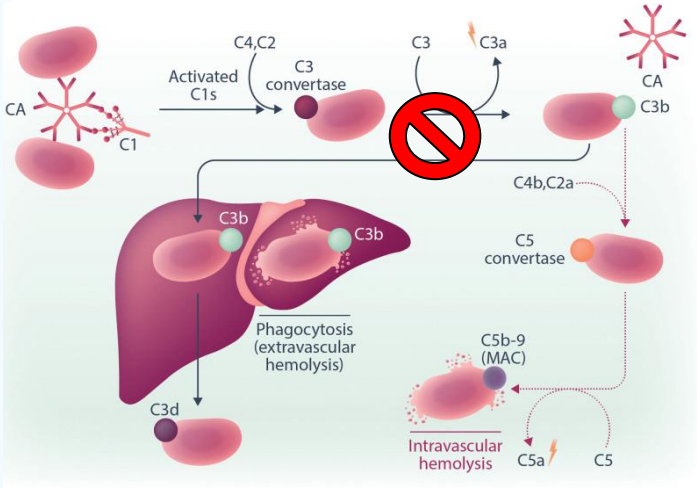
Röth A, et al. Blood Adv 2018;2:2543-9
Berentsen S. Hemasphere 2023;2;7:e879

CAD – role of complement

C3

(pegcetacoplan)
Randomized, phase 3 trial

Rationale: C5 inhibition doesn't prevent formation of C3b ➔ less effect on extravascular hemolysis



A Study to Evaluate the Efficacy and Safety of Pegcetacoplan in Patients With Cold Agglutinin Disease (CAD)

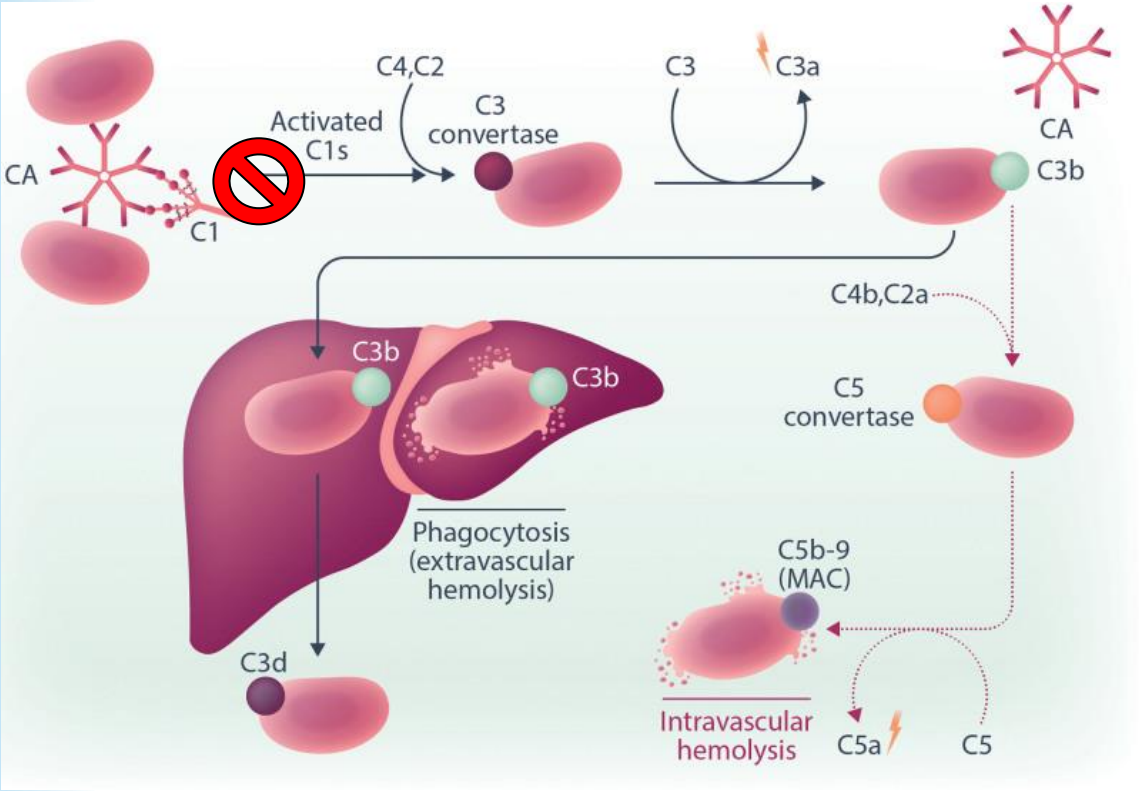
https://classic.clinicaltrials.gov/ct2/show/NCT05096403

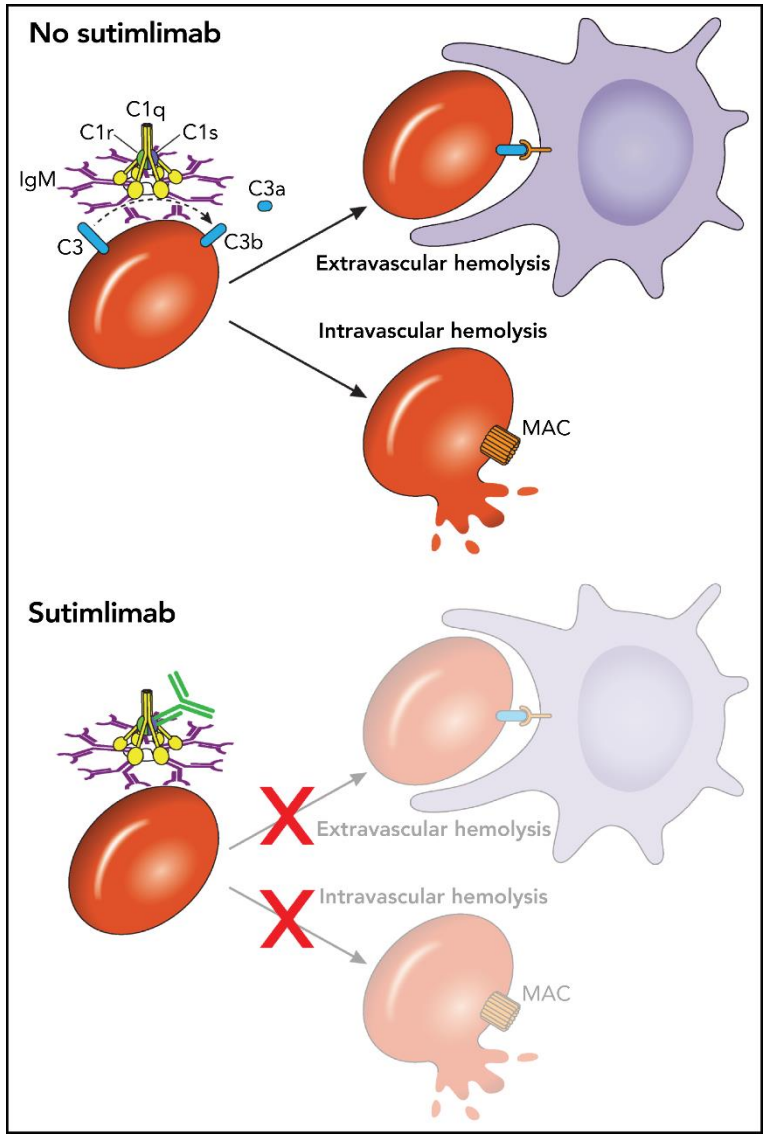
APL-2 increases Hb values in CAD and wAIHA C3+ within the first weeks of treatment with sustained benefit with longer exposure. APL-2 reduces intra- and extravascular haemolysis shown by reductions in LDH, INDBIL, and ARC. APL-2 appears to be safe and well-tolerated.

Gertz M, et al. EHA 2019, Abstract S899
Berentsen S. Hemasphere 2023;2;7:e879

CAD – role of complement

C1s



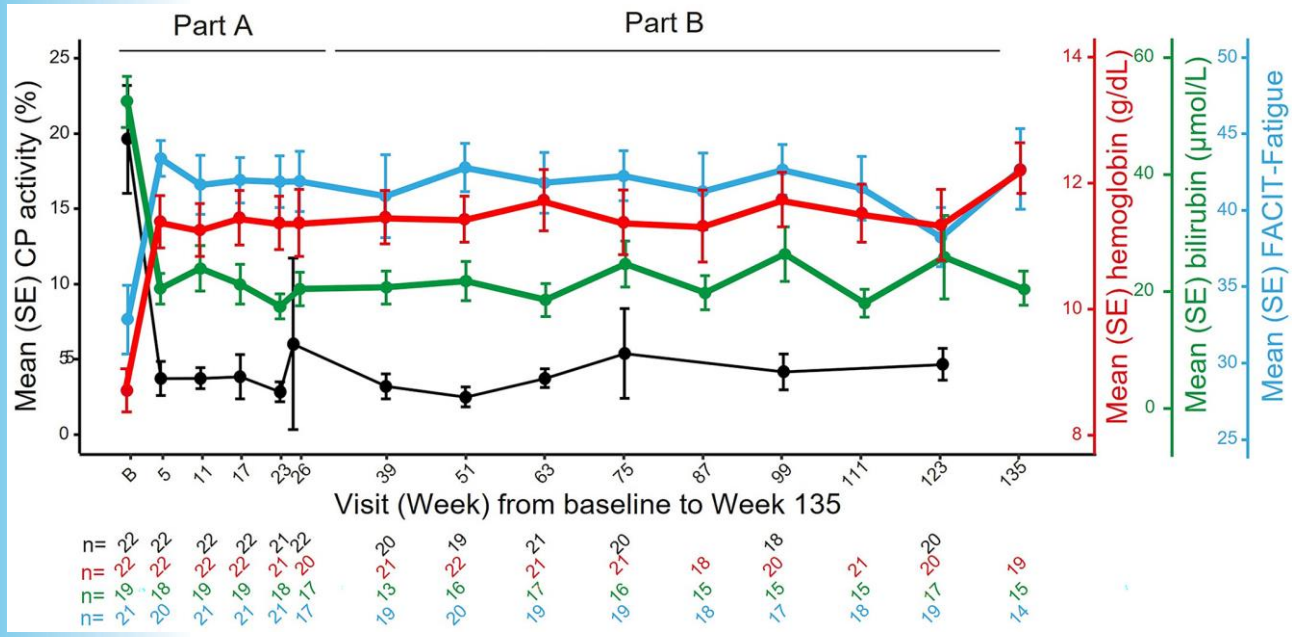


Chonat S, Stowell SR. Blood 2022;140:933-5
Berentsen S. Hemasphere 2023;2;7:e879

CAD – role of complement

C1s

CARDINAL trial (sutimlimab)
n = 24 (open label, phase 3, recent transfusion)



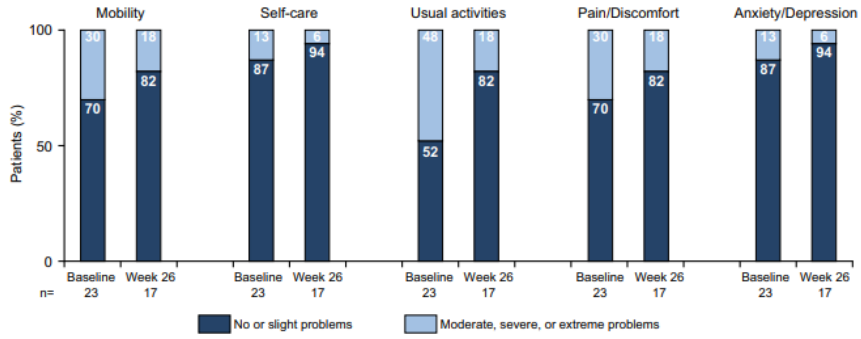


Figure 1

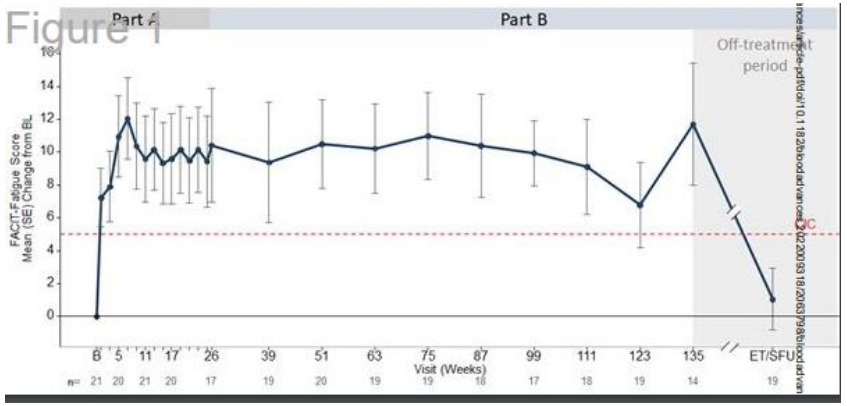


Table 2. Summary of Adverse Events.*

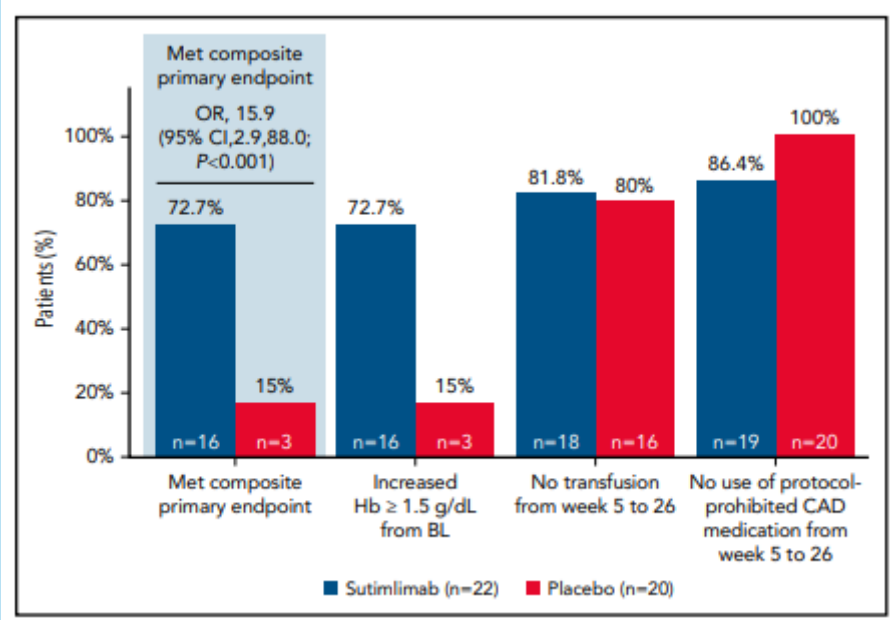
Event Category	Total (N=24)
All adverse events — no.	124
Patients with ≥1 adverse event — no. (%)	22 (92)
Patients with ≥1 treatment-related adverse event — no. (%)	9 (37)
No. of events	13
Serious adverse events — no.	16
Patients with ≥1 serious adverse event — no. (%)	7 (29)
Patients with ≥1 serious infection — no. (%)	2 (8)†
Patients who discontinued treatment or study because of an adverse event — no. (%)	1 (4)‡
Death — no. (%)	1 (4)‡

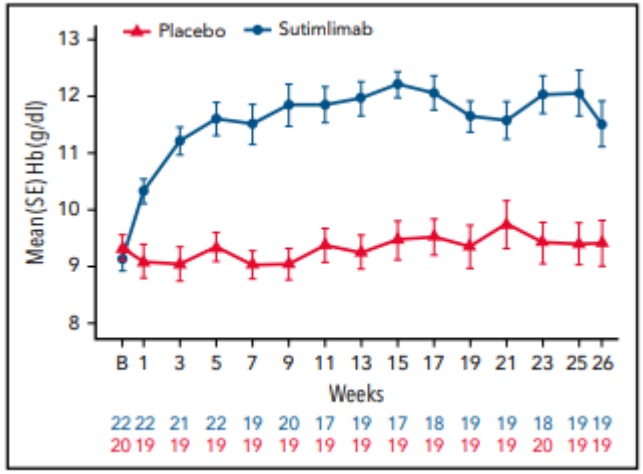
Röth A, Barcellini W. N Engl J Med 2021;384:1323-34
Röth A, et al. Ann Hematol 2022;101:2169-77
Röth A, et al. Am J Hematol 2023;98:1246-53
Röth A, et al. Blood Adv 2023;7:5890-7

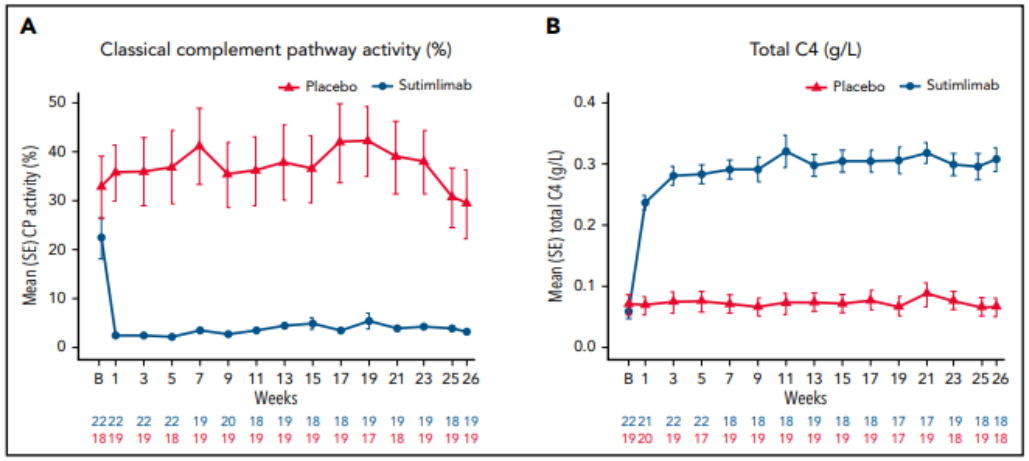
CAD – role of complement

C1s

CADENZA trial (sutimlimab)
n = 42 (randomized, phase 3, no recent transfusion)







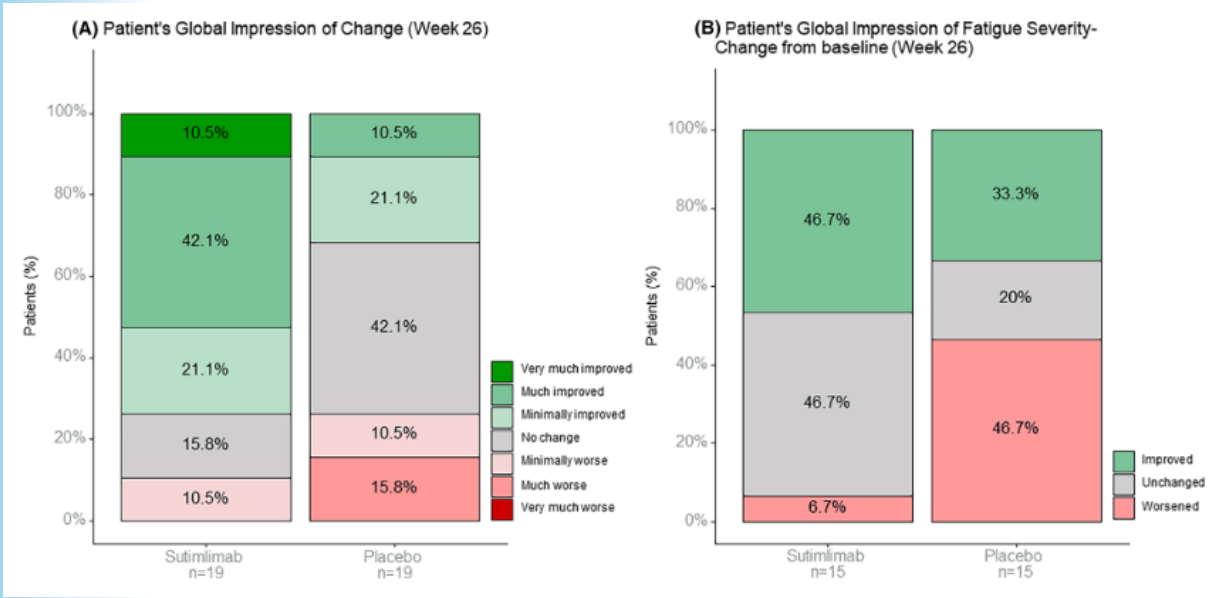
	Sutimlimab (N = 22)	Placebo (N = 20)
TEAEs, n	146	90
Patients with ≥1 TEAE, n (%)	21 (95.5)	20 (100)
Patients with ≥1 related TEAE,* n (%)	8 (36.4)†	4 (20.0)‡
Patients with ≥1 TEAE grade 3 or higher, n (%)	5 (22.7)	3 (15.0)
Patients with ≥1 TEAE infection grade 3 or higher, n (%)	2 (9.1)	1 (5.0)
TESAEs, n	4	3
Patients with ≥1 TESAE, n (%)	3 (13.6)	1 (5.0)
Patients with ≥1 related TESAE,* n (%)	1 (4.5)	0
Patients with ≥1 TESAE infection, n (%)	1 (4.5)	1 (5.0)
Total number of TESAE thromboembolic events, n	1	0
Patients with ≥1 TESAE thromboembolic event, n (%)	1 (4.5)	0
Patients who discontinued treatment and/or study owing to a TEAE, n (%)	3 (13.6)§	0
Deaths, n (%)	0	0

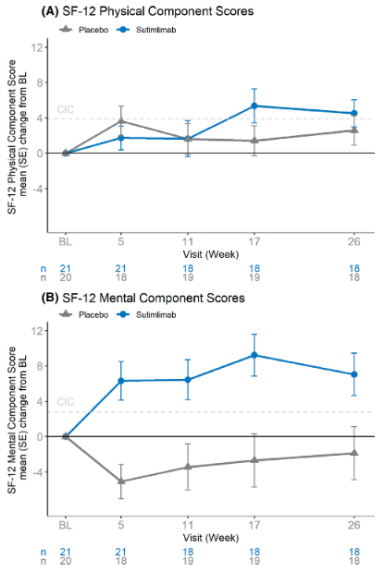
Röth A, et al. Blood 2022;140:980-91

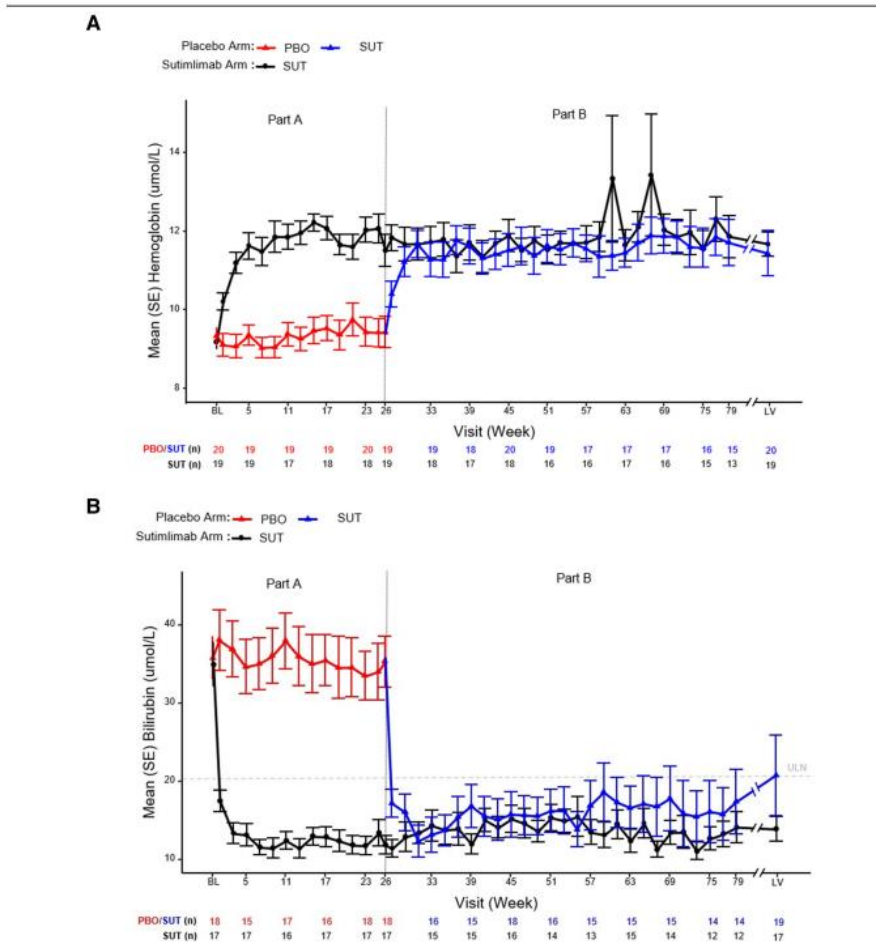
CAD – role of complement

C1s

CADENZA trial (sutimlimab)
n = 42 (randomized, phase 3, no recent transfusion)







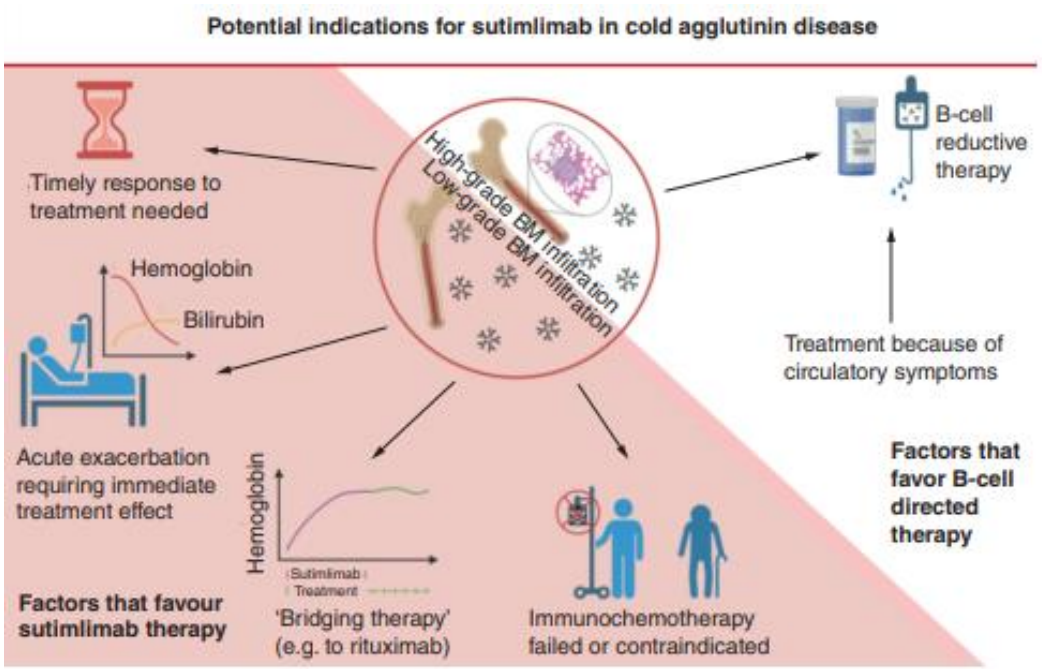
Röth A, et al. Eur J Haematol 2023;110:280-8
Berentsen S. Hemasphere 2023;2;7:e879

CAD – role of complement

C1s

Role of Complement for Specific Disease Features in CAD

Complement Involvement	Disease Feature	Pathogenetic Mechanism
Complement-mediated features	Hemolysis	Classical complement pathway activation by Ag-Ab complex→ C3b opsonization of RBCs → mainly extravascular hemolysis
	Fatigue	Classical complement pathway activation by Ag–Ab complex → soluble proinflammatory split products
	Exacerbation triggered by febrile infections, major trauma, or major surgery	Increased production of C4 (and C3) in acute phase reaction → complement enhancement→ extra/intravascular hemolysis
	Risk of thrombosis	Multiple points of interaction between the complement and coagulation cascades
Noncomplement-mediated features	Cold-induced circulatory symptoms • Acrocyanosis • Raynaud-like symptoms • Livedo reticularis (uncommon) • Gangrene (rare)	RBC surface I-antigen binds IgM-CA → RBC agglutination



Berentsen S, et al. Immunotherapy 2022;14:1191-204
Berentsen S. Hemasphere 2023;2;7:e879

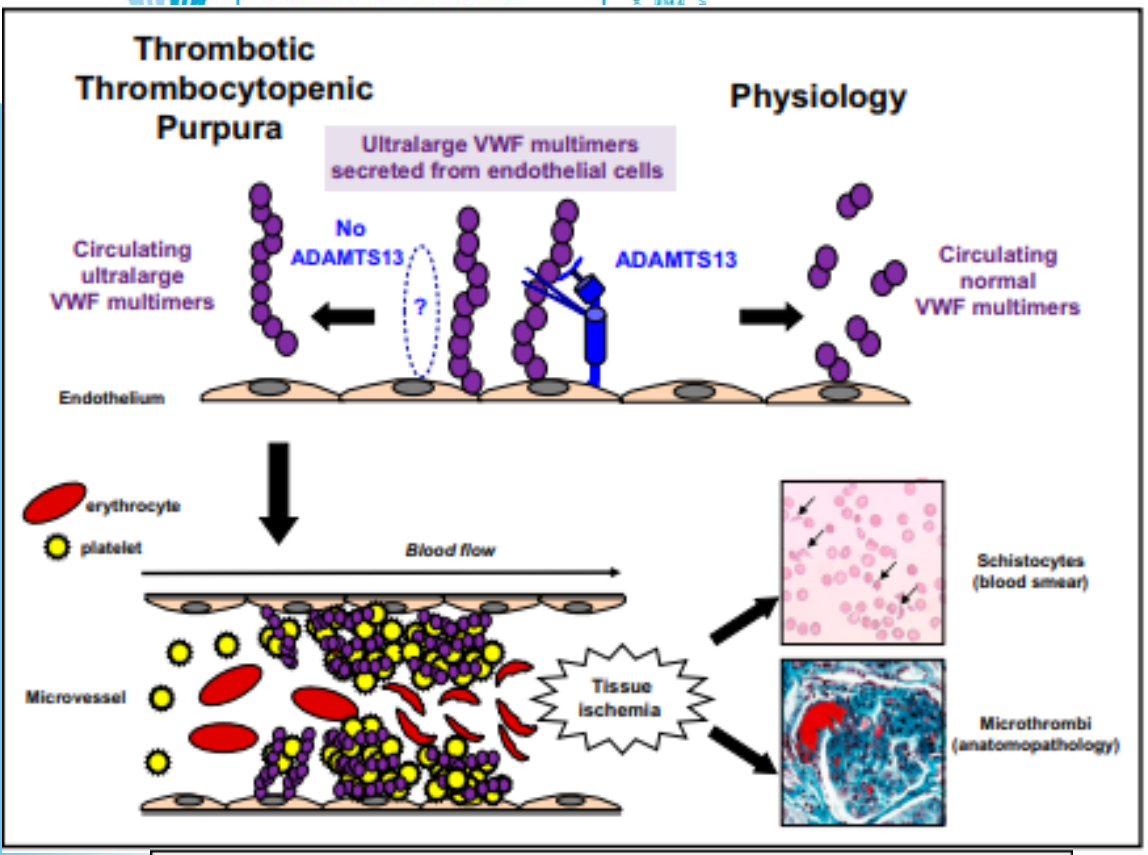
Hemolytic anemia

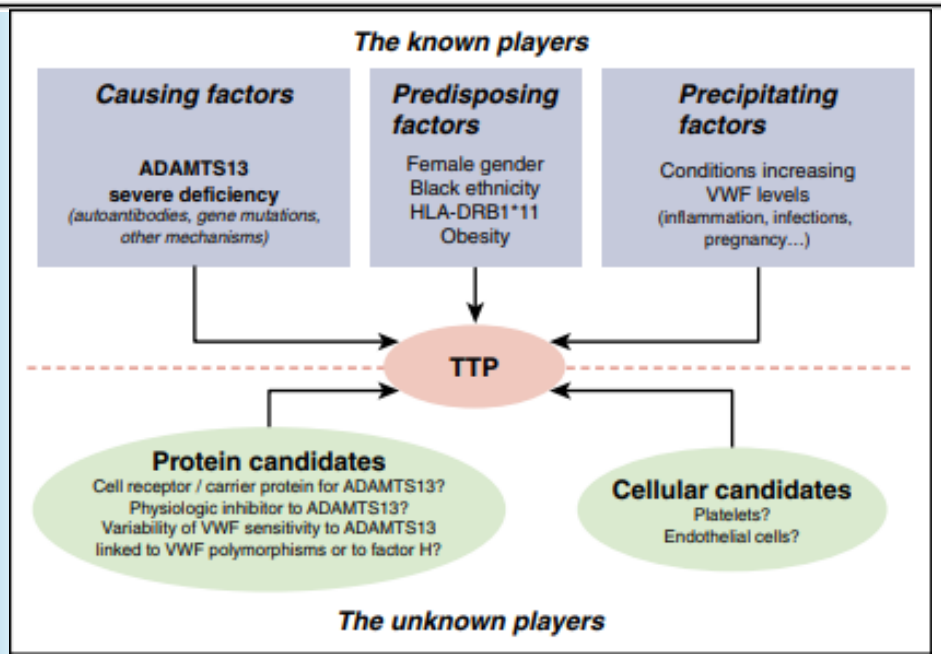
TMA

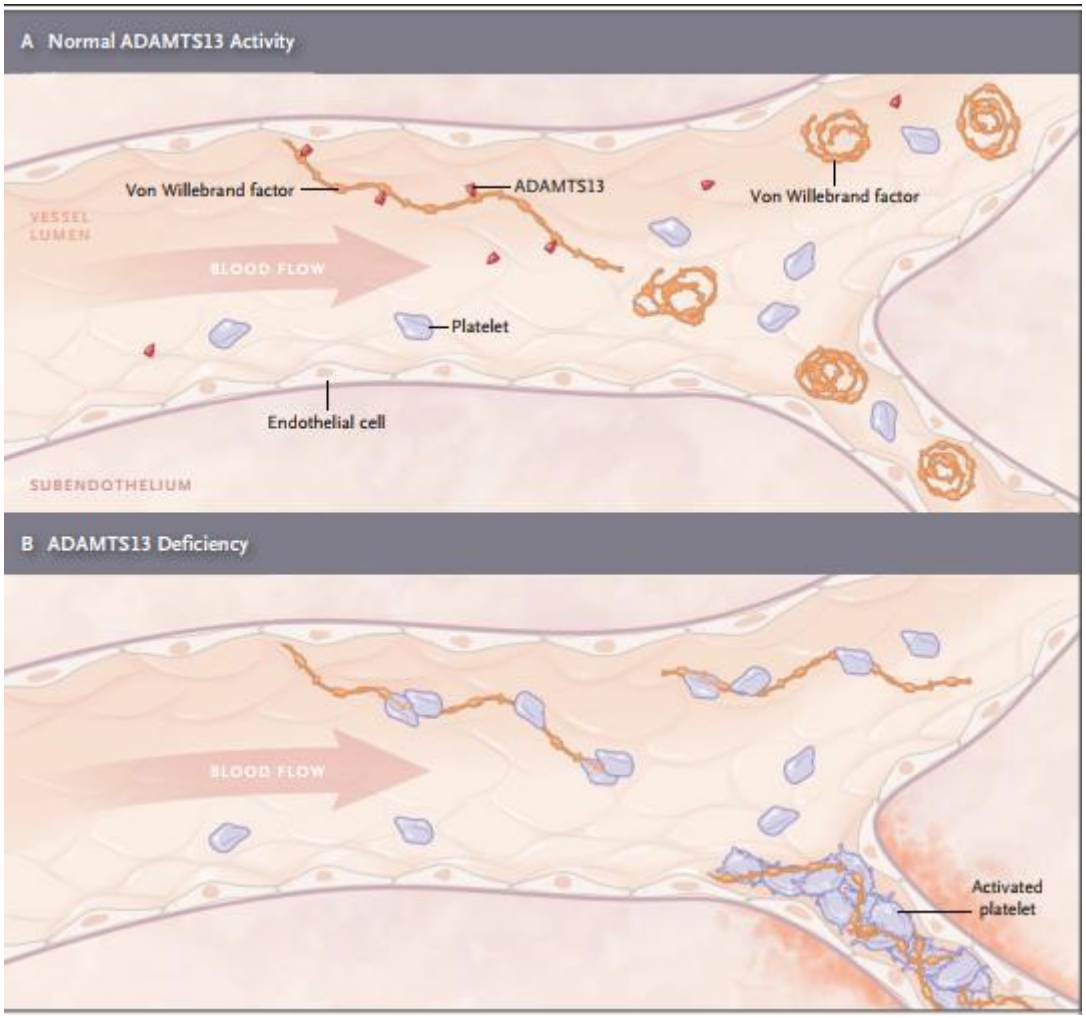
TMA - classification

- **ADAMTS13-deficiency thrombotic thrombocytopenic purpura (TTP)**
  - Acquired (auto-anti-ADAMTS13 antibodies)
  - Congenital (mutations in ADAMTS13 gene)
- **Hemolytic uremic syndrome (HUS)**
  - Typical HUS (Shiga toxin producing Escherichia coli)
  - Atypical HUS
    - Congenital (mutations in complement regulatory proteins, thrombomodulin)
    - Acquired (auto-anti-complement regulatory proteins antibodies)
- **Secondary thrombotic micro-angiopathy (TMA): associated with**
  - Solid organ transplantation
  - Hematopoietic stem cell transplantation
  - Medication (clopidogrel, ticlodipin, quinine, mitomycin C, gemcitabin, calcineurin inhibitors, proliferation signaling inhibitors,…)
  - Auto-immune disorders (antiphopsholipid syndrome, systemic lupus erythematosus,…)

TTP - physiopathology

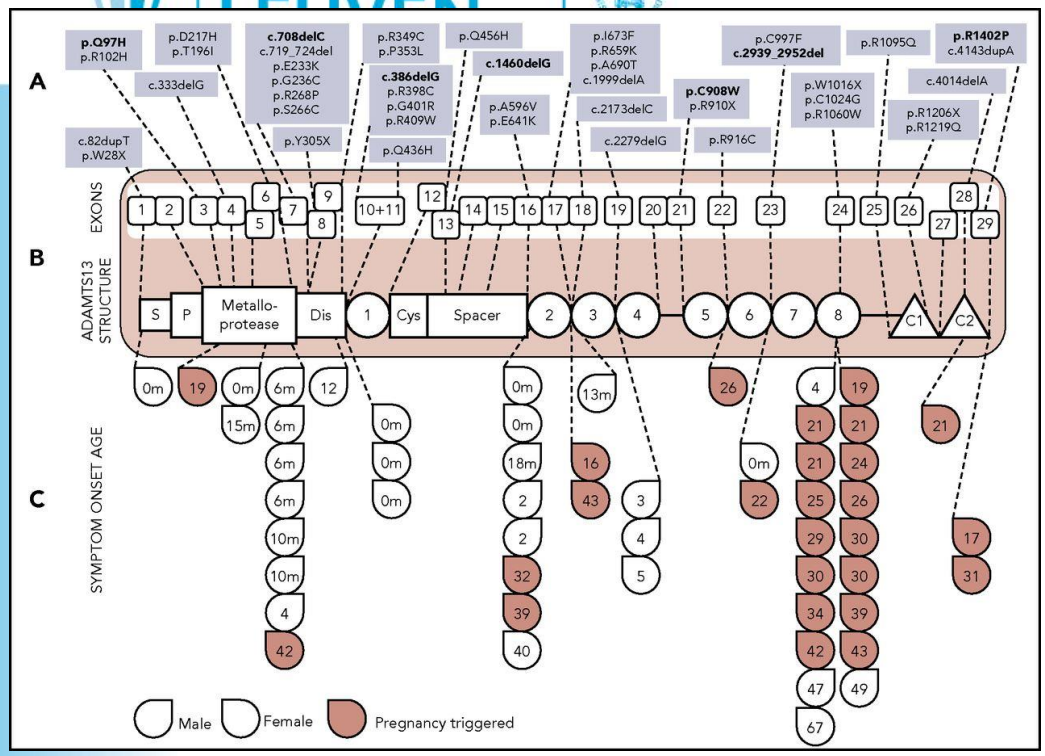


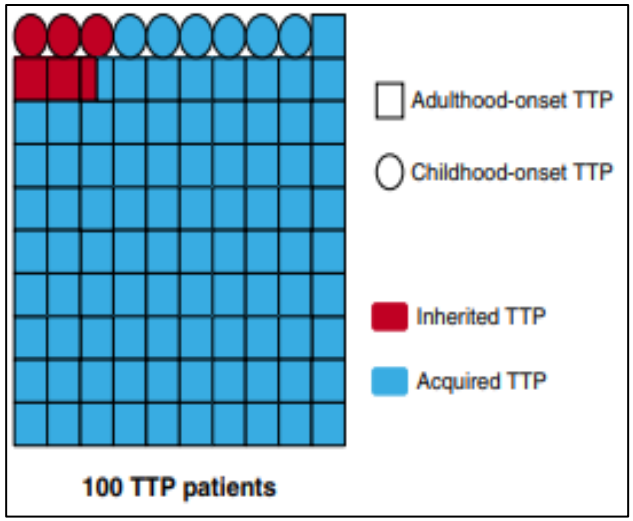


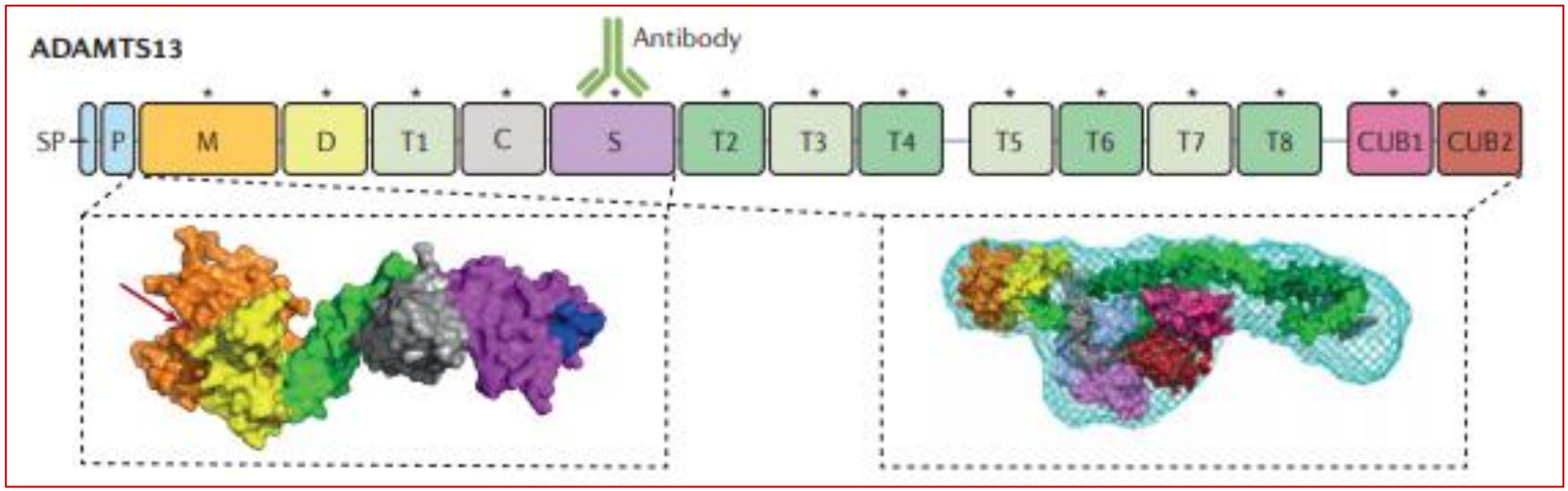


Joly BS, et al. Blood 2017;129:2836-46
Kremer Hovinga JA, George JN. N Engl J Med 2019;381:1653-62

TTP - physiopathology



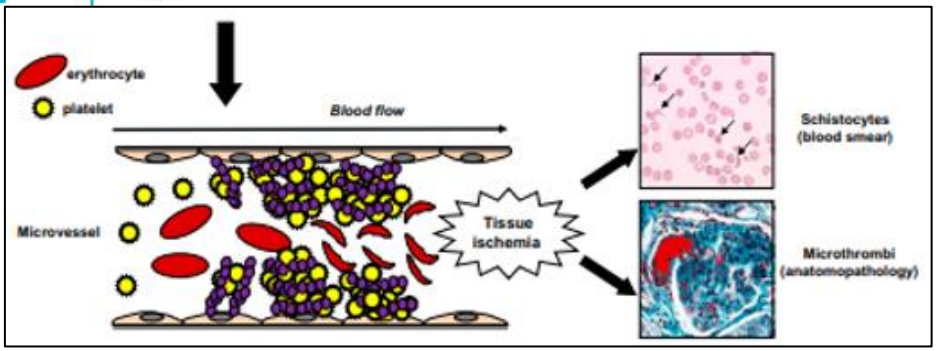




ADAMTS13

Joly BS, et al. Blood 2017;129:2836-46
Kremer Hovinga JA, et al. Nat Rev Dis Primers 2017;3:17020
Alwan F, et al. Blood 2019;133:1644-51

TTP - symptoms



Pentade:

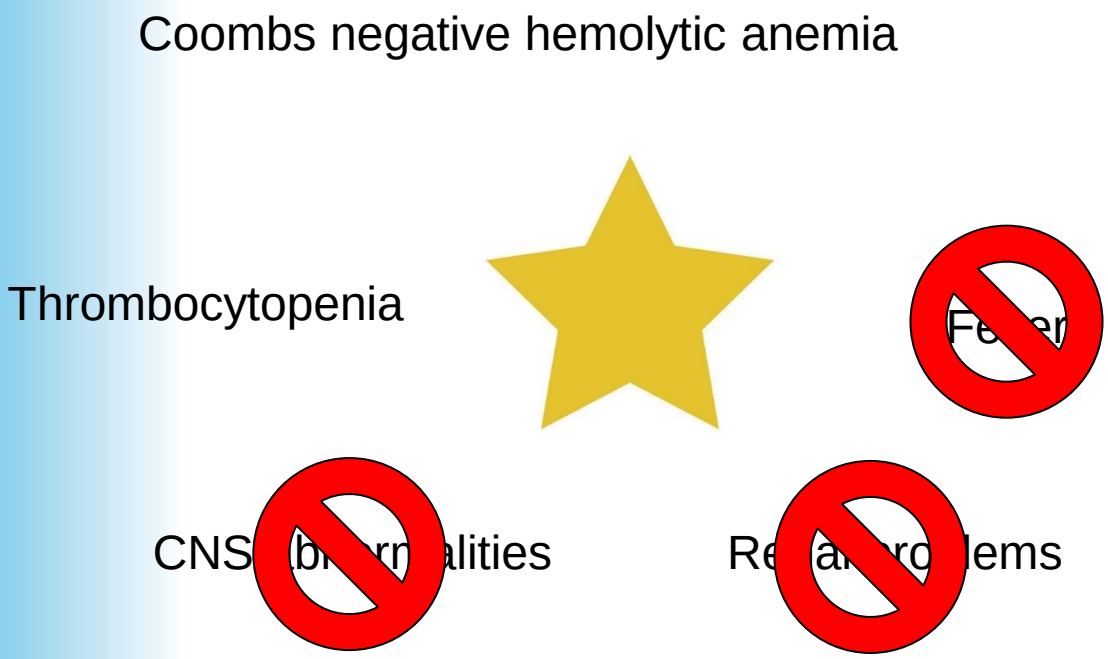
- **Coombs negative hemolytic anemia**
  - Presence of schistocytes
  - Elevated indirect bilirubin
  - Elevated LDH (hemolysis + tissue damage)
- **Trombocytopenia**
  - In acute phase very low (<20000/µL)
  - Often bleeding tendency
- **CNS abnormalities**
  - Most frequently: coma, convulsions and focal deficits
  - Often in advanced and non-diagnosed cases
- **Renal abnormalities**
  - Elevated serum creatinin, microscopic hematuria, proteinuria
- **Fever**

- Recently: often cardiac and pancreatic involvement

Symptom	N	%
MAHA	70	100
Thrombocytopenia	70	100
Neurological disturbances	60	86
Renal problems	38	54
Fever	17	24
Pentade	10	14

Sarode R, et al. Am J Haematol 1997;54:102-7
Joly BS, et al. Blood 2017;129:2836-46

TTP - symptoms



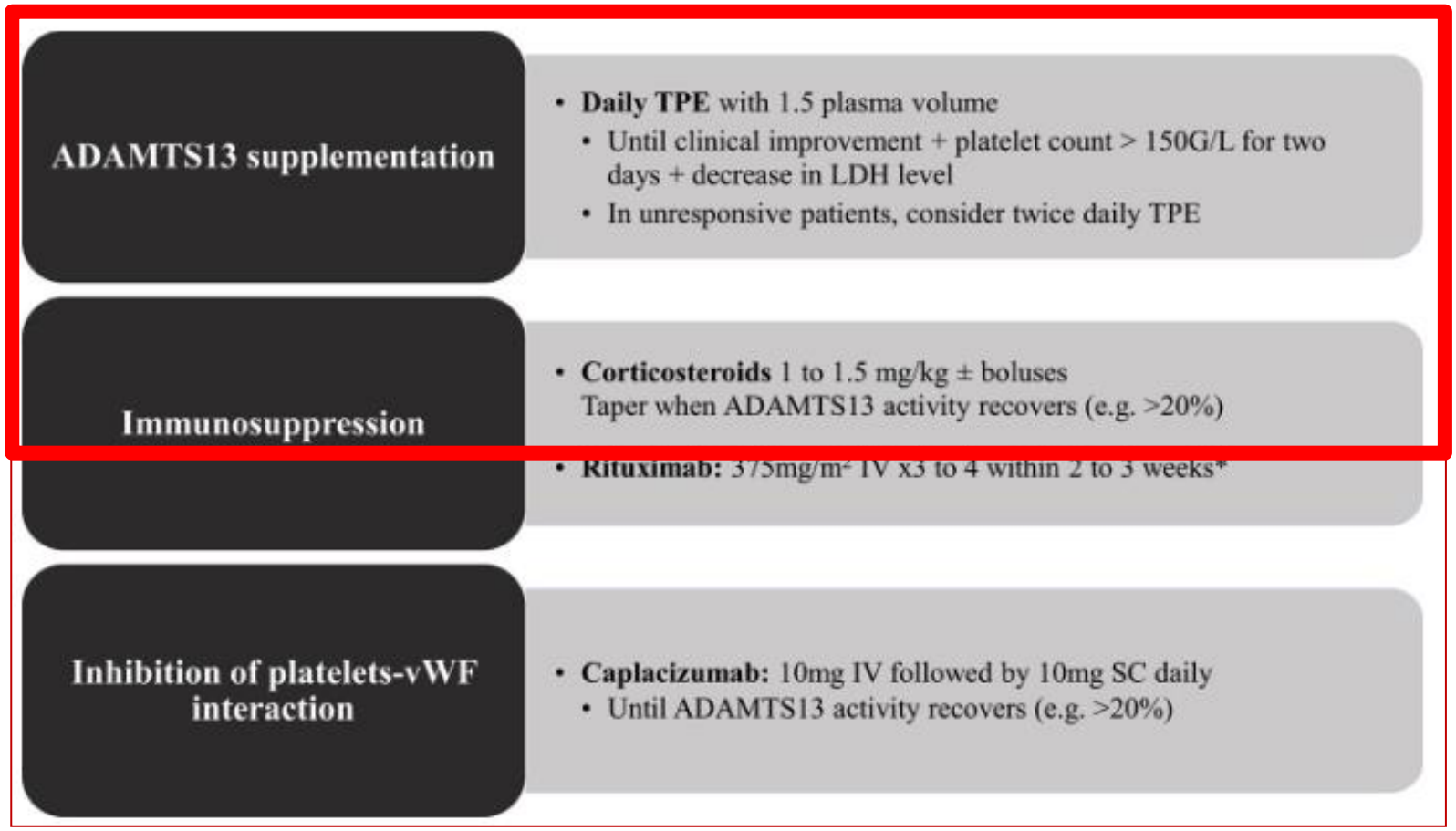
Essential for diagnosis

- Thrombocytopenia
- Coombs' negative microangiopathic hemolytic anemia
- Absence of other possible causes of microangiopathic hemolytic anemia

+ ADAMTS13 activity < 10%

Zheng XL, et al. J Thromb Haemost 2020;18:2486-95

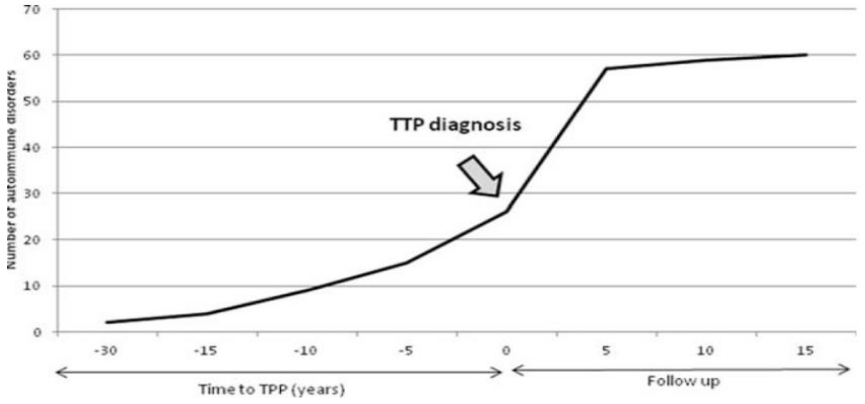
TTP - treatment



Picod A, Coppo P. Transfus Apher Sci 2019;58:273-7
Zheng XL, et al. J Thromb Haemost 2020;18:2496-502

Why do we need new treatments?

- Suboptimal respons
  - Exacerbations (50%)
  - Refractoriness (10%)
- Death: 15% (high ADAMTS IgG antibody level + low ADAMTS13 antigen level ➔ mortality 27%)
- Highest mortality: first 2 weeks (median time to death 9 days ([IQR 4-14 days])
- During follow up: 15% develop other autoimmune disorder



Number of autoimmune diseases cases according to date of TTP diagnosis.

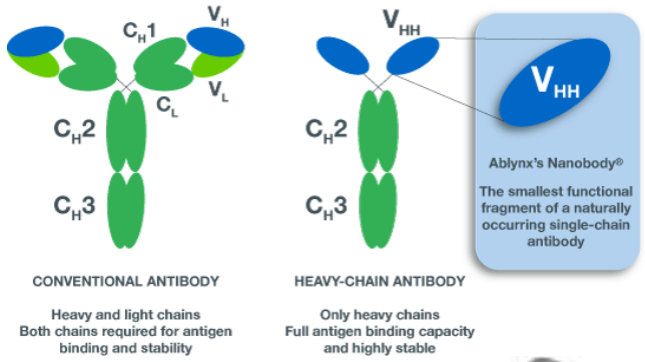
How to improve outcome of patients with TTP?

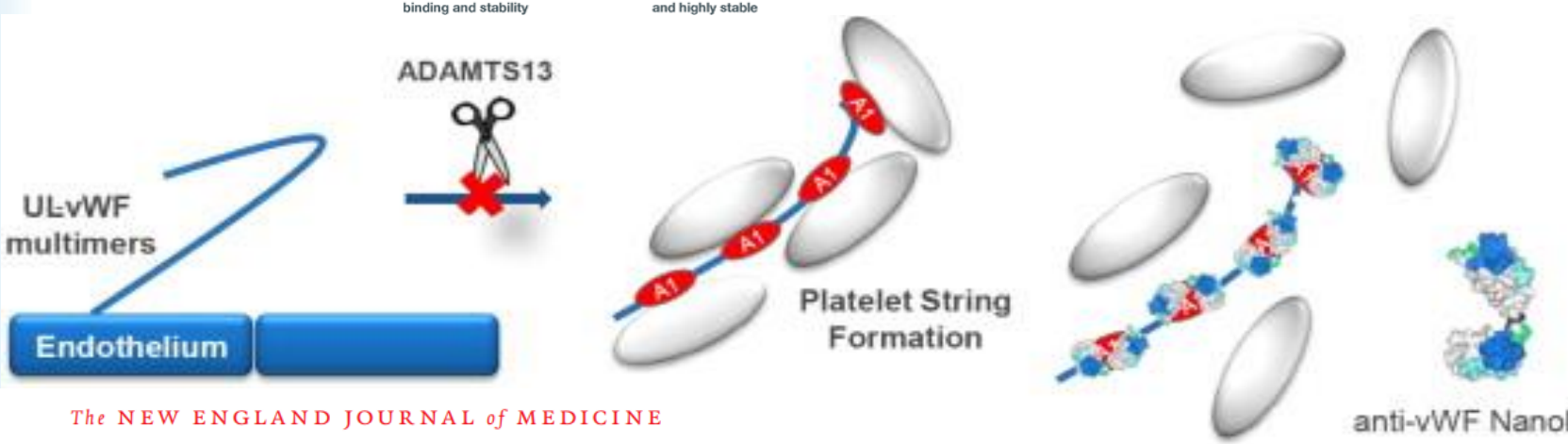
Roriz M, et al. Medicine 2015;94:e1598
Alwan F, et al. Blood 2017;130:466-7
Goel R, et al. Transfusion 2016;56:1451-81
Zheng XL, et al. J Thromb Haemost 2020;18:2496-502

TTP - treatment

1. Blocking vWF-platelet binding

Caplacizumab





The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura

M. Scully, S.R. Cataland, F. Peyvandi, P. Coppo, P. Knöbl, J.A. Kremer Hovinga, A. Metjian, J. de la Rubia, K. Pavenski, F. Callewaert, D. Biswas, H. De Winter, and R.K. Zeldin, for the HERCULES Investigators*

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 11, 2016 VOL. 374 NO. 6

Caplacizumab for Acquired Thrombotic Thrombocytopenic Purpura

Flora Peyvandi, M.D., Ph.D., Marie Scully, M.D., Johanna A. Kremer Hovinga, M.D., Spero Cataland, M.D., Paul Knöbl, M.D., Haifeng Wu, M.D.,* Andrea Artoni, M.D., John-Paul Westwood, M.D., Magnus Mansouri Taleghani, M.D., Bernd Jilma, M.D., Filip Callewaert, Ph.D., Hans Ulrichts, Ph.D., Christian Duby, M.D., and Dominique Tersago, M.D., for the TITAN Investigators†

Holz JB, Transf Apher Sci 2012;3:343-6
Peyvandi F, et al. N Engl J Med 2016;374:511-22
Scully M, et al. N Engl J Med 2019;380:335-46

TTP - treatment

Reference	Number of patients Caplacizumab vs placebo	Mean age (y) (range)	Female N (%)	CR %	Recurrence* %	Exacerbation %	Relapse %	Mortality in the acute phase %	Mean TPE duration (d)
TITAN (8)	75 (36 vs 39)	42 (19-72)	44 (59)	81 vs 46	38.9 vs 38.4	8.3 vs 28.2	30.6 vs 7.7[$]	0 vs 5.1	5.9 vs 7.9
HERCULES (9)	145 (72 vs 73)	46 (18-79)	100 (69)	NA	12.5 vs 38.3	4.2 vs 38.3	8.3 vs 0[£]	0 vs 4.1	5.8 vs 9.4

Caplacizumab

Adverse event	TITAN Caplacizumab vs placebo	HERCULES Caplacizumab vs placebo
Bleeding	54.2 vs 37.8	64.8 vs 47.9
Gingival bleeding	14.3 vs 5.4	18.3 vs 1.4
Epistaxis	31.4 vs 10.8	32.4 vs 2.7
Hematuria	0 vs 2.7	7.0 vs 2.7
Catheter site hemorrhage	NA	7.0 vs 6.8
Subarachnoid hemorrhage	2.9 vs 0	1.4 vs 0
Headache	34.3 vs 27	22.5 vs 8.2
Pyrexia	17.1 vs 16.2	14.1 vs 8.2
Myalgia	20.0 vs 2.7	NA
Urticaria	NA	16.9 vs 6.8

Peyvandi F, et al. N Engl J Med 2016;374:511-22
Scully M, et al. N Engl J Med 2019;380:335-46
Picod A, Coppo P. Transfus Apher Sci 2019;58:273-7

TTP - treatment

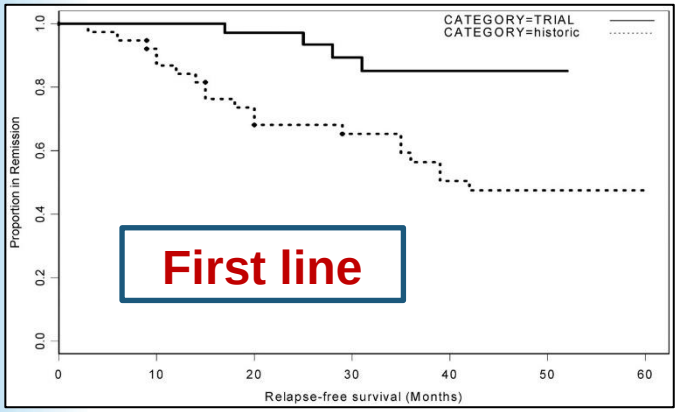
2. Anti-CD20 therapy

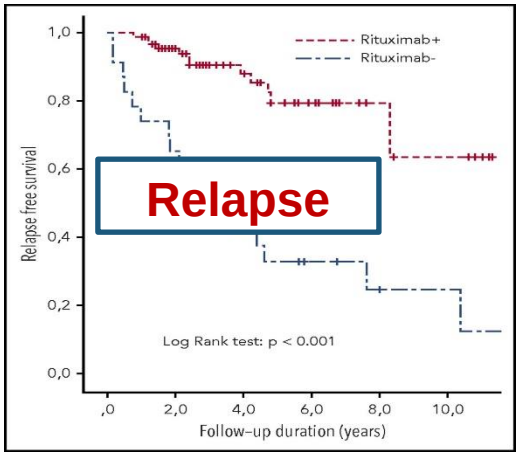
Rituximab

Table 2. Rituximab for the treatment of patients with TTP and for treatment of ADAMTS13 deficiency during remission: levels of evidence and interpretation

Indication	Key citation	Grade of recommendation and evidence*	Interpretation
Initial treatment of TTP	Scully, 2011[8]	2C	We suggest rituximab be considered for this indication. Rituximab may decrease the time to achieve remission and may delay subsequent relapse.
Treatment of refractory episodes of TTP	Froissart, 2012[17]	1C	We recommend rituximab be considered for this indication. Patients with refractory TTP require treatment in addition to PEX and conventional corticosteroid regimens, and rituximab appears to be effective.
Treatment of severe ADAMTS13 deficiency during clinical remission	Hie, 2014[25]	1C	We recommend against the use of rituximab for this indication. The benefit for relapse-free survival is marginal (P = .049). Patients in the rituximab group received multiple different treatments. The benefit of a single course of rituximab is not known. The natural history of ADAMTS13 activity following recovery from acquired TTP is not known. High-quality evidence is required before treatment of patients with no clinical evidence of TTP can be recommended.

*Grade 1 represents a strong recommendation; grade 2 represents a weak recommendation; and grade C represents the lowest quality of evidence.





Scully M, et al. Blood 2011;118:1746-53
Lim W, et al. Blood 2015;125:1526-31
Jestin M, et al. Blood 2018;132:2143-53

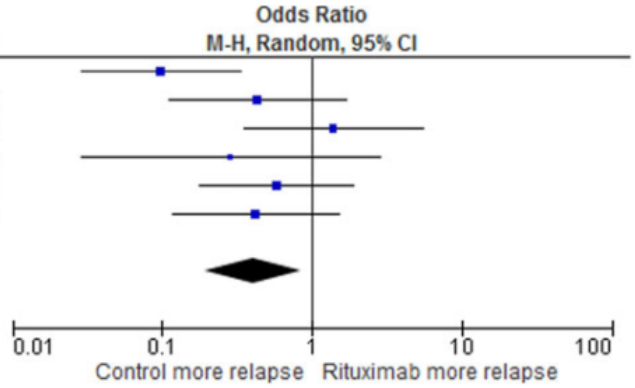
TTP - treatment

Rituximab

A

Study or Subgroup	Rituximab Events	Rituximab Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Scully 2011	4	37	21	38	19.3%	0.10 [0.03, 0.33]	2011
Froissart 2012	3	19	16	53	17.1%	0.43 [0.11, 1.70]	2012
Rinott 2015	4	14	9	40	16.9%	1.38 [0.35, 5.46]	2015
Page 2016	1	16	4	21	8.3%	0.28 [0.03, 2.82]	2016
Uhl 2017	5	36	10	46	20.0%	0.58 [0.18, 1.88]	2017
Falter 2018	5	17	14	28	18.3%	0.42 [0.12, 1.50]	2018
Total (95% CI)		**139**		**226**	**100.0%**	**0.40 [0.19, 0.85]**	
Total events	22		74				

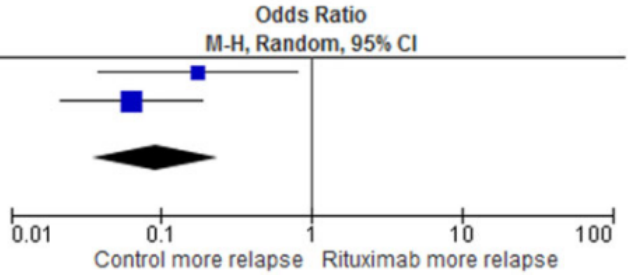
Heterogeneity: Tau² = 0.36; Chi² = 8.72, df = 5 (P = 0.12); I² = 43%
Test for overall effect: Z = 2.39 (P = 0.02)



B

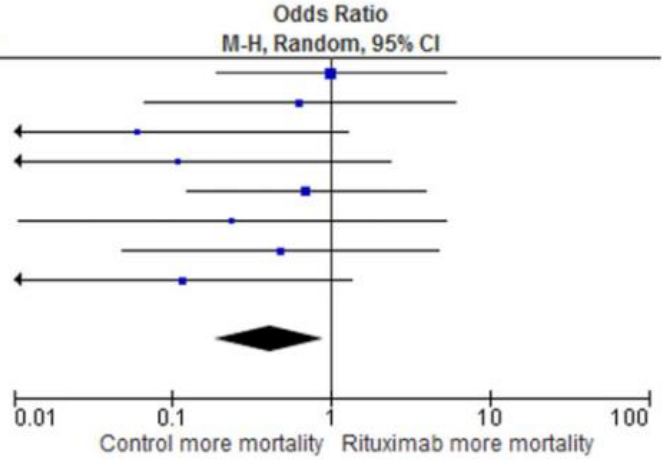
Study or Subgroup	Rituximab Events	Rituximab Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Hie 2014	3	30	7	18	35.7%	0.17 [0.04, 0.80]	2014
Jestin 2018	14	92	17	23	64.3%	0.06 [0.02, 0.19]	2018
Total (95% CI)		**122**		**41**	**100.0%**	**0.09 [0.04, 0.24]**	
Total events	17		24				

Heterogeneity: Tau² = 0.06; Chi² = 1.13, df = 1 (P = 0.29); I² = 11%
Test for overall effect: Z = 4.93 (P < 0.00001)



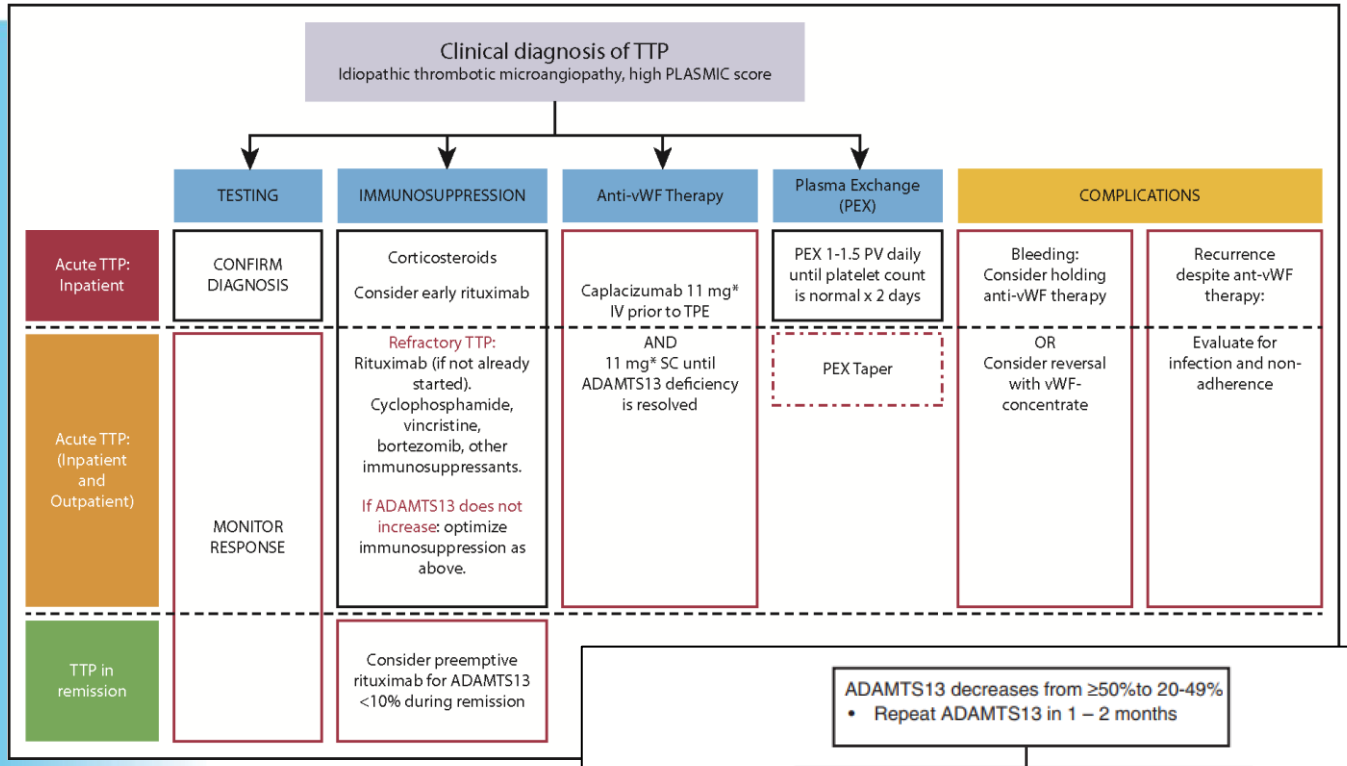
Study or Subgroup	Rituximab Events	Rituximab Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Scully 2011	3	40	3	40	23.1%	1.00 [0.19, 5.28]	2011
Froissart 2012	1	22	4	57	12.7%	0.63 [0.07, 5.98]	2012
Karim 2013	0	9	6	13	7.0%	0.06 [0.00, 1.26]	2013
Hie 2014	0	30	2	18	6.7%	0.11 [0.00, 2.39]	2014
Rinott 2015	2	14	6	31	21.1%	0.69 [0.12, 3.96]	2015
Page 2016	0	16	2	21	6.6%	0.24 [0.01, 5.28]	2016
Uhl 2017	1	40	3	59	12.1%	0.48 [0.05, 4.77]	2017
Jestin 2018	1	92	2	23	10.7%	0.12 [0.01, 1.33]	2018
Total (95% CI)		**263**		**262**	**100.0%**	**0.41 [0.18, 0.91]**	
Total events	8		28				

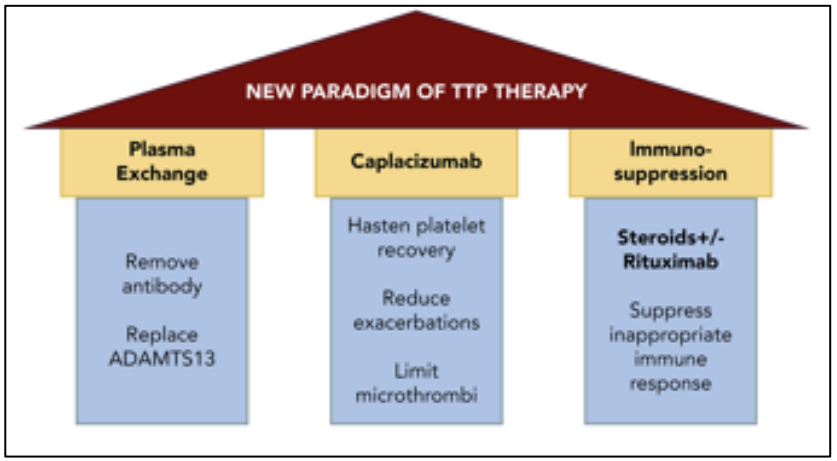
Heterogeneity: Tau² = 0.00; Chi² = 5.05, df = 7 (P = 0.65); I² = 0%
Test for overall effect: Z = 2.19 (P = 0.03)

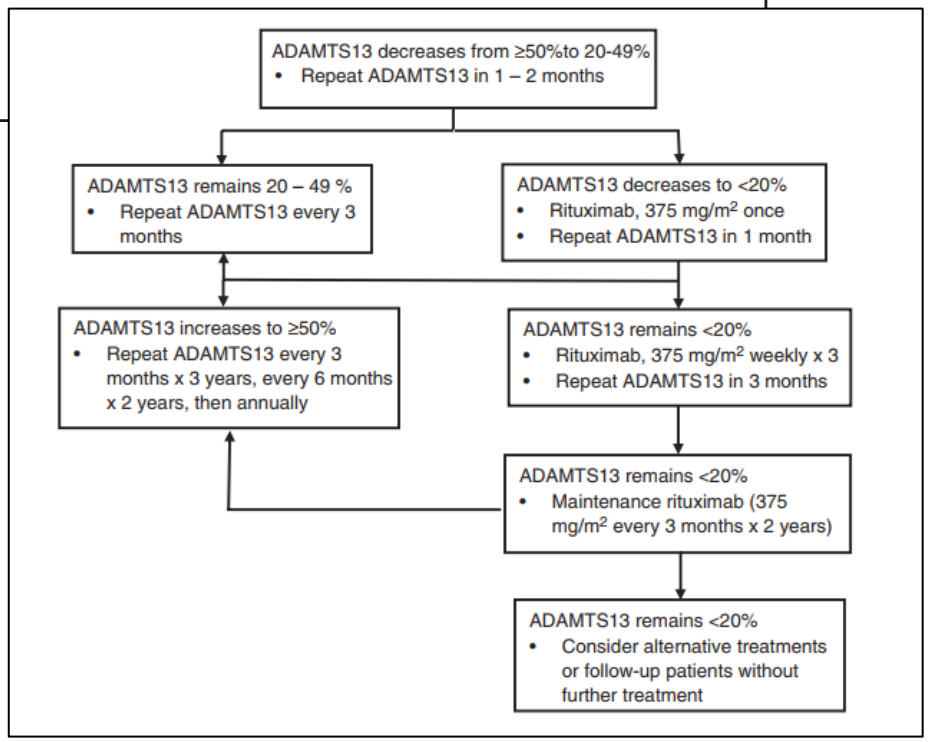


Owattanapanich W, et al. Clin Appl Thromb Hemost 2019; 25:1076029618825309

TTP – treatment & follow up

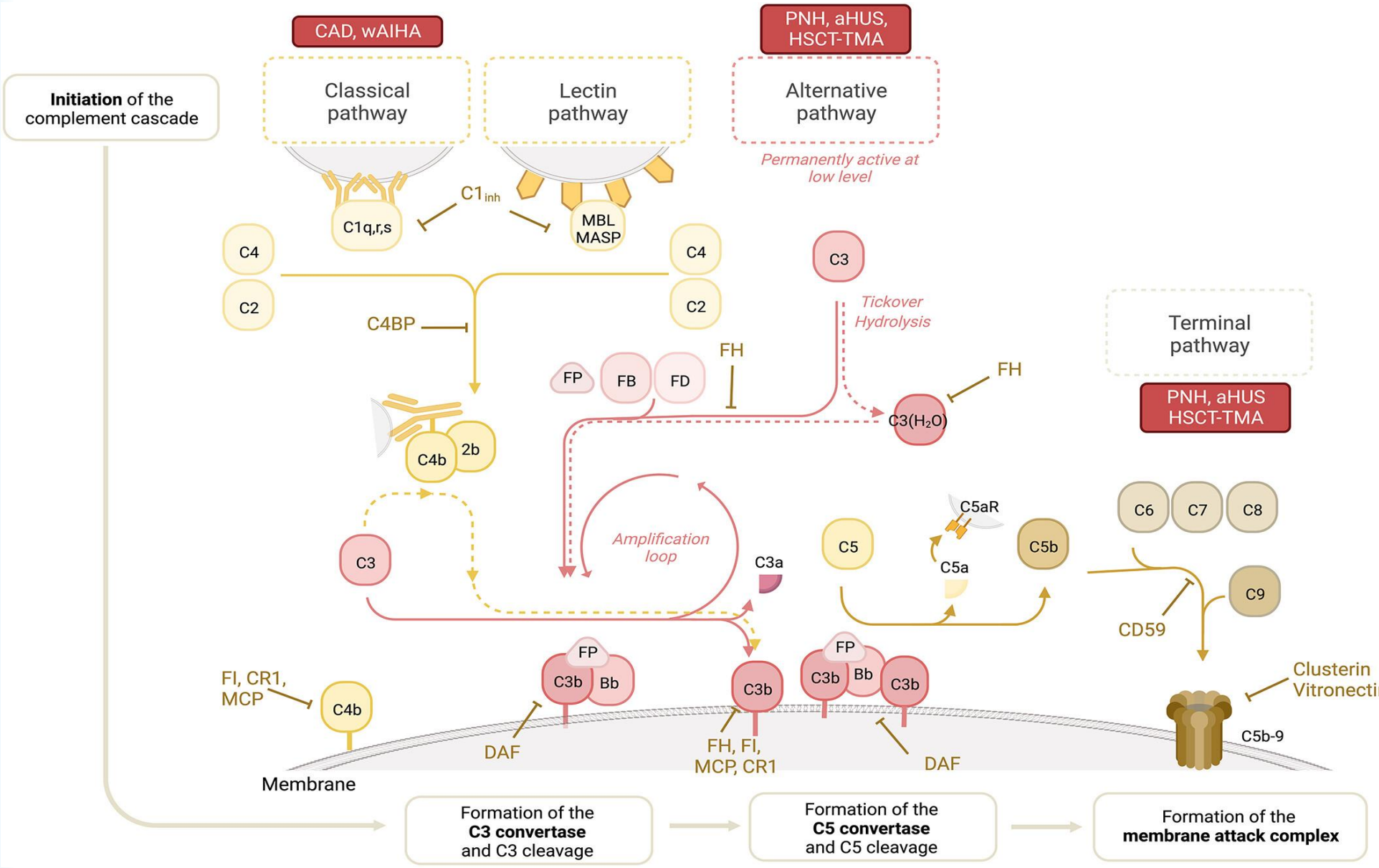






Coppo P, et al. Res Pract Thromb Haemost 2018;3:26-37
Mazepa M, et al. Blood 2019;134:415-20
Zheng XL, et al. J Thromb Haemost 2020;18:2496-502

aHUS – complement cascade



Duval A, Frémeaux-Bacchi V. Am J Hematol 2023;98:S5-S19

aHUS – complement cascade

Complement cascade: regulated from initiation till termination

- to maintain physiological balance
- To protect host surfaces against collateral damage

Regulator	Function	Regulated pathway	Main regulatory compartment
C1 inhibitor (C1-INH)	Inactivates C1r and C1s, MASP-1, and MASP-2	CP/LP	Fluid
sMAP	Binding to MBL, competition with MASPs	LP	Fluid
MAP-1	Binds to MBL/ficolins,	LP	Fluid
Vitronectin (S protein)	Binds to C5b-7/8/9	TP (MAC formation)	Fluid
Clusterin	Binds to C5b-7/8/9	TP (MAC formation)	Fluid
CD59	Binds to C8 and C9	TP (MAC formation)	Surface

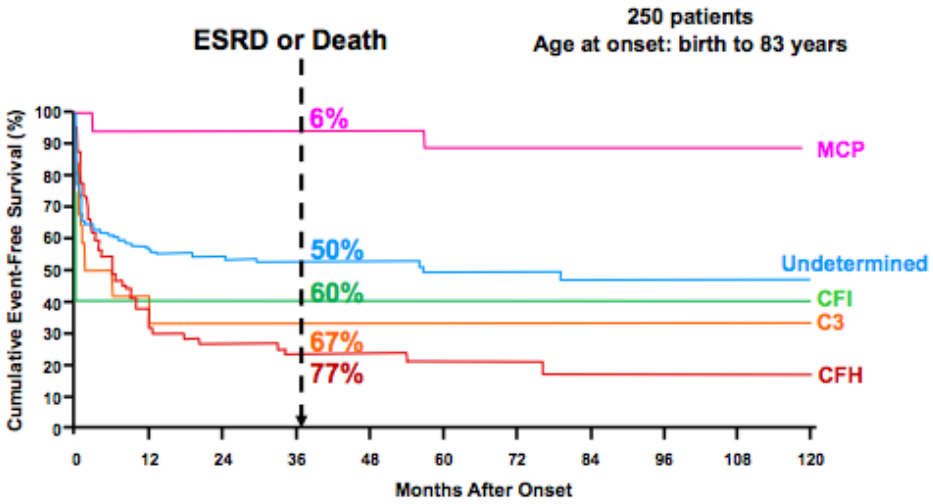
Regulator	Regulatory activity decay/ cofactor	Regulated pathway	Main regulatory compartment
CR1	DAA, CA	CP/LP and AP	Surface
DAF	DAA -	CP/LP and AP	Surface
MCP	- CA	CP/LP and AP	Surface
C4BP	DAA, CA	CP/LP	Fluid/surface
Factor H	DAA, CA	AP	Fluid/surface
FHL-1	DAA, CA	AP	Fluid/surface
Factor I	Protease for degradation of C3b or C4b in the presence of a cofactor	CP/LP and AP	Fluid (on surface only in conjunction with cofactor)

Schmidt CQ, Smith RJ. Immunol Rev 2023;313:376-401

aHUS - outcome

Prognosis of aHUS Varies According to the Genetic Defect

Noris M et al, CJASN 2010



The majority of patients received some form of plasma therapy

Table 1. Frequencies of the most common mutations identified in aHUS patients

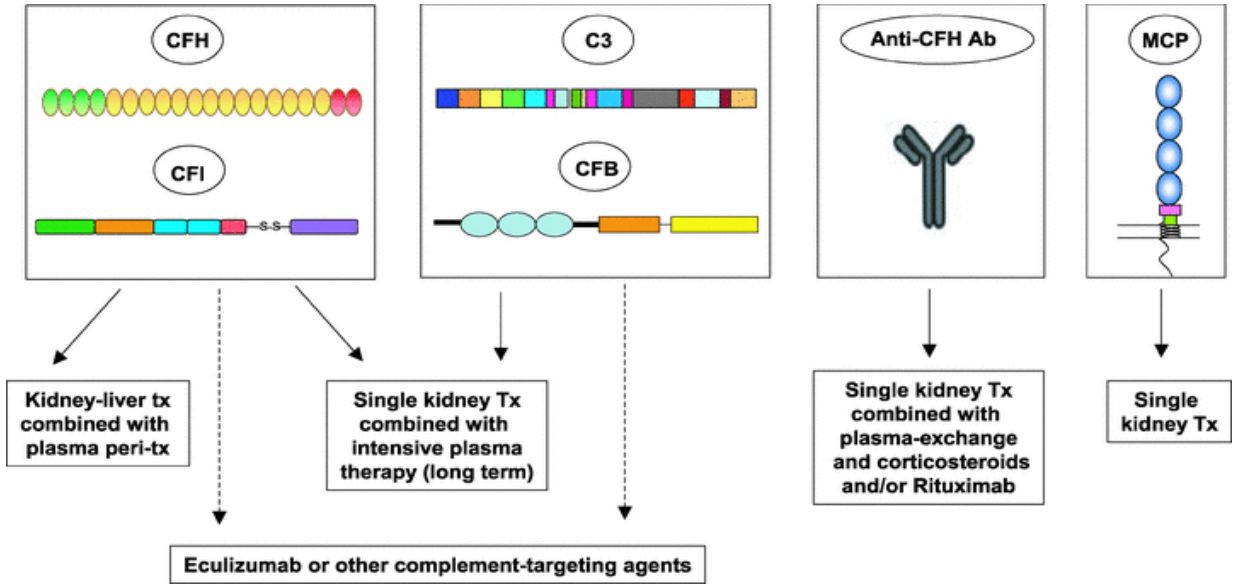
Mutated gene/protein	Type	Frequency (%)*	Death or end-stage renal disease 3-10 y after onset (%)†
Factor H (including *CFH/CFHR1* hybrid genes)	Loss of complement regulation	24-28	70-80
MCP (CD46)	Loss of complement regulation	5-9‡	<20
Factor I	Loss of complement regulation	4-8	60-70
C3	Gain of complement activation	2-8	60-70
Factor B	Gain of complement activation	0-4	70
Thrombomodulin	Possibly loss of complement regulation and procoagulative state	0-5	50-60
CFHR1/3 deficiency with anti-factor H autoantibodies	Loss of complement regulation	3-10§	30-70
Diacylglycerol kinase ε	Prothrombotic	0-3‖	46
None identified		30-48	50

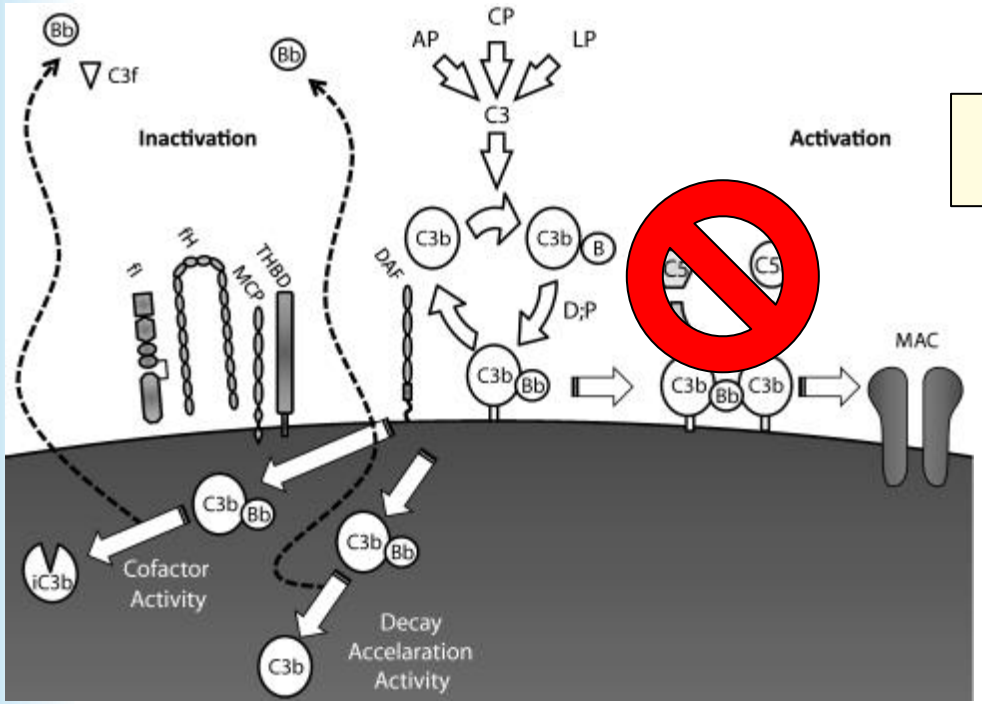
Noris M, et al. Clin J Am Soc Nephrol 2010;5:1844-59

Jokiranta TS. Blood 2017;129:2847-56

Raina R, et al.Ther Apher Dial 2019;23:4-21

aHUS - treatment



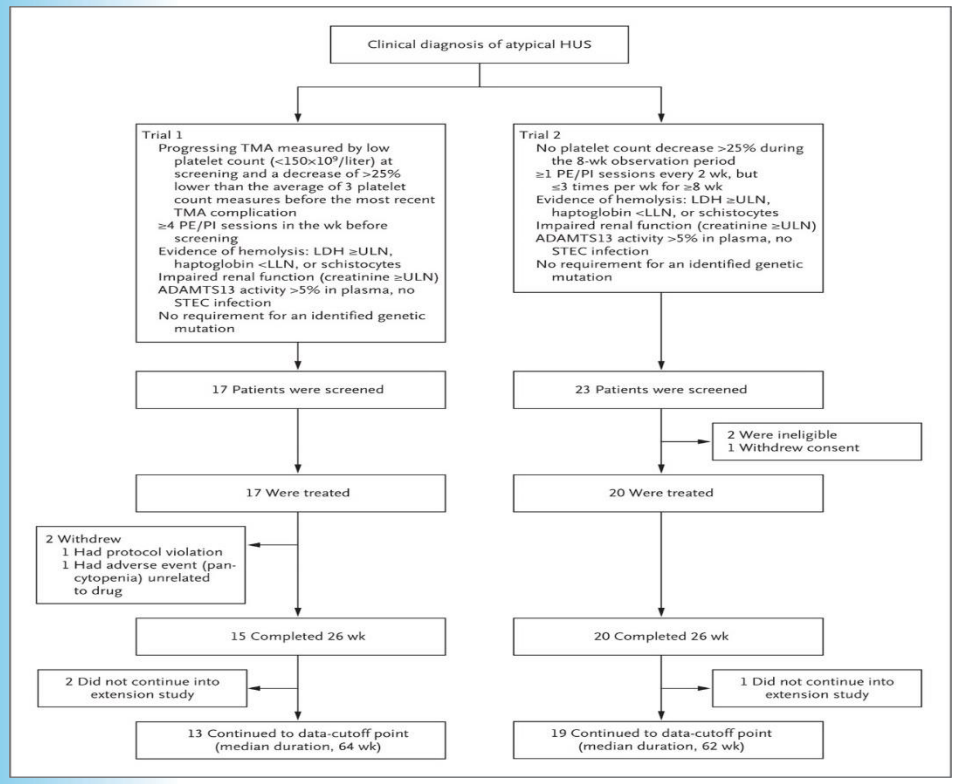


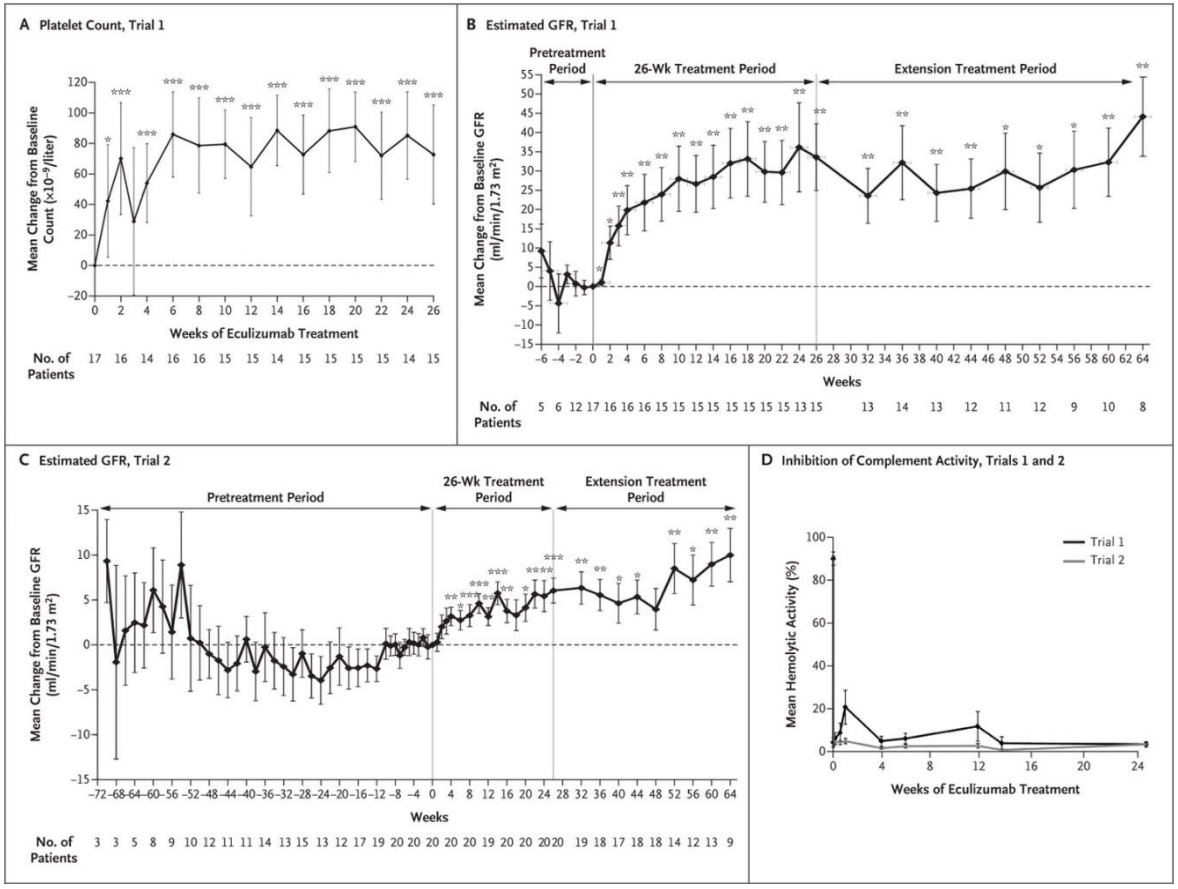
Eculizumab

Noris M, et al. Am J Transpl 2010;10:1517-23
Kavanagh D, Goodship T. Pediatr Nephrol 2010;25:2431-42

Two prospective phase 2 trials

Eculizumab





Legendre C, et al. N Engl J Med 2013;368:2169-81

Eculizumab

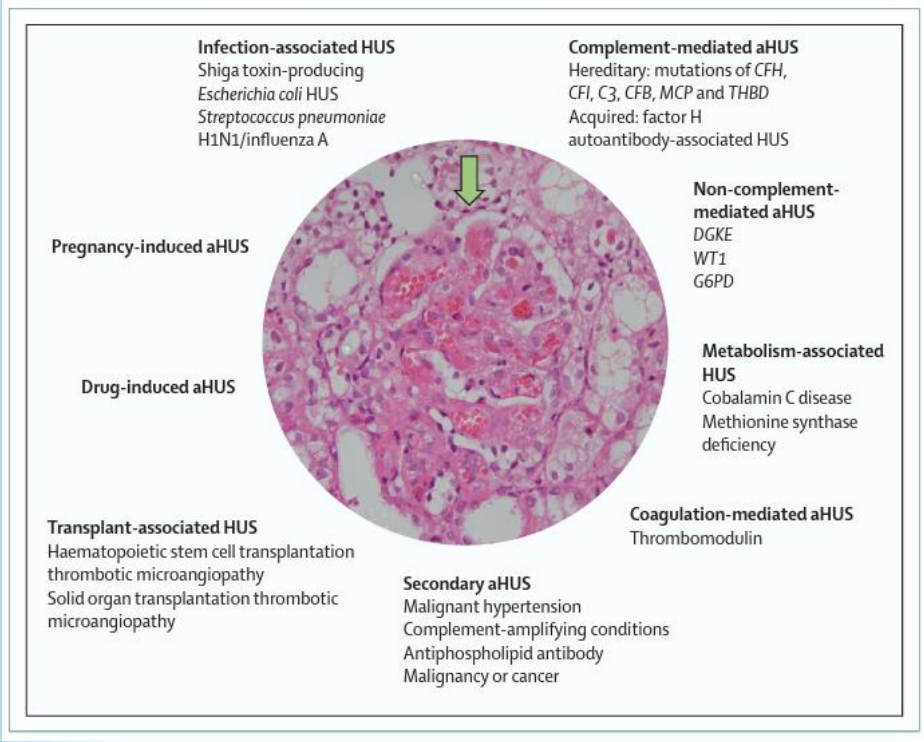
For patients ≥18 years of age, eculizumab therapy consists of:

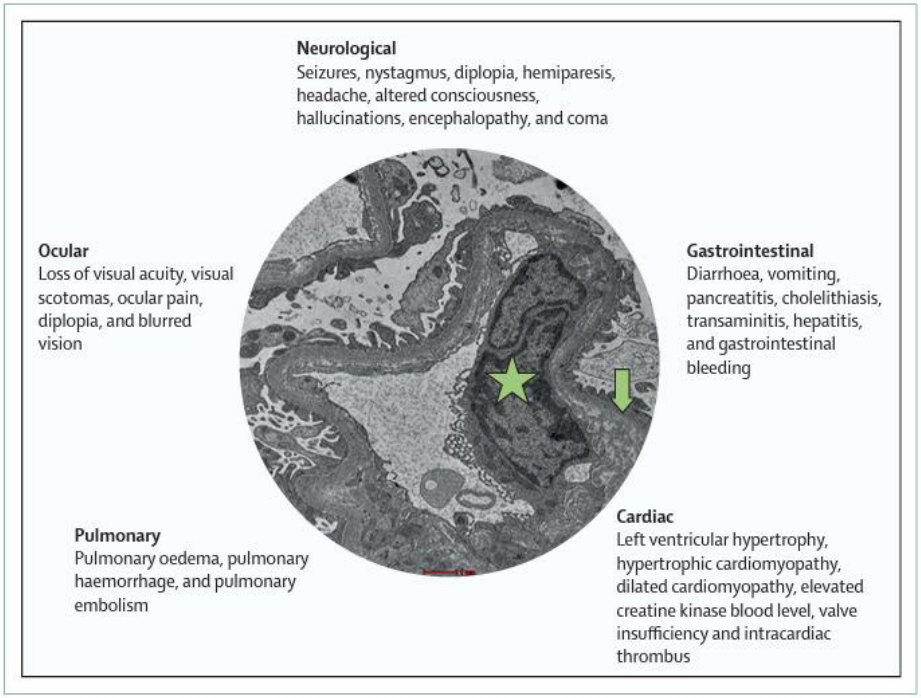
	Induction	Maintenance
	900 mg weekly x 4 doses	1200 mg at week 5; then 1200 mg every 2 weeks

For patients <18 years of age, administer eculizumab based upon body weight, according to the following schedule:

Patient Body Weight	Induction	Maintenance
40 kg and over	900 mg weekly x 4 doses	1200 mg at week 5; then 1200 mg every 2 weeks
30 kg to less than 40 kg	600 mg weekly x 2 doses	900 mg at week 3; then 900 mg every 2 weeks
20 kg to less than 30 kg	600 mg weekly x 2 doses	600 mg at week 3; then 600 mg every 2 weeks
10 kg less than 20 kg	600 mg weekly x 1 dose	300 mg at week 2; then 300 mg every 2 weeks
5 kg to less than 10 kg	300 mg weekly x 1 dose	300 mg at week 2; then 300 mg every 3 weeks

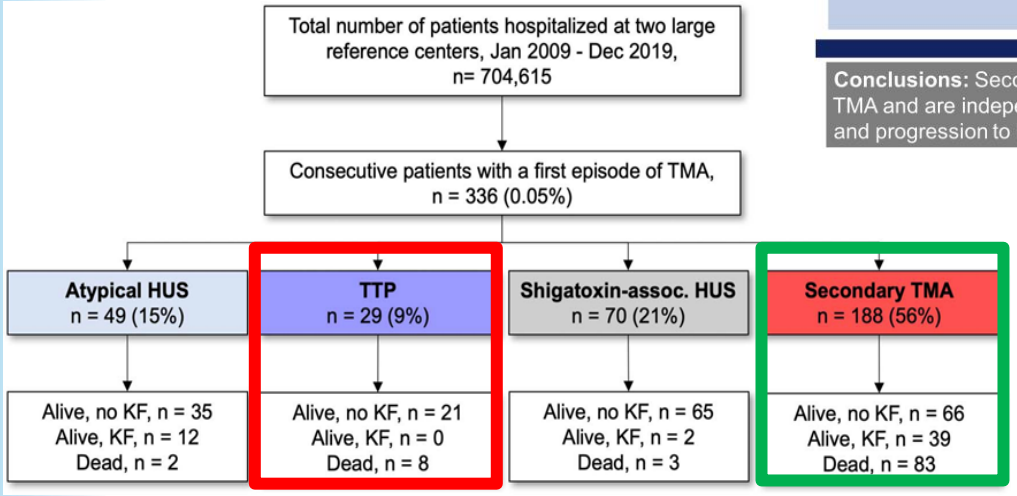
aHUS – new insights

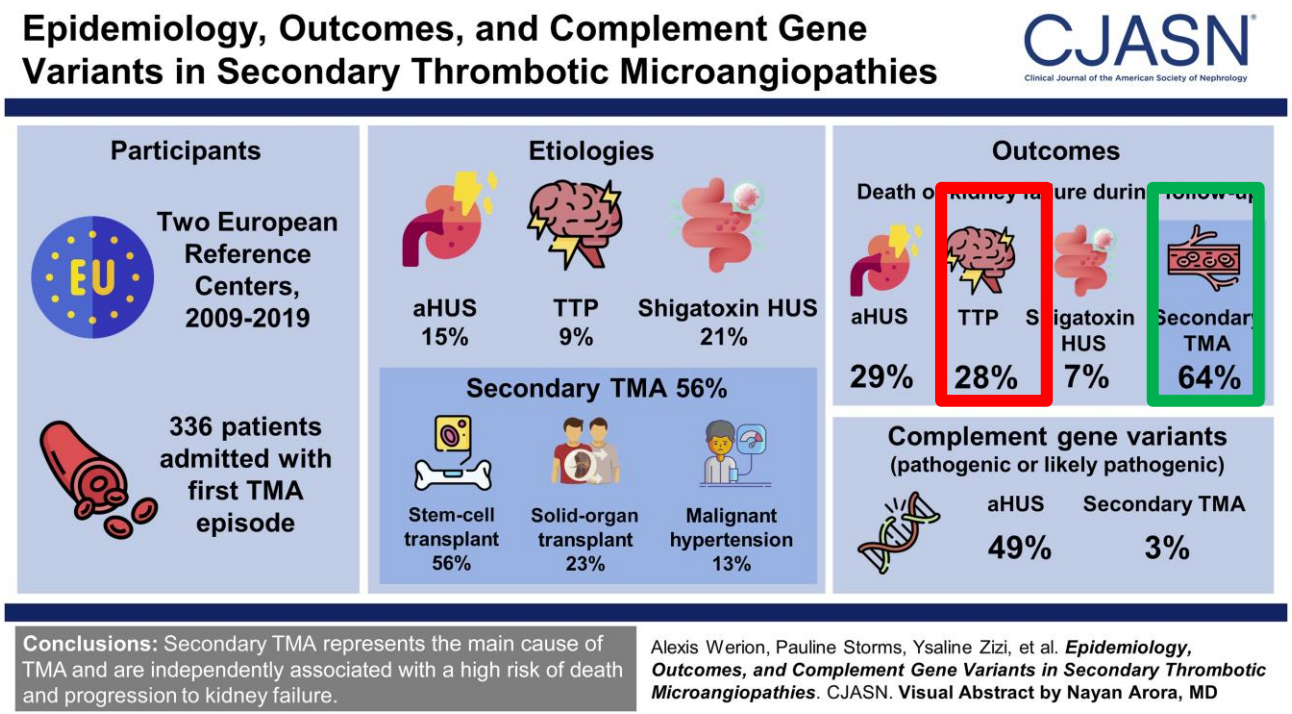




Michael M, et al. Lancet 2022;400:1722-40

TMA – real world data



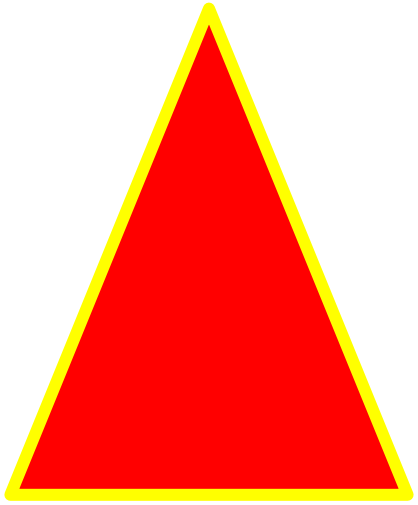


Werion A, et al. Clin J Am Soc Nephrol 2023;18:881-91

PNH - definition

Sr William Gull (London, 1866) : « anemic young tanner suffering from morning crises of intermittent haematinuria »

intravascular hemolytic anemia



bone marrow failure
(cytopenias)

thrombosis

- **It's not paroxysmal**

  Even in the absence of symptoms, progression of disease is ongoing.

- **It's not nocturnal**

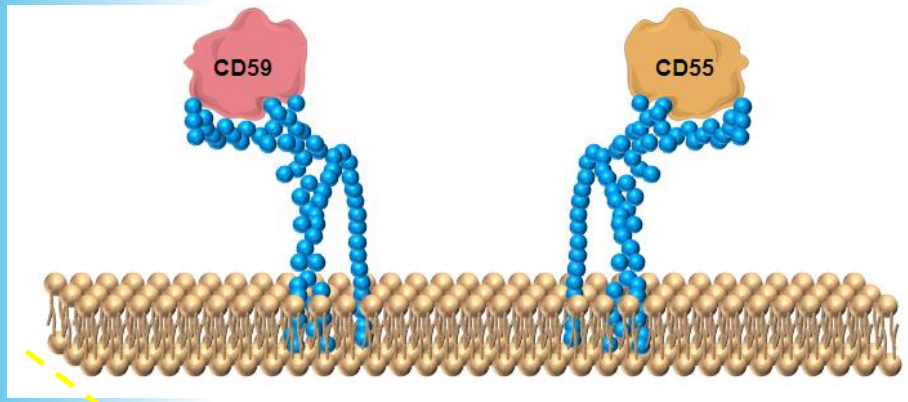
  Hemolysis in PNH is subtle and constant, 24 hours a day.

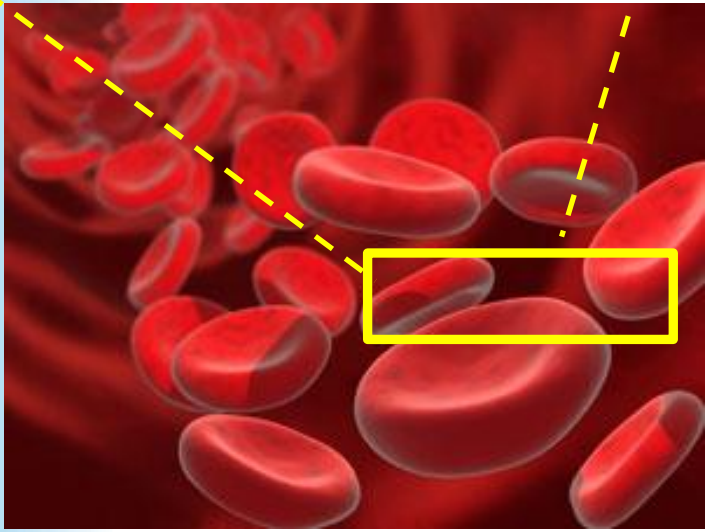
- **Hemoglobinuria is a a less common observed complication**

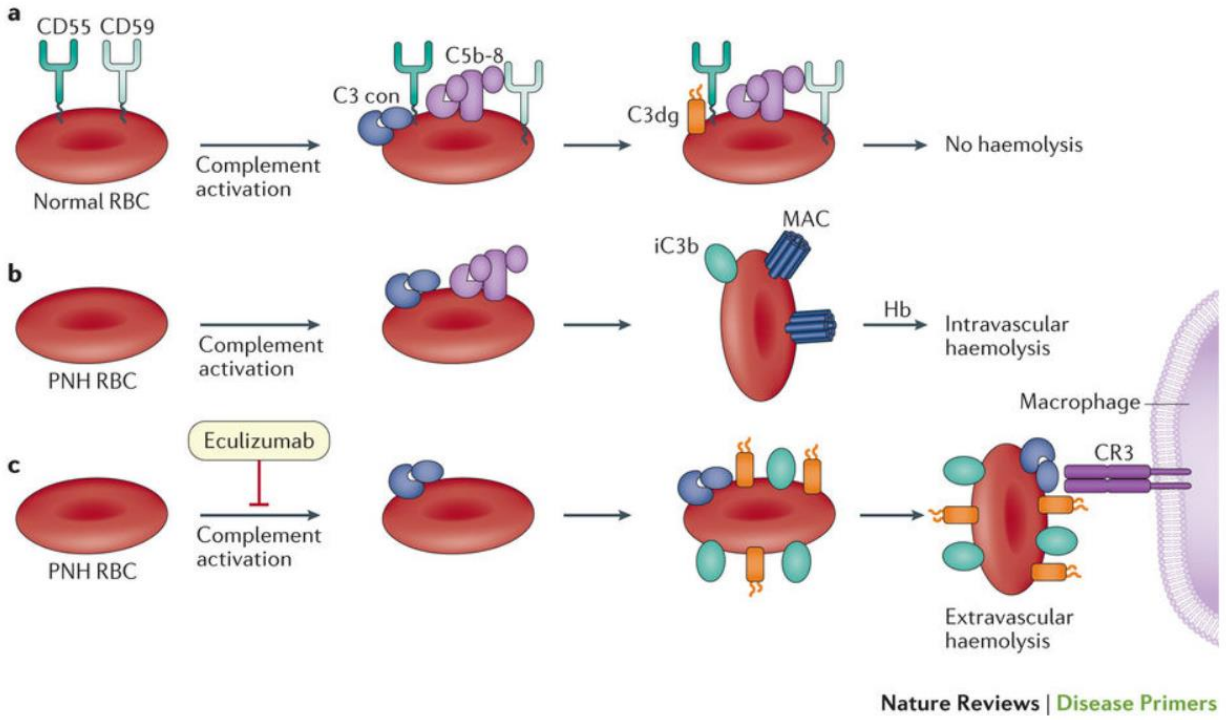
  ¾ patients present without hemoglobinuria.

Rother RP, et al, Nat Biotechnol 2007;25:1256-64
Parker C, et al. Blood 2005;106:3699-709

PNH - physiopathology







defect in the GPI anchor

Hill A, et al. Nat Rev Dis Primers 2017;3:17028

PNH - classification

Classification: International PNH Interest Group

Category	Rate of intravascular hemolysis*	Bone marrow	Flow cytometry	Benefit from eculizumab
Classic	Florid (markedly abnormal LDH often with episodic macroscopic hemoglobinuria)	Cellular marrow from erythroid hyperplasia and normal or near-normal morphology†	Large population (>50%) of GPI-AP–deficient PMNs‡	Yes
PNH in the setting of another bone marrow failure syndrome§	Mild (often with minimal abnormalities of biochemical markers of hemolysis)	Evidence of a concomitant bone marrow failure syndrome§	Although variable, the percentage of GPI-AP–deficient PMNs is usually relatively small (<50%)	Typically no, but some patients have relatively large clones and clinically significant hemolysis and may benefit from treatment
Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant bone marrow failure syndrome§	Small (<10%) population of GPI-AP–deficient PMNs detected by high-resolution flow cytometry	No

Parker C, et al. Blood 2005;106:3699-709
Parker CJ. Hematology Am Soc Hematol Educ Program 2016;2016:208-16

PNH - symptoms

Thrombosis is the leading cause of death in PNH

- **Up to 44% of patients experience clinical thrombotic events** (venous 85%)
- **Thrombosis in PNH can be life-threatening**
  - 40-67% of deaths are due to thrombosis
  - first thrombotic event can be fatal
  - first TE increases risk for death 5 to 10-fold
- Occurs **in typical** and **atypical** sites
- Is not adequately managed with anticoagulation

Atypical sites

- Abdominal (splanchnic, mesenteric, Budd-Chiari), cerebral thrombosis (sagittal sinus), CVA at young age, dermal thrombosis.

Typical sites, including one or more of the following:

- Prior thrombo-embolism in typical sites
- **Evidence of hemolysis**
- Accompanying bone marrow failure disorder
- While receiving anticoagulant therapy
- **Pancytopenia**

Parker C, et al. Blood 2005;106:3699-709
Hillmen P, et al. Blood 2007;110:4123-8
Audebert HJ, et al. J Neurol 2005;252:1379-86

PNH - treatment

Supportive Care Options Do Not Impact Progression and Risk for Severe Morbidities and Mortality

- **Transfusions**
  - Risk of iron overload
  - Transient treatment of anemia
- **Anticoagulants**
  - Risk of hemorrhage
  - Ineffective in many patients
- **Red cell supplements**
  - Folic acid, iron, erythropoiesis-stimulating agents
- **Steroids/androgen hormones**
  - No controlled clinical trials
  - AE's

Parker C, et al. Blood 2005;106:3699-709
Hillmen P, et al. Blood 2007;110:4123-8

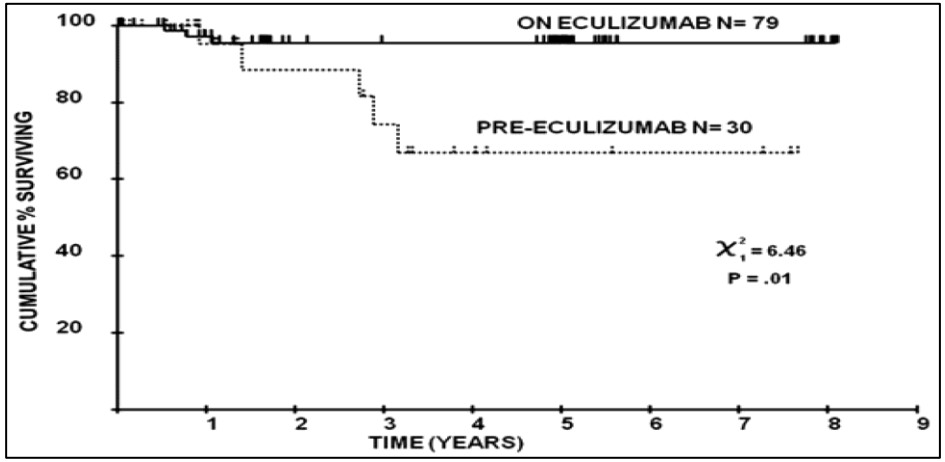
PNH - treatment

C5

TE events	TRIUMPH		SHEPHERD	Extension† (all studies)
	Placebo group	Eculizumab group		
Before treatment				
Patients, no.	44	43	97	195
TE events, no.	11	16	91	124
Patient-years, no.	470.4	309.0	718.3	1683.4
TE event rate, no. per 100 patient-years	2.34	5.18	12.67	7.37
Eculizumab treatment‡				
Patients, no.	44	43	97	195
TE events, no.	1	0	2	3§
Patient-years, no.	22.9	21.8	96.9	281.0
TE event rate, no. per 100 patient-years	4.38	0.00	2.06	1.07‖

Table 2. Stabilization of Hemoglobin Levels and the Number of Units of Packed Red Cells Transfused during Treatment.*

Primary End Point	Before Treatment†		During Treatment		P Value
	Placebo Group	Eculizumab Group	Placebo Group	Eculizumab Group	
Patients with stabilized hemoglobin levels (%)	NA	NA	0	49	<0.001‡
Packed red cells transfused (units/patient)					
Median	8.5	9.0	10	0	<0.001§
Interquartile range	7–12.5	6–12	6–16	0–6	
Mean	9.7±0.7	9.6±0.6	11.0±0.8	3.0±0.7	
Total	417	413	482	131	



Hillmen P, et al. N Engl J Med 2006.355:1233-43

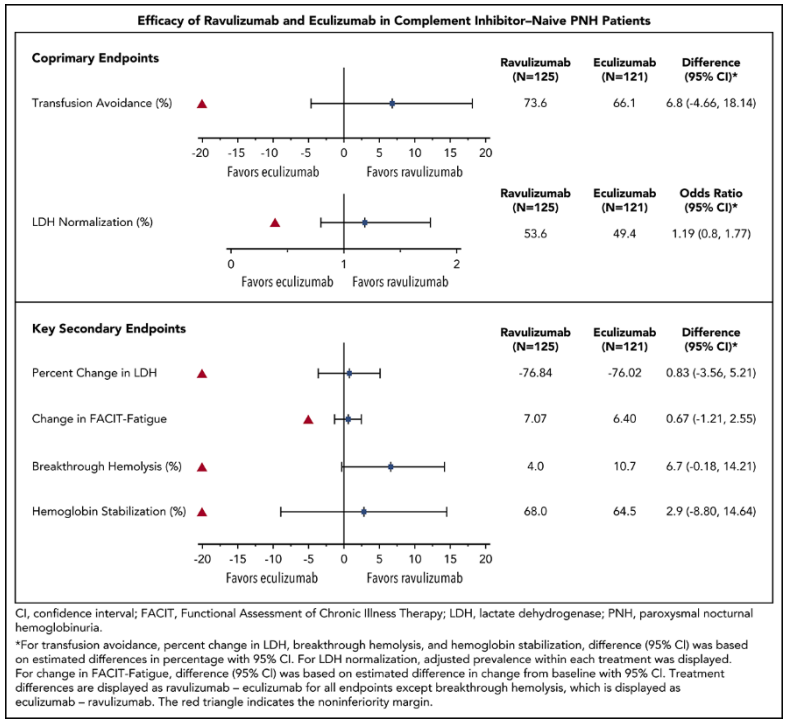
Hillmen P, et al. Blood 2007;110:4123-8

Kelly RJ, et al. Blood 2011;117:6786-92

PNH - treatment

C5

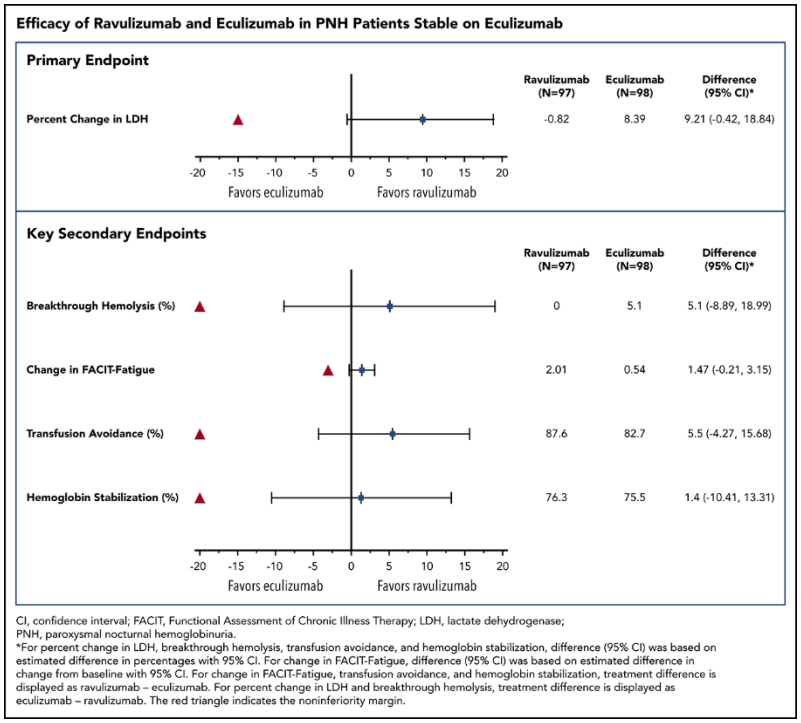
301 trial (ravulizumab)
n = 246 (open label, phase 3, C5i-naive)



CI, confidence interval; FACIT, Functional Assessment of Chronic Illness Therapy; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria.
*For transfusion avoidance, percent change in LDH, breakthrough hemolysis, and hemoglobin stabilization, difference (95% CI) was based on estimated differences in percentage with 95% CI. For LDH normalization, adjusted prevalence within each treatment was displayed. For change in FACIT-Fatigue, difference (95% CI) was based on estimated difference in change from baseline with 95% CI. Treatment differences are displayed as ravulizumab – eculizumab for all endpoints except breakthrough hemolysis, which is displayed as eculizumab – ravulizumab. The red triangle indicates the noninferiority margin.

Ravulizumab *vs* eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study

302 trial (ravulizumab)
n = 195 (open label, phase 3, , C5i-experienced



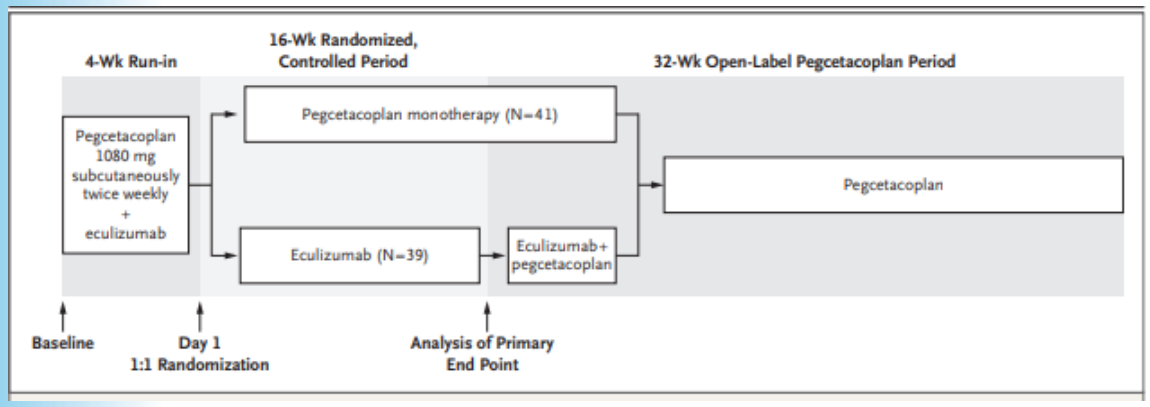
CI, confidence interval; FACIT, Functional Assessment of Chronic Illness Therapy; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria.
*For percent change in LDH, breakthrough hemolysis, transfusion avoidance, and hemoglobin stabilization, difference (95% CI) was based on estimated difference in percentages with 95% CI. For change in FACIT-Fatigue, difference (95% CI) was based on estimated difference in change from baseline with 95% CI. For change in FACIT-Fatigue, transfusion avoidance, and hemoglobin stabilization, treatment difference is displayed as ravulizumab – eculizumab. For percent change in LDH and breakthrough hemolysis, treatment difference is displayed as eculizumab – ravulizumab. The red triangle indicates the noninferiority margin.

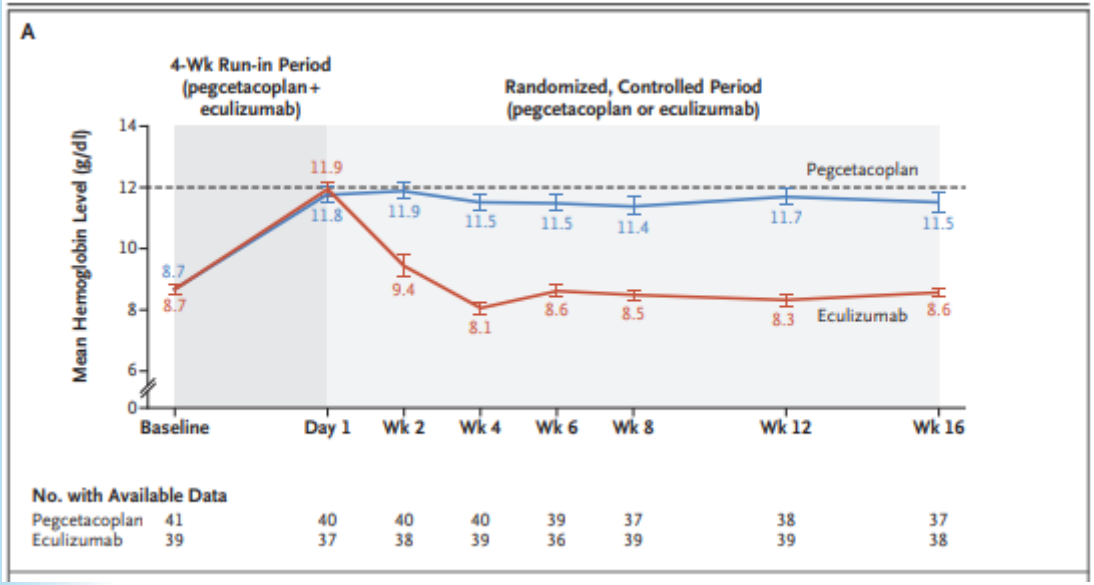
Ravulizumab *vs* eculizumab in C5-inhibitor–experienced adult patients with PNH: the 302 study

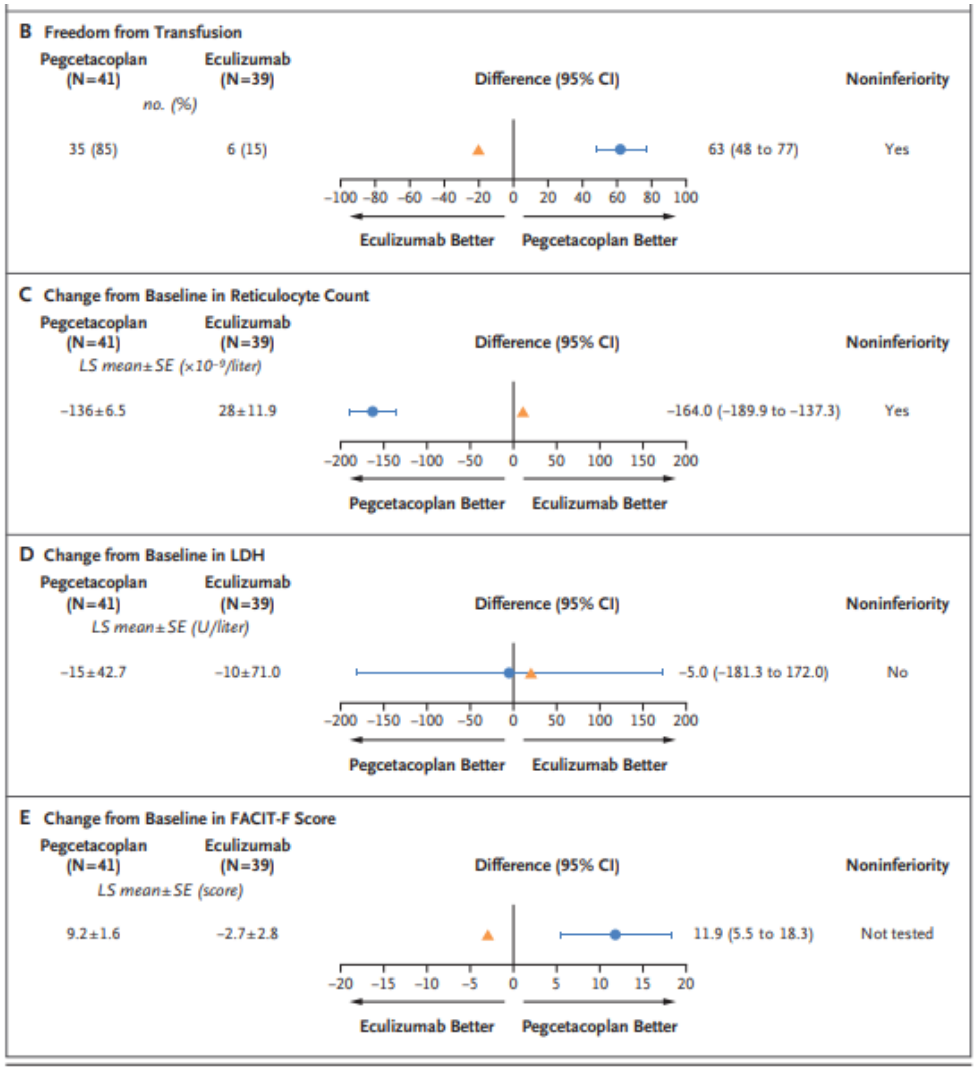
Lee JW, et al. Blood 2019;133:530-9
Kulasekaraj AG, et al. Blood 2019;133:540-9

PNH - treatment

C3





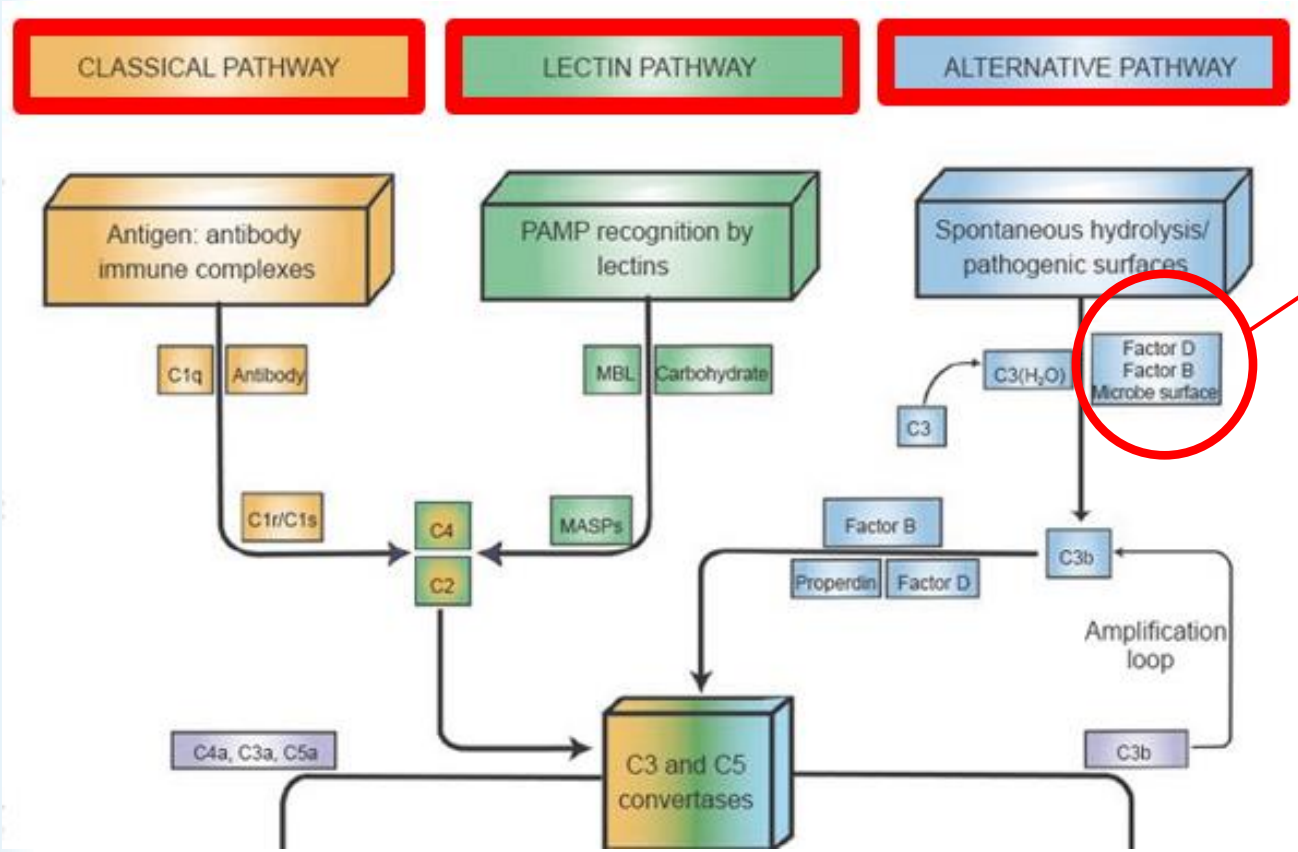


Pegasus (pegcetacoplan)
n = 80 (randomized, open label, phase 3, treatment-naive)

Hillmen P, et al. N Engl J Med 2021;384:1028-37

PNH – future treatments

CAPi

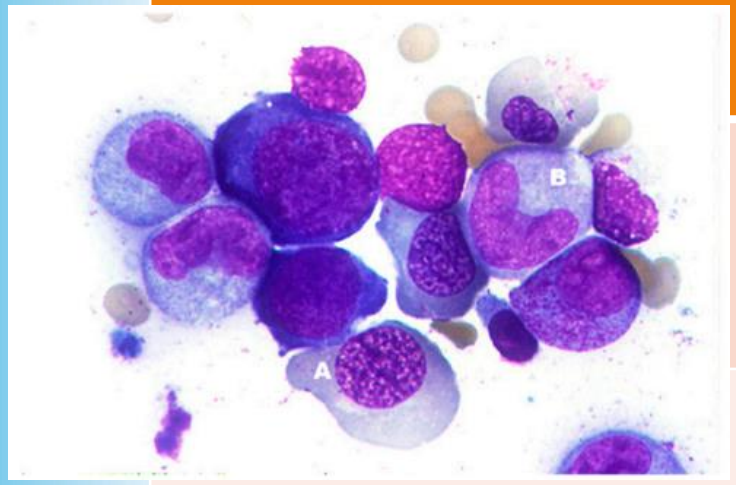


CAP inhibitors:

iptacopan (factor B)
danicopan (factor D)

Dunkelberger JR, Song WC. Cell Res 2010;20:34-50
Risitano AM, et al.Haematologica 2021;106:3188-97
Kulasekararaj AG, et al. Blood 2021;138:1928-38
Wong JJ, et al. Blood Adv 2022;6:4450-60

Acquired anemias



Hemolytic anemia

Megaloblastic anemia

Sideroblastic anemia

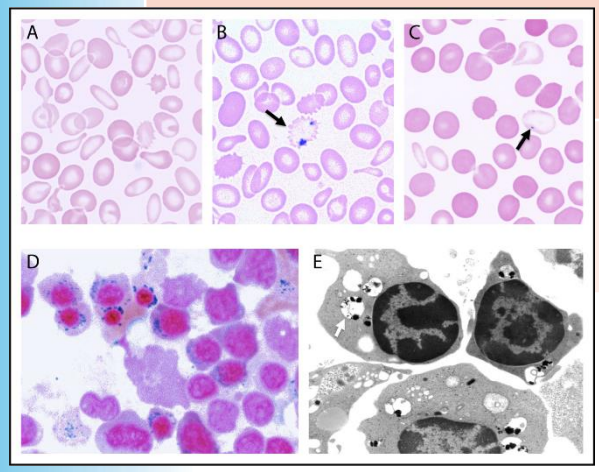
Stabler SP. N Engl J Med 2013;265:149-60
Bunn HF. N Engl J Med 2015;370:773-6
Hesdoffer CS & Longo DL. N Engl J Med 2015;373:1649-58
Green R. Blood 2017;129:2603-11
Torrez M, et al. Int J Lab Hematol 2022;44:236-47

Acquired anemias

Hemolytic anemia

Megaloblastic anemia

Sideroblastic anemia



Cazzola M & Malcovatti L. Hematology Am Soc Hematol Educ Program 2015;2015:19-25
Ducamp S, Fleming MD. Blood 2019;133:59-69
Abu-Zeinah G, DeSancho MT.J Blood Med 2020;11:305-18
Socha SD, et al. Cleve Clin J med 2020;87:153-64
Rodriguez-Sevilla JJ, et al. Genes (Basel) 2022;13:1562

Take home messages (1)

1. Difference between cold and warm AIHA is important as therapy differs.

2. Initial treatment of AIHA consists of steroids (warm) or rituximab (cold) and RBC if necessary.

3. TTP is diagnosed based on a combination of thrombocytopenia, DAT-negative microangiopathic hemolytic anemia and ADAMTS13 activity < 10%.

4. TTP is an emergency: in case of suspicion always start therapeutic plasma exchange + steroids + caplacizumab as soon as possible. Consider rituximab.

5. First line treatment of TTP with therapeutic plasma exchange and steroids was associated with a (first 2 weeks) death rate of 10-15%, making additional treatment strategies (both short and long term) necessary.

Take home messages (2)

6. Eculizumab is standard of care in both atypical HUS and PNH, but new complement therapeutics are changing the treatment landscape.

7. Thrombosis (often at unusual sites) is the leading cause of death in PNH.

8. In PNH eculizumab reduces thromboembolic event rates and transfusion needs and improves QOL.

9. Both in CAD and PNH C5 inhibition blocks intravascular hemoysis, but not extravascular hemolysis.

10. More proximal complement inhibition blocks both intravascular and extravascular hemolysis in CAD (C1s and C3) and PNH (C3 and CAP inhibitors).

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