Not So Benign Hematology

Aplastic anemia, Paroxysmal Nocturnal Hemoglobinuria, Antiphospholipid Antibody Syndrome

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Disclosures

- Dr. Brodsky serves as a Scientific Advisory Board member to:
  - Alexion Pharmaceuticals
  - Achillion Pharmaceutical

- Grant funding:
  - NHLBI
  - Alexion

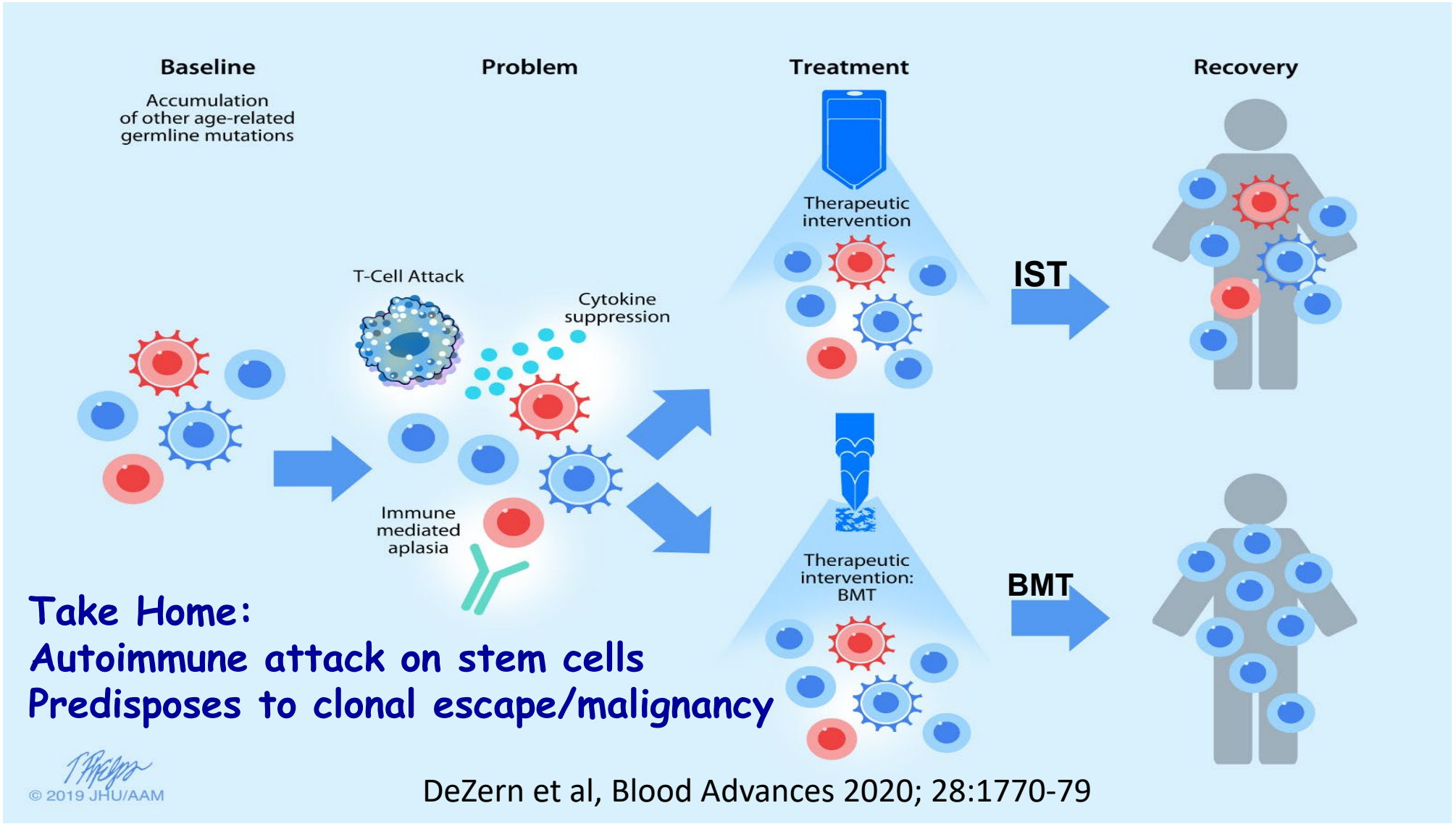
Aplastic Anemia
Diagnosis And Nomenclature

- SAA
  - Bone marrow (< 25% cellular)
  - Peripheral cytopenias (at least 2 of 3)
    - ANC < 500 per μl
    - Platelets < 20,000 per μl
    - Absolute retic < 60,000 or corrected retic < 1%
- VSAA: as above, but ANC < 200

2 year mortality > 70%

- Moderate AA or (NSAA)
  - Hypocellular marrow but does not meet criteria for SAA

SAA: Acute and Chronic Disease



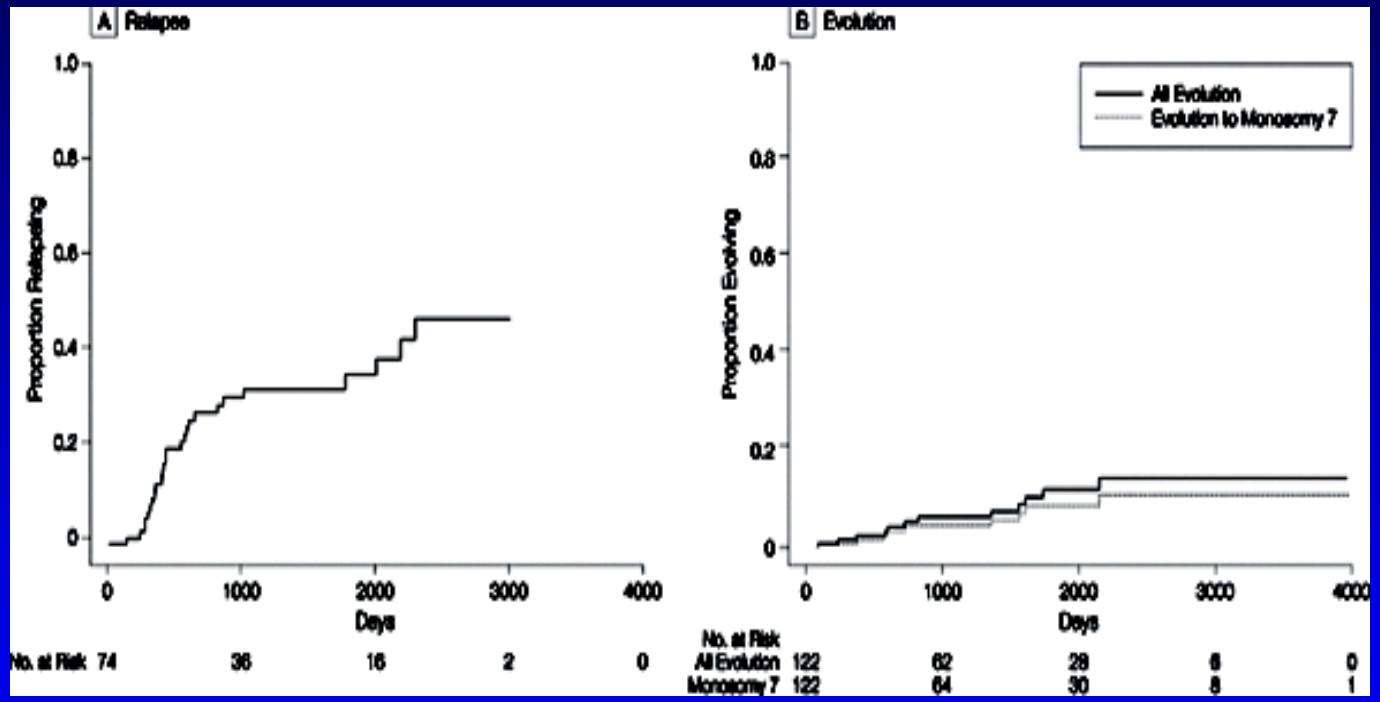
DeZern et al, Blood Advances 2020; 28:1770-79

Severe Aplastic Anemia (SAA)

- First line therapy
  - BMT (if matched sibling donor)
  - IST (ATG/CSA) +/- eltrombopag (Response rate 75%)

- Refractory Disease (poor response/prognosis)
  - BMT (usually from alternative donors)
  - Other IST

Rosenfeld et. al, Jama 2003;289: 1130-35

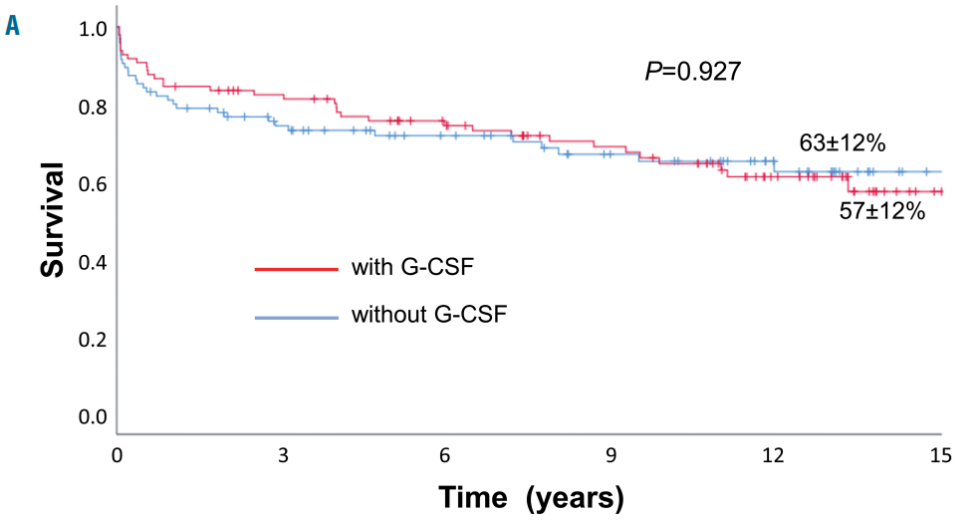


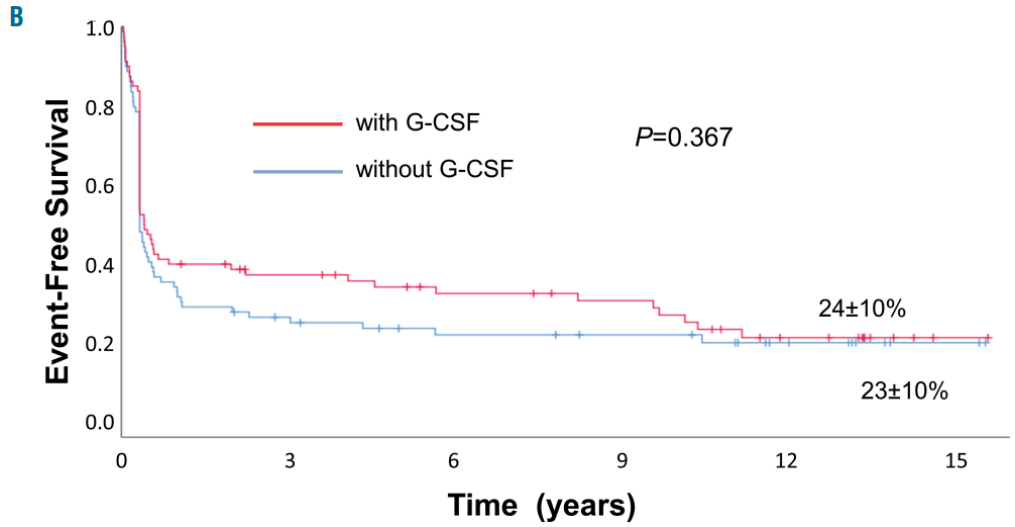
Risk of relapse > 40% in responders

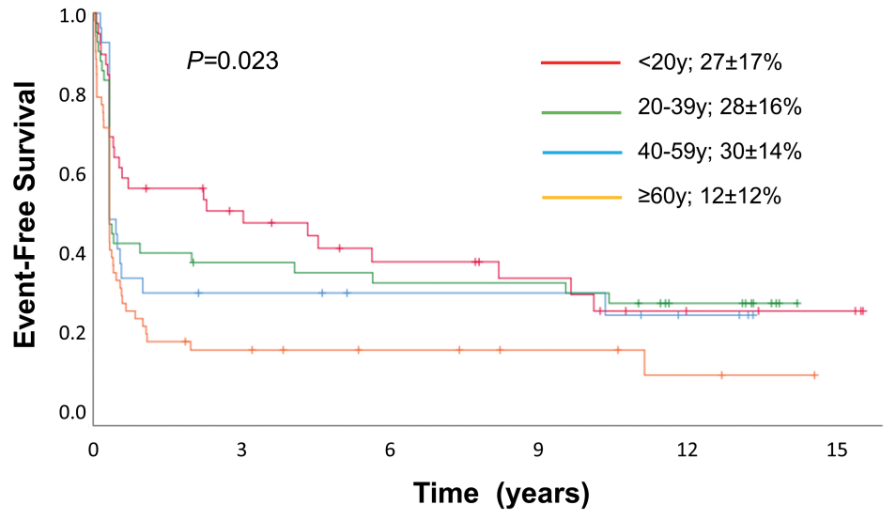
Risk of clonal evolution

30% failure-free survival

SAA: Poor Failure-free Survival at any Age with IST

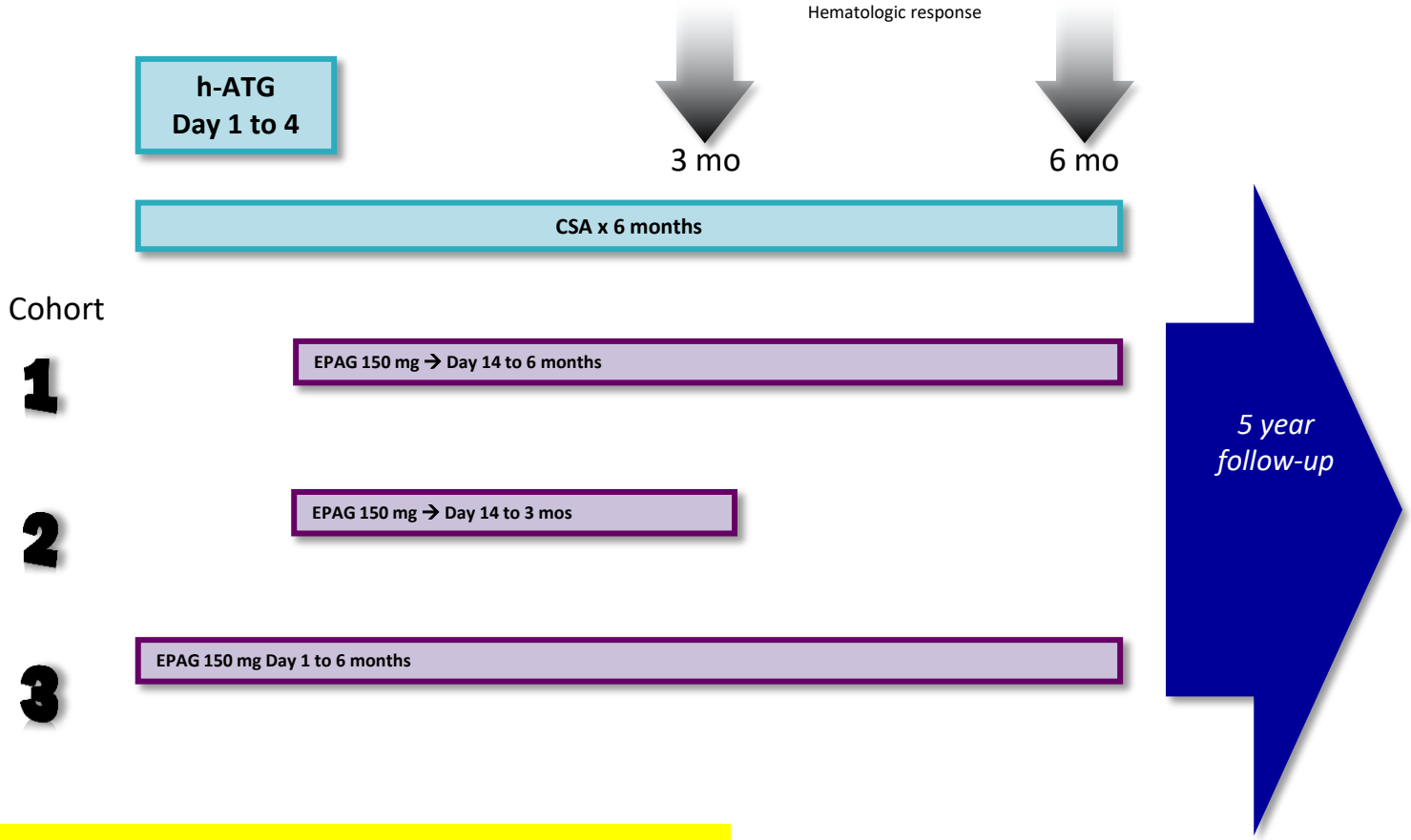






Tichelli et al, Haematologica 2020

ELTROMBOPAG ADDED TO STANDARD IMMUNOSUPPRESSION AS FIRST TREATMENT IN APLASTIC ANEMIA

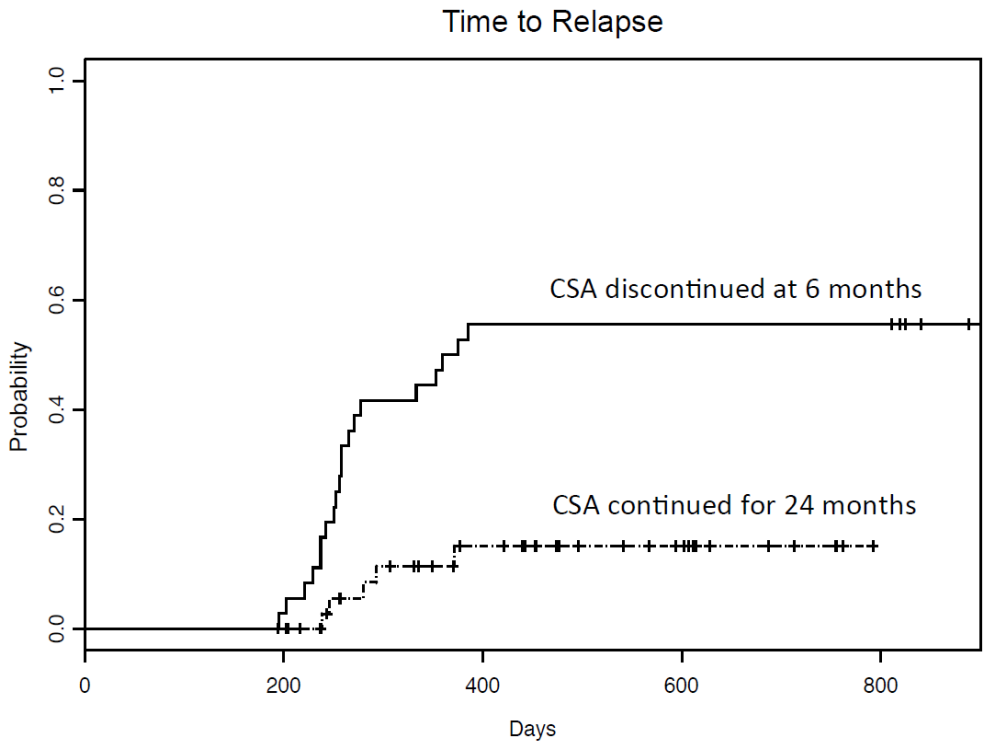


Supplemental methods:
The protocol was amended starting with subject # 46 on cohort 2, so that cyclosporine was continued at a 6 fixed daily dose, 2mg/kg/day, for an additional 18 months in order to prevent relapse.

Townsley, DM et al, *NEJM* 2017; 376:1540-50.

Supplemental Figure 4

Median follow-up 23 months



No. at risk:

CSA discontinued	92	35	17	16	16
CSA continued	92	43	24	11	1

Cytogenetic abnormality of unclear significance

68	CR	3	46, XX, del(13)(q12q22)[cp3]/46,XX[17]	No	Cytogenetics normalized
39	CR	30	48, XX +6 +15 [2]/ 46,XX[18]	No	CR stable

Chromosome 7 abnormality

64	PR	3	45,XX,t(3;3)(q21;q26),-7[3]/ 46, XX[17]	Yes	AML, death post-HSCT*
72	PR	30	45, XY, -7[20]	Yes	PR stable
48	CR	6	46,XX,del (7)(p13p15)[3]/46,XX[19]	No	HSCT
61	PR	6	45, XX,-7[7]/46,XX[16]	Yes	Awaiting HSCT
16	NR	3	45, XY,-7[6]/46,XY[14]	No	HSCT*

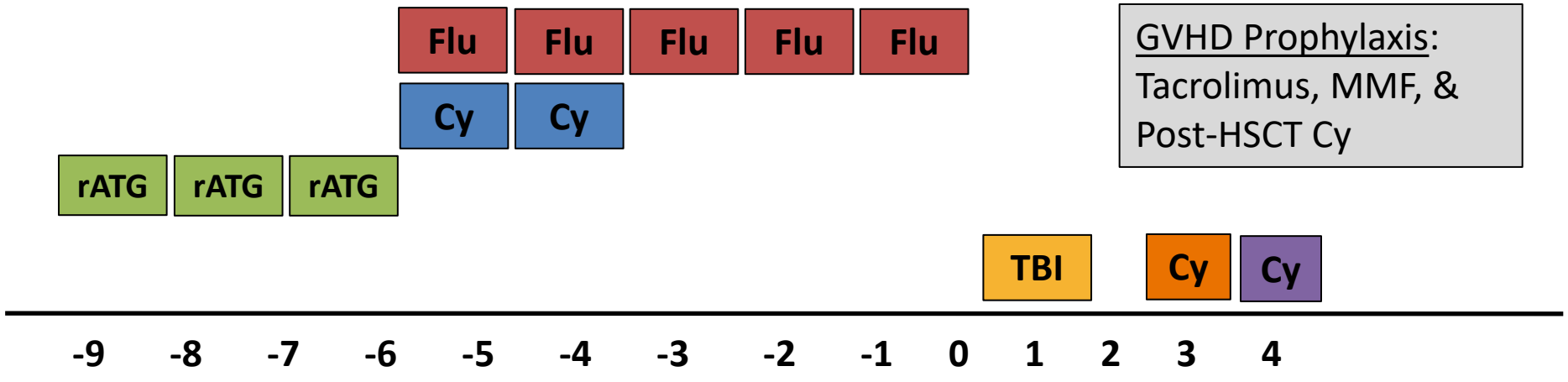
20% Risk of clonal evolution – most within 6 mos

Townsley, DM et al, *NEJM* 2017; 376:1540-50.

Reduced intensity haploidentical BMT with post-transplant Cyclophosphamide (CY)

- **Mitigates GVHD**
- **Allows for greater use of alternative donors (haplo BMT)**
  - **No difference for engraftment or GVHD btw matched sibs and HLA-haplo identical donors**
- **Average person in US has >4 HLA haplo-identical donors**

Conditioning for HLA Haplo-identical BMT



GVHD Prophylaxis: Tacrolimus, MMF, & Post-HSCT Cy

Flu = Fludarabine 30 mg/m^2 IV daily

Cy = Cyclophosphamide 14.5 mg/kg IV daily

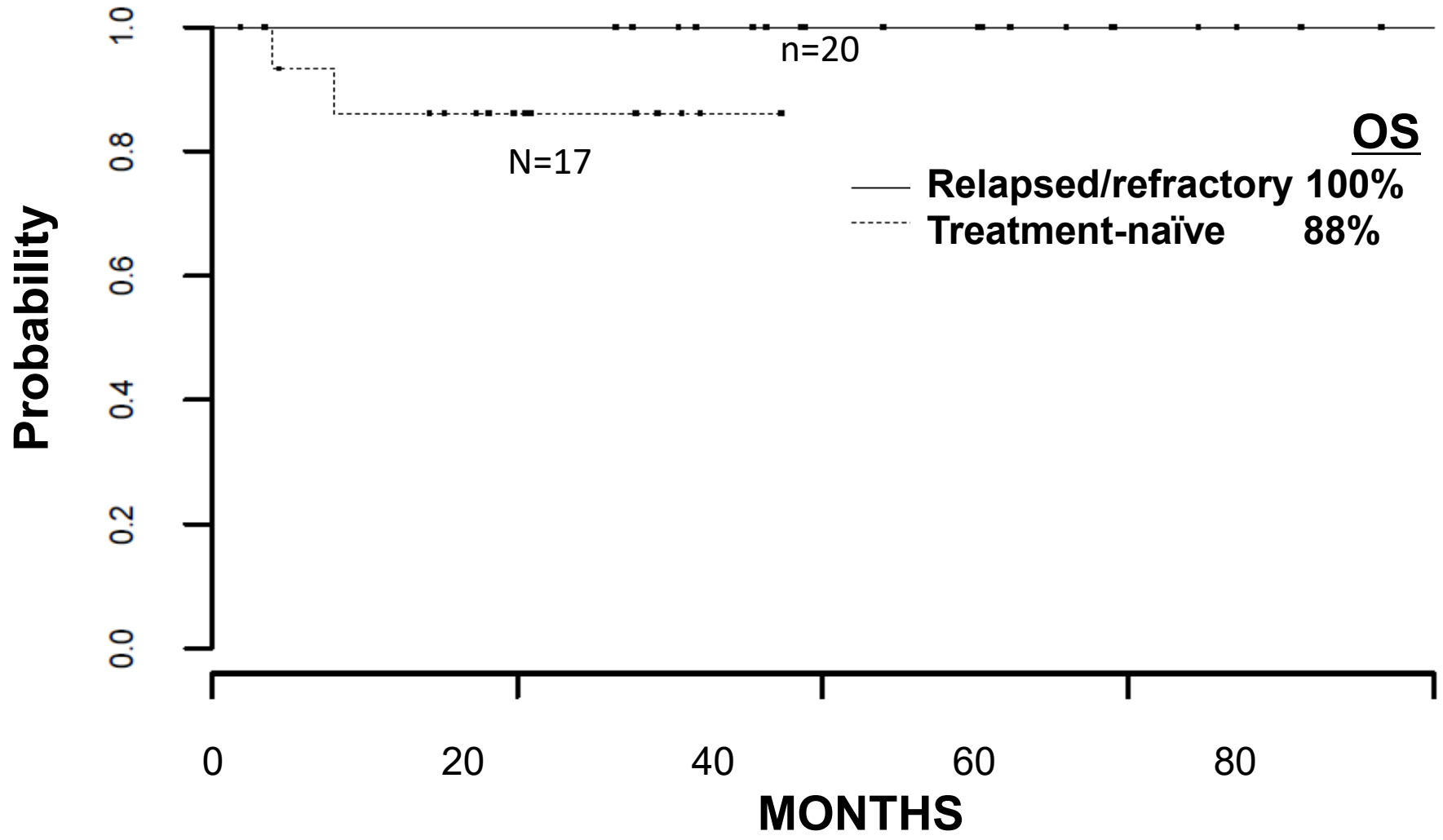
Cy = Cyclophosphamide 50 mg/kg IV daily

TBI = 200/400 cGy

rATG = Thymoglobulin 0.5 mg/kg (day-9) & 2 mg/kg (day -8,-7)

DeZern et al, Blood Advances 2020; 4:1770-79

Haplo BMT for SAA: Overall Survival



DeZern et al, Blood Advances 2020; 4:1770-79

Conclusions

- **SAA: IST vs BMT**
  - **BMT advancing faster than IST**
    - **Faster and more complete hematopoietic recovery**
    - **Early mortality now roughly the same as IST (~5%)**
    - **Cost now similar; > 50% of pts treated with IST will need a BMT anyhow**
    - **BMT cures the disease**
  - **HaploBMT now standard of care for relapsed/refractory SAA**
  - **The future?**
    - **Upfront mini-haplo BMT: requires increased TBI to 400: 10/10 engrafted.**

Paroxysmal Nocturnal Hemoglobinuria

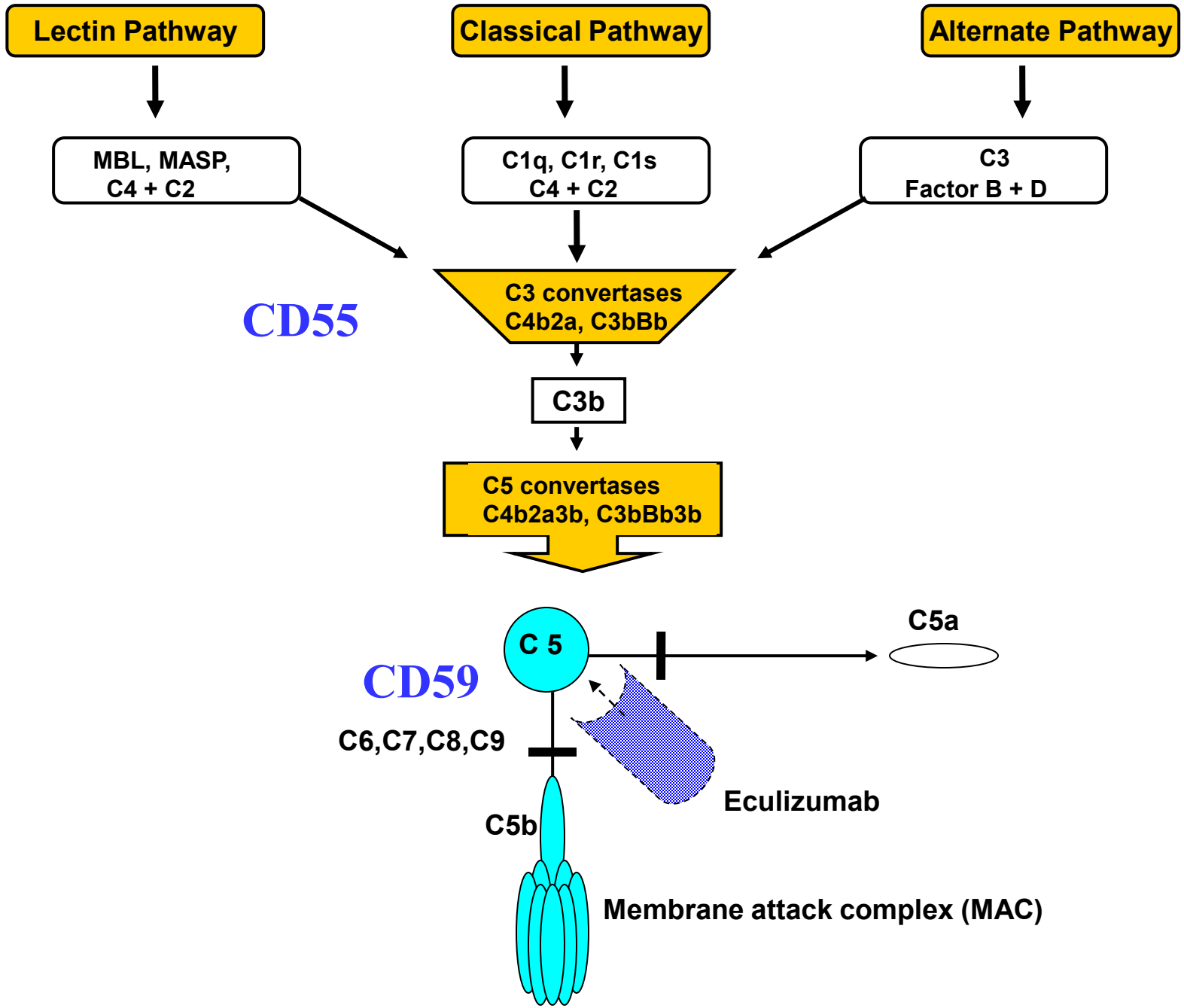
Biology

- Acquired Clonal Hematopoietic Stem Cell Disease
- *PIGA* mutation
  - X(p22.1)
- PIGA gene product necessary for 1st step in the biosynthesis of GPI anchors
- PNH cells have deficiency or absence of all GPI anchored proteins

PNH

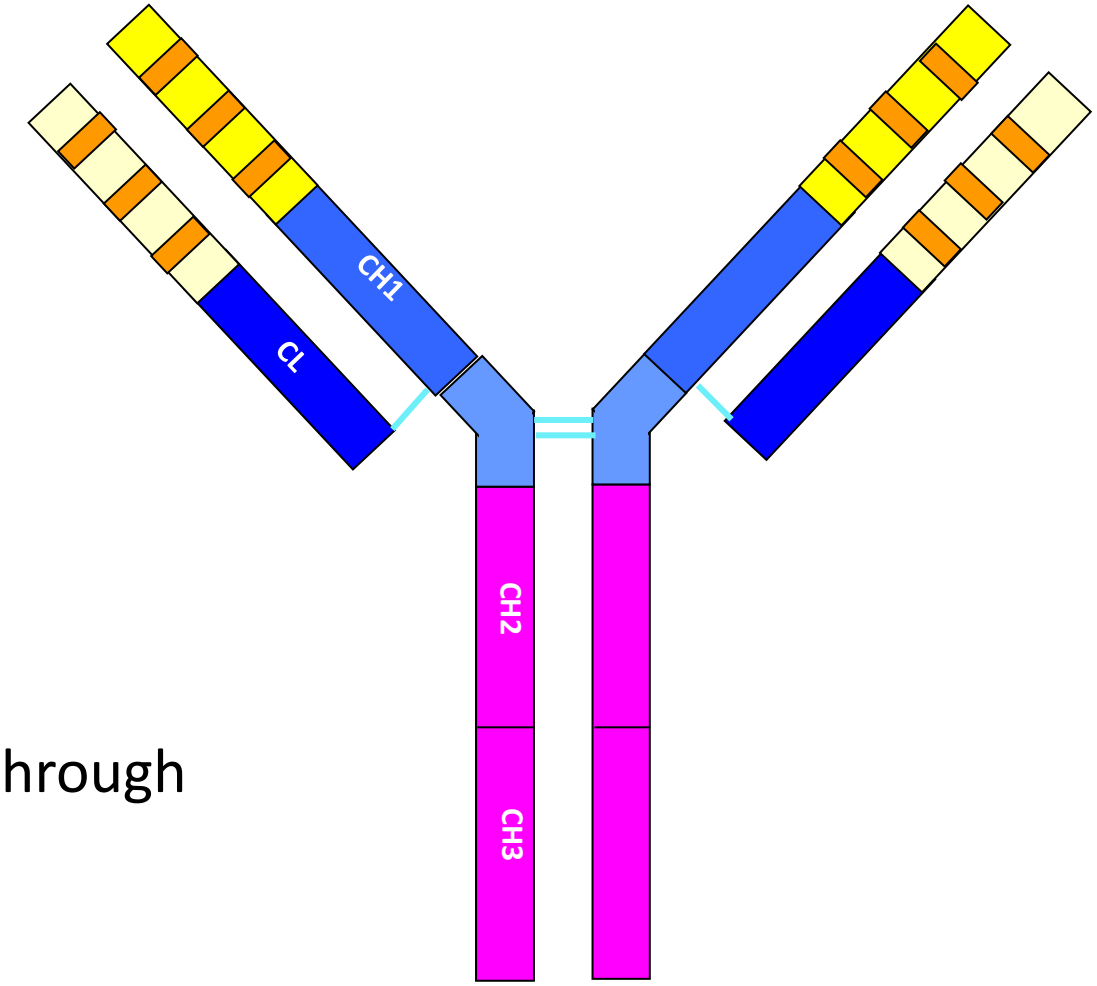
Pathogenesis of hemolytic anemia

- CD59
  - Membrane inhibitor of reactive lysis
  - Prevents incorporation of C9 into C5b-8; thus, MAC does not form
- CD55
  - Decay accelerating factor
  - Block C3 convertase
- Protect cells from complement-mediated destruction


Lectin Pathway
Classical Pathway
Alternate Pathway
MBL, MASP,
C4 + C2
C1q, C1r, C1s
C4 + C2
C3
Factor B + D
CD55
C3 convertases
C4b2a, C3bBb
C3b
C5 convertases
C4b2a3b, C3bBb3b
C 5
C5a
CD59
C6,C7,C8,C9
Eculizumab
C5b
Membrane attack complex (MAC)

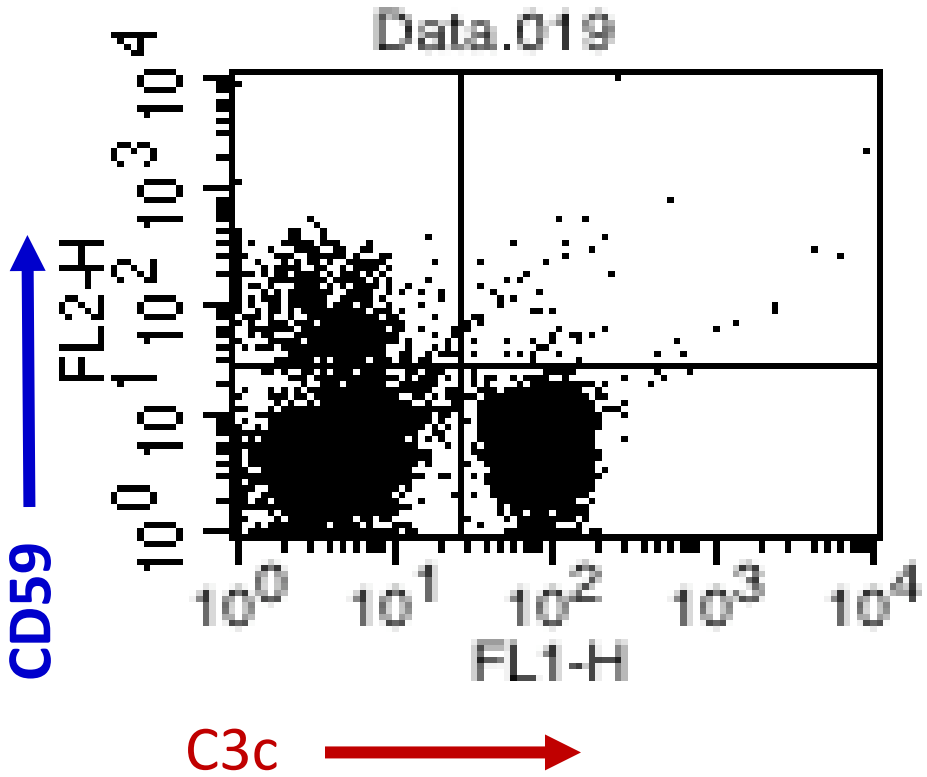
Ravulizumab

- 4 amino acids different than eculizumab
  - Extends Half-Life 4-fold
  - IV q 8 weeks in maintenance phase
- Non-inferior in two phase 3 trials
  - Better at preventing pharmacologic breakthrough
  - FDA approved 2019
  - Less expensive for long-term use

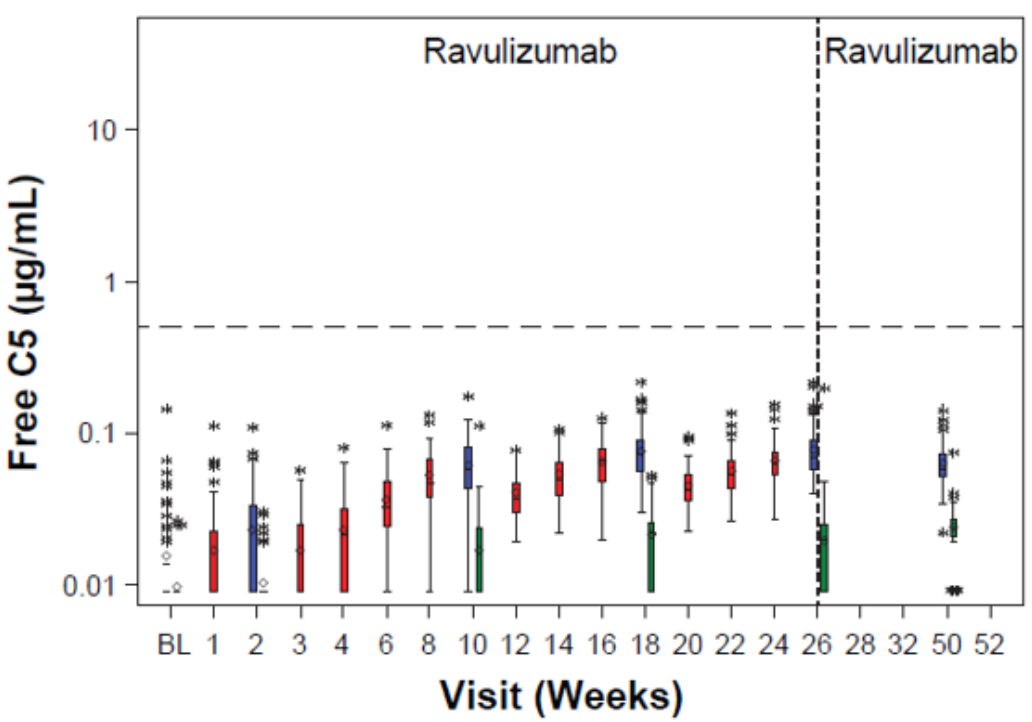


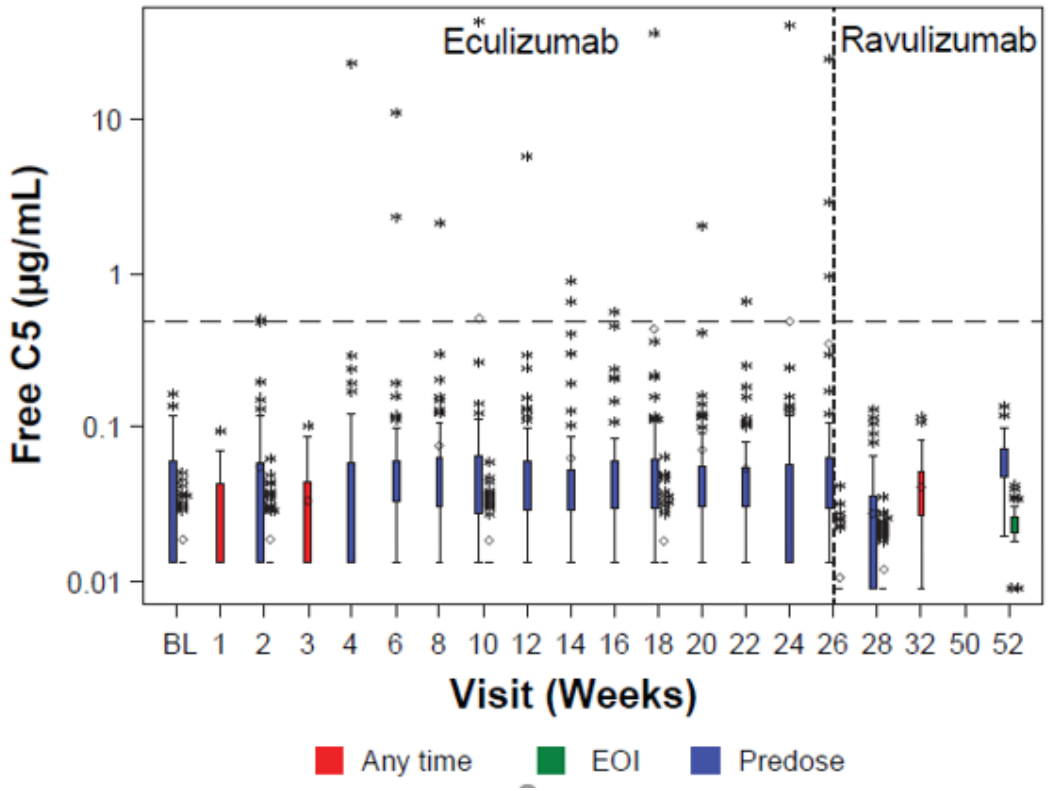
Two Mechanisms for Breakthrough Hemolysis

- Definition:
  - Return of intravascular hemolysis (hemoglobinuria increased LDH) and reappearance of classical PNH symptoms
- Causes:
  - Suboptimal C5 inhibition (**pharmacokinetic** breakthrough)
  - Complement amplifying conditions (**pharmacodynamic** breakthrough)
    - Infection
    - Pregnancy
    - Surgery



Ravulizumab Suppresses C5 more Reliably than Eculizumab



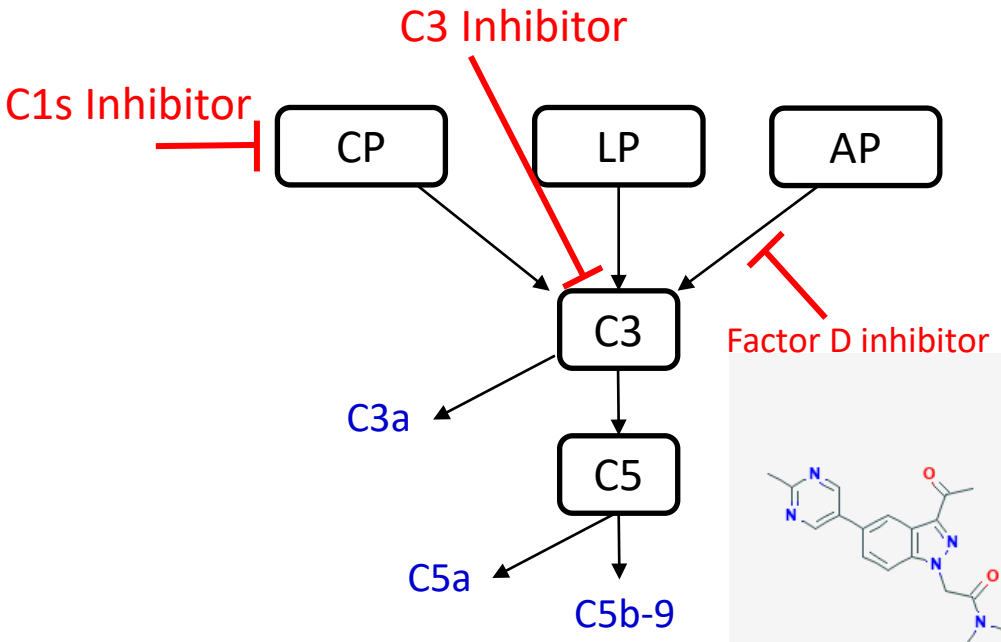


Prevents pharmacokinetic but NOT Pharmacodynamic breakthrough

Peffault de Latour et al, BJH 2020

Danicopan (Factor D inhibitor)

- Serine protease primary made in adipocytes
- FB is its only known substrate
- Among the lowest concentration of all complement proteins
- Rate limiting step of AP activation



Danicopan Stops the need for Red Cell Transfusions in PNH patients with Suboptimal Response to Eculizumab

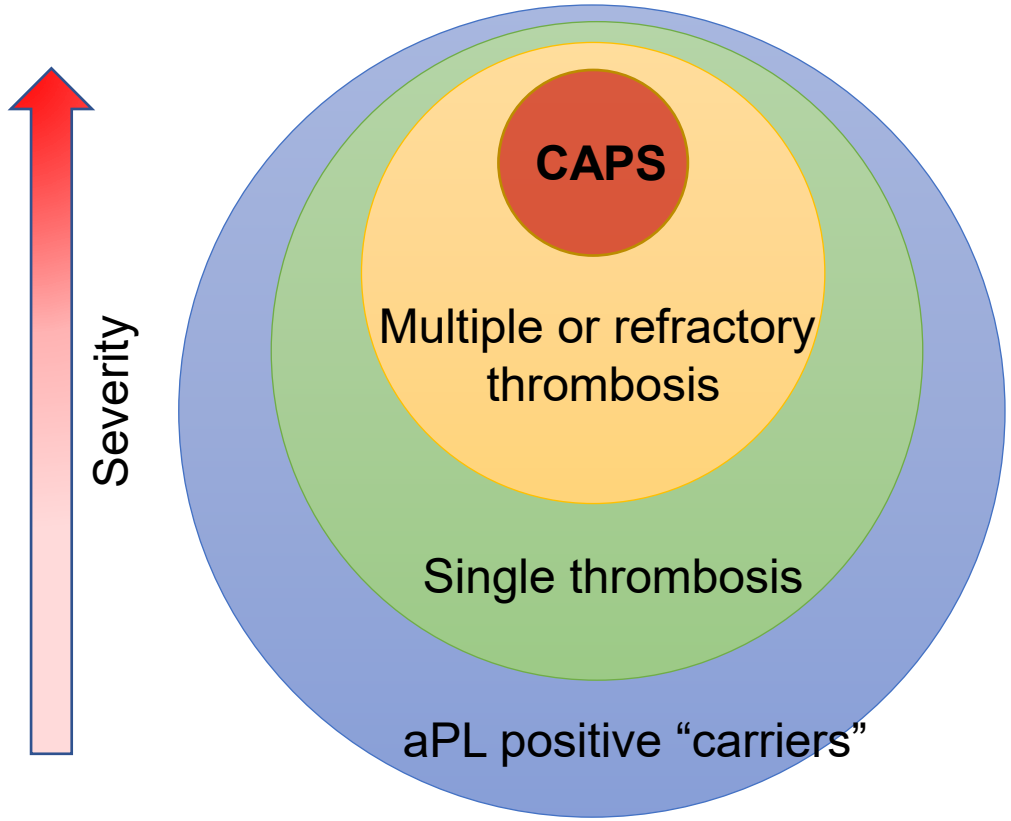
	Historical Transfusions				On Treatment
Subject*, Sex, Age	-52 to -24 Weeks	-24 to -13 Weeks	-12 Weeks to Screening	Screening to Day 1 (≤60 Days)	Day 1 to 12 Weeks
B, F 51	1 1		1		
C, M 67**		1	1		2
D, F 29	2 2 2 1	2 2	1 1 2		
E, F 22			2		
F, F 44	1 2	2	2		
G, F 35	3 3 2 3 3	3 2 2	3 1 2	2	
H, F 52	2 2 2 1 2 2	2 2 2	2 2 2		
I, F 50			1		
J, M 19		2 1 1	2 1 1 2 2	1	
K, F 57	2	1	2	1	

*Patient A excluded from table due to religious objection to receiving transfusions.

RBC symbol indicates instance with unit number inside.

Antiphospholipid syndrome (APS)

- APS is defined as **thrombosis, pregnancy morbidity,** or both along with persistently positive **antiphospholipid antibodies (aPL)**.
- Long-term anticoagulation with warfarin is the standard of care for thrombotic APS.
- Recurrent thrombosis remains common (10-20% on anticoagulation, 25-50% off anticoagulation).



Miyakis et al. J Thromb Haemost. 2006, Garcia et al. Blood 2013,
Comarmond et al. Lupus 2017, Ruiz Irastorza Arch Int Med 2002

Methods

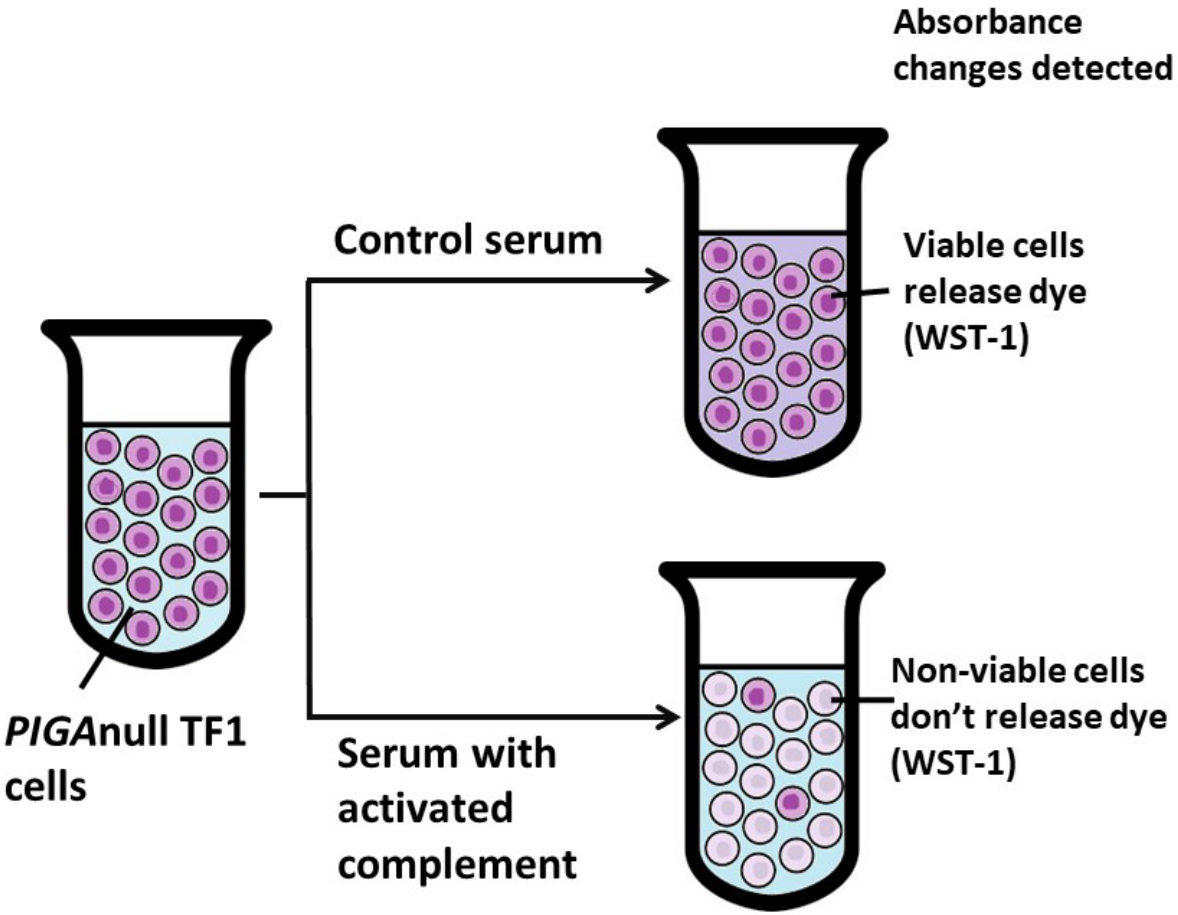
- Cross sectional study (Hopkins, Cleveland Clinic and McMaster Univ).

Diagnosis category	Number of patients
Thrombotic APS (ISTH criteria)	59
CAPS (International consensus criteria)	10 (acute sera for 7)
Lupus (SLICC criteria)	74
Additional controls for sequencing	(33 aHUS, 43 healthy controls)

- Affinity purified anti-β_2GPI from patients.
- Complement activation detected by:
  1. Functional assay: modified Ham assay
  2. Flow cytometry for C5b-9 deposition
- Targeted sequencing on a custom panel of 15 genes involved in complement regulation.

Chaturvedi et al, Blood 2020; 135:239-251

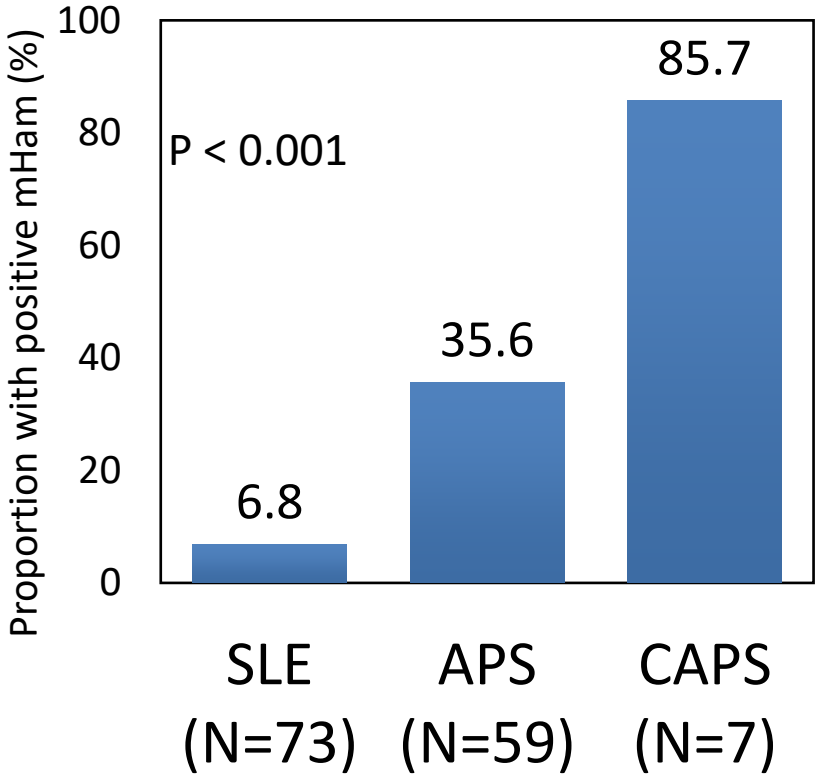
The modified Ham (mHam) assay

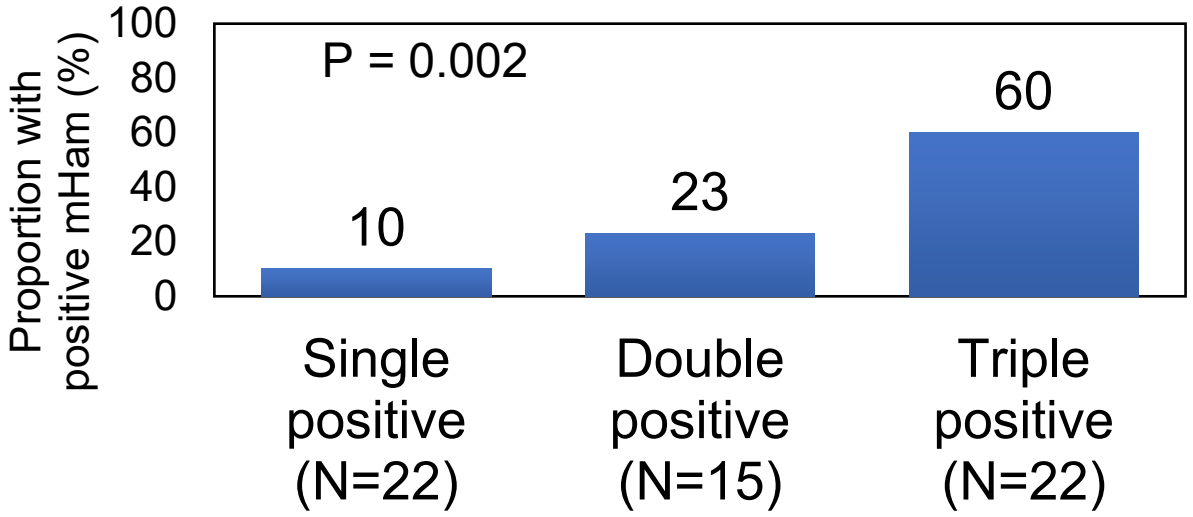


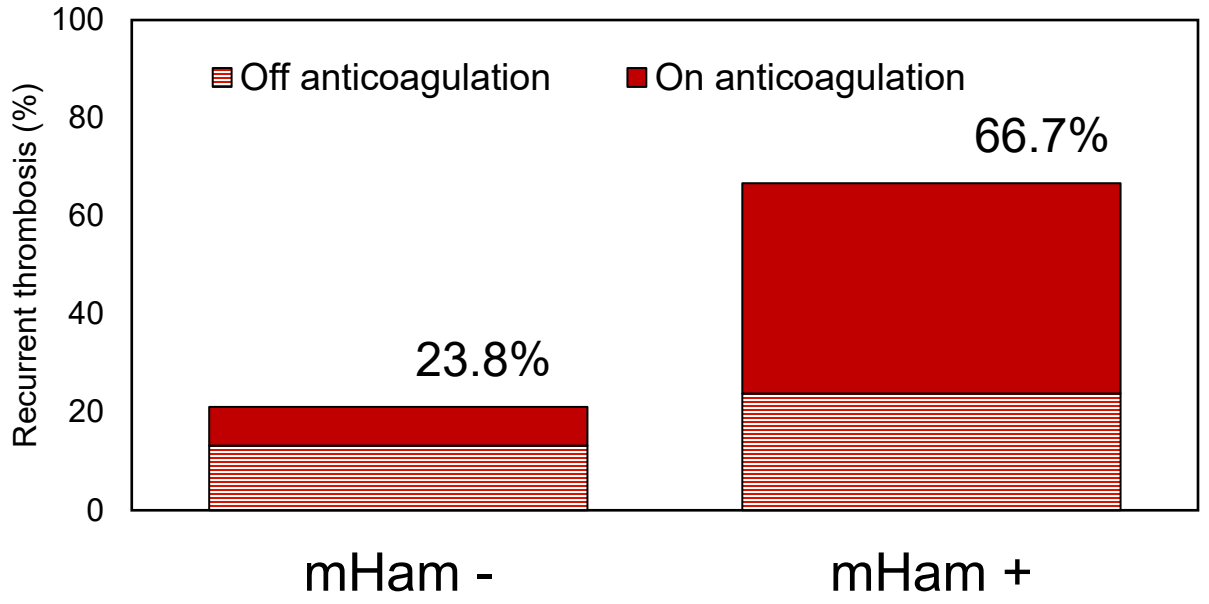
- Principle: Cell line lacking surface CD55 and CD59 is susceptible to complement mediated killing.
- 20% cell killing established as the threshold for a positive test.
- Shiga toxin as positive control, heat inactivated serum as negative control.

Gavriilaki et al. Blood 2015, Vaught et al. JCI Insight 2018, Brady et al. J Clin Exp Nephrol. 2016

Thrombotic APS is associated with a positive mHam

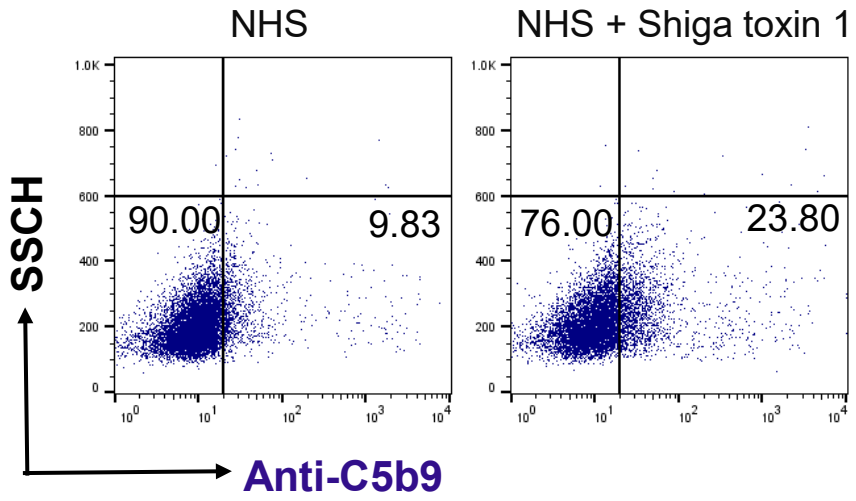


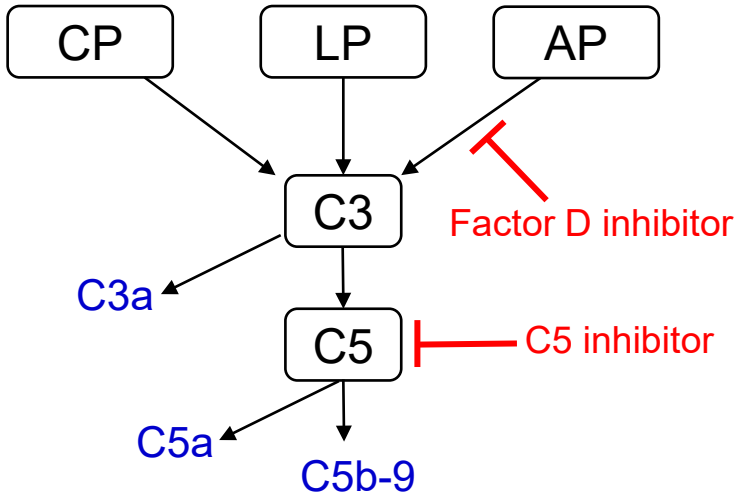


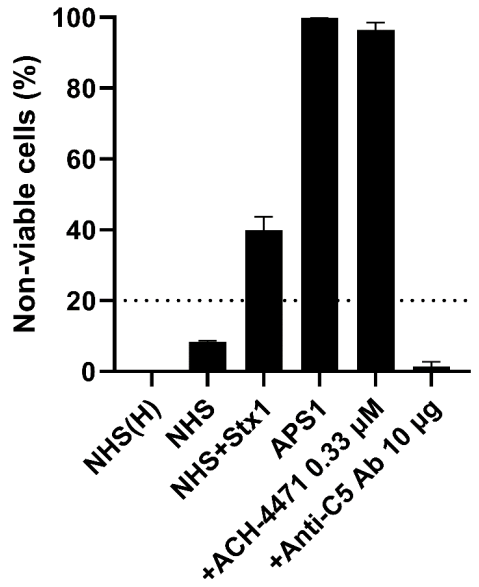


Chaturvedi S, Braunstein E and Yuan, X et al, Blood 2020

C5b-9 deposition induced by APS sera







NHS, normal human serum,
ACH4471, factor D inhibitor (Achillion)

CAPS is associated with rare variants in complement regulatory genes

Diagnosis	N	Rare germline C' mutations (%)*
aHUS	17/33	51.5%
Normal	10/43	23.3%

(i) homozygous *CFHR1-CFHR3* deletion (N = 2)

(ii) *THBD* P501L

(iii) *CR1* S1982G and homozygous *CFHR1-CFHR3* deletion

(iv) *CFHR4* R287H

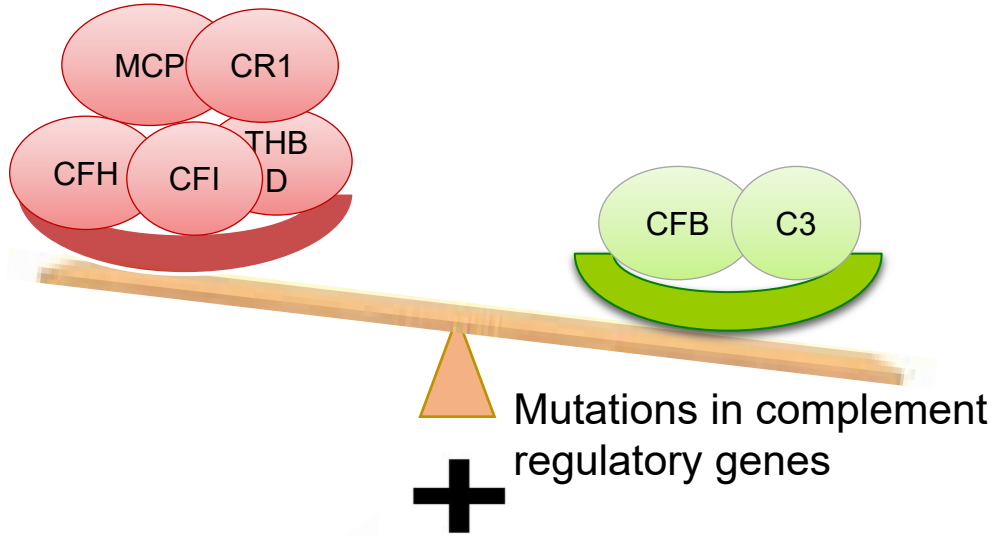
(v) *CR1* V2125L.

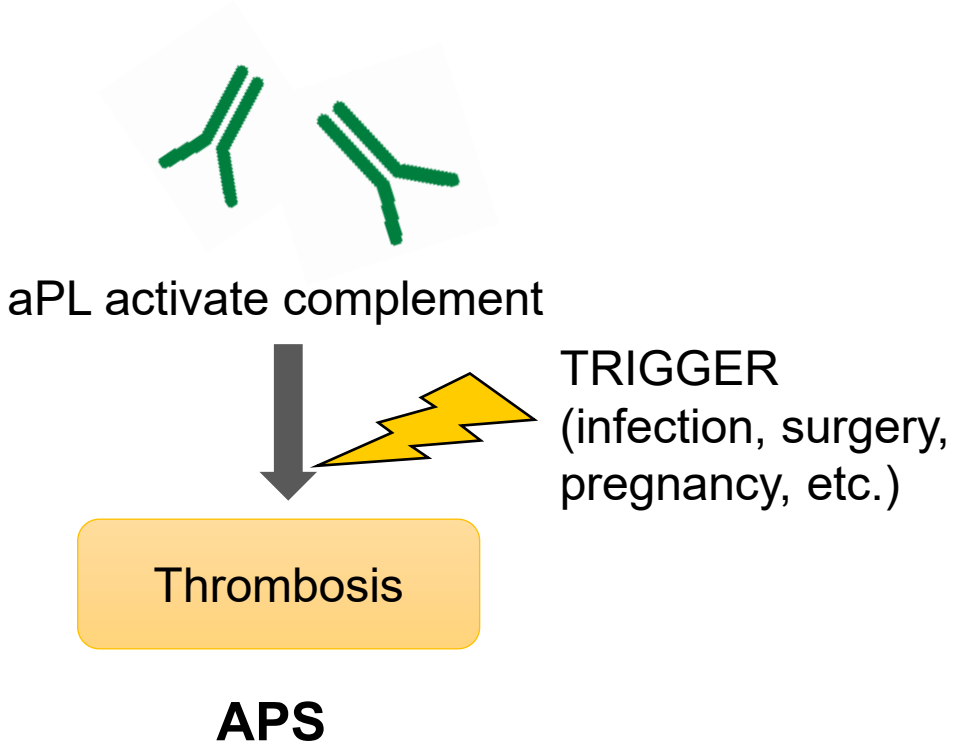
*MAF < 0.005

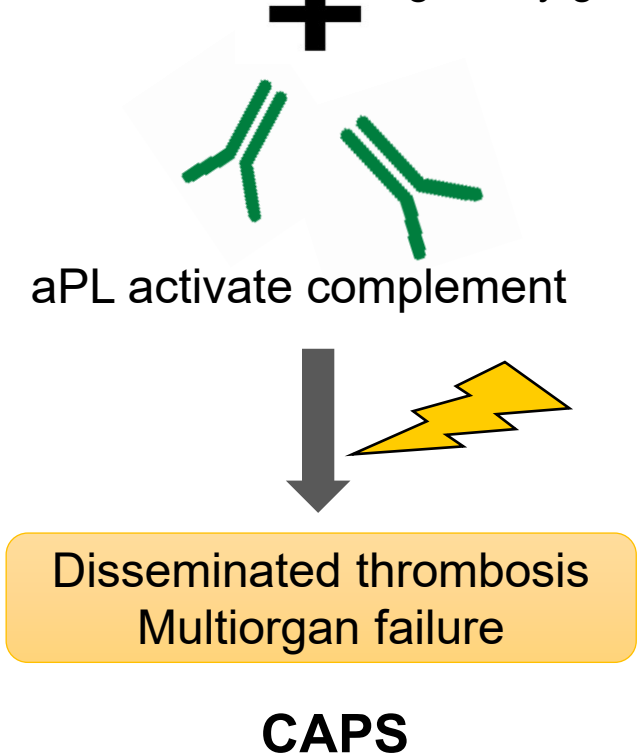
Genes on panel: *CFH*, *CFB*, *CFI*, CFD, CFP, *CFHR1*, *CFHR2, CFHR3*, *CFHR4, CFHR5*, *C3*, *CD46* (*MCP*), *THBD, CR1, DGKE*

Chaturvedi et al, Blood 2020; 135:239-251

'Multi-hit' model for CAPS







Chaturvedi S, Braunstein E and Yuan, X et al, Blood 2020

Take Home

- **SAA: IST vs BMT**
  - IST: still has only 75-80% response; high relapse and late clonality
  - Haplo BMT SOC for relapsed disease; may replace IST altogether

- **PNH:**
  - Ravulizumab new SOC
  - Non-inferior to eculizumab but much more convenient
  - Novel more effective complement inhibitors in development

- **APS/CAPS:**
  - Anti β2-GPI antibodies activate complement
  - CAPS: β2-GPI antibodies + germline mutations
  - Need for clinical trials of complement inhibitors