Advances in Classical Hematology
T. Howard Lee Keynote Lecture

Robert A. Brodsky MD

Johns Hopkins

- Curative therapy for sickle cell disease and severe aplastic anemia
- Pathophysiology, diagnosis and treatment of aHUS
- Newly approved drugs for PNH

Adults with SCD have a shortened life-span

Despite progress in the management

- prevention of pneumococcal infections and early childhood death

  - *Gaston MH NEJM 1986*

- hydroxyurea as standard care

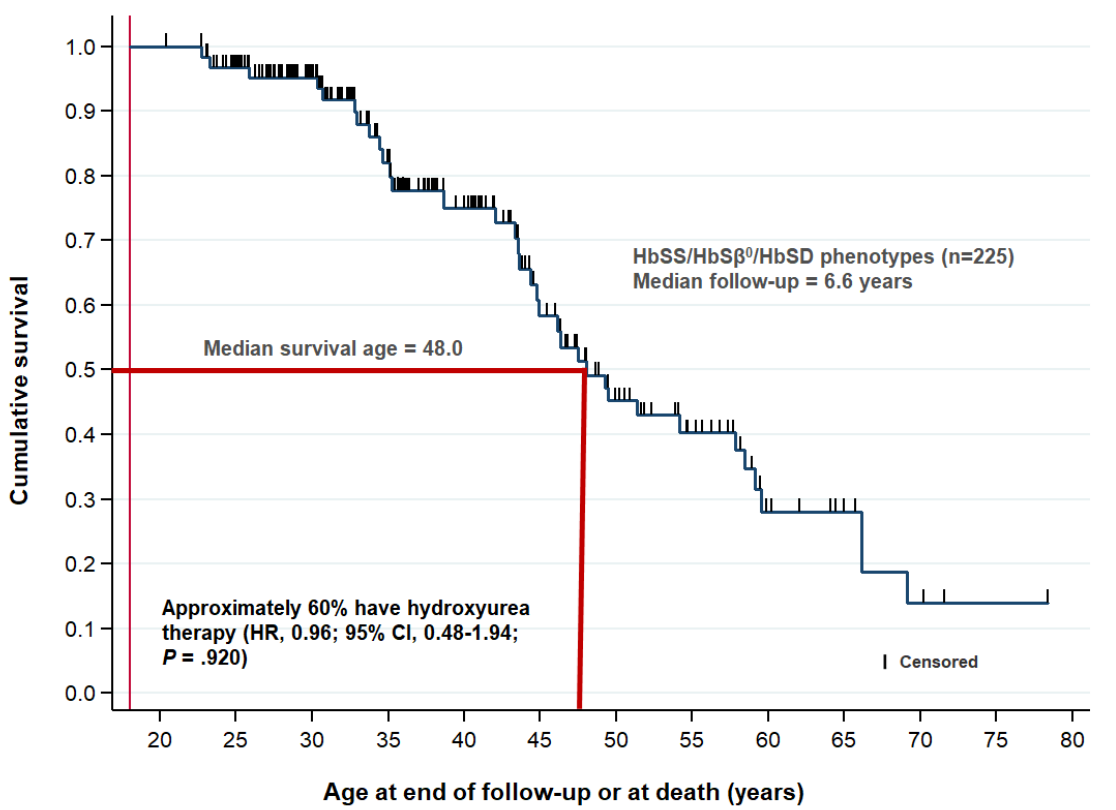
  - *Charache S NEJM 1995*

- early detection of cerebral vasculopathy and stroke prevention by TCD

  - *Adams RJ NEJM 1992*

SCA remains a disease with high risk of morbidity and early death

Median survival for HbSS: 48.0 years with no change in 25 years



DeBaun M et al. Blood, 2019. 133(6): 615-617.

BMTCTN 1507: Study design: Phase II, single-arm, multi-center trial

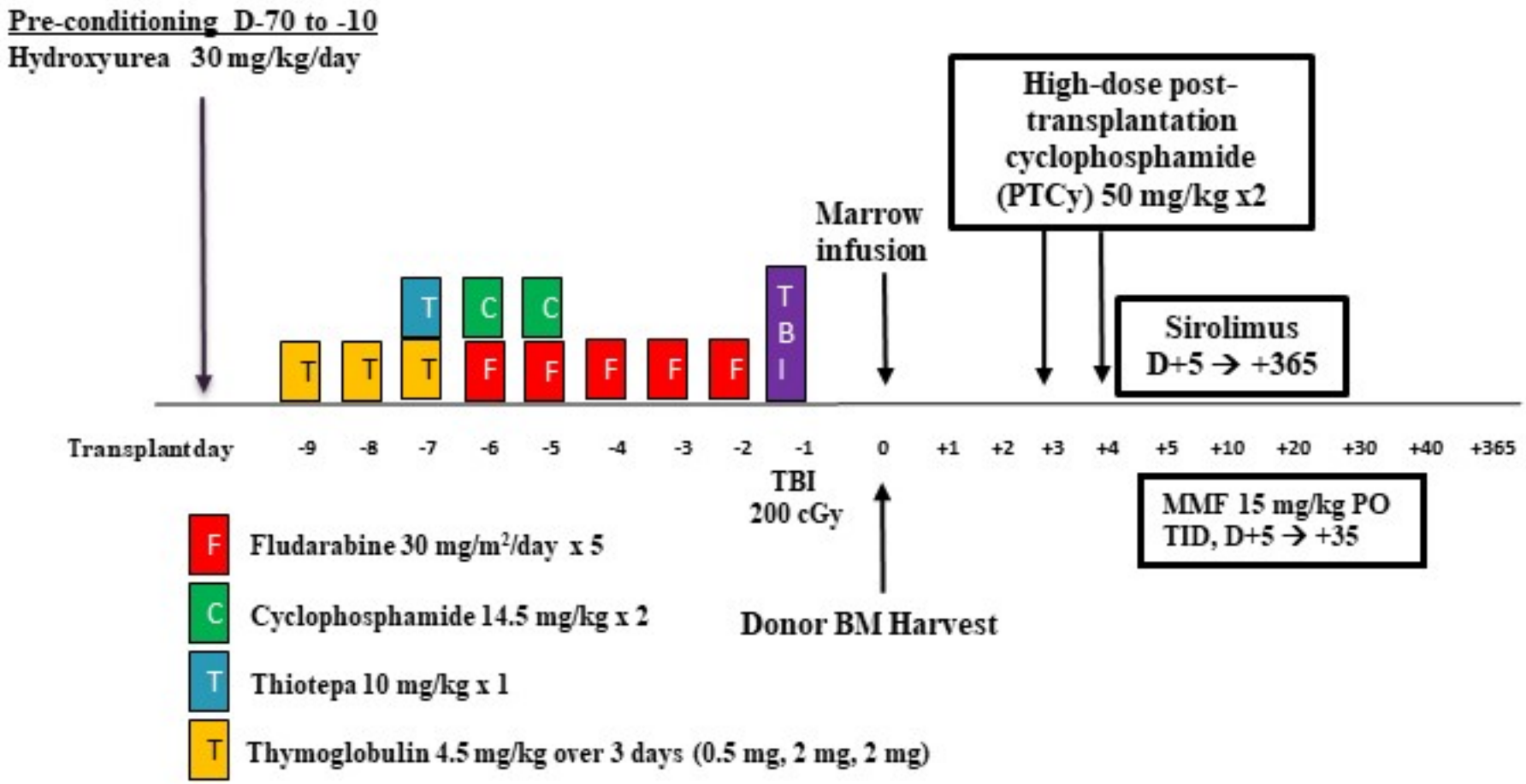
- **Primary objective** is to estimate event-free survival (EFS) at 2 years after haploidentical BMT in patients with SCD enrolled in 2 strata:
  - children 5.00 – 14.99 years of age and
  - adults 15.00 - 45.99 years of age at enrollment

- **Secondary objectives** include determining the effect of haploidentical BMT on clinical and laboratory manifestations of SCD by 2 years after transplantation and determining the incidence of other transplant-related outcomes.

Transplant indications

Clinical stroke
≥ 2 episodes of ACS in the preceding 2 years
≥ 3 episodes of VOC in the preceding 2 years
≥ 8 transfusions per year for ≥ 1 year to prevent SCD-related complications
Tricuspid valve regurgitant jet (TRJ) ≥ 2.7 m/sec

Common Conditioning Platform for Haplo-BMT

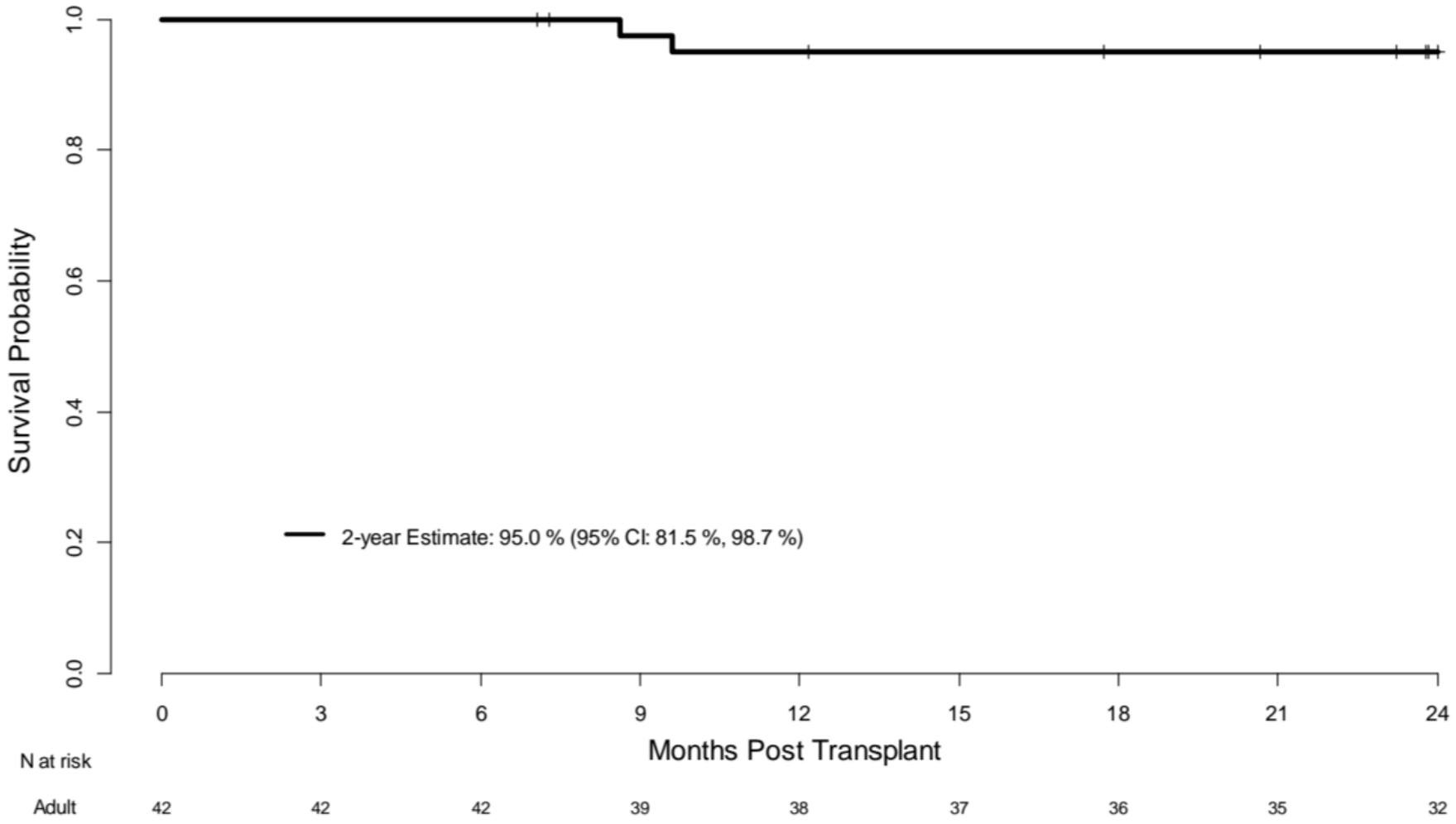


Statistical Plan

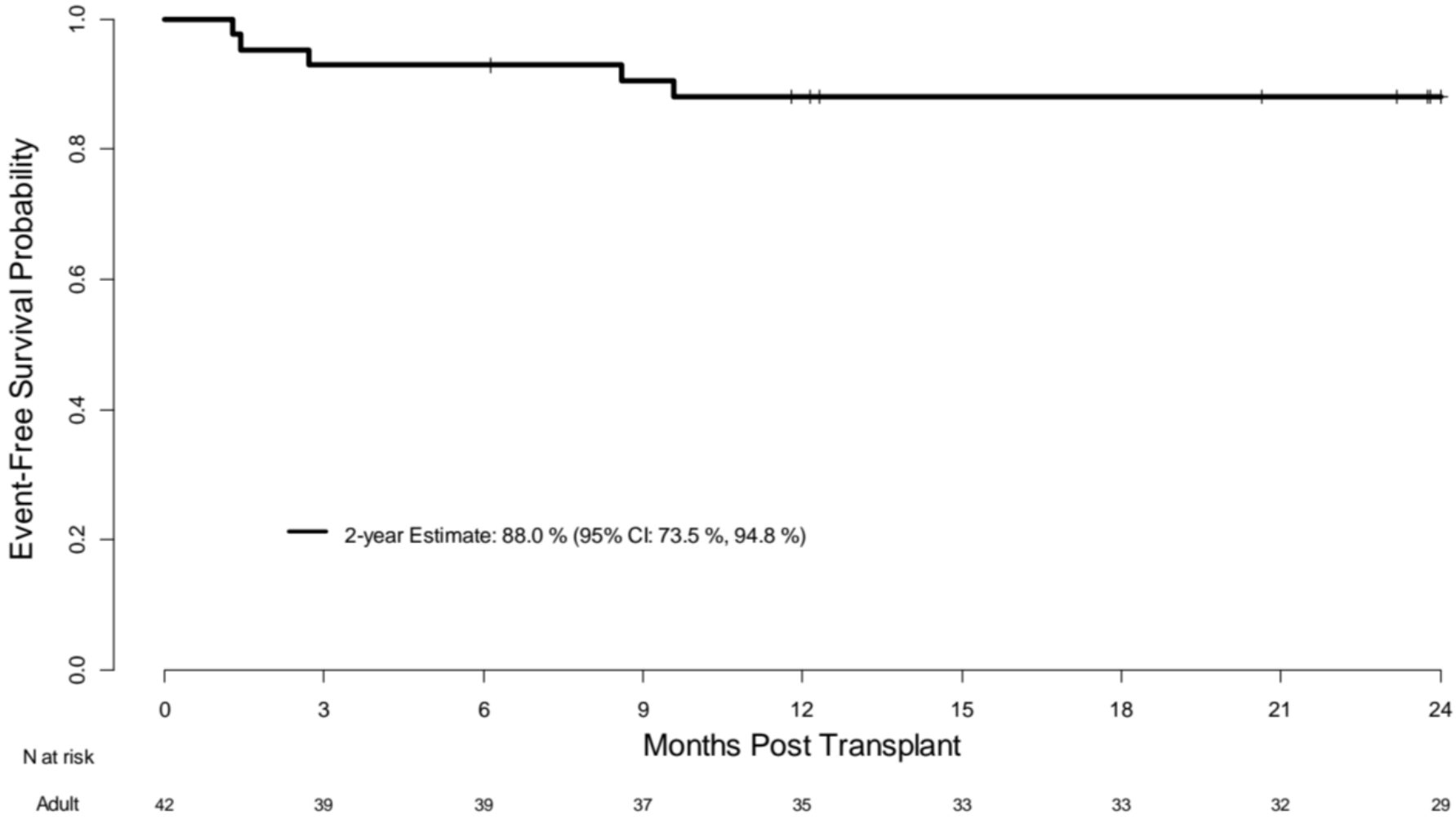
- 40 adult transplanted patients
- Two-year EFS is defined as survival without primary or secondary graft failure, second infusion of hematopoietic cells, or death

- Targeted overall survival rate - 95%
- Targeted event-free survival - 85%.

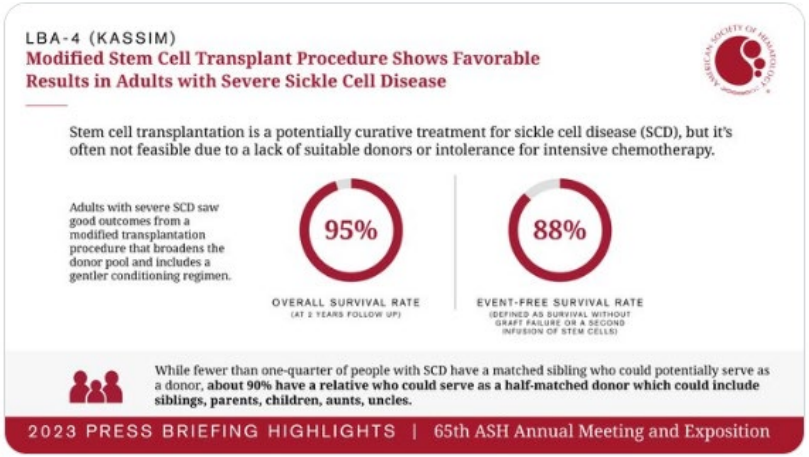
Over 95% overall survival at 2 years post-transplant



Event-free survival was 88% at 2 years post-transplant



Conclusion



- Reduced intensity haploidentical-BMT in adults with SCD shows durable donor engraftment at 2 years with low mortality.
- The 2-year EFS 88% and OS 95% are comparable to that reported after MSD myeloablative BMT.
- These results support haploidentical BMT with PTCy as a suitable and tolerable curative therapy for adults with SCD and severe end-organ toxicity such as stroke and pulmonary hypertension, a population typically excluded from participating in myeloablative gene therapy and gene editing trials.

What about Gene/gene editing?

VARIABLE	BMT/CTN	Lentiglobin	CRISPR/Cas9
Pre-transplant conditioning	Non-myeloablative	Myeloablative	Myeloablative
Neutrophil engraftment (median)	25.5	20	27
Plts (median)	34.5	36	34.5
Mean Hgb (post Rx)	13.5	11	11
EFS OS	88% 95%	85% 96%	90% 100%
COST	<300K	3.1 million	2.2 million
LOS (inpt)	~7-14 days	2-3 mos	2-3mos

CRISPR-Cas9 Editing of the *HBG1* and *HBG2* Promoters to Treat Sickle Cell Disease

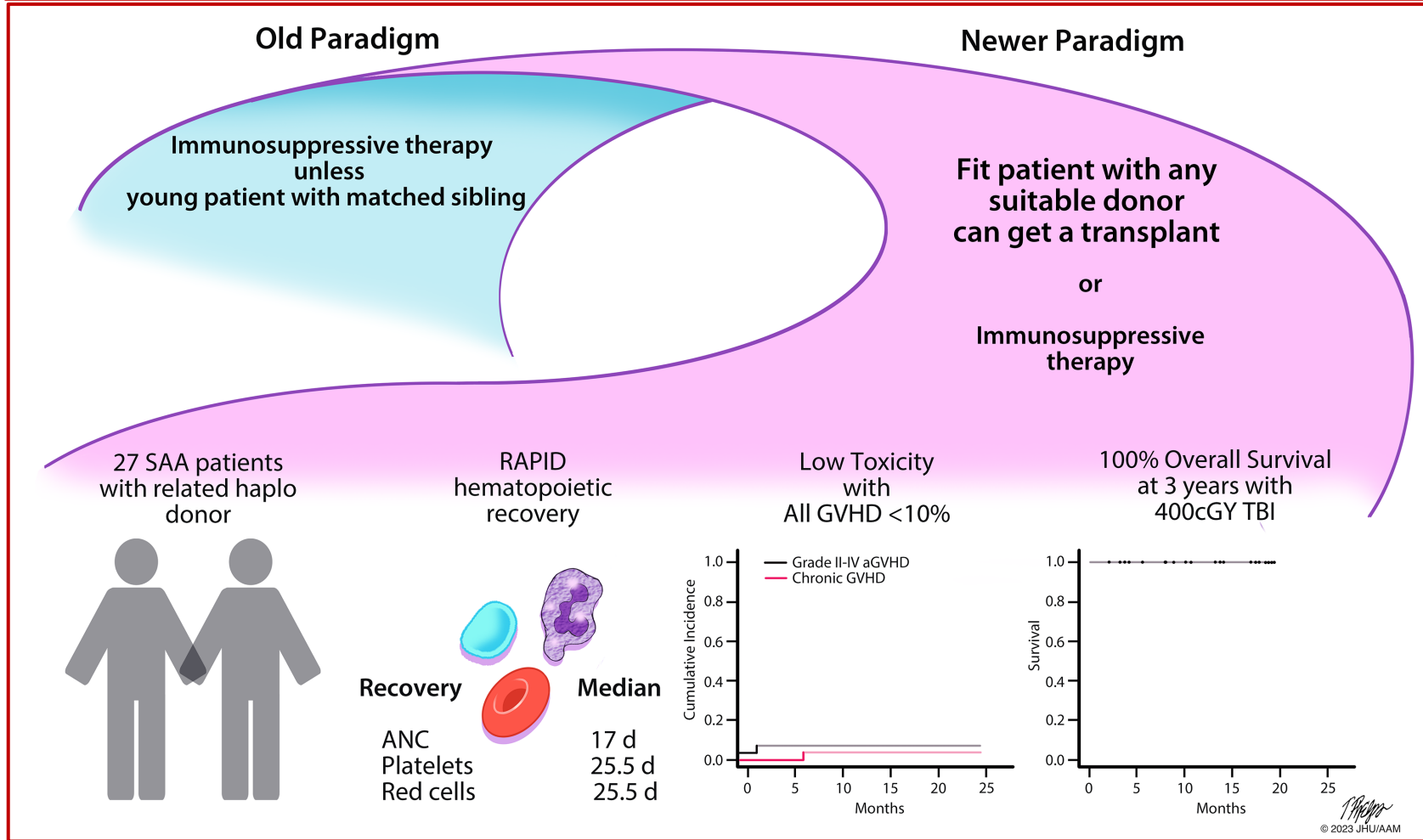
Akshay Sharma, M.B., B.S., Jaap-Jan Boelens, M.D., Ph.D., Maria Cancio, M.D., Jane S. Hankins, M.D., Prafulla Bhad, M.Sc., Marjohn Azizy, Pharm.D., Andrew Lewandowski, Ph.D., Xiaojun Zhao, Ph.D., Shripad Chitnis, Ph.D., Radhika Peddinti, M.D., Yan Zheng, M.D., Ph.D., Neena Kapoor, M.D., et al.

Table 1. Demographic Characteristics of the Participants and Outcomes.

Variable	Participant 1	Participant 2	Participant 3
Sickle cell disease genotype	β^S/β^S	β^S/β^S	β^S/β^S
Sickle cell disease–related symptoms before study enrollment	Six episodes of acute chest syndrome over the past 10 yr and a history of a silent cerebral infarction, retinopathy, and priapism	Four episodes of vaso-occlusive crisis, three episodes of acute chest syndrome, and a silent cerebral infarction during the preceding 20 yr	Twenty five episodes of vaso-occlusive pain in the 2 yr before enrollment
Treatment for sickle cell disease ongoing at study enrollment	Regular blood transfusions and hydroxyurea	Hydroxyurea	Regular blood transfusions and hydroxyurea
Apheresis collection and OTQ923 manufacture			
Mobilization cycles lasting 2–3 days each (no.)	3	2	3
Cell dose manufactured (million/kg)	2.80, a combination of two manufacturing batches, each with 84% editing efficiency	5.99, a combination of three batches with editing efficiencies of 78%, 75%, and 73%, respectively	5.04, a combination of two batches with editing efficiencies of 87% and 82%, respectively
Follow-up and outcomes			
Neutrophil engraftment	Day 26	Day 20	Day 18
Platelet engraftment	Day 44	Day 29	Day 29
Adverse events since OTQ923 infusion (no.)	36	16	45
Adverse events considered by investigators to be related to OTQ923 (no.)	0	0	0
Follow-up since OTQ923 infusion (mo)	18	12	6
Sickle cell disease–related events since OTQ923 infusion*	One episode of vaso-occlusive crisis with acute chest syndrome occurred at 17 mo after infusion; recurrent intermittent priapism; no new stroke or silent cerebral infarction; continued mild hemolysis; worsening osteonecrosis of femur	One episode of vaso-occlusive crisis occurred at 12 mo after infusion; no acute chest syndrome, stroke, or priapism; continued mild hemolysis; persistent osteonecrosis of femoral head	One episode of vaso-occlusive crisis occurred at 9 mo after infusion†; continued mild hemolysis; persistent osteonecrosis of femoral head

* The observation period for post-treatment sickle cell–related events starts on the day of the first OTQ923 infusion and ends on the day of last follow-up.
† This event happened after the data-cutoff date, and hence the rest of the follow-up is only up to 6 months.

HLA-Haploidentical Bone Marrow Transplantation (BMT) as Initial Therapy for Patients with Severe Aplastic Anemia (SAA)



Conclusion: **In SAA patients, upfront HLA-haploidentical BMT with post-transplant cyclophosphamide resulted in rapid hematopoietic recovery and low morbidity and mortality (ClinicalTrials.gov: NCT02833805).**

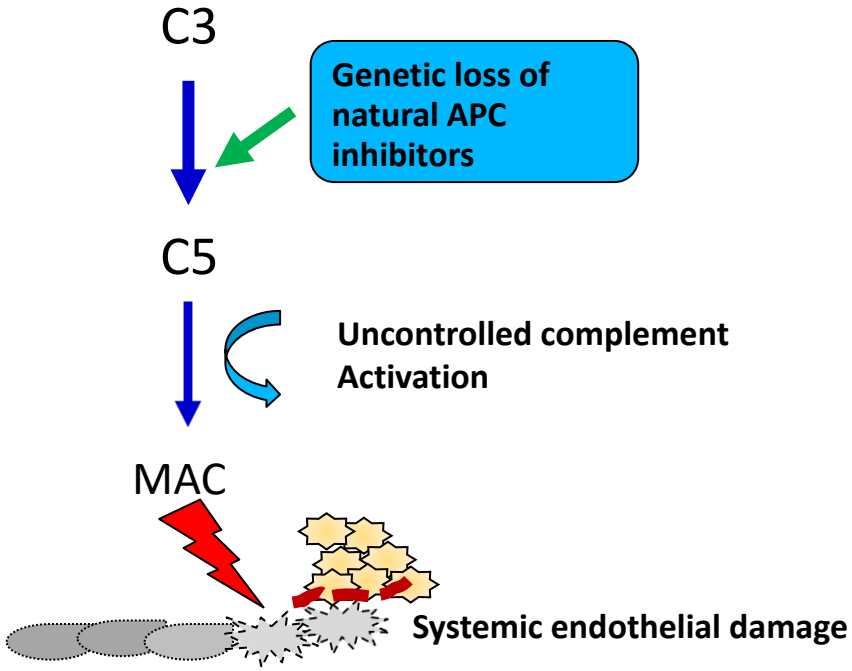
DeZern et al. Blood 2023

SAA Conclusions

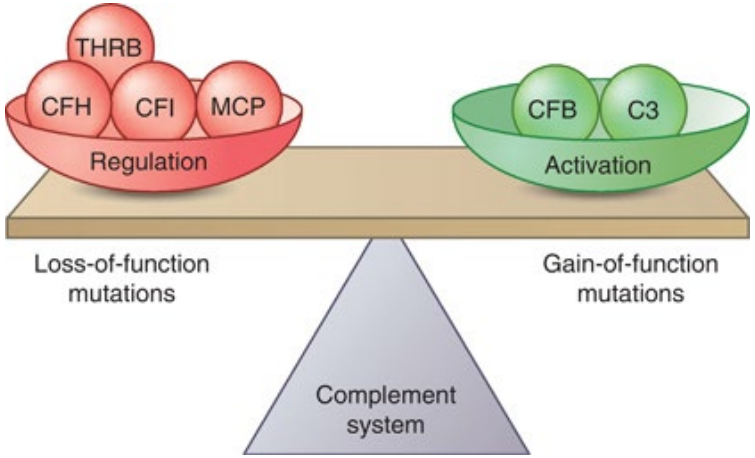
- **SAA: IST vs BMT**
  - **BMT advancing faster ATG-based therapy; solves problem of relapse and clonality**
  - **Less expensive than ATG/CSA/Epag**
  - **Faster and more complete hematopoietic recovery**

- **Post-transplant Cy is safely expanding the donor pool in SAA**
  - **Standard of care for refractory SAA**
  - **Upfront BMT even for those with only haplo donors given late relapse/malignancies that are not mitigated by Epag**
  - **Same early mortality (<5%)**

Atypical Hemolytic Uremic Syndrome: excessive activation of the APC?



1

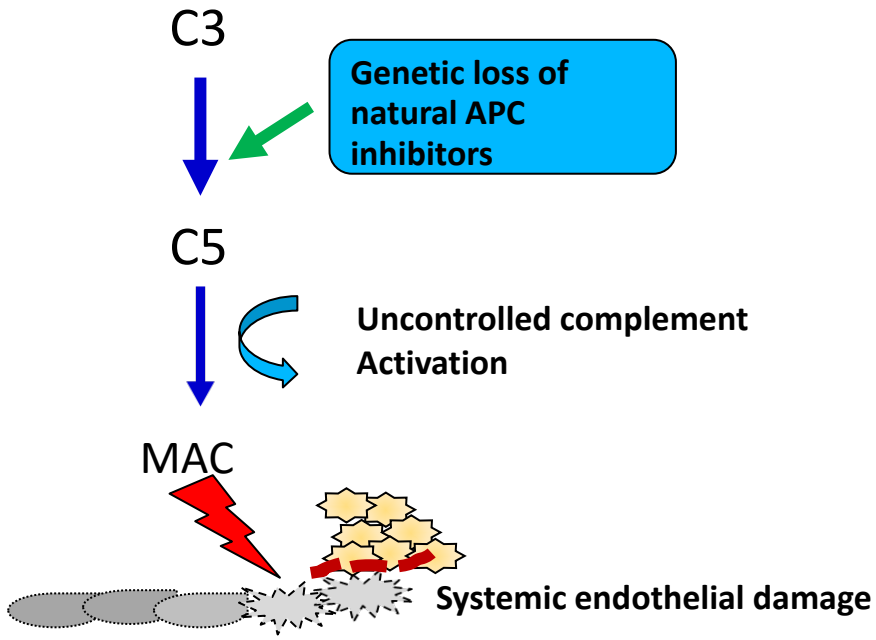


2

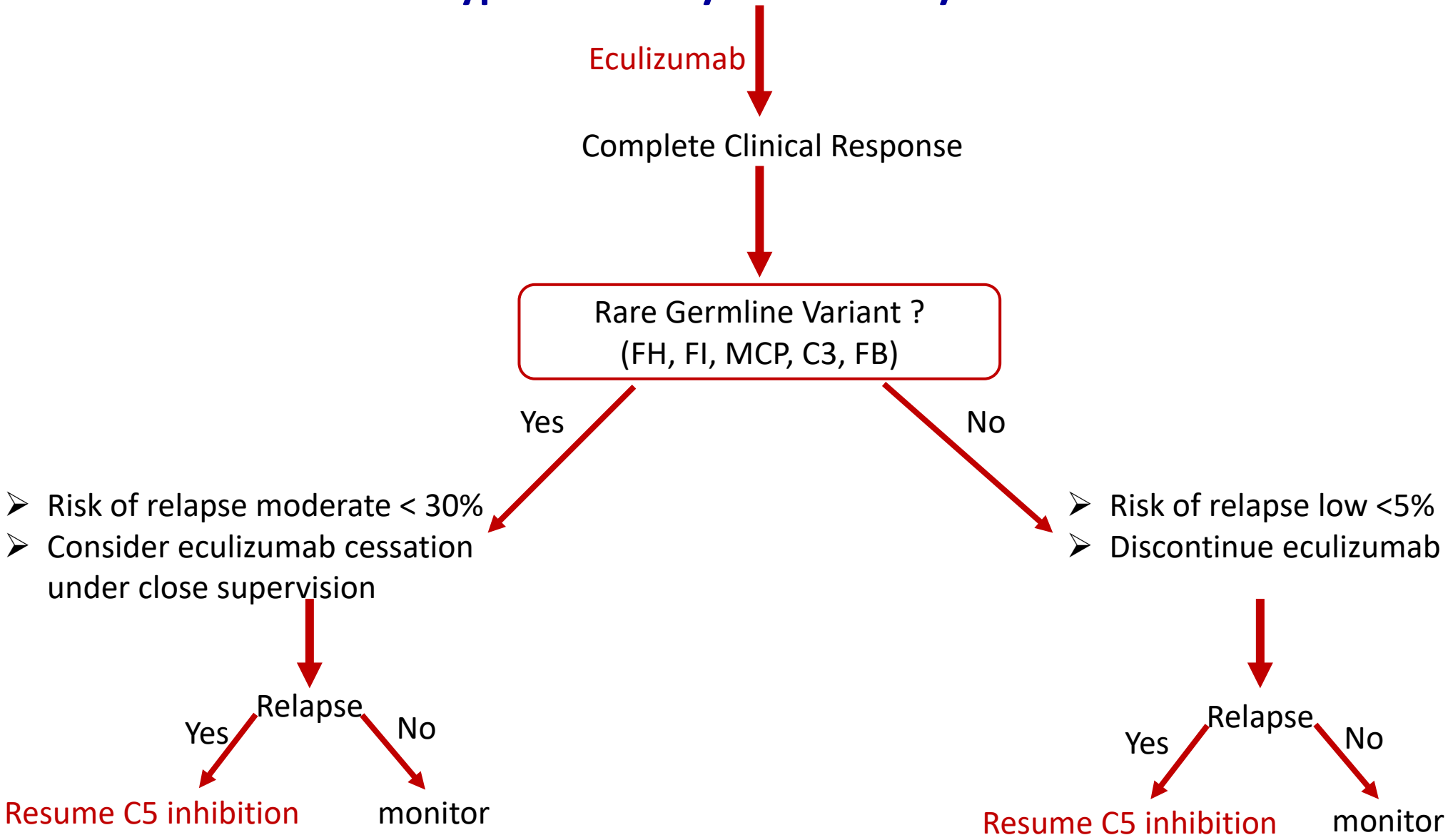
- Inflammation
- Surgery
- Pregnancy
- Autoimmunity

BIG 5 of CM-HUS

- fH: fluid and cell surface
  - Decay accelerator
  - Cofactor activity (fI)
  - Competes with fD for binding to B
- MCP(CD46) **Surface only** (renal endo)
  - Cofactor activity with fI
- fI: fluid phase
  - Inhibits all pathways by degrading C3b and C4b
- C3 and B: Fluid phase components of APC



Atypical hemolytic uremic syndrome



Brodsky RA, Blood 2021 137:2419-2420

aHUS: Genetics

- Germline mutations involving APC genes 50-60% cases
  - ~20% have 2 or more mutations, often heterozygous

- Majority are sporadic rather than familial
  - Even in familial forms penetrance is incomplete

PROBLEM:

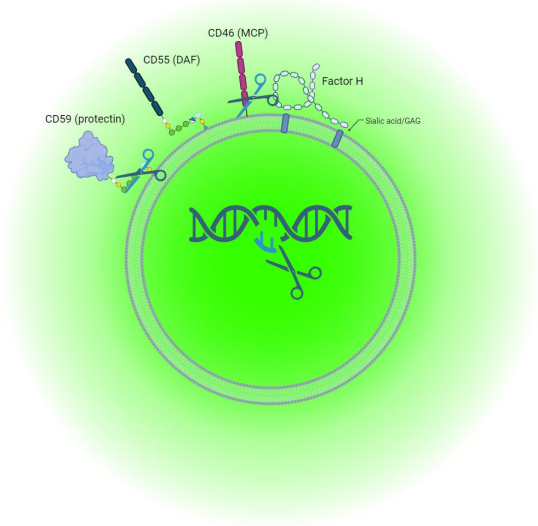
- Why no mutations or autoAbs in 40-50% of cases?
- Why do patients remain mHam positive in remission?
- Why is relapse rate so much higher in patients with mutations?

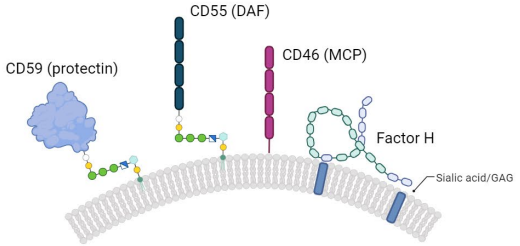
Bioluminescent mHam assay differs in three principle ways

1) Use of an autonomously bioluminescent cell line to detect viability change which obviates the need for substrate addition and washes
2) Use of HEK293 cells (as opposed to TF1 cells) which lack CR1 and more closely approximates the complement regulators on the surface of the renal endothelium
3) Potential use of three different cell lines
   - A PIGA knockout lacking both CD55 and CD59
   - A CD46 KO (CD55 and CD59 are intact)
   - A Double KO lacking CD46, CD55, and CD59

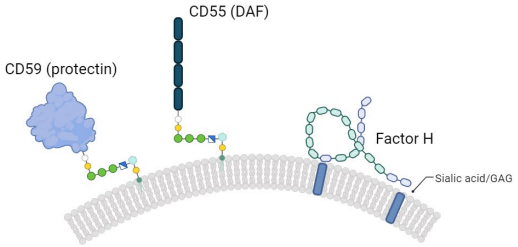
Michael Cole, MD/PhD
New Castle, Indiana

COMPLEMENT BIOSENSORS: The Bioluminescent mHam

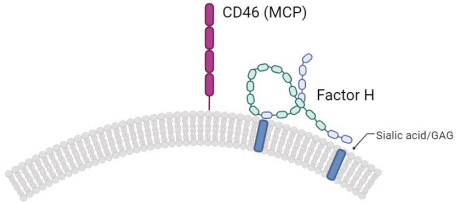




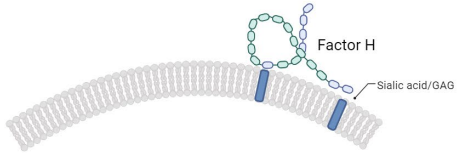
Wild type



CD46 KO



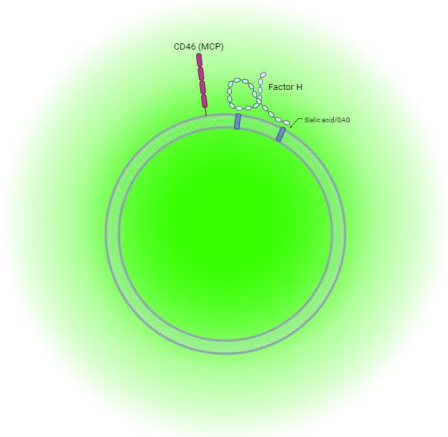
PIGA KO



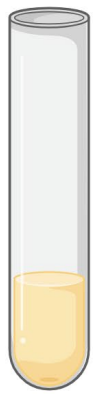
CD46 and PIGA KO (Double KO)

Downstream genetic engineering of autonomously bioluminescent cells creates complement biosensors

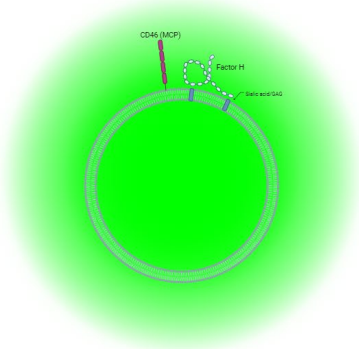
Patient serum is added to autonomously bioluminescent HEK293 cells without complement regulators



+

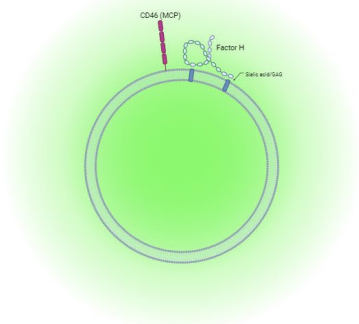


Heat inactivation or addition of complement inhibitors



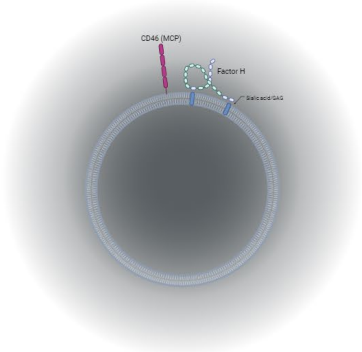
Cells glow brightest/no metabolic effects of complement

No abnormal complement activity



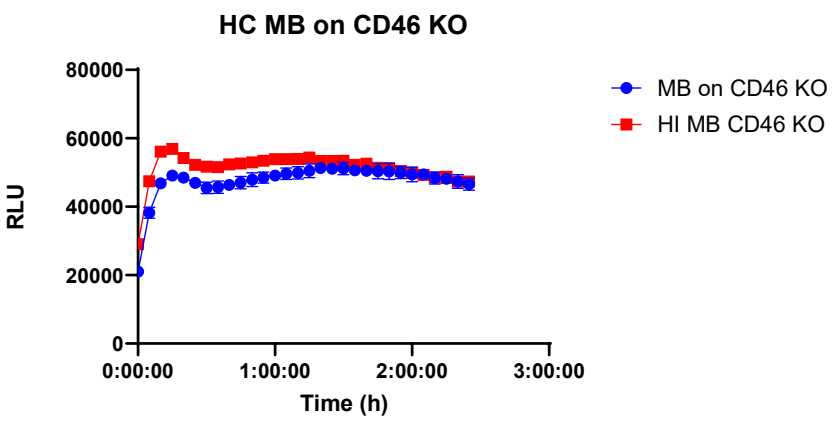
Cells keep glowing (exact effect depending upon serum and regulators present)

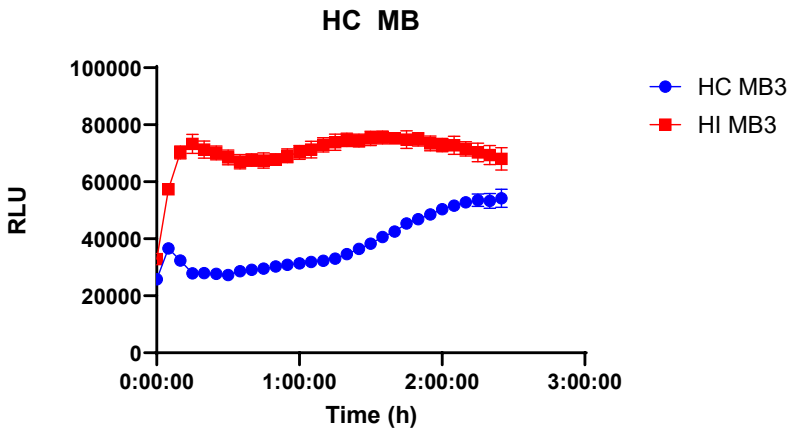
Pathologic complement activation

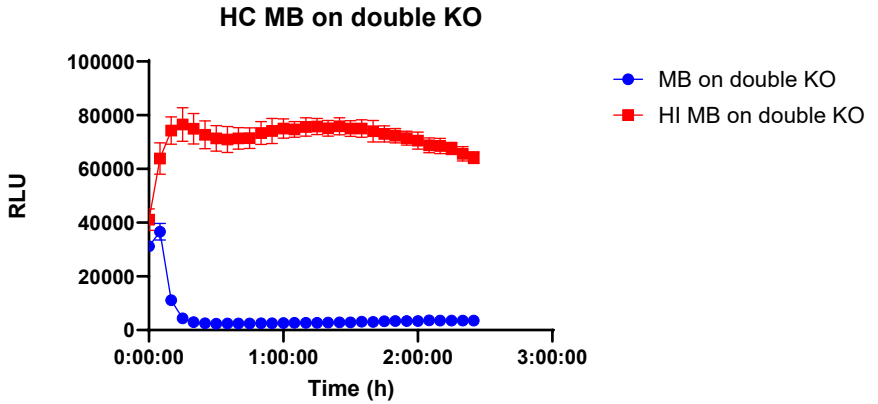


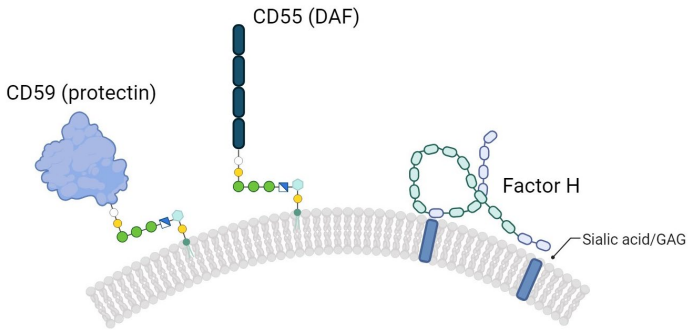
Metabolic effects of complement and cell death decrease light output

Healthy control Serum in biomHam

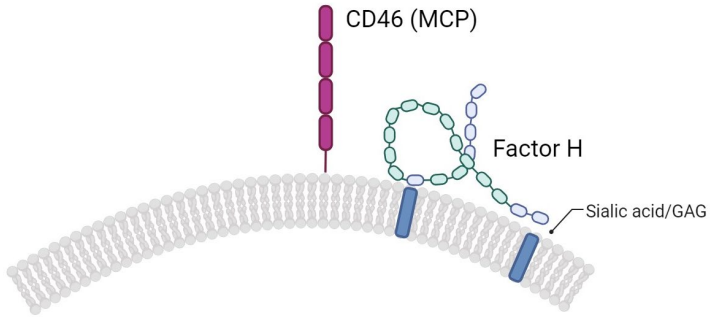




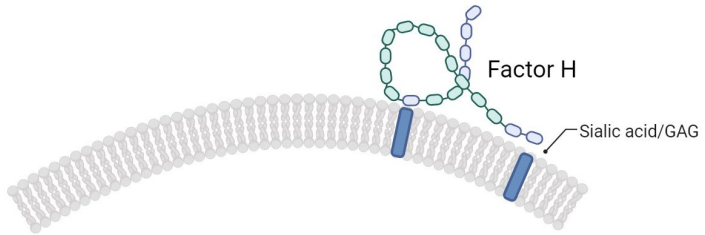




CD46 KO



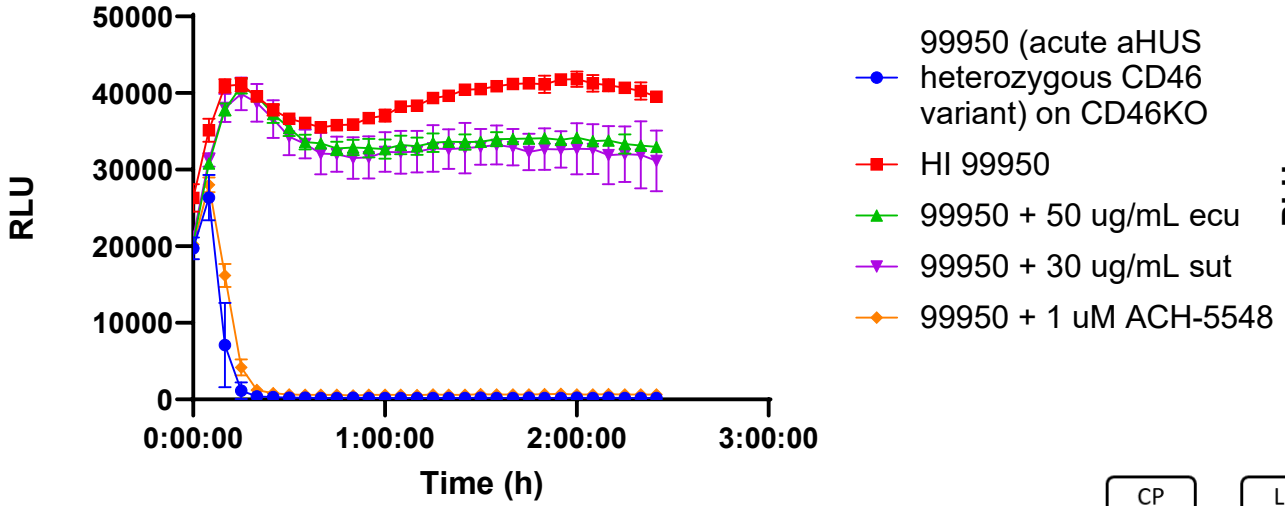
PIGA KO



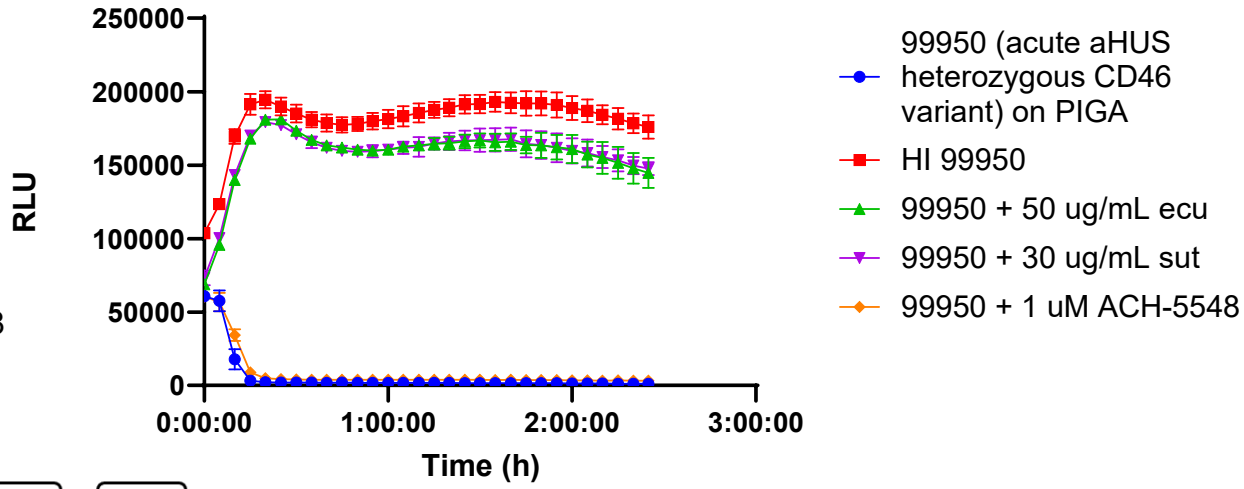
Double KO

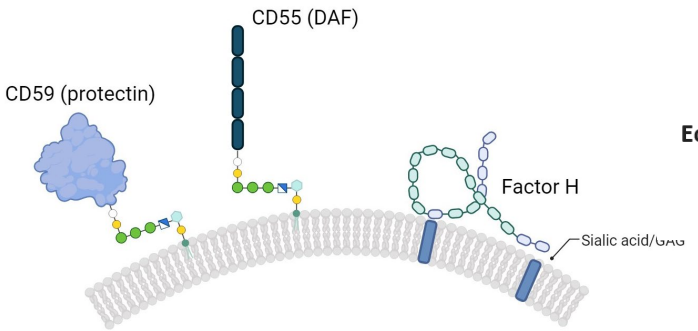
aHUS cell kill is rescued by heat inactivation, C5 and C1s inhibition, but not AP pathway inhibition

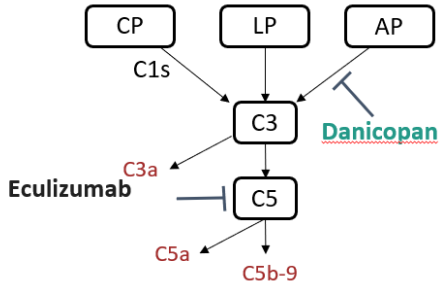
99950 (acute aHUS heterozygous CD46 variant) on CD46KO

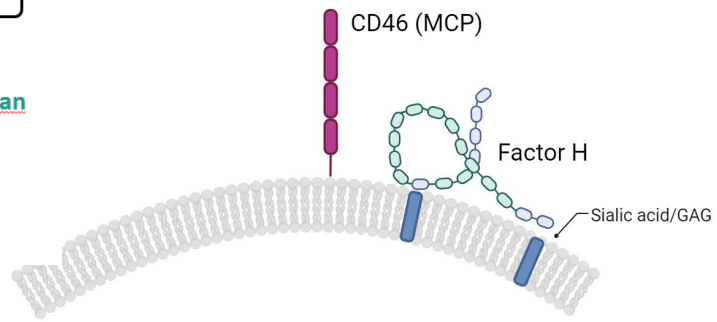


99950 (acute aHUS heterozygous CD46 variant) on PIGA





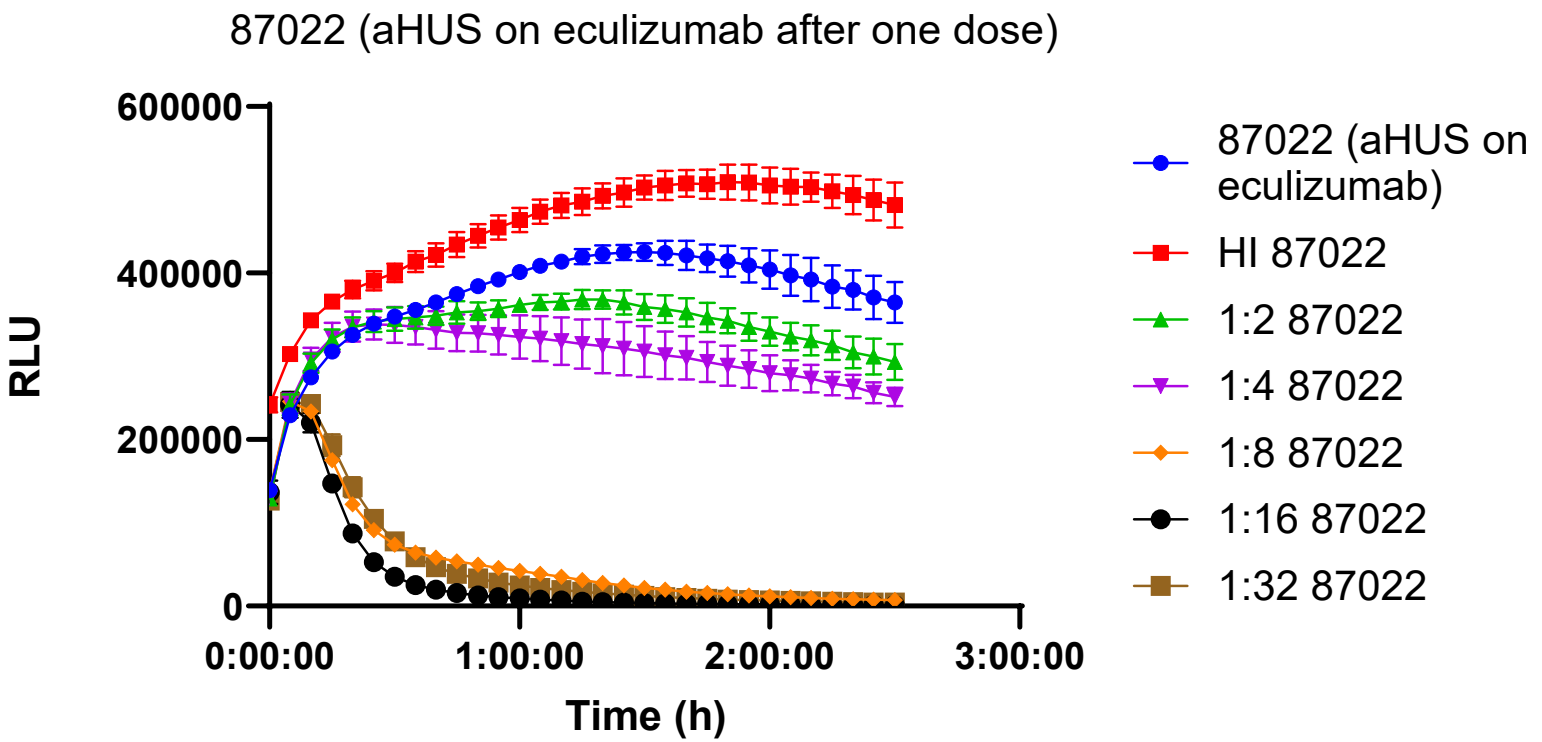




CD46 KO

PIGA KO

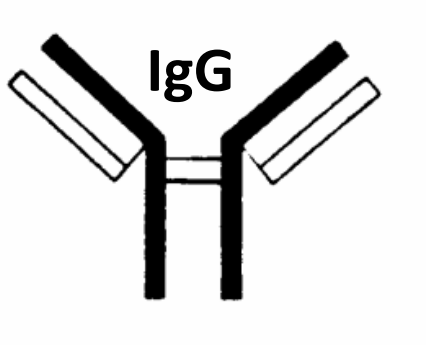
BiomHam can confirm aHUS Dx after initiation of C5i



Dilutions on aHUS patient 7 days after 1st dose of eculizumab

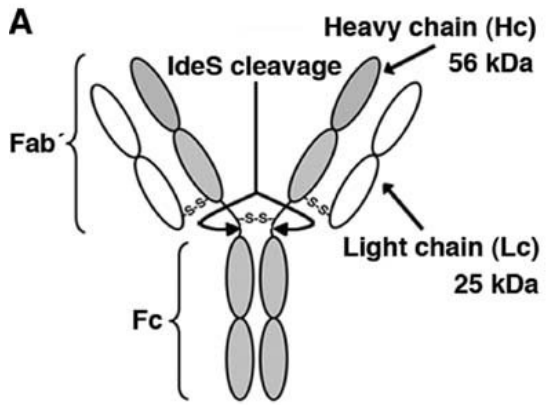
How is the Classical Pathway Activated in aHUS?

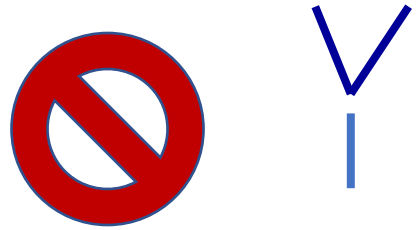
IdeS cleaves IgG; DTT cleaves IgM

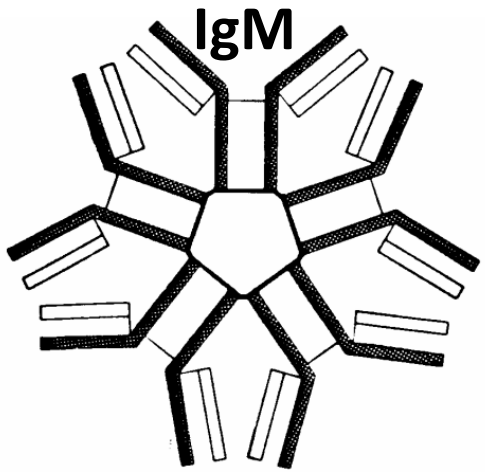


IdeS

Cleaves IgG into Fab + Fc

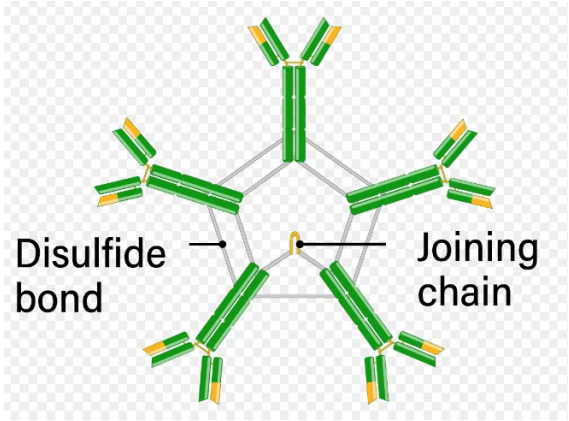


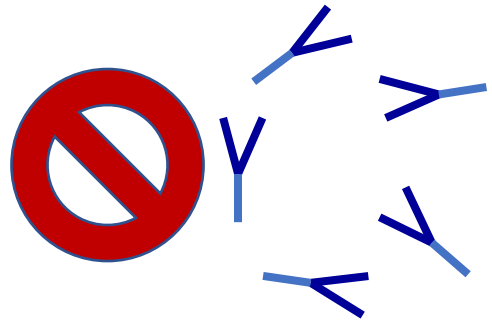




DTT

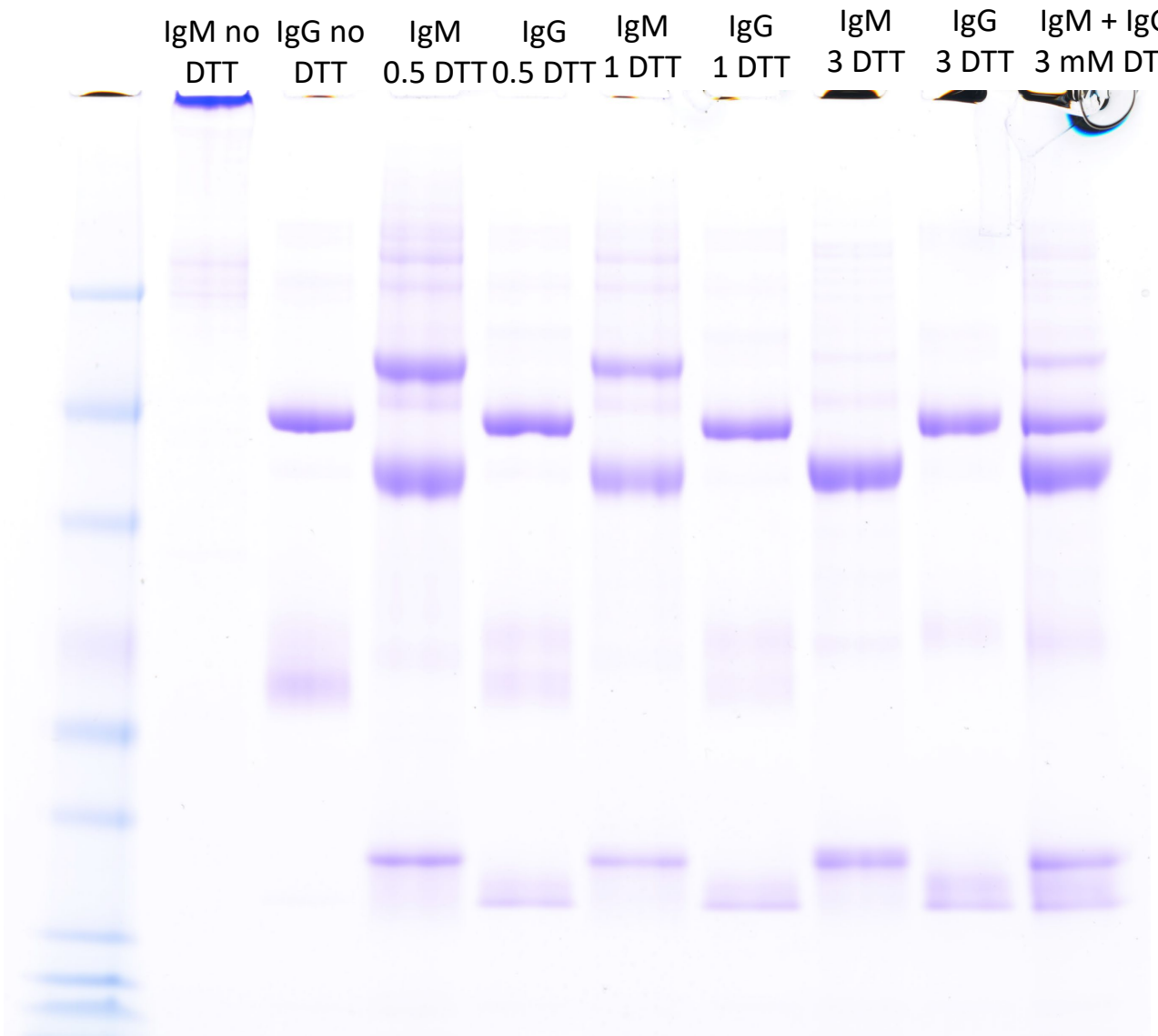
Reduces disulfide bonds



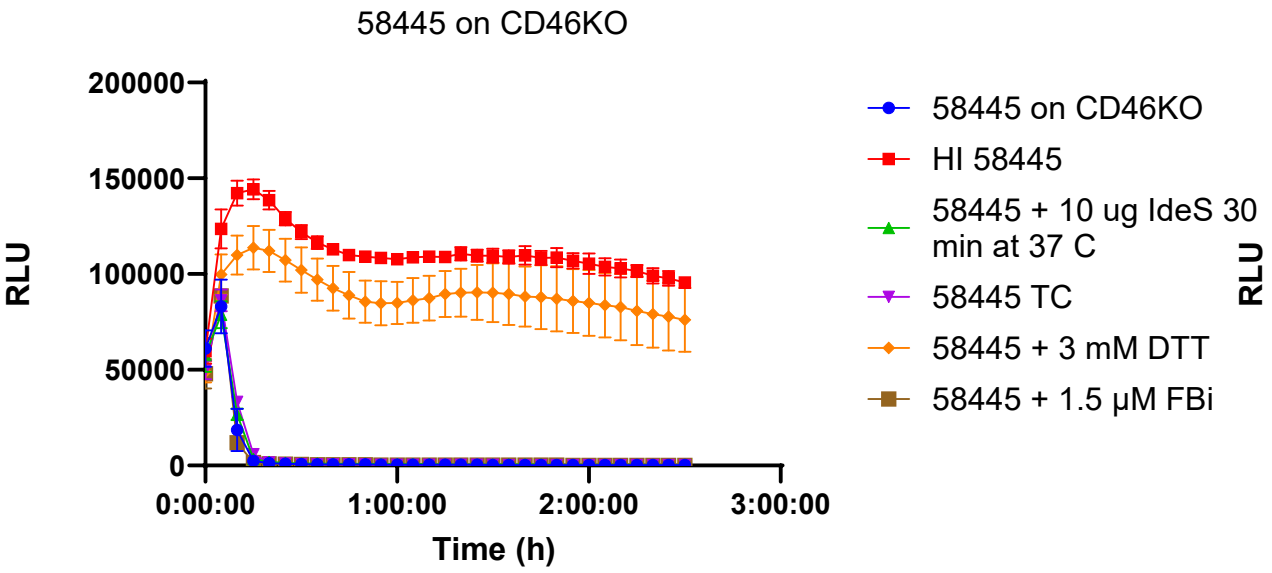


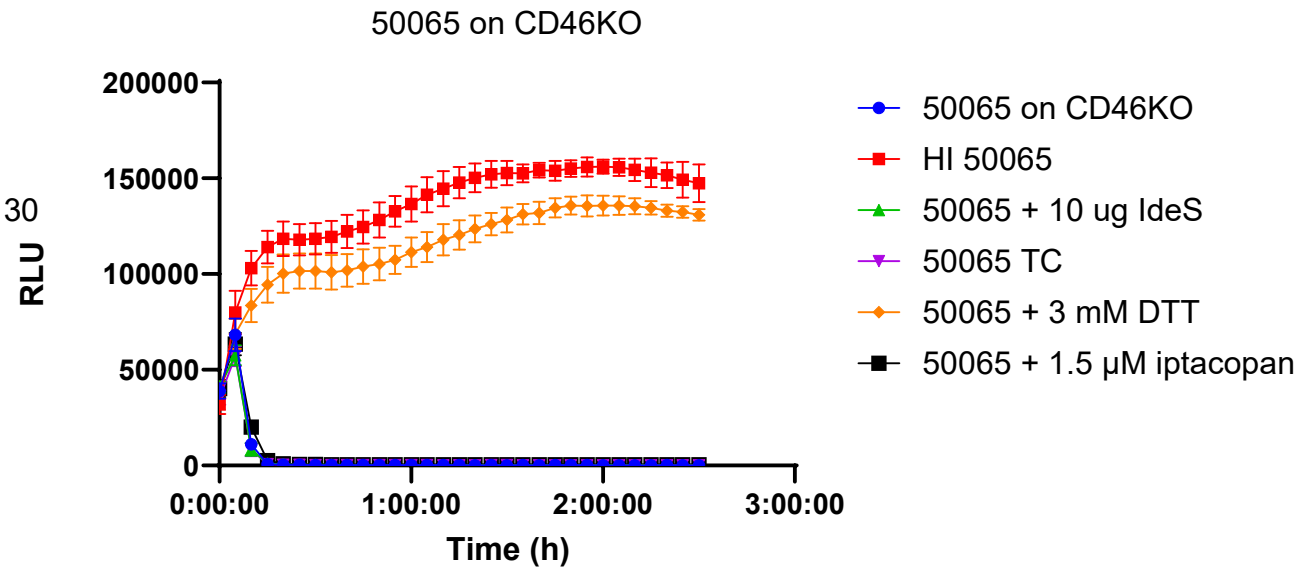
Non-reducing (no boil) SDS-PAGE following DTT treatment 30 minutes at room temperature

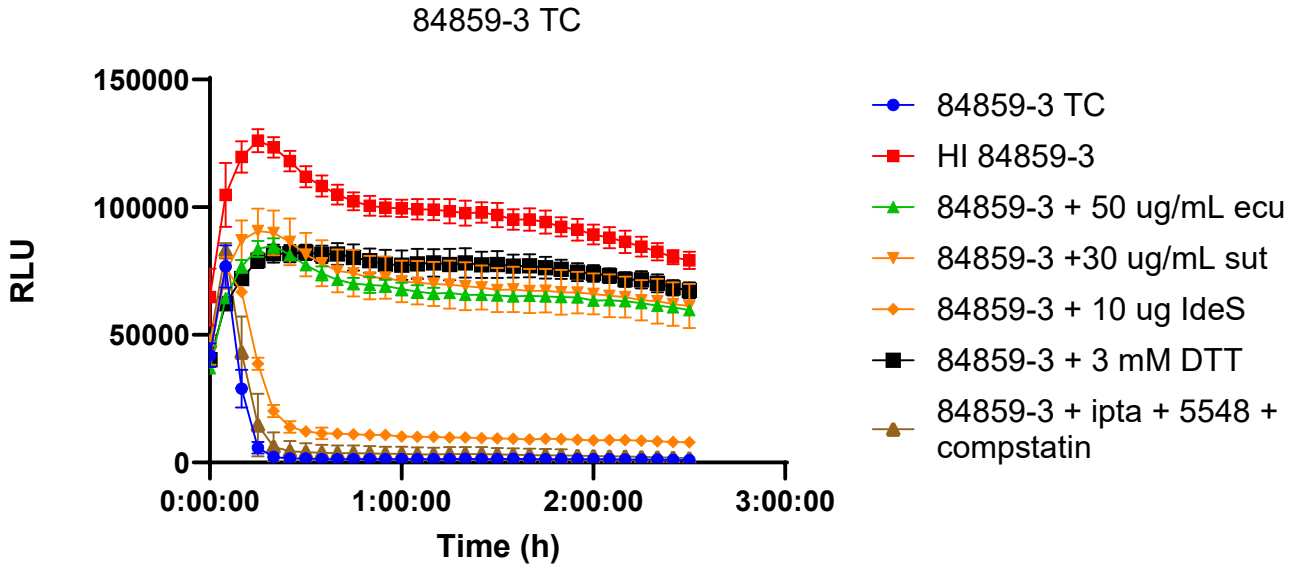
DTT reduces IgM without cleaving IgG



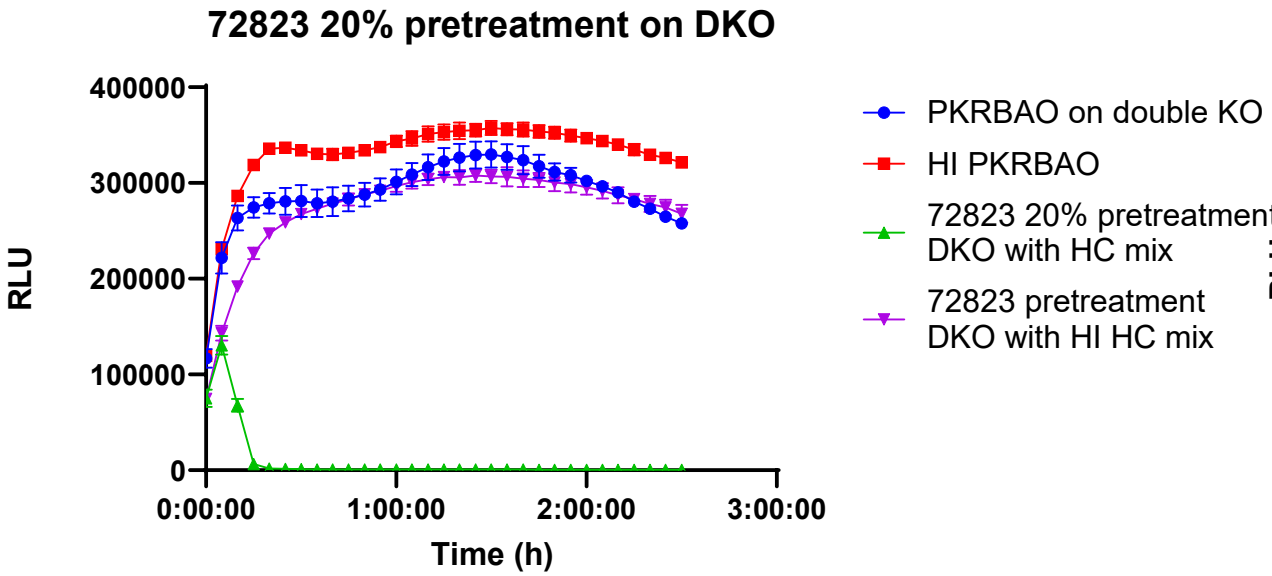
aHUS is a Classical Pathway Disease secondary to polyclonal IgM

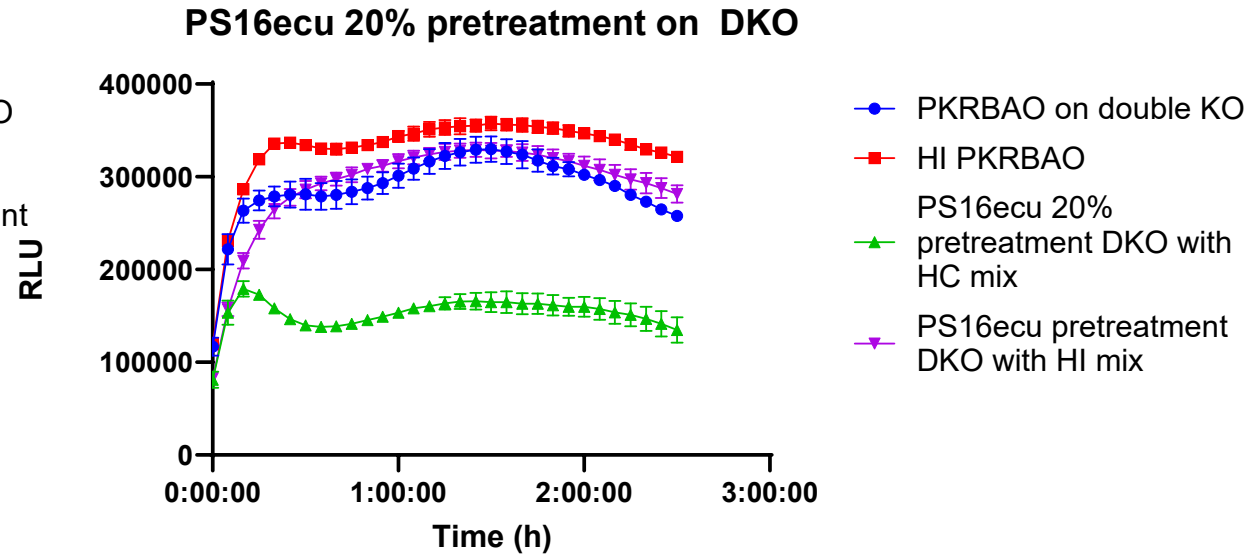






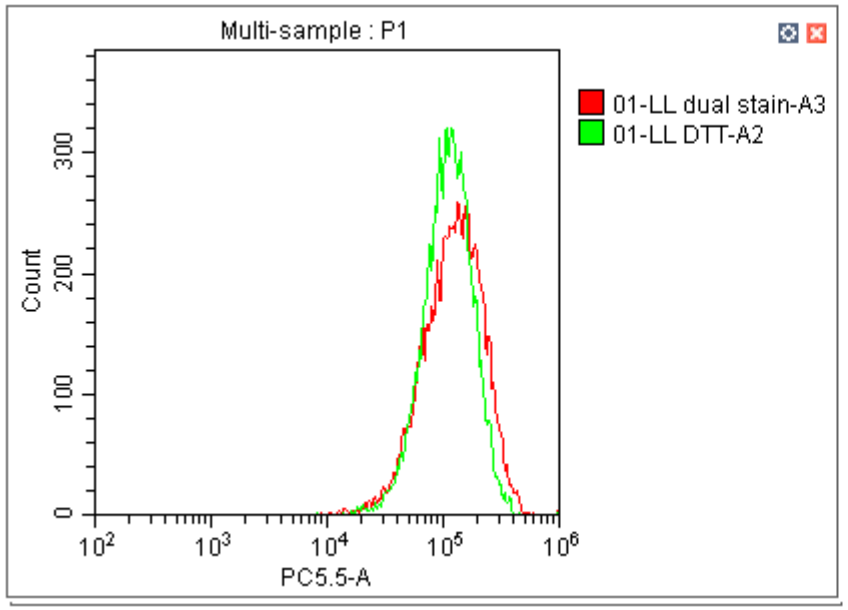
aHUS IgM Sensitizes our Biosensors to Complement-Dependent Killing from Healthy Control Serum



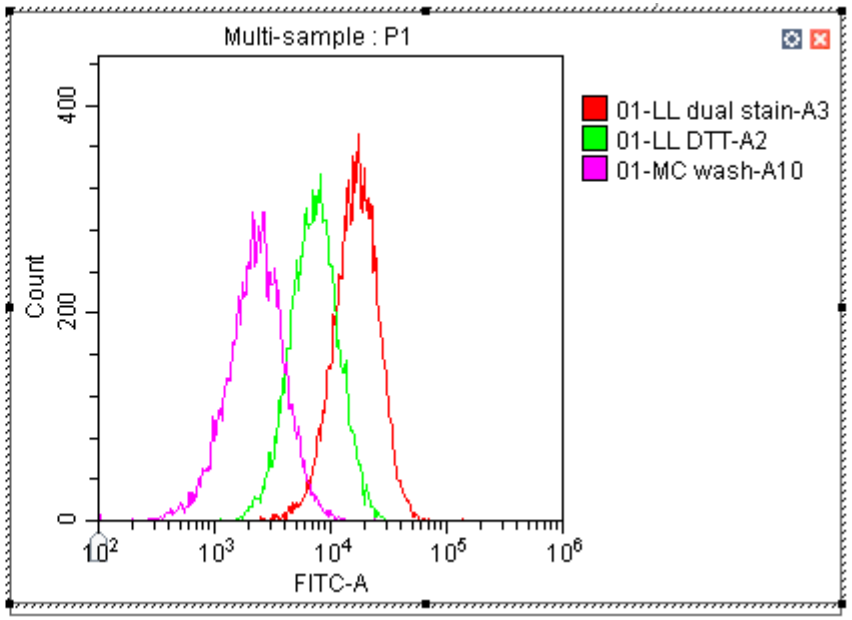


- Heat inactivate aHUS serum and incubate with cells
- Wash and replace with pooled normal serum or heat-inactivated normal serum.

aHUS serum → increased IgM on cells

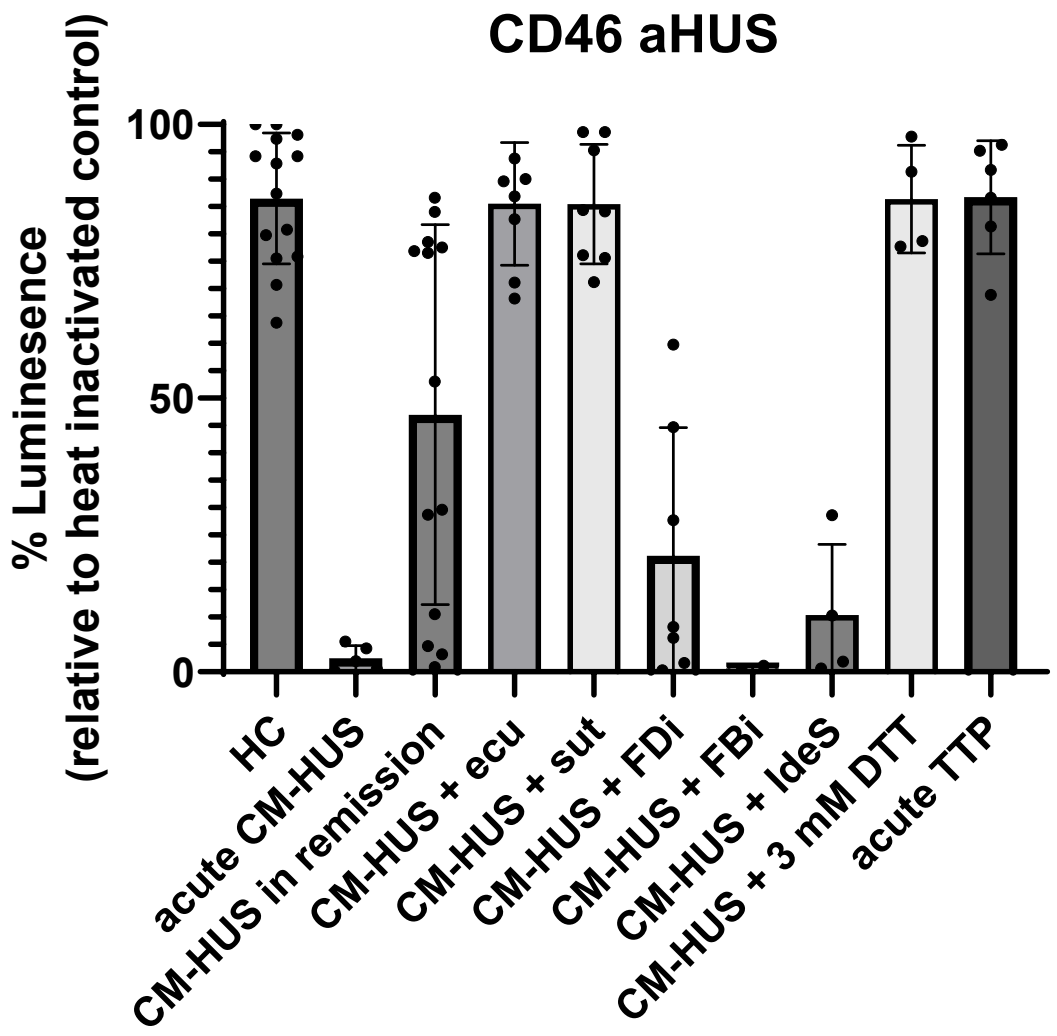


IgG



IgM

CM-HUS is now a Dx of Inclusion



Bioluminescent mHam can:

- Differentiate TTP from CM-HUS
- Dx CM-HUS even in pts on treatment
- Monitor levels of complement inhibitors
- Will become commercially available
- May inform therapy as to when to stop complement inhibition

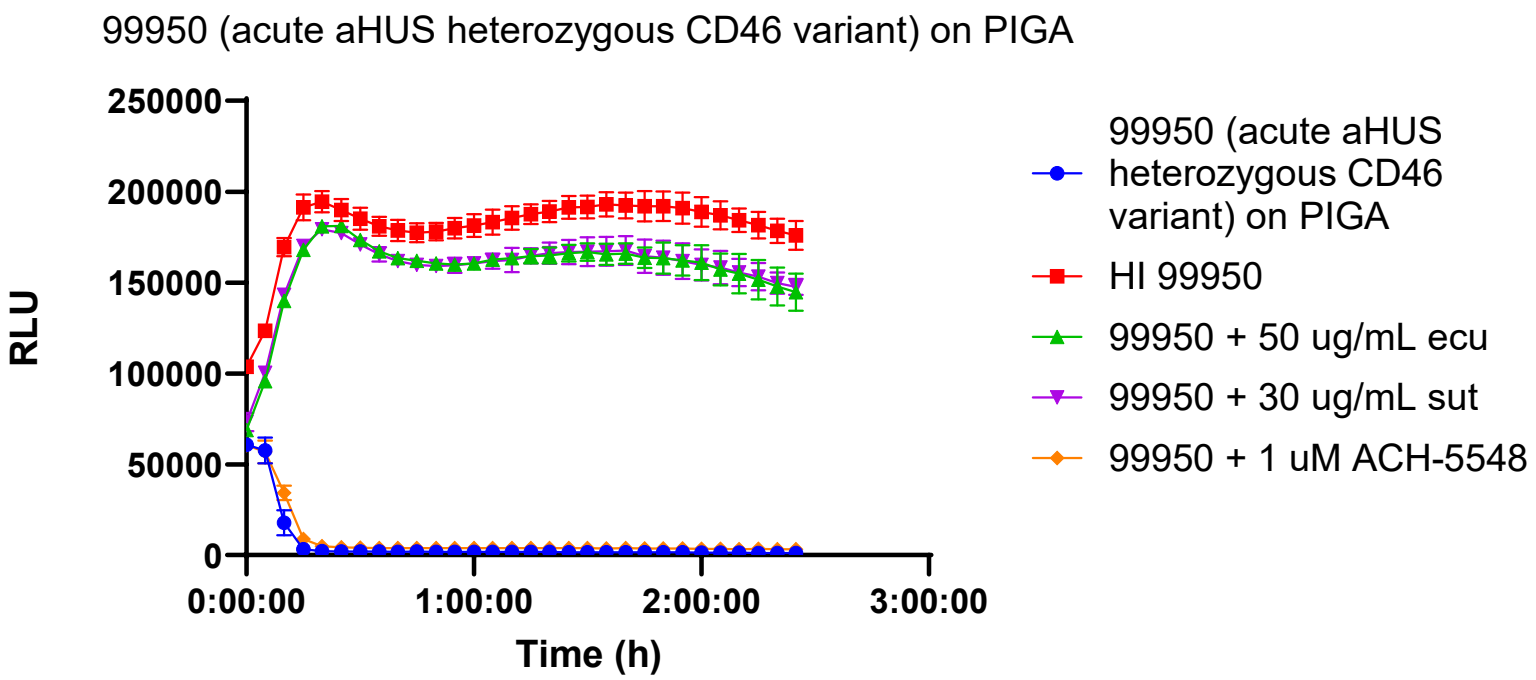
24 yo with aHUS (CD46 heterozygous) triggered by Covid-19

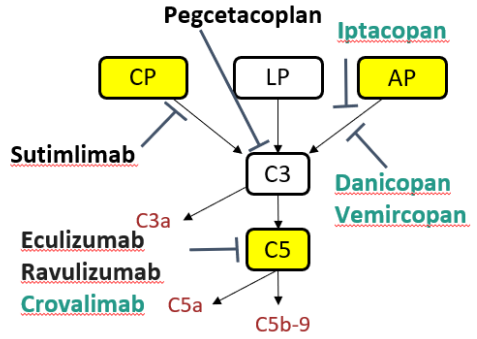
- 2022: MAHA with 8K plts, LDH >2000, Cr4.4, Retic 8%, ADAMTS13 normal
  - No family hx of TMA

- Presents 8/2023
  - Hgb12.3, plts248K, Cr 0.9, LDH 161, Retic 1.2
  - CRP 8.9

- Presents with fiancé re: future pregnancy risk

24 yo CM-HUS with heterozygous mutation in CD46 (in remission OFF all drugs)





Paroxysmal Nocturnal Hemoglobinuria

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

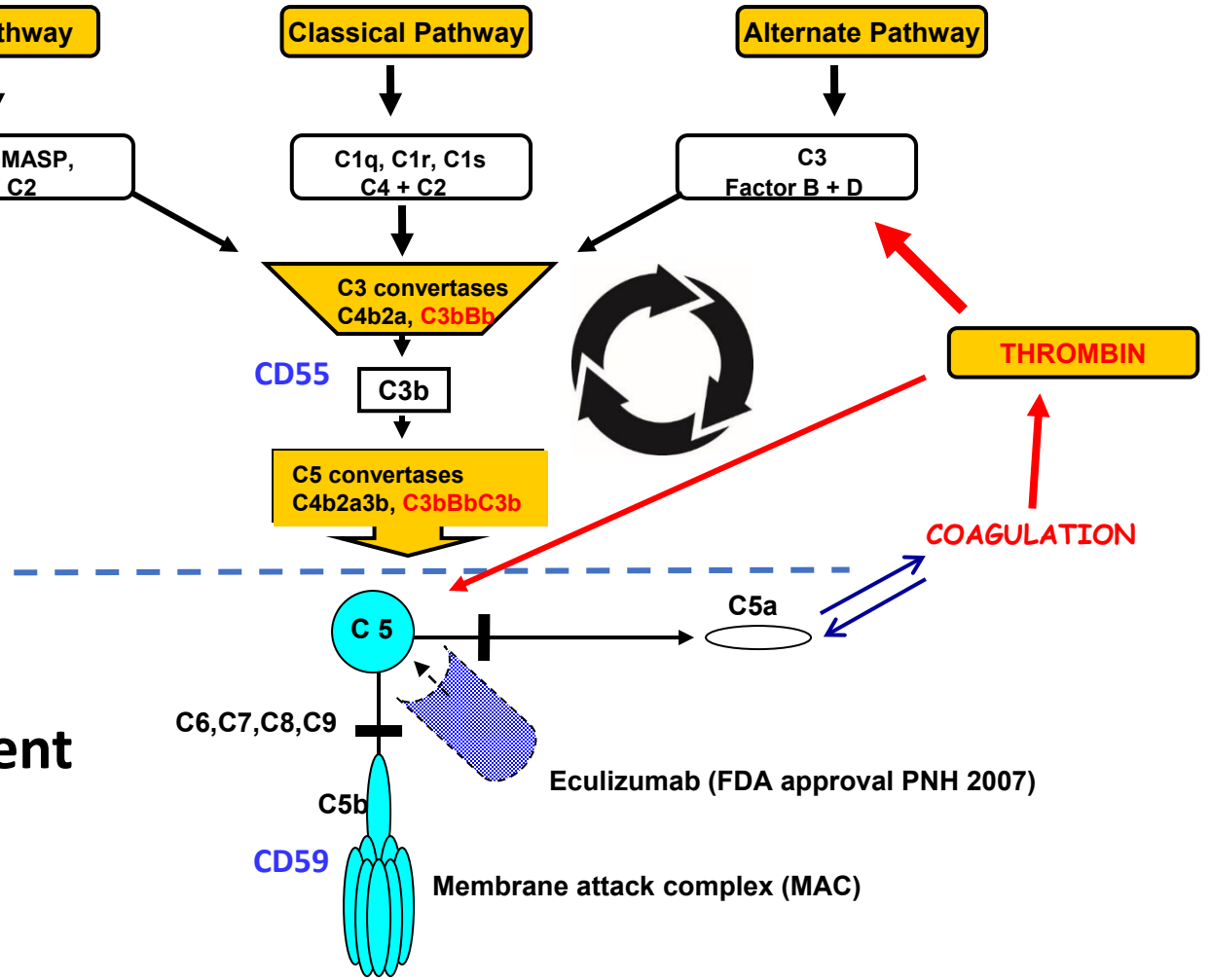
PNH

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/C5 convertase
- Protect cells from complement-mediated destruction

Eculizumab: First FDA approved Drug for PNH



Terminal complement

Ravulizumab

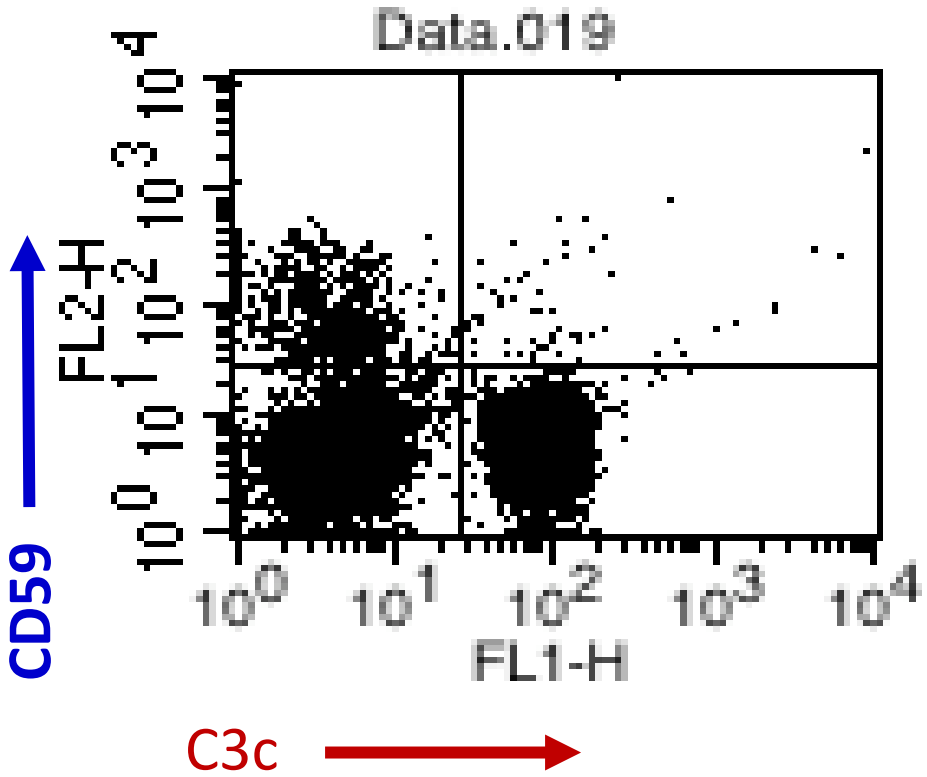
- Second FDA approved drug (2018) for PNH (targets C5)
- non inferior to eculizumab
- 4 amino acid difference explains the 4x increase in half-life
- After loading, dosed at q 8 weeks intavenously.

Kulasekararaj et al, Blood 2019;133:540-549
Lee et al, Blood 2019;133:530-539

Two Mechansims for Breakthrough Hemolysis

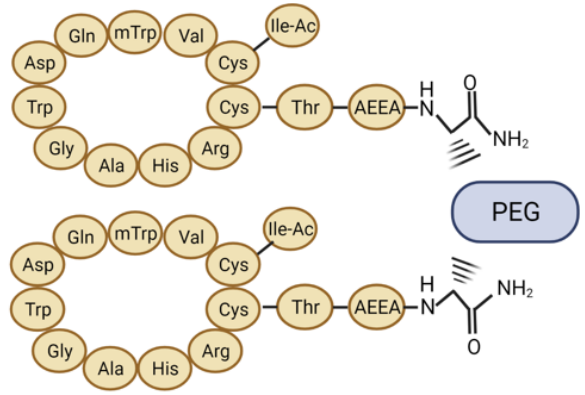
- Definition:
  - Return of intravascular hemolysis (hemoglobinuria, increased LDH,) and reappearance of classical PNH symptoms

- Causes:
  - Suboptimal C5 inhibition (**pharmacokinetic** breakthrough)
  - Complement amplifying conditions (**pharmacodynamic** breakthrough)
    - Infection
    - Pregnancy
    - Surgery

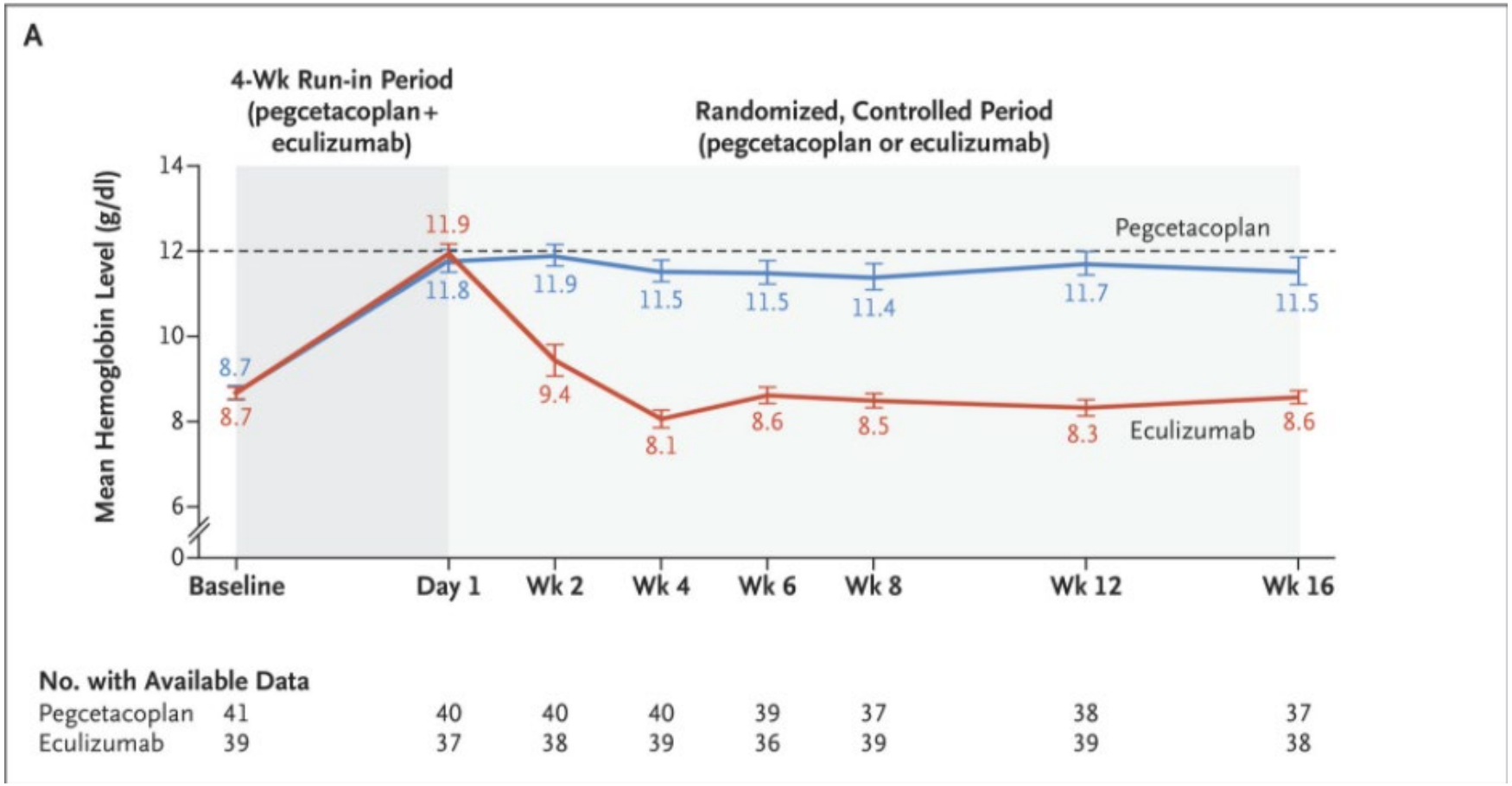


PEGCETACOPLAN

- What is it?
  - Synthetic cyclic peptide conjugated to PEG that binds to C3 and C3b to inhibit C3 cleavage and activation. Dosed to only block APC.
  - Blocks upstream to CD55 and CD59
  - Blocks intra and extravascular hemolysis
- Administration?
  - Subcutaneous infusion 1080mg twice a week



Peg vs Ecu: hgb Response



- Mean hgb 11.5 (PEG) vs 8.6 (ecu)
- 61% increased >2g/dL (PEG) vs 0% ecu
- 85% (PEG) avoided transfusion vs 15% (ecu)

Hillmen et al, NEJM 2021; 384:1028-37

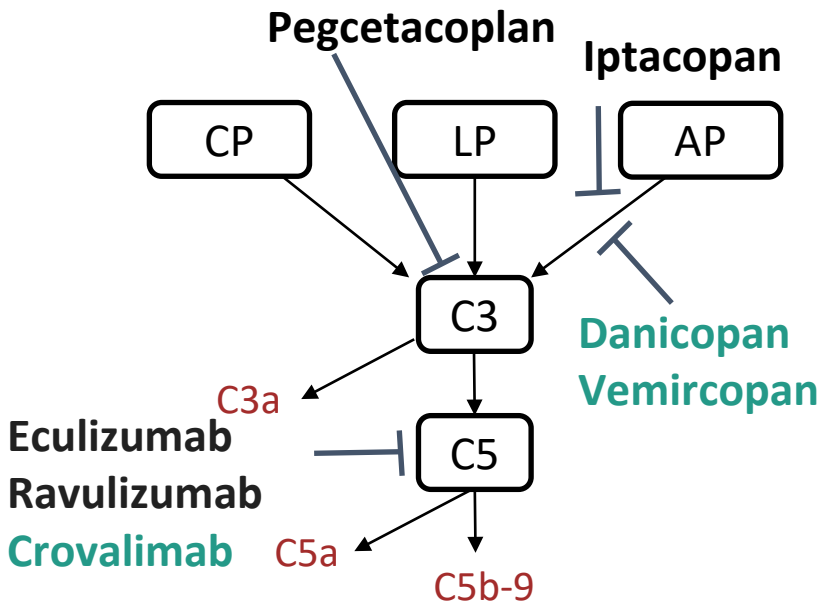
Adverse events and breakthrough hemolysis

Table S4. Patients Experiencing an AE of Hemolysis.

Patient Identification	Age, years	Sex	BMI, kg/m²	Baseline Hb, g/dl	Baseline LDH, U/l	Study Days (D) of BTH	Hb During BTH, g/dl	LDH During BTH, U/l	Discontinued From Study Due to BTH
Pegcetacoplan group									
01*	28	M	38.9	7.4	249.5	D42–47 D47–53	10.9–6.4	D44: 1539–2481‡	Yes
02	71	F	21.7	8.6	158	D49–56	8.5‡–7.2	1100‡–813	Yes
03	63	F	22.4	6.0	316.5	D36–39	7.2‡–4.8	4147‡	Yes
04	40	F	28.1	10.3	258	D106–140	6-8.3‡	3015‡–2423‡	No
Eculizumab group									
05†	66	M	29.8	9.3	193.5	D43–84 D85–126	9.1 9.8	211 153	No
06	33	F	22	9.6	191.5	D9–16	D16: 6.5	D16: 311	No
07	34	F	24.7	9.5	151.5	D29–43	6.2	266	No
08	35	M	26.9	9.2	194.5	D64–82	D70: 6.4	D84: 205	No
09	34	F	20.0	9.9	208.5	D15–43	10.9–9	379	No
10†	46	M	22.8	8.4	223	D23 D82	D28: 6.6 D83: 7.4	D28: 255 D83: 255	No
11†	53	F	33.5	8.6	226.5	D21–38 D112–126	6.1 7.8	D29: 230 D112: 199	No
12	28	M	31.5	8.8	1078.5	D15–ongoing	10.9–8.6	253–2716	No

New Drugs

- Crovalimab
  - Target: C5
  - monthly subcutaneous infusion



- Danicopan
  - Target Factor D
  - By mouth three times daily
- Vemircopan
  - Target Factor D (bid oral)
- Iptacopan
  - Target Factor B
  - By mouth twice daily

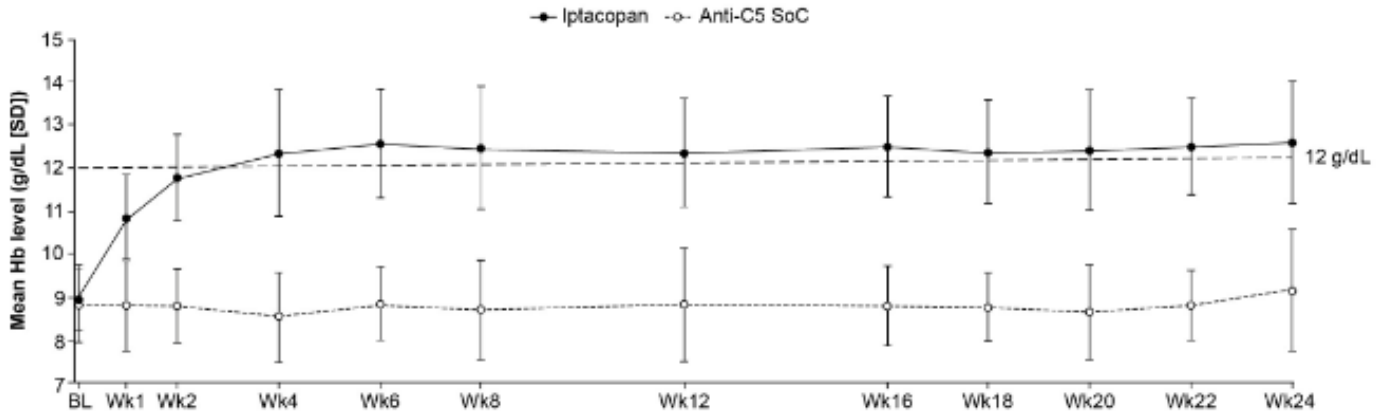
IPTACOPAN

Oral Factor B inhibitor (BID drug)

97 patients randomized Phase3
62 Ipta vs 35 SOC
Eligible pts had to have hgb <10 despite ecu or ravu

ASH LBA 2022

Figure: Mean Hb (SD) over time during the 24-week randomized treatment period of APPLY-PNH



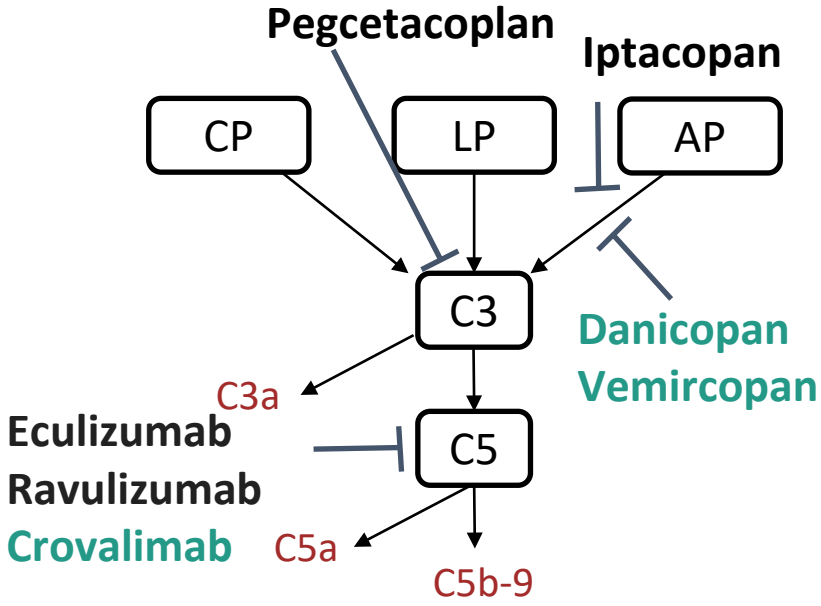
Patients with available data												
Iptacopan	62	61	59	60	59	59	60	59	59	59	57	61
Anti-C5 SoC	35	29	27	32	32	31	33	28	34	32	32	34

Drug	Mechanism	Administration	Status
Eculizumab (Soliris)	C5 inhibitor (antibody)	IV q 2 weeks	Approved 2007
Ravulizumab (Ultomiris)	C5 inhibitor (antibody)	IV q 8 weeks	Approved 2018
Pegcetacoplan (Empevali)	C3 inhibitor (pegylated peptide)	SQ Infusion (20ml) twice weekly	Approved 2021
Iptacopan	Factor B inhibitor Small molecule	Po bid	Approved 2023

PNH: My Approach as of February 2024

- **Ravulizumab weight based dosing**
  - Non-inferior to eculizumab
  - Every 8 week intravenous dosing

- **Target: C5**
  - Stops thrombosis – leading cause of death
  - >80% transfusion independent
  - Most patients have continued anemia due to EVH
  - Controls LDH
  - Extensive experience in large number of patients



- **Pegcetacoplan (Target C3) or Iptacopan (target fB)**
  - Second line agents (work up for other causes of anemia!)
  - Pt should have high retic, low LDH (< 1.5 ULN), no underlying BMF, low CH50
  - Transfusion dependent or anemic and moderately to severely symptomatic
  - Need longer follow-up re: thrombosis and safety