PNH

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PNH: A Historical Perspective

- 1882: Strübing : First reported case.
- 1928: Enneking : "paroxysmal nocturnal hemoglobinuria".
- 1938: Ham : PNH RBCs lysis in an acidified serum by an antibody-independent complement-like factors.
- 1954: Pillemer: The alternative complement pathway.
- 1961: Dacie: PNH cells in BM failure /Link to somatic mutation : "abnormal cells must have as yet, not understood biological advantage".

Parker, PNH: A historical Review. Blood 2008. 93-103

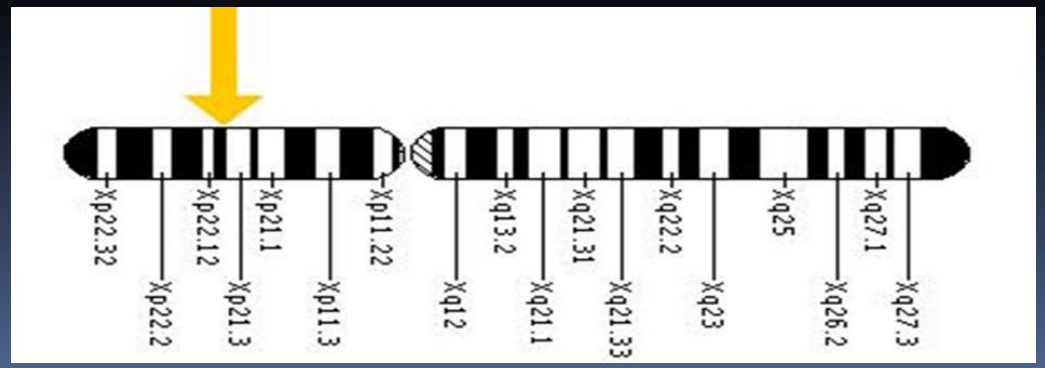
PNH: A Historical Perspective

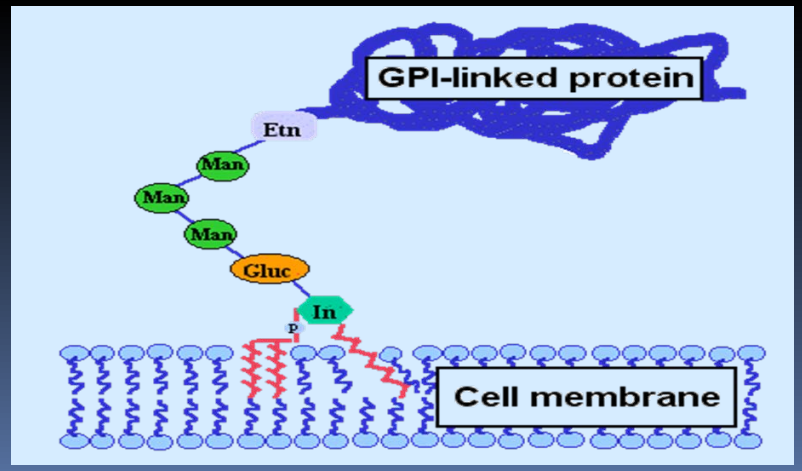
- 1966: Rosse: PNH RBC's were 25 times more sensitive to lysis by complement than normal RBC's . C_3 binding with complement activation.
- 1970: Luzzatto: Clonal nature of PNH / G6PD iso-typing.
- 1983: Weller: DAF (CD-55) deficiency in PNH cells .
- 1989: Parker: MIRL (CD-59) which inhibited reactive lysis of PNH cells.
- 1993: Kinoshita: Loss of GPI anchor is the result of a somatic mutation in *PIG-A* gene.

Parker, PNH: A historical Review. Blood 2008. 93-103

The Etiology of PNH:

- An acquired / somatic mutation in the X-linked *PIG-A* gene in hematopoietic stem cells.
- Inability to synthesize/display the GPI- anchor on cell surface.
- Loss of GPI-anchored complement-shielding CD-55/CD-59
- One mutation is required in either males or females to cause disease.





PNH : Epidemiology

- A rare disorder.
- Incidence: 1-10 cases per million.
- Median age of onset in the 30's.
- Can affect the elderly and rarely children.
- No ethnic or geographic distribution.

[1]Socie, et al. *Lancet*. 1996;348:573-577.
2 Schrezenmeier H, Haematologica. 2014 May;99(5):922-9.
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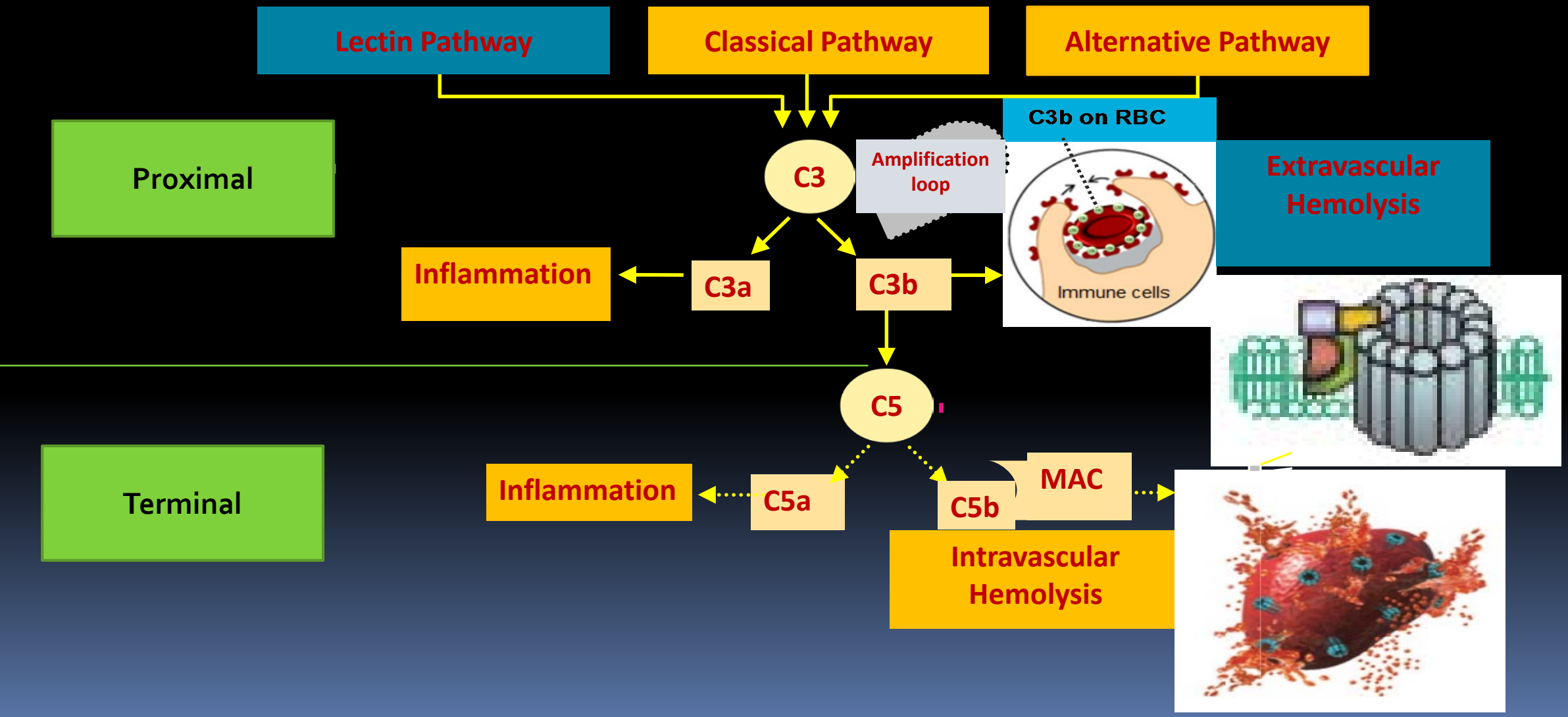
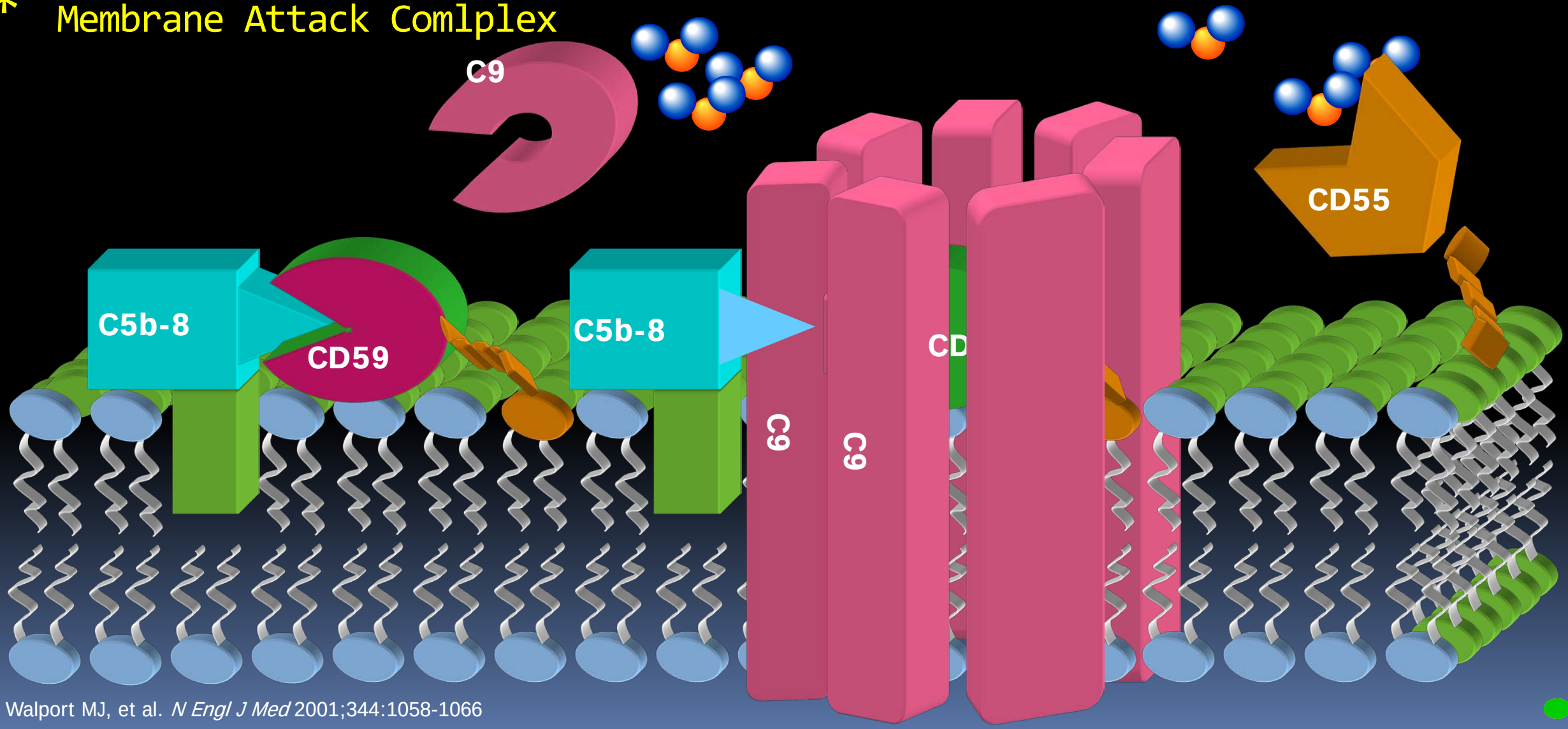
The Complement System



CD59 Deficiency and MAC* Formation

* Membrane Attack Comlplex

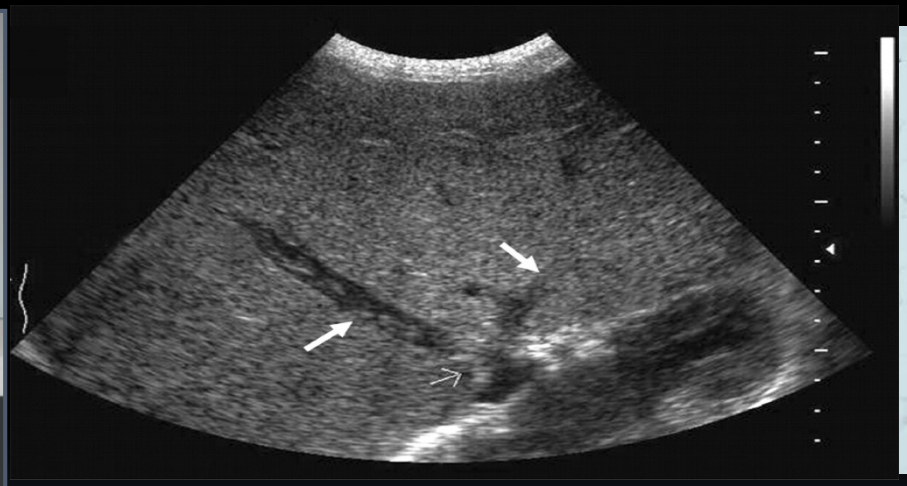


Walport MJ, et al. *N Engl J Med* 2001;344:1058-1066

The Clinical Triad of PNH

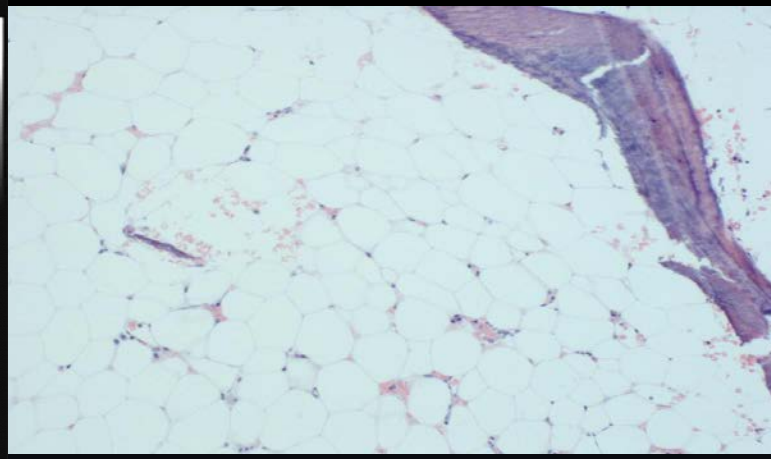
Hemolysis/Hemoglobinuria

Thrombosis



Budd-Chiari Syndrome

Bone Marrow Failure*



Clinical Manifestations of PNH

- Intravascular Hemolysis
  - Anemia and fatigue
  - Hemoglobinuria
  - Renal failure
  - Esophageal spasms
  - Abdominal pain
  - Headache
  - Erectile dysfunction

- Thrombotic Events
  - Venous thromboembolism DVT/PE
  - Intra-abdominal vein thrombosis
    - Budd-Chiari
    - Splenic/mesenteric/renal vein thrombosis
  - Cerebral vein thrombosis
  - Retinal and dermal vein thromboses
  - Arterial thromboses: MI/Strokes

Bone Marrow Failure

Pancytopenia

Propensity for infection

bleeding

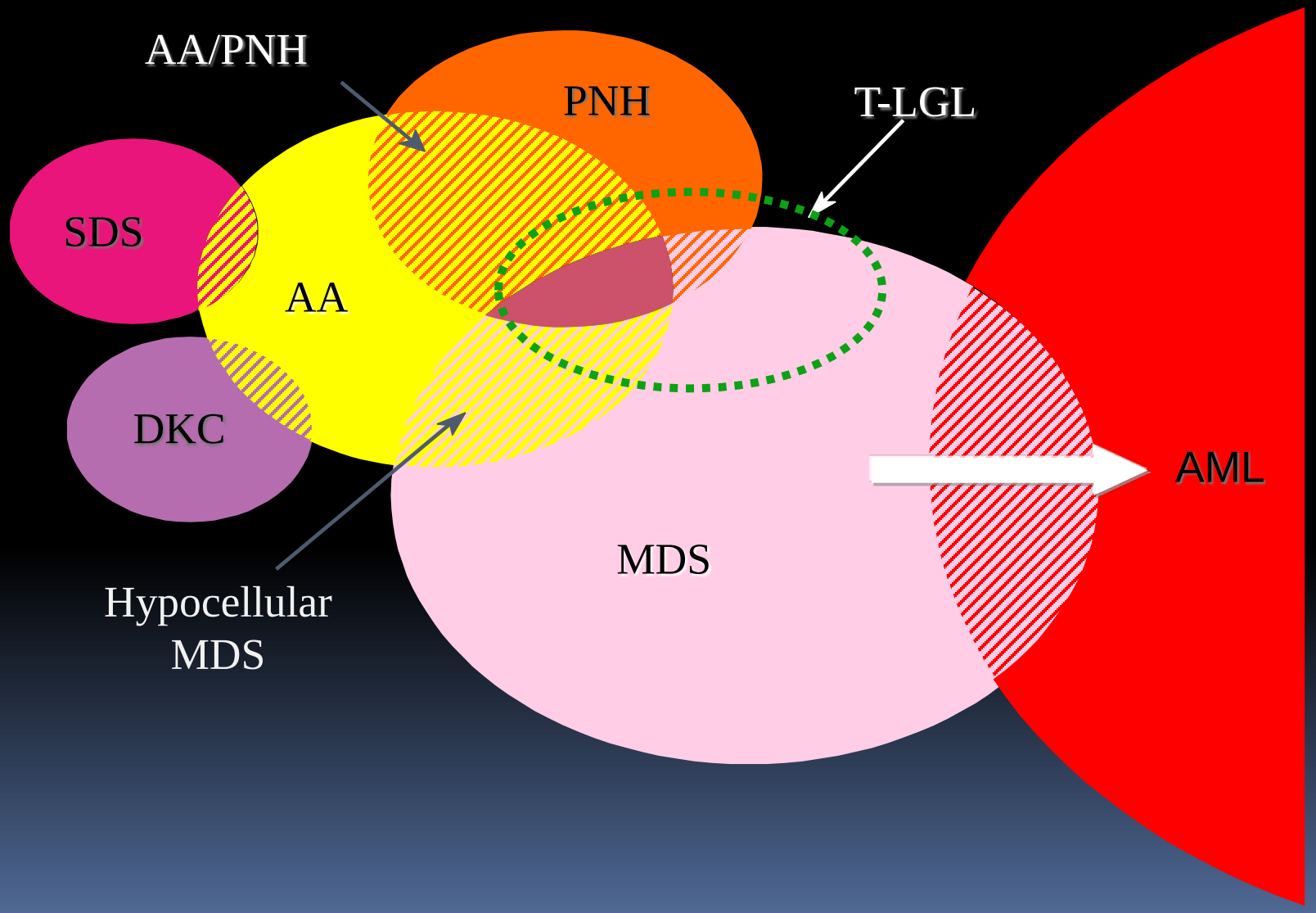
Classification of PNH

Classic	Florid (macroscopic hemoglobinuria is frequent or persistent)	Cellular marrow with erythroid hyperplasia and normal or near-normal morphology
PNH in the setting of another marrow failure syndrome	Mild to moderate (macroscopic hemoglobinuria is intermittent or absent)	Evidence of a concomitant marrow failure syndrome
Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant marrow failure syndrome

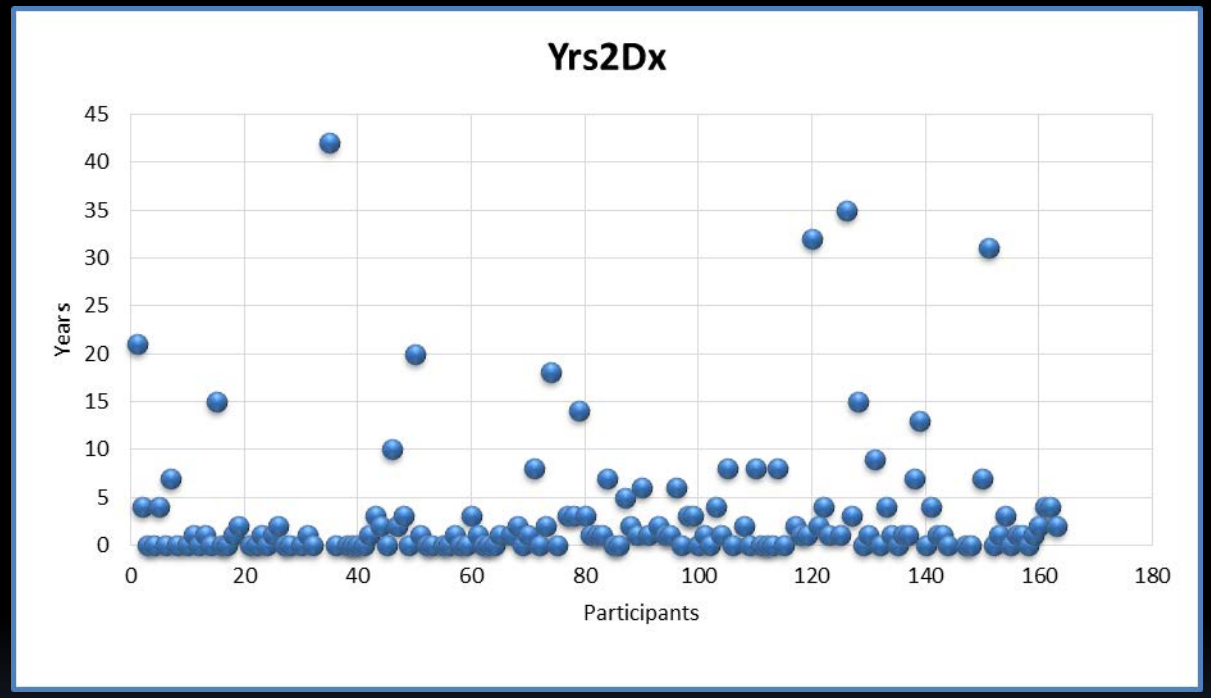
International PNH Interest Group (*Blood* 106:3699, 2005)

PNH: An Approach To Diagnosis

Bone Marrow Failure Syndromes



Time to Diagnosis of PNH



Average time to diagnosis from onset of symptoms <2 years

Shammo et al. SM Clin Med Oncol. 2017; 1(1): 1001-1005

Providers Consulted Prior to PNH Diagnosis

PROVIDER	n	1st	2nd	3rd	4th	5th	Never
Cardiologist	114	4 (4%)	3 (3%)	4 (4%)	3 (3%)	5 (5%)	95 (83%)
Emergency	124	24 (19%)	27 (22%)	3 (2%)	3 (2%)	5 (2%)	52 (42%)
Hematologist	150	19 (13%)	50 (33%)	42 (28%)	17 (11%)	12 (8%)	10 (6%)
Nephrologist	102	2 (2%)	7 (7%)	7 (7%)	7 (7%)	3 (3%)	76 (74%)
Neurologist	99	0 (0%)	4 (4%)	4 (4%)	2 (2%)	5 (5%)	84 (85%
OB/GYN	82	11 (13%)	10 (12%)	5 (6%)	1 (1%)	1 (1%)	53 (66%)
PCP	148	90 (61%)	18 (12%)	4 (3%)	4 (3%)	5 (4%)	19 (12.8%)
Mental Health	94	2 (2%)	1 (1%)	1 (1%)	2 (2%)	4 (4%)	84 (89%)
Pulmonologist	99	2 (2%)	1 (1%)	0 (0%)	3 (3%)	2 (2%)	91 (92%)
Urologist	107	5 (5%)	8 (8%)	6 (6%)	2 (2%)	3 (3%)	83 (77%)
Other	65	7 (11%)	10 (15%)	8 (12%)	4 (6%)	5 (7%)	30 (46%)

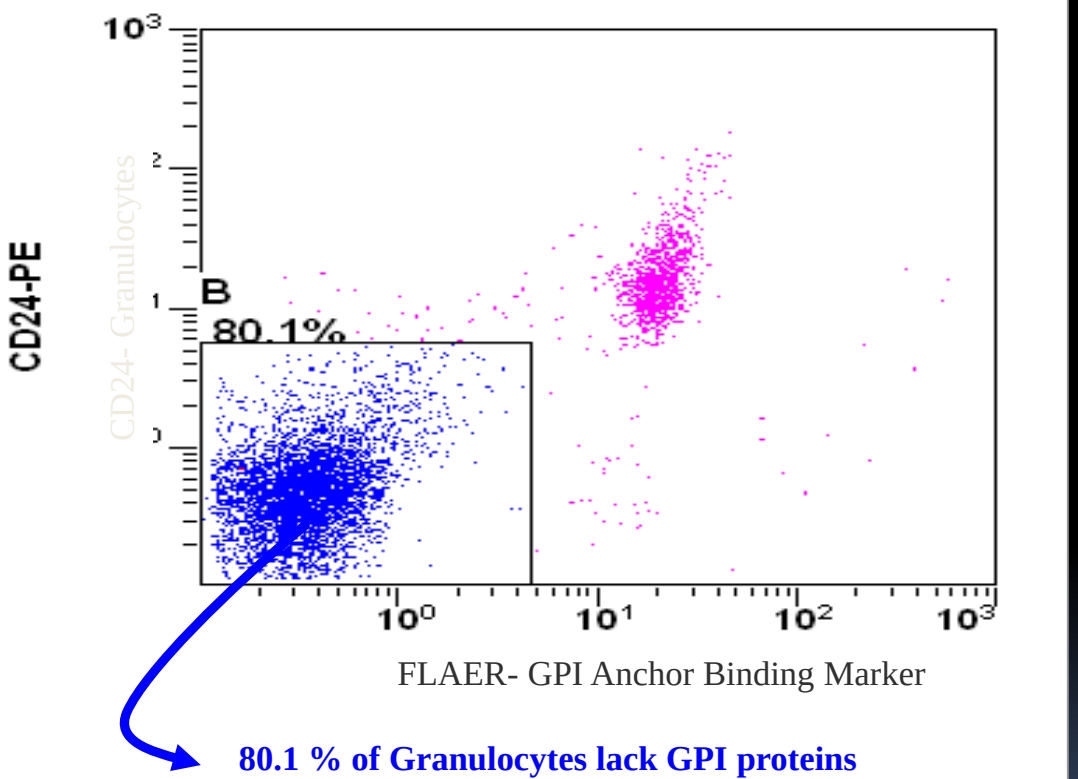
Shammo et al. SM Clin Med Oncol. 2017; 1(1): 1001-1005

Diagnostic Testing for PNH

- Flow cytometry of peripheral blood:
- Gold standard test for diagnosis of PNH
  - Stains with monoclonal antibodies for CD55 and CD59
  - FLAER (fluorescein-tagged pro-aerolysin variant) that binds a portion of GPI anchor
- More than one cell lineage should be evaluated
  - Granulocytes
  - Monocytes
  - Red Blood Cells (RBC)
- Sensitivity Level ~ 0.01%

Flow Cytometric Analysis in PNH

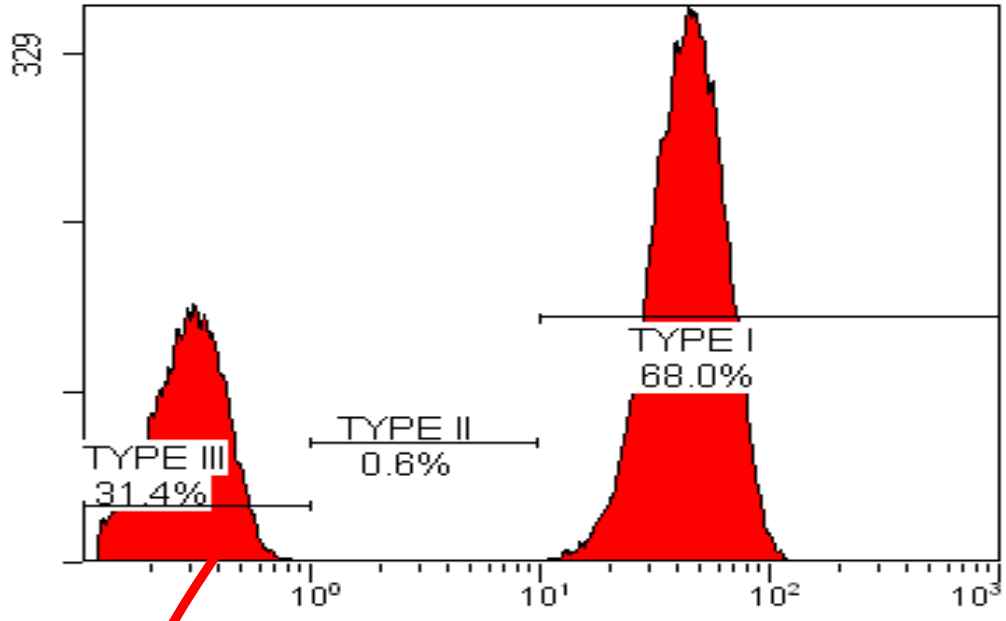
WBC



FLAER- GPI Anchor Binding Marker

80.1 % of Granulocytes lack GPI proteins

RBC



CD59 – GPI Anchored Protein

31.4% RBCs are Type III PNH cells

Identifying patients for PNH Testing

- All Patients with AA
- Select patients with MDS.
- Unexplained Coombs- negative hemolytic anemia.
- Hemoglobinuria/ Intravascular hemolysis.
- Young patients with Unexplained thromboses.
- Thromboses at unusual sites.
- Thrombotic events despite adequate anticoagulation.
- Thrombotic events with iron deficiency, granulocytopenia , and or hemolytic anemia.
- Patients with unexplained cytopenias

Borowitz et al. Clinical cytometry, 78b: 211-230 (2010)
Dezern et al. clinical cytometry, 94b: 16-22 (2018)

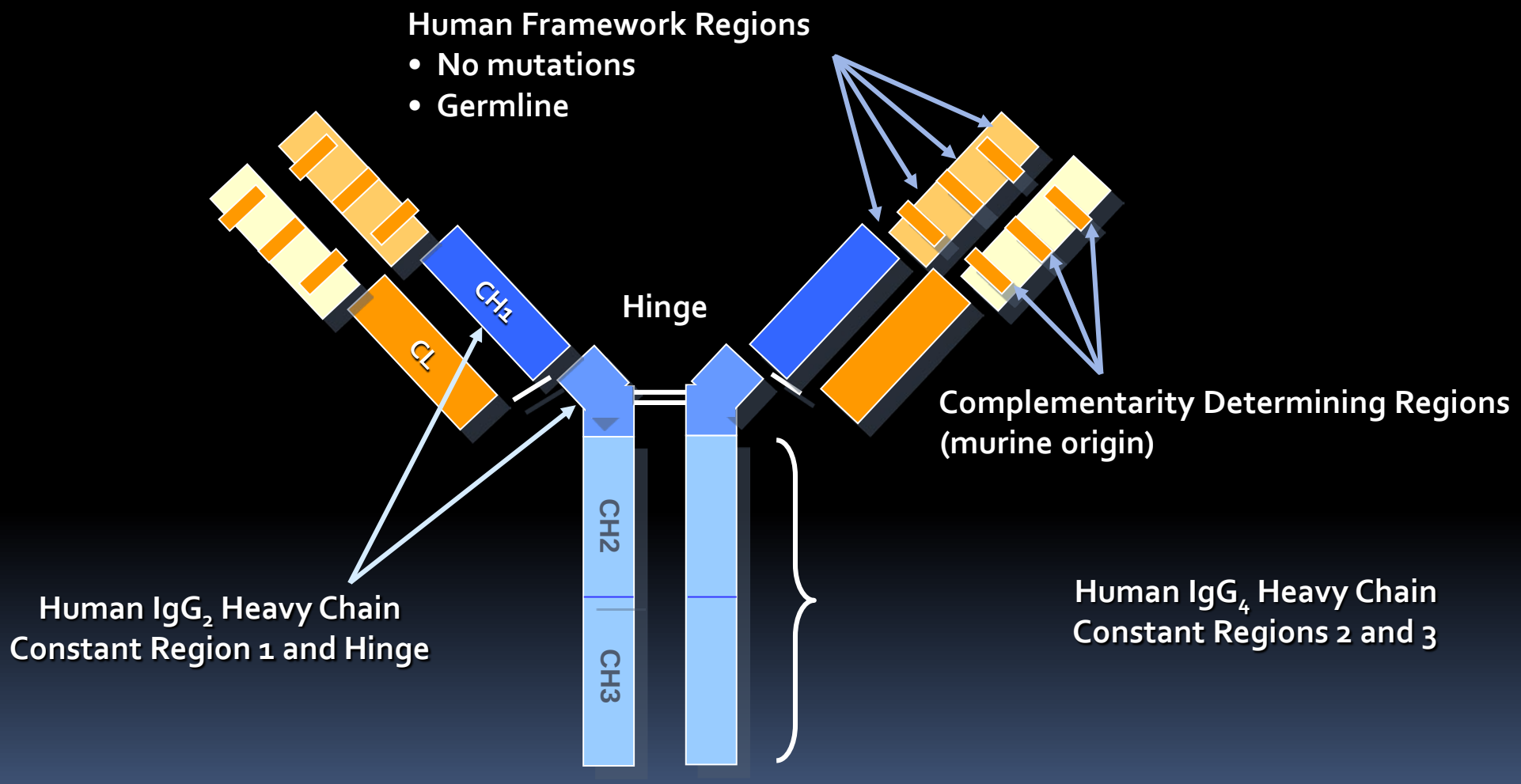
PNH: Treatment

Treatment options for PNH

- **Supportive care**
  - Transfusions.
  - Iron/folate.
  - Androgens.
  - Erythropoiesis stimulating agents.
- **Prednisone**
  - Dose required (20-30 mg/day).
- **Allogeneic Bone marrow transplantation.**
- **Complement Inhibition:**
  - **Eculizumab (Soliris).**
  - **Ravulizumab (ultomiris)**
- **Clinical trials/Novel agents.**

1. International PNH Interest Group. *Blood.* 2005;106:3699-3709.
2. Brodsky *Blood* 2009; 113:6522-6527.
3. Hillmen et al. N Engl J Med. 2006 Sep 21;355(12):1233-43

Eculizumab : Anti-C5 Antibody



Rother R et al. Nat Biotech 2007;25:1256

Eculizumab Clinical Studies

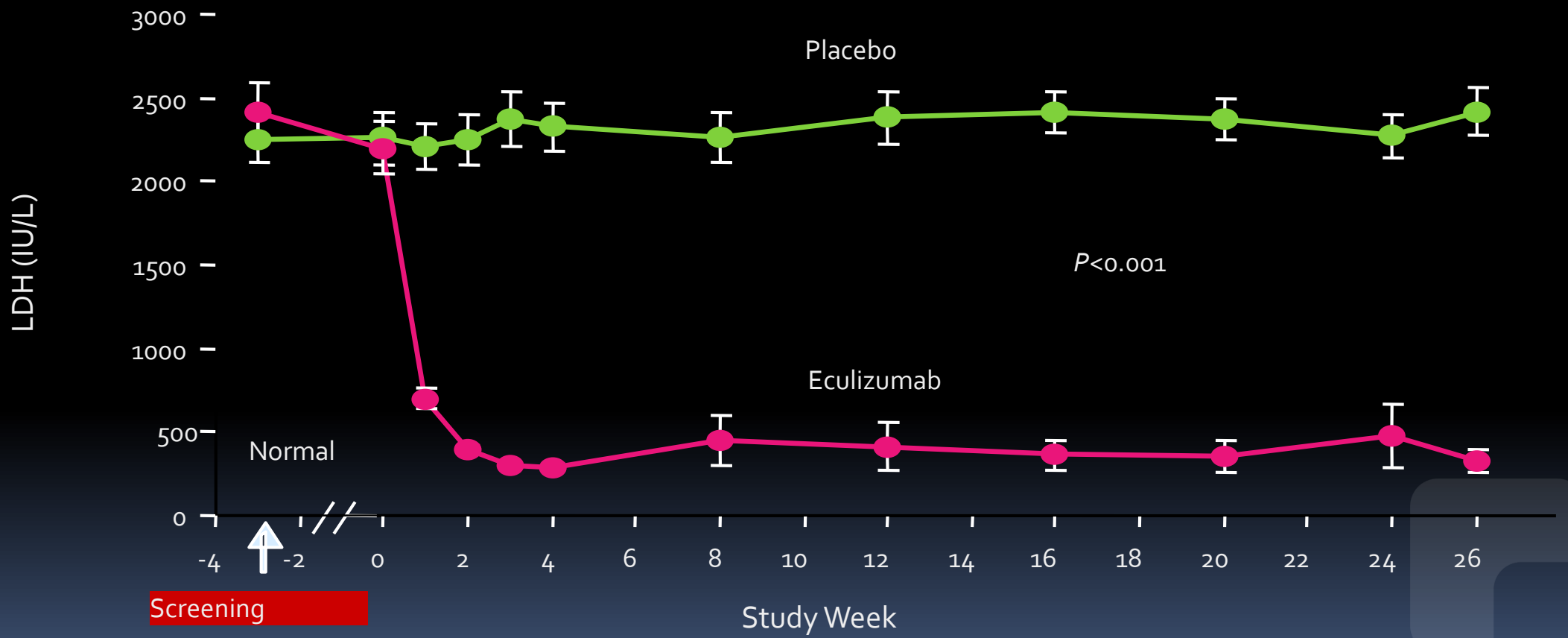
Pilot Study – *NEJM*. 2004
N = 11
Primary endpoint: reduction of hemolysis

TRIUMPH – *NEJM*. 2006
Pivotal Phase III, Double-Blind, Placebo-Controlled Trial, N = 87

SHEPHERD – *Blood*. 2008
Broader patient population, including those receiving minimal transfusions or with thrombocytopenia, N = 97

Long-Term Extension Trial Hillmen *Blood*. 2007
Evaluated long-term safety, efficacy and effect on thrombosis; Placebo patients switched to Eculizumab
N = 187

TRIUMPH – LDH



Hillmen et al. N Engl J Med. 2006 Sep 21;355(12):1233-43.

CASE 1

- A 55 year old man diagnosed with aplastic anemia in 1998.
- Treated with immunosuppressive therapy.

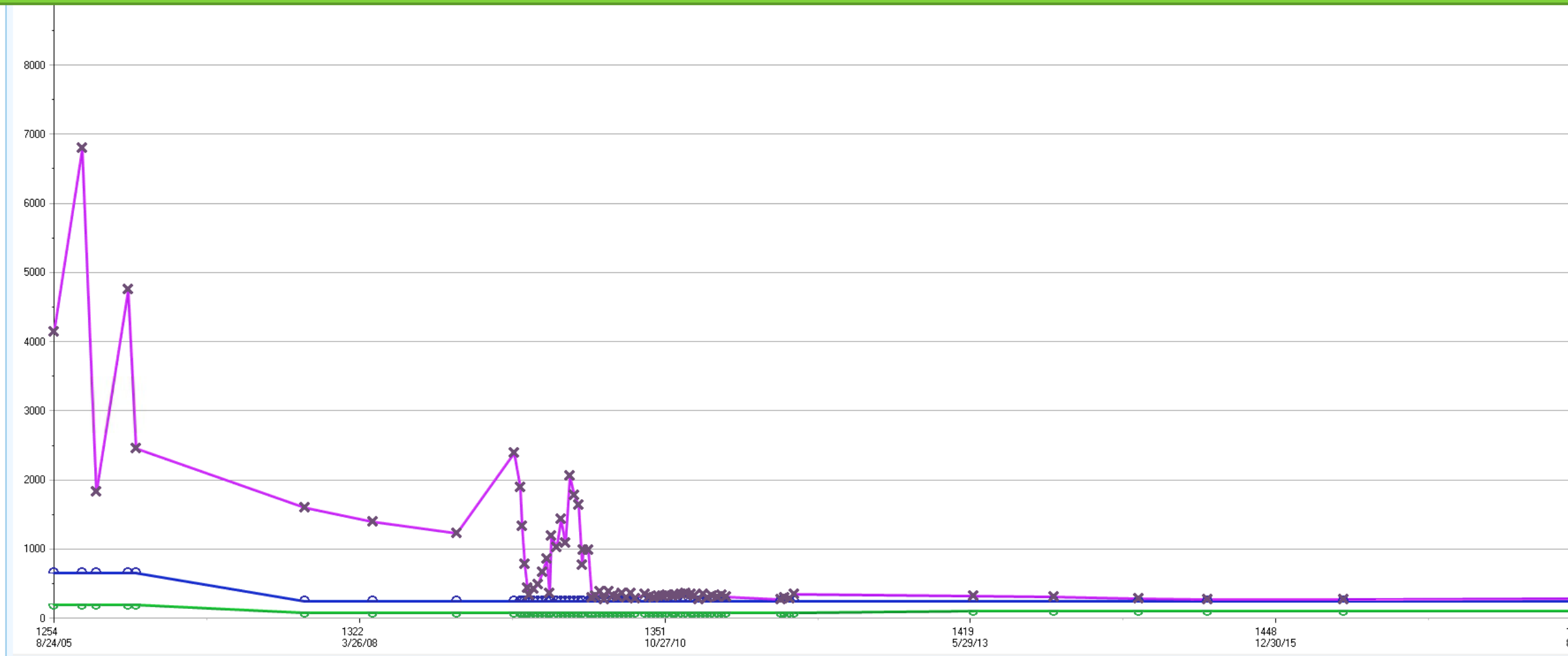
- In 2005, he presented with worsening anemia:
  - HGB: 9.8 gm/dl, MCV:115, WBC/ANC/Plt. WNL
  - T. Bili: 2.2, mostly unconjugated. LDH: >4000.

CASE 1: Results of Diagnostic Testing

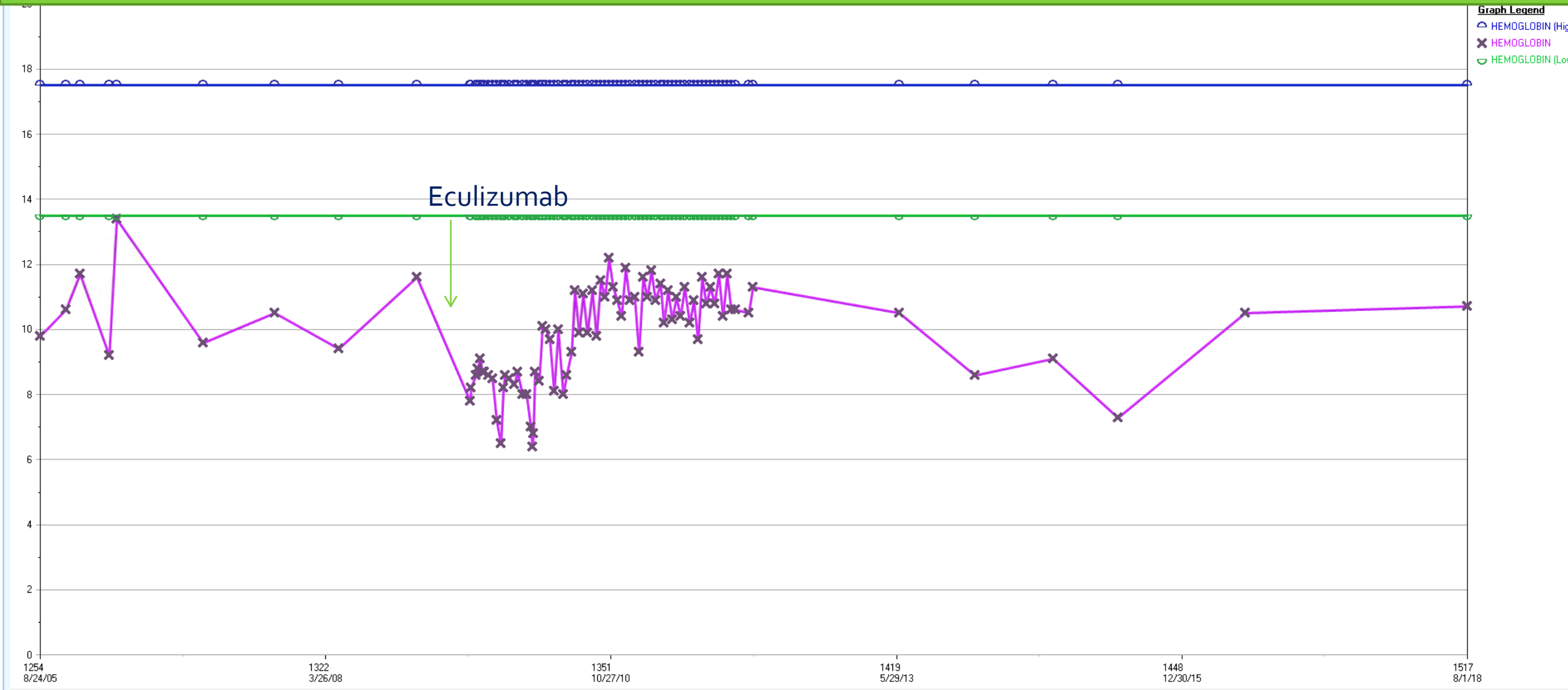
- Bone marrow biopsy:
  - Normocellular bone marrow
  - Erythroid hyperplasia.
  - No overt dysplasia seen.
  - **Absent storage iron.**

- Flow cytometry results:
  - RBC Type I: Normal CD59 level. 74.81%
  - RBC Type II: Partial CD59 deficiency 1.13%
  - **RBC Type III: Complete CD59 deficiency 24.06%**
- **Granulocytes FLAER/CD24 deficiency 76.06%**
- **Monocytes FLAER/CD14 deficiency 83.65%**

CASE 1: LDH


8000
7000
6000
5000
4000
3000
2000
1000
0
1254
8/24/05
1322
3/26/08
1351
10/27/10
1419
5/29/13
1448
12/30/15

CASE 1: Hemoglobin

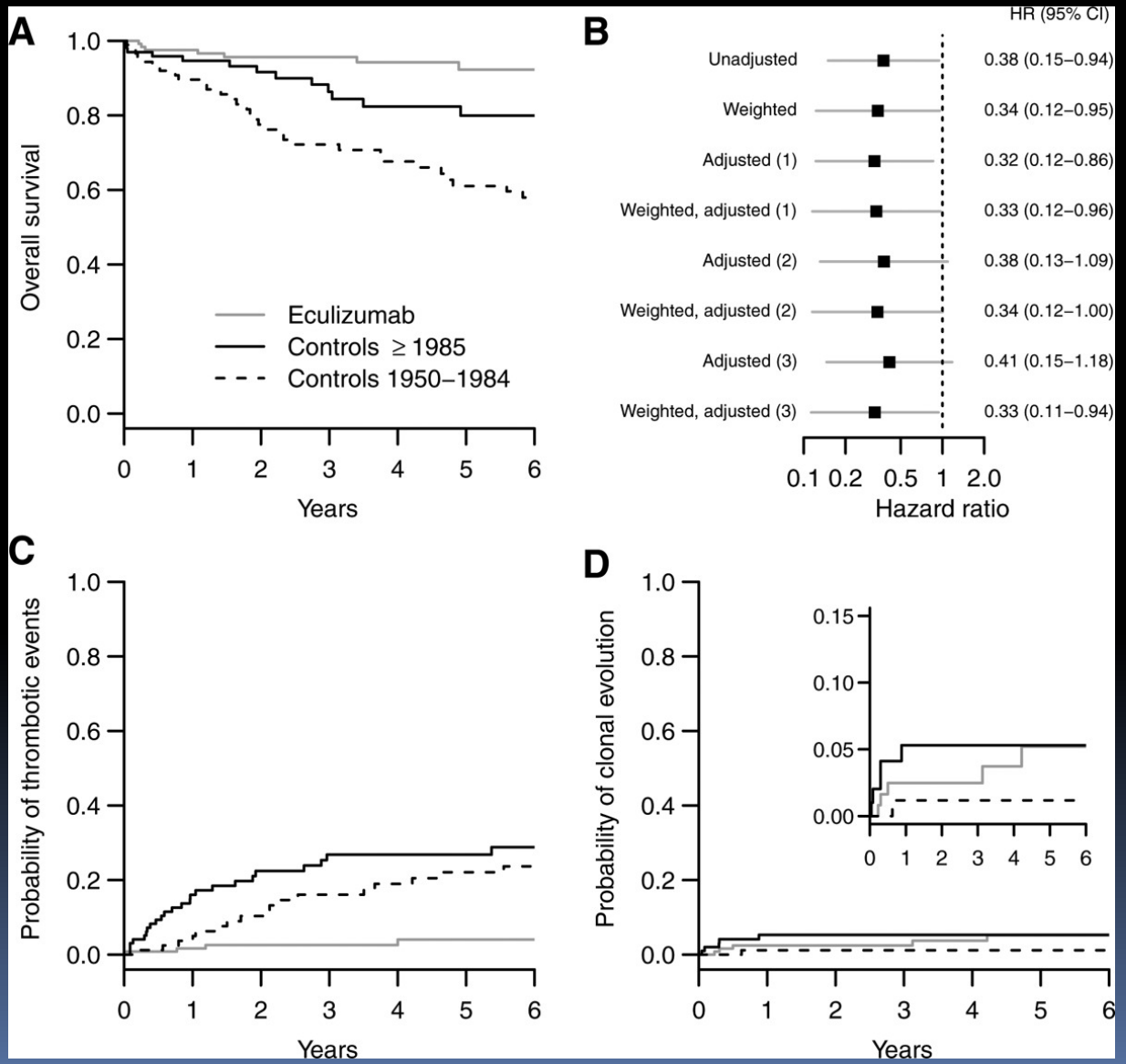


Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: a treatment versus no-treatment study

- A retrospective comparison study between 123 patients treated with eculizumab in the recent period (>2005) and 191 historical controls (from the French registry).

- Overall survival (OS) at 6 years was 92% in the eculizumab cohort versus 80% in historical controls diagnosed after 1985 .
(HR 0.38 [0.15 to 0.94], P = 0.037).

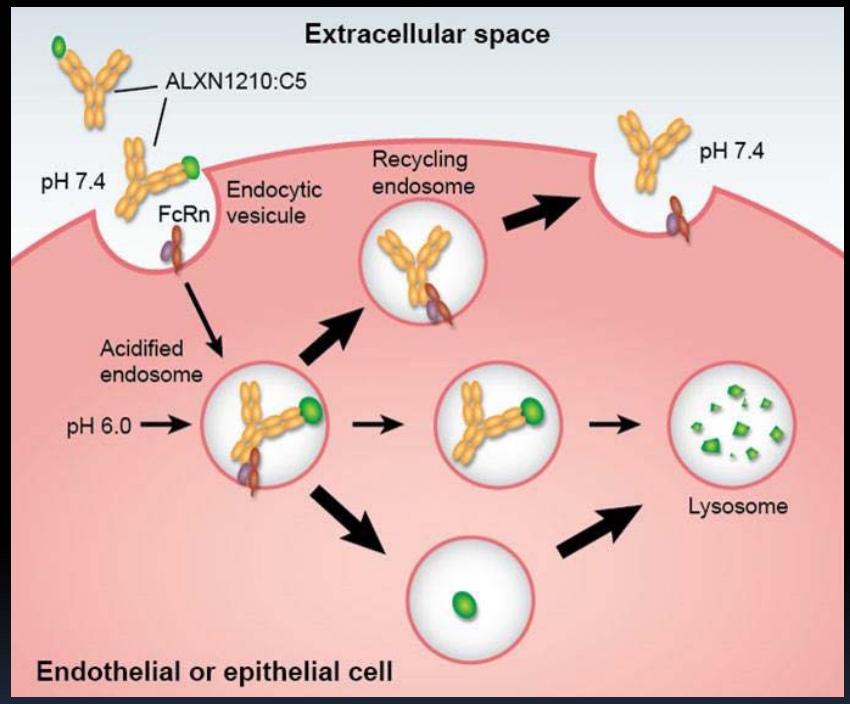
- There were significantly fewer thrombotic events in the group of patients treated with eculizumab (4%) as compared to the historical cohort (27%).



Loschi et al. Am J Hematol. (2016) 91:366–70. doi: 10.1002/ajh.24278

ALXN -1210: Ravulizumab

- A humanized monoclonal antibody to C5.
- Enhanced Fc receptor recycling.
- Half-life of 42 days (4x longer than Eculizumab).
- Administered IV q 8 weeks.
- Weight-based dosing.
- A sq. version is under investigation.



Ravulizumab: Phase 3 Trials

- **PNH-301:**
  - A randomized, open-label trial in treatment-naïve PNH patients (n: 246) .
  - To evaluate the efficacy and safety of ALXN1210 VS. Eculizumab
  - Co-primary endpoint : Transfusion avoidance, and LDH normalization.
  - ALXN 1210 reported to be noninferior to eculizumab (Lee, EHA 2018)
- **PNH-302 :**
  - A randomized noninferiority trial of ALXN1210 versus eculizumab.
  - Previously–treated adult patients with PNH (n: 197).
  - At least 6 months of eculizumab therapy, LDH < 1.5 X ULN
  - Primary efficacy endpoint was hemolysis /percentage change in LDH level from baseline to day 183.

Ravulizumab (ALXN1210) vs eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study

by Jong Wook Lee, Flore Sicre de Fontbrune, Lily Wong Lee Lee, Viviani Pessoa, Sandra Gualandro, Wolfgang Füreder, Vadim Ptushkin, Scott T. Rottinghaus, Lori Volles, Lori Shafner, Rasha Aguzzi, Rajendra Pradhan, Hubert Schrezenmeier, and Anita Hill

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Volume 133(6):530-539
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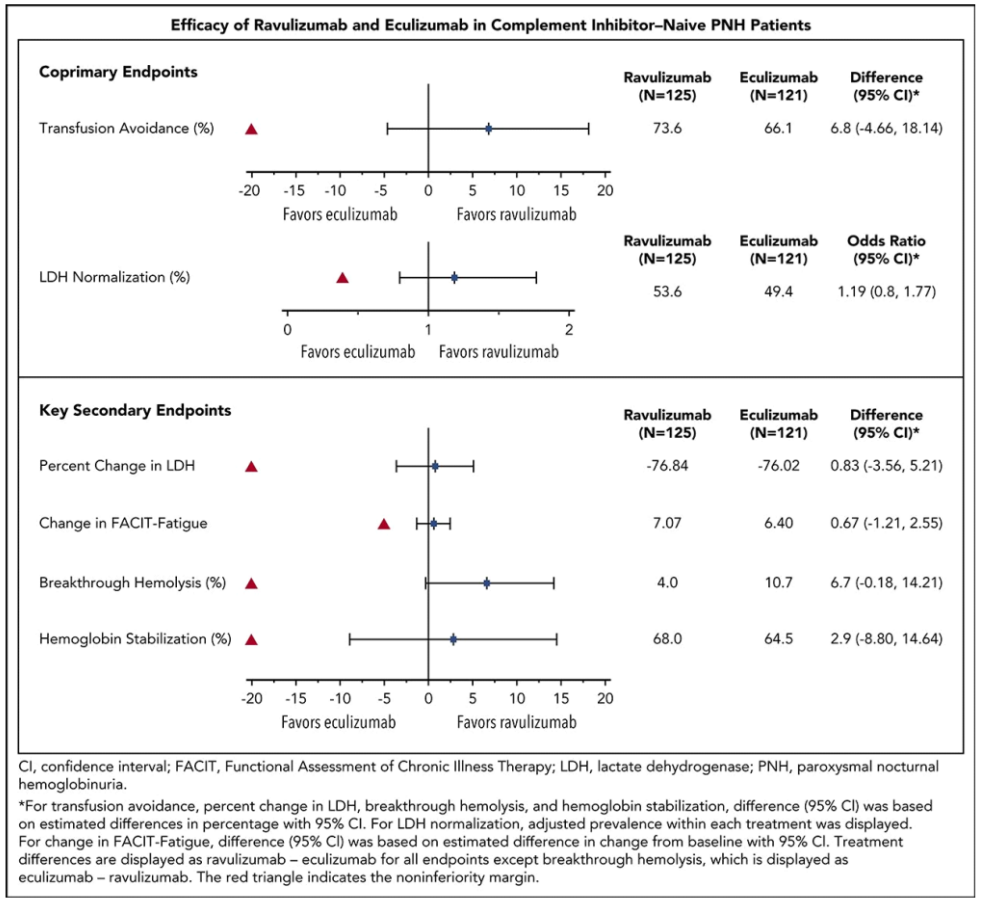
Efficacy of Ravulizumab and Eculizumab in Complement Inhibitor–Naive PNH Patients



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.

Jong Wook Lee et al. Blood 2019;133:530-539

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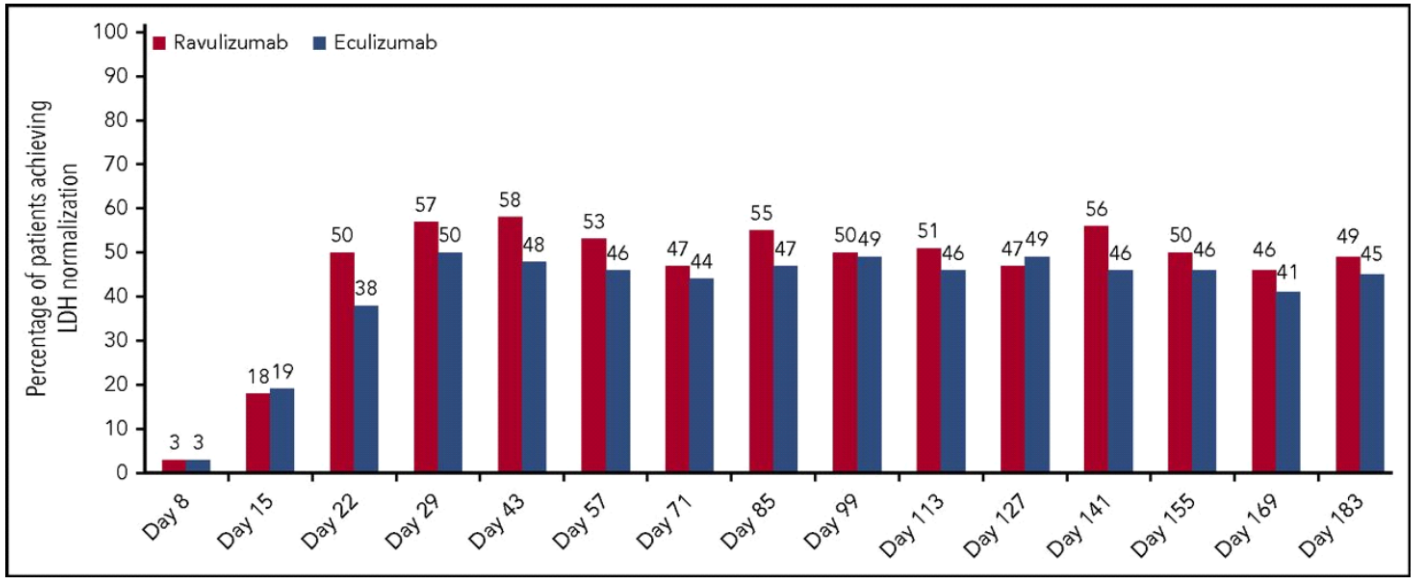
Proportion of patients achieving LDH-N over time in the ravulizumab and eculizumab treatment groups.



Jong Wook Lee et al. Blood 2019;133:530-539

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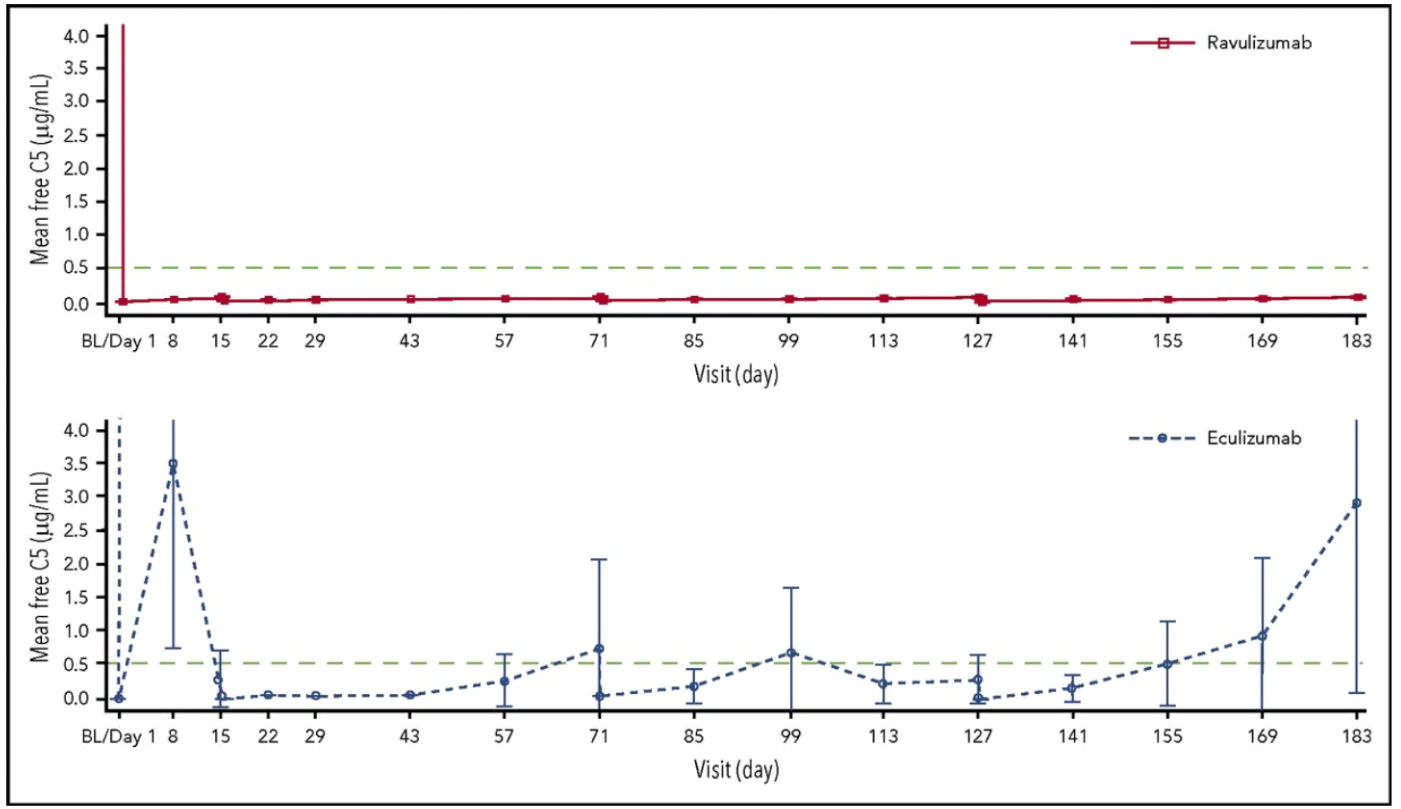
Mean (95% CI) free C5 concentrations in the ravulizumab and eculizumab groups over time.



Jong Wook Lee et al. Blood 2019;133:530-539

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Ravulizumab (ALXN1210) vs eculizumab in C5-inhibitor–experienced adult patients with PNH: the 302 study

by Austin G. Kulasekararaj, Anita Hill, Scott T. Rottinghaus, Saskia Langemeijer, Richard Wells, F. Ataulfo Gonzalez-Fernandez, Anna Gaya, Jong Wook Lee, Emilio Ojeda Gutierrez, Caroline I. Piatek, Jeff Szer, Antonio Risitano, Shinji Nakao, Eric Bachman, Lori Shafner, Andrew I. Damokosh, Stephan Ortiz, Alexander Röth, and Regis Peffault de Latour

Blood
Volume 133(6):540-549
February 7, 2019

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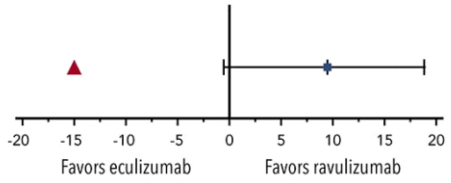
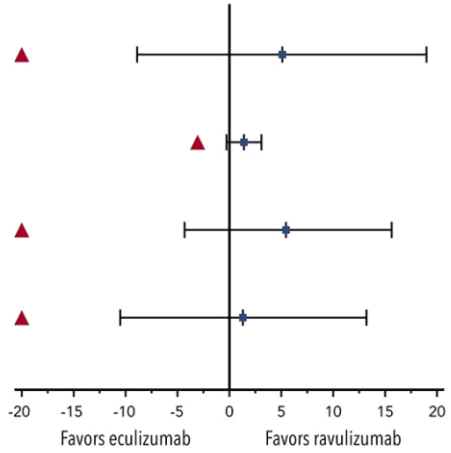
Efficacy of Ravulizumab and Eculizumab in PNH Patients Stable on Eculizumab

Primary Endpoint



	Ravulizumab (N=97)	Eculizumab (N=98)	Difference (95% CI)*
Percent Change in LDH	-0.82	8.39	9.21 (-0.42, 18.84)

Key Secondary Endpoints



	Ravulizumab (N=97)	Eculizumab (N=98)	Difference (95% CI)*
Breakthrough Hemolysis (%)	0	5.1	5.1 (-8.89, 18.99)
Change in FACIT-Fatigue	2.01	0.54	1.47 (-0.21, 3.15)
Transfusion Avoidance (%)	87.6	82.7	5.5 (-4.27, 15.68)
Hemoglobin Stabilization (%)	76.3	75.5	1.4 (-10.41, 13.31)

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.

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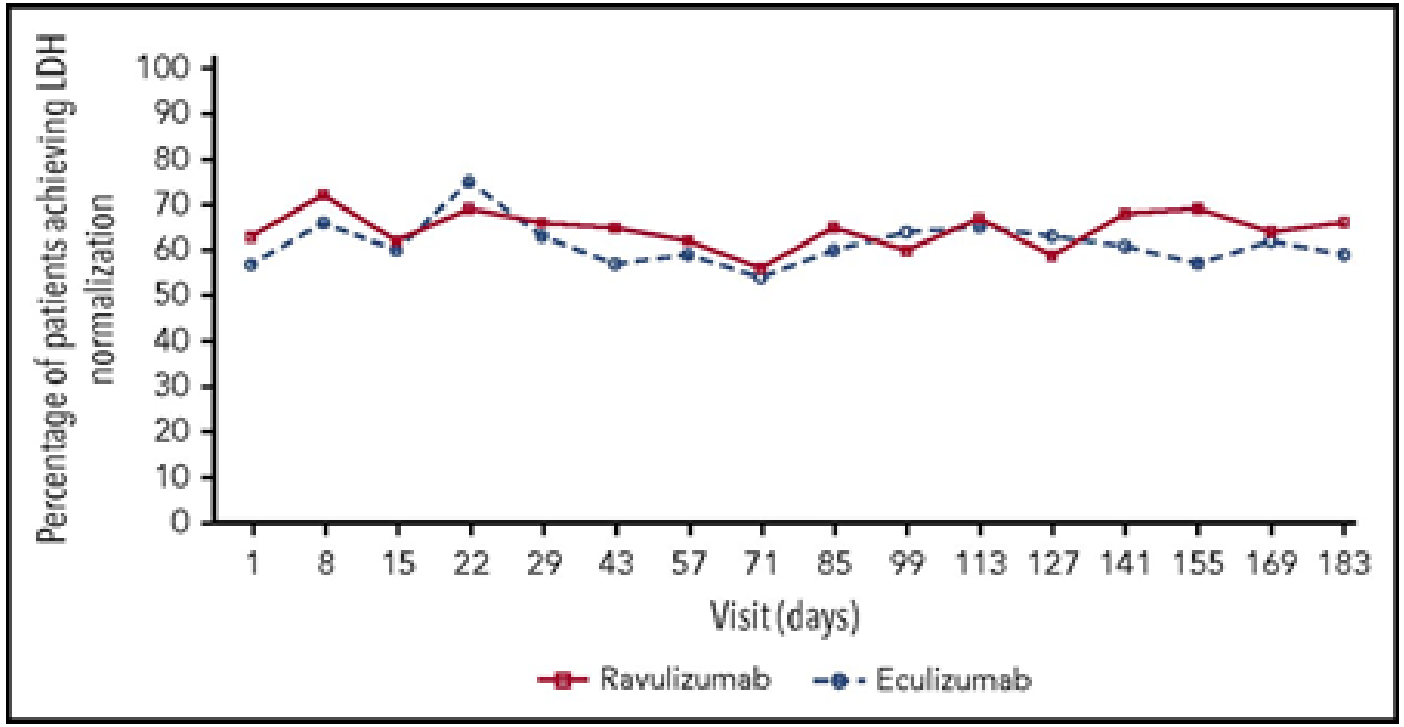
Percentage of patients achieving LDH normalization over time in the ravulizumab and eculizumab treatment groups.



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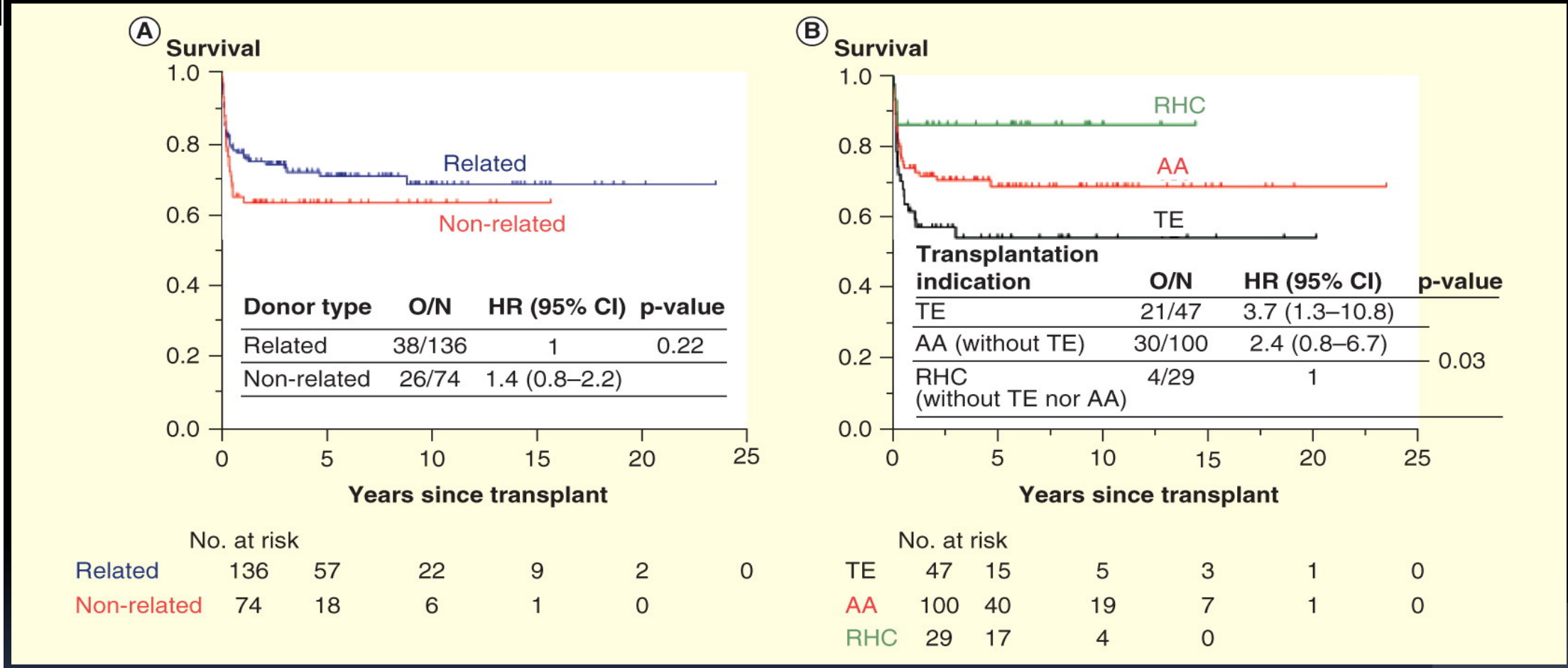
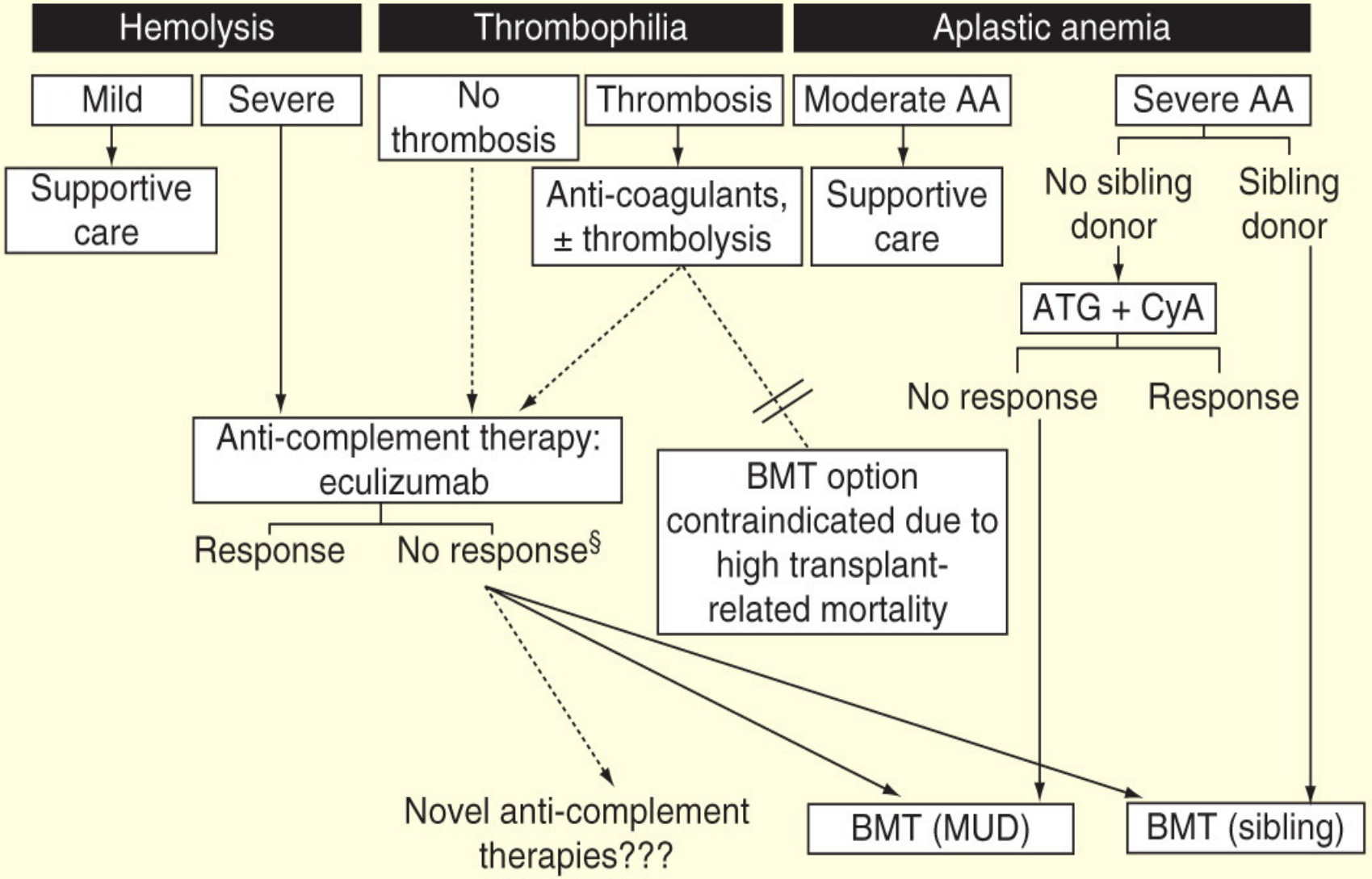
Bone Marrow Transplantation for PNH



(A) Impact of donor: matched sibling versus unrelated donors. **(B)** Impact of transplant indication: BMT performed RHC, AA or TE.

De Latour et al: Hematologica. 2012;97(11):1666-1673.

PNH Treatment Algorithm



Risitano; *Expert Review of Hematology* **2014,** 7, 775-789.

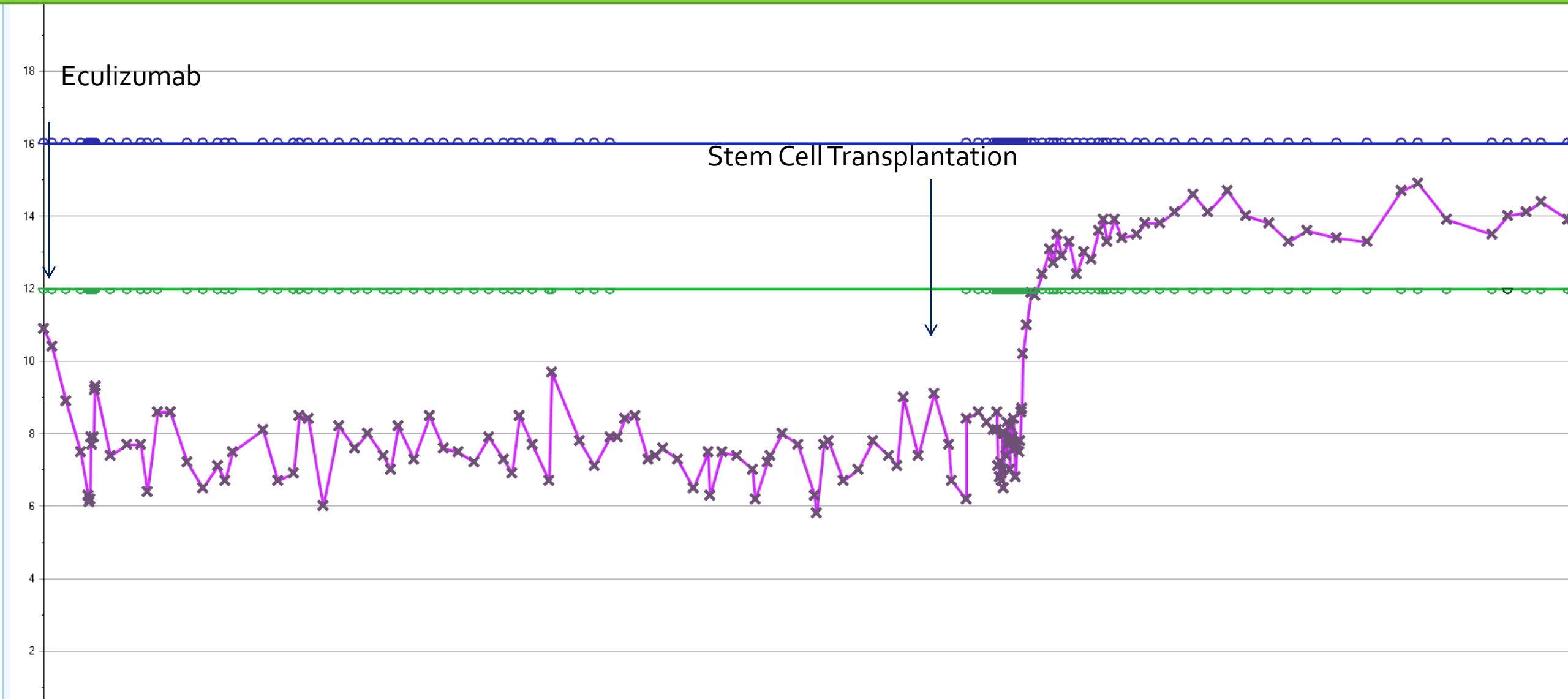
CASE 2

- A 25 year old woman with no prior medical history presented to PCP with fatigue and exercise intolerance.
- A CBC was performed:
  - Hemoglobin 4.5 g/dl, MCV 103.8 Fl
  - WBC 2.8 th/mL, ANC: 1.18 th/mL
  - Platelets 59 th/mL.
- LDH (> 3000), Total bilirubin: 3.5, mostly unconjugated
- Coombs negative.
- Extensive workup for causes of hemolytic anemia was negative.

CASE 2: Results of Diagnostic Testing

- Bone marrow biopsy: Normocellular marrow with erythroid hyperplasia. No dysplasia present.
- Flow cytometry on a peripheral blood sample for PNH was performed:
  - Type I RBC 69.29%
  - Type II RBC 20.26%
  - Type III RBC 10.45%
- Granulocytes FLAER/CD24 deficiency: 86%
- Monocytes FLAER/CD14 deficiency: 84.99%

CASE 2: Hgb



CASE 2: Platelets

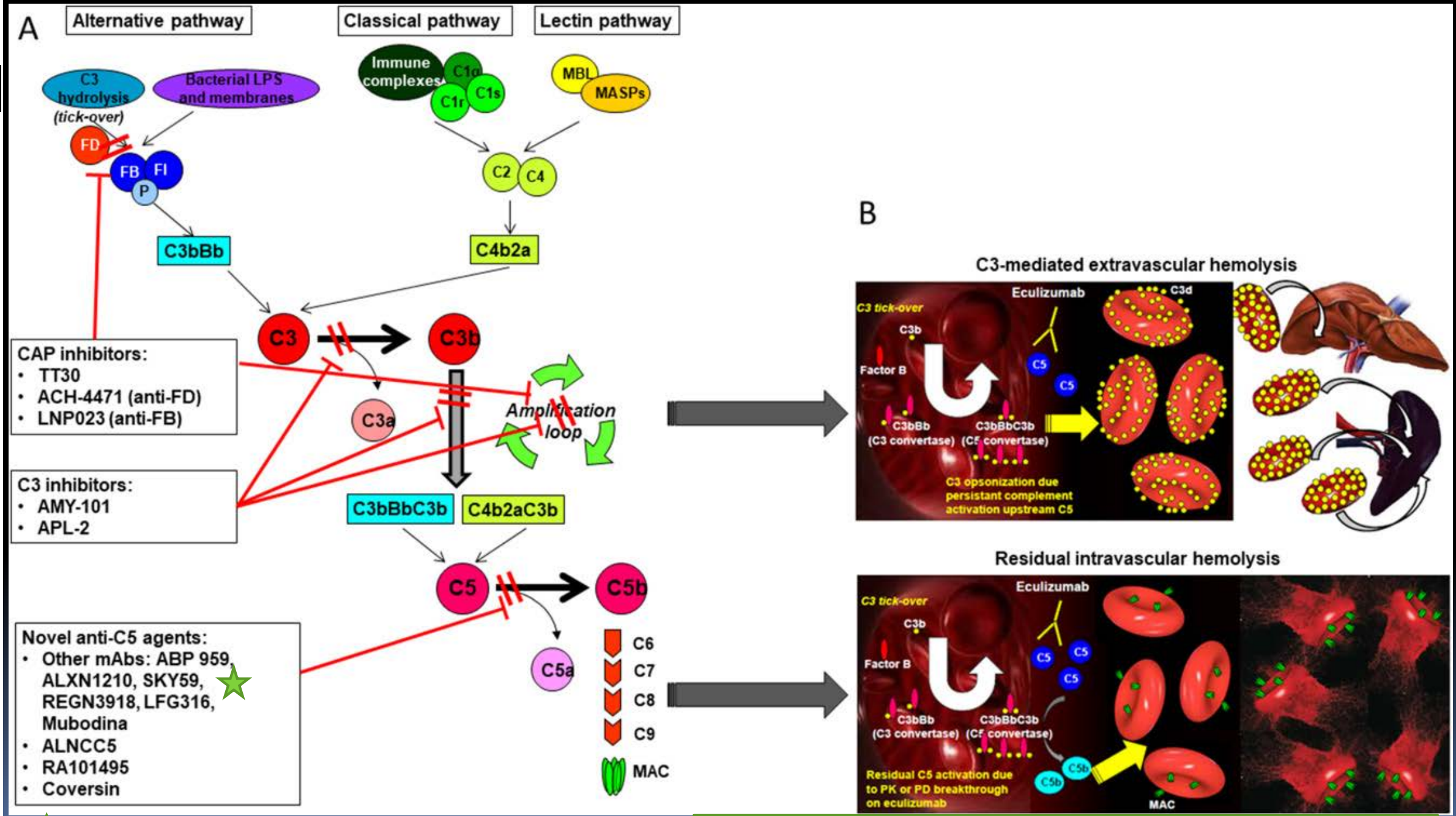
CASE 2: PNH Clone Kinetics

- **Pre-BMT:**
- RBCS
  - Type I: Normal CD59 level. 54.64%
  - Type II: Partial CD59 deficiency 6.49%
  - Type III: Complete CD59 deficiency 38.87%
- GRANULOCYTES FLAER/CD24 deficiency 94.34%
- MONOCYTES FLAER/CD14 deficiency 96.93%
- **1 Month Post BMT**
- RBCS
  - Type I: Normal CD59 level. 99.08%
    Type II: Partial CD59 deficiency 0.59%
    Type III: Complete CD59 deficiency 0.33%
- GRANULOCYTES FLAER/CD24 deficiency 1.11%
- MONOCYTES FLAER/CD14 deficiency 0.66%
- **4 Months Post BMT**
  - **No flow cytometric evidence of paroxysmal nocturnal hemoglobinuria (PNH).**

PNH: Novel Agents

PNH: Ongoing Dilemmas

- C5 polymorphism/lack of response to Eculizumab
- Breakthrough hemolysis
- Concomitant bone marrow failure
- Extravascular hemolysis (+ DAT/C3 deposits)
- Increased risk for meningococcal infection.
- Need for frequent infusions (q 2 week).
- Cost of therapy.



Oral presentation on Monday, abstract 535

Risitano et al. Am J Hem. 2018 . 4;(93) : 564-577

Novel Agents

Anti-C5 mAb

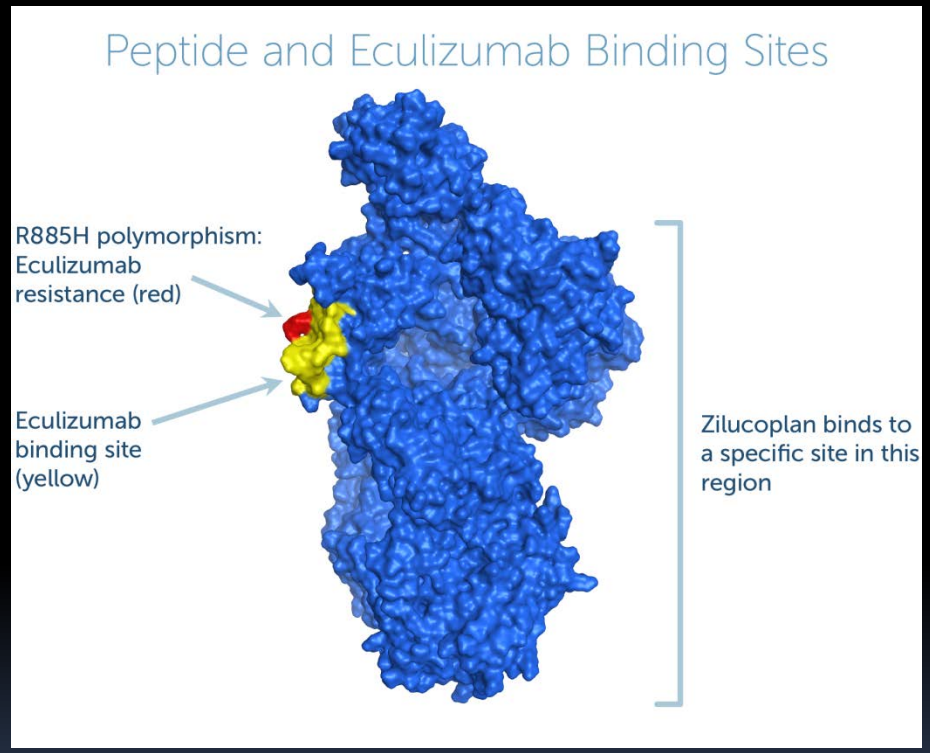
- REGN3918
- LFG316
- Eculizumab biosimilars
- Coversin
- SKY59/RO7112268/Crovalimab

Interference with C5 production

ALN-CC5

Zilucoplan: C5 Complement Inhibitor

- Zilucoplan (RA101495 SC): self-administered, subcutaneous C5 inhibitor
- Binds to C5 and C5b
- Phase 2 studies completed
- Phase 3 studies in paroxysmal nocturnal hemoglobinuria (PNH) planned for 2019



Inhibitors of the Proximal Complement

Broad inhibitors of C3 : two compstatin analogs

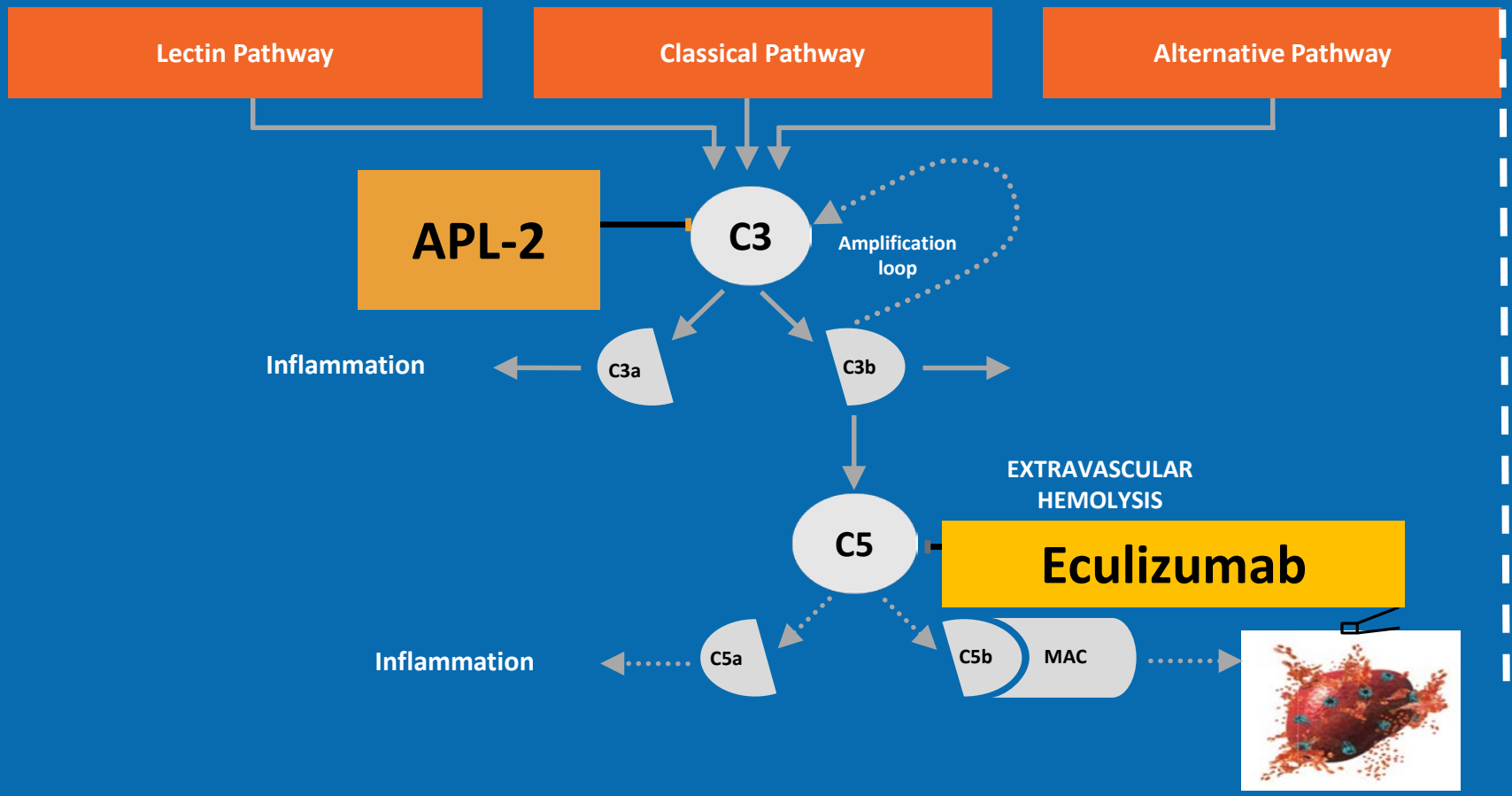
- AMY-101
- APL-2) a

Selective inhibitors of the alternative pathway

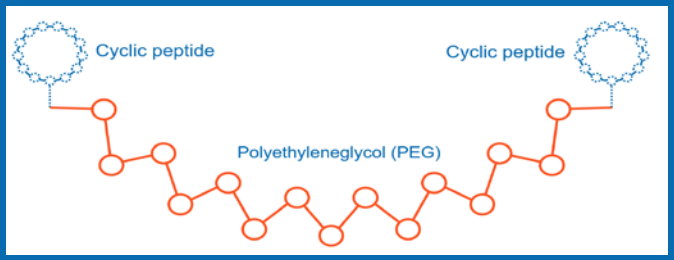
ACH-4471: Anti complement factor D (FD)

LNP023: Anti complement factor B (FB).

APL-2: Central Inhibition of Complement



APL-2
Subcutaneous C3 Inhibitor



Peptides of the APL-2 family bind to a pocket of C3 and inhibit activation

Janssen, J. Biol. Chem., 282(40), 29241-29247, 2007

Phase Ib APL-2 PNH Trials

PHAROAH: APL-2 Add-on Therapy in Eculizumab Tx'd Pts *

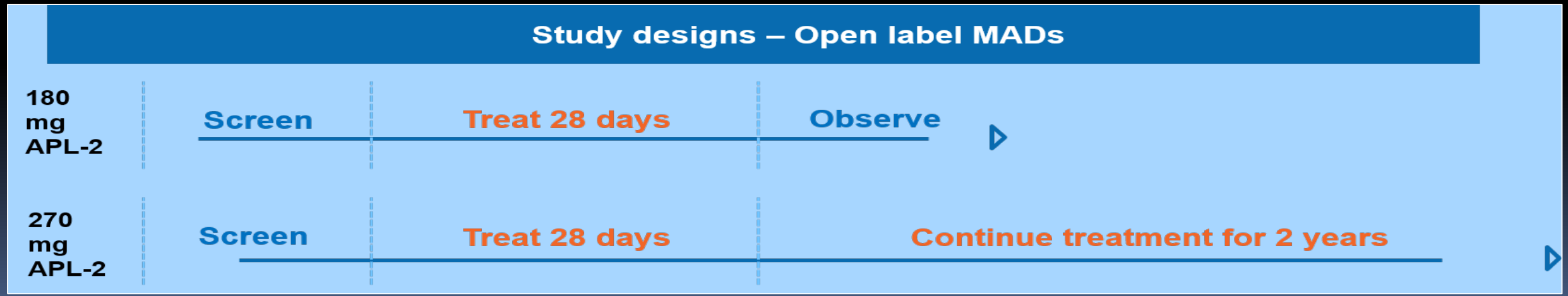
Patient Population:

- Diagnosis of PNH (WBC clone > 10%)
- **At least 3 months of eculizumab treatment**
- **Hb < 10.5 g/dL at screening, LDH > 2X ULN OR** have received at least one transfusion with 12 months prior to screening
- Platelet count of > 30,000/mm^3 and ANC > 500 x 10^9/L

PADDOCK: APL-2 Monotherapy in eculizumab-Naïve PNH

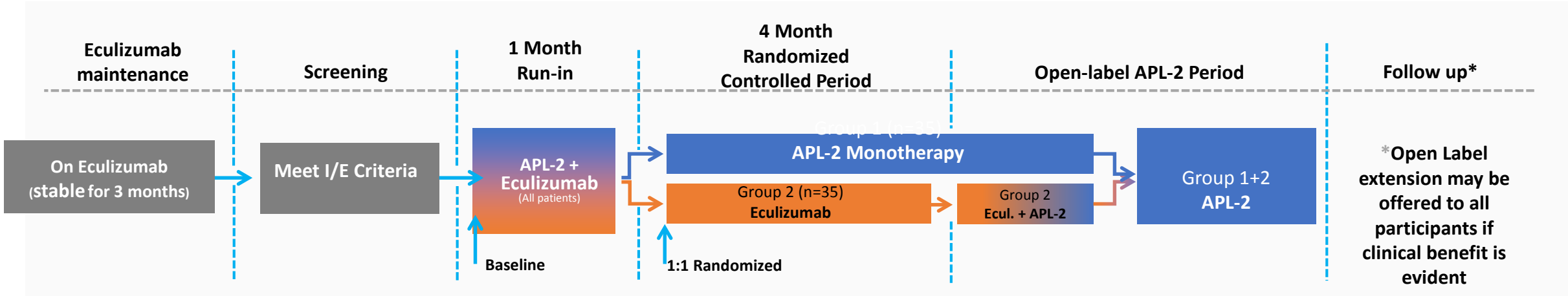
Patient Population:

- Diagnosis of PNH (WBC clone > 10%)
- **LDH > 2X ULN**
- **Last transfusion within 12 months prior to screening**
- Platelet count of > 30,000/mm3 and ANC > 500 x 109/L



* Poster presentation on Sunday , abstract 2314

PEGASUS Phase III Ongoing Study (n=70)



Parameter	
Population	PNH patients on eculizumab who continue to be anemic (Hb < 10.5 g/dL at screening), elevated retic counts
Primary Endpoint	Week 16 change from baseline in hemoglobin level
Design	Randomized 1:1 APL-2:eculizumab. 1 Month Run In; 4 Month Randmoization;6 Month Open Label; 1 Month Follow Up
Dosing	Dose 1080 mg 2/week, SC infusion via injection pump
Sample size	70 patients (35/group) randomized 1:1

Key Takeaways

- PNH is a rare disease with serious consequences.
- Testing for PNH should be performed in high-yield patient populations
- Flow cytometry of the peripheral blood is the diagnostic procedure of choice for PNH.
- PNH clones should monitored periodically.
- SCT is reserved for patients with PNH/ severe bone marrow failure, or those who do not respond to complement inhibition.
- Multiple novel agents are being evaluated.

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