Hemolytic Anemia: Autoimmune Hemolytic Anemia and PNH

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Hemolytic Anemia--Causes

Hereditary

- RBC Membrane
  - HS, HE, pyropoikilocytosis
- RBC Metabolic Defects
  - EM pathway
  - HMP shunt
  - Nucleotide synthesis
- Hemoglobin Defects
  - Thalassemia
  - Abnormal variants

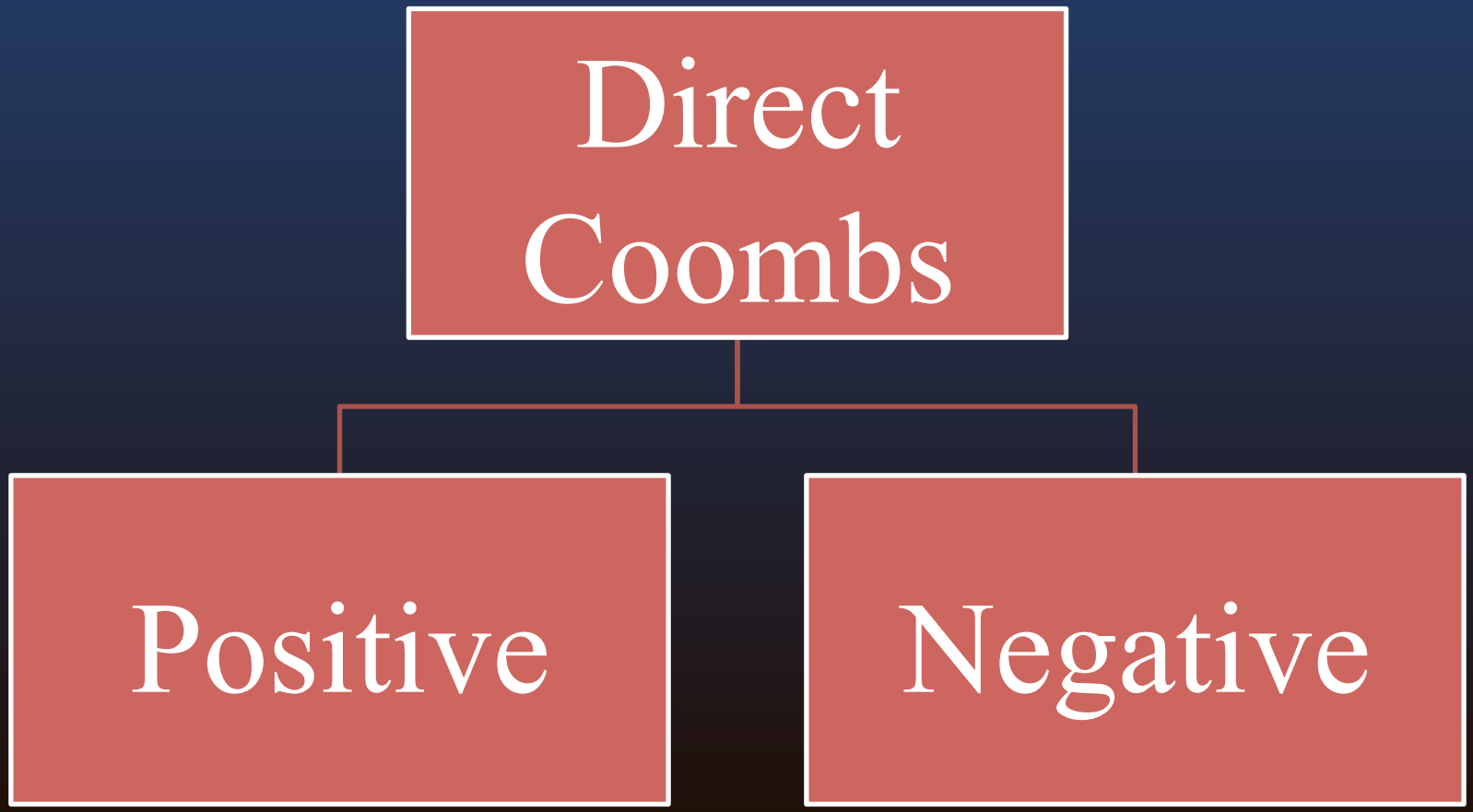
Acquired

- Immune
  - Autoimmune
  - Isoimmune
  - Drug
- RBC fragmentation syndromes
- PNH
- Secondary
  - Renal and liver disease
- Misc
  - Drugs, infections, chemicals, toxins, physical agents

Laboratory approach to the patient with suspected hemolytic anemia

- Laboratory
  - CBC
  - Reticulocyte Count
  - Blood smear
  - LDH
  - Bilirubin-direct and indirect
  - Haptoglobin
  - Urinalysis

Laboratory evaluation of hemolytic anemia



Coombs +

- Warm autoimmune hemolytic anemia
- Cold agglutinin disease
- Drug induced
- Paroxysmal Cold Hemoglobinuria

Coombs -

- Hemoglobinopathies
- Enzymopathies
- Membrane Defects
- Microangiopathic
- Drugs
- Toxins/Wilson's disease
- PNH
- Etc….

Autoimmune Hemolytic Anemia (AIHA)-Classification

Warm-reacting antibodies

optimally bind red blood cells at 37°C

- Idiopathic
- Secondary
  - Autoimmune disorders
  - Immunodeficiencies
  - Lymphoproliferative disorders
  - Nonlymphoid malignancies
  - Viral infections
- Mixed warm and cold antibodies
- Drug Induced

Cold-reacting antibodies

optimally bind red blood cells <37°C

- Cold agglutinins
  - Idiopathic
  - Secondary (associated clinical conditions)
    - Infections
    - Lymphoproliferative disorders
    - Nonlymphoid malignancies
- Paroxsymal cold hemoglobinuria (Donath-Landsteiner antibodies)
  - Syphilis
  - Viral infections

Audience Response Question: Coombs test

Which of the following statements about the Coombs test and the blood bank evaluation of autoimmune hemolytic anemia is most accurate?

A.)A positive Coombs test implies at least low grade hemolysis

B.)Cold agglutinins are typically IgG positive on the Coombs test

C.)The thermal amplitude of a cold agglutinin is the best predictor of clinical significance

D.)Autoadsorption of autoantibodies are useful to define the specificity of the antibody

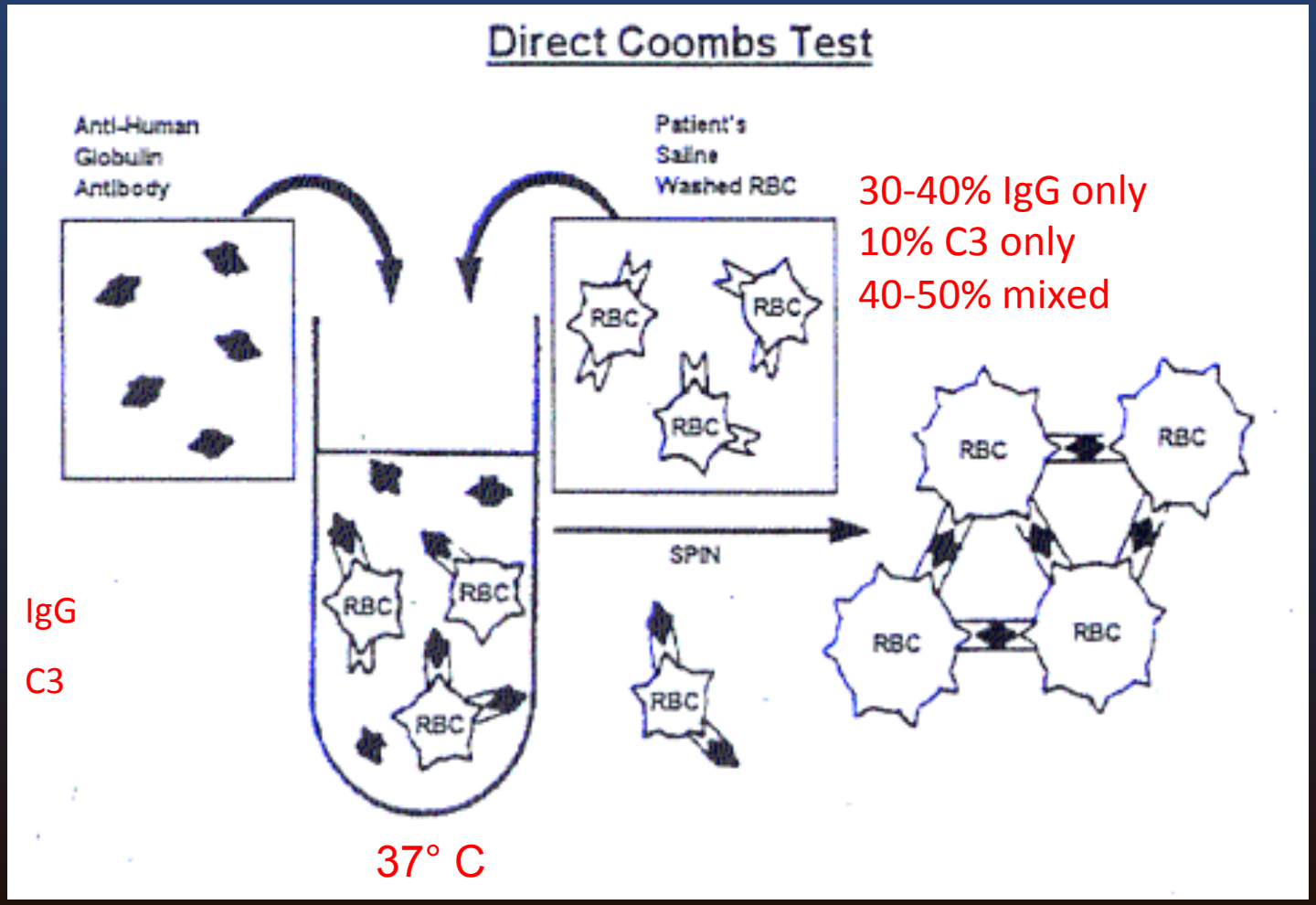
E.)The eluate of concentrated IgG from patients with warm autoimmune hemolytic anemia is used to rule out alloantibodies

Direct Coombs test

- Detects antibody coating the RBC surface
- Positive test isn't necessarily diagnostic of hemolysis
  - As many as 0.1% of healthy blood donors are positive
  - 1-2% of hospitalized patients are positive
- Degree of hemolysis doesn't always correlate with degree of positivity

Reardon. Am J Clin Pathol 2006:125(supl1) S71-S77

Direct Coombs Test



30-40% IgG only
10% C3 only
40-50% mixed

Patterns

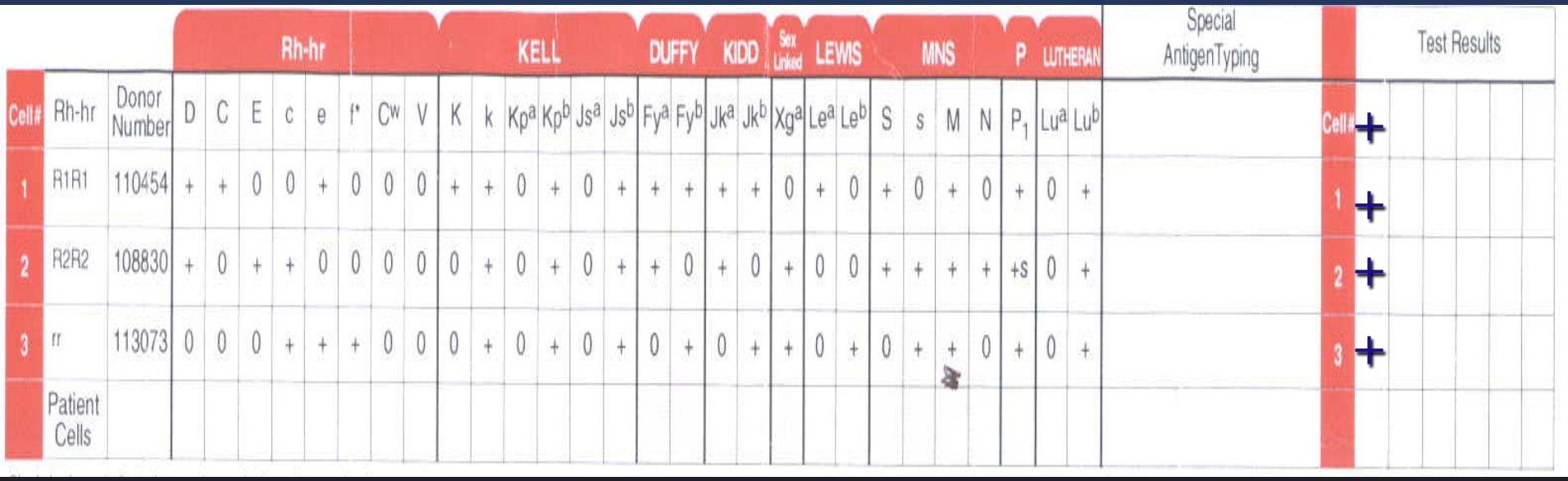
	IgG	C3
Warm AIHA		
(67%)	++	+
(20%)	+	-
(13%)	-	+
Cold agglutinin disease	-	+
Paroxsymal Cold Hemoglobinuria	-	+

Adapted from Petz and Swisher. Immunology and Its Relation to Blood Transfusion. In:Clinical Practice of Transfusion Medicine 3rd ed NY, NY. Churchill-Livingstone. 1996. P.49

Elution: Detection of specificity of the autoantibody

- Elute off IgG from patient RBCs
  - Incubate with reagent RBCs to test for activity and specificity of the antibody
  - Antibody most commonly reacts to a full RBC panel with similar agglutination strengths
  - Less commonly may show a relative specificity within the Rh system such as the e antigen (WAHIA) or I (cold agglutinin)

Difficulty performing a Type and Screen



			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing	Test Results							
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f*	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #	+							
1	R1R1	110454	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	0	+	0	+	0	+		1	+							
2	R2R2	108830	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	+s	0	+		2	+							
3	rr	113073	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+		3	+							
	Patient Cells																																						

Autoadsorption: Detection of Alloantibodies

- Goal is remove the autoantibody from the serum to allow the detection of alloantibodies
  - 32% of patients with AIHA have alloantibodies
  - 1 ml of packed autologous RBCs treated to remove the Ab and than incubate with patient's serum at 37°C

Antibody Screen Results for Patients With Autoimmune Hemolytic Anemia Without (Example A) or With (Example B) an RBC Alloantibody Before and After Autoadsorption*

	Example A		Example B	
Screening Cell	Unadsorbed Serum (Autoantibody Only)	Adsorbed Serum	Unadsorbed Serum (Autoantibody Plus Alloantibody)	Adsorbed Serum
I	2+	0	2+	0
II	2+	0	4+	2+
III	2+	0	2+	0

* Agglutination is read from 0, which is a negative result, to 4+, which is the strongest possible agglutination.

Reardon AM J Clin Pathol 2006;125(Suppl 1):S71-77

Transfusion in AIHA

- Proceed with caution-consider risk/benefit ratio
  - Transfused blood often has a short half-life
  - Use phenotype matched blood if available
    - Rh groups, Kell, Duffy, Kidd antigens
    - If antibody shows specificity for a given antigen use antigen negative blood
  - If cold agglutinin disease transfuse through a blood warmer

Reardon AM J Clin Pathol 2006;125(Suppl 1):S71-77

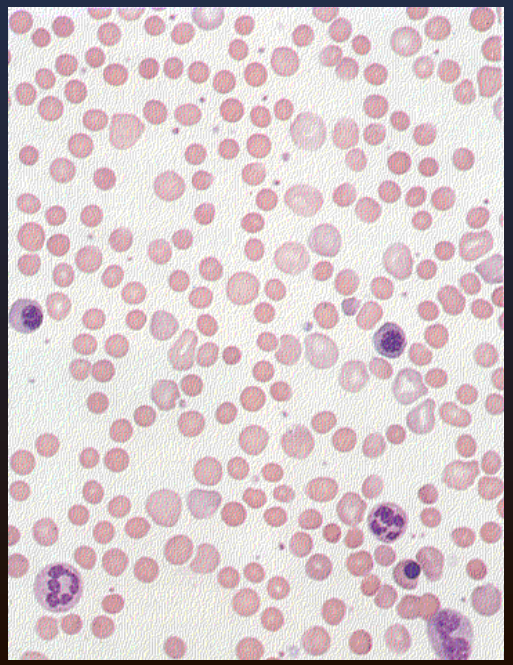
Case 1

A 63 year-old previously healthy man is admitted with angina and dyspnea on exertion. He has had a lack of energy for 2 months and has also complained of some intermittent yellowing of his eyes.

No prior blood counts are available. He is taking no medications and does not recall any recent illness. He denies alcohol consumption, tobacco, or illicit drug use.

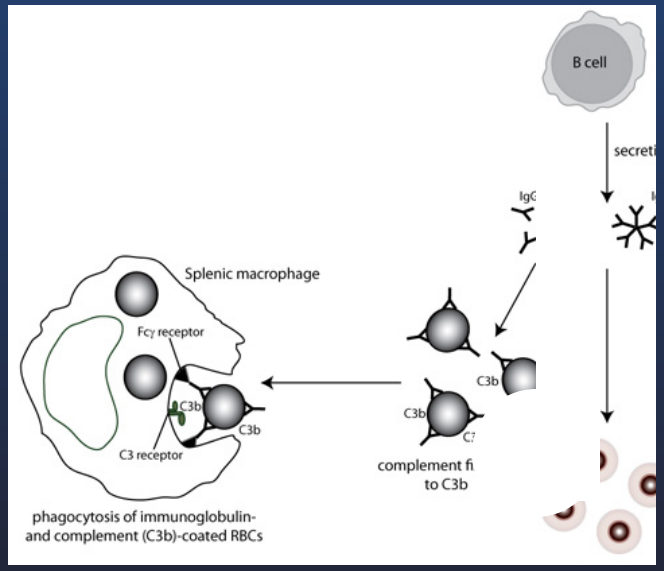
Laboratory data and blood smear are as follows:

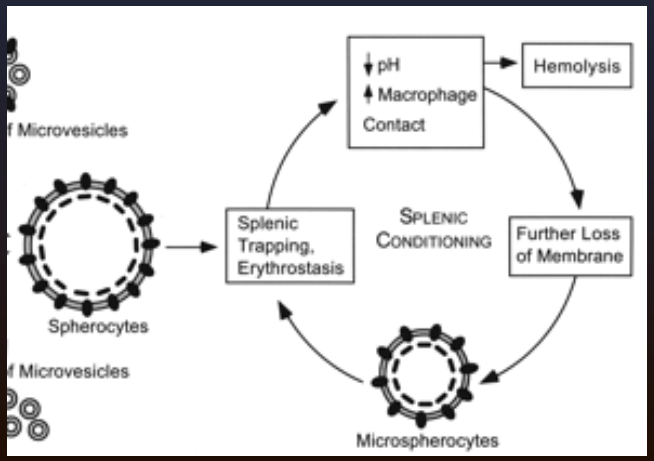
Hct	26%
MCV	103 fL
WBC count	5900/ul
Retic count	7.1 %
LDH	949 IU/L
Coombs	IgG+++, C3+



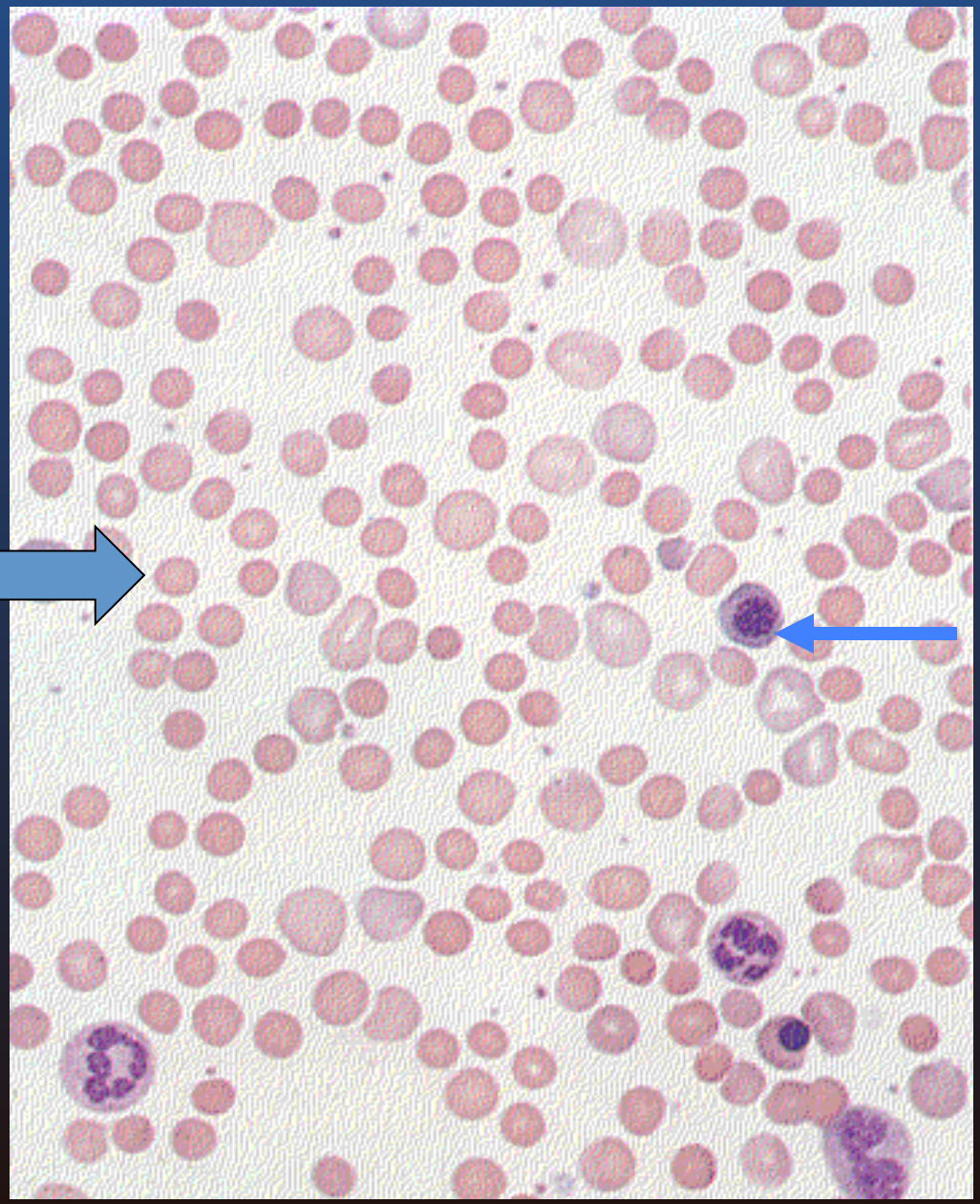
Warm Autoimmune Hemolytic Anemia (W-AIHA)

- IgG panagglutinating antibody directed against "public" epitope often on the Rh system
  - Optimally bind red cells at 37°C
  - Primarily removed by Fc receptor macrophages in the reticuloendothelial system
    - Partial phagocytosis leads to spherocytes removed by the spleen
  - Less commonly or weakly fixes complement





Packman CH. Blood Rev. 2008 Jan;22(1):17-31. PMID: 17904259



Positive Coombs Test

Diagnosis: Warm Autoimmune hemolytic Anemia

- Investigation
  - Rule out lymphoproliferative disorder
    - Consider bone marrow aspirate and biopsy
  - Rule out autoimmune disorder
    - ANA, etc…
  - Rule out immunodeficiency
    - Quantitative immunoglobulins
  - Rule out drugs

Warm autoimmune hemolytic anemia-Treatment

- Treat underlying disease if identified
- Steroids first line (1mg/kg) for one to three weeks
  - Interfere with ability of macrophages to clear IgG coated RBC's
  - Decreases antibody production
  - 60-85% initial response (20% CR), but frequent relapses
  - Initial quick taper, than slowly when down to 20mg (for 2-3 months)
  - Pulses of high dose glucocorticoids may be useful in some who fail

Kessler. Clin Advances in Hemaotol Onc. 2008; 6(10):739-41
King. Semin Hematol. 2005; 42:131-136

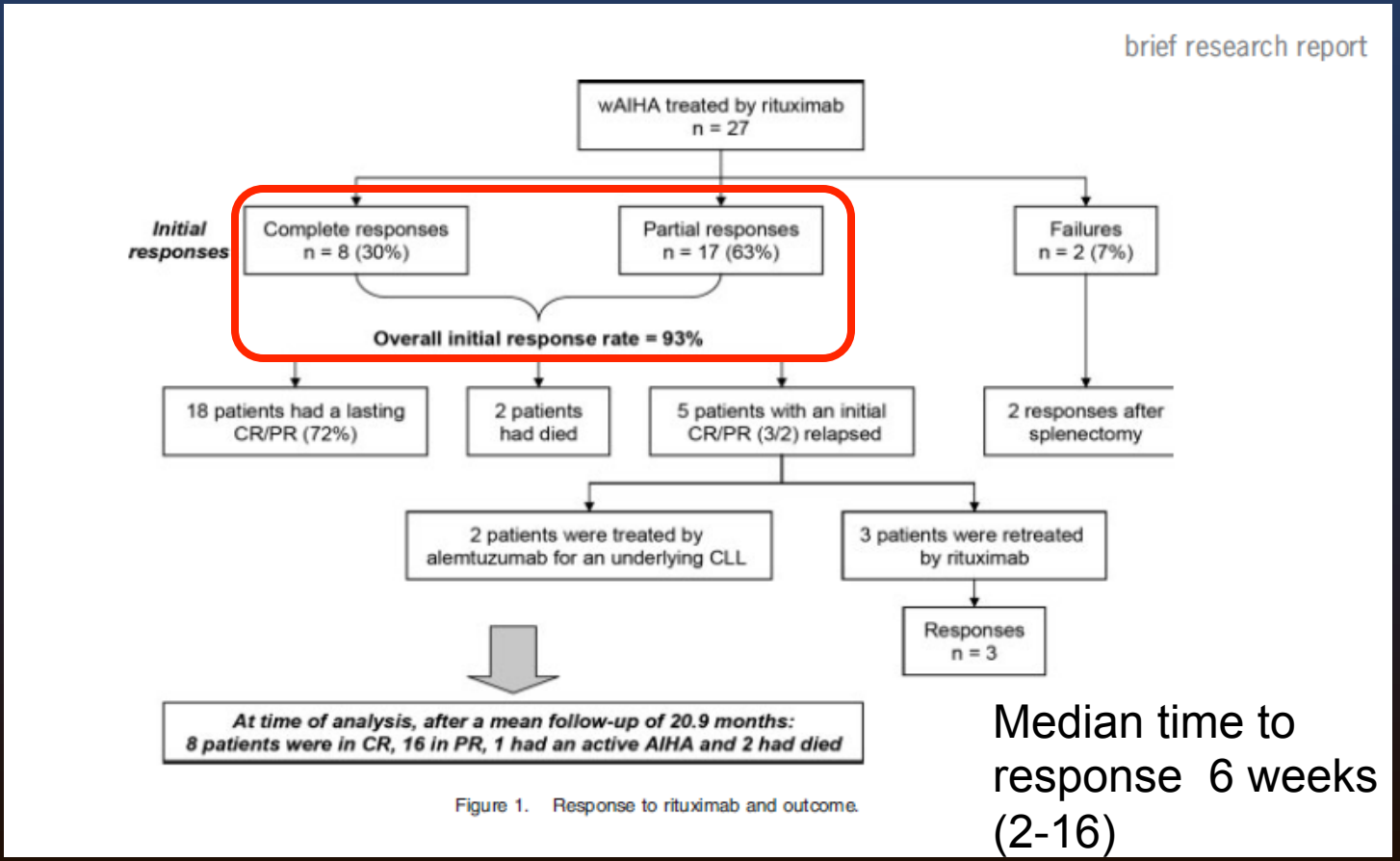
Warm autoimmune hemolytic anemia- Treatment

- Splenectomy
  - Consider in two-three weeks if no response to steroids
  - 2/3 respond, but relapses occur
  - Ensure immunizations and education about infectious risk
    - *Pneumoccus, meningococcus*, and *hemophilis influenza type B*
    - ??? Prophylactic antibiotics
  - Higher incidence of post-splenectomy venous thrombembolism and pulmonary hypertension
    - High incidence of antiphospholipid antibodies

Kessler. Clin Advances in Hemaotol Onc. 2008; 6(10):739-41
Crary. Blood. 2009 Oct 1;114(14):2861-8

Rituximab

- Chimeric monoclonal antibody targeting CD-20 on mature B Cells



Median time to response 6 weeks (2-16)

Bussone . AM J Hematol. 2009: 84:153-157

Treatment-Other

10% refractory to both steroids and splenectomy

- Other immunosuppressants
  - Immuran
  - Cyclophosphamide
  - Cyclosporine
  - Danazol
  - IVIG
    - Not as effective as in ITP
- Paucity of randomized trials to suggest which agent is superior
- Responses may be delayed several months

Kessler. Clin Advances in Hemaotol Onc. 2008; 6(10):739-41

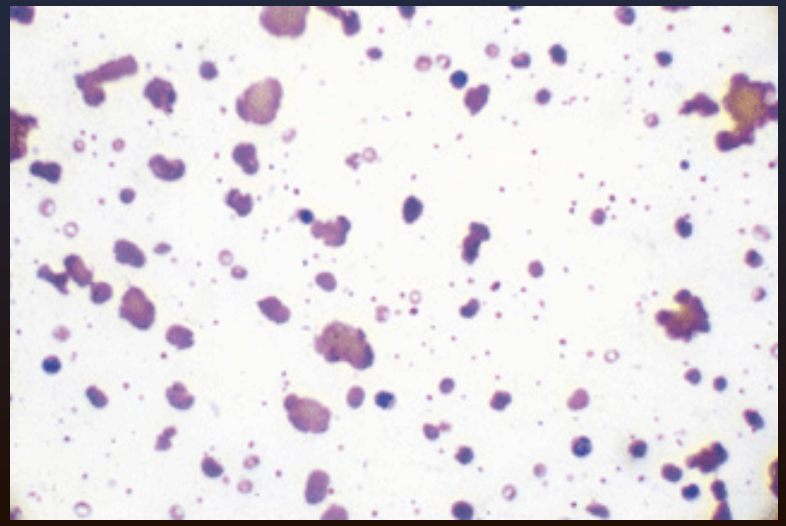
Case 2

A 63 year-old previously healthy male complains of fatigue and lack of energy for 2 months. He denies a history of fever, chills, night sweats or weight loss, but reports painful blue digits in the cold.

A routine CBC drawn 1 year ago was normal. He is taking no new medications and does not recall any recent illness. He denies alcohol consumption, tobacco, or illicit drug use

Laboratory data and blood smear are as follows:

Hct	26%
MCV	133 fL
WBC count	5900/ul
Retic count	7.1 %
LDH	949 IU/L
Coombs	IgG-, C3++



Cold agglutinin Disease

- IgM antibody optimally binds to RBCs at lower temperatures
  - Fixes complement
    - Direct lysis of red blood cells
    - Removal of C3b-coated RBC's by the liver
  - Titers <1:64 usually not clinically significant
    - Titer should increase at lower temperatures
  - Most commonly with I (or i in infections) specificity

Berntsten. Hematology. 2007; 12(5): 361-70

Blood Bank evaluation-Thermal Amplitude Test

- Keep blood at 37°C from point of bedside collection to testing
  - initial screening is often at 20°C or room temperature

Example of Thermal Amplitude Results for a Patient With Cold Agglutinin Disease*

Serum Dilution	4°C	22°C	30°C	37°C
Undiluted	4+	3+	3+	2+
2	4+	3+	2+	2+
4	3+	2+	2+	1+
8	3+	2+	1+	1+
16	2+	1+	1+	0
32	2+	1+	0	0
...	2+/1+	0	0	0
1,024	1+	0	0	0
2,048	0	0	0	0
Titer anti-I	1,024	32	16	8

* Agglutination is read from 0, which is a negative result, to 4+, which is the strongest possible agglutination.

Reardon. Am J Clin Pathol 2006:125(supl1) S71-S77

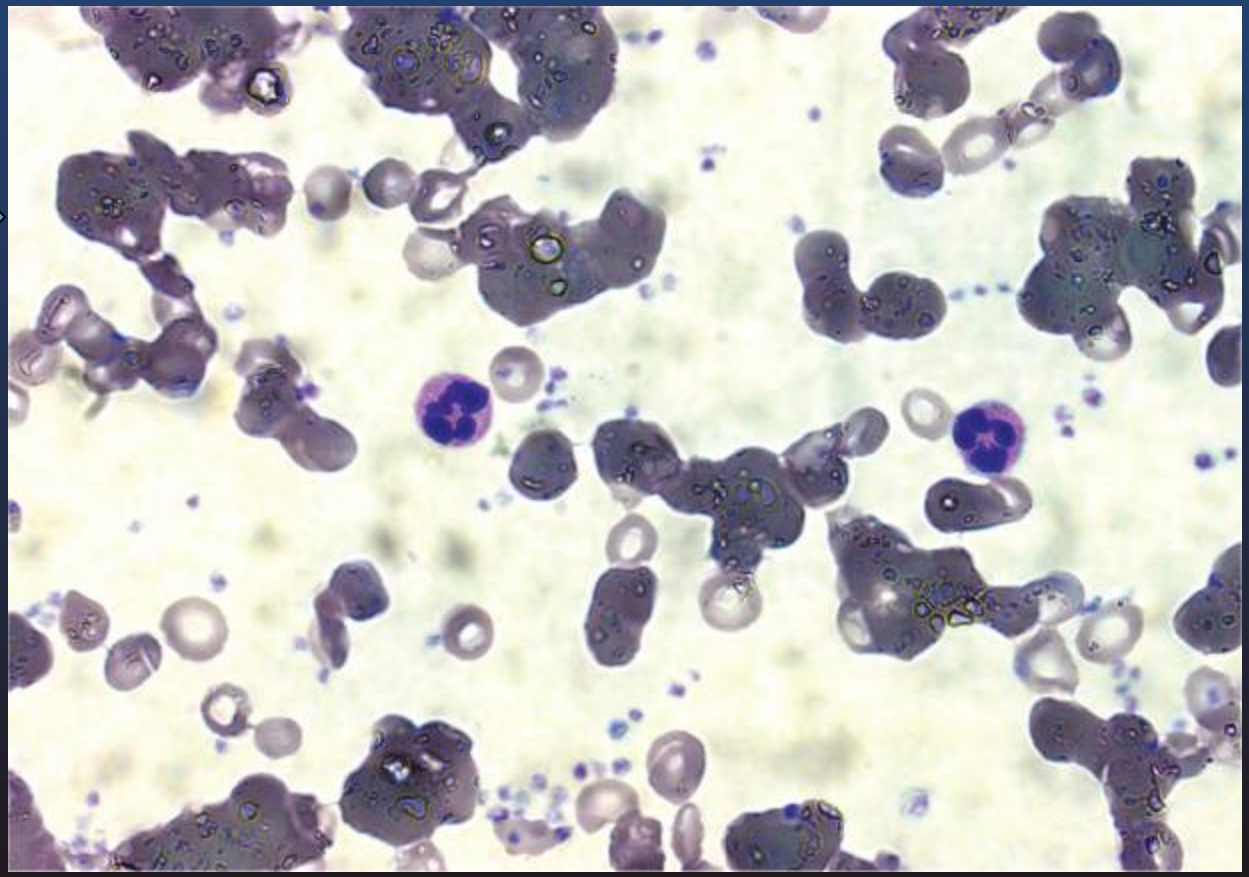
Cold Agglutinin Disease: Diagnostic Criteria

- 1.) Clinical evidence of an acquired hemolytic anemia
- 2.) Positive Coombs test using anti-C3
- 3.) Negative Coombs test using anti-IgG
- 4.) Presence of cold agglutinin with reactivity up to 30°C
- 5.) Cold agglutinin titer at 4°C $\geq$ 256

Petz. Blood Reviews. 2008; 22: 1-15

Cooling of blood during passage through acral parts of the body allows cold agglutinins to bind to the RBC leading to agglutination, complement binding, and hemolysis

RBC agglutination



- Spurious marked elevation of MCV, MCHC may occur
- Agglutination will abate with warming

Berntsten. Hematology. 2007; 12(5): 361-70

Investigation of Cold Agglutinin Disease

- Consider infectious etiologies such as Mycoplasma or Epstein-Barr virus

- Consider lymphoma or other lymphoproliferative disorders
  - Serum protein electrophoresis (SPEP)
  - Bone marrow biopsy if no obvious infection
  - Consider radiologic imaging

Berntsten. Hematology. 2007; 12(5): 361-70

Cold Agglutinin Disease

- Monoclonal band can be detected in the majority of patients on serum protein electrophoresis/ immunofixation
  - Majority IGM κ
    - Kappa 94% in one study
  - Most encoded by IGHV4-34 gene

- Clonal B lymphocytes often found by flow even if bone marrow morphology is negative and no other evidence of lymphoma

Berntsten. Hematology. 2007; 12(5): 361-70

Clinical

- Moderate chronic hemolytic anemia
  - Hb usually 9-12 g/dL
- Most lack physical findings
  - Acrocyanosis
  - Raynaud's phenomenon
  - Gangrene

Petz. Blood Reviews. 2008; 22: 1-15

Cold agglutinin Disease Treatment

- Avoid cold environments (temperatures reach <30° in exposed skin vessels)
  - Wear socks, mittens, ear muffs in cold temperature
  - Transfuse through a blood warmer when necessary
  - Caution with bypass surgery or hypothermic surgeries
- Treat underlying disease
  - Lymphoma
  - If secondary to mycoplasma (anti-I), mono (anti-i) or other virus treatment is supportive and hemolysis transient
- Steroids and splenectomy are not generally effective
  - Unless secondary to steroid responsive disease
    - lymphoma

Petz. Blood Reviews. 2008; 22: 1-15
Gertz. Br J Haem. 2007; 138(4): 422-9

Cold agglutinin Disease Treatment

- Cytotoxic therapy
  - Chlorambucil
    - Begin with low daily doses of 2-4 mg/day
    - Follow counts closely
  - Cyclophosphamide
  - Favorable responses in the minority of cases
  - Risk of suppressing bone marrow and reticuloctye response

Petz. Blood Reviews. 2008; 22: 1-15
Gertz. Br J Haem. 2007; 138(4): 422-9

Cold Agglutinin Disease: Rituximab

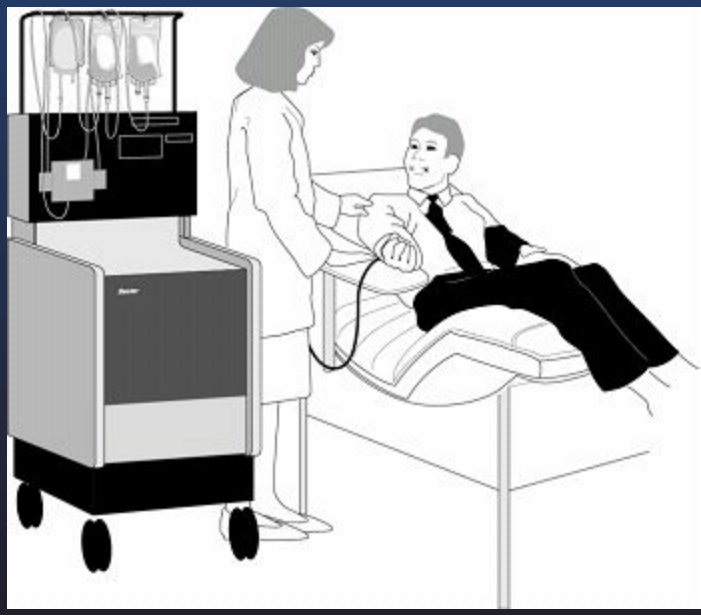
- Gaining popularity with recognition of most cases of CAD as a clonal B cell disorder

- Biggest series
  - 27 patients with 37 courses
  - OR response rate of 54%, mostly partial
  - Responders had a median increase in Hb of 4g/dL and decrease in IgM by 54%
  - Median response time was 1.5 months

Berensten. Blood. 2004; 103:2925-8

Cold Agglutinin Disease: Plasma exchange

- IgM is primarily intravascular



- Used with life-threatening hemolysis or acrocyanosis
  - Rapid, but transient responses
  - Need to use a blood warmer
  - Consider prior to procedures requiring hypothermia

Petz. Blood Reviews. 2008; 22: 1-15

Audience Response Question: Autoimmune hemolytic anemia treatment

Which of the following statements is true concerning the treatment of autoimmune hemolytic anemia?

A.)Splenectomy is an effective second line treatment in cold agglutinin disease

B.)Rituximab has proven effective in trials of cold agglutinin disease, but not warm autoimmune hemolytic anemia

C.)Steroids usually lead to complete, but transient responses in warm autoimmune hemolytic anemia

D.)Cytotoxic therapy is used more frequently in cold agglutinin disease as compared to warm autoimmune hemolytic anemia

Treatment comparison between IgG and IgM mediated hemolysis

	IgG (WAIHA)	IgM (cold aggl.)
Severity	May be severe	moderate
Mechanism of hemolysis	Fc mediated	Complement mediated
Blood smear	Spherocytes	Agglutination
Temperature	Typically warm	cold
Steroids	Yes	No
Rituximab	Yes	Yes
Splenectomy	Yes	No
Cytotoxic	Rarely	Yes
Plasmapheresis	No	Yes

Case 3

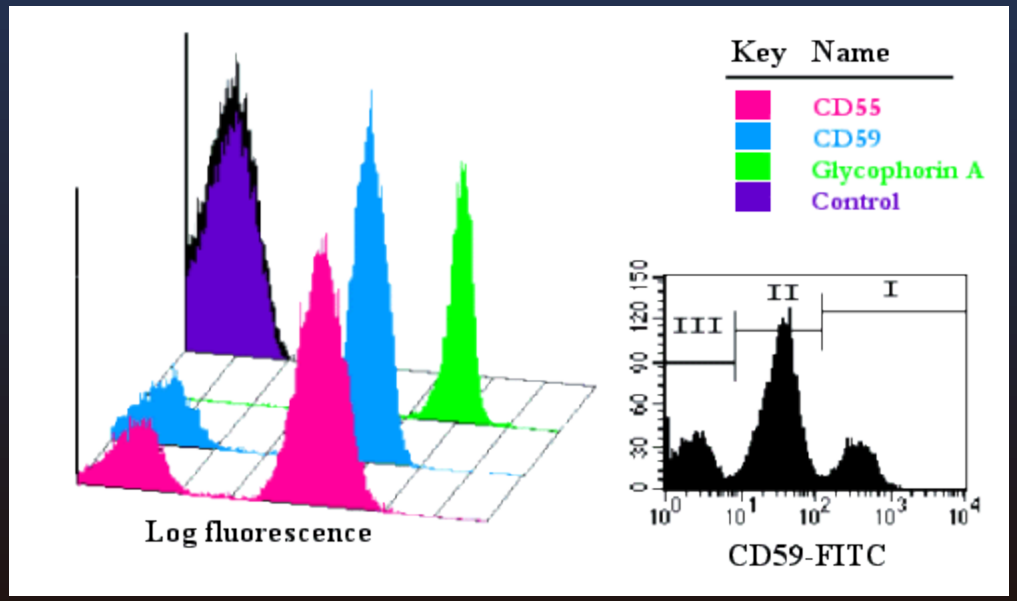
A 43 year-old male is referred with a 3 month history of transfusion dependant anemia. Prior history 1 year ago is significant for an unprovoked DVT. His only medication is warfarin. Evaluation has been negative for GI bleeding. Blood counts 3 years ago were normal

WBC count	3900/ul
Hb	9.3 g/dL
Hct	29%
Plt	127k/cc mm
MCV	107 fL
Retic count	5.1 %
LDH	849 IU/L
Haptoglobin	undetectable
Coombs	negative
Peripheral smear	normal morphology

Case

- Given Coombs negative, non spherocytic hemolytic anemia, recent DVT, and pancytopenia, PNH is suspected and peripheral blood is sent for CD-55 and CD-59 on RBCs

Diagnosis: PNH

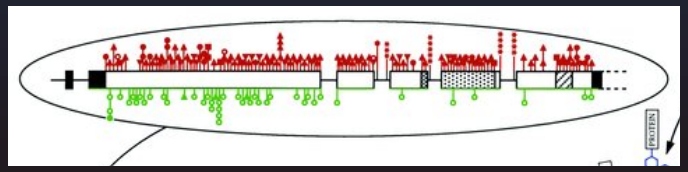


Acquired clonal hematopoietic stem cell disorder

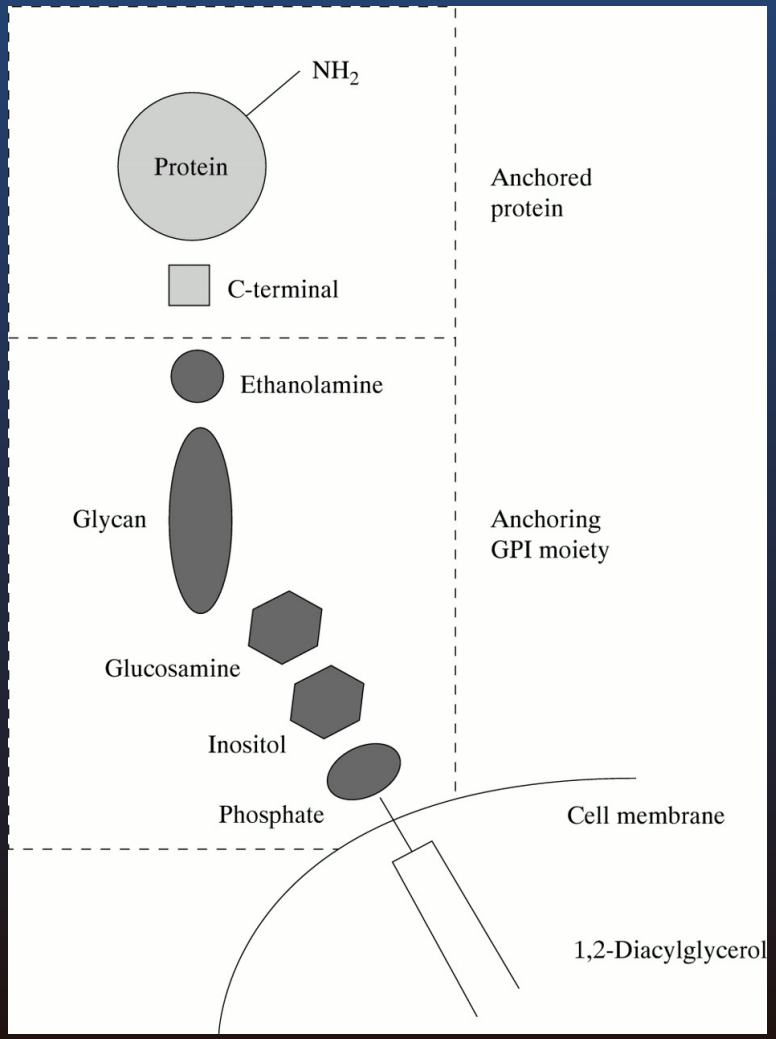
The glycosyl phosphatidylinositol (GPI) anchor

Due to a mutation in the phosphatidylinositol glycan complementation class A gene (PIGA)

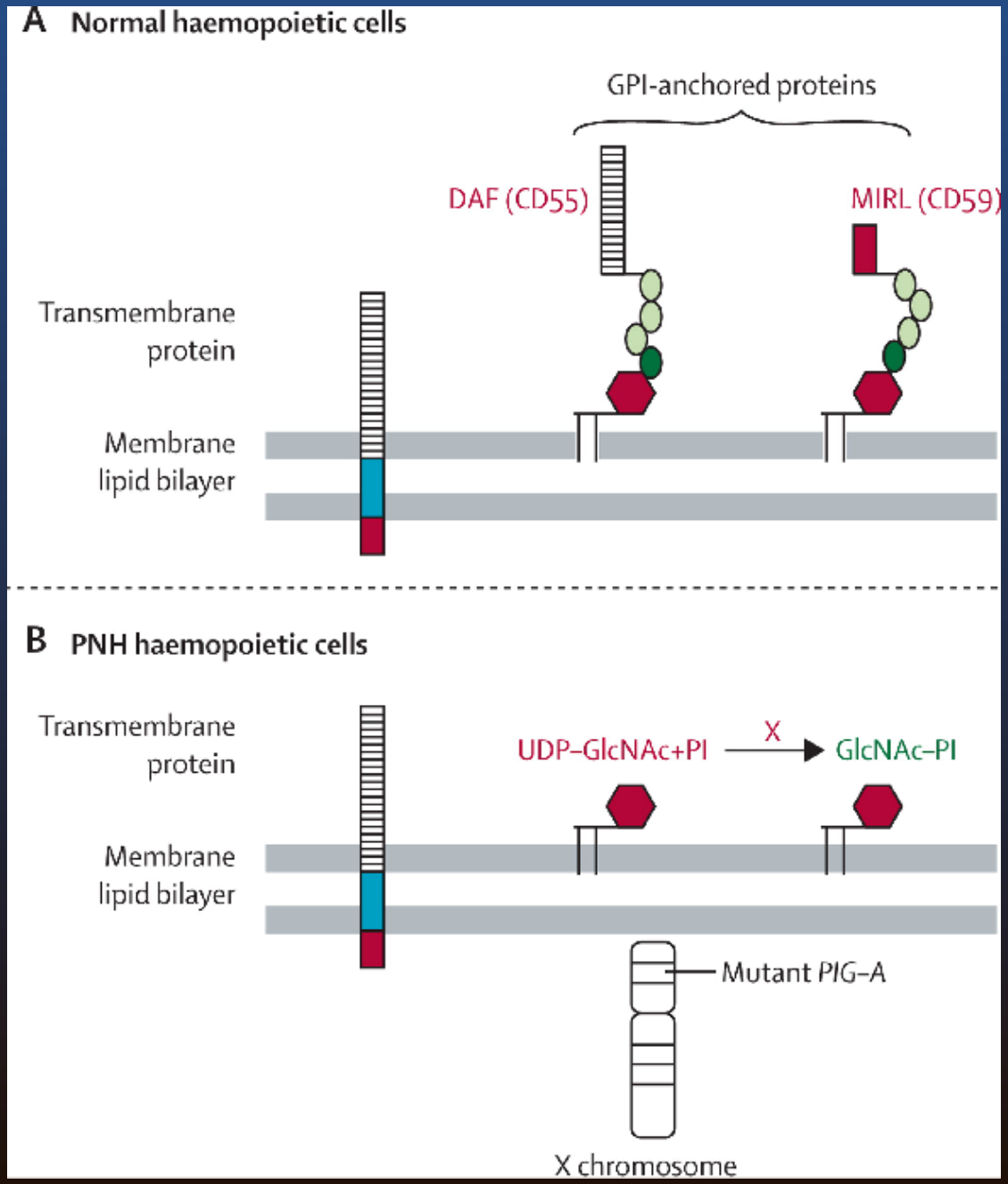
- Needed to make glycosyl phosphatidylinositol (GPI), a molecule that anchors specific proteins to the cell membrane
- Lack of this anchor leads to a variety of clinical sequela



PIG A gene
Xp22.1

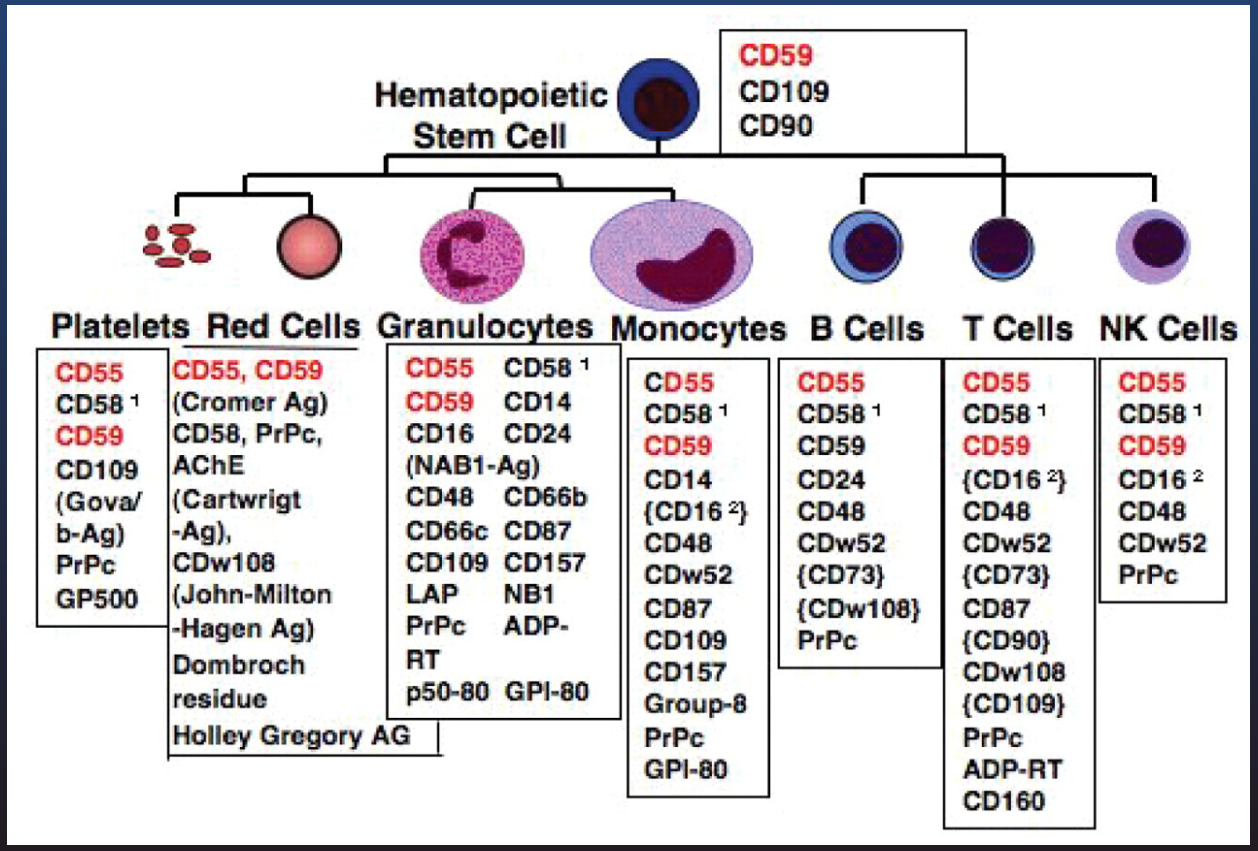


Johnson R J , Hillmen P Mol Path 2002;55:145-152



Parker. Lancet. 2009 Feb 28;373(9665):759-67

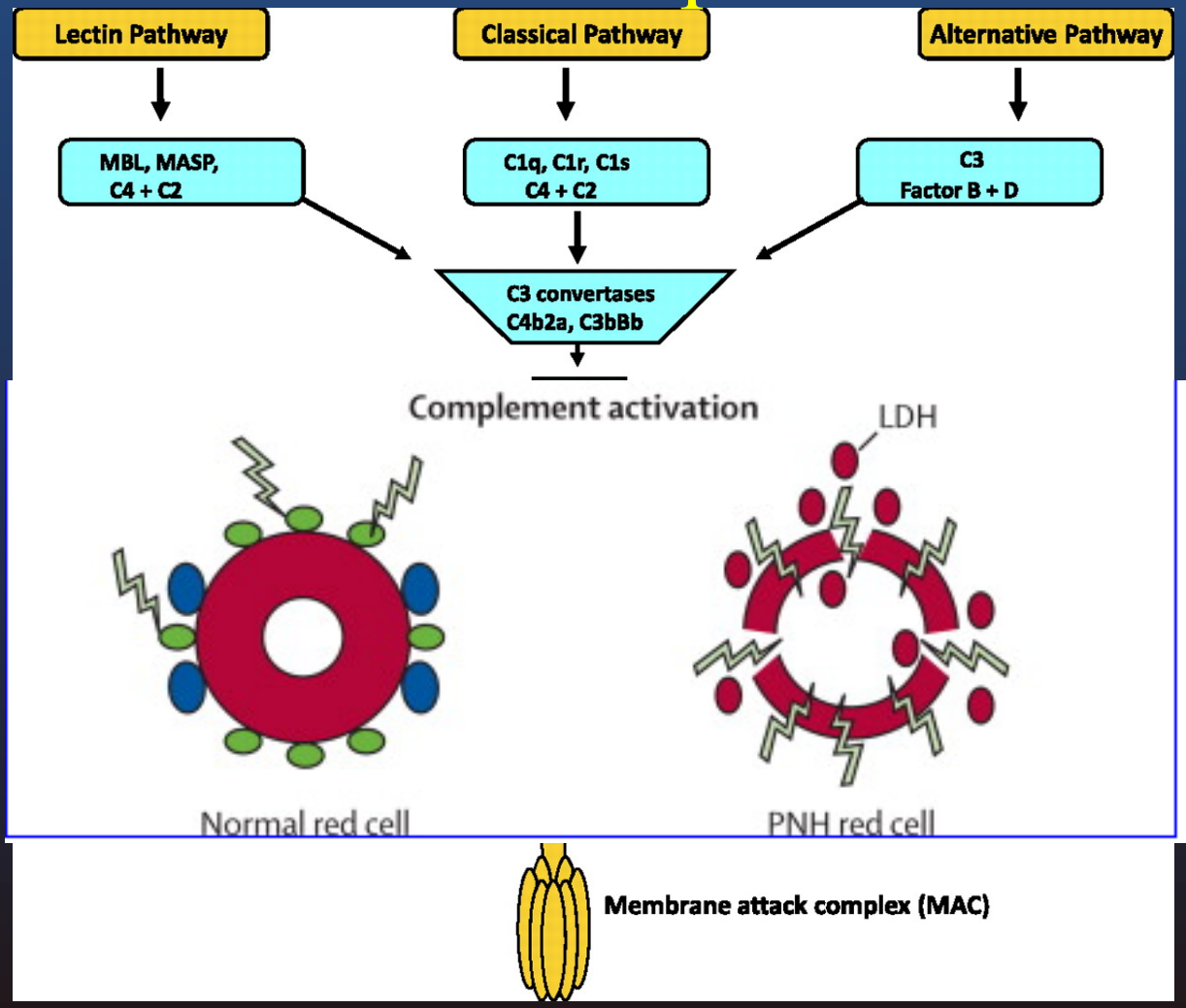
GPI-anchored surface proteins on human hematopoietic cells



Hematology 2008;2008:491-506

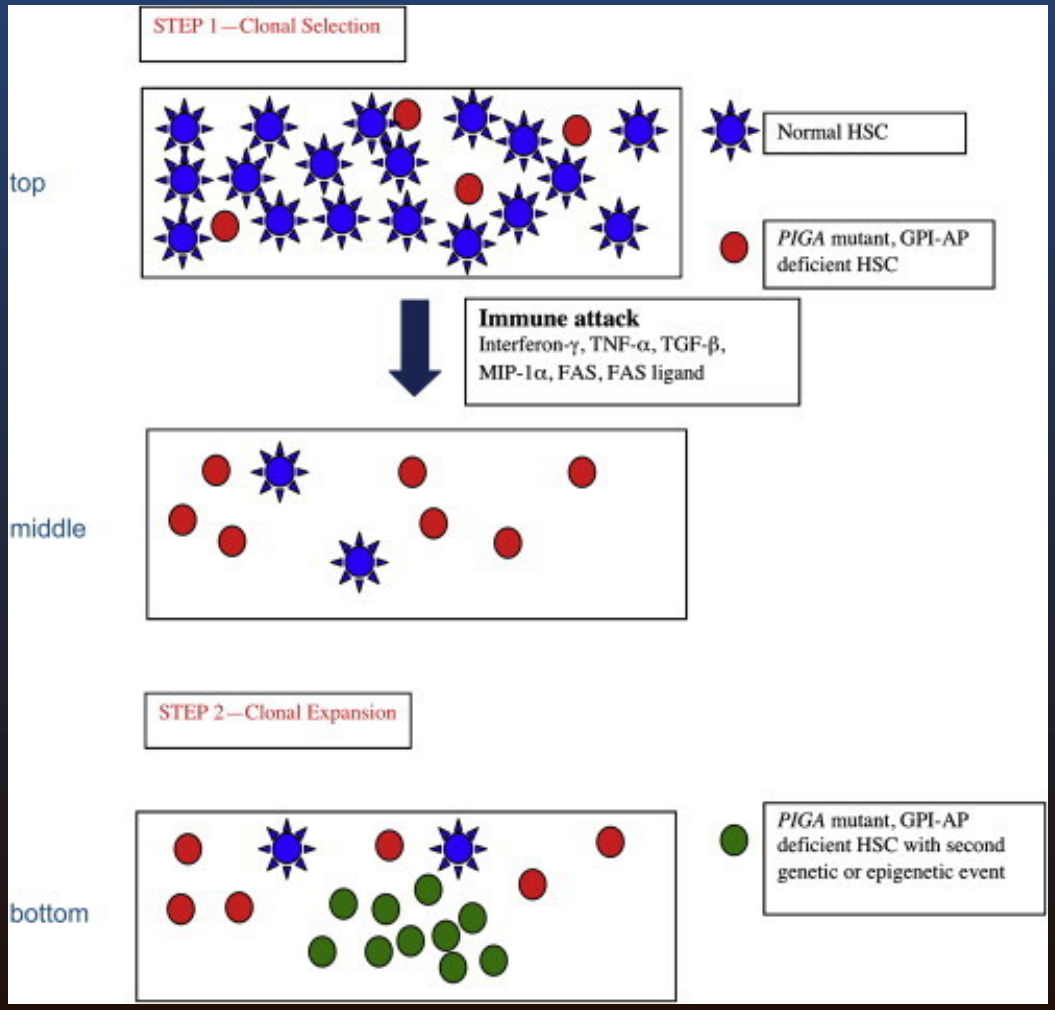
blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Overview of the complement cascade



Brodsky, R. A. Blood 2009;113:6522-6527
Parker. Lancet. 2009 Feb 28;373(9665):759-67

PNH: Selective Survival Advantage



Parker. Hematol Oncol Clin North AM 2009; 23(2):333-46

PNH: Clinical

- Median survival is 10-15 years
- No current cure other than bone marrow transplantation
- 10-15% have spontaneous remission

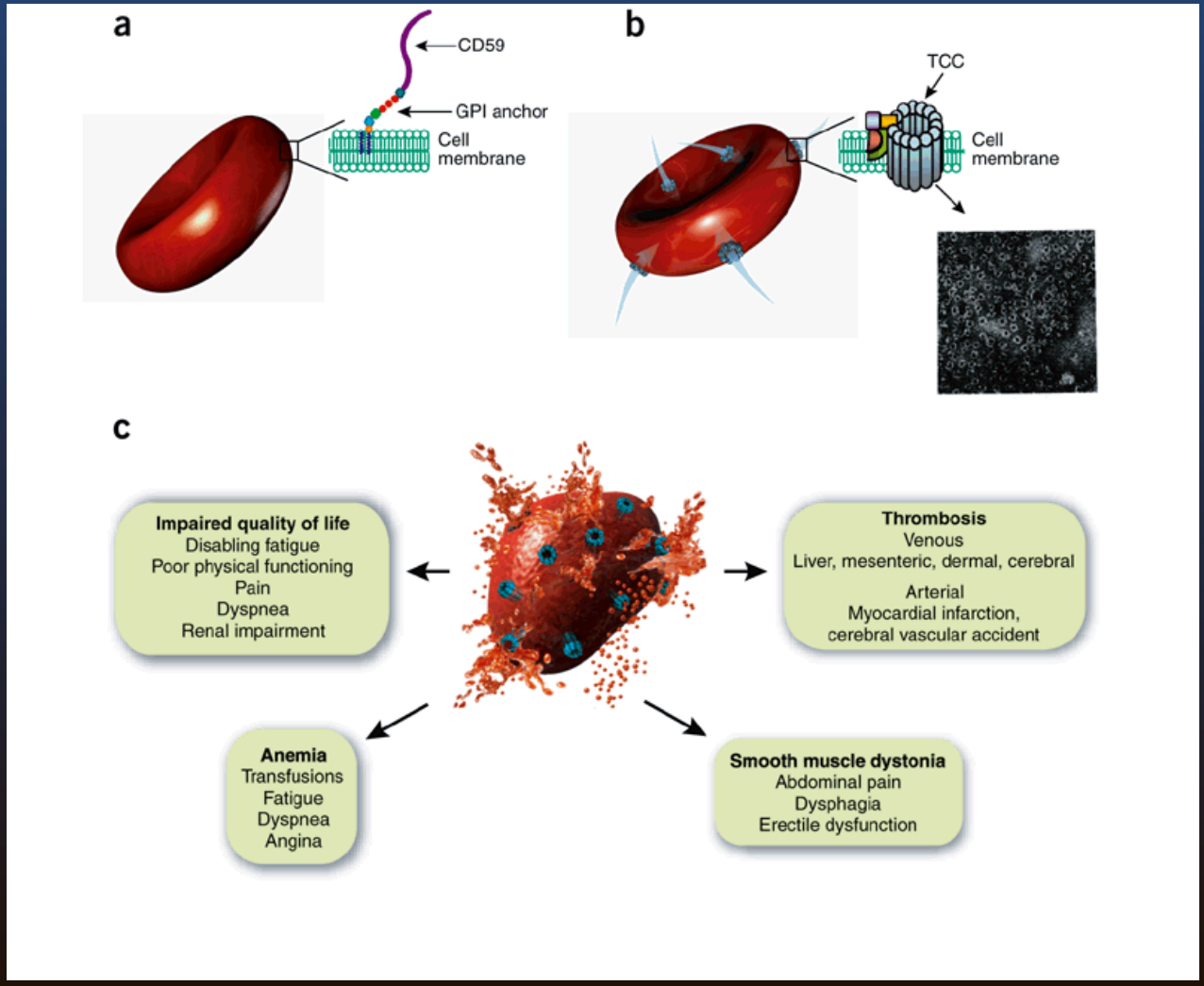
Bessler. Hematology Am Soc Hematol Educ Progam 2008: 104-110

Audience Response Question: Clinical Manifestations

- Which of the following is not a well described clinical sequela of PNH

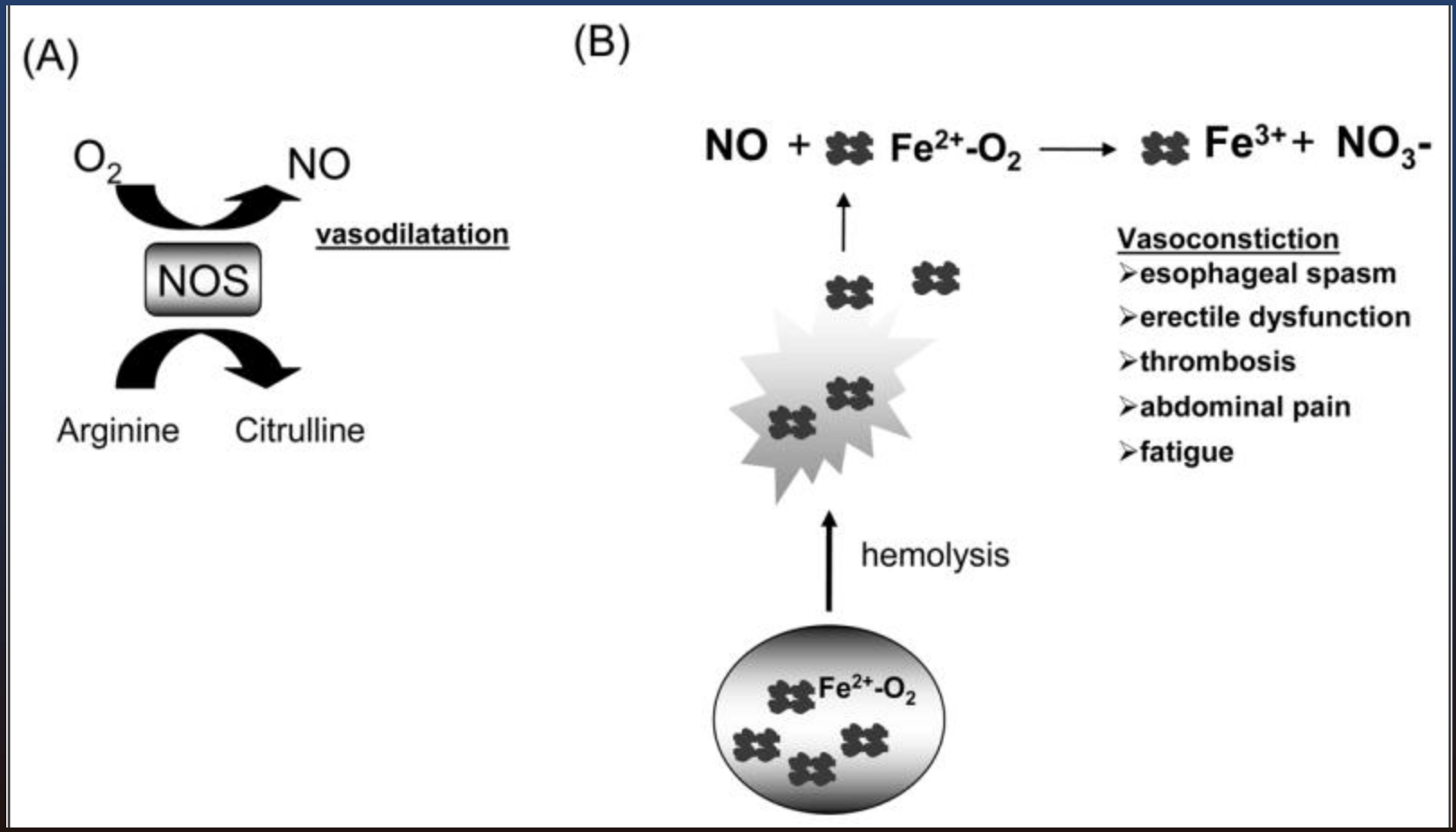
- A.)Thrombosis of the hepatic vessels
- B.)Transfusion dependent anemia
- C.)Esophageal spasm
- D.)Pancytopenia
- E.)Pulmonary fibrosis
- F.)Renal dysfunction
- G.)Erectile dysfunction

Clinical Manifestations



Nature Biotechnology 25, 1256 - 1264 (2007) Published online: 7 November 2007

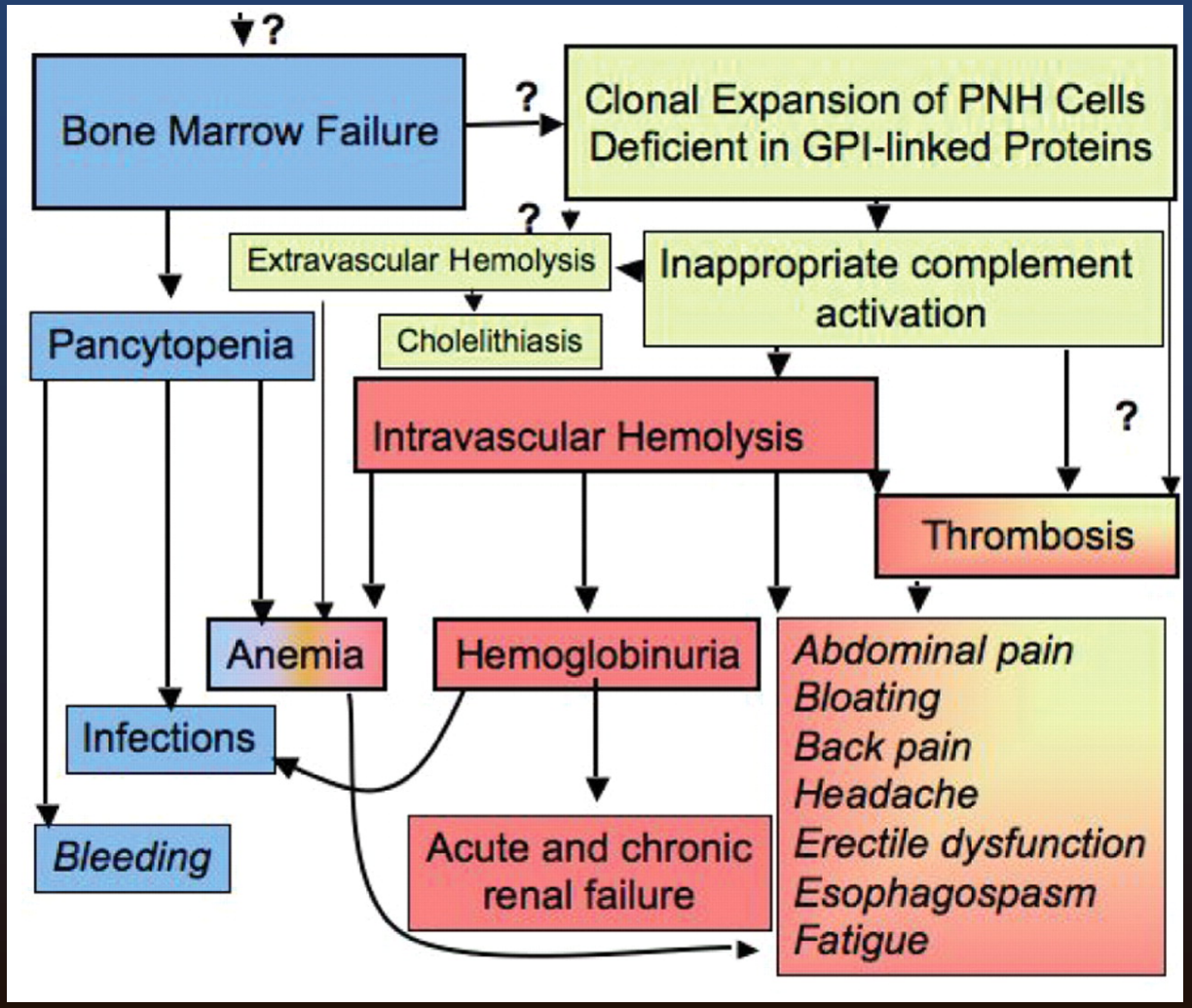
PNH: Consequences of chronic hemolysis



Brodsky. Blood Reviews. 2008. 22:65-74

HEMATOLOGY
ASH Education Program Book

The pathophysiology of disease in patients with paroxysmal nocturnal hemoglobinuria (PNH)



Hematology 2008;2008:491-506

PNH: Thrombosis

- Increased propensity for life threatening thrombosis
  - Cerebral, hepatic, portal, mesenteric, splanchnic, and renal veins
  - Approximately 40% of patients have a thrombotic event during their illness
    - Higher risk with larger PNH clone (>60%)
    - Venous greater then arterial
    - Main cause of death

Brodsky. Blood Reviews. 2008. 22:65-74

PNH: Bone Marrow Failure

- Small to moderate PNH clones found in up to 70% of patients with aplastic anemia
  - Usually less than 30% PNH granulocytes
  - May evolve into clinical PNH
  - 20% of MDS patients have small clones

- Small populations of PNH cells can be found in virtually all healthy controls
  - Approx 1 in 50,000 granulocytes
  - Arise from a more differentiated colony forming cell
  - No self renewal capacity

Brodsky. Hematology Am Soc Hematol Educ Program 2008.; 104-110

PNH: Bone Marrow Failure

- Most patients with clinical manifestations of PNH who do not have overt signs of marrow aplasia have evidence of diminished hematopoiesis
  - Two-thirds exhibit granulocytopenia and/or thrombocytopenia at some time during the course of their disease

- Leukemia develops in up to 5% of patients
  - The average onset occurs at about five years (range is from a few months to 22 years)

Dacie, JV. Proc R Soc Med 1963; 56:587
Rosse. Up-To-Date. Clinical manifestations of paroxysmal nocturnal hemoglobinuria. January 2010 last update

PNH: Classifications

Table 1 -- Classification of PNH [a]

Category	Rate of Intravascular Hemolysis [b]	Bone Marrow	Analysis of Glycosyl Phosphatidylinositol–Anchored Protein Expression by Flow Cytometry
Classic	Florid (macroscopic hemoglobinuria is frequent or persistent)	Cellular marrow with erythroid hyperplasia and normal or near-normal morphology [c]	Large population (> 50%) of GPI-AP–deficient PMNs [e]
PNH in the setting of another bone marrow failure syndrome [d]	Mild to moderate (macroscopic hemoglobinuria is intermittent or absent)	Evidence of a concomitant bone marrow failure syndrome [d]	Although variable, the percentage of GPI-AP–deficient PMNs [e] usually is relatively small (< 30%)
Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant bone marrow failure syndrome [d]	Small (< 1%) population of GPI-AP–deficient PMNs detected by high-resolution flow cytometry

Parker. Hematol Oncol Clin North AM 2009; 23(2):333-46

Who should be tested for PNH

- All patients with unexplained:

All patients with unexplained hemoglobinuria
All patients with unexplained Coombs negative nonspherocytic hemolytic anemia
All patients with unexplained visceral or cerebral vein thrombosis
All patients with unexplained cytopenias
Unexplained iron deficiency
PNH symptoms—fatigue, esophageal spasms,
Unexplained erectile dysfunction

Rosse. HemeOnc Today. Feb 10, 2010. 4-5

Audience Response Question: Diagnostic Testing

Which of the following is a true statement regarding diagnostic testing in PNH?

A.)Screening of only red blood cells can lead to falsely negative tests

B.)FLAER is the most sensitive reagent to detect PNH on red blood cells

C.)The percentage of PNH red blood cells more accurately reflects the size of the clone as compared to white blood cells

D.)CD 55 and CD 4 are the most common GPI anchored protein antigens evaluated on RBCs of PNH patients

Laboratory Testing

Bessler. Hematology Am Soc Hematol Educ Program 2008.;

Table 1 Laboratory tests for the diagnosis of PNH

Laboratory tests	Principle	Advantages	Disadvantages
Ham test Sucrose lysis test	The lysis of PNH red blood cells exposed to activated complement	Cheap and simple to perform	Labor intensive, low sensitivity and specificity, not quantitative
Sephacryl gel card test	Haemagglutinin test using the gel microtyping system	Cheap and simple to perform	Low sensitivity and not quantitative
Flow cytometric analysis CD 59 and CD55 on RBC	Study expression of GPI-AP using monoclonal antibodies by flowcytometry	Rapid, sensitive, and quantitative	Lower estimate of clone size because of shorter life span of RBC
CD 59, CD55, CD24, CD66b, and CD16 for granulocytes		Better estimate of clone size	Require fresh blood sample Difficult in patients with AA with neutropenia
FLAER for granulocytes	Lack of FLAER binding to PNH granulocytes	Highly sensitive as single reagent for diagnosis	

PNH, paroxysmal nocturnal haemoglobinuria; RBC, red blood cells; GPI-AP, GPI-anchored proteins; AA, aplastic anemia; FLAER.

Madkaikar. Eur J of Haem. 2009; 83: 503-11

Flow Cytometry on RBCs: Three different phenotypes

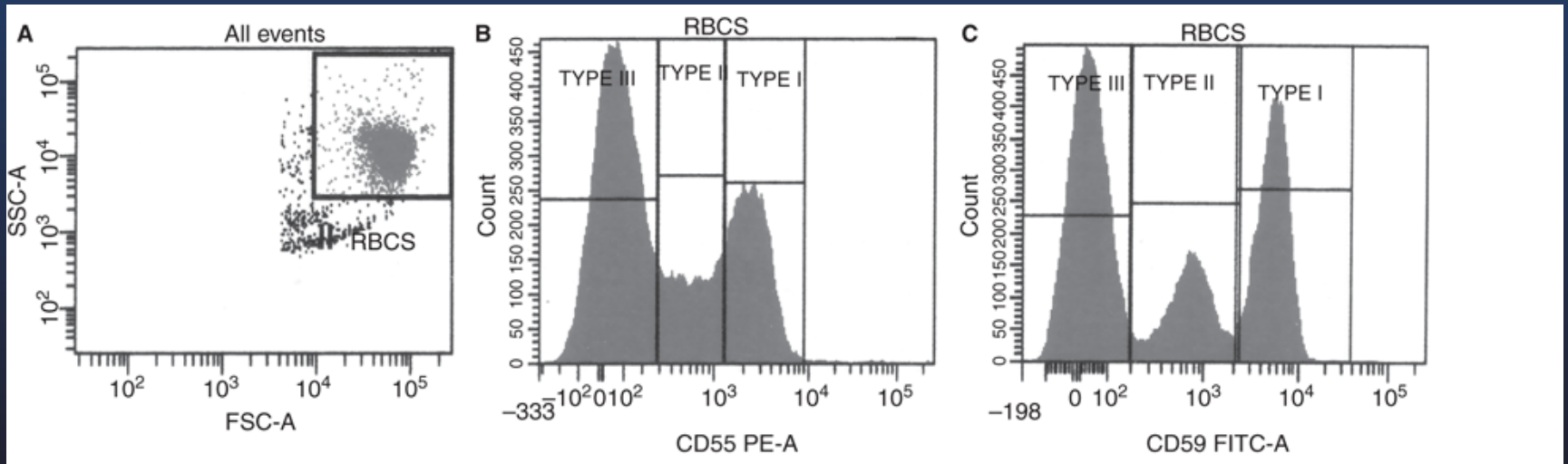


Figure 1 Expression of the GPI-linked antigens on RBC in patient with paroxysmal nocturnal haemoglobinuria (PNH). (A) Gating of RBC using forward scatter (FSC) and sideways scatter (SSC) amplification in log mode. (B) Histogram showing expression of CD59 on RBC with clear separation of Type I, II and III cells. (C) Histogram showing expression of CD55 on same patient with poor separation between Type I, II, & III cells compared to CD59.

Madkaikar. Eur J of Haem. 2009; 83: 503-11

Flow Cytometry: RBC vs. WBC

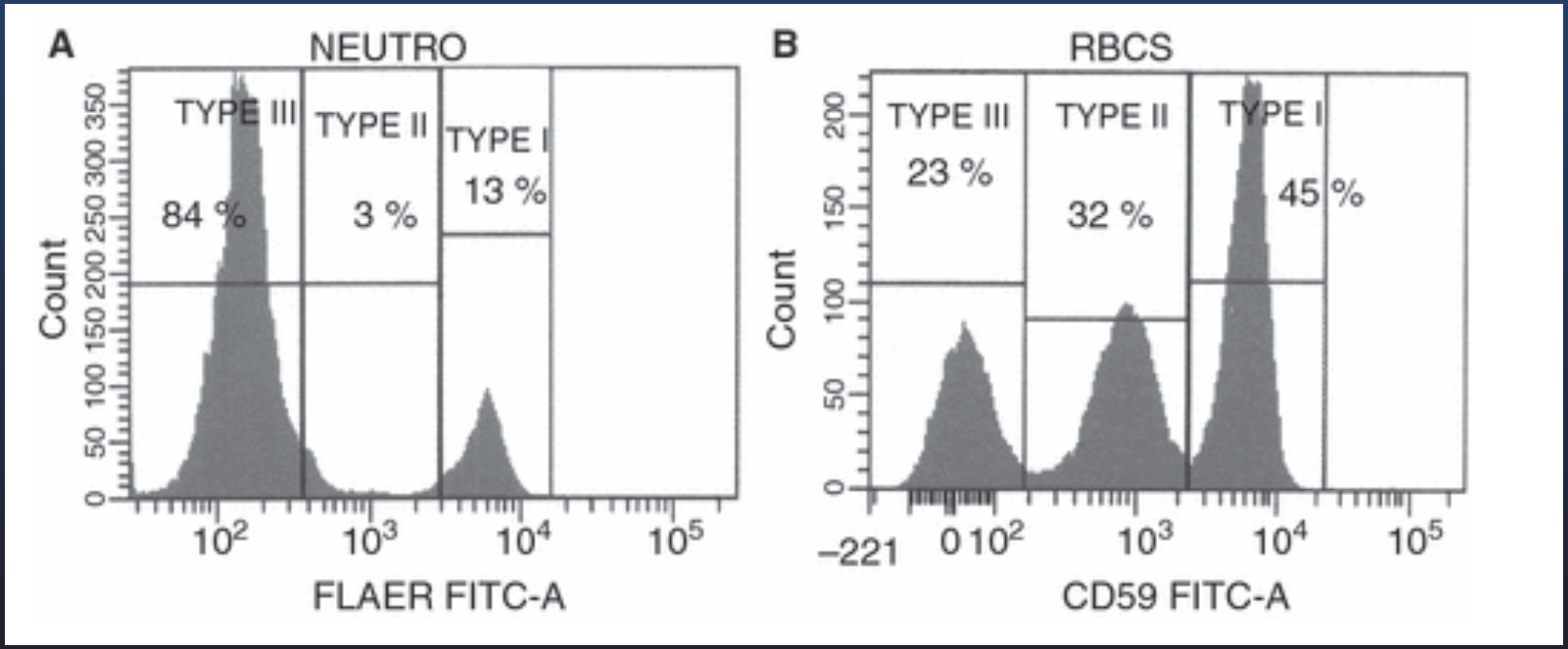
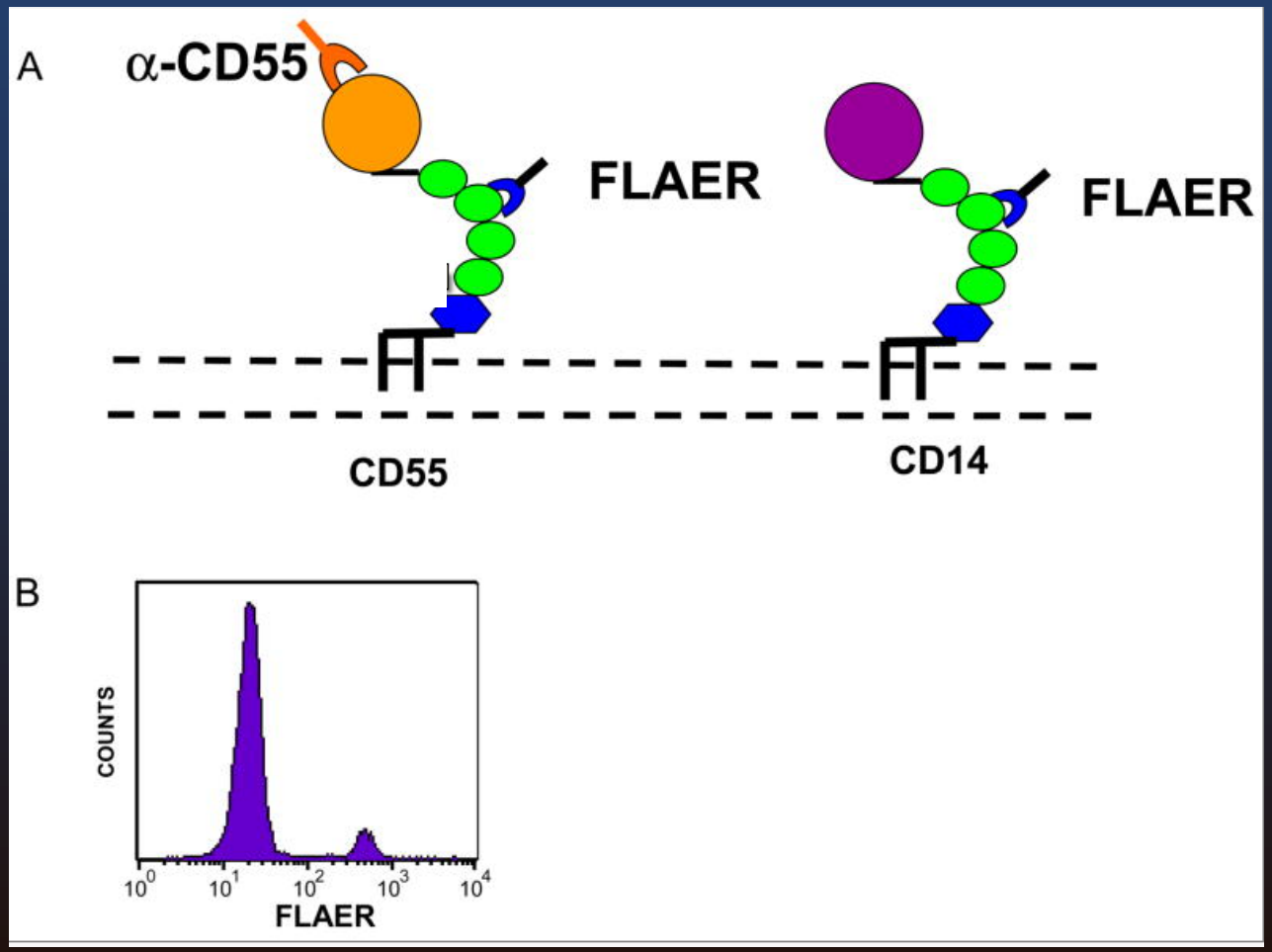


Figure 2 PNH clone size and the proportion of Type I, II, and III cells differs on neutrophils and RBC done simultaneously on the same patient. (A) Shows analysis of granulocyte with FLAER showing predominantly Type III cells. (B) Shows analysis on RBC with CD59 on the same sample showing markedly reduced Type III cells and higher proportion of Type II cells.

Madkaikar. Eur J of Haem. 2009; 83: 503-11

FLAER



Brodsky. Blood Reviews. 2008. 22:65-74

PNH:
FLAER vs. immunophenotyping

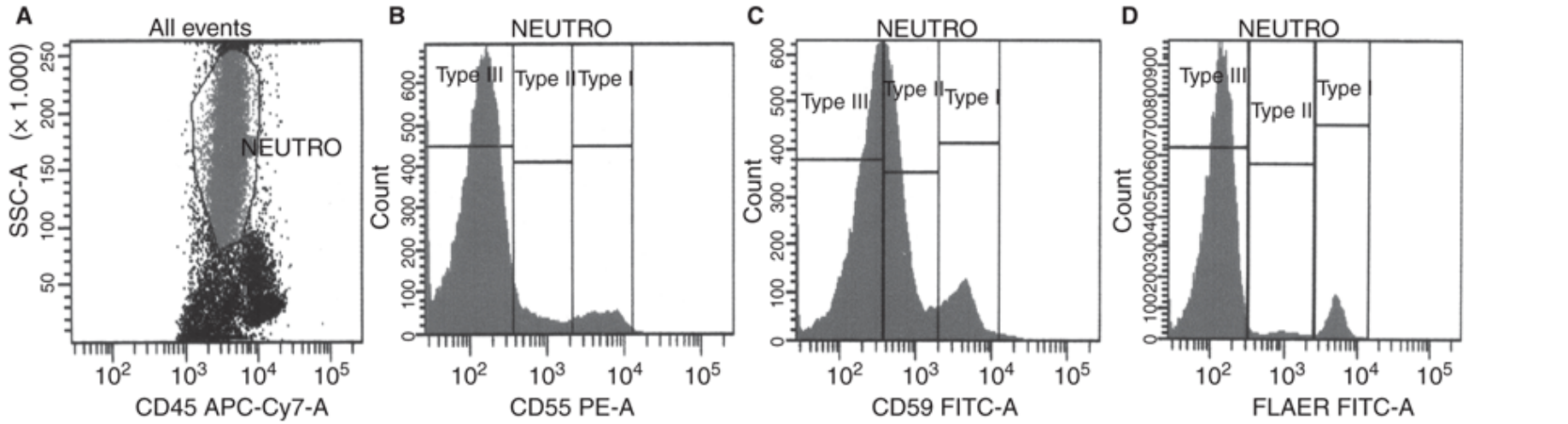


Figure 3 Expression of CD55, CD59 and FLAER on neutrophils in a patient with PNH. (A) Shows gating of neutrophils using SSC/CD45 gating strategy. (B) Histogram showing expression of CD55 on neutrophils with Type I, II, and III cells. (C) Histogram shows expression of CD59 on same patient showing poor separation between Type I, II, & III cells compared to CD55. (D) Histogram shows FLAER expression with clear separation of Type I, II, & III cells.

Madkaikar. Eur J of Haem. 2009; 83: 503-11

PNH: FLAER vs. immunophenotyping

Table 2 Comparison between FLAER and immunophenotyping for the diagnosis of PNH

FLAER	Immunophenotyping using monoclonal antibodies against GPI-AP
Sensitive as a single agent and hence more economical as screening test	At least two antibodies required
Detection of PNH clone only on leukocytes	Detection of PNH clone on all peripheral blood cells
Better separation Type I, II, and III cells on granulocytes	Separation of Type I, II, and III cells on granulocytes is not always clear
Better estimation of clone size on granulocytes and monocytes and hence useful for estimation of small clone on granulocyte in AA and MDS using multiparametric assay	Essential for estimation of clone size on RBCs and monitoring of RBC clone size in patients on Eculizumab therapy
More robust assay for detection of clone on granulocytes, can be performed on samples stored up to 48 h	Analysis on granulocyte needs to be performed within 8 h of collection, but analysis on RBCs can be done in samples stored up to 21–30 d

PNH, paroxysmal nocturnal haemoglobinuria; GPI-AP, GPI-anchored proteins; AA, aplastic anemia; MDS, myelodysplastic syndrome.

Madkaikar. Eur J of Haem. 2009; 83: 503-11

Historical Management of PNH

Palliative options do not impact progression and carry risk for severe morbidity and mortality

- Steroids/androgen hormones
  - No controlled clinical trials
- Red cell supplements
  - Folic acid, iron, erythropoiesis-stimulating agents
  - ESAs may expand clones and elevate hemolysis
- Transfusions
  - Transient treatment of anemia
  - Risk of iron overload
- Anticoagulants
  - Prophylaxis is debated, especially if >50% clone
  - Warfarin is recommended for all with VTE
  - Maybe ineffective in certain patients

1. Hillmen, et al. *N Engl J Med*. 1995;333:1253-1258.
2. Parker, et al. *Blood*. 2005;106:3699-3709.
3. Saso, et al. *Brit J Haem*. 1999;104:392-396

ESA=erythropoietin stimulating agents

Historical Management of PNH

Bone Marrow Transplant

- Allogeneic bone marrow transplant
  - 44% mortality at 2 yrs with HLA-matched sibling donor
  - Acute GVHD in 34%; chronic GVHD in 33%
  - GVHD-free survival in 14% of patients

1. Parker, et al. *Blood*. 2005;106:3699-3709.
2. Saso, et al. *Brit J Haem*. 1999;104:392-396.
3. Hegenbart et al. Biology of Blood and Marrow Tplt. 2003;9:689-697

Audience Response Testing: Ecluzimab

Which of the following is the most likely consequence in a PNH patient treated with Ecluzimab?

A.)Increase in hemoglobin to normal

B.) *Neisseria meningitides* infection

C.)Increase in the percentage of Type III (totally deficient of GPI linked proteins) erythrocytes

D.)Deep vein thrombosis

E.)Stabilization of LDH

blood
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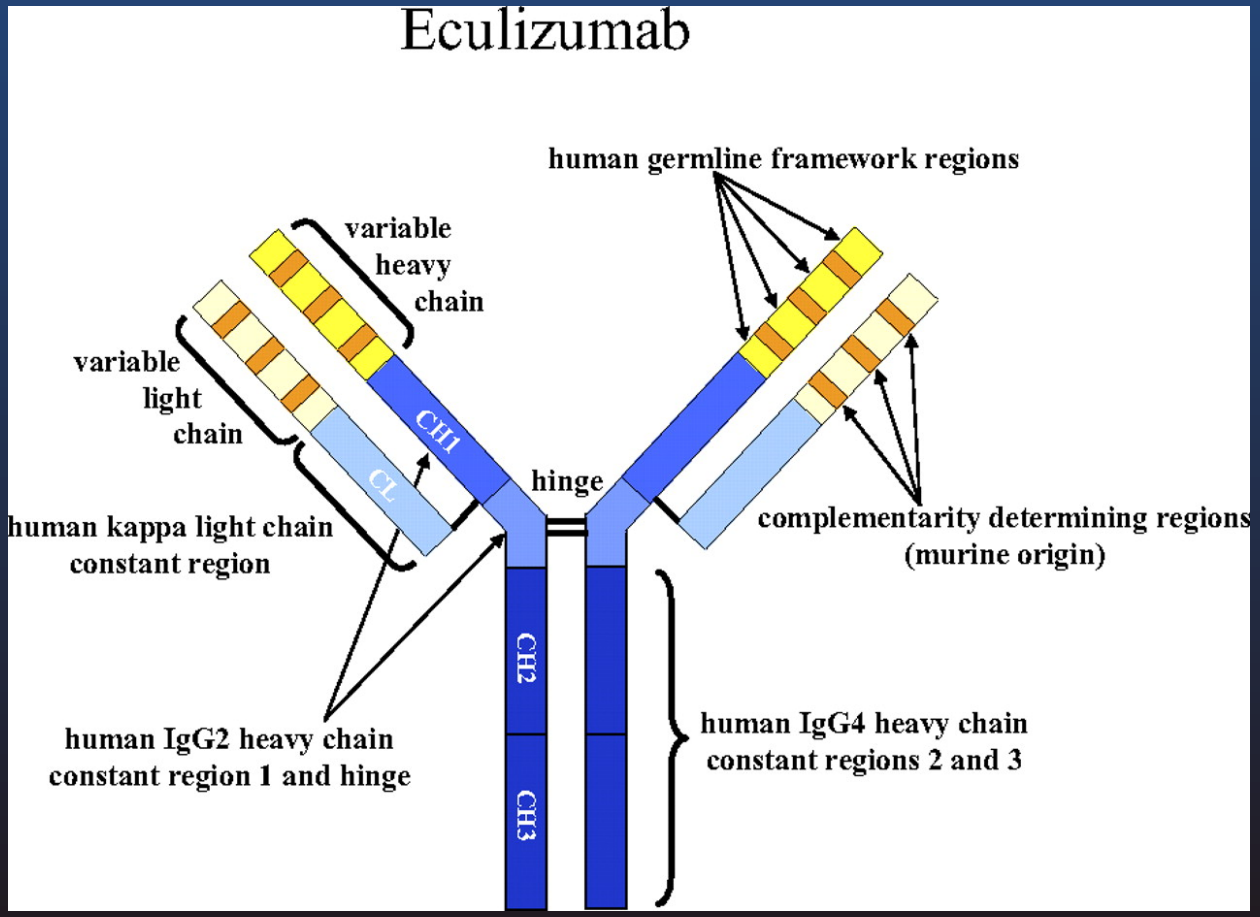
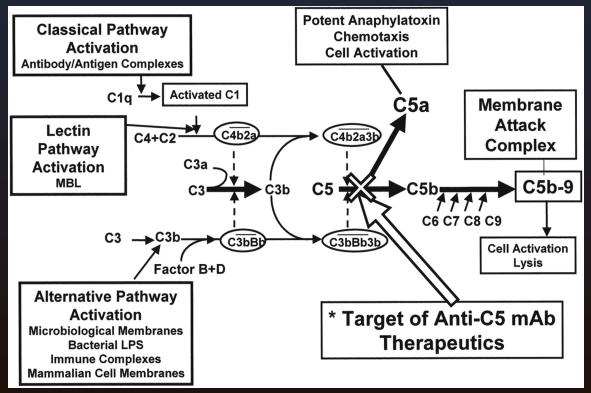
Structure of eculizumab



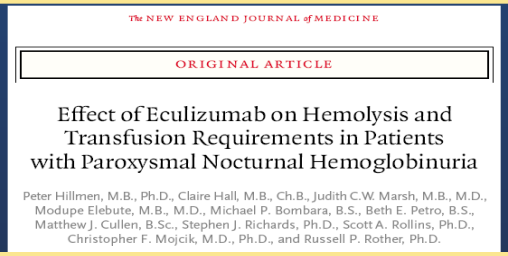


Brodsky, R. A. Blood 2009;113:6522-6527

Hillmen, P. Hematology 2008;2008:116-123

Ecluzimab PNH Clinical Studies

Pilot Study – *NEJM*. 2004
N = 11

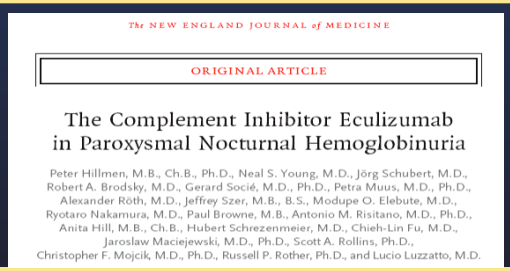

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ph.D., Claire Hall, M.B., Ch.B., Judith C.W. Marsh, M.B., M.D., Modupe Elebute, M.B., M.D., Michael P. Bombara, B.S., Beth E. Petro, B.S., Matthew J. Cullen, B.Sc., Stephen J. Richards, Ph.D., Scott A. Rollins, Ph.D., Christopher F. Mojcik, M.D., Ph.D., and Russell P. Rother, Ph.D.

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


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

The Complement Inhibitor Eculizumab in Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ch.B., Ph.D., Neal S. Young, M.D., Jörg Schubert, M.D., Robert A. Brodsky, M.D., Gerard Socié, M.D., Ph.D., Petra Muus, M.D., Ph.D., Alexander Röth, M.D., Jeffrey Szer, M.B., B.S., Modupe O. Elebute, M.D., Ryotaro Nakamura, M.D., Paul Browne, M.B., Antonio M. Risitano, M.D., Ph.D., Anita Hill, M.B., Ch.B., Hubert Schrezenmeier, M.D., Chieh-Lin Fu, M.D., Jaroslaw Maciejewski, M.D., Ph.D., Scott A. Rollins, Ph.D., Christopher F. Mojcik, M.D., Ph.D., Russell P. Rother, Ph.D., and Lucio Luzzatto, M.D.

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


blood

2008 111: 1840-1847
Prepublished online Nov 30, 2007;
doi:10.1182/blood-2007-06-094136

Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria

Robert A. Brodsky, Neal S. Young, Elisabetta Antonioli, Antonio M. Risitano, Hubert Schrezenmeier, Jörg Schubert, Anna Gaya, Luke Coyle, Carlos de Castro, Chieh-Lin Fu, Jaroslaw P. Maciejewski, Monica Bessler, Henk-André Kroon, Russell P. Rother and Peter Hillmen

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

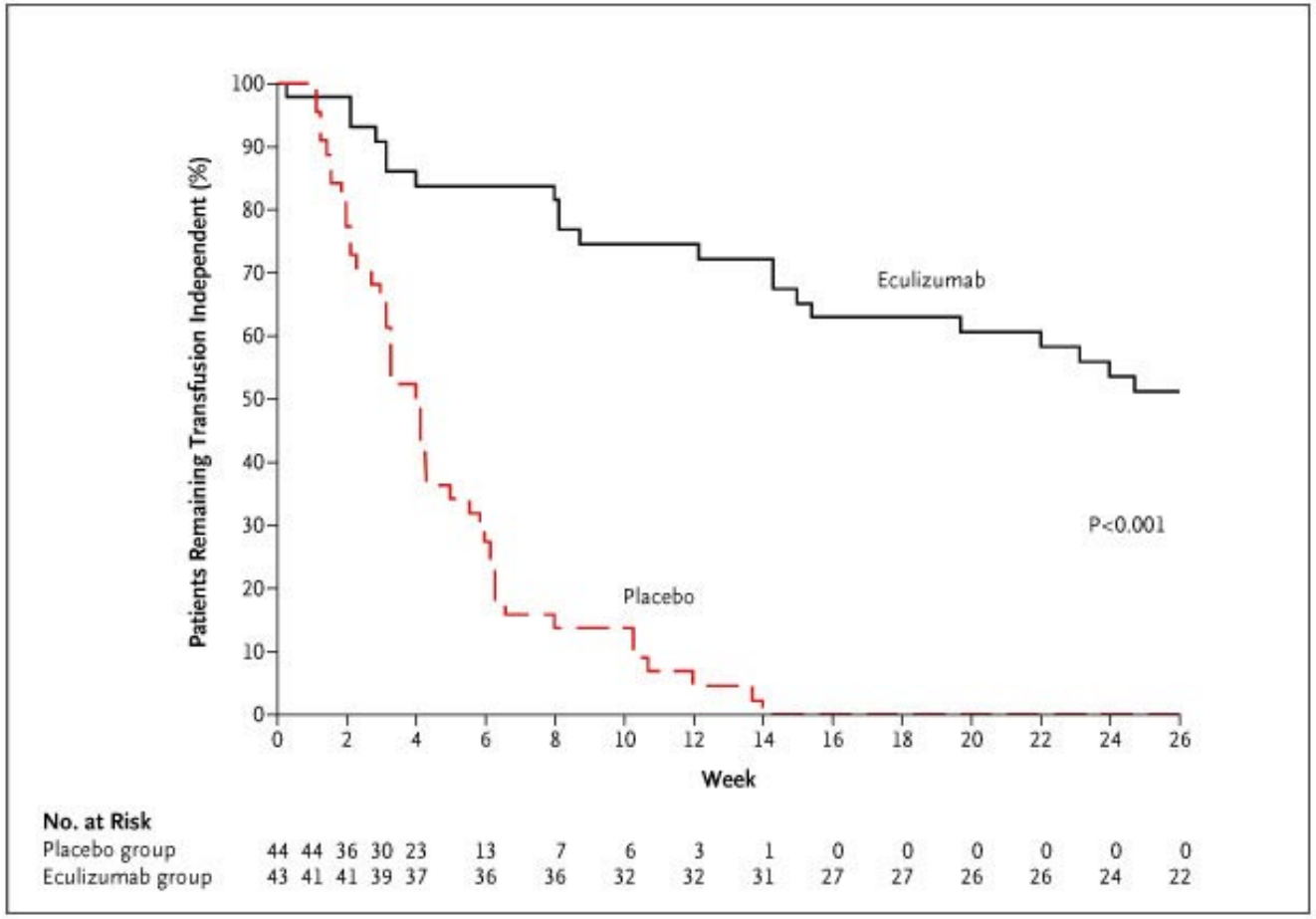

Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria

Peter Hillmen,[1] Petra Muus,[2] Ulrich Dührsen,[3] Antonio M. Risitano,[4] Jörg Schubert,[5] Lucio Luzzatto,[6] Hubert Schrezenmeier,[7] Jeffrey Szer,[8] Robert A. Brodsky,[9] Anita Hill,[1] Gerard Socié,[10] Monica Bessler,[11] Scott A. Rollins,[12] Leonard Bell,[12] Russell P. Rother,[12] and Neal S. Young[13]

[1]Leeds General Infirmary, Leeds, United Kingdom; [2]Radboud University Medical Center, Nijmegen, The Netherlands; [3]University Essen, Essen, Germany; [4]Federico II University, Naples, Italy; [5]Saarland University Medical School, Homburg-Saarland, Germany; [6]Istituto Toscano Tumori, Florence, Italy; [7]Institute of Transfusion Medicine, University Hospital, Ulm, Germany; [8]Royal Melbourne Hospital, Melbourne, Australia; [9]Johns Hopkins School of Medicine, Baltimore, MD; [10]Hôpital Saint-Louis and Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France; [11]Washington University, St Louis, MO; [12]Alexion Pharmaceuticals, Cheshire, CT; and [13]National Heart, Lung, and Blood Institute, Bethesda, MD

Modified Alexion slide deck

Kaplan-Meier Curves for the Time to the First Transfusion during Treatment



The NEW ENGLAND JOURNAL of MEDICINE

Hillmen P et al. N Engl J Med 2006;355:1233-1243

Stabilization of Hemoglobin Levels and the Number of Units of Packed Red Cells Transfused during Treatment

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

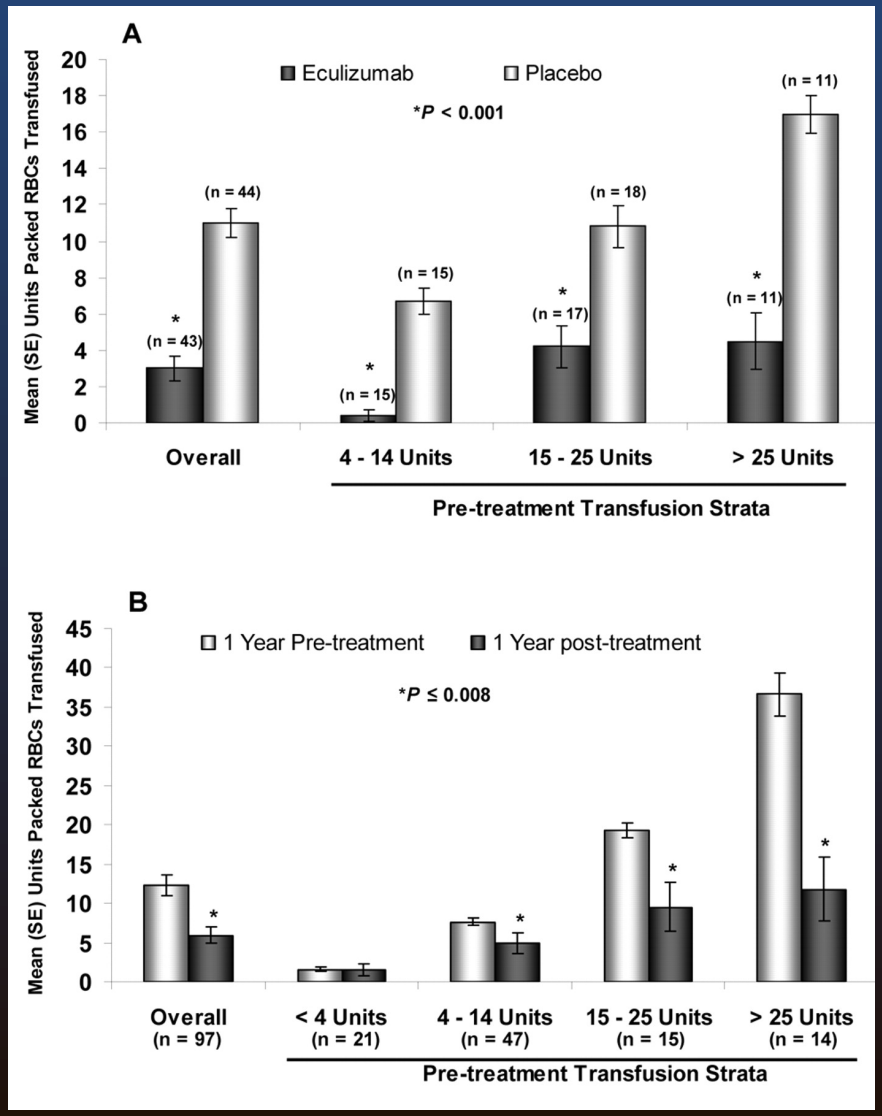
Primary End Point	Before Treatment†		During Treatment		P Value
	Placebo Group	Eculizumab Group	Placebo Group	Eculizumab Group	
Patients with stabilized hemoglobin levels (%)	NA	NA	0	49	<0.001‡
Packed red cells transfused (units/patient)					
Median	8.5	9.0	10	0	<0.001§
Interquartile range	7–12.5	6–12	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	

* Plus–minus values are means ±SE. NA denotes not applicable.
† Transfusion data obtained during 12 months before treatment were normalized to a value equivalent to the value for a 6-month period.
‡ The P value is for the comparison between groups during treatment, calculated with the use of a two-tailed Fisher's exact test.
§ The P value is for the comparison between groups during treatment, calculated with the use of the Wilcoxon rank-sum test.

Hillmen P et al. N Engl J Med 2006;355:1233-1243

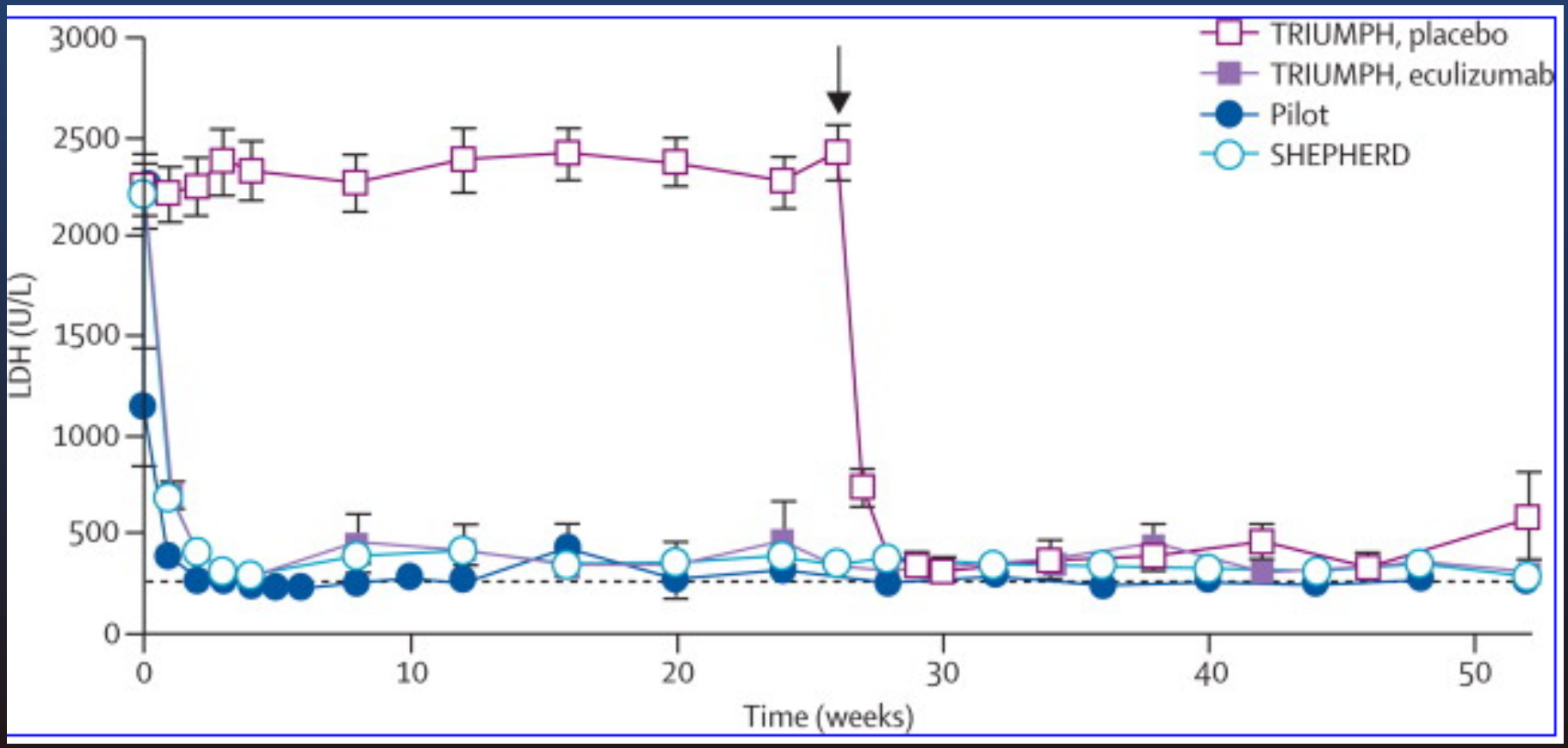
The NEW ENGLAND JOURNAL of MEDICINE

Mean (SE) units packed red blood cells transfused by pre-treatment transfusion strata during the TRIUMPH and SHEPHERD studies



Hillmen, P. Hematology 2008;2008:116-123

Levels of lactate dehydrogenase during treatment with Eculizumab



Hillmen, P. Hematology 2008;2008:116-123

Parker. Lancet. 2009 Feb 28;373(9665):759-67

blood
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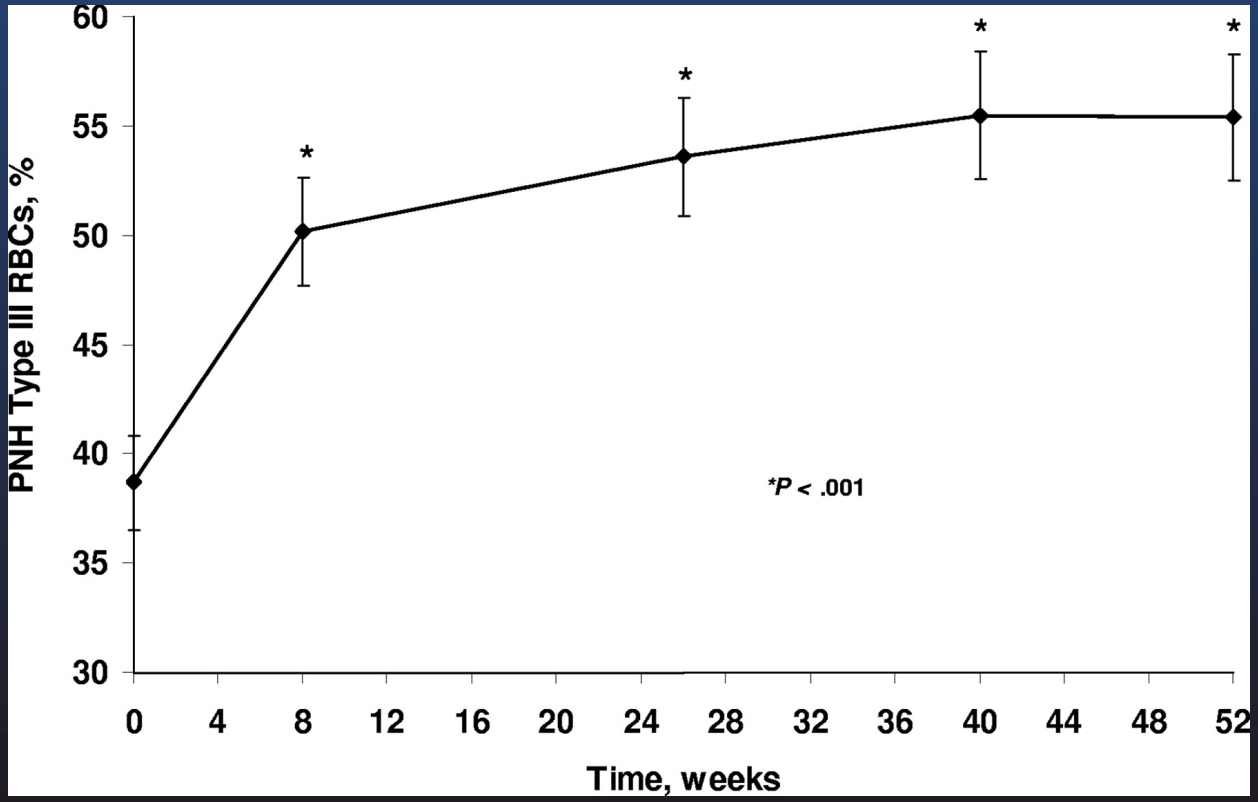
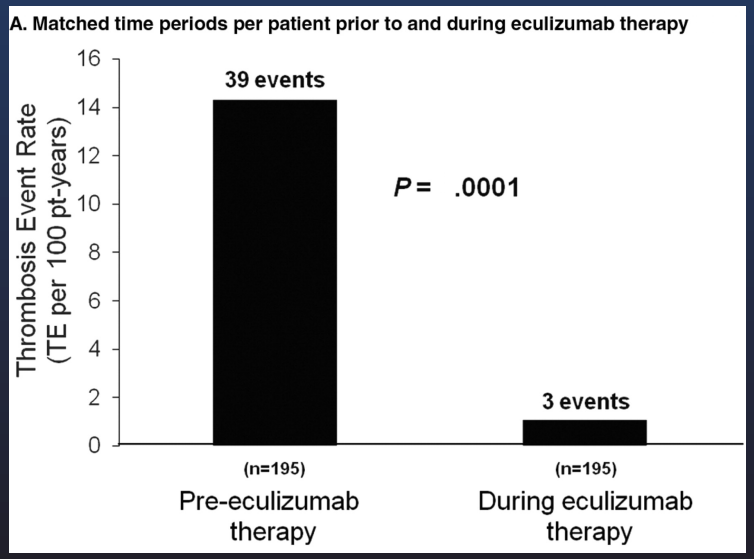
PNH RBC proportions (mean {+/-} SE) during Eculizumab treatment

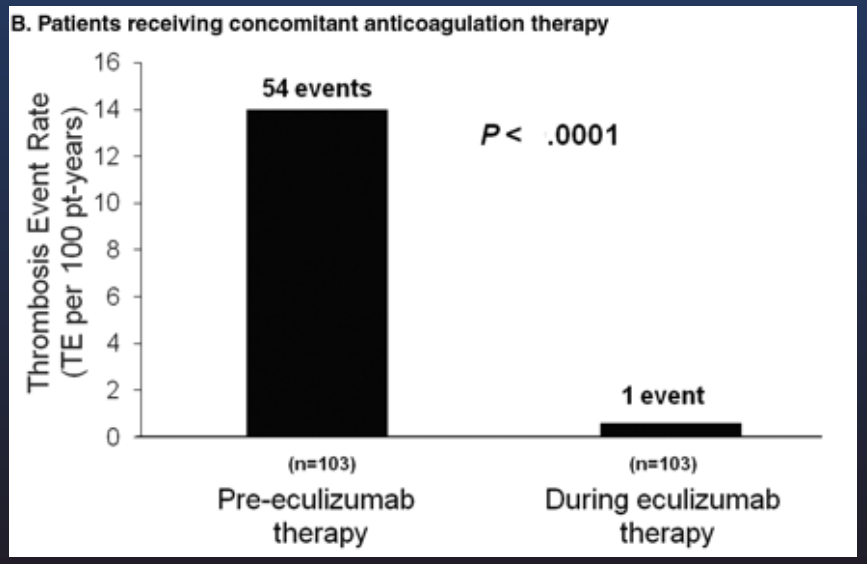


Brodsky, R. A. et al. Blood 2008;111:1840-1847

HEMATOLOGY
ASH Education Program Book

Analysis of thrombosis before and during Eculizumab therapy





Hillmen, P. Hematology 2008;2008:116-123

Ecluzimab: Expectations of therapy

Table 2 -- Clinical activity of eculizumab

What It Does	What It Does Not Do	What It May Do
Blocks complement-mediated intravascular hemolysis	Eliminate anemia and reticulocytosis [e]	Reduce the risk of thromboembolism [f]
Reduces transfusion requirement [a]	Affect the underlying marrow dysfunction	—
Improves quality of life (particularly fatigue)	Affect the *PIGA*-mutant stem cell clones	—
Increases the proportion of circulating type III PNH erythrocytes [d]	Increase the risk for a catastrophic hemolytic crisis if the drug is discontinued [c]	—
Increases the risk of infection with *Neisseria meningitides* [b]	—	—

Parker. Hematol Oncol Clin North AM 2009; 23(2):333-46

Safety

- In three major trials no patients withdrew due to an adverse event
  - 2/195 patients developed meningococcal sepsis
    - Vaccinations required
    - Alert cards suggested
  - Nasopharyngitis
  - Headache
  - Upper respirator tract infections

Future therapy?

- Inhibitor of complement C9
  - Block MAC and control hemolysis
  - Leave intact downstream functions of complement including the ability to generate C5a
    - Reduce risk of *Neisseria* sp infections

Parker. Lancet. 2009; 373:759-67