Update on the Diagnosis and Management of Paroxysmal Nocturnal Hemoglobinuria

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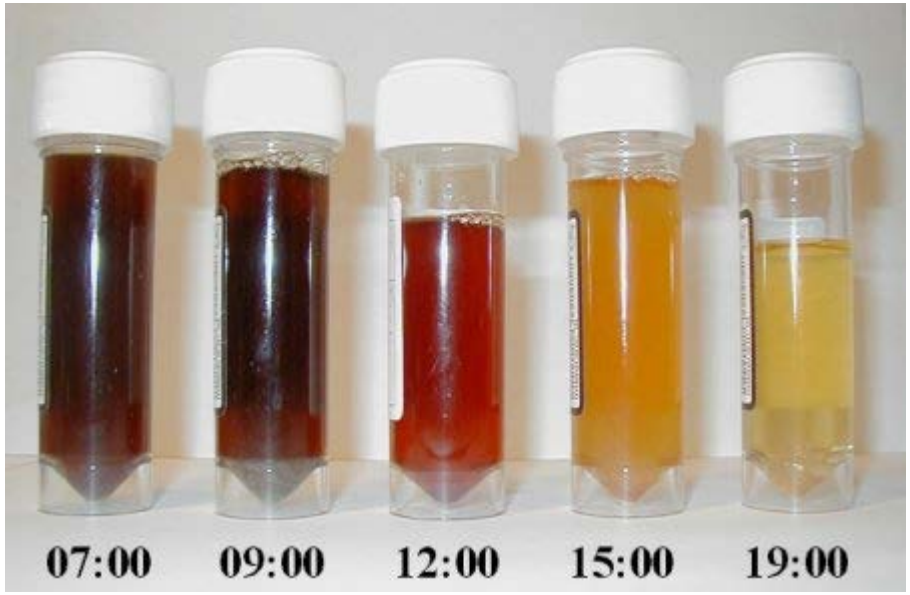
THE UNIVERSITY OF UTAH

Case Presentation

- A 31 years old female presented to an ER with complaints of fever and dark urine.
- Hgb 3.8 gm/dl; Hct 12%, WBC 4,100/µl; plt count 171,000; LDH 1,872 (ULN 240 IU/L); reticulocyte count 11.5%; haptoglobin <6 mg/dl.
- A diagnostic test was done
    - Was it the
        - "windowsill" test?
        - Coombs' test
        - Ham's Test
        - Peripheral blood flow cytometry for expression of GPI-anchored proteins?

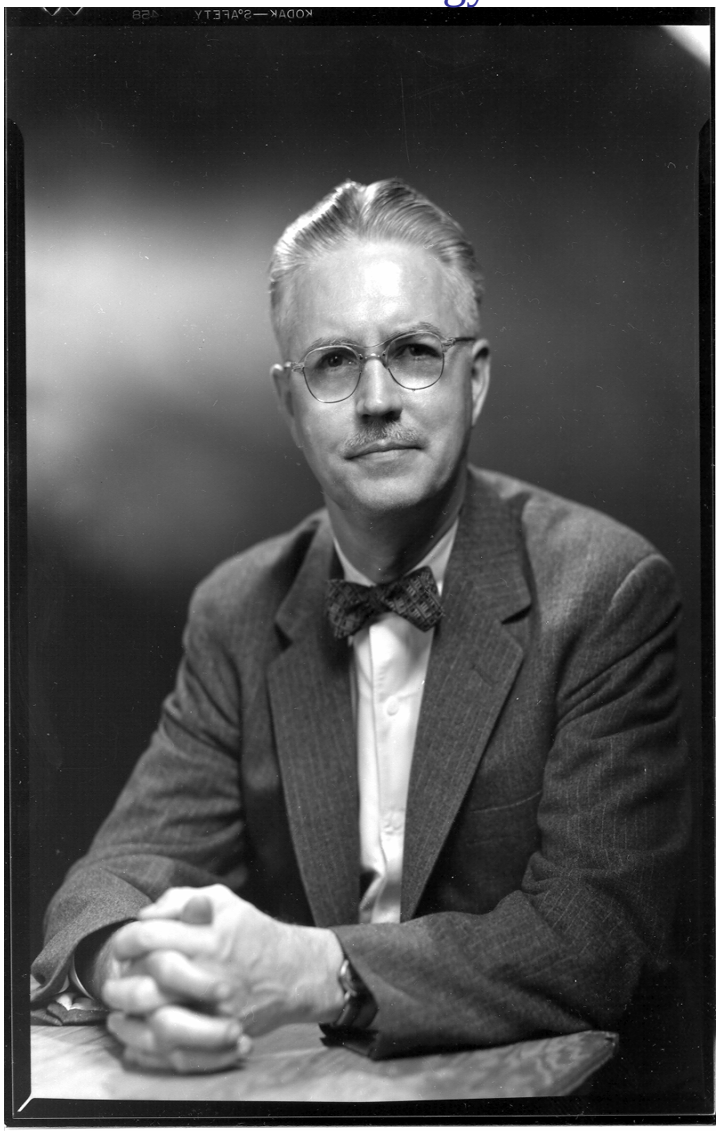
Diagnosis of PNH Using the Windowsill Method



porto rose´ Chablis

Thomas Hale Ham (1905-1987)

7th President of the American Society of Hematology



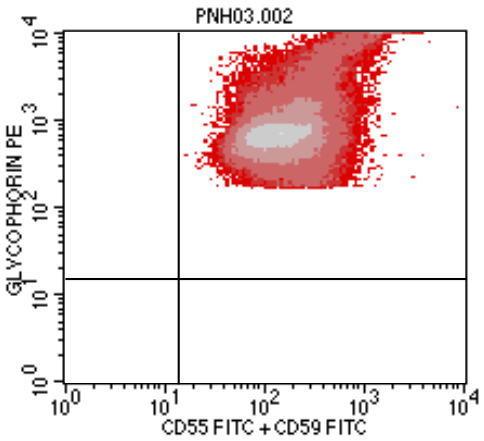
Archives of Case Western Reserve University

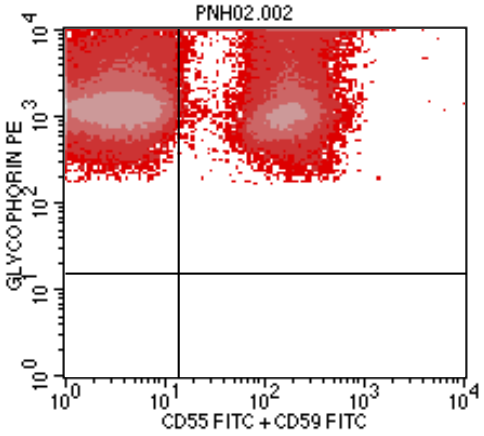
Flow Cytometric Diagnosis of PNH

Normal Control

Patient

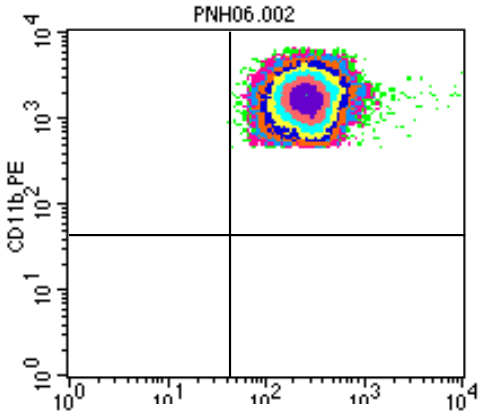
RBCs

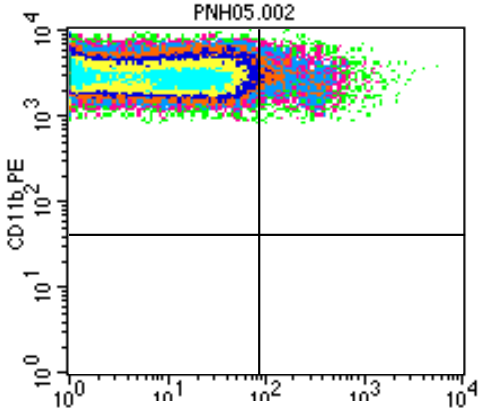




72%

PMNs





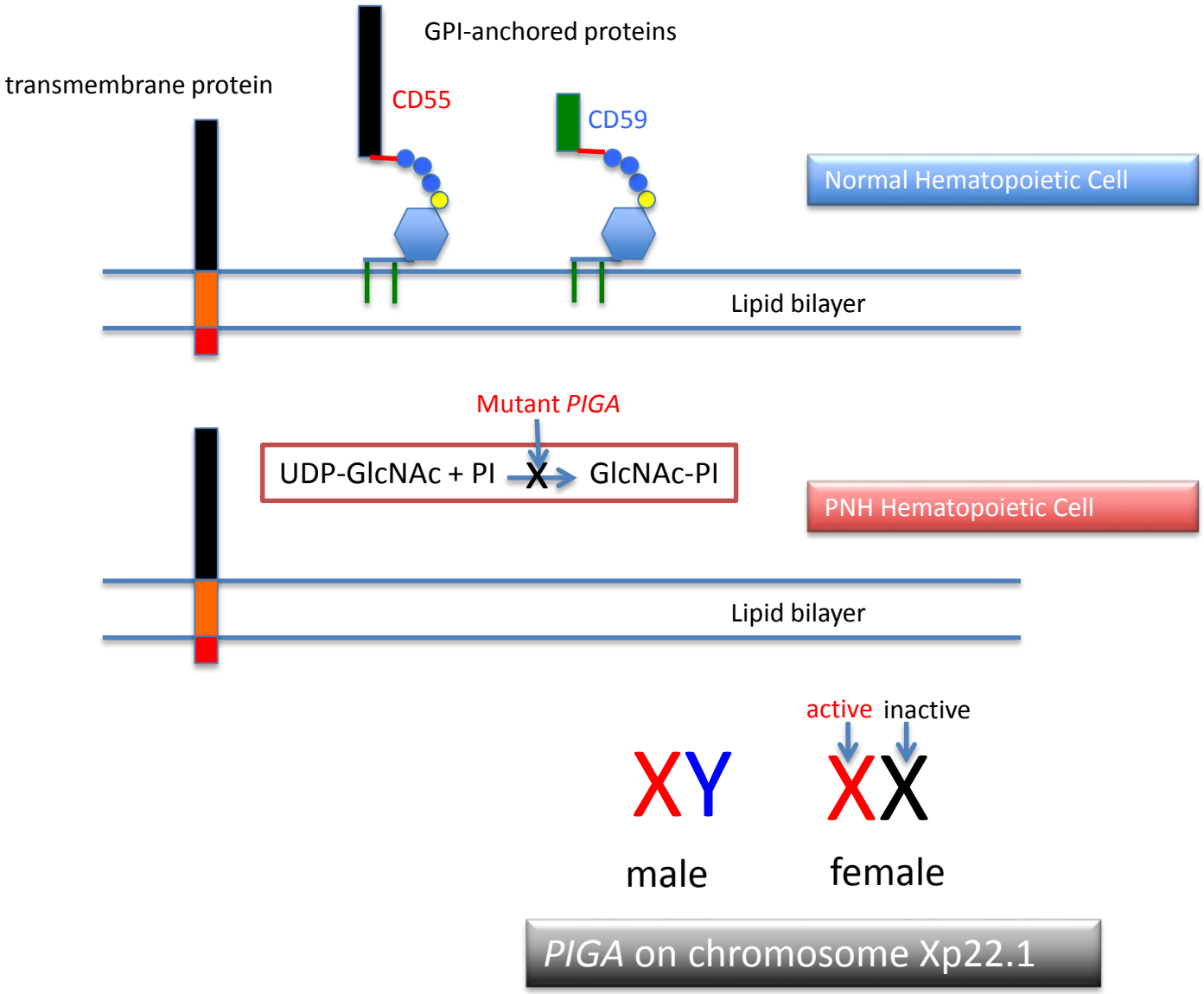
96%

What Is PNH?

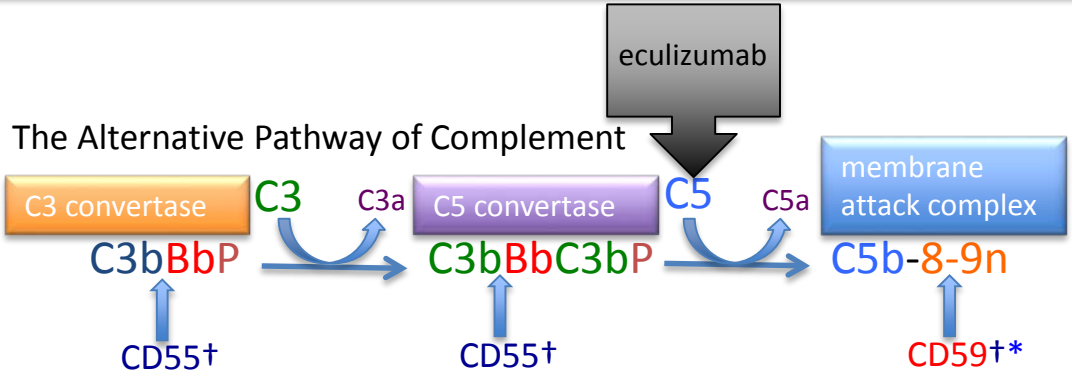
(more than a hemolytic anemia)

- A disorder of the *hematopoietic stem/progenitor cell (HSPC)*
- PNH is a consequence of *nonmalignant* clonal expansion of *one or several HSPCs* that have acquired a *somatic mutation of* PIGA
- Because of mutant-*PIGA*, progeny of affected HSPC's are deficient in *all* glycosyl phosphatidylinositol-anchored proteins (GPI-APs) that are normally expressed on hematopoietic cells
- Major clinical manifestations in addition to hemolytic anemia: *bone marrow insufficiency or failure* and *thrombophilia*

Pathophysiology of PNH



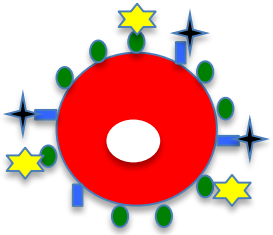
The Hemolytic Anemia of PNH Is Mediated by the APC



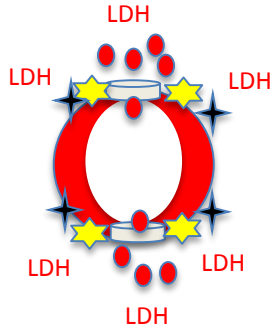
†GPI-anchored complement regulatory proteins

*CD59 also appears to participate in regulation of the APC C3/C5 convertase

Complement-Mediated Hemolysis



Normal RBC



PNH RBC

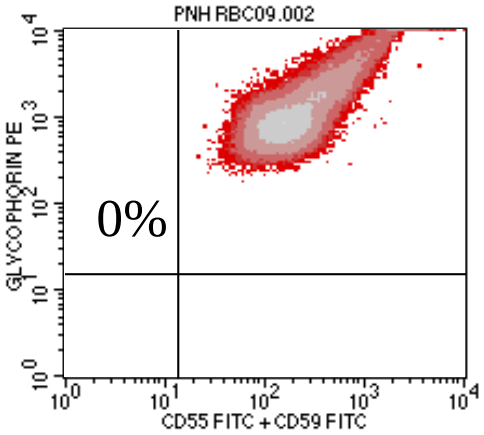
Characteristics of PNH

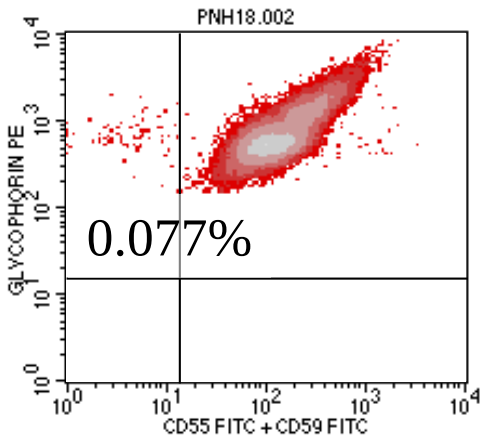
- Once suspected, diagnosis of PNH is straightforward, however, PNH is a heterogeneous disease because the size of the PNH clones vary among patients
  - The percentage of circulating PNH cells (determined by the size of the PNH clones, along with the PNH phenotype of the RBCs, is the major determinant of the clinical manifestations of the disease

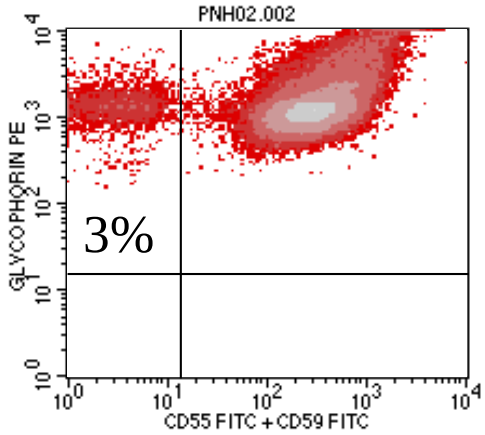
Flow Cytometric Analysis of Peripheral Blood for Diagnosis of PNH

Normal Control | Subclinical PNH | PNH/BMF

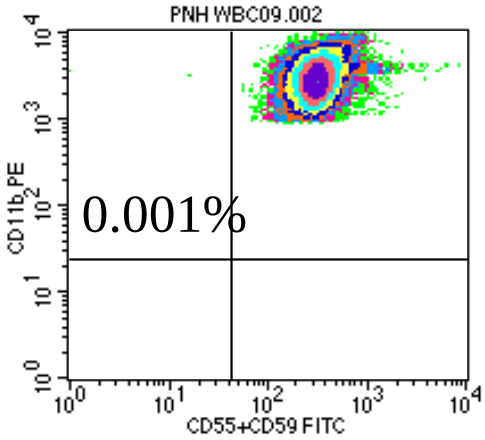
RBCs

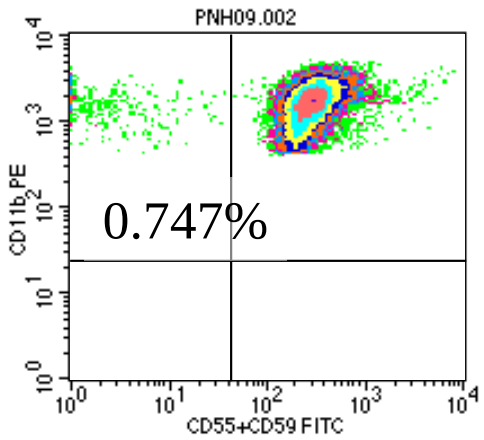


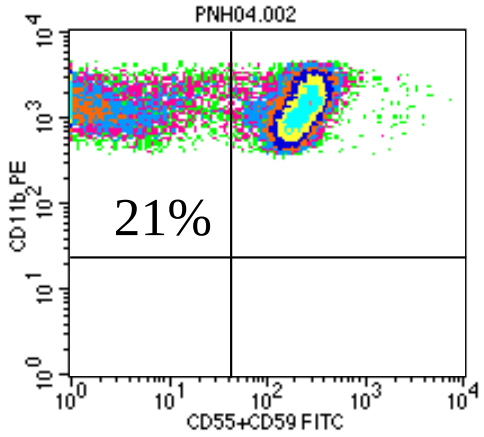




PMNs







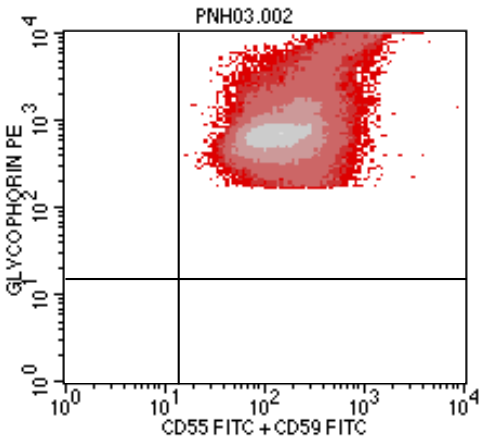
The FLAR reagent can be used for analysis of GPI-AP expression on PMNs

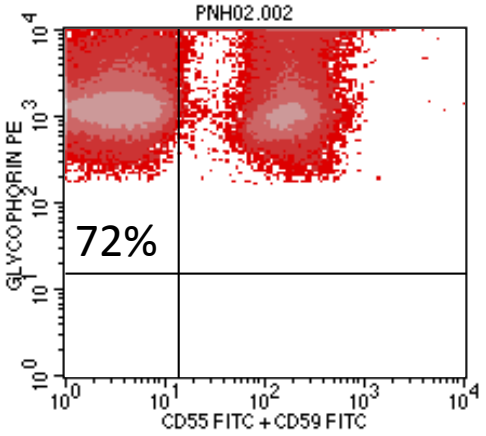
Flow Cytometric Analysis of Peripheral Blood for Diagnosis of PNH

Normal Control

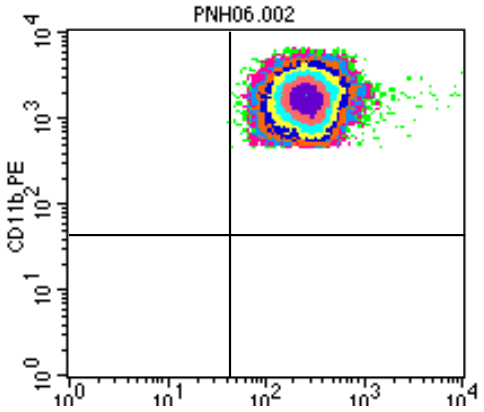
Classic PNH

RBCs

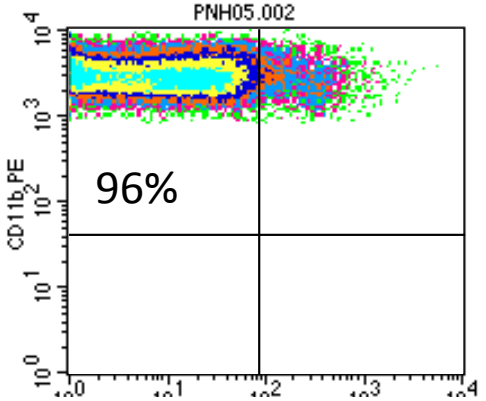




PMNs



anti-CD55-FITC + anti-CD59-FITC



anti-CD55-FITC + anti-CD59-FITC

Basic Evaluation for PNH

Flow cytometric evidence of a population of peripheral blood erythrocytes and granulocytes deficient in multiple GPI-APs*

Complete blood count; *reticulocyte count*; biochemical markers of hemolysis [serum concentration of lactate dehydrogenase (LDH)†, bilirubin (fractionated) and haptoglobin]; determination of iron stores

Bone marrow aspirate, biopsy, and genetic analysis§

*PNH *clone size* is determined by the percentage of GPI-AP deficient PMNs, and *phenotype* is determined by analysis of peripheral blood RBCs

†Clinically useful metric for assessing *intravascular* hemolysis and response to therapy

§Bone marrow analysis is used to distinguish classic PNH from PNH in the setting of another bone marrow failure syndrome. Genetic analysis may help distinguish hypoplastic MDS from aplastic anemia.

Classification of PNH Guides Management

Clinical PNH

*Classification of PNH**

Category	Rate of Intravascular Hemolysis†	Bone Marrow Characteristics	Flow Cytometry	Benefit from Eculizumab
Classic	Florid (markedly abnormal LDH, often with episodic macroscopic hemoglobinuria)	Cellular marrow due to erythroid hyperplasia and normal or near-normal morphology	Large population (>50%) of GPI-AP deficient PMNs	Yes
PNH in the setting of another bone marrow failure syndrome§	Usually mild (often with minimal to modest abnormalities of biochemical markers of hemolysis)	Evidence of a concomitant bone marrow failure syndrome§	Although variable, the percentage of GPI-AP deficient PMNs is usually relatively small (25-50%)	Variable. Some patients have clinically significant hemolysis and benefit from treatment
Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant bone marrow failure syndrome§	Small (usually <1%) population of GPI-AP deficient PMNs detected by high-resolution flow cytometry	No

* Based on recommendations of the International PNH Interest Group (*Blood* 2005;106:3699-3709)
† Based on episodes of macroscopic hemoglobinuria, serum LDH concentration, and reticulocyte count
§ Aplastic anemia or low risk myelodysplastic syndrome

Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, hemolytic parameter (reticulocyte count, serum LDH concentration), and bone marrow analysis

↓

Subclinical PNH

↓

No specific PNH therapy—focus on underlying bone marrow failure syndrome*†

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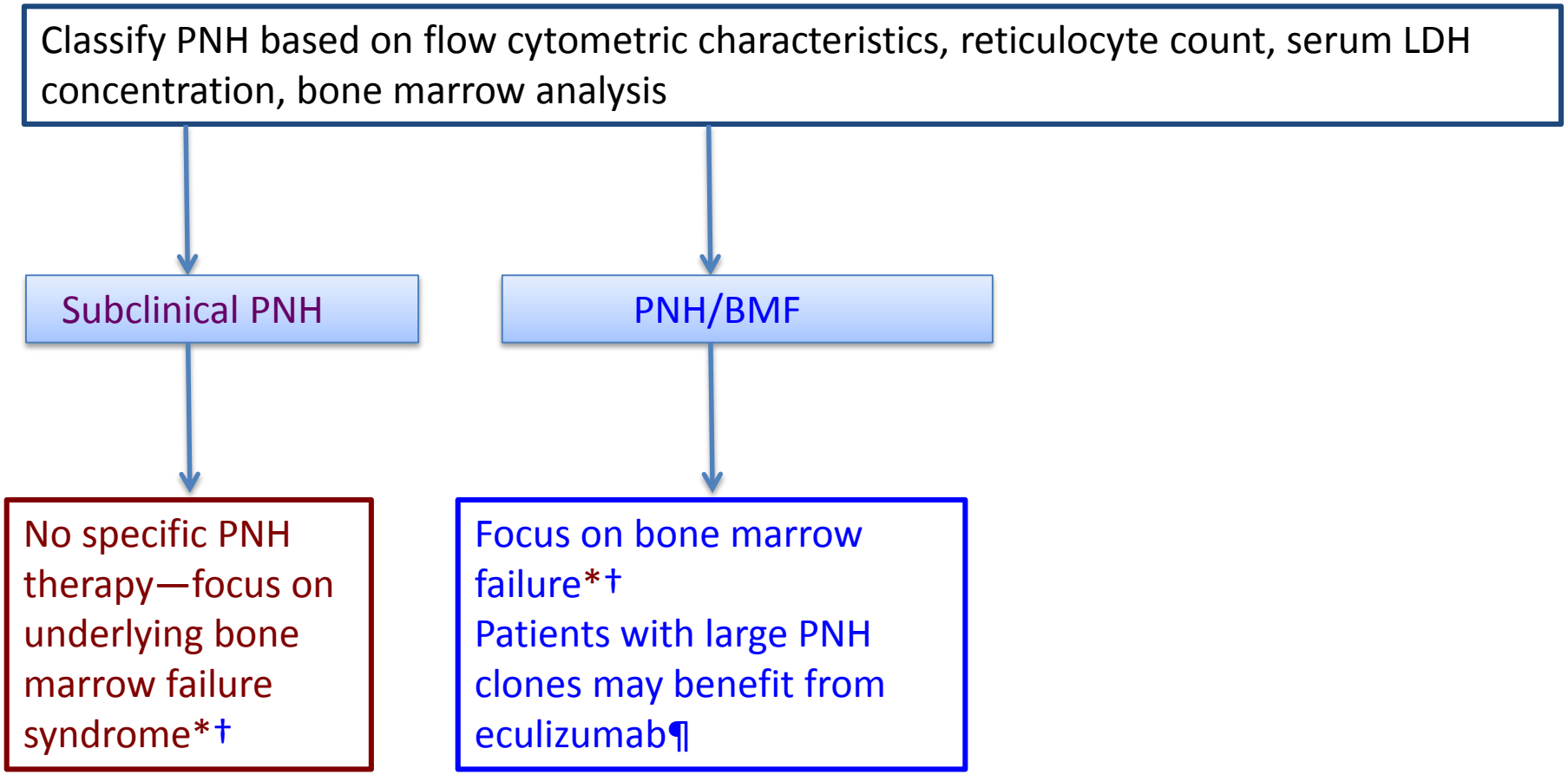
*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST). Treatment with IST does not affect PNH clone size

†Hematopoietic stem cell transplant eradicates the PNH clone

Management of PNH Based on Disease Classification



BMF, bone marrow failure (aplastic anemia and low risk MDS)

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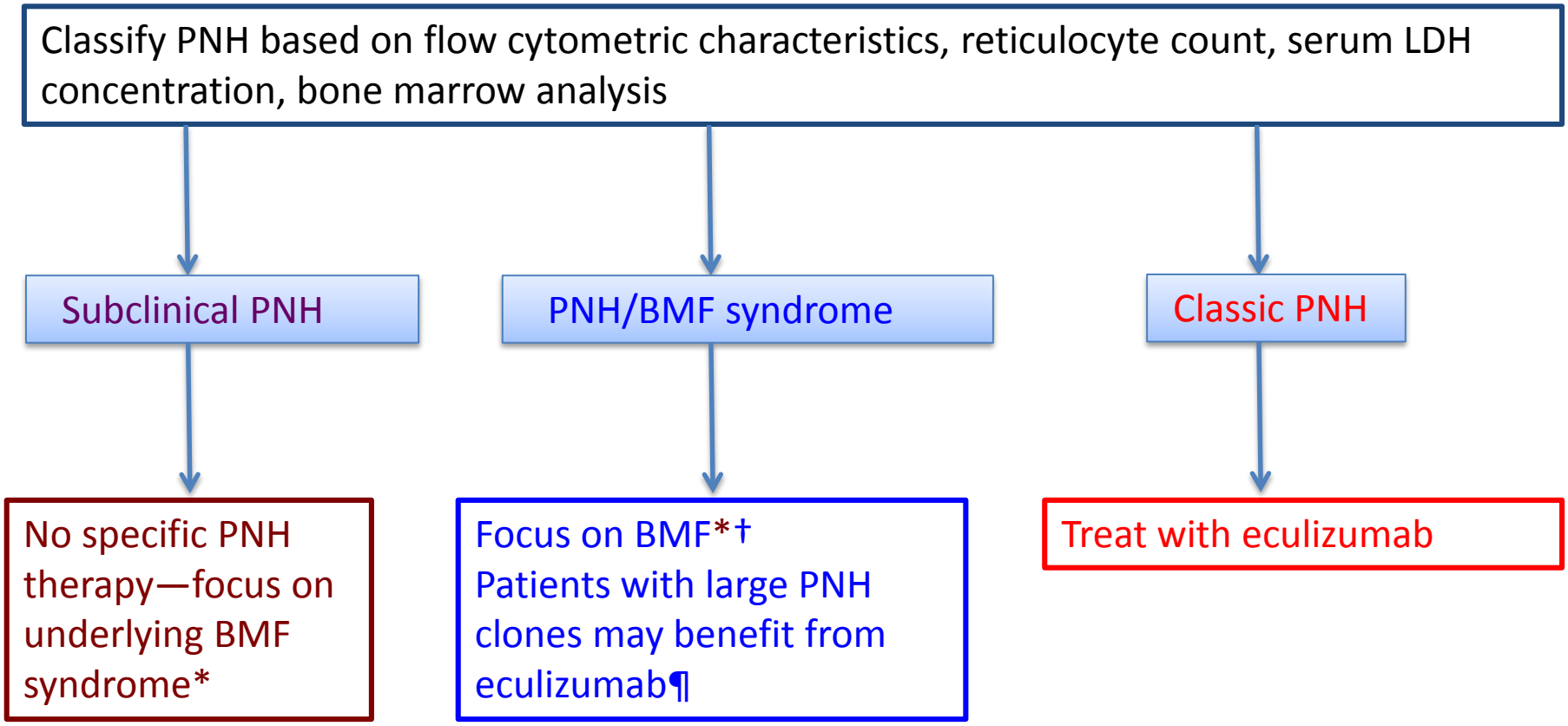
¶Approximately 50% of patients with PNH/BMF require treatment for hemolysis or thrombosis

Management of PNH Based on Disease Classification



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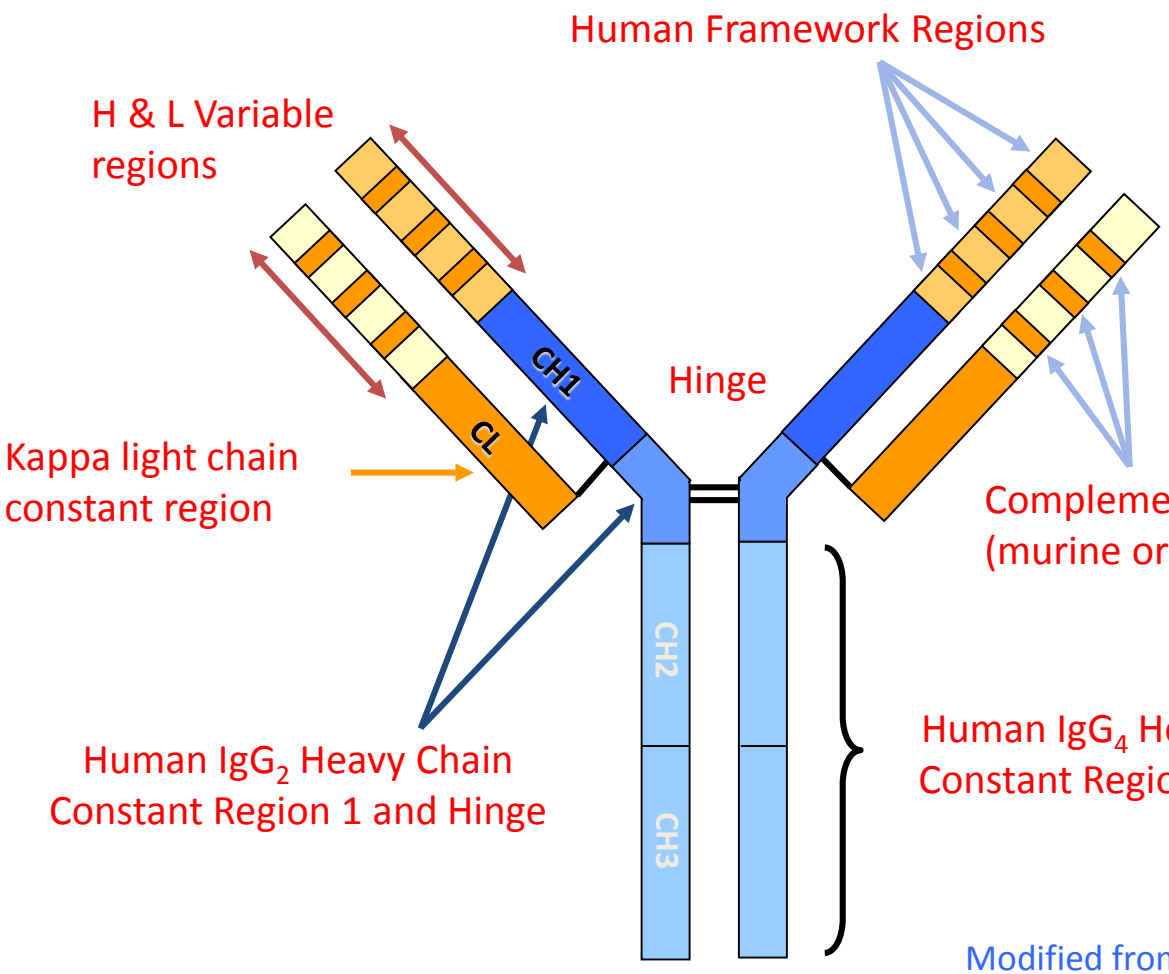
Complement Inhibitory Therapy for Treatment of the Hemolysis of PNH

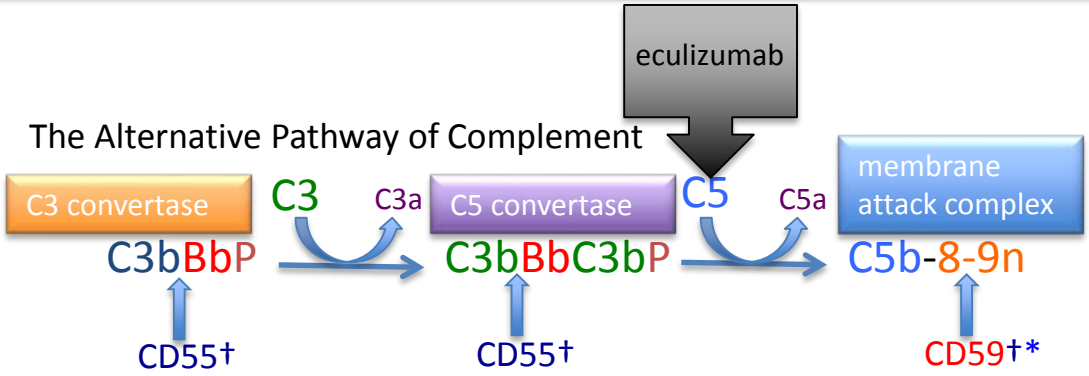
Eculizumab Is a Humanized Anti-C5 Antibody



- Patients must be vaccinated against *Neisseria meningitides*
- After an initial loading period, given as an every two-week infusion *continuously*
- Generally well-tolerated

Modified from R. Rother (Alexion Pharmaceuticals)

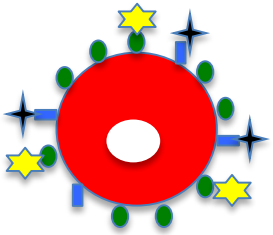
Mechanism of Action of Eculizumab



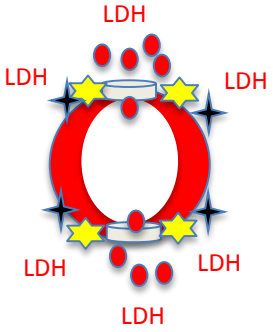
*CD59 also appears to participate in regulation of the APC C3/C5 convertase

†GPI-anchored proteins deficient in PNH

Complement-Mediated Hemolysis



Normal RBC



PNH RBC

What Does Eculizumab Do?

- Blocks Intravascular Hemolysis†
  - Ameliorates symptoms associated with chronic intravascular hemolysis
    - malaise, lethargy, fatigue, asthenia, dysphagia, male impotence*
  - Reduces transfusion requirements
    - ~65% become transfusion independent
    - Prolongs transfusion intervals in those who remain transfusion dependent
- Reduces the Risk of Thrombosis§

†Normalization or near normalization of serum LDH

*Symptom control improves quality of life (treatment can be transformative)

§Based on retrospective data

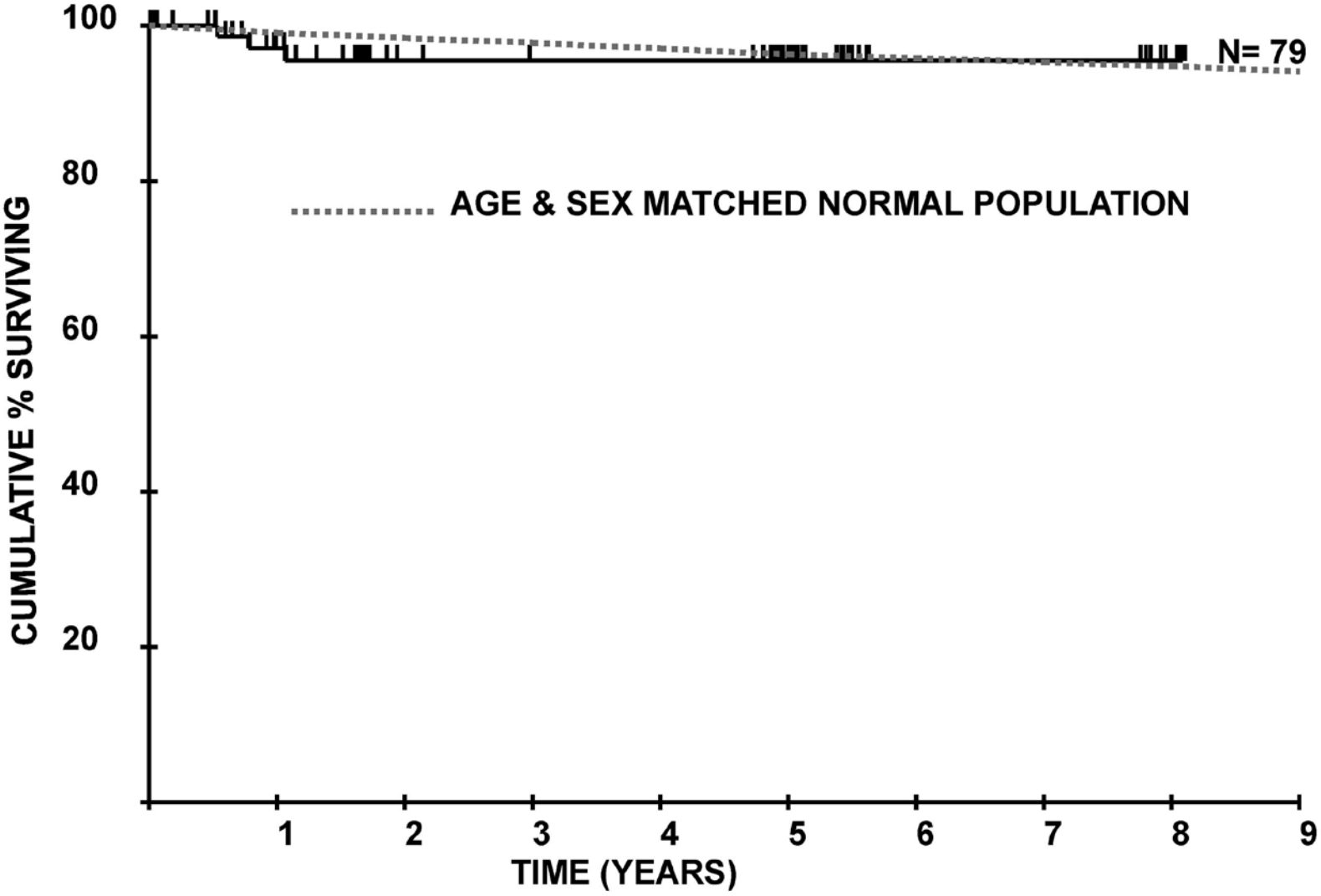
What Doesn't Eculizumab Do?

- Eliminate Transfusion Requirements in All Patients
- Block *Extra-Vascular Hemolysis* Mediated by Complement Opsonization of PNH RBC's
- Affect the Underlying Disease Process
  - Bone marrow failure persists
  - Clonal hematopoiesis persists
  - Symptomatic therapy in the form of eculizumab is beneficial long-term because *PNH is not a malignant, progressive disease*

Allogeneic SCT for PNH

- The PNH clone can be eradicated by allogeneic hematopoietic stem cell transplant
- In the era of complement inhibitory therapy, there is little enthusiasm for allogeneic BMT

Survival of Patients with PNH Treated with Eculizumab

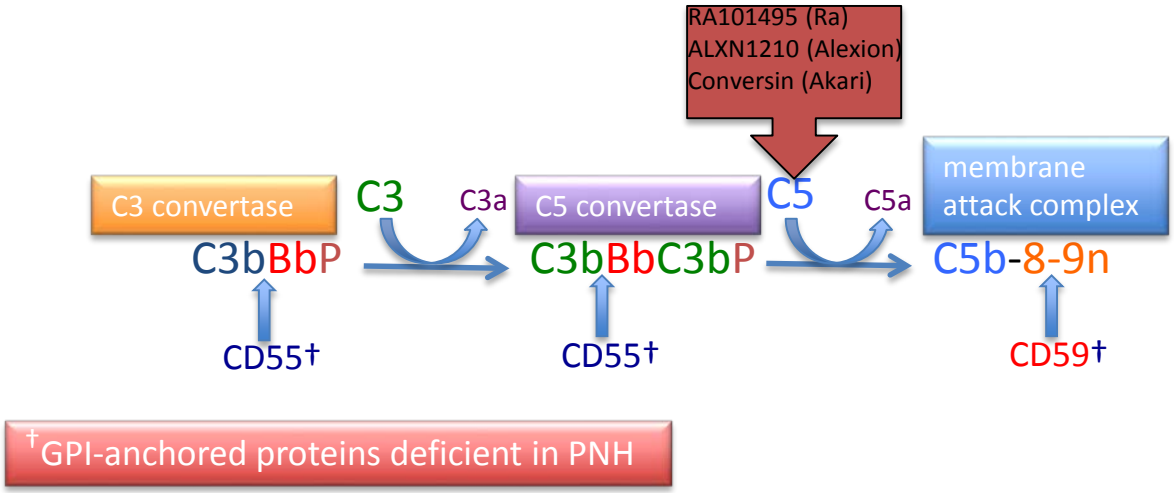


Kelly et al. *Blood* 2011: 117, 6786-6792

PNH

- Pathophysiology
- Diagnosis
- Management
- What's on the horizon for treatment of PNH

Because of the Success of Eculizumab, Other Products Are in Development for Treatment of PNH



- Like eculizumab, some are aimed at direct inhibition of C5
- Their efficacy and safety are likely to be equivalent to eculizumab, but delivery systems and dosing intervals may be more convenient
  - Synthetic peptides (Ra Pharmaceuticals)
  - Anti-C5 monoclonal antibodies engineered for extended duration of complement inhibition (every 8 week dosing interval for ravulizumab*), SKY 50 (Novartis), subcutaneous injection
  - Recombinant forms of naturally occurring inhibitors of C5, Conversin, Akari
  - Biosimilars (Amgen)

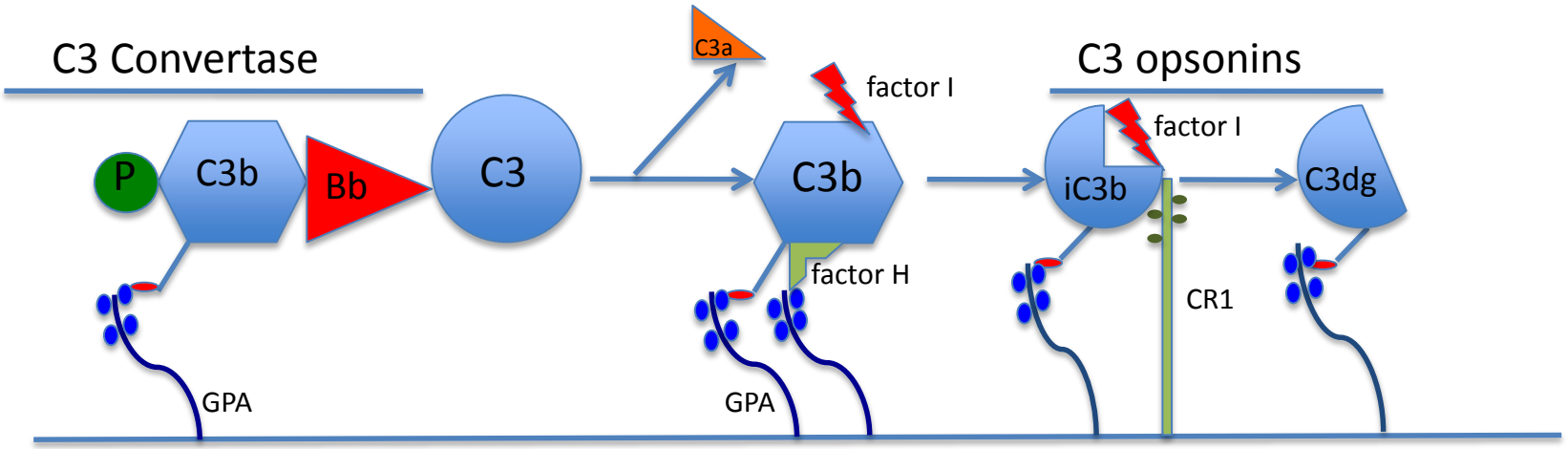
* December 2018, ravulizumab (Ultomiris, Alexion Phamaceuticals) approved for treatment of PNH. Patients can be switched from eculizumab to ravulizumab.

Suboptimal Response to Eculizumab

Extravascular Hemolysis in Patients Treated with Eculizumab

- Although serum LDH concentration returns to normal or near normal in most PNH patients treated with eculizumab, *reticulocytosis and some degree of anemia persists* in most patients with classic PNH and some remain transfusion dependent
  - Extravascular hemolysis due to C3 opsonization of PNH erythrocytes likely explains the suboptimal response in eculizumab-treated patients

Generation of C3 Opsonins* on PNH Erythrocytes† In Patients Treated with Eculizumab



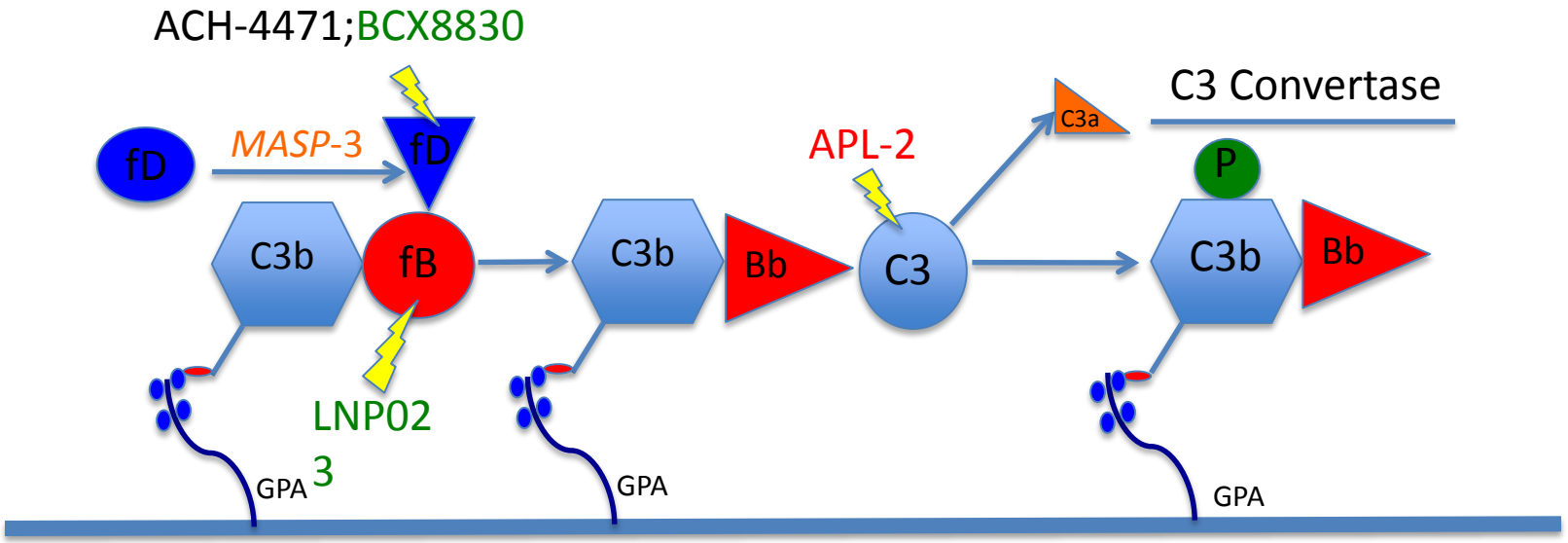
*C3 opsonins, iC3b and C3dg, target RBCs for destruction by reticuloendothelial cells expressing complement receptors:

CR2 ➡ C3dg

CR3 ➡ iC3b

†In eculizumab-treated patients, the direct antiglobulin test (Coombs' test) can become positive for C3 (but not IgG)

Inhibition of APC C3 Convertase Formation by Blocking Complement Factor D or Complement C3



- C3 Convertase Inhibitors
  - APL-2 (Apellis), synthetic peptide inhibitor of C3
  - ACH-4471 (Achillion), BCX8830 (BioCryst), small molecule factor D inhibitor being developed for *oral* administration
  - LNP023 (Novartis), smal molecule factor B inhibitor
  - Natural history of patients with congenital deficiencies of APC C3 convertase components raises concerns about the safety of chronic inhibition of the APC
    - Loss of C3 opsonization may increase the risk for microbial infections
  - Burden of proof of safety of APC C3 convertase inhibitors will likely be high

John V. Dacie (1912-2005)

Professor Dacie at 87

"I saw my first case of PNH over 25 years ago now, and I must confess I still look upon it as *the* blood disease, unique in its pathology and remarkable in its clinical diversity and haematological interrelationships."

–1963 Address to the Royal Society as President of the Pathology Section

Photograph provide by Dr. Wendell Rosse