

Bone Marrow Failure: Aplastic Anemia and PNH

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Disclosures

- I have the following financial relationships to disclose relevant to the content of this presentation:
- Alexion Pharmaceuticals: Chair, International Advisory Committee
- Vanderbilt has determined that there are no conflicts of interest.

Objectives

- Discuss diagnosis and prognosis of acquired severe aplastic anemia
- Discuss the pathophysiology of PNH
- Recognize assays to diagnose PNH
- Recognize the relevance of PIGA mutations in PNH and in healthy controls
- Discuss novel therapeutic strategies for PNH

Aplastic Anemia
Diagnosis And Nomenclature

- **SAA**
 - Bone marrow (< 25% cellular)
 - Peripheral cytopenias (at least 2 of 3)
 - ANC < 500 per μ l
 - Platelets < 20,000 per μ l
 - Absolute retic < 60,000 or corrected retic < 1%
- **VSAA: as above, but ANC < 200**
- **Moderate AA or (NSAA)**
 - Hypocellular marrow but does not meet criteria for SAA

2 year mortality > 70%

Aplastic Anemia
Etiology

- **Acquired AA (most cases idiopathic)**
 - Drugs (5 – 10%)
 - Infection (hepatitis –usually seronegative)
 - Toxic (benzene, radiation)
- **Congenital**
 - Fanconi anemia
 - Shwachman-Diamond
 - Dyskeratosis congenita
 - Amegakaryocytic thrombocytopenia

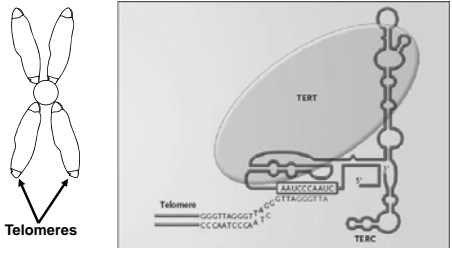
Fanconi Anemia

- **Presents as AA, usually in 1st decade of life**
- **Physical abnormalities**
 - Short stature, café au lait spots, thumb/radial deformities
 - 10 % have no stigmata
- **Premalignant**
 - DNA repair defect predisposes to MDS/leukemia and solid tumors

Dyskeratosis Congenita

- **Clinical Triad: abnormal skin pigmentation, nail dystrophy, mucosal leukoplakia**
 - Pulmonary fibrosis, cirrhosis, osteoporosis
- **Genetics: short telomeres and low telomerase activity**
 - Telomeres are structural elements that seal the ends of chromosomes, protecting them from recombination, end to end fusion, and recognition of damaged DNA
- **X-linked recessive: DKC1 (dyskerin)**
- **Autosomal dominant**
 - *TERC*: RNA component of telomerase
 - *TERT*: Telomerase reverse transcriptase

Structure and Function of Telomerase



TERC: RNA component
TERT: Telomerase reverse transcriptase
Dyskerin: Protein important for stabilizing telomerase complex
-- autosomal recessive form of DKC


} **Autosomal dominant**

Schwachman-Diamond Syndrome

- **Usually presents with neutropenia, bony abnormalities and pancreatic dysfunction**
- **Genetics:**
 - Autosomal recessive
 - SBDS mutations in 90%

Aplastic Anemia: Differential Dx

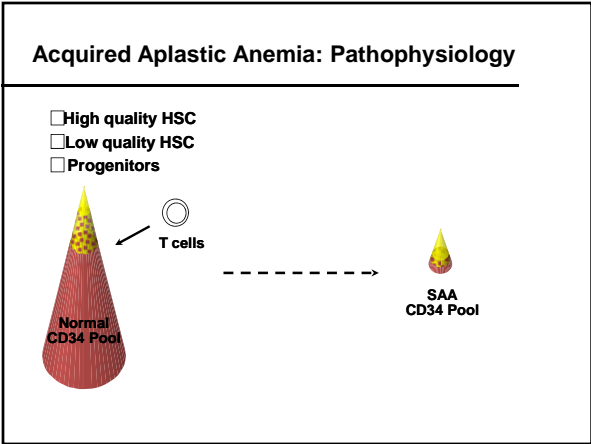
- **Congenital disorders**
 - Fanconi: all patients < 40yo (DEB,MMC)
 - Others: careful history
- **PNH - flow cytometry**
- **Hypoplastic MDS – morphology, cytogenetics, CD34 count**
- **LGL - flow cytometry**



Moderate AA vs. MDS

Characteristic	MDS	Aplastic anemia
Cellularity	Increased or nl*	decreased
CD34 count	nl or increased	Decreased (< 0.1%)
Dyserythropoiesis	common	common
Ringed sideroblasts	common	never
Myeloid dysplasia or blasts	common	never
Dysplastic megas	common	never
PNH population	rare	common
Abnormal karyotype	common	rare

* Up to 15% of cases have a hypocellular bone marrow



Initial Management - Essentials

- CBC, diff & retic, biochem profile
- Bone marrow aspirate, bx, and cytogenetics
- Peripheral blood for PNH and Fanconi
- Careful history
- Transfuse CMV-neg., Irradiated blood products (avoid family members)
- Consider prompt referral to specialized center

Severe Aplastic Anemia

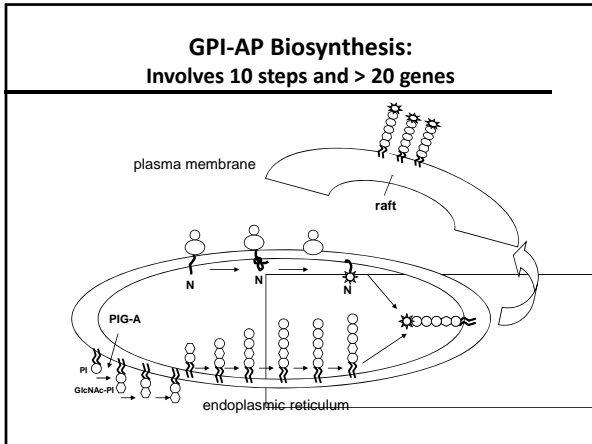
Definitive management

- **Allogeneic bone marrow transplantation**
 - HLA-identical
 - unrelated
 - mismatched
- **Immunosuppressive therapy**
 - ATG/CSA
- **High-dose cyclophosphamide**

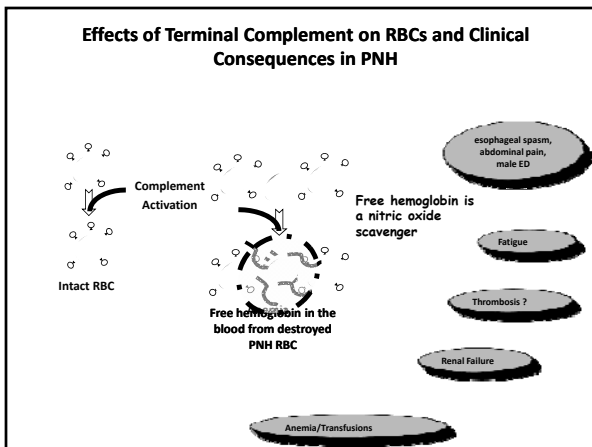
Brodsky and Jones, Lancet 2005, 365:1647-56

PNH

- **Acquired Clonal Hematopoietic Stem Cell disease with protean manifestations**
 - hemolytic anemia
 - pancytopenia
 - thrombosis
 - smooth muscle dystonias
- **PIGA mutation**
 - X(p22.1)
- **PIGA gene product necessary for 1st step in the biosynthesis of GPI anchors**
- **PNH cells have deficiency or absence of all GPI anchored proteins**



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



GPI-AP serve as Receptors for Proaerolysin

- Pore-forming protoxin secreted by *Aeromonas hydrophila*
- PNH cells uniquely resistant to aerolysin because they lack GPI-anchors
- FLAER - Fluoresceinated AERolysin variant that binds GPI-anchors but does not form channels.

Brodsky et. al., Blood 1999 93:1749
Brodsky et. al., Am. J. Clin. Pathol. 2000:457
Mukhina et. al., Brit. J. Haematol. 2001 115:482

FLAER (FLuorescent AERolysin)

- Binds to GPI-anchor rather than surrogate protein

The diagram illustrates the binding of FLAER to GPI-anchors. On the left, a GPI-anchored protein (CD59) is shown with an alpha-CD59 antibody binding to its extracellular domain. On the right, a GPI-anchored protein (CD14) is shown with FLAER binding to its GPI-anchor. An inset flow cytometry plot shows two quadrants: the top-left quadrant is dark, indicating high alpha-CD59 and low FLAER binding, while the top-right quadrant is lighter, indicating high alpha-CD59 and high FLAER binding.

PNH: Size of Clone Correlates With Risk for Thrombosis

Odds ratio for thrombosis is 1.64 (p= 0.008) for every 10% rise in size of PNH clone

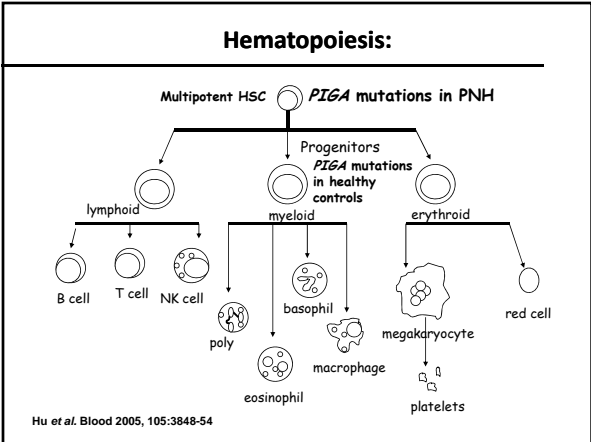
Moyo et. al, Br J Haematol. 2004 126:133-8.

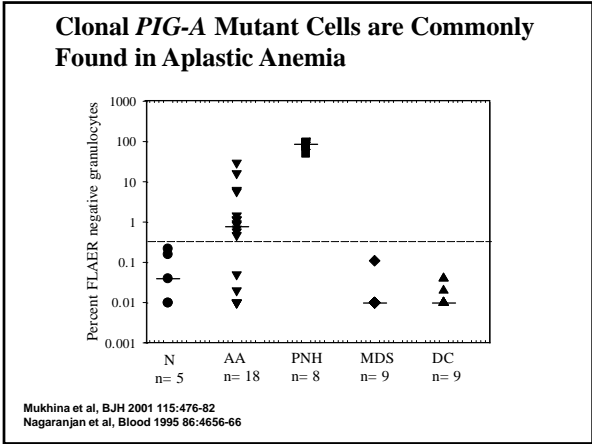
We all have *PIGA* mutations. Why don't we all have PNH?

- **GPI-AP^{neg} lymphocytes appear after treatment with CAMPATH**
 - Hertenstein et. al., Blood, 1995 86:1487
- **Roughly 1 in 50,000 granulocytes from healthy controls harbor *PIG-A* mutations**
 - Araten et. al., PNAS, 1999 96:5209
- **Circulating *PIGA* mutant lymphocytes in healthy controls (~ 1 per 50,000)**
 - Ware et. al., Exp Hematol 2001, 29:1403

Possible explanations for *PIGA* mutations in healthy controls

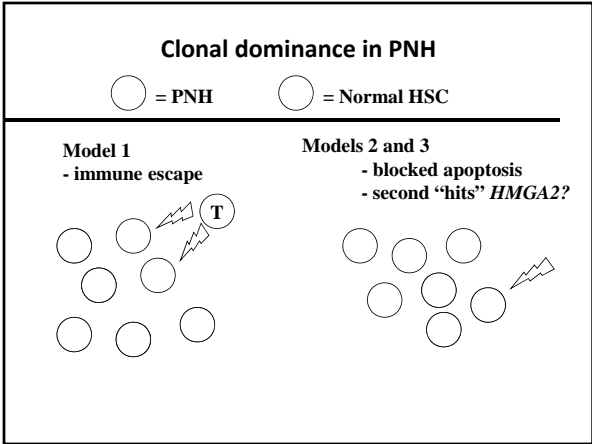
- ***PIGA* mutations are common events in HSC and are necessary but not sufficient to cause the disease**
 - Immune selection
 - Second mutations
- ***PIGA* mutations in healthy controls do not occur in cells with self-renewal capacity**



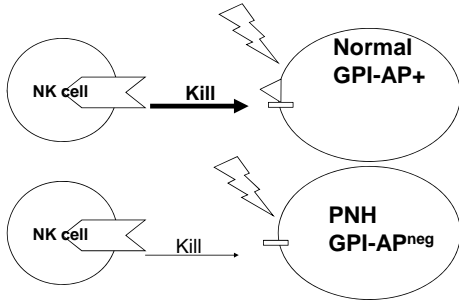


PNH Cells in MDS?

- Reported to occur in ~ 10% of patients
- Is it MDS or Aplastic Anemia?
- Most cases have the following characteristics
 - Type: hypocellular or RA
 - Normal cytogenetics
 - HLA-DR15 positive
 - Highly likely to respond to immunosuppression



Alternative Mechanism: PNH cells with an absence of stress inducible membrane proteins (ULBPs) would preferentially survive an immune mediated attack.



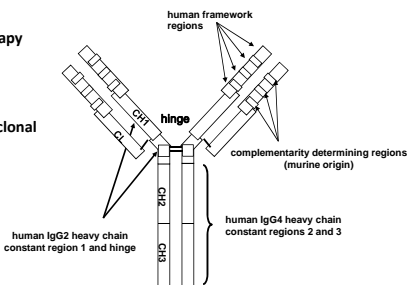
Hanaoka et al. Blood 2007, 107:1184-91
Savage et al. Exp. Hematol. 2009, 37:42-51.

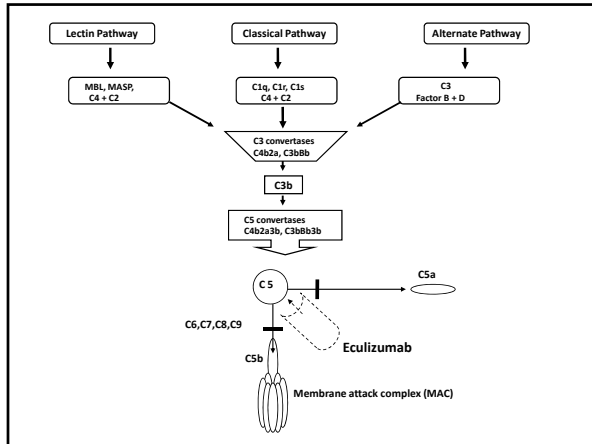
Mechanism of Clonal Dominance

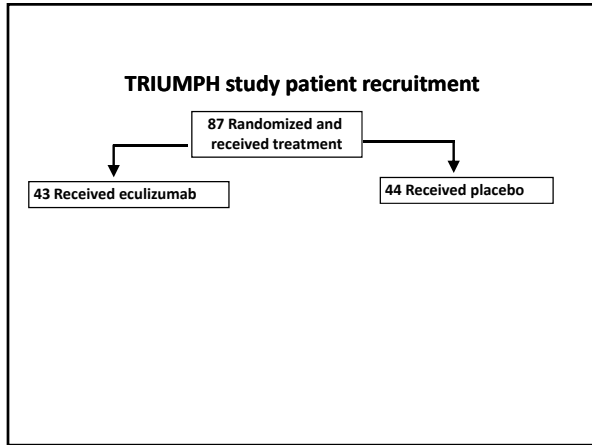
- **PIG-A mutation must occur in a cell with self-renewal capacity**
- **PNH cells display less apoptosis in response to an immune attack**
 - Partly because they don't express GPI-anchored stress inducible ULBPs
- **Additional survival mutations or other epigenetic hits may give a growth advantage to some patients**

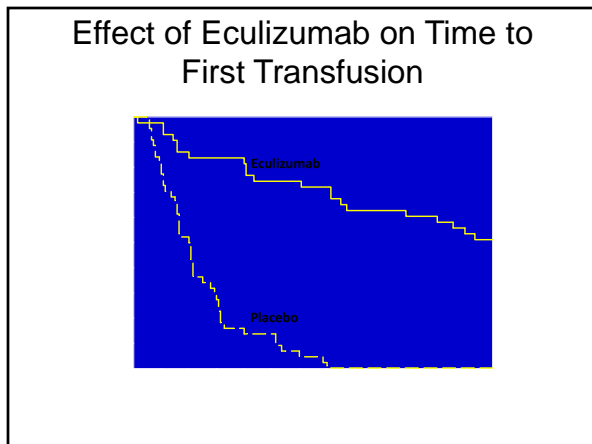
Complement inhibition is a highly effective therapy for classical PNH

- **Allogeneic BMT**
 - Only curative therapy
- **Eculizumab**
 - Humanized monoclonal
 - Antibody to C5

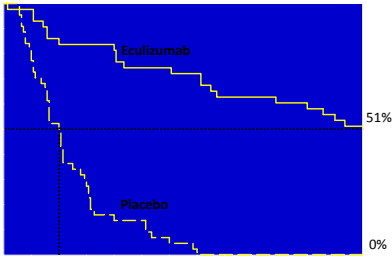




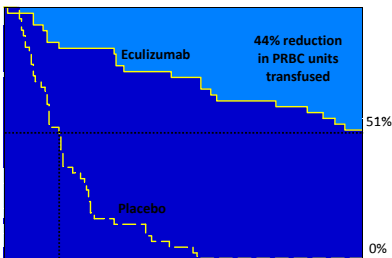




Effect of Eculizumab on Time to First Transfusion



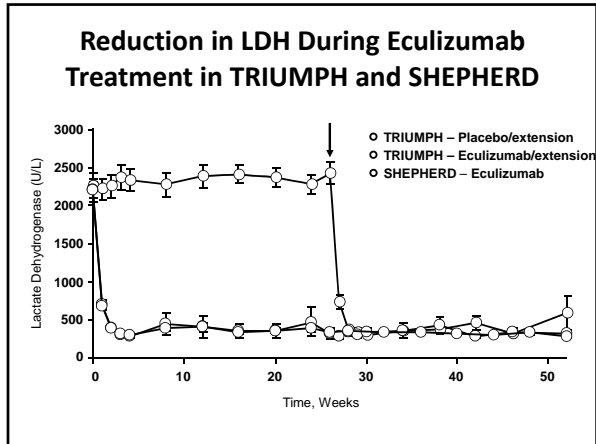
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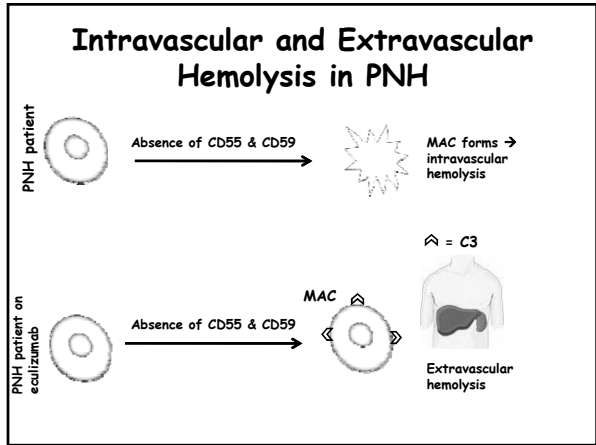


SHEPHERD

- **Open-label study of eculizumab broader PNH population**
 - Platelet cut-off 30,000
 - Lower transfusion requirements than TRIUMPH
- **Decreased intravascular hemolysis**
 - Improved anemia
 - Decreased need for transfusions
 - Improved quality of life

Brodsky et al. BLOOD 2008, 111:1840-47





- ### Lessons from Eculizumab Trials (TRIUMPH, SHEPHERD, EXTENSION)
- Safe
 - Mild side-effects
 - Increased risk for Neisserial infections (~0.5% per year)
 - Effective
 - Decreases intravascular hemolysis
 - Decreases (>90%) or eliminates (50%) need for PRBC
 - Improves quality of life
 - Reduces the risk for thrombosis by >90%

**Lessons from Eculizumab Trials – cont.
(TRIUMPH, SHEPHERD, EXTENSION)**

- **Drawbacks**
 - Lifelong therapy intravenous therapy
 - Cost (> 350K a year)
- **Not as effective in patients with AA/PNH**
 - Does not treat bone marrow failure
- **Ideal PNH patient (classical PNH)**
 - Large PNH populations (>10% type III red cells, > 50% PNH granulocytes)
 - LDH > 1.5x upper limit of normal
 - Retics > 3%
 - Bone marrow: normal to hypercellular

Eculizumab: Unanswered questions

- **Eculizumab**
 - Overall survival advantage?
 - Influence on clonal progression (MDS/leukemia)
 - Long term safety
 - Pregnancy
 - How to manage patients on anticoagulation
 - Who should be considered for allo BMT?
