# PNH

AA-MDS Patient Conference
Raleigh / Durham
July 2016
PNH: Current Thinking on the Disease, Diagnosis, and Treatment

## PNH

- What is PNH?
- What causes PNH?
- What are the clinical symptoms of PNH?
- How is PNH diagnosed?
- What are the long-term risks and complications of PNH?
- How is PNH treated?
- What is new or on the horizon for treatment?

## What is PNH?

## What is PNH?

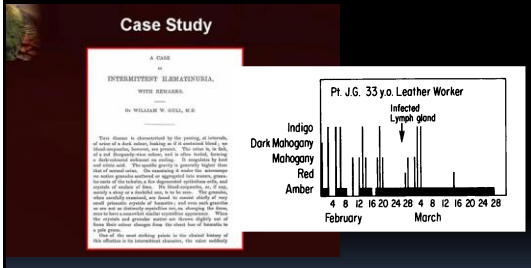
- Paroxysmal – sudden onset
- Nocturnal – occuring at night (or early in morning upon awakeneing)
- Hemoglobinuria

Despite the name, the majority of patients do not present this way.

## What is PNH?

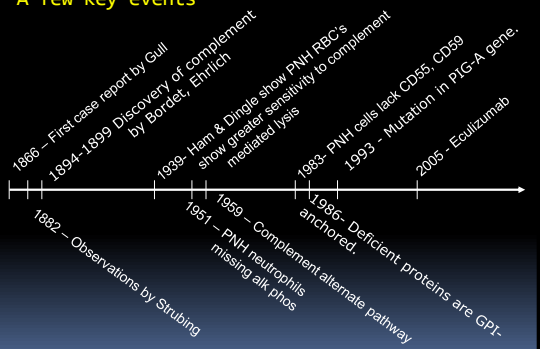
- A rare and unusual acquired hematologic disorder characterized by
  - Intravascular hemolysis (breaking apart of red cells in the blood vessels)
  - Bone marrow failure (cytopenias= low blood counts)
  - Thrombosis (Blood clots)
- There is an incredible amount of clinical heterogeneity amongst patients with PNH.

## 1st published case report of PNH - 1866




Gull WW. Guys Hospital Reports 12:381-392, 1866.

## PNH – A historical view
## A few key events




Parker CJ. Historical Aspects of Paroxysmal Nocturnal Hemoglobinuria: "Defining the Disease." Brit J Haematol 117:3-22, 2002.

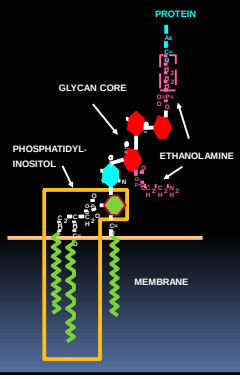
## What causes PNH?

## What causes PNH?

- PNH requires "two-hits"
  1) A mutation must occur in a hematopoietic stem cell.
     - Partial or complete deficiency of the GPI anchor
  2) PNH is due to a condition that allows this mutated cell to become the dominant cell in the bone marrow.

## What causes PNH?

- The mutation in the PIG-A gene in a hematopoietic stem cell leads to a defect in the production of an anchor protein that ties other proteins to the cell surface.
  - Sometimes the mutation leads to a partial decrease in the amount of anchor protein that is made and the cells have a partial deficiency (Type II cells); sometimes the mutation completely knocks out the GPI anchor
  - Some patients have more than one stem cells with different mutations in PIG-A gene




**A case of paroxysmal nocturnal hemoglobinuria caused by a germline mutation and a somatic mutation in *PIGT***

Peter M. Krawitz,[1] Britta Höchsmann,[2] Yoshiko Murakami,[3] Britta Teubner,[1] Ulrike Krüger,[1] Eva Klopocki,[4] Heidemarie Neitzel,[1] Alexander Hoellein,[5] Christina Schneider,[6] Dmitri Parkhomchuk,[1] Jochen Hecht,[6] Peter N. Robinson,[1] Stefan Mundlos,[1] Taroh Kinoshita,[3] and Hubert Schrezenmeier[2]

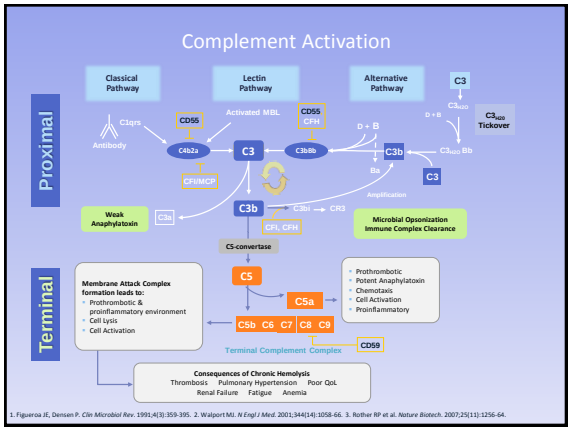
(*Blood.* 2013;122(7):1312-1315)

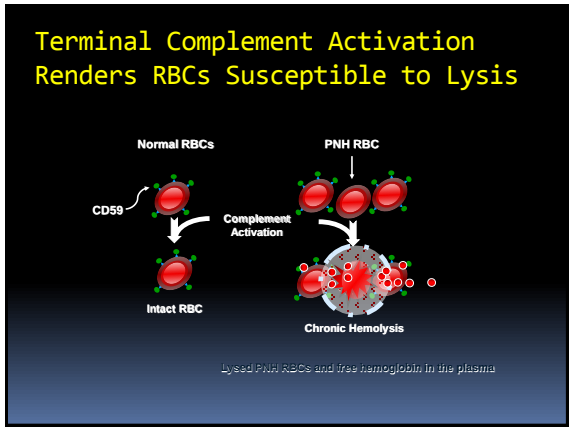
**Key Points**

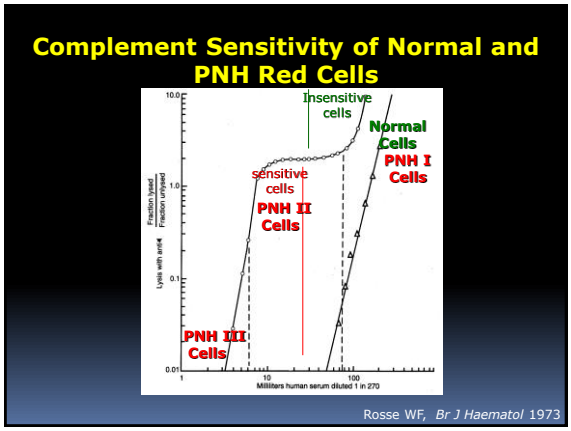
- A carrier of a deleterious splice site mutation in *PIGT* acquired a second hit in *PIGT* and developed PNH.

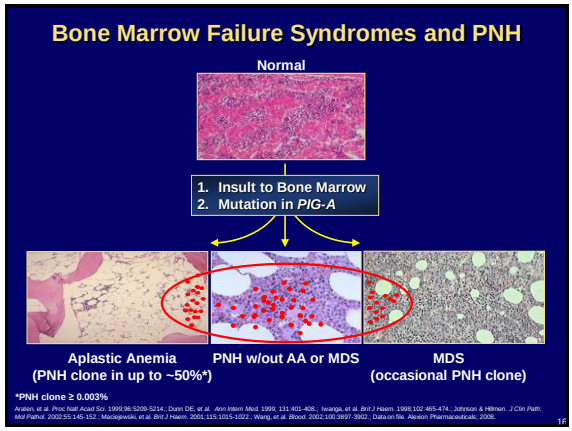
## The Missing Proteins in PNH

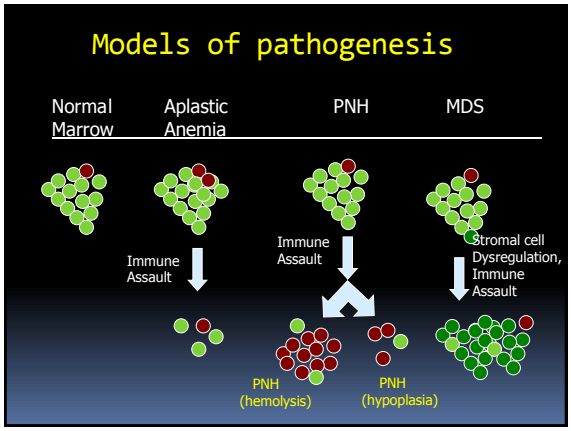
- Complement defense proteins
  - CD55 (decay accelerating factor, DAF)
  - CD59 (membrane inhibitor of reactive lysis)
- Enzymes
  - Acetylcholinesterase
  - Alkaline phosphatase
- Immune system ligands
- Adhesion molecules
  - NCAM
  - Fibronectin receptor
- Growth Factors and receptors
- Differentiation antigens
  - CD14 (monocytes)
  - CD52 (T cells)
- Anti-procoagulant proteins
  - uPAR (CD87)

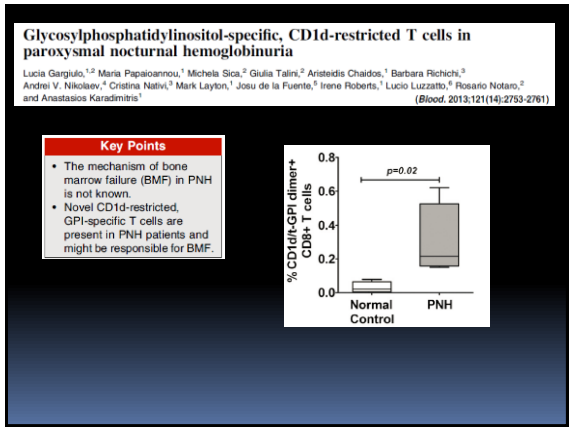

Complement Activation
Classical Pathway
Lectin Pathway
Alternative Pathway
Proximal
Terminal
C3
C4b2a
C3bBb
C3b
CD55
CFH
CFI/MCP
Weak Anaphylatoxin
Microbial Opsonization
Immune Complex Clearance
C5-convertase
C5
C5a
C5b C6 C7 C8 C9
Terminal Complement Complex
CD59
Membrane Attack Complex formation leads to:
Prothrombotic & proinflammatory environment
Cell Lysis
Cell Activation
Prothrombotic
Potent Anaphylatoxin
Chemotaxis
Cell Activation
Proinflammatory
Consequences of Chronic Hemolysis
Thrombosis Pulmonary Hypertension Poor QoL
Renal Failure Fatigue Anemia


Terminal Complement Activation Renders RBCs Susceptible to Lysis
Normal RBCs
PNH RBC
CD59
Complement Activation
Intact RBC
Chronic Hemolysis
Lysed PNH RBCs and free hemoglobin in the plasma


Complement Sensitivity of Normal and PNH Red Cells
Insensitive cells
Normal Cells
PNH I Cells
sensitive cells
PNH II Cells
PNH III Cells
Fraction lysed
Fraction unlysed
Lysis with anti-4
Milliliters human serum diluted 1 in 270
Rosse WF, Br J Haematol 1973


Bone Marrow Failure Syndromes and PNH
Normal
1. Insult to Bone Marrow
2. Mutation in PIG-A
Aplastic Anemia (PNH clone in up to ~50%*)
PNH w/out AA or MDS
MDS (occasional PNH clone)
*PNH clone ≥ 0.003%


Models of pathogenesis
Normal Marrow
Aplastic Anemia
PNH
MDS
Immune Assault
Immune Assault
Stromal cell Dysregulation, Immune Assault
PNH (hemolysis)
PNH (hypoplasia)

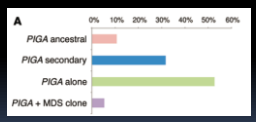

Glycosylphosphatidylinositol-specific, CD1d-restricted T cells in paroxysmal nocturnal hemoglobinuria
Lucia Gargiulo,[1,2] Maria Papaioannou,[1] Michela Sica,[2] Giulia Talini,[2] Aristeidis Chaidos,[1] Barbara Richichi,[3] Andrei V. Nikolaev,[4] Cristina Nativi,[3] Mark Layton,[1] Josu de la Fuente,[5] Irene Roberts,[1] Lucio Luzzatto,[6] Rosario Notaro,[2] and Anastasios Karadimitris[1]
(Blood. 2013;121(14):2753-2761)
Key Points
The mechanism of bone marrow failure (BMF) in PNH is not known.
Novel CD1d-restricted, GPI-specific T cells are present in PNH patients and might be responsible for BMF.
% CD1d/GPI dimer+ CD8+ T cells
p=0.02
Normal Control
PNH

Clonal selection – T cell mediated process
Clonal dominance - ?
Clonal expansion _ ?

**Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria**

Wenyi Shen,[1,2] Michael J. Clemente,[1] Naoko Hosono,[1] Kenichi Yoshida,[3] Bartlomiej Przychodzen,[1] Tetsuichi Yoshizato,[3] Yuichi Shiraishi,[4] Satoru Miyano,[4,5] Seishi Ogawa,[3] Jaroslaw P. Maciejewski,[1] and Hideki Makishima[1]

*J Clin Invest*. 2014;124(10):4529–4538



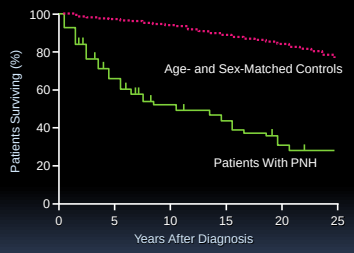

*STAC3, DHX29, TET2, JAK2, SUZ12, NTNG1*

## Clinical Aspects of PNH

## The clinical picture of PNH

- Hemolysis due to complement activation
  - Anemia and fatigue
  - Hemoglobinuria, kidney damage
  - Nitric oxide trapping >> Esophageal spasm, abdominal pain, pulmonary hypertension, impotence, fatigue?
- Thrombosis – Cause of blood clots is still unknown
  - Unusual sites of blood clots
- Bone marrow failure
  - Decreased blood counts (cytopenias)

## Significant Mortality in PNH




- 5 year mortality: 35%
- Diagnosed at all ages - median time from diagnosis to death: 10-15 yrs

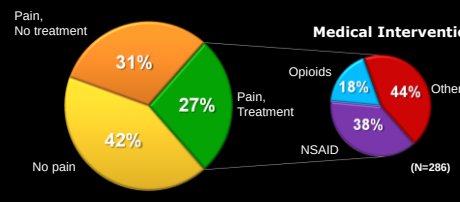
Hillmen P, et al. N Engl J Med. 1995;333:1253-8.

## Fatigue in PNH is significant

- Rosse book chapter (Hoffman-*Hematology*)[1]
  - "Many patients note a feeling of fatigue that may be disabling during periods of hemoglobinuria."
  - This is not related to hemoglobin level (anemia), as it disappears when the hemoglobinuria stops."
- Brodsky book chapter (Hoffman-*Hematology*)[2]
  - "PNH patients frequently complain of disabling fatigue that is often out of proportion to the degree of anemia."

1. Rosse. Paroxysmal nocturnal hemoglobinuria In: R Hoffman; EJ Benz; SJ Shattil et al., eds. *Hematology: Basic Principles and Practice*. 3rd ed. New York: Churchill-Livingstone; 2000:331-342.
2. Brodsky. Paroxysmal nocturnal hemoglobinuria. In: R Hoffman; EJ Benz; SJ Shattil et al., eds. *Hematology. Basic Principles and Practice*. 4th ed. Philadelphia: Elsevier Churchill Livingstone; 2005:419-427.

## Pain is a Common Symptom in PNH Patients

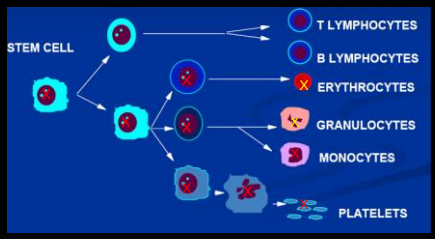



**Almost 3 out of 5 (58%) patients reported significant pain**
**27% of patients with pain required medical intervention**

South Korean National Registry

Lee JW et al. *Hematologica* 2010. 95 (s2): Abstract #506.

## Clone size




Clone size refers to how many of the bone marrow stem cells have the mutation. In PNH, since the PNH red cells are being destroyed, the % of red cells that are CD59 – (PNH cells) does not give an accurate estimate of clone size. The white cells (granulocytes or monocytes) are not destroyed. Therefore the % of abnormal granulocytes is a more accurate estimate of the percentage of abnormal stem cells in the bone marrow.

## Incidence of symptoms or complications of PNH Correlation with clone size

| | PNH Clone | | | | Bone Marrow Disorder | | |
|---|---|---|---|---|---|---|---|
| Symptom or complication | <10% | 10-49% | >50% | P-value* | Aplastic or Hypoplastic Anemia | No Bone Marrow Disorder | P-value** |
| TE | 5% | 5% | 22% | <.01 | 18% | 19% | 0.89 |
| Abdominal Pain | 41% | 53% | 46% | 0.58 | 48% | 47% | 0.92 |
| Shortness of breath | 49% | 44% | 53% | 0.63 | 53% | 61% | 0.23 |
| Chest pain | 14% | 31% | 24% | 0.21 | 31% | 27% | 0.43 |
| Fatigue | 59% | 72% | 76% | 0.15 | 75% | 76% | 0.99 |
| Discolored urine | 30% | 56% | 75% | <.01 | 60% | 72% | 0.05 |

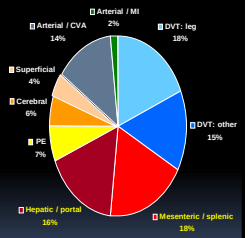
*Chi-square test for differences across three PNH clone size categories.
** Chi-square test for differences across two BMD categories.

International PNH Registry data – 524 patients

Urbano-Ispizua A, et al. EHA meeting 2010. Haematologica 95(s2): Abstract 1022

## What about thrombosis (blood clots) in PNH?

- Blood clots are a presenting sign in 10-20% of patients with PNH.
- Can occur in up to 40% of patients with PNH.
- Occur in unusual locations – veins of the liver (Budd-Chiari syndrome), spleen, brain, and skin.
- Associated with a very bad prognosis
- Cause of these blood clots is unknown – possibly related to complement activation.




## Chronic Renal Insufficiency in PNH

- Associated with hemolysis and/or microvascular thrombosis[1,2]
- Insidious and progressive chronic renal insufficiency (CRI, GFR <60/ml/min) in up to ~ 30% of patients[2]
- May be acute renal failure, which is frequently reversible[2]
- Renal failure reported as cause of death in ~ 8% of US PNH patients[3]

1. Brodsky. Paroxysmal Nocturnal Hemoglobinuria. In: Hoffman, et al, eds. *Hematology - Basic Principles and Practices*. 4th ed. Philadelphia, PA: Churchill Livingstone; 2005:419-427.
2. Clark DA, et al. *Blood*. 1981;57:83-89.
3. Nishimura J et al. *Medicine*. 2004;83:193-207.

## Diagnosis of PNH

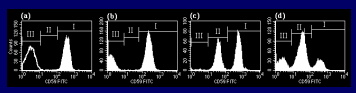
**Average delay to diagnosis exceeds 3 years; may be greater than 10 years[1]**

- **PNH continues to be primarily a clinical diagnosis, which can be confirmed by laboratory analyses**
- **Signs and symptoms are highly variable and may mirror other conditions**
- **Most common symptoms at presentation are not unique to PNH**
  - **Hemolytic anemia, often requiring transfusions**
  - **Fatigue**
  - **Dyspnea**
  - **Abdominal pain or dysphagia**

[1]Hillmen, et al. *New Engl J Med*. 1995;333:1253-1258.
[2] Dacie & Lewis. *Sem Haemat*. 1972;5:3-23.

## Flow Cytometry: Diagnostic Test for PNH

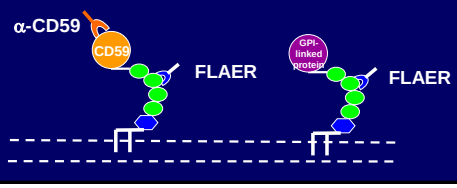
- **Perform on peripheral blood**
- **Test both granulocytes and erythrocytes[2]**
  - **Erythrocytes alone are not sufficient due to hemolysis and the dilution effect of transfusions**
- **Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55[1,2]**
- **PNH blood cells (PNH clone) are cells missing GPI-anchored proteins**




[1]Parker, et al. *Blood*. 2005;106:3699-3709.
[2]Hall & Rosse. *Blood*. 1996;87:5332-5340.

## Fluorescent AERolysin (FLAER)

- FLAER binds to the GPI-anchor itself, rather than to a single protein such as CD55 or CD59
- FLAER provides much greater signal noise and better accuracy than an antibody against a single target




## Who Should Be Screened For PNH?

- Patients with:
  - Hemoglobinuria[1]
  - Hemolytic anemia[1]
  - Bone marrow dysfunction[1]
    - Aplastic anemia (AA) or MDS screened annually
  - Coombs-negative intravascular hemolysis[1]
    - Elevated serum LDH
  - Unusual or unexplained venous thrombosis[1]
    - Budd-Chiari syndrome
    - Mesenteric, portal, cerebral, or dermal veins
  - Unexplained arterial thrombosis[2,3]

LDH=lactate dehydrogenase; MDS=myelodysplastic syndrome.

[1]Parker, et al. *Blood.* 2005;106:3699-3709. [2]Hillmen, et al. *N Engl J Med.* 1995;333:1253-1258. [3]Nishimura, et al. *Medicine.* 2004;83:193-207.

## What happens to PNH patients?

## PNH – What do patients die from?

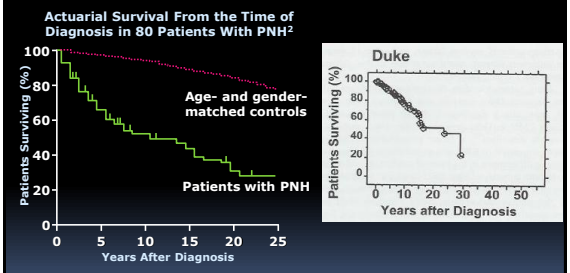
| Cause of death | Duke | Japan |
|---|---|---|
| Thrombosis | 16 (42%) | 3 (8%) |
| Abd site | 8 | 1 |
| Other site | 7 | 0 |
| Arterial | 3 | 2 |
| Hemorrhage | 4 (10.5%) | 9 (24%) |
| Severe Infection | 14 (36.5%) | 14 (36.8%) |
| MDS/AML | 3 (8%) | 6 (16%) |
| Renal failure | 3 (8%) | 7 (18%) |
| Other malignancy | 2 (5%) | 2 (5%) |
| Unknown | 2 (5%) | 0 |

Nishimura et al Medicine 83: 193-207, 2004.

## Possible long term effects of Eculizumab

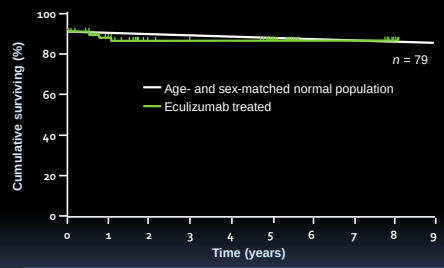
- Improve kidney function
- Prevent pulmonary hypertension
- Increase survival

## PNH Survival – Pre-eculizumab



## Eculizumab Has a Major Impact on Survival in PNH

*Survival is comparable to age and gender-matched control population out to 8 years*




- 96% (76/79) patient survival
- There was no difference in mortality between patients on eculizumab and the normal population (*P*=0.46)

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

bjh research paper

### Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria

British Journal of Haematology, 2013, **162**, 62–73

Peter Hillmen,[1] Petra Muus,[2] Alexander Röth,[3] Modupe O. Elebute,[4] Antonio M. Risitano,[5] Hubert Schrezenmeier,[6] Jeffrey Szer,[7] Paul Browne,[8] Jaroslaw P. Maciejewski,[9] Jörg Schubert,[10] Alvaro Urbano-Ispizua,[11] Carlos de Castro,[12] Gérard Socié[13] and Robert A. Brodsky[14]

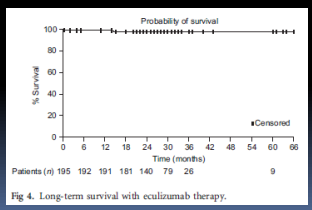



Fig 4. Long-term survival with eculizumab therapy.