Thrombosis in Paroxysmal Nocturnal Haemoglobinuria 'PNH'

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UMR UA11 INSERM-University of Montpellier IDESP, France;
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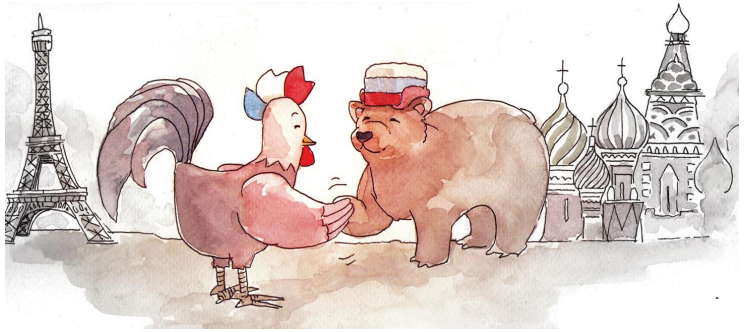
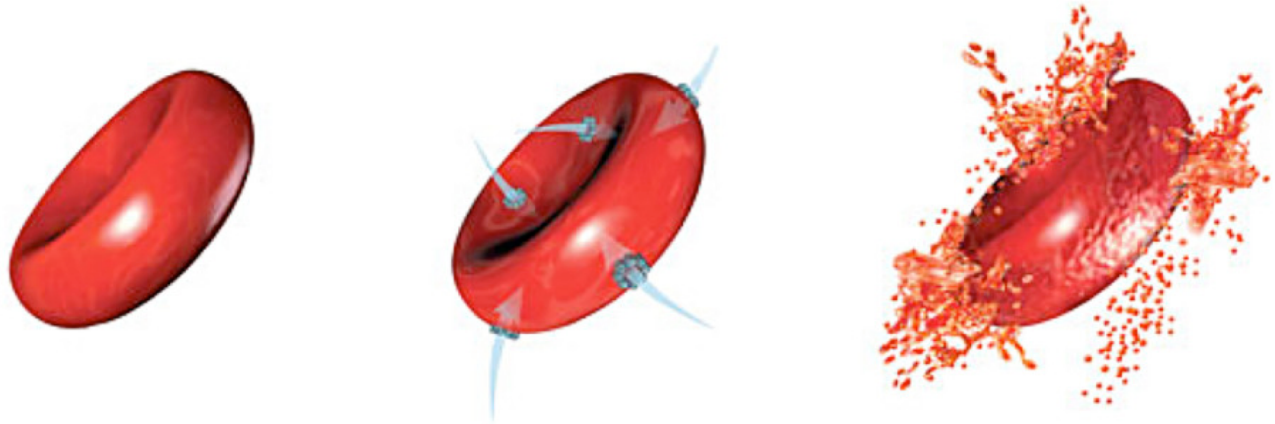
Meet the Hemostasis Experts V, Athens, October 7, 2021

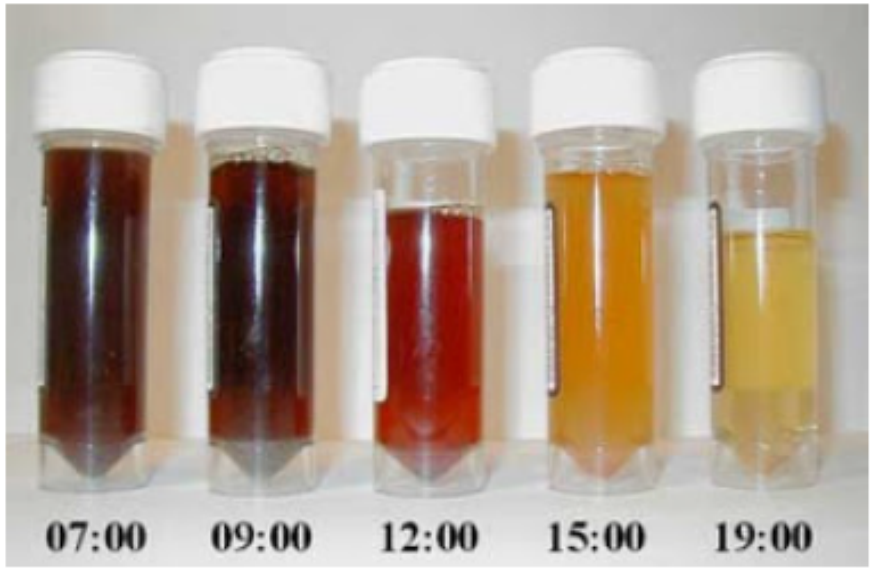
Disclosures



- Grants:
  - Bayer Healthcare SAS, Sanofi SA, Stago
- Scientific Advisory Boards:
  - Sanofi SA, LFB, Novo Nordisk,
    Coagulant Therapeutics
- Speaker / speaker bureau member, supports:
  - Alexion Pharma France, Amgen, Astra-Zeneca, Bayer,
    Bristol-Myers-Squibb, Boehringer-Ingelheim, Daïchi-Sankyo,
    Fumouze diagnostics-Sofibel, Glaxo-Smith-Kline,
    Horiba ABX SAS, Laboratoire Français des Biotechnologies (LFB),
    Léo Pharma, Novo Nordisk, Oséus, Pfizer, Sanofi SA, Shire, Stago



PNH: *total recall*



HISTORICAL REVIEW

Paroxysmal Nocturnal Hemoglobinuria

A Classic Description by Paul Strübing in 1882, and a Bibliography of the Disease

By WILLIAM H. CROSBY, LT. COL., M.C., A.U.S.

Blood 1951; 6 (6): 270-284

Strübing P.
Deutche Med. Wehnschr. **1882**; 8:1-17

Lecture for habilitation as Dozent in Greifswald, Pomerania, Germany.
Paroxysmale Haemoglobinurie.

« After his discharge from the military service in 1876, the patient observed that his urine sometimes looked dark brown or black. This color change only showed itself in the morning in the urine, passed after getting up and by noon. Fatigue, abdominal pain »

Enneking J.
Klin Wochenschr. 1928; 7:2045.

Haemoglobinuria paroxysmalis nocturia

CHRONIC HEMOLYTIC ANEMIA WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Study of the Mechanism of Hemolysis in Relation to Acid-Base Equilibrium

THOMAS H. HAM, M.D.†

BOSTON

THE clinical syndrome of chronic hemolytic anemia associated with paroxysmal nocturnal hemoglobinuria, first recognized as a disease entity by the two Italian investigators, Marchiafava[1] and Micheli,[2] has been reviewed recently by Witts[3] and Hamburger and Bernstein.[4] The present communication deals with the preliminary results of a study of the mechanism of hemolysis in three patients exhibiting this syndrome, of whom one* has already been reported as such by Hamburger and Bernstein.[4]

The blood picture of these three adult subjects, two males and a female, was characteristic of increased blood destruction, as evidenced by severe anemia, increased reticulocytes, elevated serum bilirubin and especially the constant finding of free hemoglobin in the plasma by spectroscopy. The plasma varied in color from light brown to reddish-brown. In two of the patients there was mild leukopenia and thrombocytopenia. Moderate splenomegaly was present in one. In the third patient,* whose spleen had been removed twenty-six months before these observations, the white blood-cell and platelet counts were normal or slightly increased. The bloods of all the patients belonged in Group O (International), and their red blood cells showed a normal fragility in hypotonic salt solutions. The Wassermann reactions were negative. At some time during the period of observation of each patient the urine appeared red, dark reddish-brown or almost black and contained large amounts of free hemoglobin, as demonstrated by spectroscopic examination. Variable amounts of albumin but no red blood cells were found in the urine. Hemosiderin, identified by the method of Cook,[5] was a constant urinary finding.

Quantitative determinations of the amounts of hemoglobin in the plasma and urine were made by a modification of the benzidine method of Bing and Baker[6] upon specimens obtained every three hours during day and night for periods of several days. In the two patients whose spleens had not been removed, an increase in hemoglobinemia was always observed during sleep, whether at night or during the day, and an increase in hemoglobinuria was frequently present. These phenomena did not occur if the patient was kept awake for twenty-four hours or kept awake under basal conditions in positions assumed during sleep. There was no apparent relation to the time or amount of ingested food or fluid. In the patient whose spleen had been removed, there was no increase in hemoglobinemia or hemoglobinuria during sleep, although definite nocturnal hemoglobinuria had been observed by Hamburger and Bernstein[4] before splenectomy.

Because of the elevation in the carbon-dioxide content of the arterial blood and the decrease in pH known to occur during sleep,[7, 8] it was suspected that a change in the acid-base equilibrium was related to the increased hemoglobinemia of two of the patients during sleep. When two subjects were given 40 gm. of sodium bicarbonate daily the hemoglobinemia and hemoglobinuria decreased; withdrawal of the alkali produced an increase in plasma and urine hemoglobin. When a large single dose of ammonium chloride (10 or 12 gm.) was given, the hemoglobinemia and hemoglobinuria increased, and in one patient the pH of the arterial blood fell to 7.25. In one patient the pH of the arterial blood during natural sleep was 7.3, with a carbon-dioxide partial pressure of 42 mm. of mercury; the usual increase in hemoglobinemia and hemoglobinuria occurred. In the same twenty-four-hour period, however, hyperventilation in a Drinker artificial respirator during natural sleep caused a rise in the arterial-blood pH to 7.47, a fall in the carbon-dioxide partial pressure to 28 mm. and a decrease in plasma and urine hemoglobin.

It was noted that on standing for four hours at room temperature or in the incubator, samples of whole clotted blood, defibrinated blood and blood containing heparin as anticoagulant, all developed progressive hemolysis. At ice-box temperatures hemolysis was minimal. Comparable samples of the blood of normal subjects showed no significant hemolysis under these conditions. Equilibration of the patients' defibrinated blood or blood containing heparin with mixtures of oxygen and

This investigation was aided in part by a grant from the J. K. Lilly gift to the Harvard Medical School.

From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

*Study of this patient was made possible through the courtesy of Dr. William B. Porter, of Richmond, Virginia.

†Assistant in medicine, Harvard Medical School.

N Engl J Med **1937**; 217(6): 915-7

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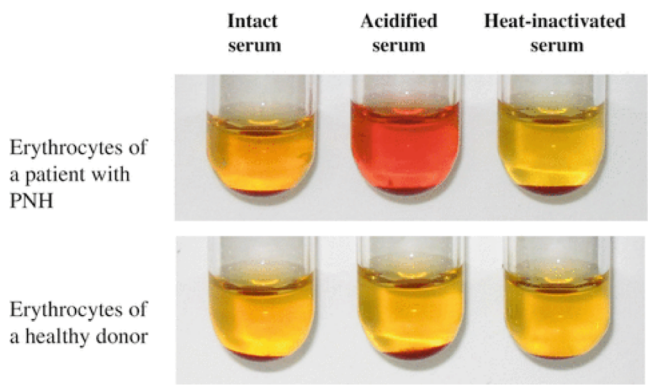
STUDIES ON DESTRUCTION OF RED BLOOD CELLS. II. CHRONIC HEMOLYTIC ANEMIA WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: CERTAIN IMMUNOLOGICAL ASPECTS OF THE HEMOLYTIC MECHANISM WITH SPECIAL REFERENCE TO SERUM COMPLEMENT[1]

BY THOMAS HALE HAM AND JOHN H. DINGLE[2]

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital, the Department of Medicine, and the Department of Bacteriology and Immunology, Harvard Medical School, Boston)

J Clin Invest **1939**; 18(6):657-72.

**« The mechanism
is that of abnormal red blood cells
hemolysed in presence
of human complement,
the susceptibility to cell lysis
varying with the degree of acidity of the serum »**



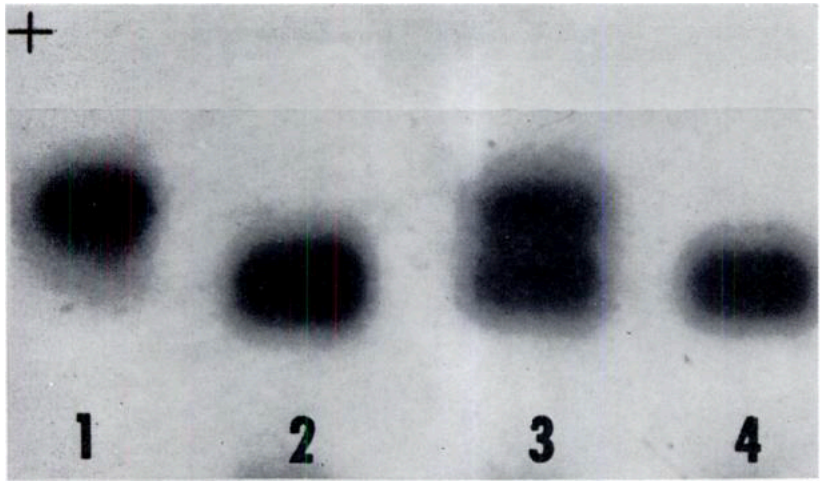
Paroxysmal Nocturnal Hemoglobinuria: Evidence for Monoclonal Origin of Abnormal Red Cells

By S. B. Oni, B. O. Osunkoya and L. Luzzatto

Blood, Vol. 36, No. 2 (August), 1970

PNH in a 26-years old Nigerian woman,
died of amebic colitis.

Patient's red cells:
mosaicism with respect to G6PDH.
(A and B variants).
Red cells bearing PNH abnormality:
only the B variant.



1: G6PDH type A, control
2: Type B, control
3: Patient's whole hemolysate
4: Patient's PNH cells

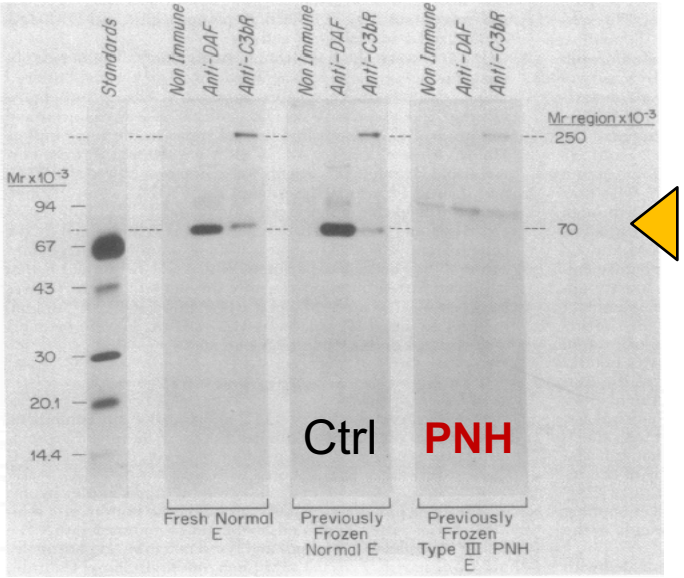
Affected erythrocytes of patients with paroxysmal nocturnal hemoglobinuria are deficient in the complement regulatory protein, decay accelerating factor

(complement regulation/C3 convertases)

ANNE NICHOLSON-WELLER*†‡, JONATHAN P. MARCH‡, STEPHEN I. ROSENFELD§, AND K. FRANK AUSTEN*‡

Proc. Natl. Acad. Sci. 1983; 80:5066-70.

D.A.F.: Decay Accelerating Factor; CD 55



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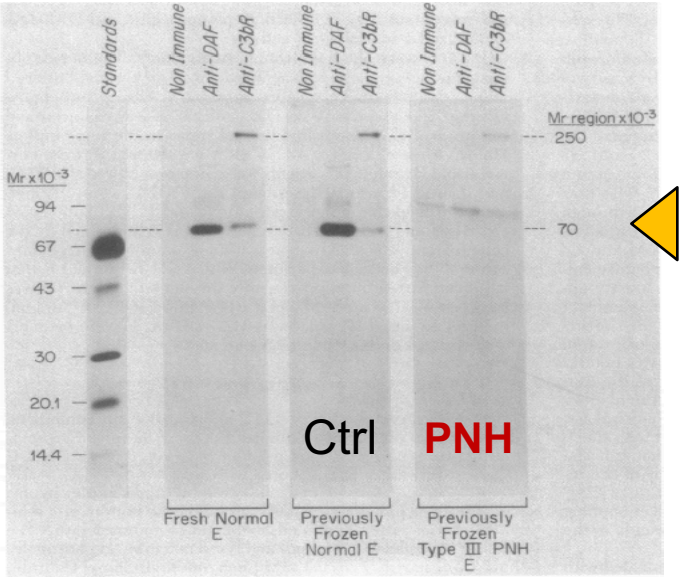
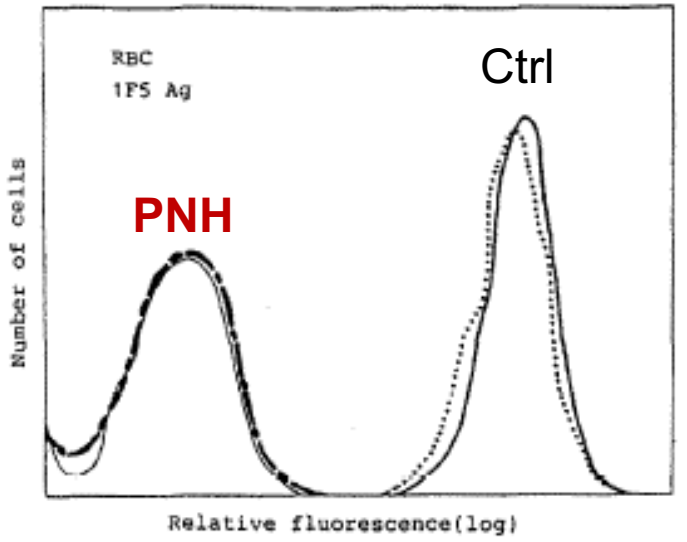


Analysis of PI (phosphatidylinositol)-anchoring antigens in a patient of paroxysmal nocturnal hemoglobinuria (PNH) reveals deficiency of 1F5 antigen (CD59), a new complement-regulatory factor

Ryo Taguchi, Yasuhiro Funahashi, Hiroh Ikezawa and Izumi Nakashima*

FEBS Lett. 1990; 261(1):142-6.

M.I.R.L.: Membrane Inhibitor of Reactive Lysis, CD 59



DISTRIBUTION OF DECAY-ACCELERATING FACTOR IN THE PERIPHERAL BLOOD OF NORMAL INDIVIDUALS AND PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

By TAROH KINOSHITA, M. EDWARD MEDOF, ROBERT SILBER, AND VICTOR NUSSENZWEIG

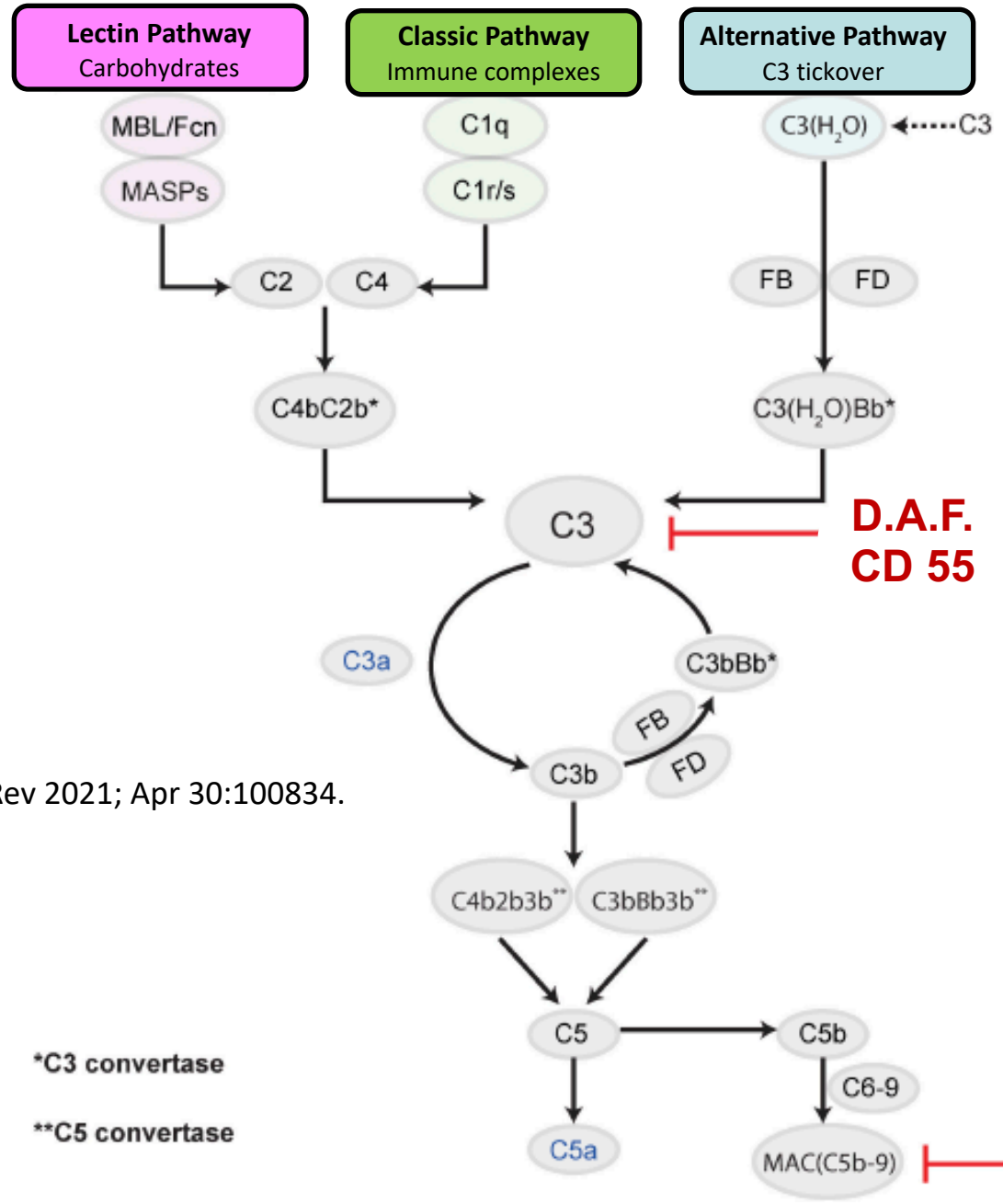
J. Exp. Med. 1985; 162:75-92.

Variable DAF deficiency
in **erythrocytes**,
but also in
platelets,
PMN, monocytes, lymphocytes

« Additional support
for the
clonal origin of PNH cells,
derived from an
abnormal bone marrow progenitor »

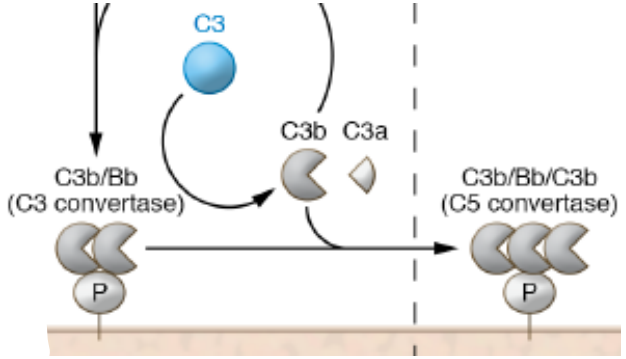
Distribution of DAF in Cells from Peripheral Blood of PNH Patients

Cell type	Expression of DAF in PNH patients		
	Patient SB	Patient GC	Patient VR
Erythrocytes	60% Undetectable 40% Normal	40% Undetectable 60% Below normal*	30% undetectable 70% low levels
Platelets	78% Undetectable 22% Normal	Undetectable (by IRMA)	ND‡
Monocytes	80% Undetectable 20% Normal	Undetectable	Very low
PMN	77% Undetectable 23% Normal	Undetectable	Very low
Lymphocytes	Normal	Very low or undetectable	Very low

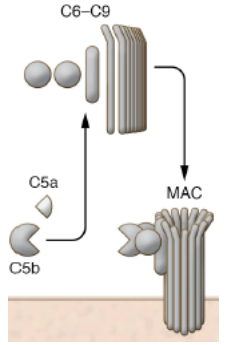


Blood Rev 2021; Apr 30:100834.

CD 55:
accelerate the decay
of cell-surface bound C3 and C5 convertases;



CD 59: inhibits pore formation
of MAC; membrane attack complex



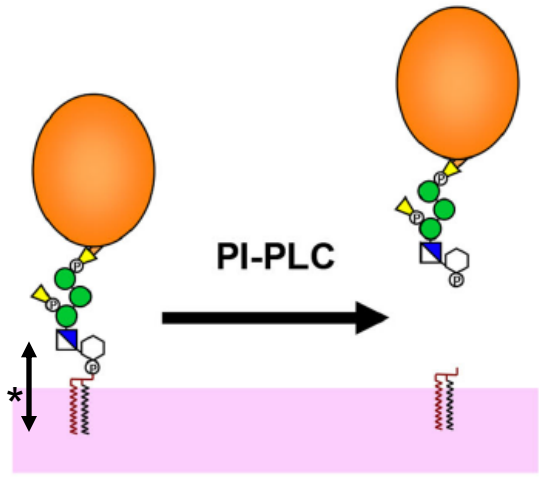
1976: M.G. Low and coll. purify a novel **phospholipase**
from *Bacillus cereus*
that acts upon phosphatidylinositol;
It releases alkaline phosphatase from tissues

Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein

Martin G. Low & Paul W. Kincade

Nature 1985; 318(6041):62-4.

THY-1:
CD 90



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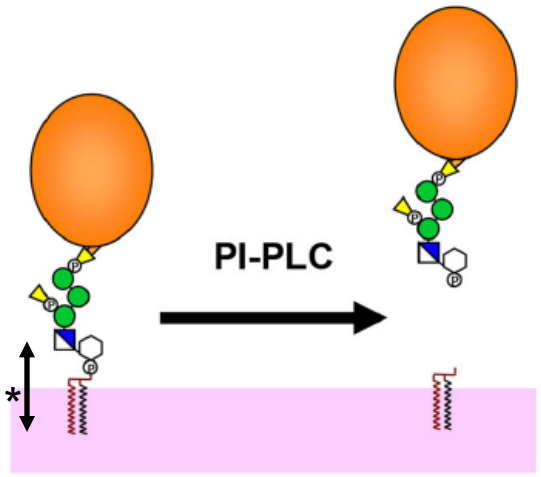

*** Phosphatidylinositol**

Glycosylphosphatidylinositol-anchored proteins
GPI-APs

Complement defense proteins

Decay Accelerating Factor DAF, CD55

Membrane Inhibitor of Reactive Lysis MIRL, CD59

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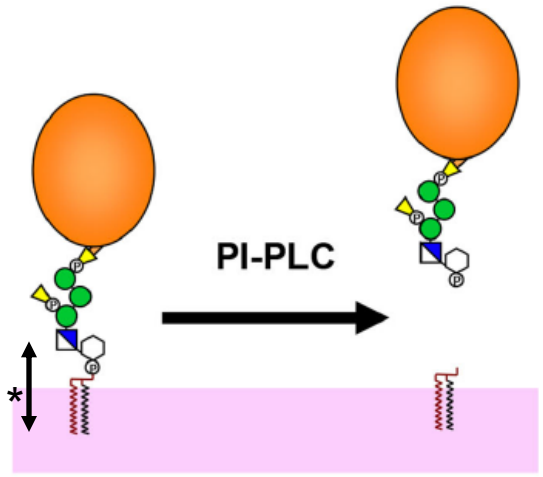


*** Phosphatidylinositol**

Glycosylphosphatidylinositol-anchored proteins *GPI-APs*

Abnormal PNH cells are deficient in GPI-APs

Complement defense proteins

- **Decay Accelerating Factor DAF, CD55**
- **Membrane Inhibitor of Reactive Lysis MIRL, CD59**
- C8 Binding Protein, HRF

Ecto-enzymes

- Acetylcholinesterase, erythrocytes
- Alkaline phosphatase, leukocytes
- 5'-ectonucleotidase, lymphocytes

Receptors

- Fcγ receptor III, CD16b
- Urokinase receptor, u-PAR, CD87
- Folate receptor
- Endotoxin binding receptor CD14
- Immunologic contact receptors
  - LFA-3: CD58 , all cells
  - BLAST-1: CD48, lymphocytes
  - CAMPATH-1: CDw52, lymphocytes

Other proteins

- JMW-bearing protein, erythrocytes
- CD24, a P-selectin ligand, lymphocytes
- Granulocyte-specific activation Ag CD66
- Granulocyte-specific activation Ag CD67

Biosynthesis and biology of mammalian GPI-anchored proteins

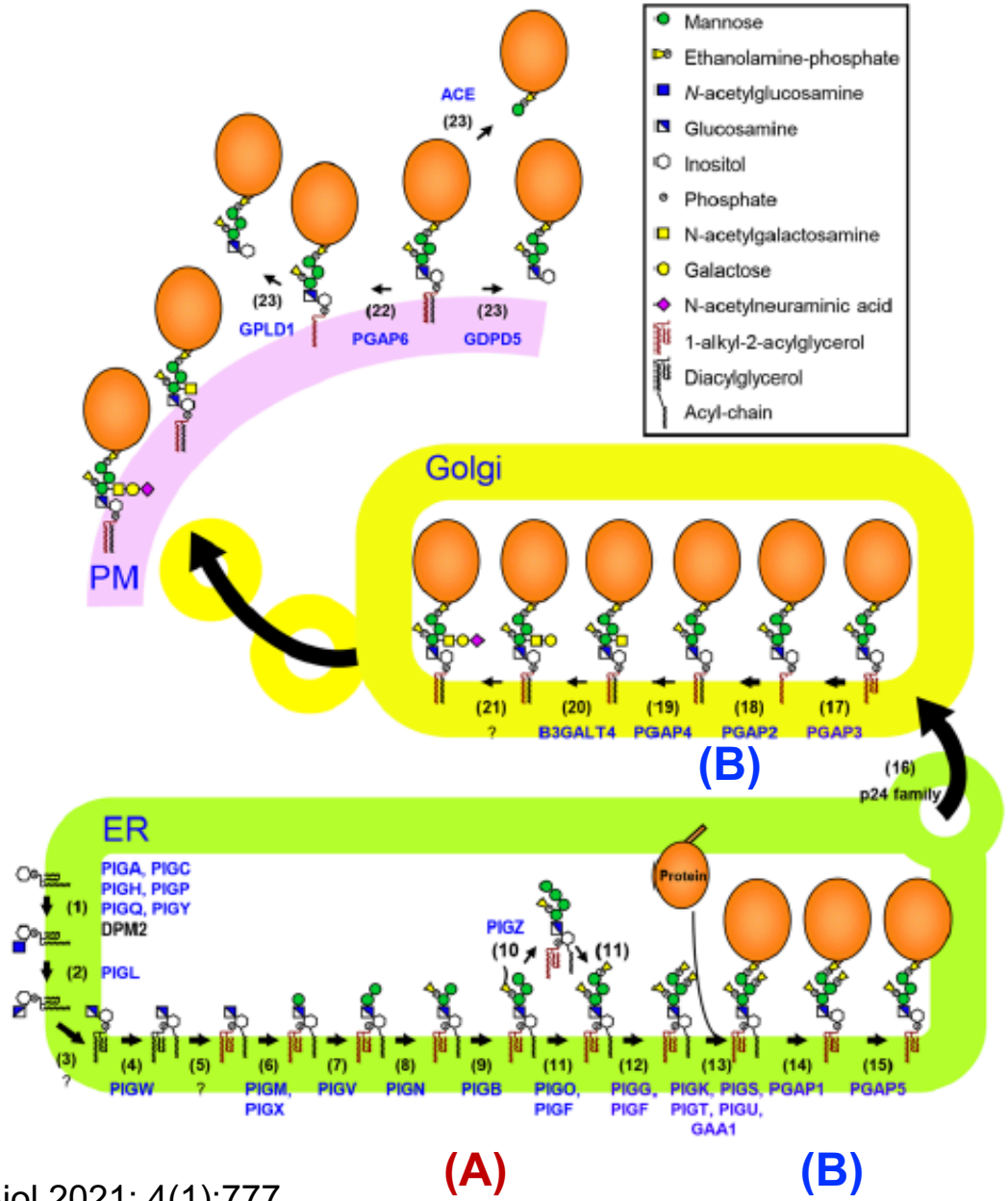
Taroh Kinoshita

Open Biol. 2020; 10(3):190290

GPI-AP synthesis: very complex; 3 parts:
**** biosynthesis***
**** protein attachment to GPI***
**** GPI-AP remodelIng***

Whole process: 15 stages;
26 genes > 15 enzymes;

22 **phosphatidylinositol glycan (PIG) genes (A)**:
biosynthesis and polypeptide chain attachment to GPI,
endoplasmic reticulum ER
4 **post-GPI attachment to protein (PGAP) genes (B)**:
GPI modifications,
ER-Golgi-Plasma membrane transport

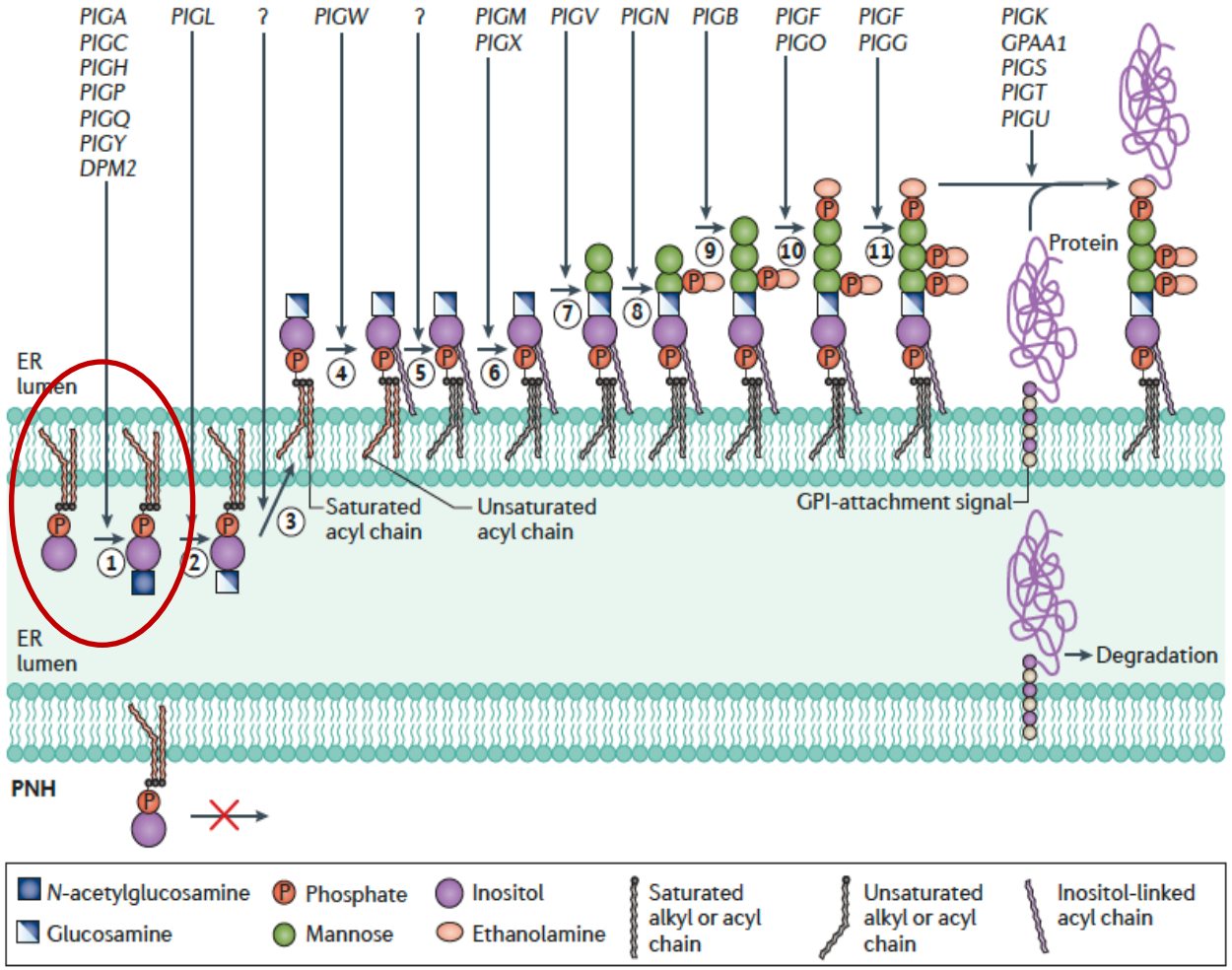


Com Biol 2021; 4(1):777

Deficient Biosynthesis of *N*-Acetylglucosaminyl-Phosphatidylinositol, the First Intermediate of Glycosyl Phosphatidylinositol Anchor Biosynthesis, in Cell Lines Established from Patients with Paroxysmal Nocturnal Hemoglobinuria

By Minoru Takahashi,* Junji Takeda,* Shinichi Hirose,§ Robert Hyman,‖ Norimitsu Inoue,* Toshio Miyata,* Etsuko Ueda,‡§ Teruo Kitani,‡ M. Edward Medof,§ and Taroh Kinoshita*

J Exp Med 1993; 177(2):517-21



Nat Rev Dis Primers 2017; 3:17028

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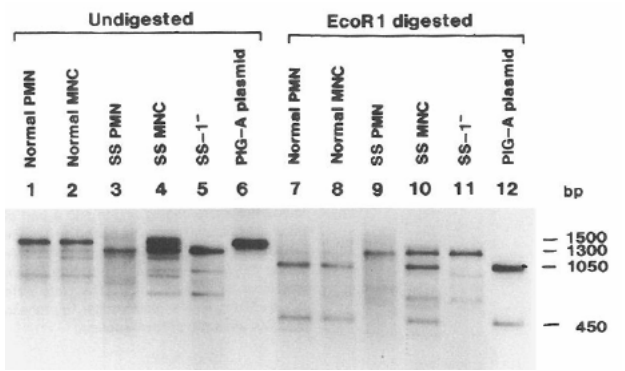
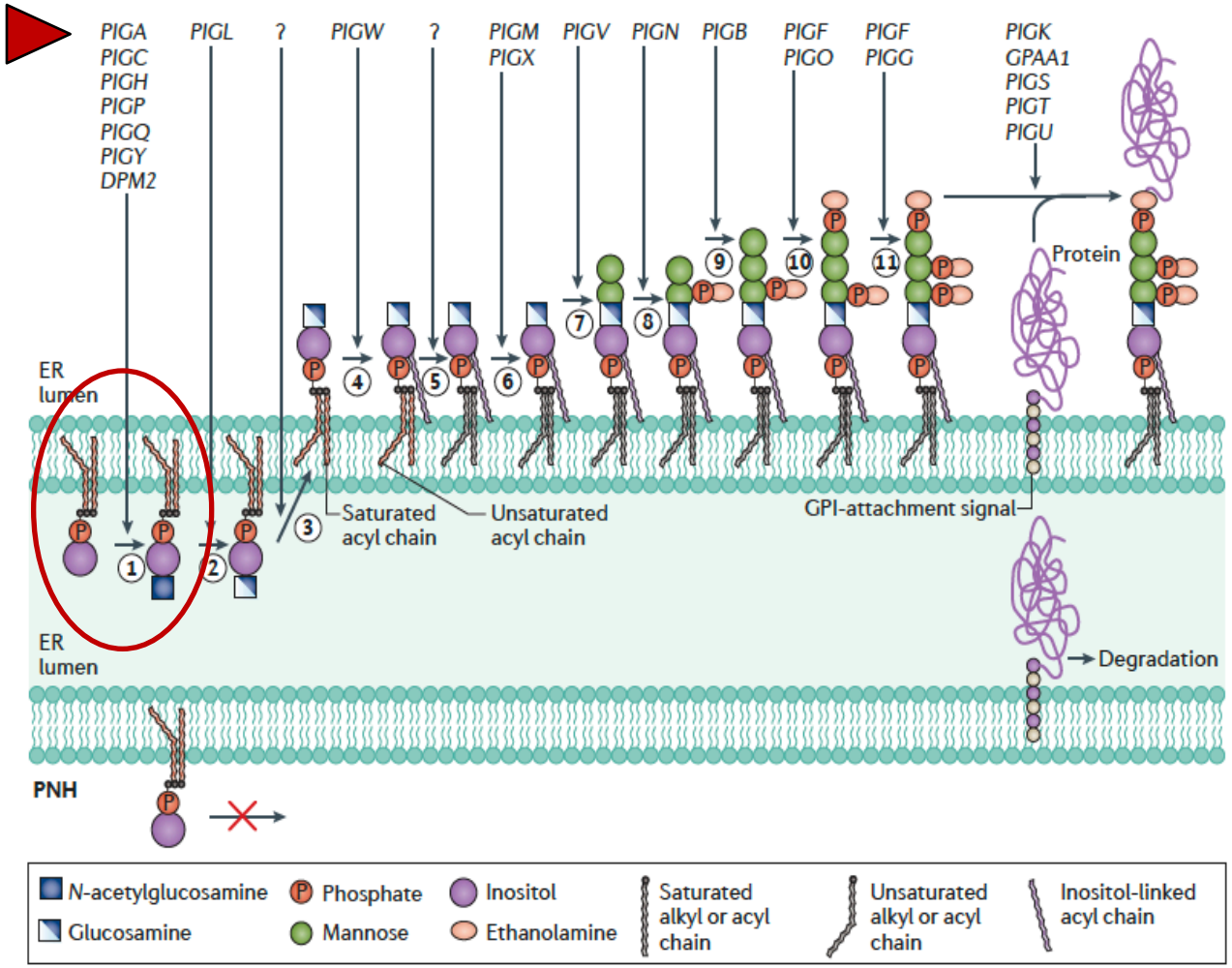
J Exp Med 1993; 177(2):517-21

Deficiency of the GPI Anchor Caused by a Somatic Mutation of the *PIG-A* Gene in Paroxysmal Nocturnal Hemoglobinuria

Cell 1993; 73(4):703-11





Nat Rev Dis Primers 2017; 3:17028

PIGA (Phosphatidylinositol Glycan Anchor biosynthesis class A gene):

X-linked gene (Xp22.2)

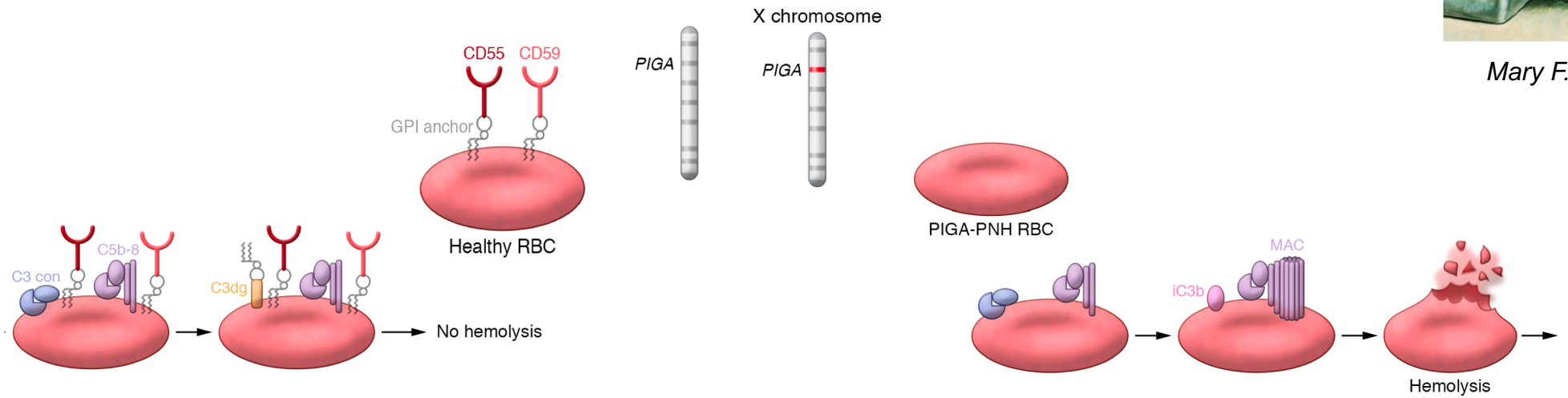
Only one allele is functional;

in men: (X,Y)

in women: (X,X) but X inactivation (lyonization)

A single somatic mutation is sufficient to cause GPI-AP deficiency

Mary F. Lyon



Germ-line *PIGA* mutations:

Loss-of-function mutations: thought embryonic lethal.

Hypomorphic *PIGA* mutations: X-linked form of the multiple congenital anomalies-hypotonia-seizure syndrome 2 (MIM 300818)

Severe intellectual disability, dysmorphic facial features, seizures, early death.

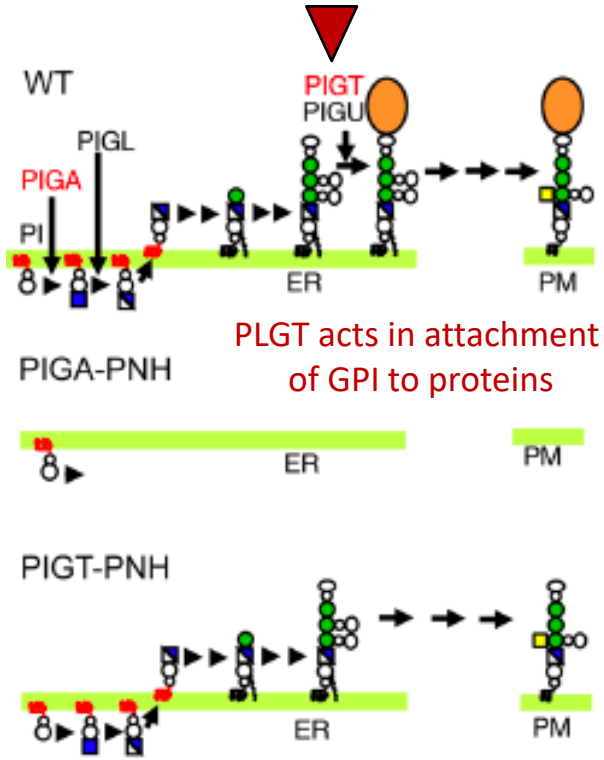
Most conspicuous on granulocytes

Red cells: little or no GPI anchor deficiency and no haemolysis

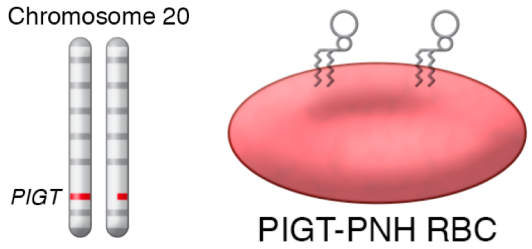
Complement and inflammasome overactivation mediates paroxysmal nocturnal hemoglobinuria with autoinflammation

Britta Höchsmann,[1,2] Yoshiko Murakami,[3,4] Makiko Osato,[3,5] Alexej Knaus,[6] Michi Kawamoto,[7] Norimitsu Inoue,[8] Tetsuya Hirata,[3] Shogo Murata,[3,9] Markus Anliker,[1] Thomas Eggermann,[10] Marten Jäger,[11] Ricarda Floettmann,[11] Alexander Höllein,[12] Sho Murase,[7] Yasutaka Ueda,[5] Jun-ichi Nishimura,[5] Yuzuru Kanakura,[5] Nobuo Kohara,[7] Hubert Schrezenmeier,[1] Peter M. Krawitz,[6] and Taroh Kinoshita[3,4]

J Clin Invest 2019; 129(12):5123-36



PLGT acts in attachment of GPI to proteins

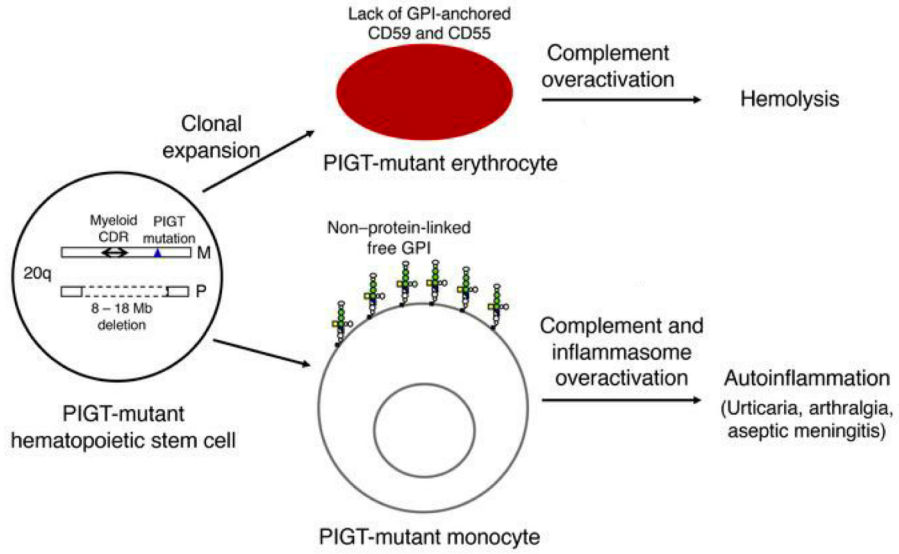


Describe two types of patients
in whom PNH results from
biallelic mutations of *PIGT* on chromosome 20
and of *PLGB* on chromosome 15
(germ-line mutation in one allele + somatic mutation in the other)

Typical PNH

+

prominent autoinflammatory features,
urticaria, arthralgia
including recurrent attacks of aseptic meningitidis
(free GPI stimulates the inflamasome)



Advances in the creation of animal models of paroxysmal nocturnal hemoglobinuria

Yingying Chen and Fu Rong

HEMATOLOGY
2021, VOL. 26, NO. 1, 491–496

Successful creation of PNH mouse and PNH rhesus macaque models

Detection of GPI-APs deficient cells with shorter lifespans and inceased sensitivity to complement-activated haemolysis in vitro

NO clinical manifestations such as haemolysis and/or thrombosis

Suggests that the PIG-A mutation is one of the several conditions required for PNH, but it alone is not enough to cause PNH

Mutational landscape and its clinical significance in paroxysmal nocturnal hemoglobinuria

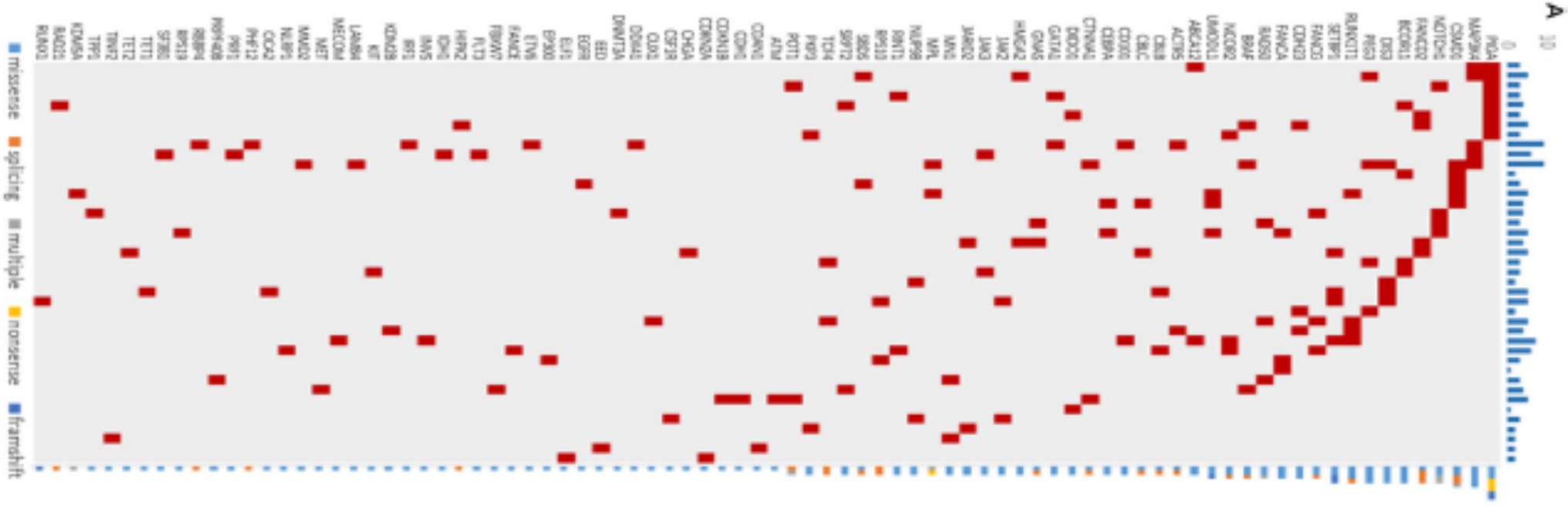
Fangfei Chen[1,2], Shimin Hu[3], Jing Ruan[1], Miao Chen[1] and Bing Han[1]

Blood Cancer Journal (2021)11:58

41 patients with newly diagnosed PNH
WES, 178 myeloid cancer-related genes

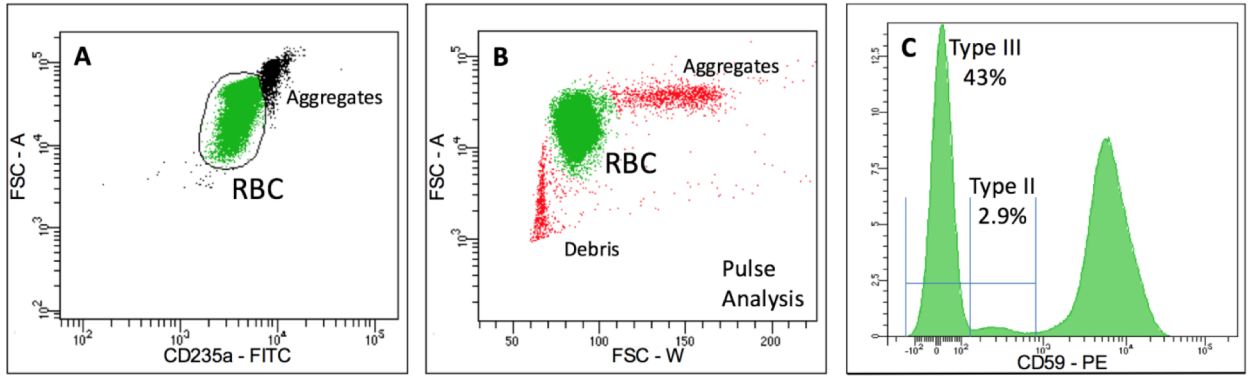
10 most frequently mutated genes:
PIGA, BCORL1, RUNX1T1, MAP3K4, CSMD1, NOTCH1, FANCD2, PEG2, DIS3, SETBP1.

Associations:
PIGA: larger PNH clones, female sex.
BCORL1: younger age
RUNX1T1: larger PNH clones, lower Hb levels, higher bilirubin
SRRD: visceral thrombosis *(regulation heme synthesis)*
EGR4: myocardial infarction *(zinc finger transcription factor)*



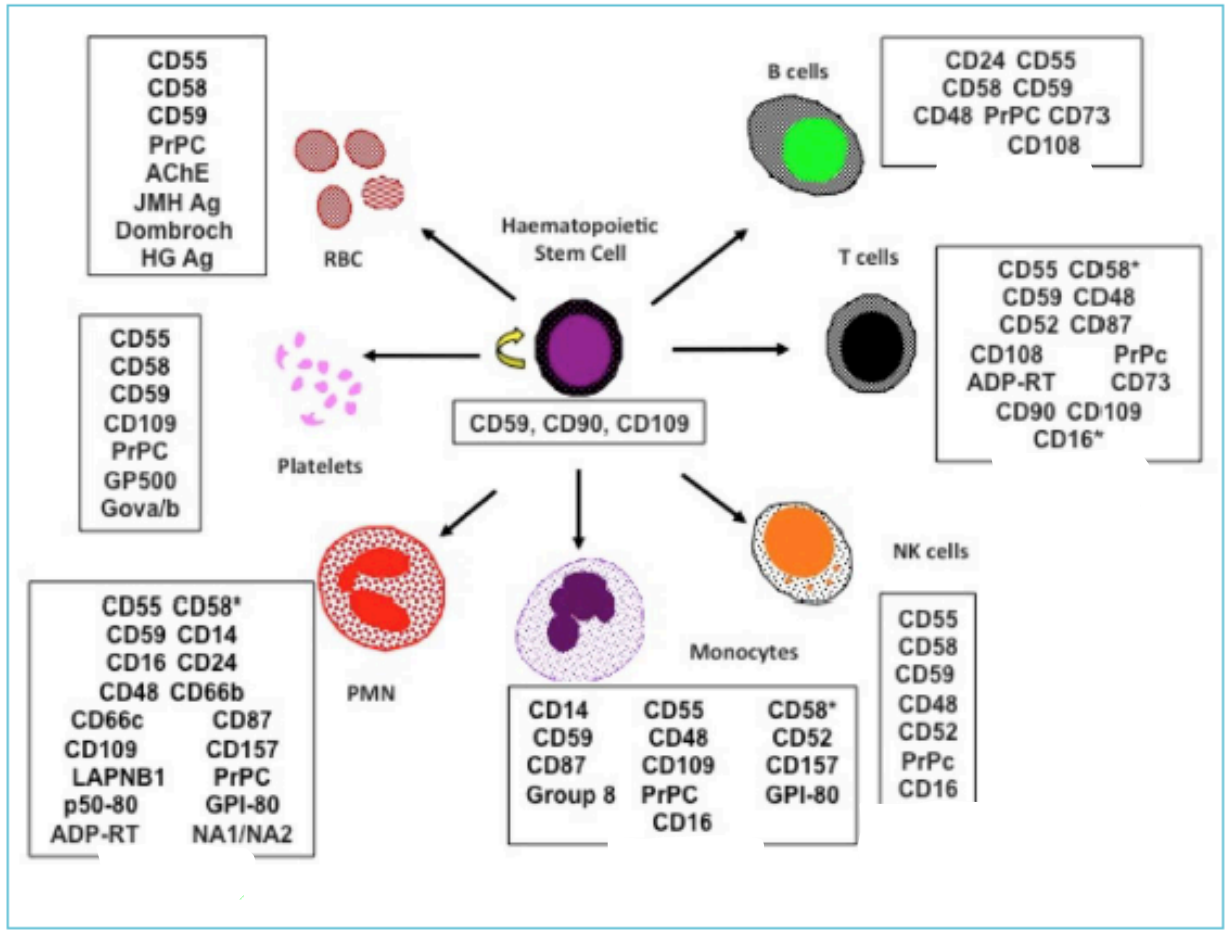
Diagnosis: flow cytometry

1- Anti-GPI-APs mAbs



RBC analysis using CD235a (Glycophorin A) for gating

Type I: normal
Type II: partial deficiency
Type III: total deficiency



Diagnosis: flow cytometry

1- Anti-GPI-APs mAbs

2- *Aeromonas hydrophila* toxin: Aerolysin

Glycosylphosphatidylinositol Anchors of Membrane Glycoproteins Are Binding Determinants for the Channel-forming Toxin Aerolysin*

Dzung B. Diep, Kim L. Nelson, Srikumar M. Raja, Erin N. Pleshak, and J. Thomas Buckley‡

J Biol Chem 1998; 273(4):2355-60.

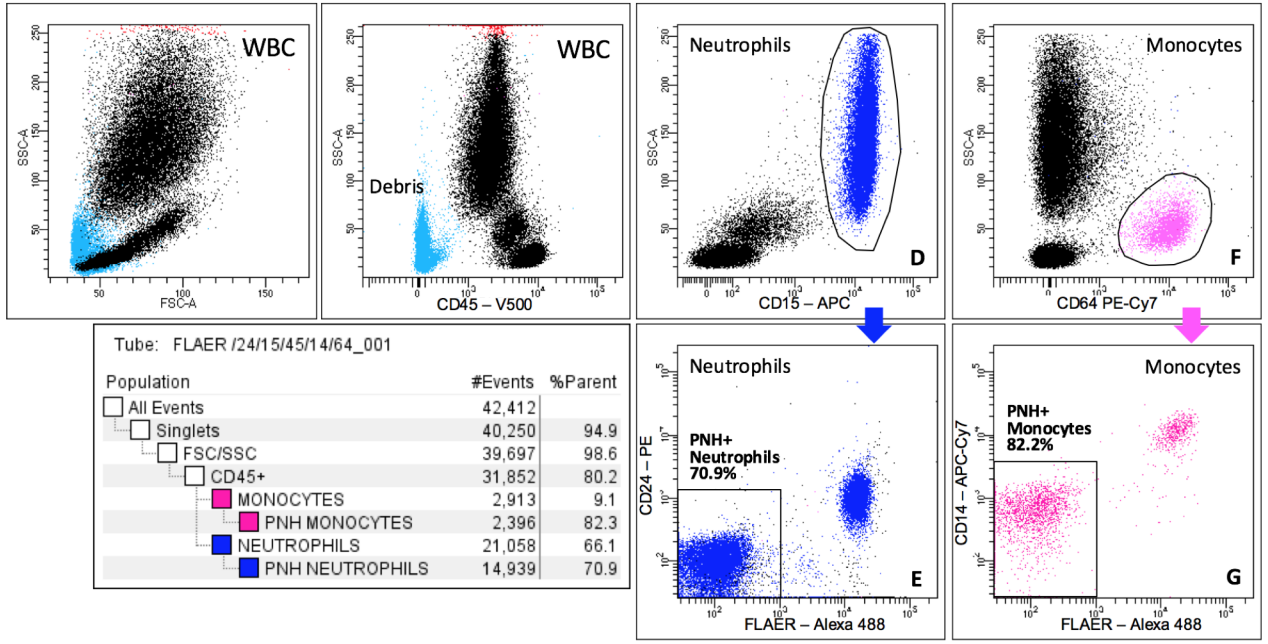
Improved Detection and Characterization of Paroxysmal Nocturnal Hemoglobinuria Using Fluorescent Aerolysin

Robert A. Brodsky, MD,[1] Galina L. Mukhina, MD,[1] Shiyong Li, MD, PhD,[2] Kim L. Nelson, PhD,[3] Patricia L. Chiurazzi, MT(ASCP),[2] J. Thomas Buckley, PhD,[3] and Michael J. Borowitz, MD, PhD[2]*

Am J Clin Pathol 2000 ; 114(3):459-66.

Aerolysin:
specific ligand for the GPI molecule ***on white blood cells****.*

Use of flurescent Aerolysin for diagnosis,
FLAER: highly effective,
suitable indicator of GPI-deficient leucocytes in PNH.



WBC analysis
using CD15 as the gating marker for neutrophils (D)
and CD64 as the gating marker for monocytes (F).

Diagnosis: flow cytometry

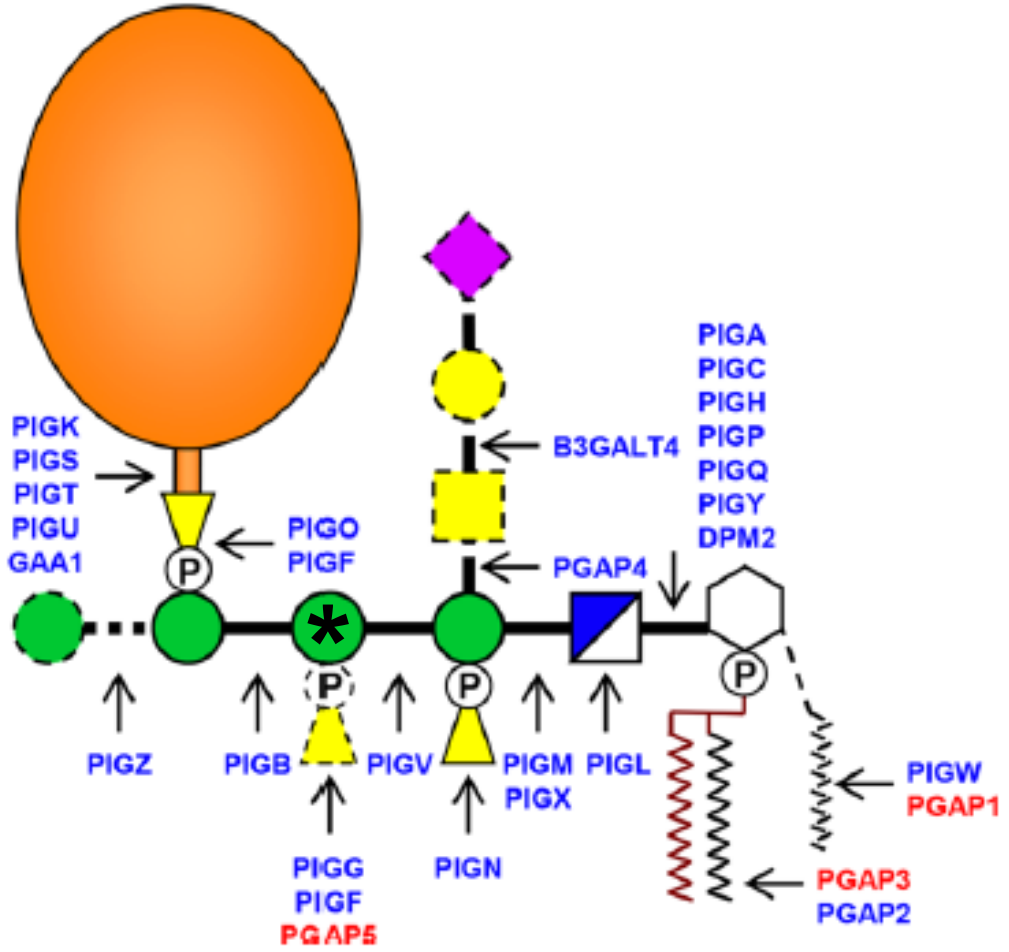
1- **Anti-GPI-APs mAbs**

2- ***Aeromonas hydrophila* toxin: Aerolysin**

A knockout cell library of GPI biosynthetic genes for functional studies of GPI-anchored proteins

Si-Si Liu[1], Yi-Shi Liu[1], Xin-Yu Guo[1], Yoshiko Murakami[2,3], Ganglong Yang[1], Xiao-Dong Gao[1], Taroh Kinoshita[2,3] & Morihisa Fujita[1✉]

Com Biol 2021; 4(1):777



Aerolysin recognizes the second mannone (*) without modification

PNH heterogeneity

- **3 main categories**
  - **Classical PNH**
    - Haemolytic, thrombotic
    - No evidence of bone marrow deficiency
  - **PNH in other bone marrow diseases**
    - Haemolytic, thrombotic
    - Evidence of bone marrow deficiency
      - Aplastic anemia, myelodysplasia,…
  - **Subclinical PNH**
    - No evidence of haemolysis or thrombosis
    - Small PNH clones
      - < 10% PNH granulocytes
      - *Commonly detected with another bone marrow disorder*

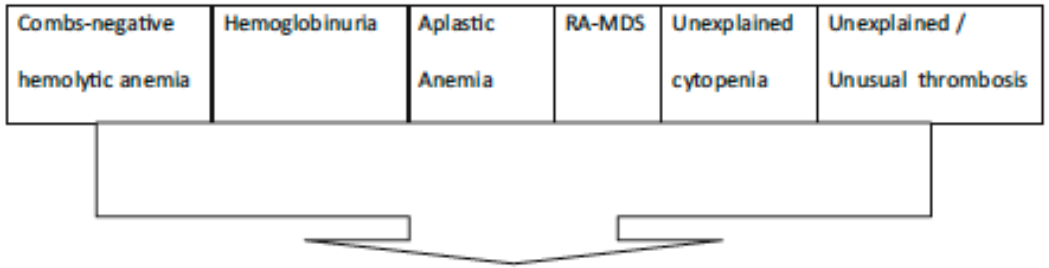
- **3 main clinical manifestations**
  - **Anemia**
    - Intravascular haemolysis
      - *Extravascular on C5 inhibitors*
    - Iron deficiency, bone marrow failure if…
  - **Thrombosis**
    - Any site, uncommon sites
  - **Smooth muscle dystonia**
    - Abdominal pain, oesophageal spasms, dysphagia, erectile dysfunction
  - ***Other***
    - Fatigue +++
    - Chronic kidney disease
    - Pulmonary hypertension

Diagnostic Screening of Paroxysmal Nocturnal Hemoglobinuria: Prospective Multicentric Evaluation of the Current Medical Indications

Marta Morado,[1] Alex Freire Sandes,[3] Enrique Colado,[4] Dolores Subirá,[5] Paloma Isusi,[6] María Soledad Noya,[7] María Belén Vidriales,[8] Amparo Sempere,[9] José Ángel Díaz,[10] Alfredo Minguela,[11] Beatriz Álvarez,[12] Cristina Serrano,[13] Teresa Caballero,[14] Mercedes Rey,[15] Ana Pérez Corral,[16] María Cristina Fernández Jiménez,[17] Elena Magro,[18] Angelina Lemes,[19] Celina Benavente,[20] Helena Bañas,[21] Juana Merino,[22] Celine Castejon,[23] Olivier Gutierrez,[24] Pilar Rabasa,[25] Matheus Vescosi Gonçalves,[3] Martin Perez-Andres,[2] and Alberto Orfao,[2*] on behalf of the PNH working group of the Iberian Society of Cytometry (SIC)

Cytometry part B (Clinical Cytometry) 2017; 92B:361

3,938 peripheral blood samples
submitted for FMC testing,
24 laboratories in Spain,
one reference centre in Brazil



Incidence of positive cases:

Based on consensus medical indications: 14%

Aplastic anemia:	44%
Bonne marrow failure syndrome:	33%
Myelodysplastic syndromes:	10%
Haemoglobinuria:	48%
Intravascular haemolytic anemia:	19%
Unexplained cytopenia:	09%

Unexplained thrombosis:

+ nonhaemolytic anemia and/or other cytopenia:	14%
without cytopenia:	**00.4%**

Current medical indications: highly efficient;
Improved screening algorithm are needed for patients presenting with thrombosis and normal blood cell counts

Screening of Patients with Idiopathic Venous Thromboembolism for Paroxysmal Nocturnal Hemoglobinuria Clones

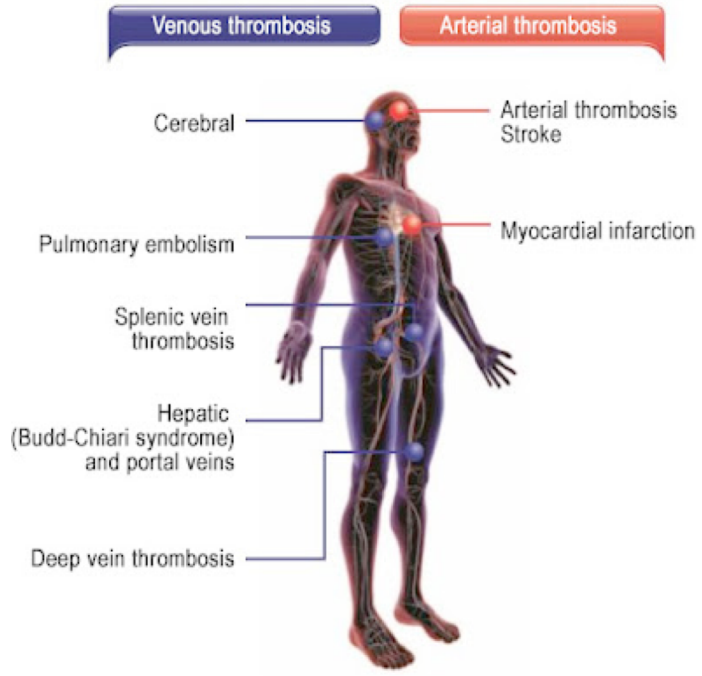
Alejandro Lazo-Langner [a,b,*], Michael J. Kovacs [a], Ben Hedley [c], Fatimah Al-Ani [a], Michael Keeney [c], Martha L. Louzada [a,b], Ian Chin-Yee [a]

Thromb Res 2015; 135(6):1107

Canada, Western Ontario
Cross-sectional study
Venous thromboembolism VTE
N=388

Only one patient had a detectable PNH clone in the neutrophil population
and no detectable erythrocyte clone…

Screening for PNH clones among patients with VTE:
better reserved for patients with signs of haemolysis.



THROMBOSIS IN PNH

The most common, most feared complication of PNH

Review Article

Thrombosis in paroxysmal nocturnal hemoglobinuria

Anita Hill, Richard J. Kelly, and Peter Hillmen

Department of Haematology, St. James's University Hospital, Leeds, United Kingdom

Blood 2013; 121(25):4985

Before the complement inhibitors era:

Mortality:

Thrombosis: most common cause (40%-65%)
Poor survival if thrombotic complications (8 years' RR: 10)
Thrombosis at presentation: 40% survival rate at 4 years

Incidence:

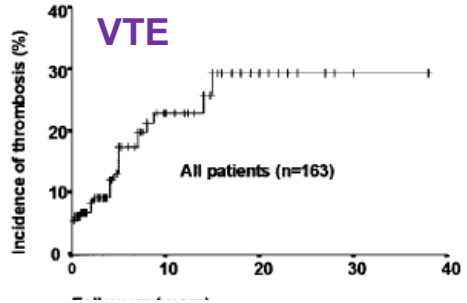
Thrombotic event during the course of the disease: 30%-45%
Thrombosis preceding the diagnosis of PNH: 20% of patients
After diagnosis: visceral thrombosis at a median of 5 years (0-24)

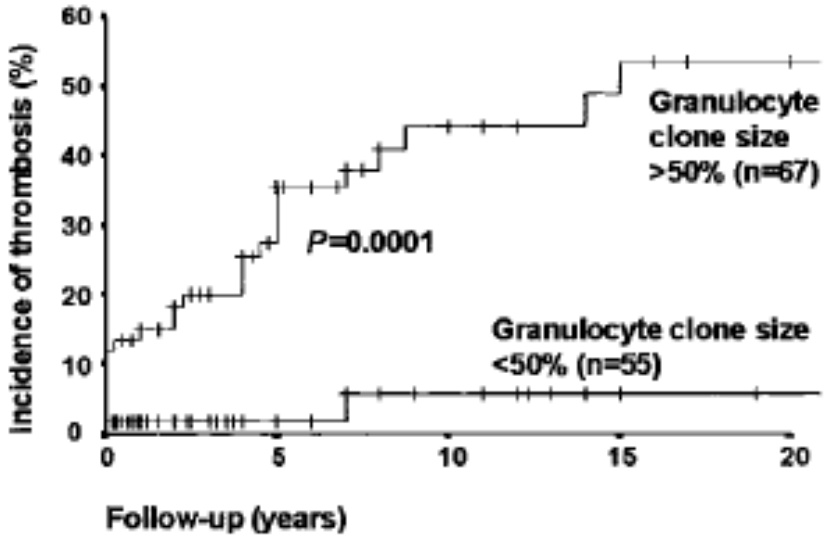
Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH)

Claire Hall, Stephen Richards, and Peter Hillmen

Blood 2003; 102(10):3587

163 patients





Thrombosis: impact of PNH clone size

Baseline clinical characteristics and disease burden in patients with paroxysmal nocturnal hemoglobinuria (PNH): updated analysis from the International PNH Registry

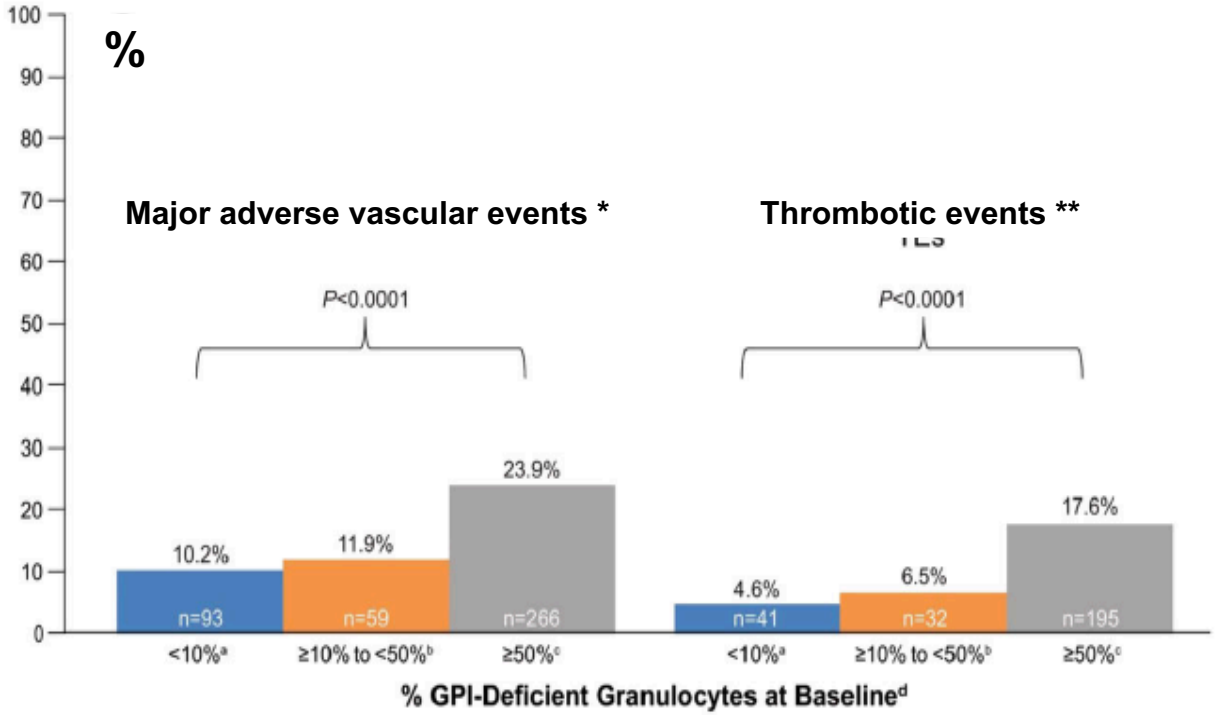
Hubert Schrezenmeier[1,2] · Alexander Röth[3] · David J. Araten[4] · Yuzuru Kanakura[5] · Loree Larratt[6] · Jamile M. Shammo[7] · Amanda Wilson[8,9] · Gilda Shayan[8,10] · Jaroslaw P. Maciejewski[11]

Ann Hematol 2020; 99(7):1505-1514

4,439 patients, baseline,
not eculizumab-treated

2,701: GPI-AP-deficient granulocyte clone size data

Mean age at onset: 40 years, at baseline: 45 years.



* venous and arterial thrombosis, atherothrombosis, amputation, gangrene
** venous and arterial thrombosis

Significant correlation with clone size

Larger GPI-deficient granulocyte clone size: higher disease burden

Substantial proportion of patients with smaller clone size have a vascular event/thrombotic history

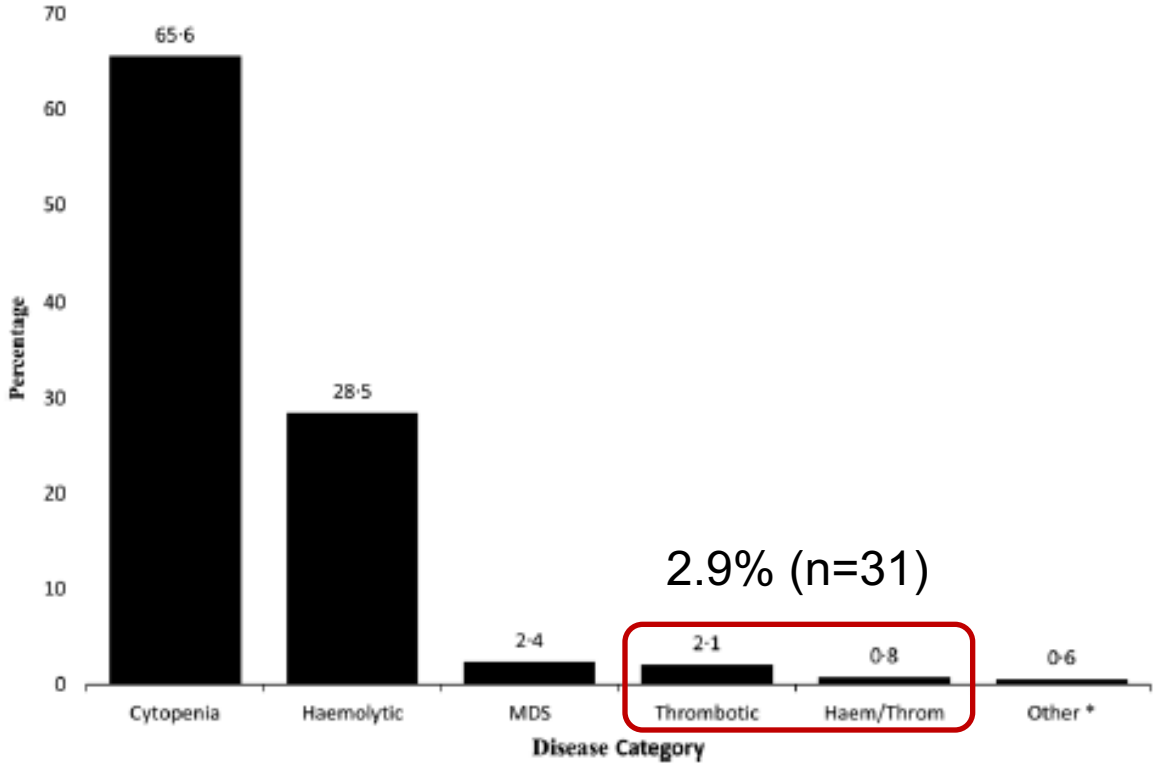
bjh research paper

Presentation clinical, haematological and immunophenotypic features of 1081 patients with GPI-deficient (paroxysmal nocturnal haemoglobinuria) cells detected by flow cytometry

Stephen J. Richards,[1,2] Anita J. Dickinson,[2] Matthew J. Cullen,[3] Morag Griffin,[4] Tahla Munir,[4] Claire McKinley,[1] Lindsay D. Mitchell,[5] Darren J. Newton,[1] Louise Arnold,[4] Anita Hill[4] and Peter Hillmen[1,4]

Br J Haematol 2020; 189(5):954

Single-centre study over 25 years, 1,081 PNH patients



Thrombosis: rare at presentation

bjh research paper

Presentation clinical, haematological and immunophenotypic features of 1081 patients with GPI-deficient (paroxysmal nocturnal haemoglobinuria) cells detected by flow cytometry

Stephen J. Richards,[1,2] Anita J. Dickinson,[2] Matthew J. Cullen,[3] Morag Griffin,[4] Tahla Munir,[4] Claire McKinley,[1] Lindsay D. Mitchell,[5] Darren J. Newton,[1] Louise Arnold,[4] Anita Hill[4] and Peter Hillmen[1,4]

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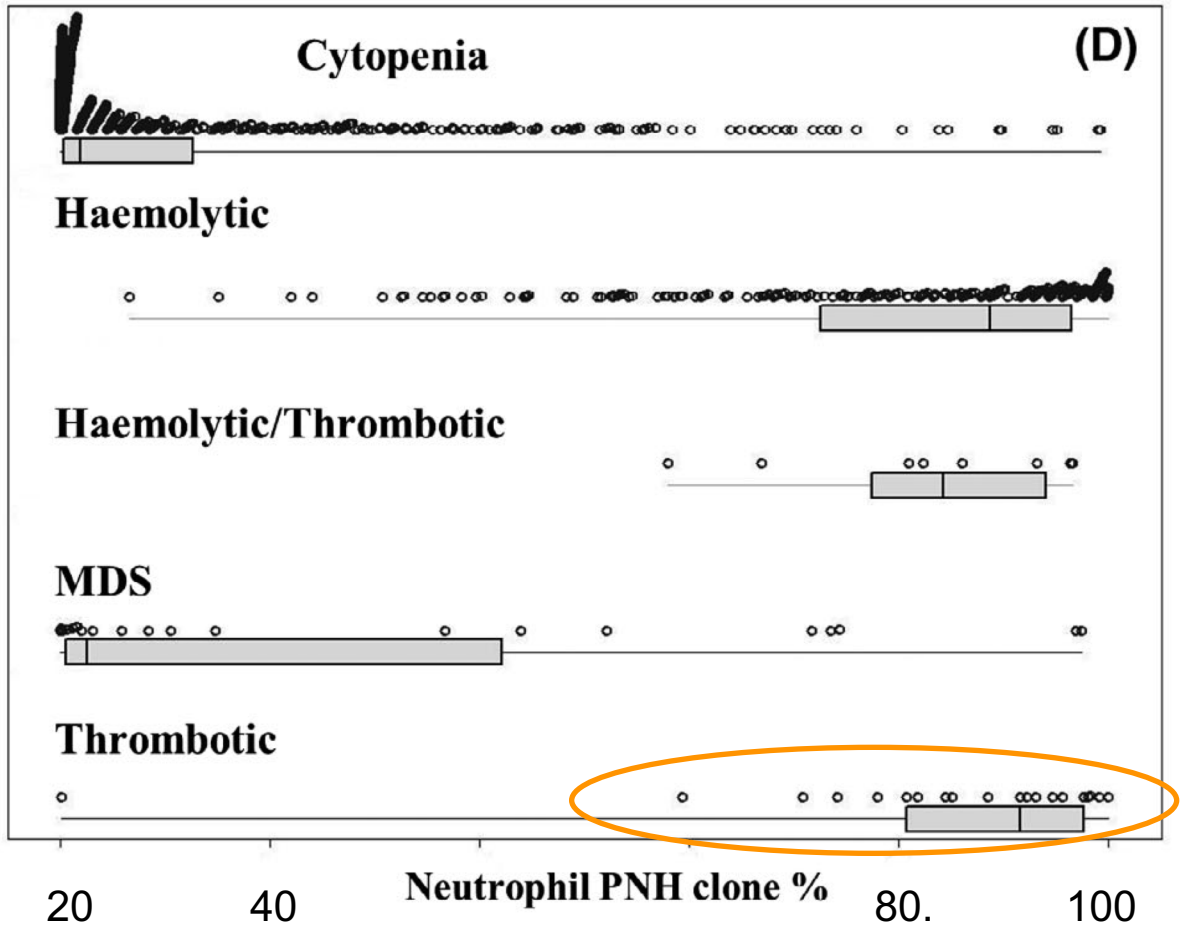


Thrombosis: large PNH neutrophil clones

bjh research paper

Presentation clinical, haematological and immunophenotypic features of 1081 patients with GPI-deficient (paroxysmal nocturnal haemoglobinuria) cells detected by flow cytometry

Stephen J. Richards,[1,2] Anita J. Dickinson,[2] Matthew J. Cullen,[3] Morag Griffin,[4] Tahla Munir,[4] Claire McKinley,[1] Lindsay D. Mitchell,[5] Darren J. Newton,[1] Louise Arnold,[4] Anita Hill[4] and Peter Hillmen[1,4]

Br J Haematol 2020; 189(5):954

Single-centre study over 25 years, 1,081 PNH patients

PNH	Type II cells	Type III cells
Haemolytic	9.4%	26.9%
Haemotytic/thrombotic	18.2%	16.3%
Thrombotic	21.9%	12.3%

Thrombosis: higher rate of type II, lower rate of type III cells;
confirmed with the immature (CD71+: transferin receptor) RBC

Significant hemolysis is not required for thrombosis in paroxysmal nocturnal hemoglobinuria

Morag Griffin, Peter Hillmen, Talha Munir, Stephen Richards, Louise Arnold, Kathryn Riley and Anita Hill

Haematologica 2019; 104(3):e94

Patient	Age at thrombosis (years)	Pre-thrombotic co-morbidities	PNH granulocytes	Total PNH red cells	Type II red cells	Type III red cells	LDH (IU/L) *	Thrombosis
1	49	No	98%	0.77%	0.77%	0	158 (Normal)	Budd Chiari CVA with extension
2	58	AA, receiving ATG	58%	1.72%	0.18%	1.54%	410 (Normal)	NSTEMI
3	63	NLPHD (no treatment)	49%	1.68%	0.15%	1.53%	482 (1.1 x ULN)	Ischemic colitis
4	31	No	98%	0.1%	0.02%	0.08%	428 (1.01 x ULN)	CVA
5	21	AA, receiving ATG	89.7%	3.27%	1.22%	2.07%	550 (1.22 x ULN)	DVT
6	26	AML post chemotherapy	97%	0.53%	0.04%	0.49%	228 (Normal)	Mesenteric
	40		93%	1.2%	0.4%	1.26	1.01 x ULN	
7	67	No	100%	99%	71%	28%	460 (1.9 x ULN)	NSTEMI with stent thrombosis x2
8	49	No	70.8%	22.8%	13.4%	9.4%	442 (1.6x ULN)	STEMI Critical limb ischemia Splanchnic vein

First case series of patients with PNH who experieced **thrombosis** **with low levels of haemolysis** (LDH < 2 x upper limit of normal)

high PNH WBC proportion
and
low PNH RBC proportion

WBC and platelets:
a more pivotal role
than previously thought?

Review Article

Thrombosis in paroxysmal nocturnal hemoglobinuria

Anita Hill, Richard J. Kelly, and Peter Hillmen

Department of Haematology, St. James's University Hospital, Leeds, United Kingdom

Blood 2013; 121(25):4985

REVIEW ARTICLE

The prothrombotic state in paroxysmal nocturnal hemoglobinuria: a multifaceted source

Barnaby Peacock-Young,[1] Fraser L. Macrae,[1] Darren J. Newton,[2] Anita Hill[3]* and Robert A S Ariëns[1]*

Haematologica 2018; 103(1):9

Thrombotic events:

venous origin **85%**

arterial origin **15%**

more than one site 20%

may occur at any site

DVT, PE, in situ pulmonary thrombosis

Myocardial infarction, stroke

Review Article

Thrombosis in paroxysmal nocturnal hemoglobinuria

Anita Hill, Richard J. Kelly, and Peter Hillmen

Department of Haematology, St. James's University Hospital, Leeds, United Kingdom

Blood 2013; 121(25):4985

REVIEW ARTICLE

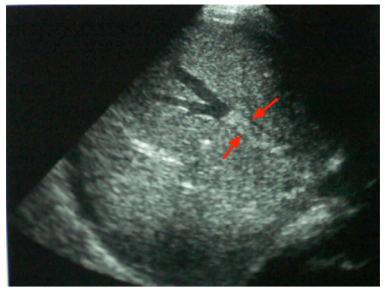
The prothrombotic state in paroxysmal nocturnal hemoglobinuria: a multifaceted source

Barnaby Peacock-Young,[1] Fraser L. Macrae,[1] Darren J. Newton,[2] Anita Hill[3]* and Robert A S Ariëns[1]*

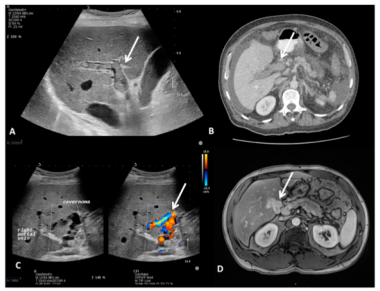
Haematologica 2018; 103(1):9

Thrombotic events:

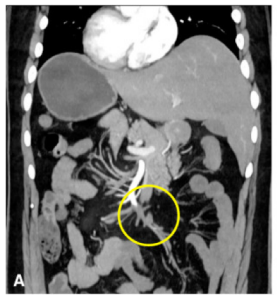
increased incidence of thrombosis at **atypical sites**

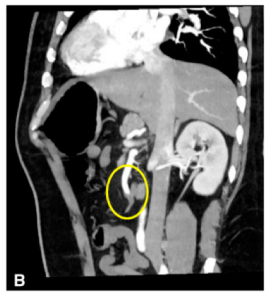


Hepatic veins: Budd-Chiari syndrome

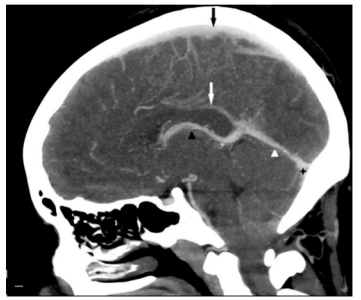


Portal vein occlusion

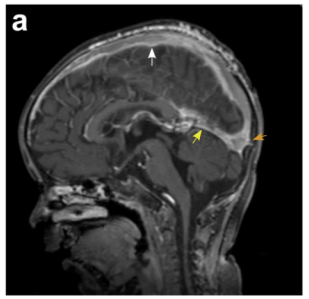


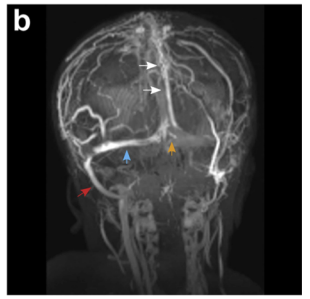


Mesenteric vessels occlusion



Cerebral vein thrombosis





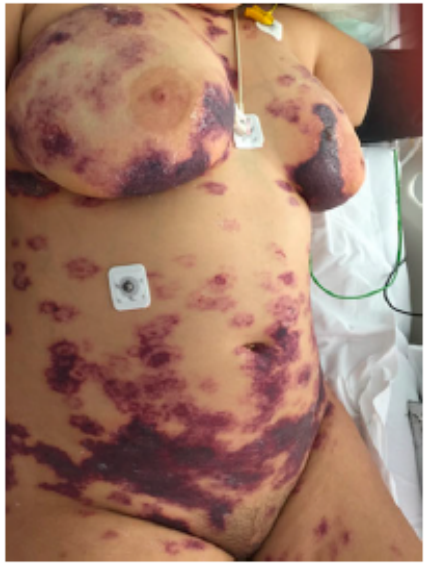
Cerebral venous sinus thrombosis

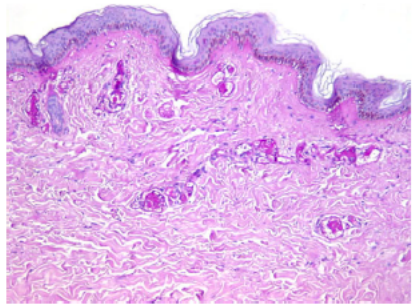
bjh images in haematology

Cutaneous thrombosis as the presenting finding of paroxysmal nocturnal haemoglobinuria

Ozan Salim[1], Orhan K. Yücel[1], Gülay Karatas[1], Sevil Alan[2], Cumhur I. Bassorgun[3] and Levent Undar[1]

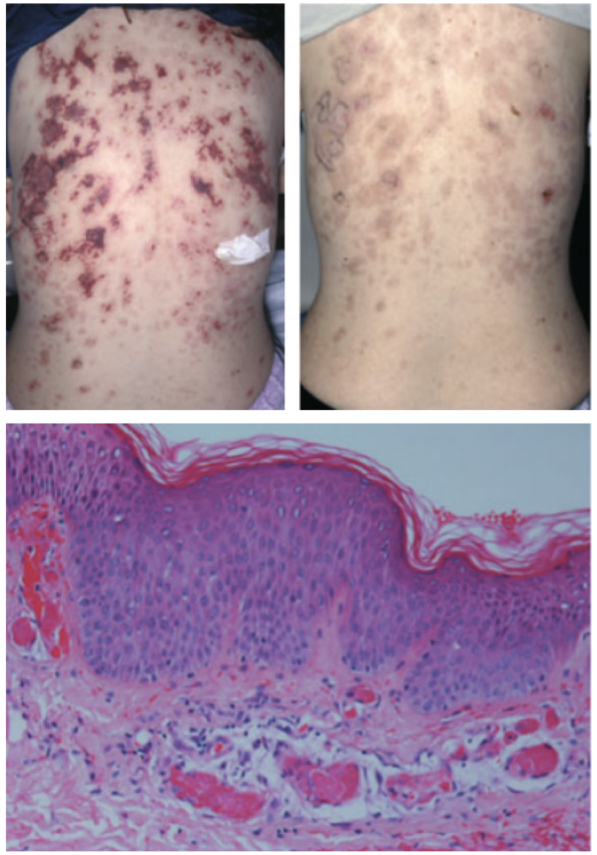
Br J Haematol 2015; 171(3):296





bjh

Br J Haematol 2007; 137(4):271



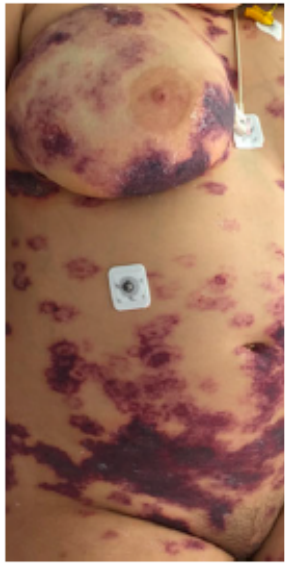
Purpura fulminans-like lesions

bjh images in haematology

Cutaneous thrombosis
nocturnal haemoglobi

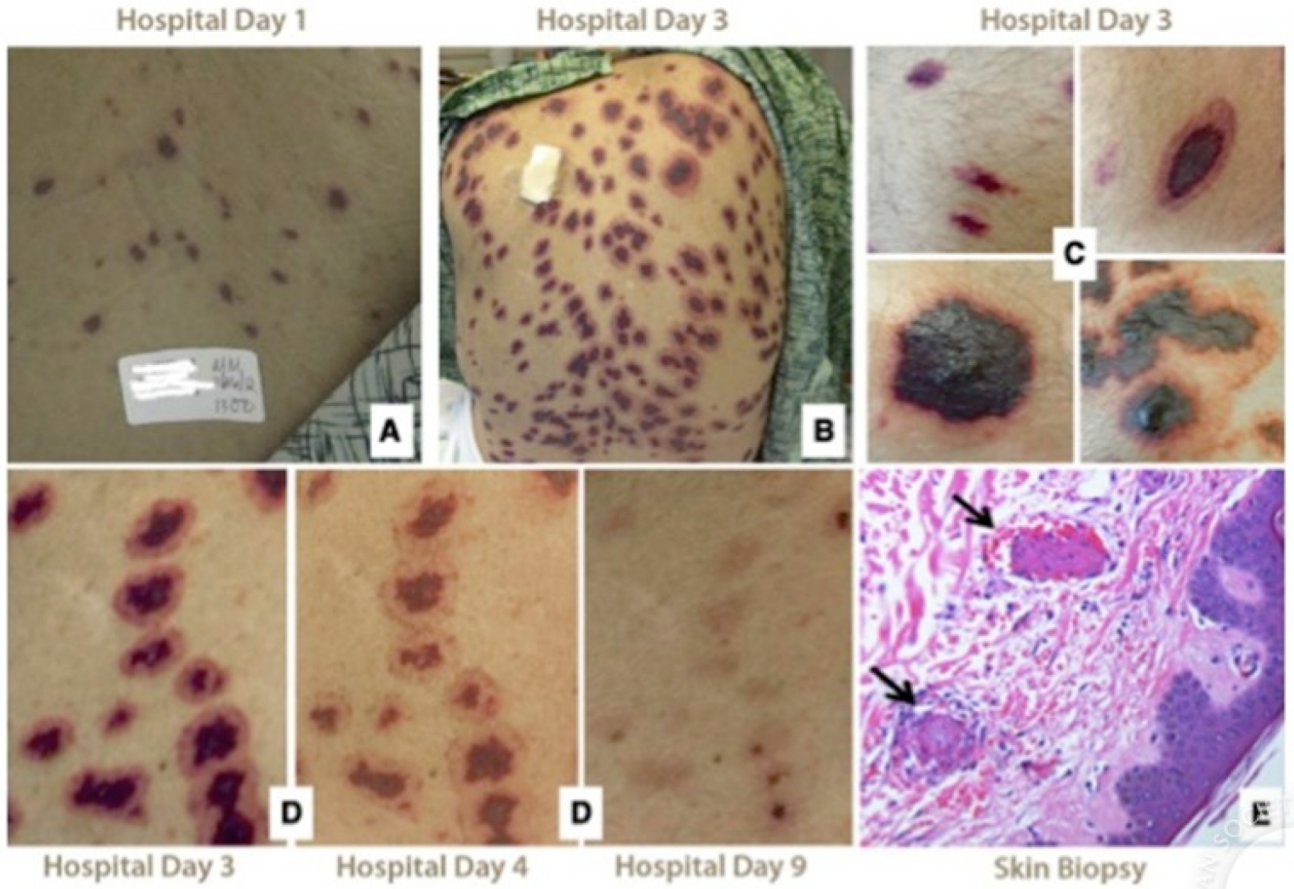
Ozan Salir
Cumhur I.

Br J H

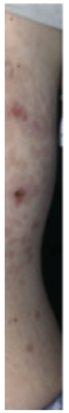


blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

66-year-old man with a 7-year history of untreated paroxysmal nocturnal hemoglobinuria (PNH) reported new onset fever, malaise, joint pains, and skin rash (panel A).



Zhao H , and Shattil S Blood 2013;122:3249



ions

A comparative analysis of clinical characteristics of patients with paroxysmal nocturnal hemoglobinuria between Asia and Europe/America

Fan Yu[1] · Yali Du[2] · Bing Han[2]

Int J Hematol 2016; 103(6):649.

PNH cases
with a median follow-up period > 60 months
published after 2000

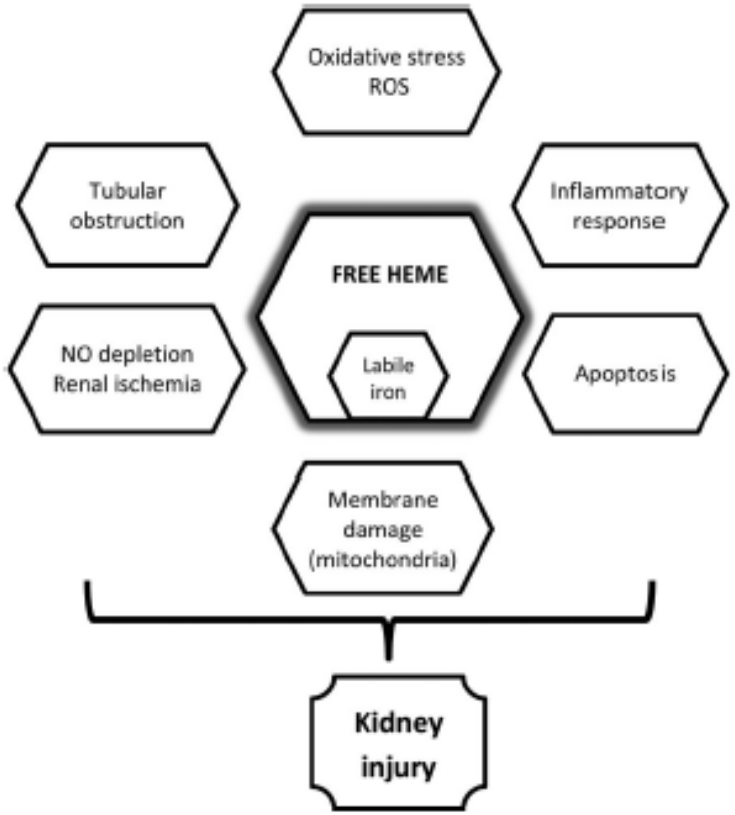
Outcomes	Asia % (n/N)	Europe/America % (n/N)	*P* value
Death of TE	6.9 (7/102)	43.7 (66/151)	0.000

Patients with thrombotic events TE:
Asian: higher proportion of **arterial thrombosis**
Western: higher proportion of **abdominal venous thrombosis**

Renal involvement in paroxysmal nocturnal hemoglobinuria: an update on clinical features, pathophysiology and treatment

Styliani I Kokoris[a], Eleni Gavriilaki[b], Aggeliki Miari[c], Anthi Travlou[c,d], Elias Kyriakou[a], Achilles Anagnostopoulos[b] and Elissavet Grouzi[e]

Hematology 2018; 23(8):558



Acute kidney injury AKI
or
chronic kidney disease CKD.

Up to 65% of PNH patients: CKD (stages 1-5).
Renal insuficiency (CKD stages 3-5): 20% of the patients.
Renal failure a a cause of death: 8-18%;
8-fold increase in mortality risk.

Kidney injury in PNH:

underdiagnosed
poorly investigated clinical feature
affects a significant portion of patients
to be early screened and recognised

Paroxysmal Nocturnal Haemoglobinuria and Pregnancy

John Michael Svigos[1,3] FRACOG, FRCOG and John Norman[2,4] FRACP

Aust N Z J Obstet Gynaecol 19094; 34(12):104

Review, *pre-eculizumab era*

Intrapartum and Postpartum Maternal Complications in 65 Pregnancies

Complications	Number	(%)
Obstetric haemorrhage	7	(10.8%)
Septicaemia/infection	5	(7.7%)
Acute haemolysis	4	(6.1%)
Pulmonary embolus/deep vein thrombosis	3	(4.6%)
Hepatic vein thrombosis	3	(4.6%)
Intracranial haemorrhage	2	(3.1%)
Cerebral thrombosis	1	(1.5%)

. Pregnancy Outcome with Paroxysmal Nocturnal Haemoglobinuria

Total of 65 reported pregnancies	Number	(%)
First trimester miscarriage	10	(16.2%)
Second trimester miscarriage	10	(16.2%)
Elective termination of pregnancy	8	(12.3%)
Total of 37 pregnancies more than 20 weeks' gestation		
Preterm delivery	11	(29.7%)
Term delivery	25	(67.6%)
Unrecorded gestation	1	(2.7%)
Caesarean section	7	(18.9%)
Perinatal deaths	5	(13.5%)

Gene mutations associated with thrombosis detected by whole-exome sequencing in paroxysmal nocturnal hemoglobinuria

Liyan Li | Honglei Wang | Hui Liu | Zhaoyun Liu | Lijuan Li | Kai Ding | Guojin Wang | Jia Song | Rong Fu

Int J Lab Hematol 2019; 41(3):424

Potential gene mutations associated with thrombosis?

13 PNH patients:
CD59-neg granulocytes, whole exome sequencing;
then 22 PNH patients:
expression of targeted gene mutations

The expression level of mutation genes' mRNA in thrombus group, nonthrombus group, and normal controls

	Thrombosis group	Nonthrombosis group	Normal control group
BMPR2	2.00 ± 1.48	3.75 ± 6.51*	7.66 ± 7.67**
THBD	1.34 ± 0.79	5.78 ± 2.36***	8.05 ± 4.93†
F8	2.03 ± 1.41	2.01 ± 2.98	1.31 ± 1.15
ITGA2B	1.01 ± 0.87	1.47 ± 1.23	1.47 ± 1.57
THBS1	10.78 ± 4.02	5.22 ± 4.34‡	1.90 ± 3.70§

Thrombotic PNH:

decreased ***BMPR2***
bone morphogenic protein receptor 2
gatekeeper to protect endothelial cells from increased TGFβ responses

decreased ***THBD***
thrombomodulin
maintains intravascular patency

decreased ***ITGA2B***
integrin subunit α2b CD41
adhesion, cell-surface mediated signalling

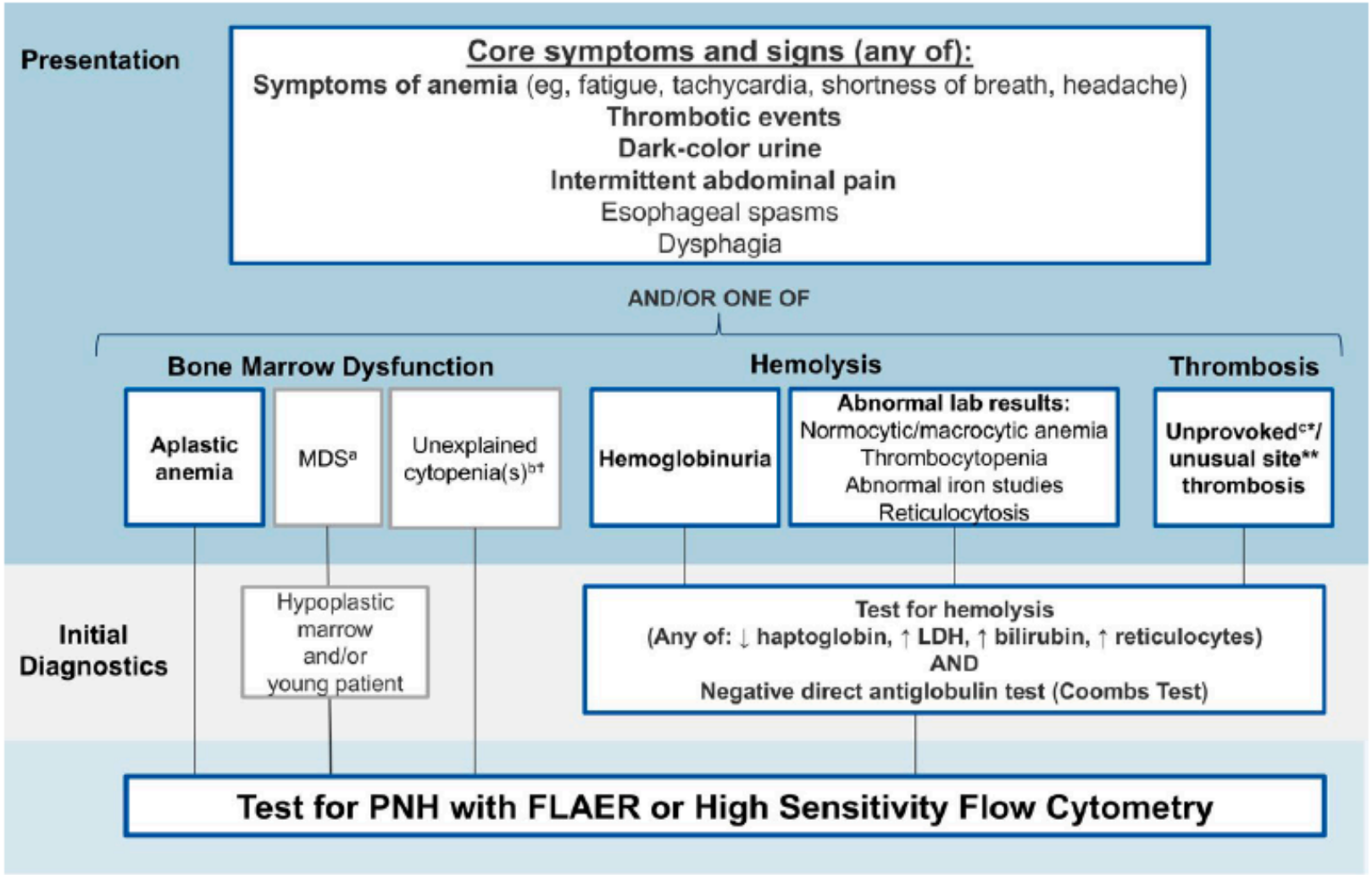
increased ***THBS1***
thrombospondin 1
platelet-vessel wall interractions correlated with D-dimers and su-PAR levels)

Screening and diagnostic clinical algorithm for paroxysmal nocturnal hemoglobinuria: Expert consensus

Alexander Röth[1] | Jaroslaw Maciejewski[2] | Jun-Ichi Nishimura[3] | Deepak Jain[4] | Jeffrey I. Weitz[5]

Eur J Hematol 2018; 101(1):3

Expert advisory committee of PNH experts from North America, Europe and Japan; Delphi methodology.



Proposed mechanisms of thrombosis in PNH

Thrombotic state in PNH:

Complex,
Multifaceted,
Relative contribution of each mechanism difficult to quantify,
Subject to continued research,

But therapeutic C5 inhibition lowers the thrombotic risk.

Thrombotic state in PNH:

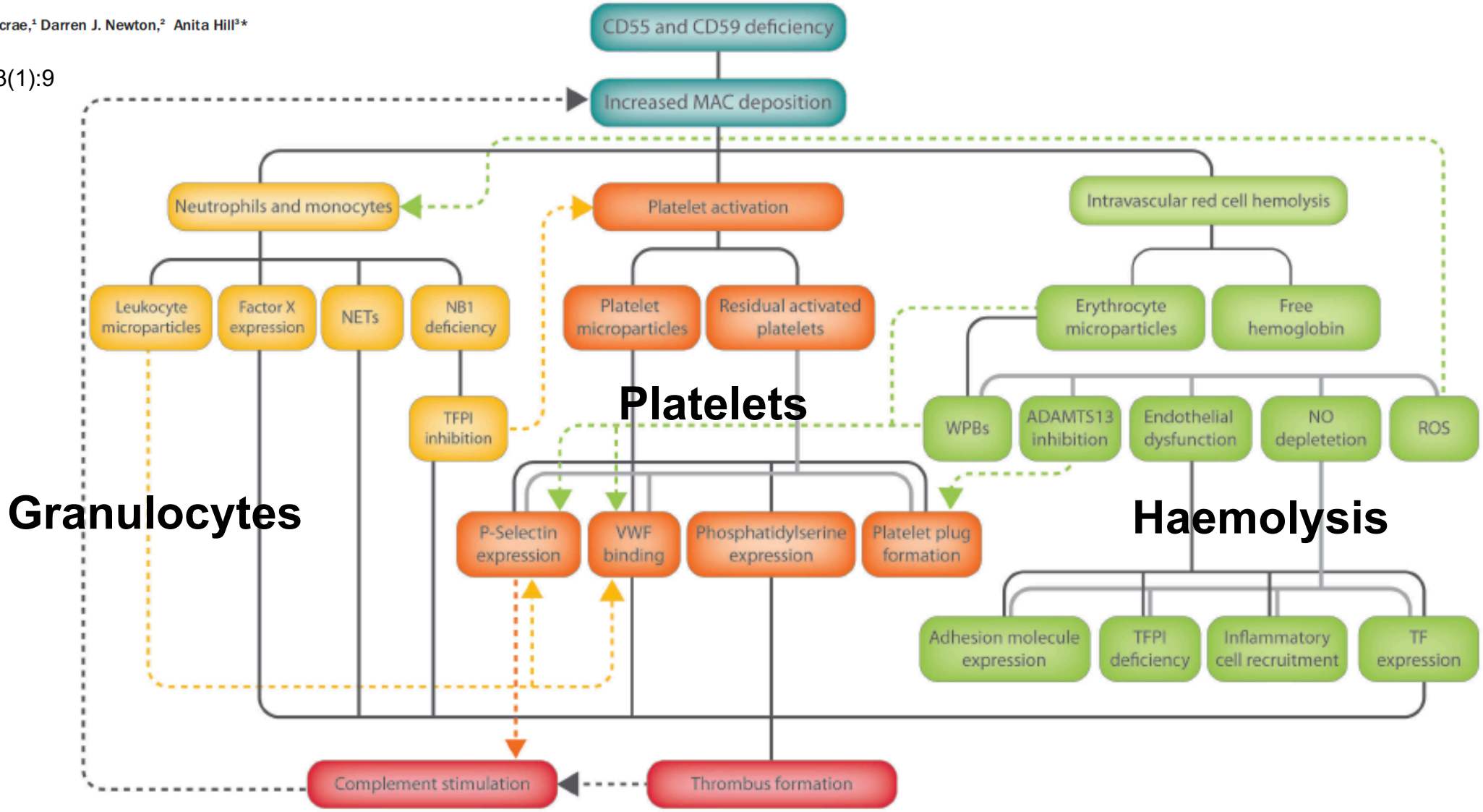
Interactions between the complement system, platelets and coagulation
Coagulation activation
Prothrombotic feedback mechanisms
Platelet activation
Platelet-derived microparticles and residual activated platelets
Haemolysis
Free haemloglobin and endothelial dysfunction
Reactive oxygen species
Neutrophils and monocytes; netosis;
Leukocyte micoparticles
Extracellular DNA, nucleosomes, histones;
Nitric oxyde depletion
Fibrin clot structure
Impaired fibrinolysis

Wow! My cat also wants to play.

The prothrombotic state in paroxysmal nocturnal hemoglobinuria: a multifaceted source

Barnaby Peacock-Young,[1] Fraser L. Macrae,[1] Darren J. Newton,[2] Anita Hill[3]* and Robert A S Ariëns[1]*

Haematologica 2018; 103(1):9



The multiple factors thought to contribute to the prothombotic state in PNH and interactions

Thrombosis in paroxysmal nocturnal hemoglobinuria

Anita Hill, Richard J. Kelly, and Peter Hillmen

Department of Haematology, St. James's University Hospital, Leeds, United Kingdom

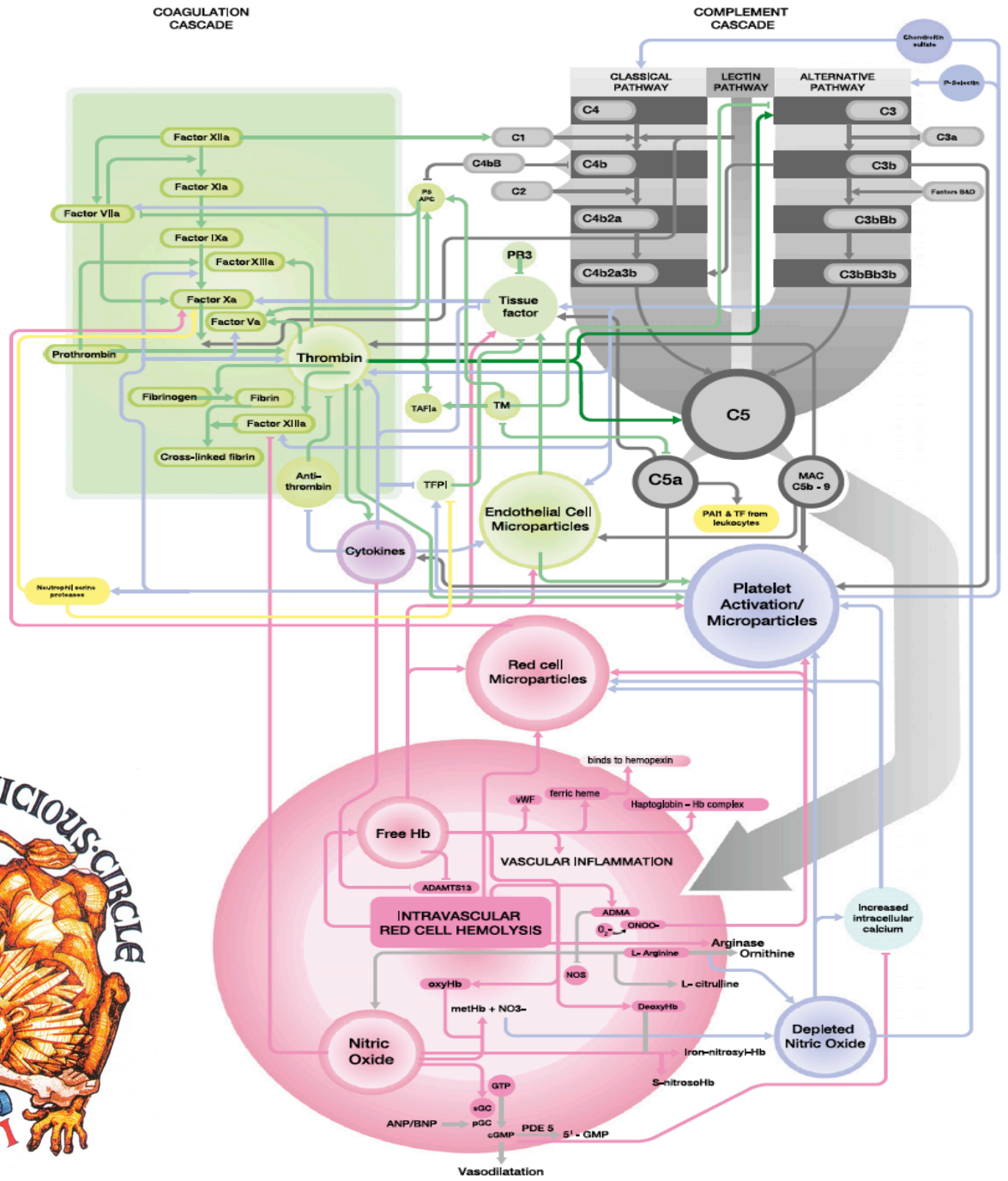
Blood 2013; 121(25):4985

Complement and coagulation systems: *common ancestral genes*, strong interactions.

C5a activates FII, FX, FXI, plasminogen;
their derived serine proteases
in turn activate complement.

Thrombin above all acts as a C5a convertase;
thrombin and C5 convertase
enhance the terminal complement pathway

Thrombomodulin, C4b-BP and C1-inhibitor
both regulate the 2 systems.



Mechanisms of thrombosis in PNH:

Extremely complex
Far from being clear

Likely to be different from those of other thrombotic disorders

Mechanisms of thrombosis in PNH:

Extremely complex
Far from being clear

Likely to be different from those of other thrombotic disorders

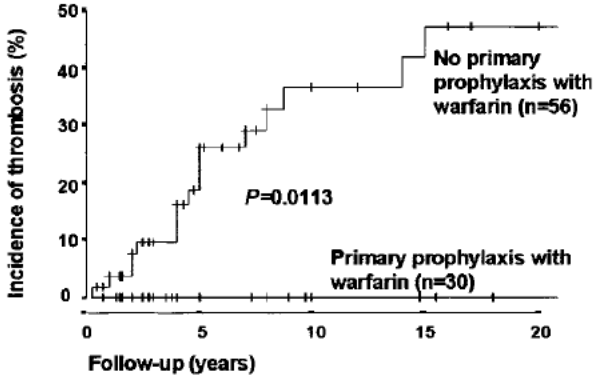
But an old drug can work!

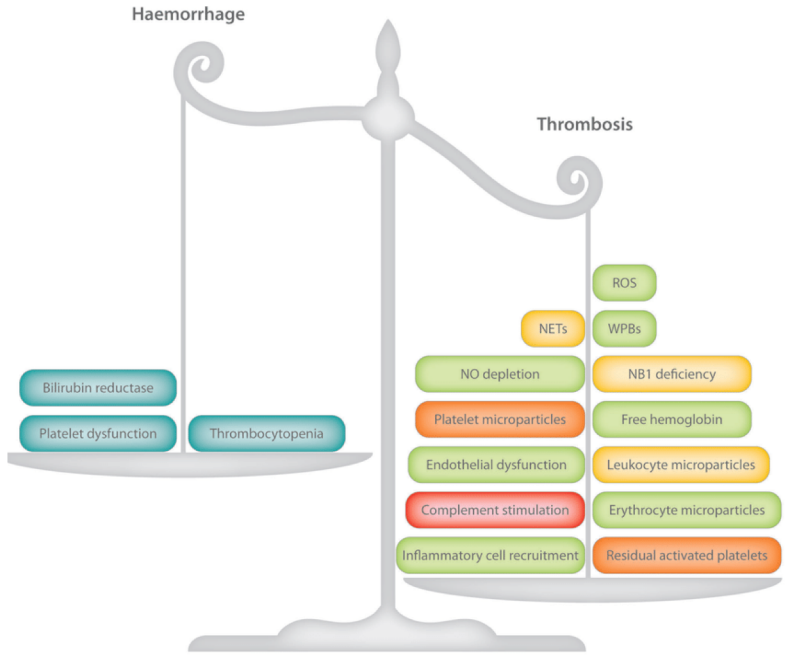
Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH)

Claire Hall, Stephen Richards, and Peter Hillmen

Blood 2003; 102(10):3587

163 PNH patients

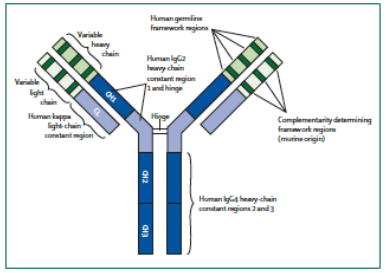


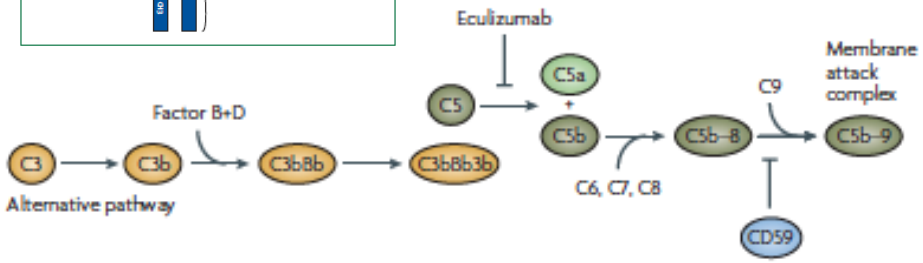


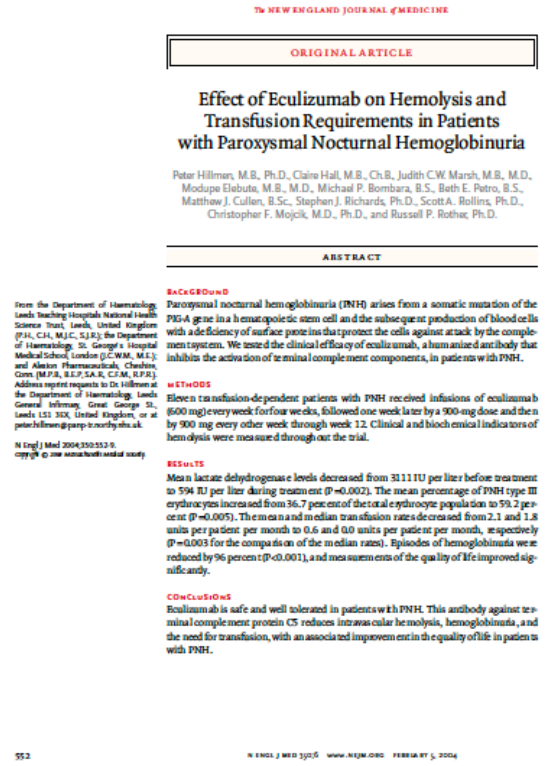
TREATMENT OF THROMBOSIS IN PNH

Pathophysiologic treatment of PNH reduces the incidence of thrombosis

Eculizumab (Soliris®):
humanised, first-in class, anti-C5 antibody







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.

ABSTRACT

BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the PIG-A gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect the cells against attack by the complement system. We tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH.

METHODS

Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Clinical and biochemical indicators of hemolysis were measured throughout the trial.

RESULTS

Mean lactate dehydrogenase levels decreased from 3111 IU per liter before treatment to 594 IU per liter during treatment ($P=0.002$). The mean percentage of PNH type III erythrocytes increased from 36.7 percent of the total erythrocyte population to 59.2 percent ($P=0.005$). The mean and median transfusion rates decreased from 2.1 and 1.8 units per patient per month to 0.6 and 0.0 units per patient per month, respectively ($P=0.003$ for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96 percent ($P<0.001$), and measurements of the quality of life improved significantly.

CONCLUSIONS

Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

From the Department of Haematology, Leeds Teaching Hospitals National Health Science Trust, Leeds, United Kingdom (P.H., C.H., M.J.C., S.J.R.); the Department of Haematology, St. George's Hospital Medical School, London (J.C.W.M., M.E.); and Alexion Pharmaceuticals, Cheshire, Conn. (M.P.B., B.E.P., S.A.R., C.F.M., R.P.R.). Address reprint requests to Dr. Hillmen at the Department of Haematology, Leeds General Infirmary, Great George St., Leeds LS1 3EX, United Kingdom, or at peter.hillmen@parp-tr.northy.nhs.uk.

N Engl J Med 2004;350:552-9.

552

N ENGL J MED 350;6 WWW.NEJM.ORG FEBRUARY 5, 2004

First human data: 2004

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]

Blood 2007; 110(12):4123.

All the patients
from the 3 eculizumab PNH clinical studies
between 2002 and 2005
(N=195)

Thrombotic events:

before treatment:
14.3 events for 100 patient-years;

ontreatment:
1.07 events for 100 patient-years

TE events	Pilot*	TRIUMPH	SHEPHERD	Extension† (all studies)
Before treatment				
Patients, no.	11	43	97	195
Patient-years, no.	33.0	21.8	93.6	272.1
TE events	5	0	21	39
Eculizumab treatment				
Patients, no.	11	43	97	195
Patient-years, no.	34.2	21.8	96.9	281.0
TE events	0.00	0.00	2	3‡

‡$P < .001$ for comparisons of eculizumab treatment versus before treatment, signed rank test.

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]

Blood 2007; 110(12):4123.

All the patients
from the 3 eculizumab PNH clinical studies
between 2002 and 2005
(N=195)

Substantial improvement with eculizumab
even in patients with less severe disease:

lowest pretreatment LDH quartile:
from 10.8 to 2.9 events per 100 patient-years;

minimal pretreatment anemia
(0 or 1 transfusion per year):
from 4.9 to 0 events per 100 patient-years

Review Article

Thrombosis in paroxysmal nocturnal hemoglobinuria

Anita Hill, Richard J. Kelly, and Peter Hillmen

Department of Haematology, St. James's University Hospital, Leeds, United Kingdom

Blood 2013; 121(25):4985

To summarise:

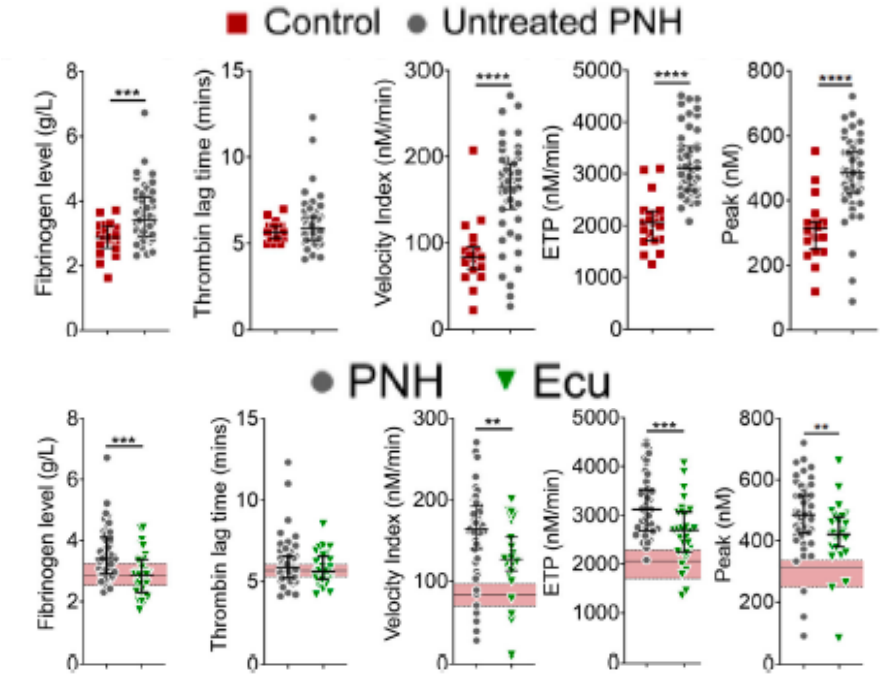
PNH patients on eculizumab
have a 85% relative risk reduction
of thrombotic events.

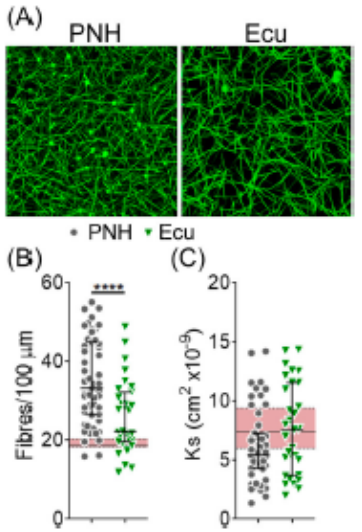
Patients with paroxysmal nocturnal hemoglobinuria demonstrate a prothrombotic clotting phenotype which is improved by complement inhibition with eculizumab

Fraser L. Macrae[1] | Barnaby Peacock-Young[1] | Polly Bowman[1] | Stephen R. Baker[1,2] | Sam Quested[1] | Emma Linton[1] | Peter Hillmen[3] | Morag Griffin[3] | Talha Munir[3] | Daniel Payne[3] | Claire McKinley[4] | Deborah Clarke[4] | Darren J Newton[4] | Anita Hill[3] | Robert A. S. Ariëns[1]

Am J Hematol 2020; 95(8):944

Antithrombotic effect of eculizumab
in part associated with
reductions
in fibrinogen
and thrombin generation,
with downstream effets
on clot stucture





Clot structure

Risk Analysis of Eculizumab-Related Meningococcal Disease in Japan Using the Japanese Adverse Drug Event Report Database

Drug Healthc Patient Saf 2020;12:207-215

Report database, 2010-2019
3559 person-years of eculizumab-exposed patients;
17 patients died
with symptoms of meningococcal disease.

Related mortality rate:
13,000 to 114,000 times the mortality rate
from meningococcal disease
in the general population of Japan.

Eculizumab and C5 inhibitors:
increase the risk of infection with *Neisseria meningitidis*

Risk Analysis of Eculizumab-Related Meningococcal Disease in Japan Using the Japanese Adverse Drug Event Report Database

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17 patients died
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Related mortality rate:
13,000 to 114,000 times the mortality rate
from meningococcal disease
in the general population of Japan.

Eculizumab and C5 inhibitors:
increase the risk of infection with *Neisseria meningitidis*

vaccination against *N. meningitidis* is mandatory before treatment *
ciprofloxacin antibiotic prophylaxis for the first 2 weeks after starting
penicillin V prophylaxis if long-term prophylaxis depending on countries
extreme vigilance to the risk (cases in vaccinated patients)

absolue risk up to 0.5% per year

Eculizumab PNH Dosing Schedule

Pretreatment		Induction phase				Maintenance phase				
≥2 weeks before induction	Week →	1	2	3	4	5	6	7	8	9
Neisseria meningitidis vaccination	Eculizumab dose, mg	600	600	600	600	900	X	900	X	900

q14d

Dose within ±2 days.

IV within 25-45 min.
Shortening of the time interval to 12 days,
or increasing the dosse to 1,200 mg
if signs of worsening hemolysis

** Tetravalent vaccine against serotypes ACYW135 and serogroup B at least 2 weeks before treatment*

Other anti-C5 antibodies

Ravulizumab: Ultomiris® Alexion

Terminal half-life 4 times the one of eculizumab:

IV every 8 weeks *after the 2-week induction phase*

Not inferior to eculizumab

CLINICAL TRIALS AND OBSERVATIONS

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

Austin G. Kulasekararaj,[1] Anita Hill,[2] Scott T. Rottinghaus,[3] Saskia Langemeijer,[4] Richard Wells,[5] F. Ataulfo Gonzalez-Fernandez,[6] Anna Gaya,[7] Jong Wook Lee,[8] Emilio Ojeda Gutierrez,[9] Caroline I. Piatek,[10] Jeff Szer,[11] Antonio Risitano,[12] Shinji Nakao,[13] Eric Bachman,[3] Lori Shafner,[3] Andrew I. Damokosh,[3] Stephan Ortiz,[3] Alexander Röth,[14] and Regis Peffault de Latour[15-17]

Blood 2019; 133(6):540.

KEY POINTS

- Ravulizumab every 8 weeks is noninferior to eculizumab every 2 weeks across all efficacy end points in eculizumab-experienced PNH patients.
- Patients with PNH may be safely and effectively switched from labeled-dose eculizumab every 2 weeks to ravulizumab every 8 weeks.

Crovalimab: Hoffmann-La Roche and Chugai

Subcutanous, phase II

Blood 2020;135(12):912.

- PNH patients, treatment naive or switching from SoC, were stably controlled on up to every 4-week subcutaneous self-administered injections of crovalimab.

Tesidolumab: Novartis Pharmaceutical

Pozelimab: Regeneron Pharmaceuticals

Eculizumab biosimilars (ABP959, Elizaria®)

Other anti-C5 antibodies

Ravulizumab: Ultomiris® Alexion

Terminal half-life 4 times the one of eculizumab:

IV every 8 weeks *after the 2-week induction phase*

KEY POINTS

- Ravulizumab every 8 weeks is noninferior to eculizumab every

self-administered injections of crovalimab.

Novel anti-C5 agents:

better pharmacological properties, possibly allowing a deeper C5 inhibition;

may reduce the risk of pharmacokinetics breakthrough haemolysis;

transient autoimmune manifestations, due to drug-target-drug immune complexes

in patients switching from eculizumab to other C5 Abs targeting different C5 epitopes

Tesidolumab: Novartis Pharmaceutical

Pozelimab: Regeneron Pharmaceuticals

Eculizumab biosimilars (ABP959, Elizaria®)

Management of acute thrombosis in patients not on eculizumab before

Eculizumab, induction phase, 2 weeks;
Full dose anticoagulation: UFH or LMWH
DOACs: no data

Eculizumab *or* ravulizumab (crovalimab?), maintenance phase
3 to 6 months,
as long as there are no other provoking factor

Anticoagulation discontinued if no thrombotic symptom, and LDH < 1.5 x ULN under a complement inhitor

Hematol Transfus Cell Ther 2021; 43(3):341.

Management of acute thrombosis in patients not on eculizumab before

Eculizumab, induction phase, 2 weeks;
Full dose anticoagulation: UFH or LMWH
DOACs: no data

Eculizumab *or* ravulizumab (crovalimab?), maintenance phase
3 to 6 months,
as long as there are no other provoking factor

Anticoagulation discontinued if no thrombotic symptom, and LDH < 1.5 x ULN under a complement inhitor

Management of acute thrombosis while on eculizumab

Immediate additional dose of C5 inhibitor
Eculizumab: increase the dose by 300 mg.
Full dose anticoagulation.

Hematol Transfus Cell Ther 2021; 43(3):341.

Recommendations for long-term thromboprophylaxis:

1- Granulocyte clone > 50% & no indication for eculizumab

2- Previous VTE & eculizumab not available

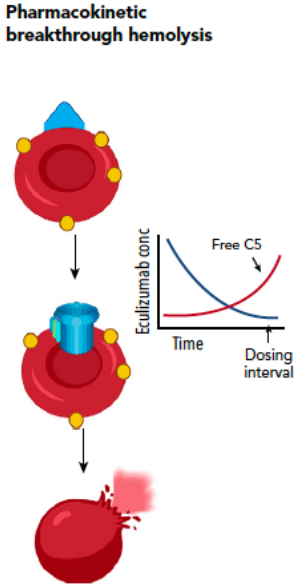
3- Pregnancy/puerperium in association with eculizumab

Exculizumab therapy recommended for patients with a large PNH granulocyte clone
who have disabling fatigue, thrombosis, red cell transfusion dependence due to haemolysis,
frequent pain paroxysms, renal insufficeincy, pulmonary hypzertension
or other end-organ complication from disease.

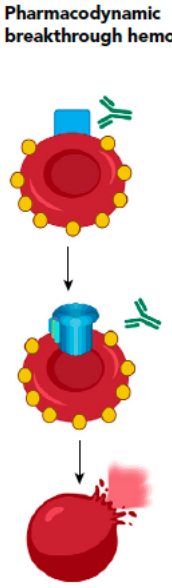
Residual haemolysis in C5-inhibitor treated patients:

Intravascular haemolysis:

Pharmacokinetic:
insufficient drug dosing



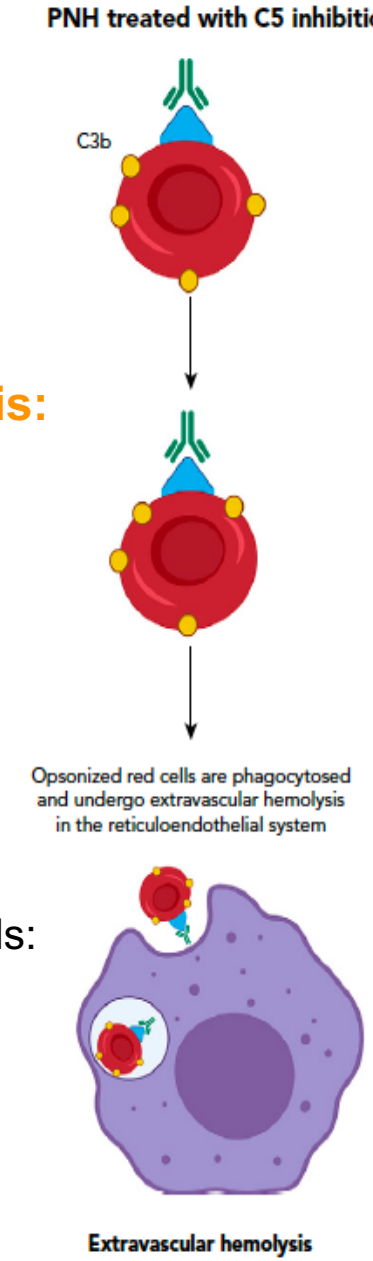
Pharmacodynamic:
complement amplifying conditions
(pregnancy, infection, major surgery)



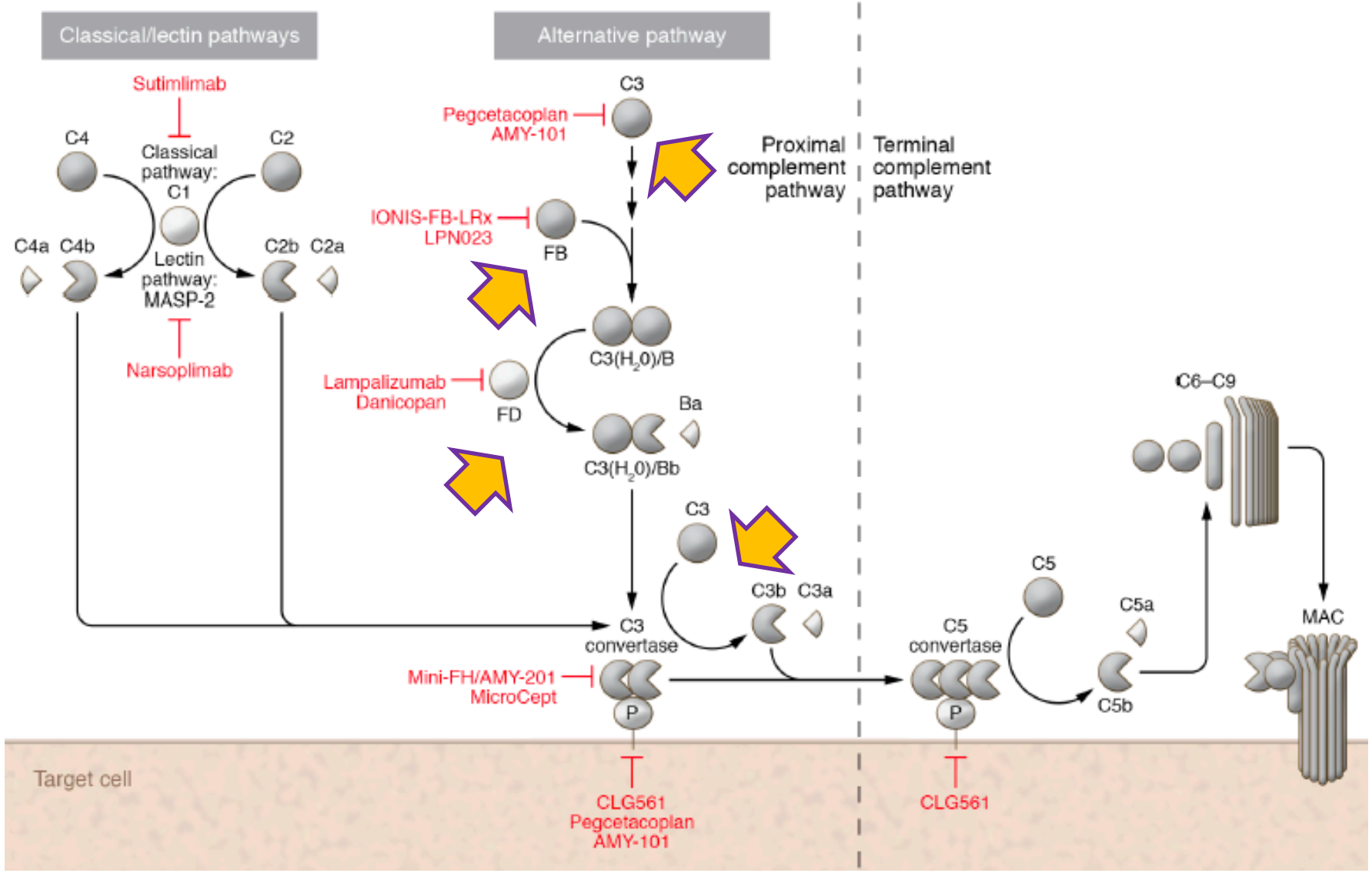
Rare inherited
C5 variants (Japan))

Extravascular haemolysis:

Correct C5 inhibition;
excess C3b accumulation on cells:
opsonised red cells;
extravascular macrophages.



Next generation treatments: proximal complement inhibitors targeting Factor B, Factor D, C3



- **Factor B inhibitors**
  - LN023 Iptacopan (Novartis)
    - small molecule, *peros*
      - *preserves increased meningococcal killing in vitro in vaccinated volunteers; ?*
- **Factor D inhibitors**
  - ACH4471 Danicopan (Achillion)
    - small molecule, *peros*
- **C3 inhibitors**
  - APL2 Pegcetacoplan (Apellis Pharmaceuticals) Empaveli®
    - 15 AA cyclic peptide conjugated to PEG, *subcutaneous*
  - AMY-101 Compstatin (Amyndas)
    - peptide, *subcutaneous*

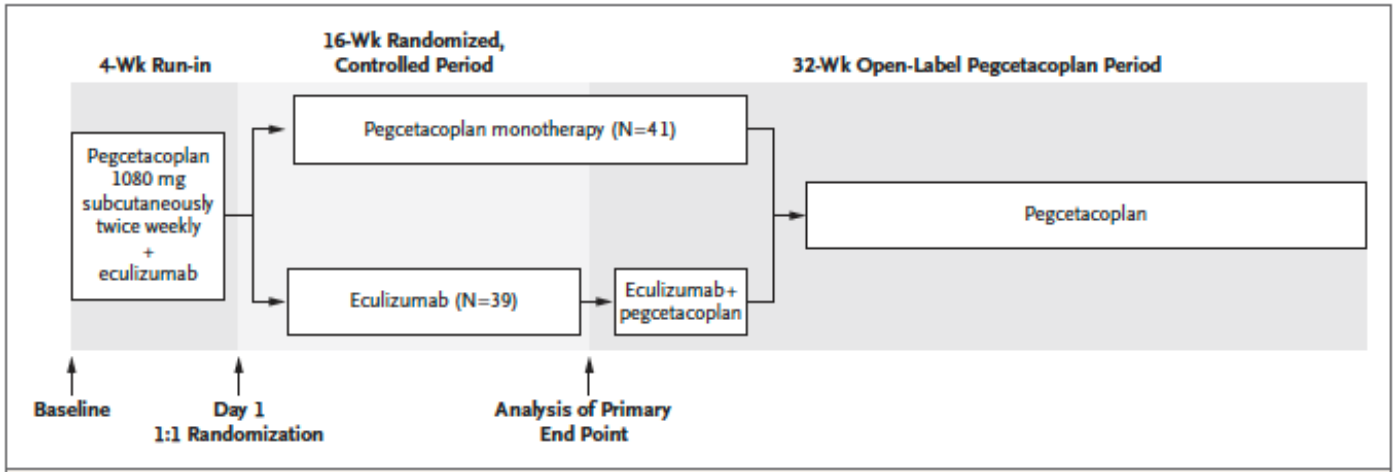
ORIGINAL ARTICLE

Pegcetacoplan versus Eculizumab in Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ch.B., Ph.D., Jeff Szer, M.B., B.S., Ilene Weitz, M.D., Alexander Röth, M.D., Britta Höchsmann, M.D., Jens Panse, M.D., Kensuke Usuki, M.D., Ph.D., Morag Griffin, B.M.Sc., M.B., Ch.B., Jean-Jacques Kiladjian, M.D., Ph.D., Carlos de Castro, M.D., Hisakazu Nishimori, M.D., Ph.D., Lisa Tan, R.N., Mohamed Hamdani, M.S., Pascal Deschatelets, Ph.D., Cedric Francois, M.D., Ph.D., Federico Grossi, M.D., Ph.D., Temitayo Ajayi, M.D., Antonio Risitano, M.D., Ph.D., and Régis Peffault de la Tour, M.D., Ph.D.

N Engl J Med 2021; 384(11):1028.

Phase III open-label RCT



Pegcetacoplan superior to eculizumab
in improving hemoglobin
and clinical and hematological outcomes
by providing broad hemolysis control,
ìncluding intravascular
and extravascular
hemolysis.

Thrombosis ???

How I treat paroxysmal nocturnal hemoglobinuria

Robert A. Brodsky

Blood 2021; 137(10):1304

How we('ll) treat paroxysmal nocturnal haemoglobinuria: diving into the future

Antonio Maria Risitano[1,2,3] and Régis Peffault de Latour[3,4,5]

Br J Haematol 2021; Aug 5. doi: 10.1111/bjh.17753.

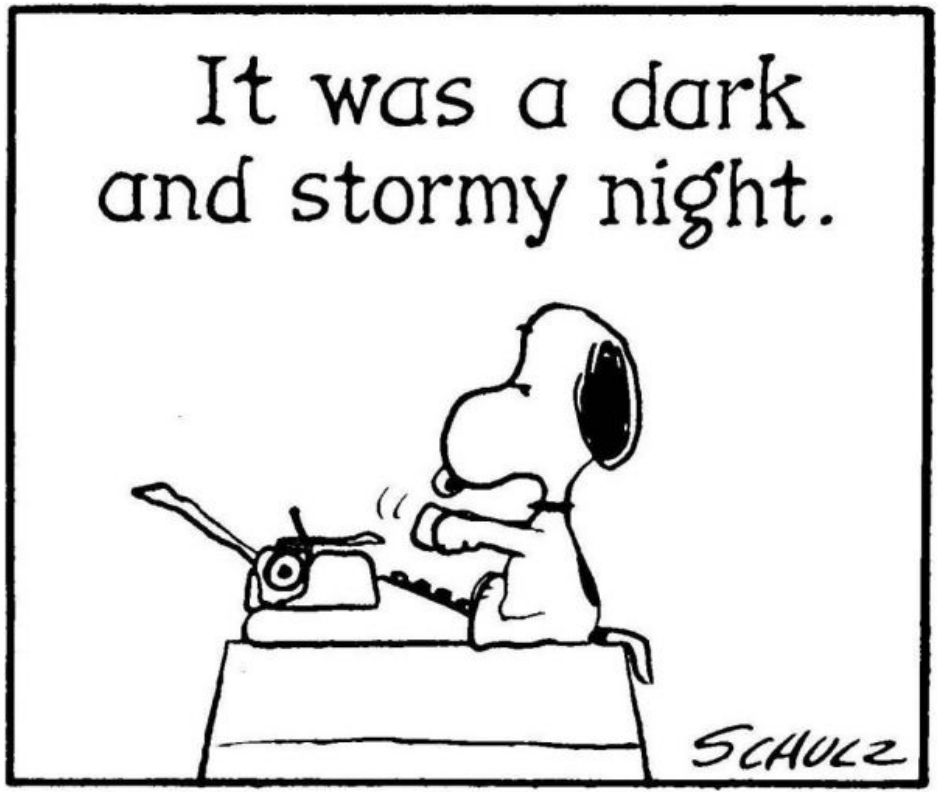
Once added to anti-C5 therapies,
proximal complement inhibitors
effective in preventing
C3-mediated extravascular haemolysis

Given their
broader impairment
of the complement cascade,
a *broader vaccination schedule* is likely
as well as *possible antimicrobial prophylaxis*

Induce a large increase of PNH erythrocytes:
novel condition;
possible peculiar clinical course
and complications

Impact on thrombosis? No data; to be cautiously followed…

Conclusion



- **Thrombosis in PNH:**
  - After years of guilt on haemolysis (PNH v1.0),
    now increasing complexity, nothing final: do we really understand?
- **PNH in thrombosis:**
  - think about it, then forget it, then finally look for it adequately
- **No more thrombotic PNH:**
  - C5 inhibitors, first-in-line eculizumab: PNH v2.0; infectious risk
  - Antithrombotics: acute thrombosis, pregnancy,
    granulocyte clone > 50% + eculizumab not indicated,
    previous VTE + eculizumab unavailable
- **PNH v3.0**
  - The rise of proximal complement inhibitors: but impact on thrombosis, but…?
  - Towards personalised treatments?

- Haematology
- *UMR UA11 INSERM-UM*
- Gynaecology Obstetrics
- Oncology

The NOHA network

- Intensive Care Unit
- Vascular Medicine
- Internal Medicine
- Clinical Research Unit