PATHOGENESIS OF PNH

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University of Florence, Firenze, ITALY

Disclosures of LUCIO LUZZATTO

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
	NO	NO	NO	NO	NO	NO	NUMEROUS DISCUSSIONS WITH SEVERAL COMPANIES

THE HÆMOLYTIC ANÆMIAS

Congenital and Acquired

By

J. V. DACIE

M.D.(Lond.), M.R.C.P.(Lond.)

Reader in Haematology, Department of Pathology, Postgraduate Medical School of London

With 98 Illustrations

LONDON

J. & A. CHURCHILL LTD.

104 GLOUCESTER PLACE, W.1.

1954

CONTENTS

HEMOLYTIC ANEMIAS WITH MAINLY INTRAVASCULAR HEMOLYSIS

1. Blackwater fever (severe malaria)
2. ABO-incompatible blood transfusion
3. *Clostridium perfringens* septicemia
4. AHA in G6PD deficiency
5. March hemoglobinuria
6. Paroxysmal cold hemoglobinuria
7. Paroxysmal nocturnal hemoglobinuria

HAEMOGLOBINURIA

*Indicates **intravascular** haemolysis*

Teil v. Melancholischer Saft

Johann Schmidt, Danzig (1623–1690)

Observatio LXXXVII in: Miscellane

De urina nigra nil funesti inc

[144] Vir apud nos Prae-co
nus mihi maximus G.K. nu
nam dum redderet, nigram
insolitum urinae suae colo
iubet: accedens video solo

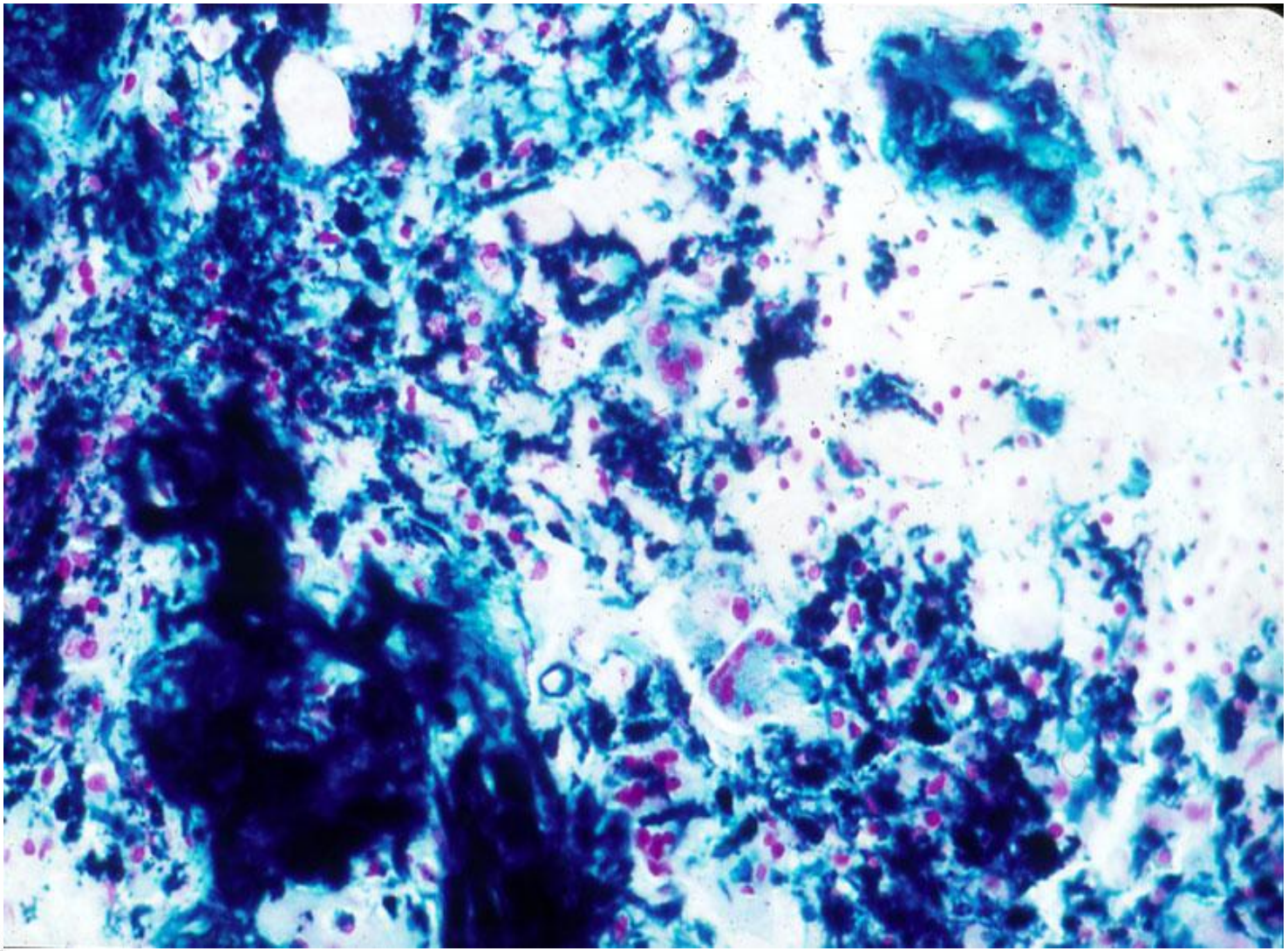
LANDMARKS IN THE EARLY HISTORY OF PNH

1678. Johan Schmidt (Danzig) reports intermittent black urine in a patient who did not have cancer

1882. Classic paper by Paul Strübing in *Deutsche Medizinische Wochenschrift*

1928. Ettore Marchiafava discovers *perpetual hemosiderinuria* in PNH patients

HEAVY HAEMOSIDERIN DEPOSITS IN THE URINE (HAEMOSIDERINURIA) OF A PATIENT WITH PNH



*Indicates **chronic** intravascular haemolysis*

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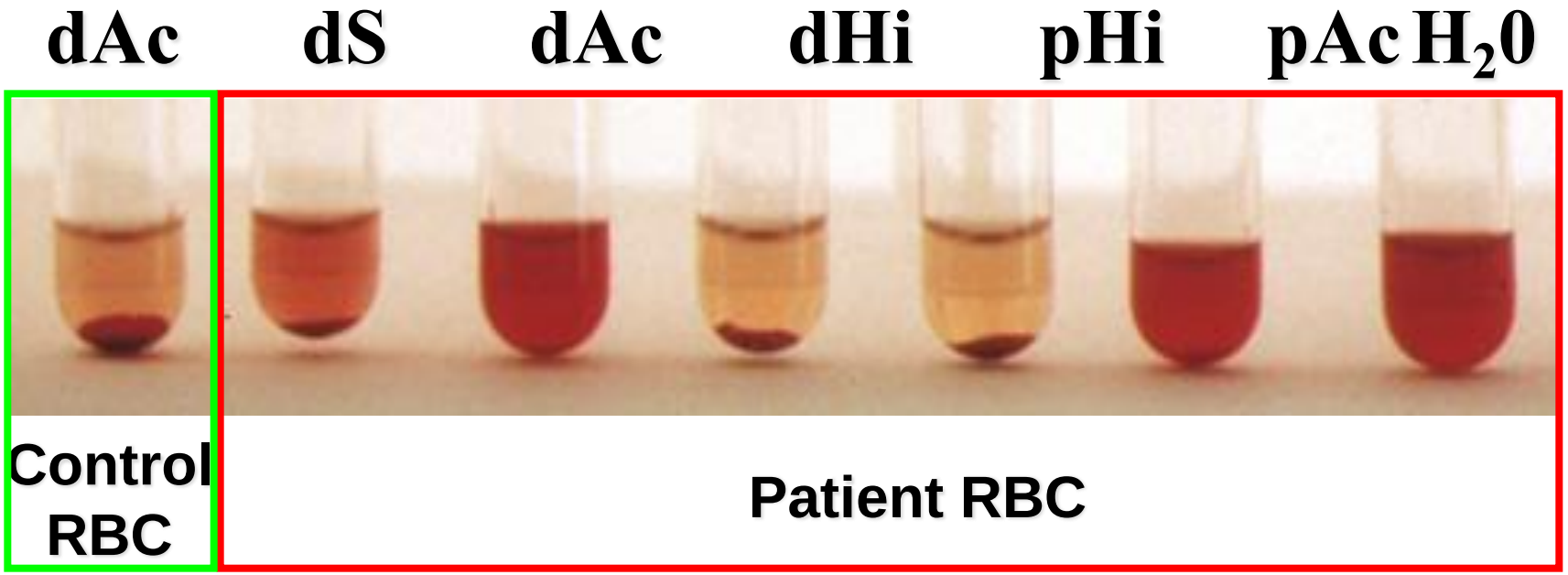
Ham Test in a PNH Patient



PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA:

Haemolytic anaemia
with characteristic clinical triad:

1. Intravascular haemolysis
2. Thrombosis
3. Cytopenias (bone marrow failure)

An ultra-rare disorder: estimated Prevalence 1-10 per million

A chronic disorder

LANDMARKS IN THE EARLY HISTORY OF PNH

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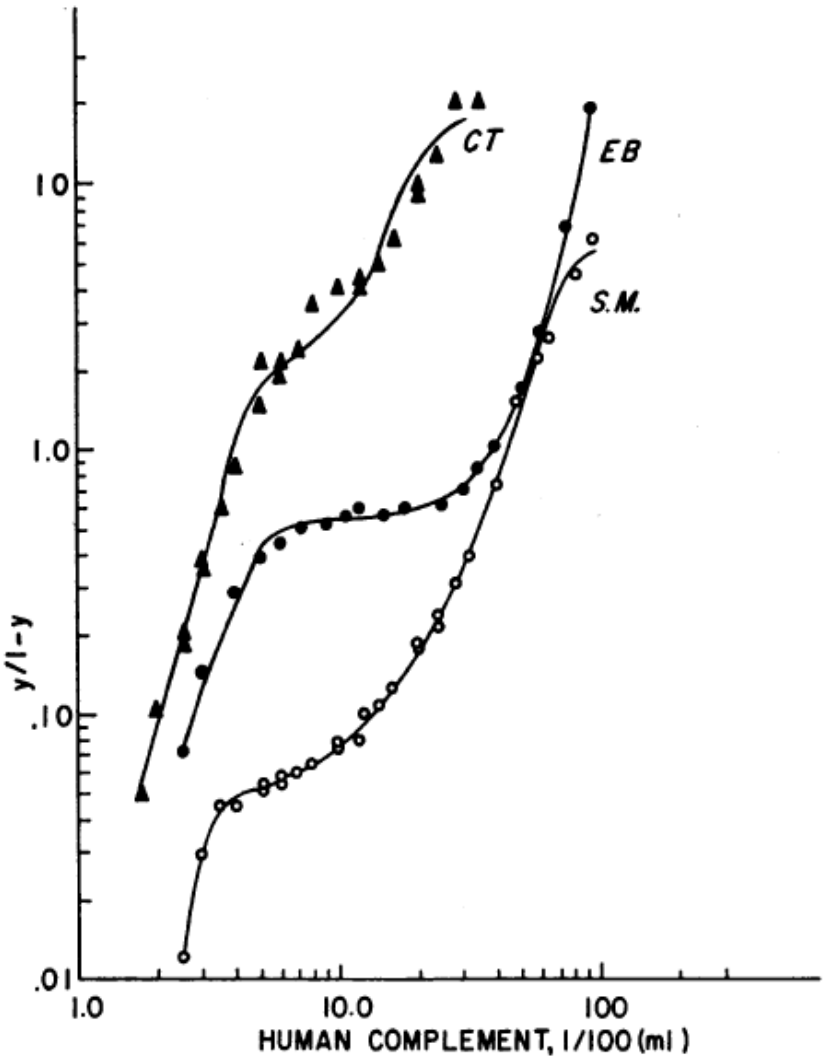
1963-66. Wendell Rosse and John Dacie demonstrate two populations of cells in PNH patients

Bimodal pattern of complement sensitivity of red cells in patients with PNH

In 1963 J V Dacie (in *Proc Roy Soc Med*. **56**:587) had hypothesized that the population of red cells hypersensitive to complement **might have arisen through a somatic mutation**.

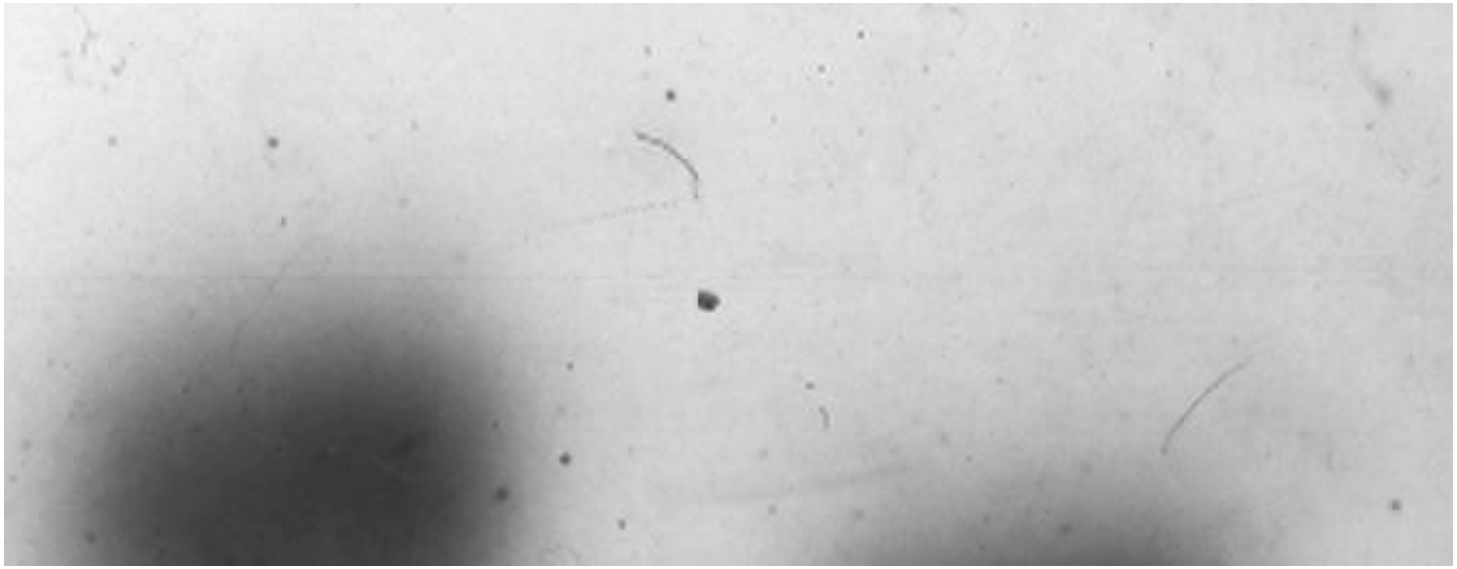


(From Rosse & Dacie, *JCI* **45**:736,1966)

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



G6PD-A G6PD-B Whole RBC PNH RBC

Controls **Patient**

From the Subdepartment of Hematology, Department of Pathology, University College Hospital, Ibadan, Nigeria.

LANDMARKS IN THE EARLY HISTORY OF PNH

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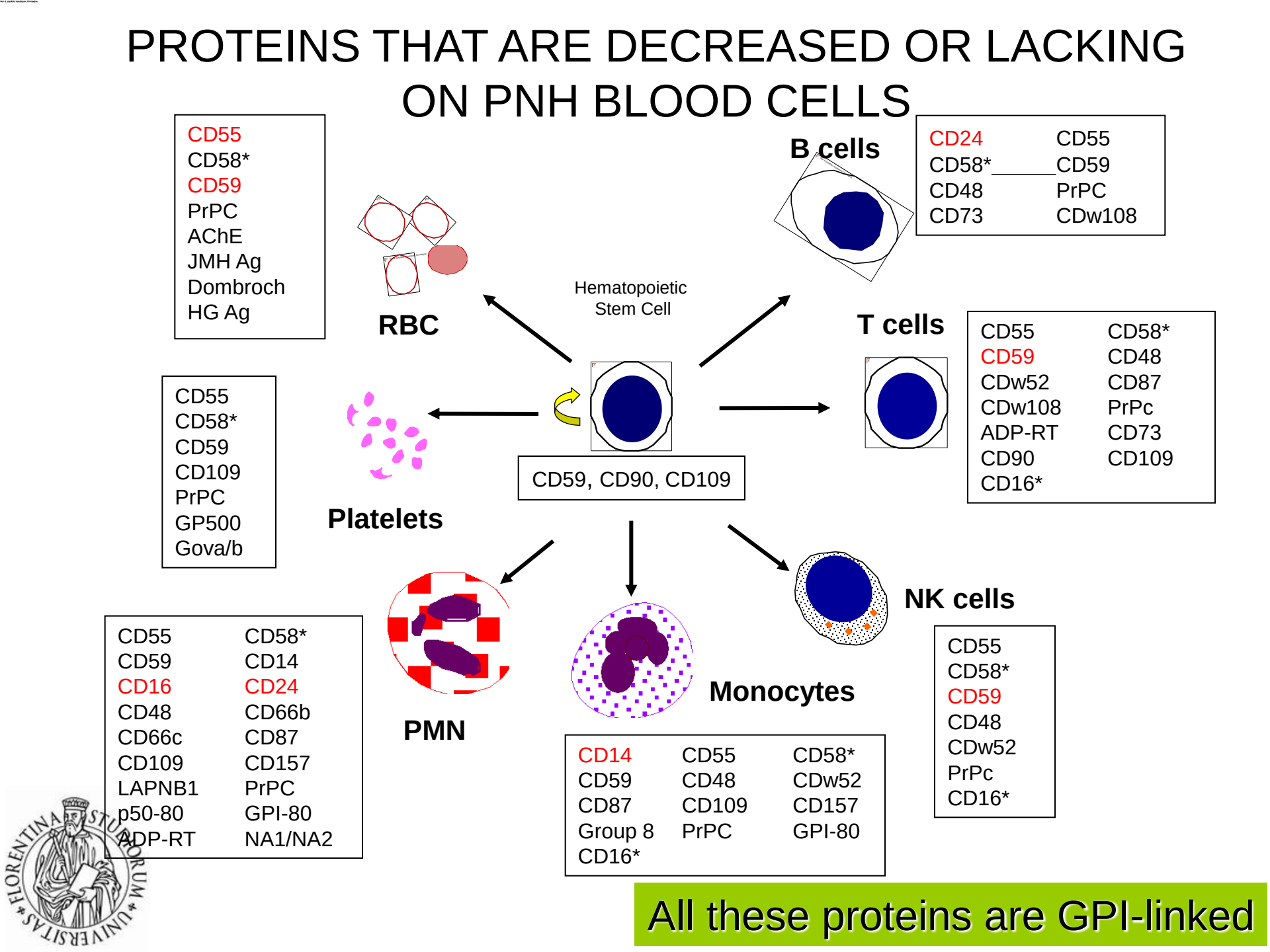
1938. Thomas Ham introduces the acidified serum test for the diagnosis of PNH

1963-66. Wendell Rosse and John Dacie demonstrate two populations of cells in PNH patients

1970. As Dacie had hypothesized, PNH must be caused by a somatic mutation; thus **PNH is a *non-neoplastic* clonal disorder**, the first example of what is referred to today as *clonal hematopoiesis*

PROTEINS THAT ARE DECREASED OR LACKING ON PNH BLOOD CELLS



All these proteins are GPI-linked

EIGHTH EDITION

Practical Haematology

SIR JOHN V. DACIE
S. M. LEWIS

Churchill Livingstone 1995

15. Laboratory methods used in the investigation of paroxysmal nocturnal haemoglobinuria (PNH)

Revised by L. Luzzatto and P. Hillmen

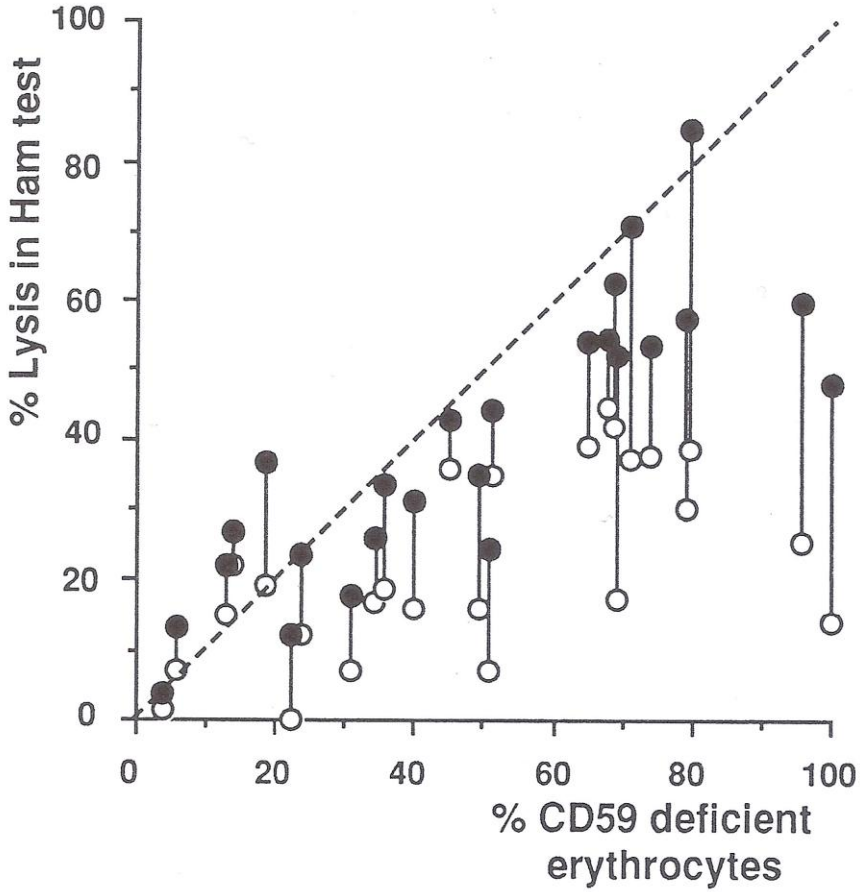
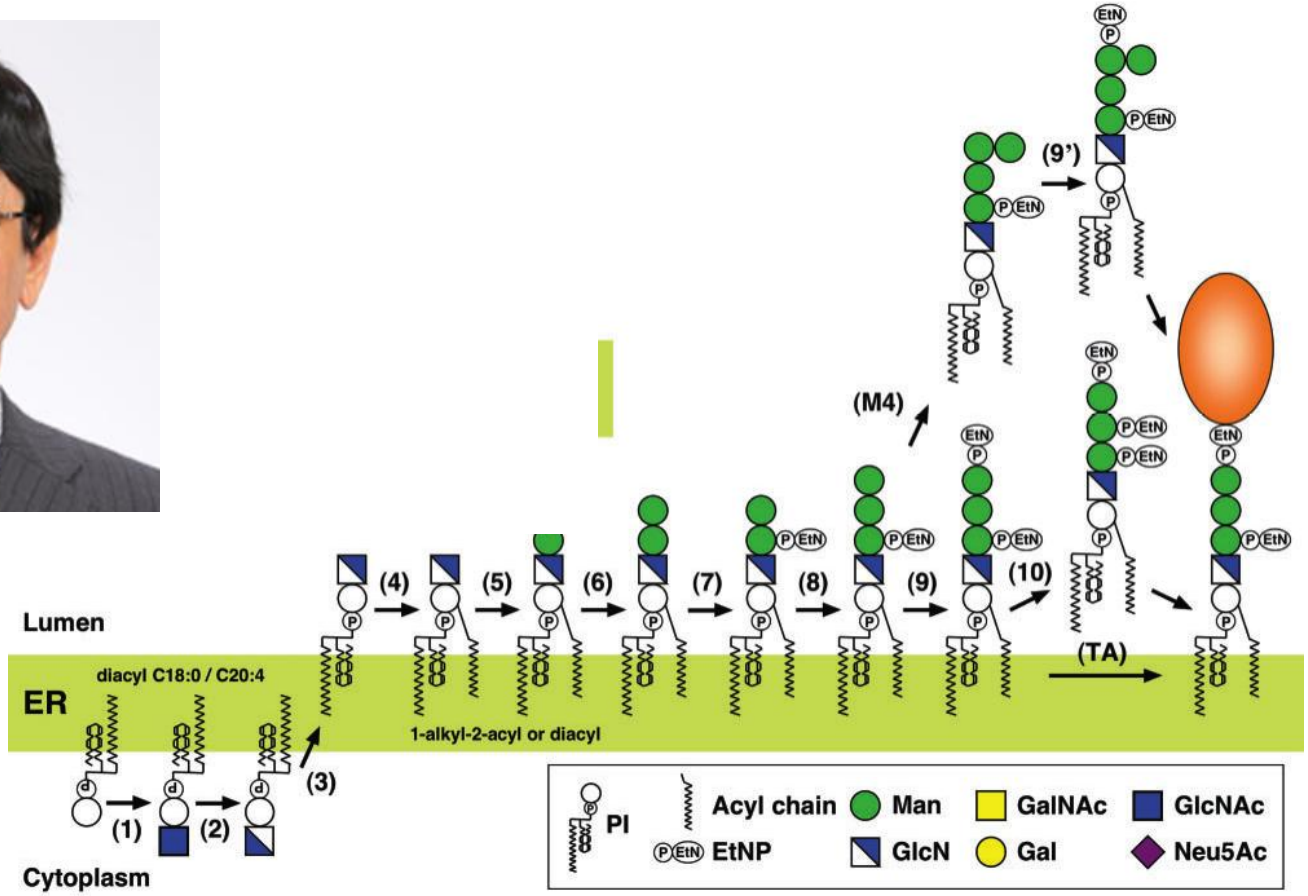


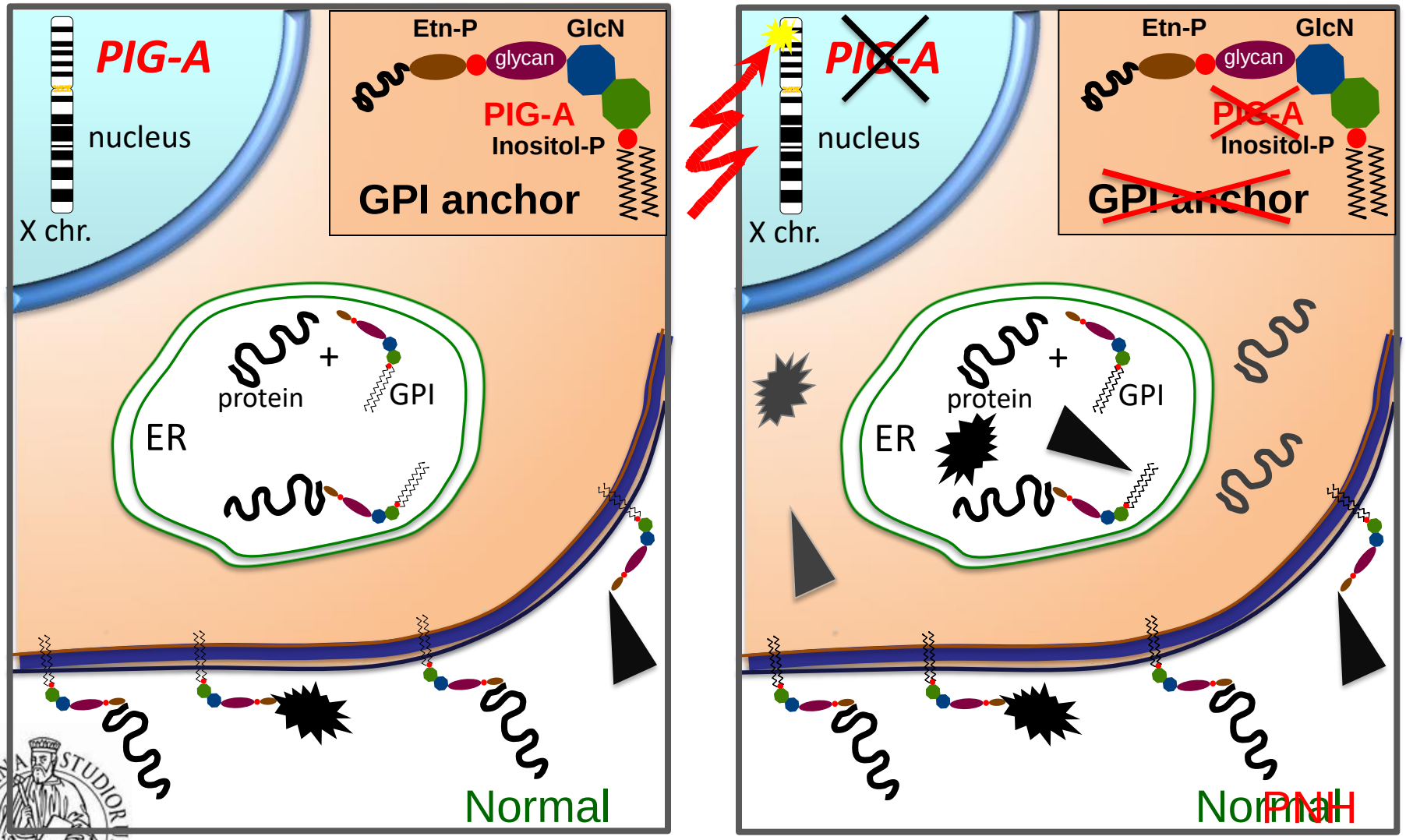
Fig. 15.2 Comparison of the proportion of CD59 deficient red cells with the lysis in the Ham test. The percentage lysis in the Ham test with added magnesium (●) and without added magnesium (○) is plotted against the proportion of CD59 deficient red cells in the same samples from 25 patients with PNH (P. Hillmen, M. Bessler, D. Roper and L. Luzzatto, unpublished observation).

THE BIOSYNTHETIC PATHWAY OF THE GPI ANCHOR INVOLVES AT LEAST TEN DISCRETE STEPS



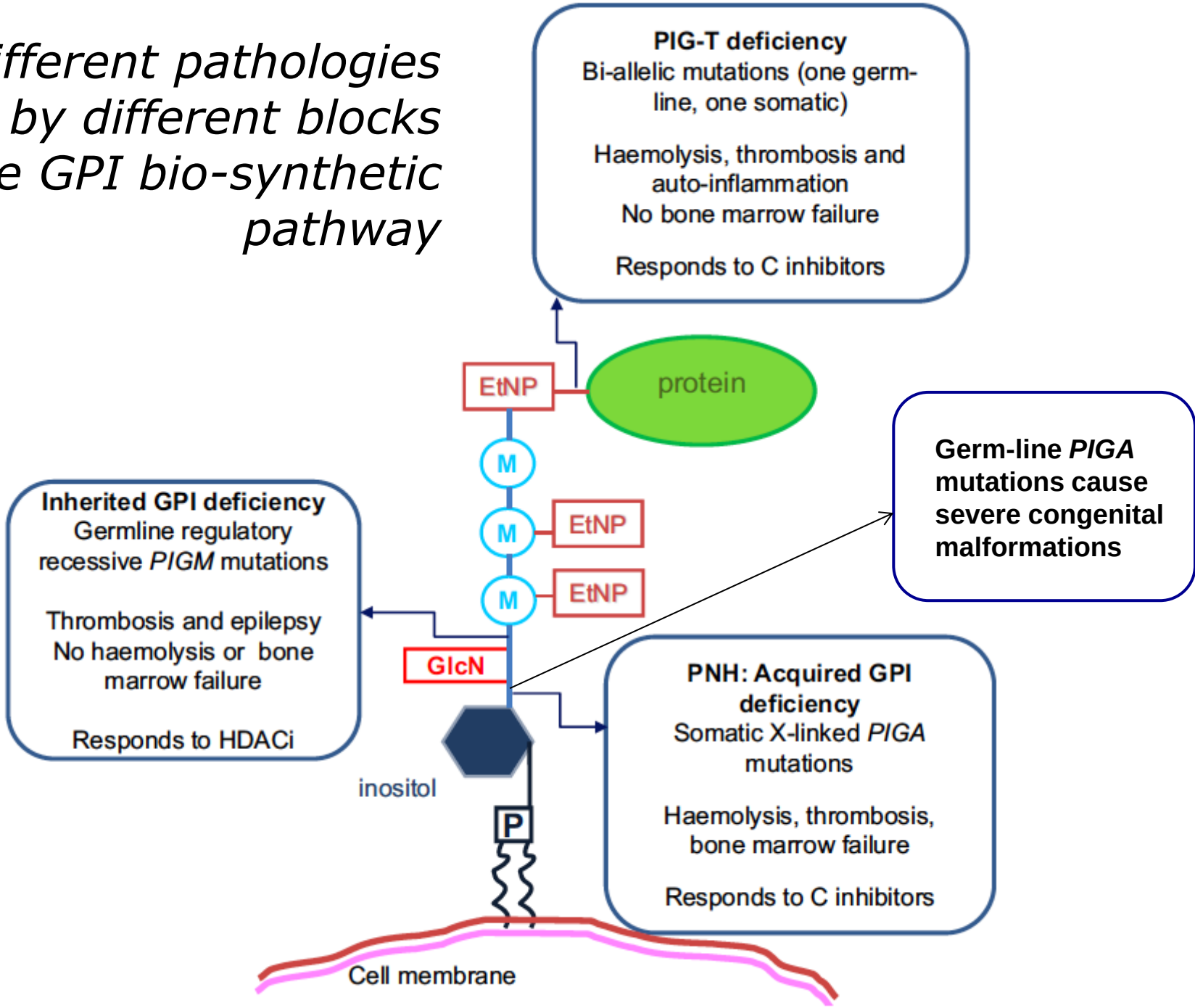
[From Kinoshita et al., *J Biochem* (JPN) **144**:287,2008]

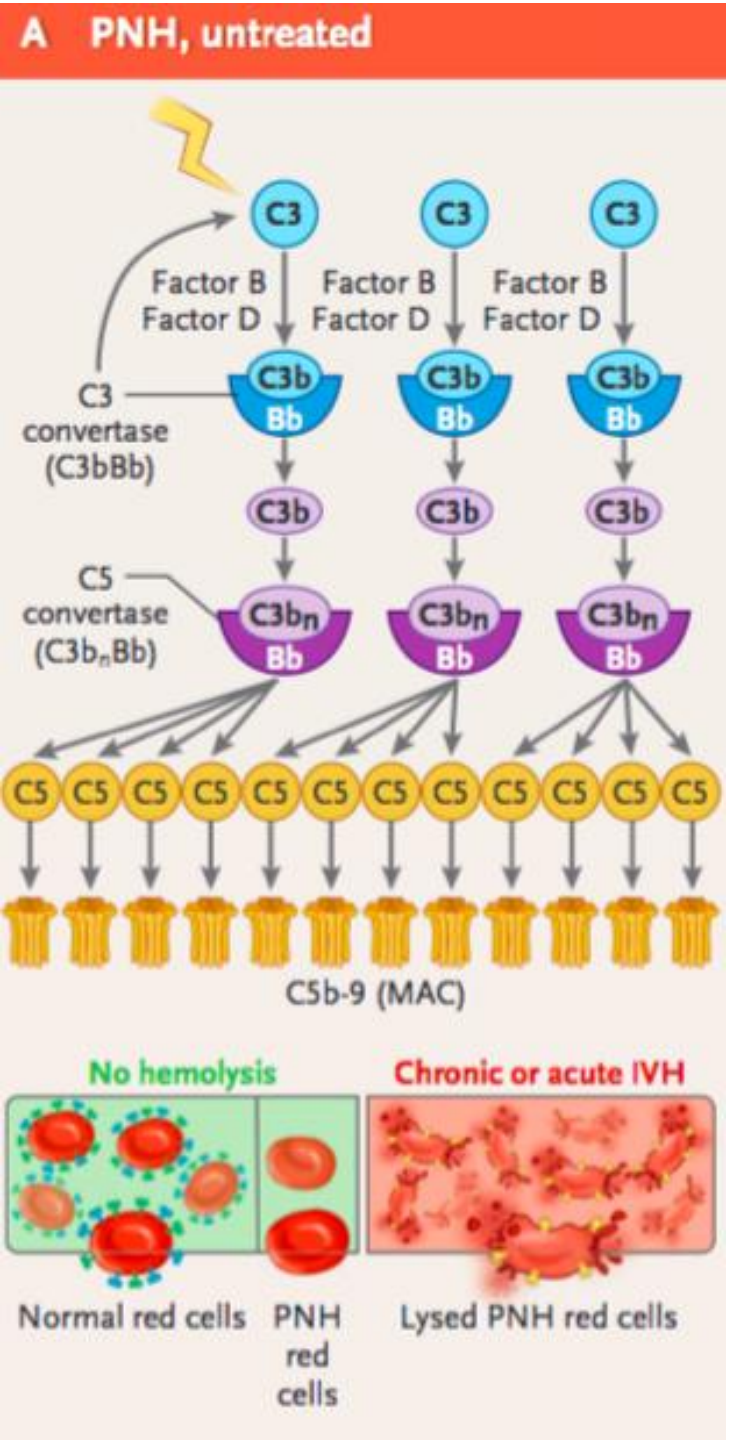
PATHOGENESIS OF A PNH CELL



Haematopoietic stem cell

Different pathologies caused by different blocks in the GPI bio-synthetic pathway



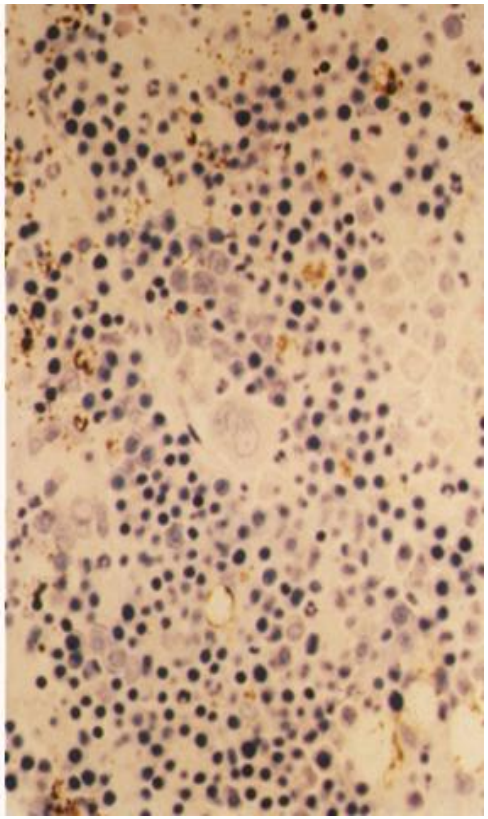


(From Notaro & Luzzatto
New Eng J Med **387:**160,2022)

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA:

Haemolytic anaemia
with characteristic clinical triad:

1. Intravascular haemolysis ✓
2. Thrombosis (✓)
3. Bone marrow failure ?

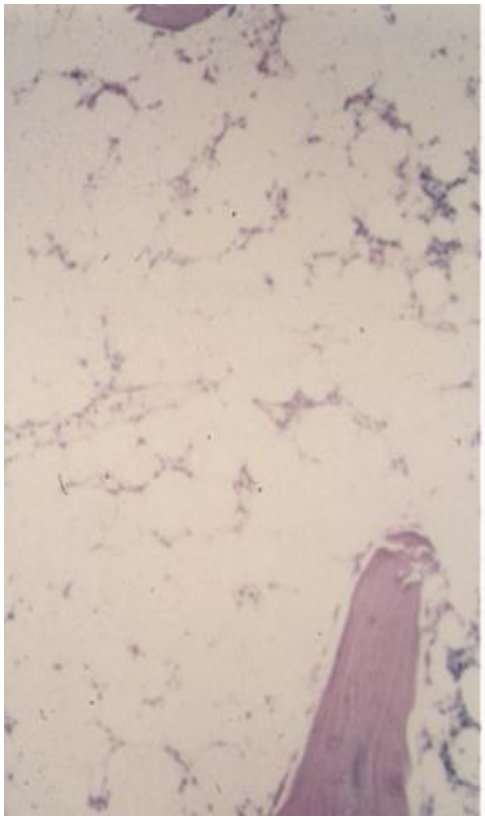


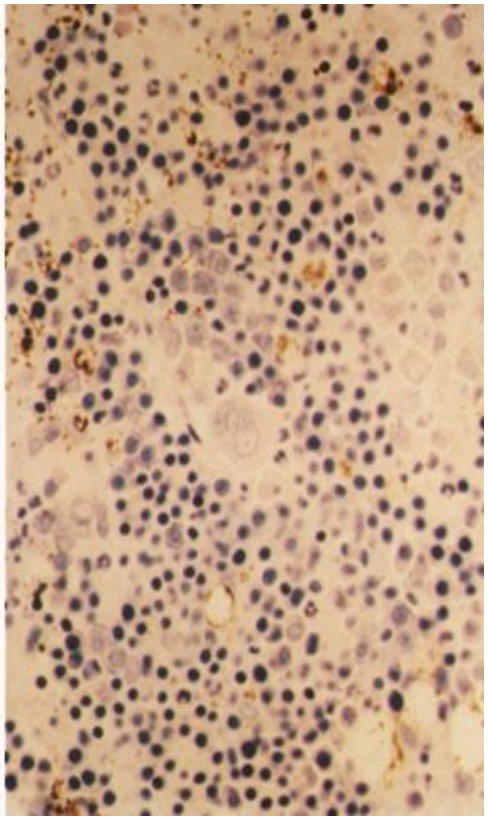
Patient WXY
1992

- Intravascular haemolysis
- Thrombosis
- Cellular marrow with erythroid hyperplasia

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

Patient WXY
1988





Patient WXY
1992

- Intravascular haemolysis
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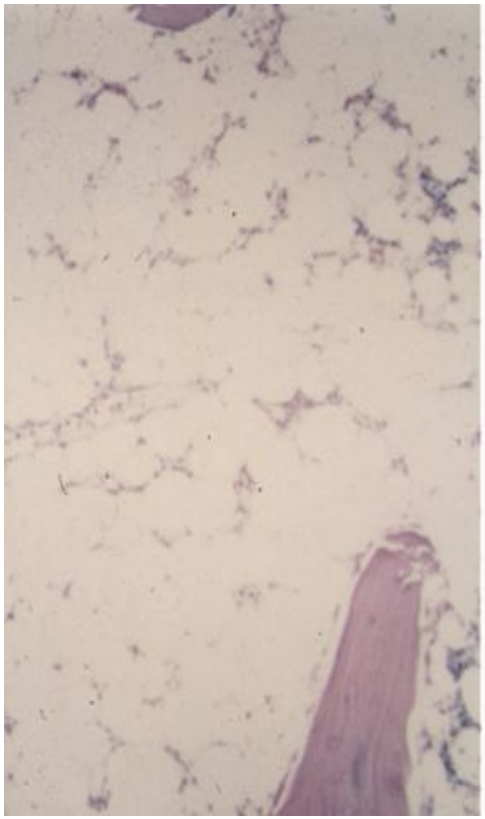
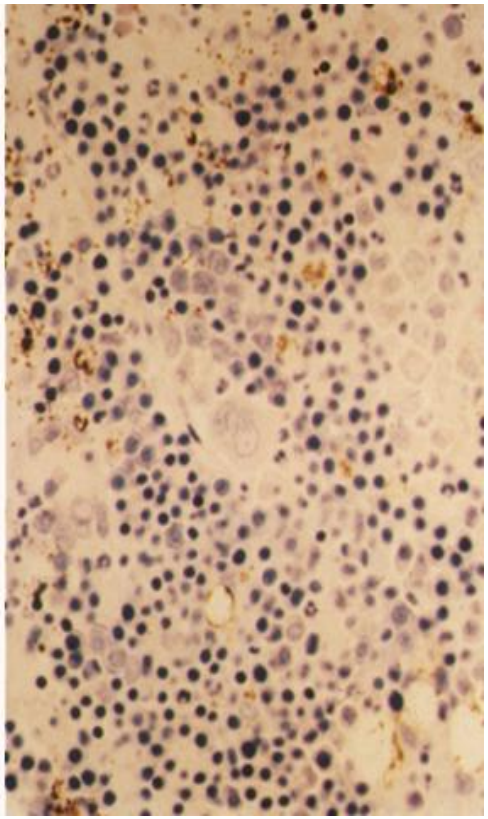
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

Patient WXY
1988





Patient WXY
1992

- Pancytopenia
- Hypocellular bone marrow

APLASTIC ANEMIA (AA)

- Intravascular haemolysis
- Thrombosis
- Cellular marrow with erythroid hyperplasia

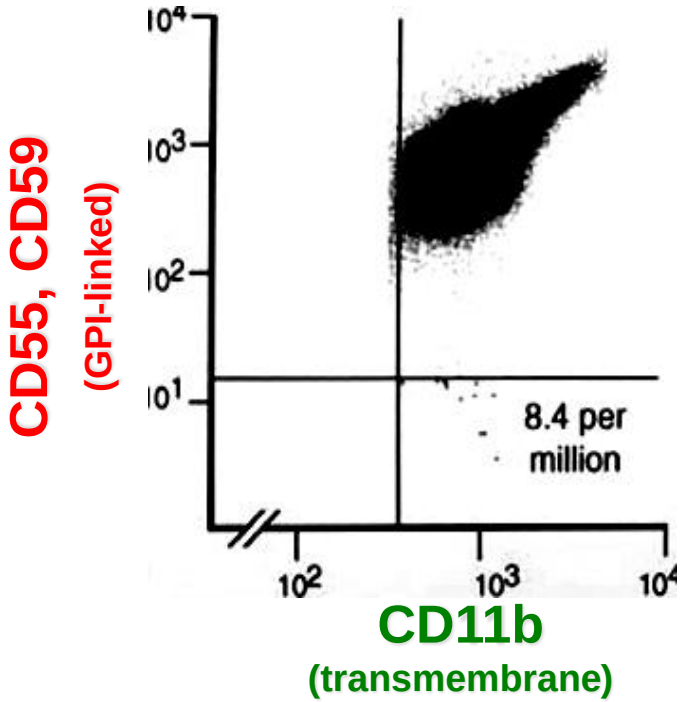
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

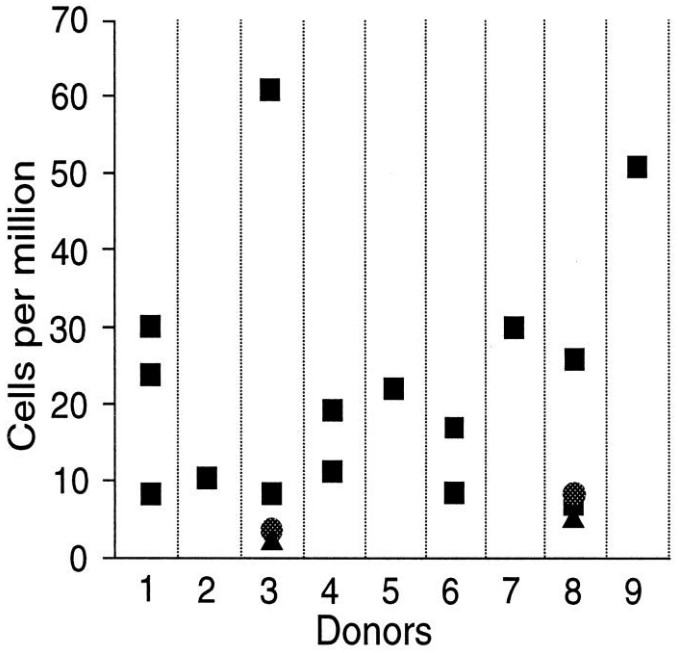
PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA:

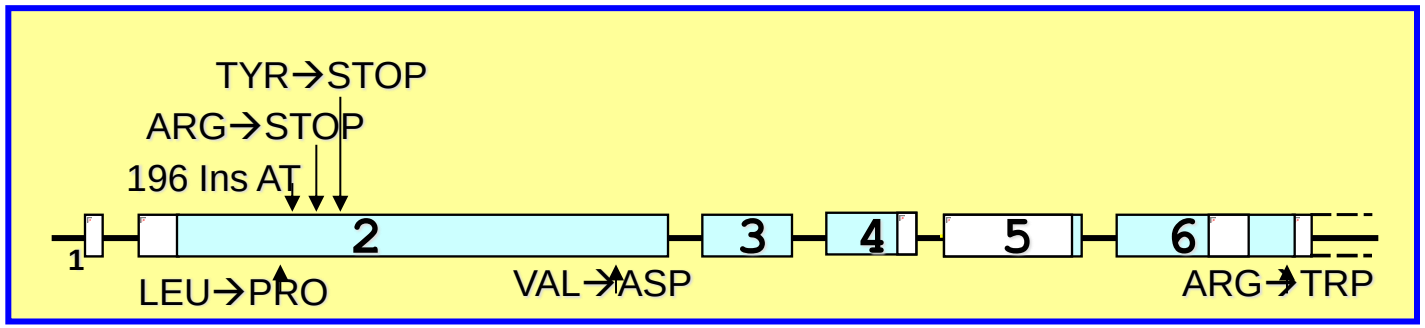
How to explain two paradoxical features:

1. Expansion of a non-neoplastic clone
2. Haemolytic anaemia arising in an aplastic bone marrow

GPI(–) micro-clones are common and they have inactivating PIGA *mutations*

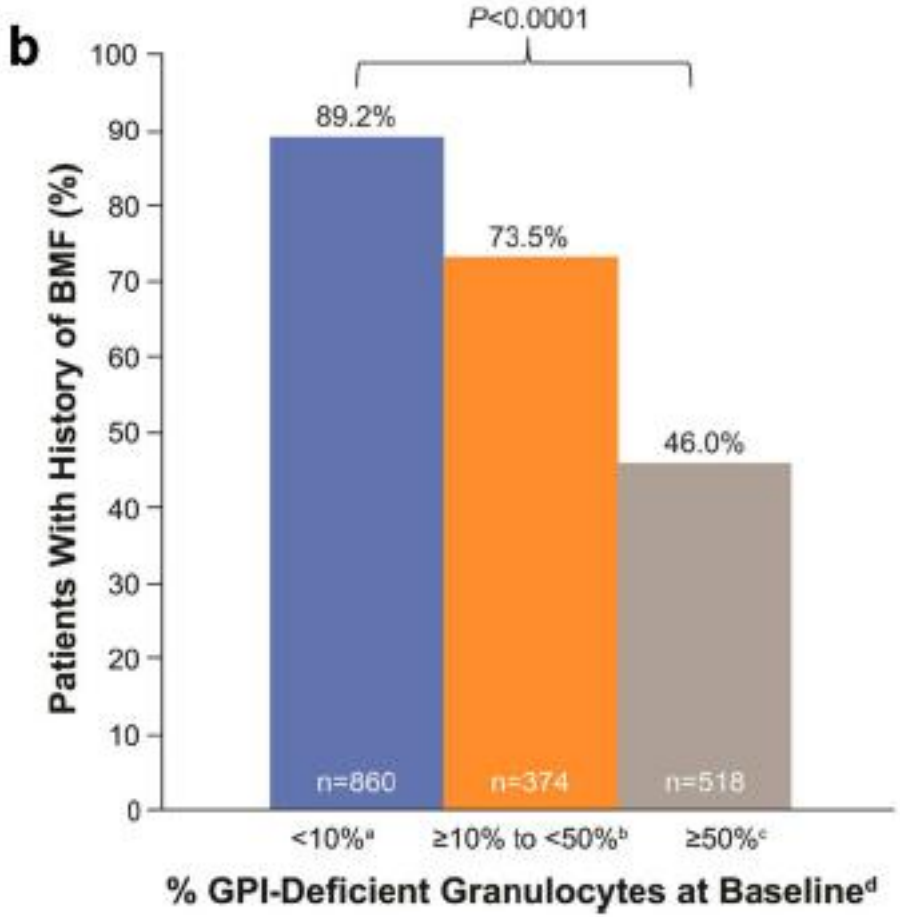






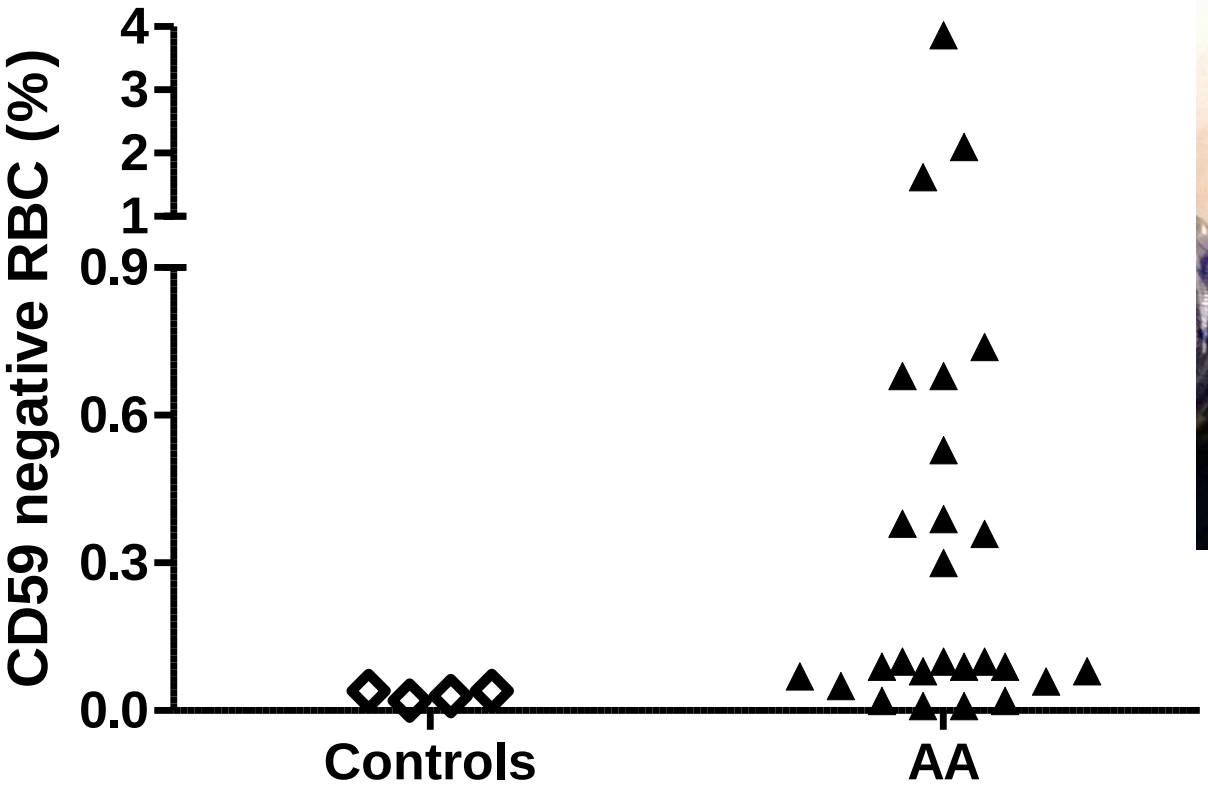
(From Araten etal., *PNAS* **96**:5209,1999)

In a PNH International Registry "2206 out of 4201 (53%) patients had a history of aplastic or hypoplastic anemia*"; and* ***63% had a history of bone marrow failure****.*

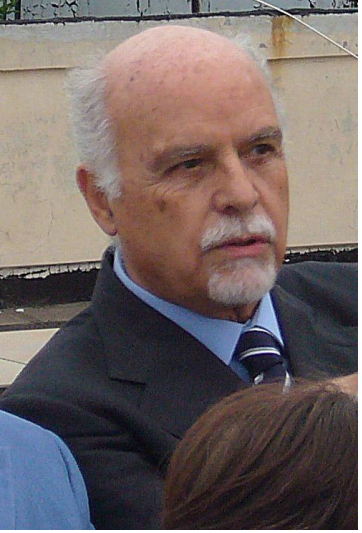


(From Schrezenmeier et al, *Ann Haematol* **99:**1505,2020)

‘PNH’ RED CELLS IN AA PATIENTS IN TANZANIA



(From Ally, Magesa & Luzzatto, *Am J Hematol* **94**:E86,2019)



Cell, Vol. 88, 1–4, January 10, 1997, Copyright ©1997 by Cell Press

Somatic Mutations in Paroxysmal Nocturnal Hemoglobinuria: A Blessing in Disguise?

Minireview

Lucio Luzzatto, Monica Bessler, and Bruno Rotoli*
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Memorial Sloan Kettering Cancer Center
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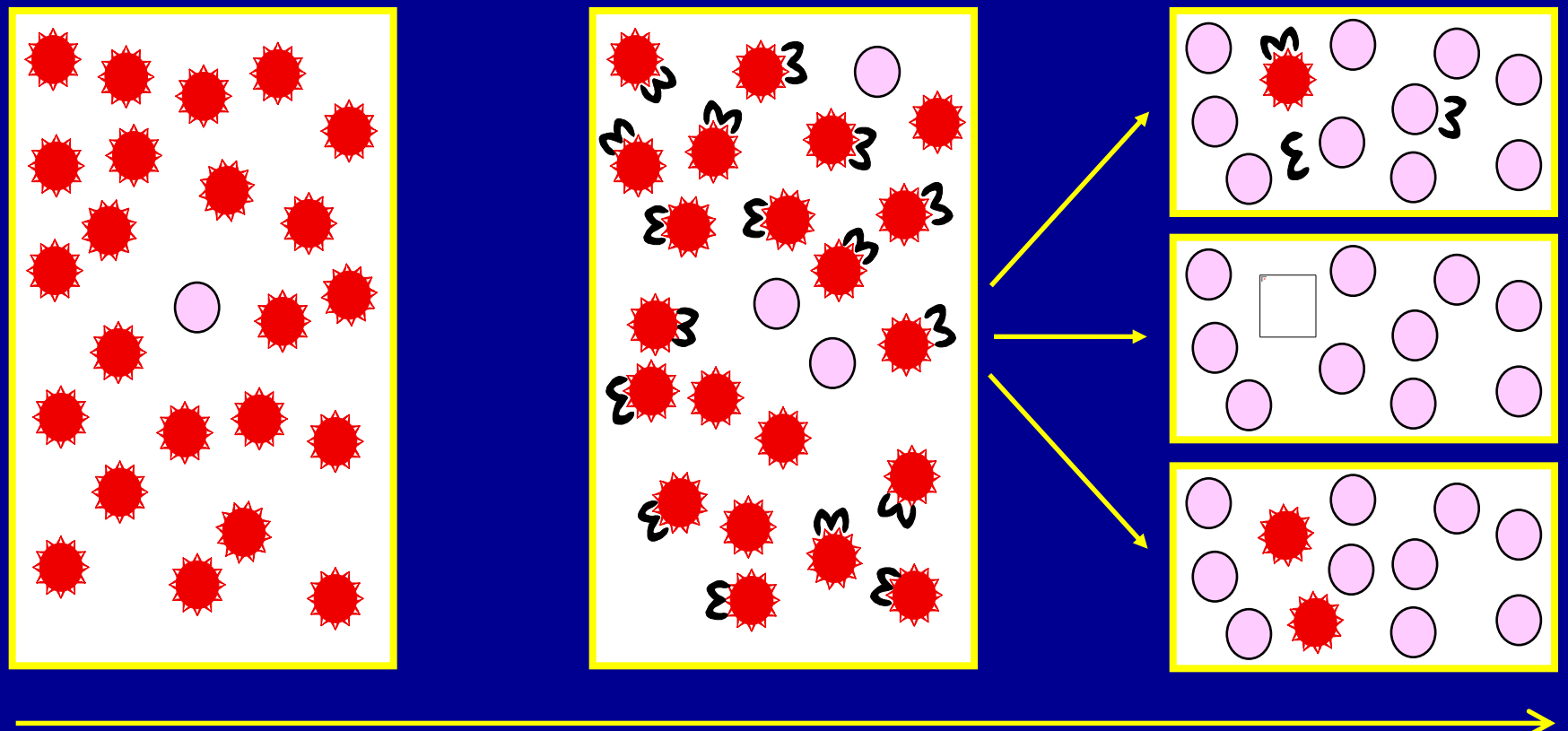
Division of Hematology
Federico II University Medical School
Via S Pansini 5
80100 Napoli
Italy

PNH Arises through a Somatic Mutation in an X-Linked Gene

This set of remarkable features led to the notion that PNH might arise through a somatic mutation in a multipotent hematopoietic stem cell. In support of this notion, the PNH red cells of women heterozygous for two electrophoretically distinguishable alleles of the X-linked gene encoding glucose 6-phosphate dehydrogenase were found all to express the same allele, indicating that they belonged to one clone (Oni et al., 1970). Subsequently, extensive characterisation of the abnormal cells—which we will refer to for brevity as PNH cells—

Pathogenesis of PNH

(Rotoli & Luzzatto, 1989)



What is the fate of a mutant clone?

	In populations of organisms	*In populations of somatic cells*
Lethal mutation	No offspring	No clonal growth
Neutral mutation	No visible change	
	Frequency of mutant could increase through genetic drift	
Mutation with absolute advantage	Mutant people will gradually take over	Clone will grow faster than other cells
Mutation with conditional advantage	Mutant people will increase in a certain environment	Clone will grow faster under certain conditions

(See Luzzatto & Risitano, *BJH* **182:**758,2018)

TWO FACTORS IN THE PATHOGENESIS OF PNH

- **Somatic mutation of *PIGA* in a HSC**
- **Expansion of *PIGA* mutant clone associated with GPI-targeted T cell-mediated auto immune attack**

'**Darwinian selection**' model:
Micro-environment dependent selection favors mutant clone (a.k.a. *immune escape*)

The mutation rate of PIG-A is normal in patients with PNH

Table 2. Analysis of cell lines from patients

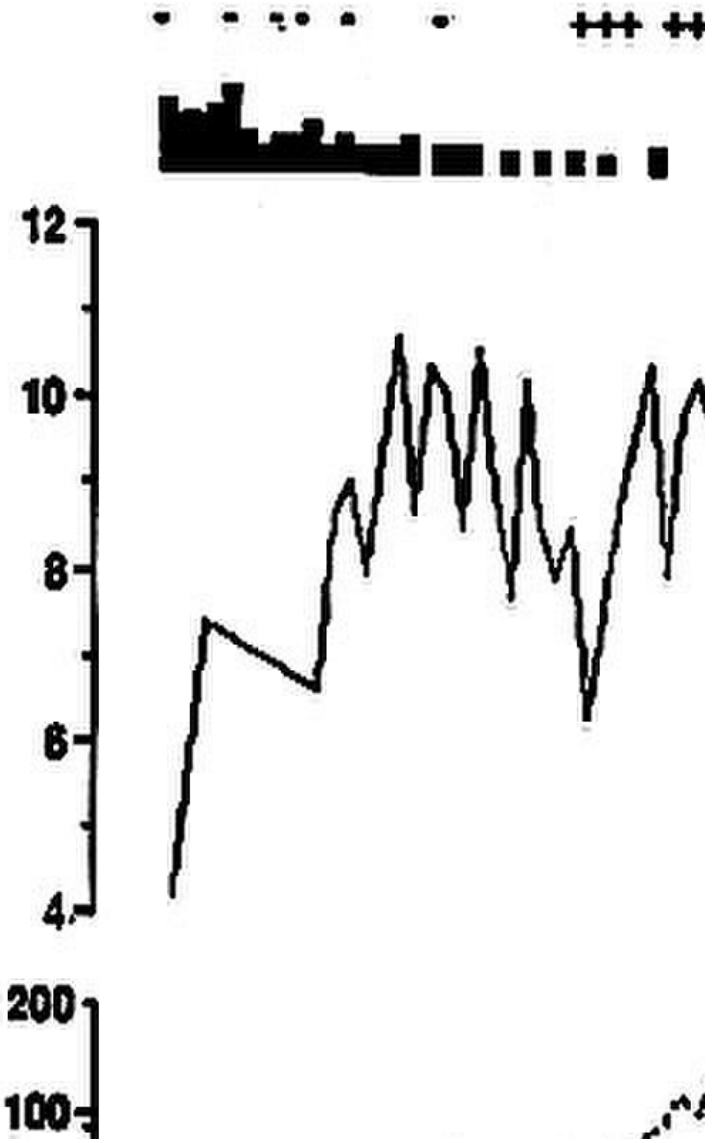
Patient	*d**	No. of GPI$^-$ cells	No. of GPI$^+$ cells	*f*,† × 10^6	μ,‡ × 10^7
1	4.24	4	846 083	4.73	11.2
2	10.6	4	792 311	5.05	4.76
3	9.5	1	849 779	1.18	1.24
4	6.49	4	2 934 988	1.36	2.10
5	6.07	5	2 342 270	2.13	3.51

Normal range for μ: 2.4-29.6 × 10^{-7} mutations per cell division

(From Araten & Luzzatto, *Blood* **108:**734,2006)

Litron Laboratories

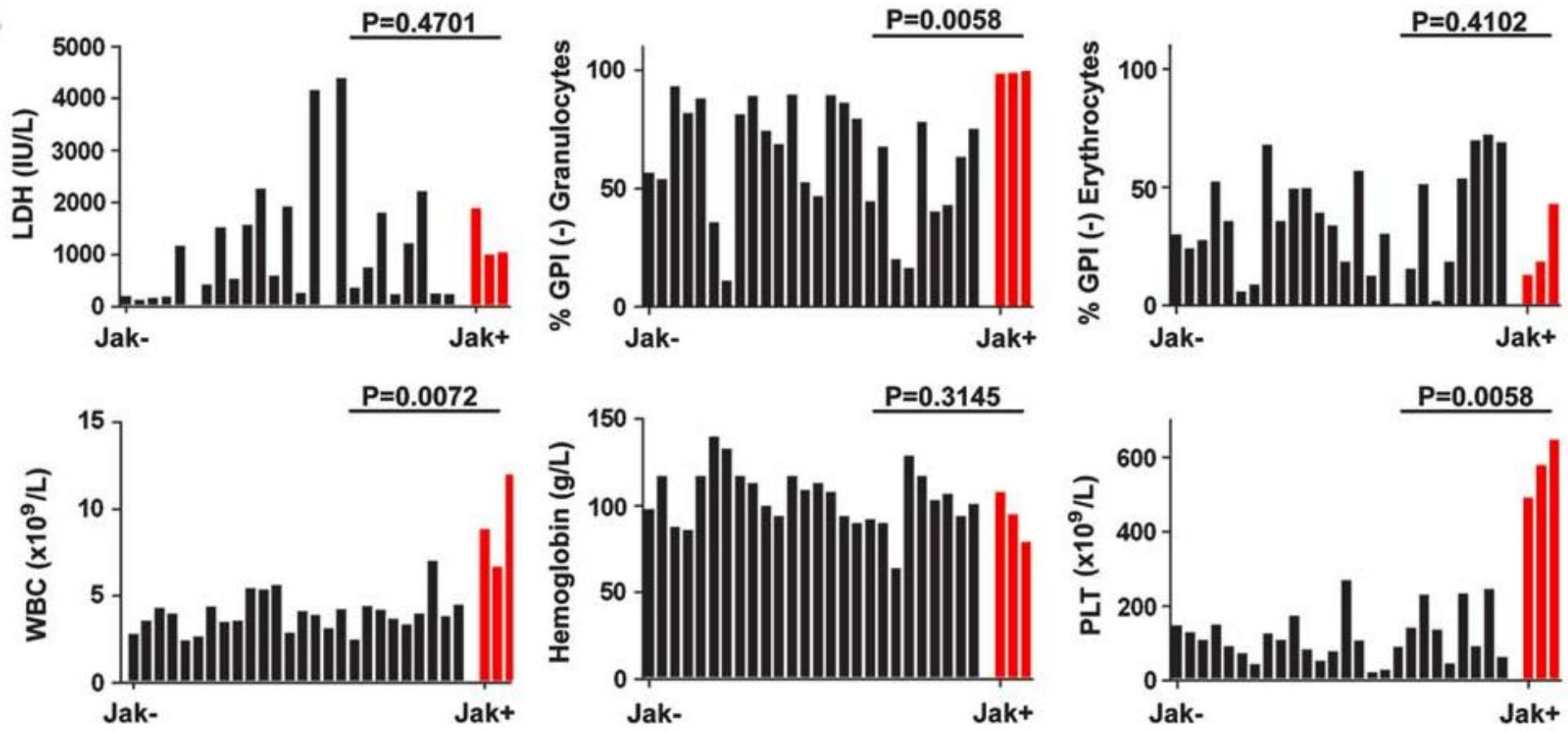
PNH EVOLVING FROM APLASTIC ANEMIA



(From Hows & Luzzatto, 1993)

PNH can cure aplastic anemia

HEMATOLOGIC FEATURES OF PATIENTS WHO HAVE BOTH A *PIGA* MUTATION AND THE *JAK2*^{V617F} MUTATION: a PNH/MPN overlap syndrome



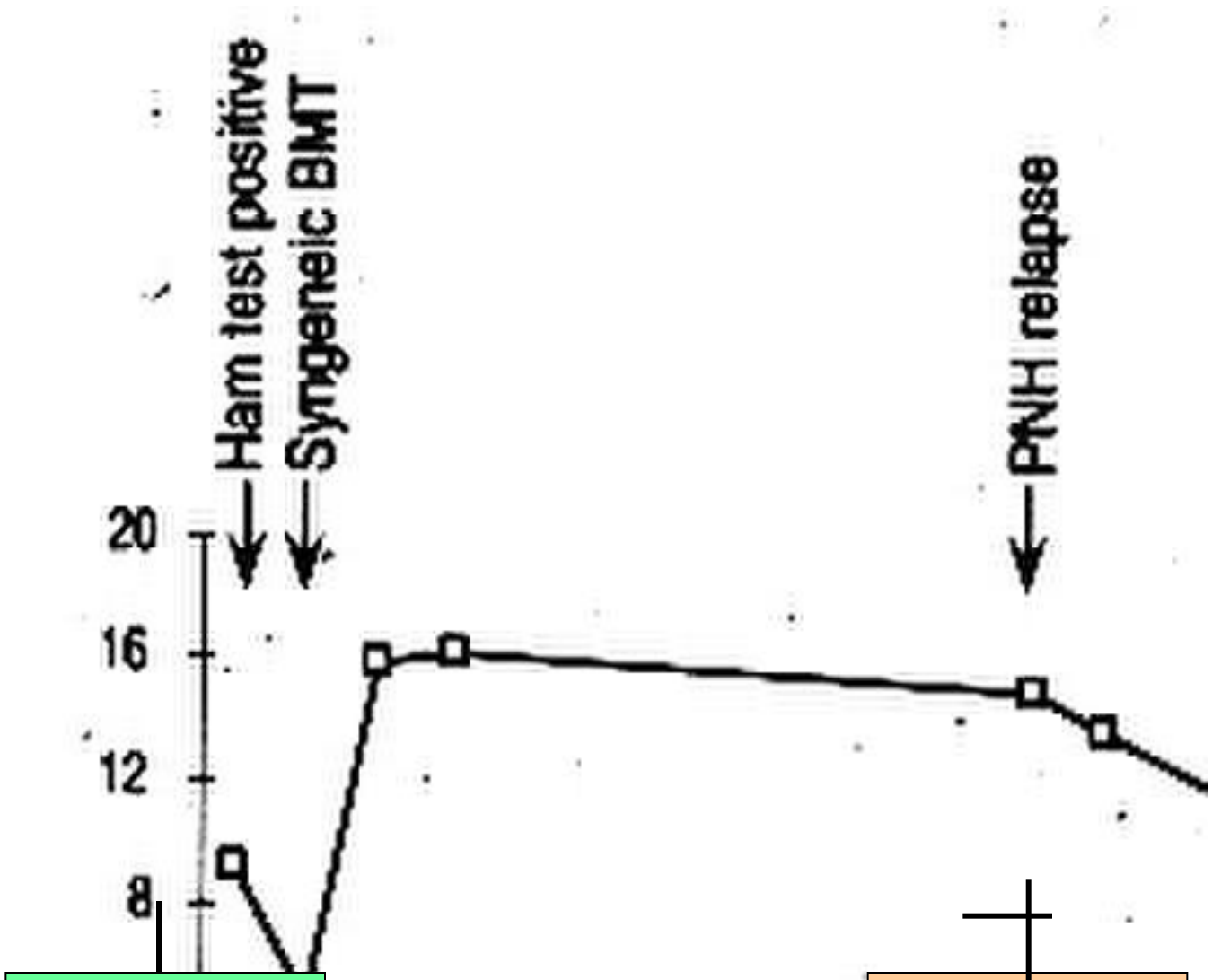
(From Sugimori et al, *Blood Cancer J* **2**:e63,2010)

A SINGLE STEM CELL CAN SUPPORT HEMATOPOIESIS FOR A LONG TIME

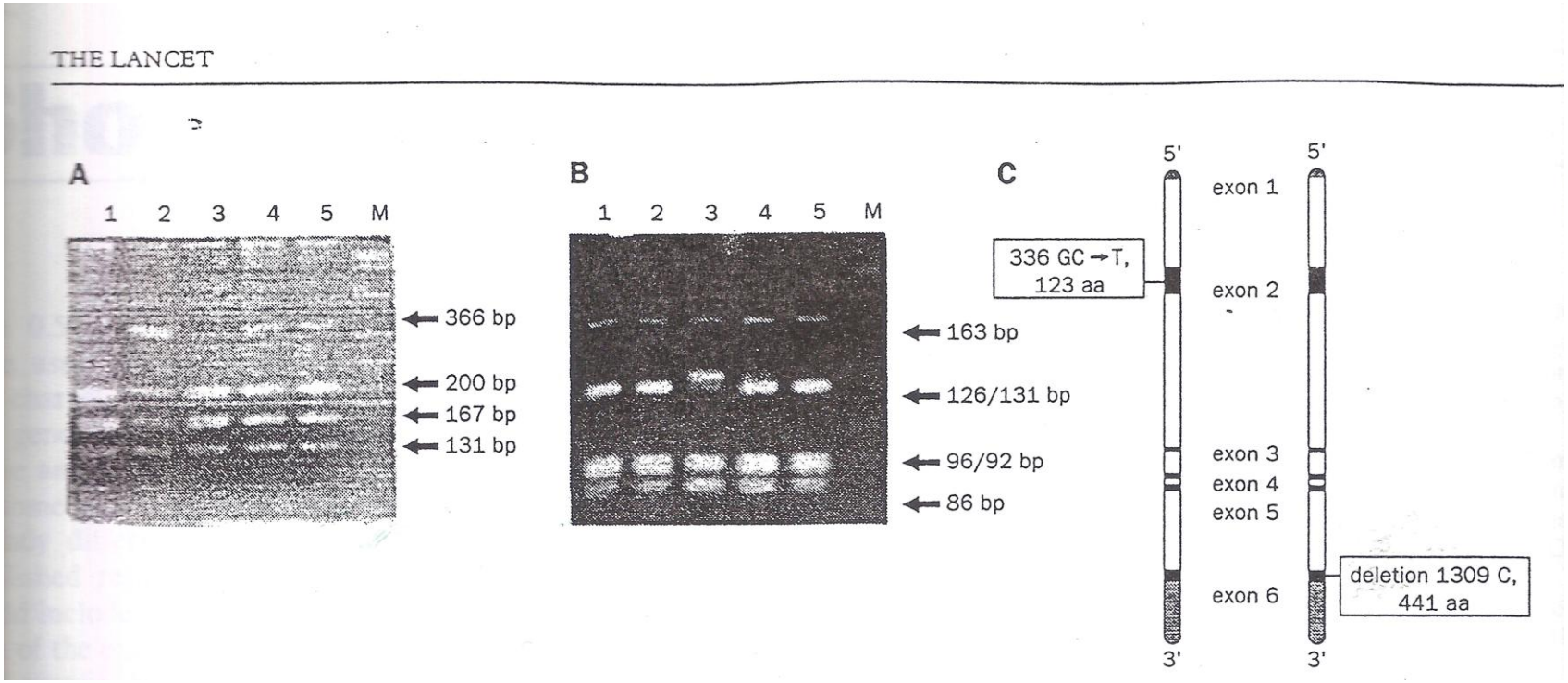
Patient	*Years follow-up*	*Size of predominant clone*		*Clinical course*
J12	17	14/20	4/16	Stable
J13	8	18/20	4/10	Stable
J16	24	5/5	4/18	PNH to AA

(From Nishimura et al., 2004)

THE COURSE OF PNH CAN SPAN DECADES



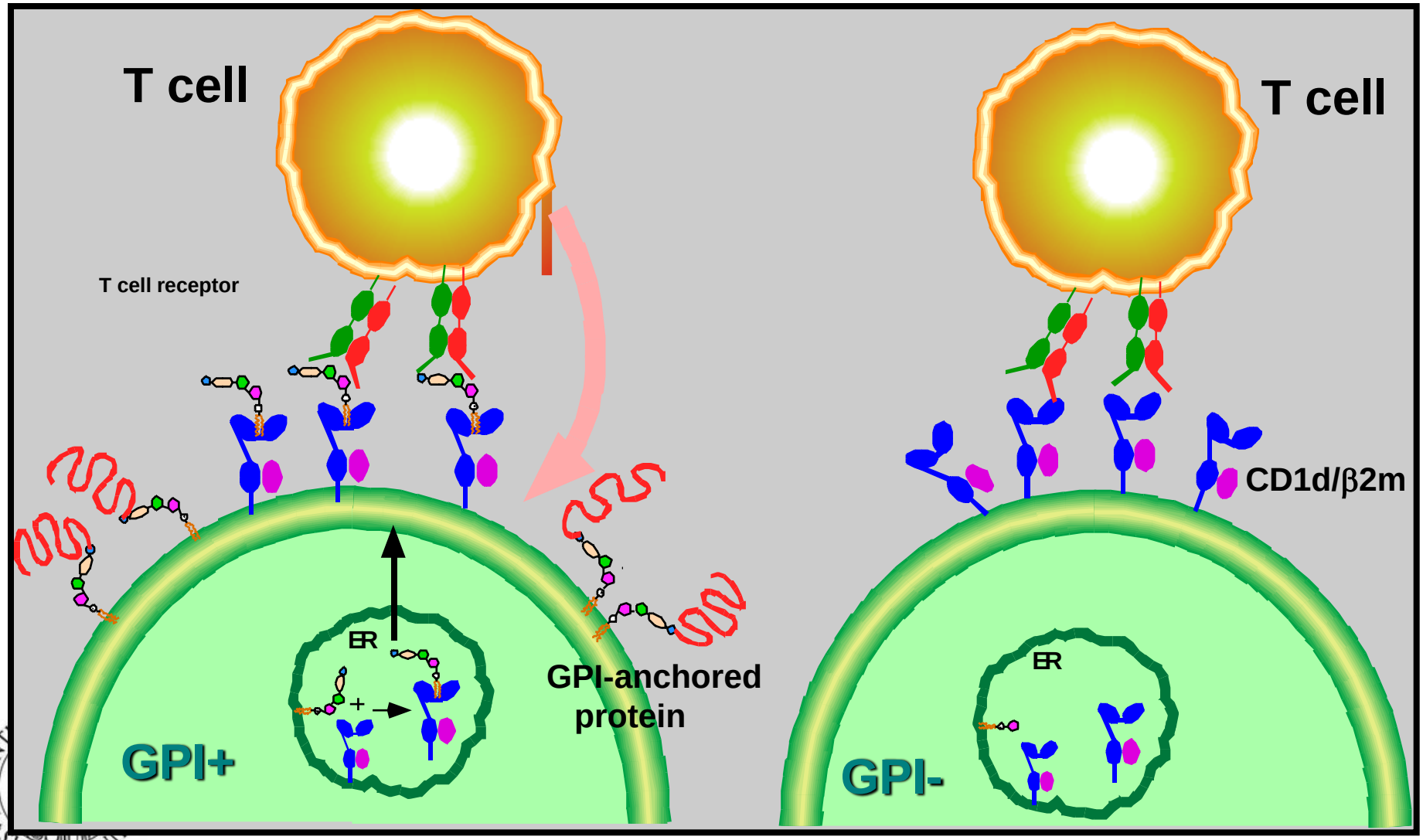
Two different PIGA *mutations in two separate lymphoblastoid cell lines obtained from the same patient with PNH*



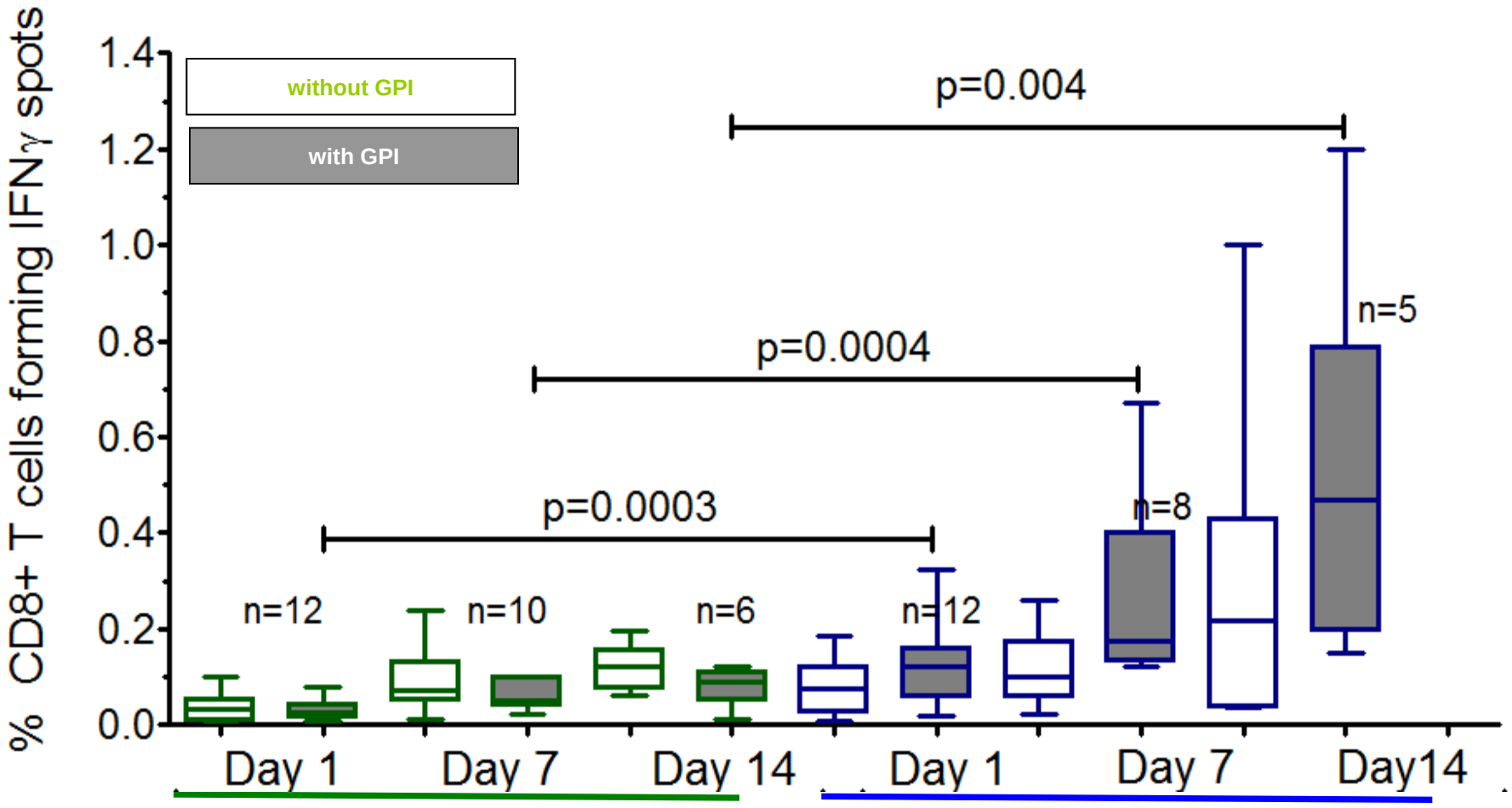
AN EXAMPLE OF **CONVERGENT EVOLUTION**

(From Bessler et al, *Lancet* **343**:951,1994)

GPI-specific, CD1d-restricted T cells in the pathogenesis of PNH



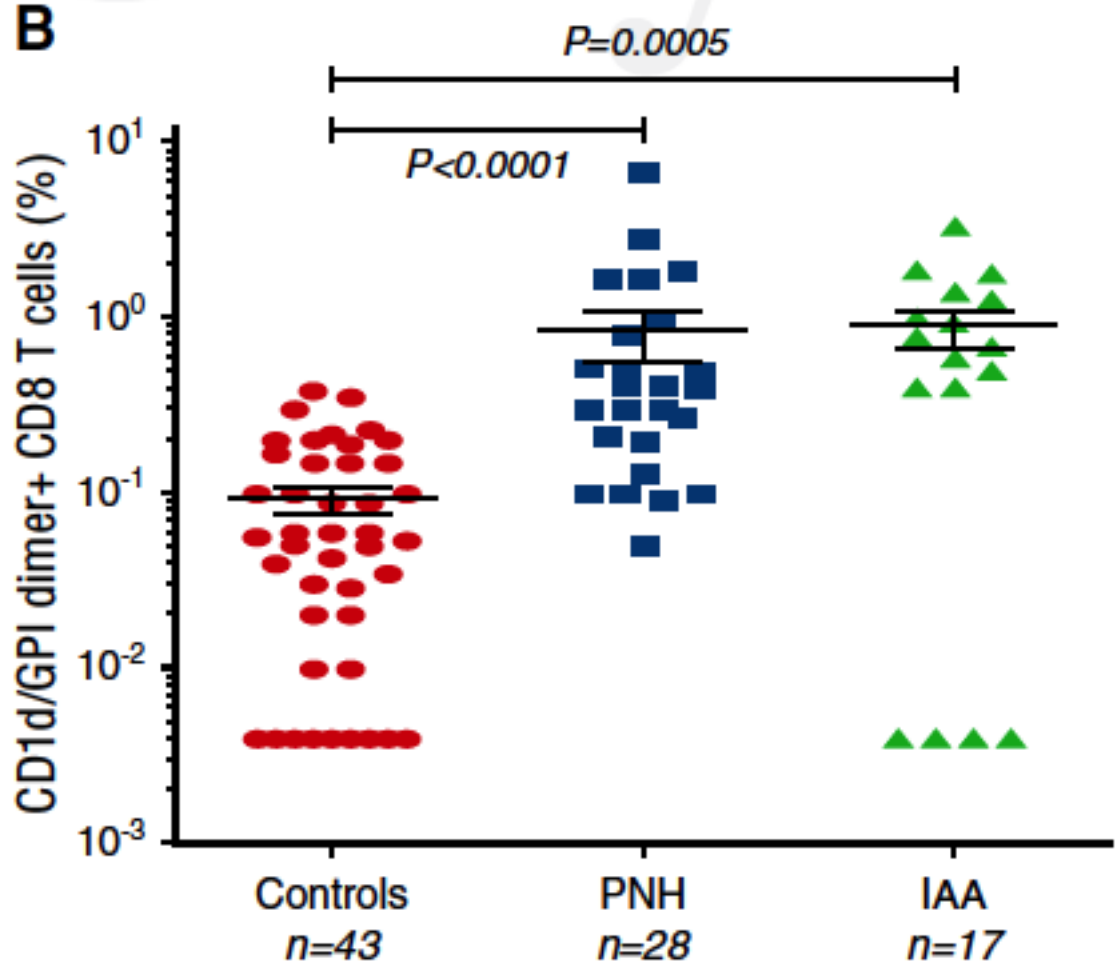
GPI-SPECIFIC T CELLS IN PNH PATIENTS



Normal Controls **PNH Patients**

(From Gargiulo *et al. Blood* **121:** 2753, 2013)

GPI-specific CD1d-restricted T cells are markedly increased in patients with PNH and in a subset of patients with idiopathic aplastic anaemia



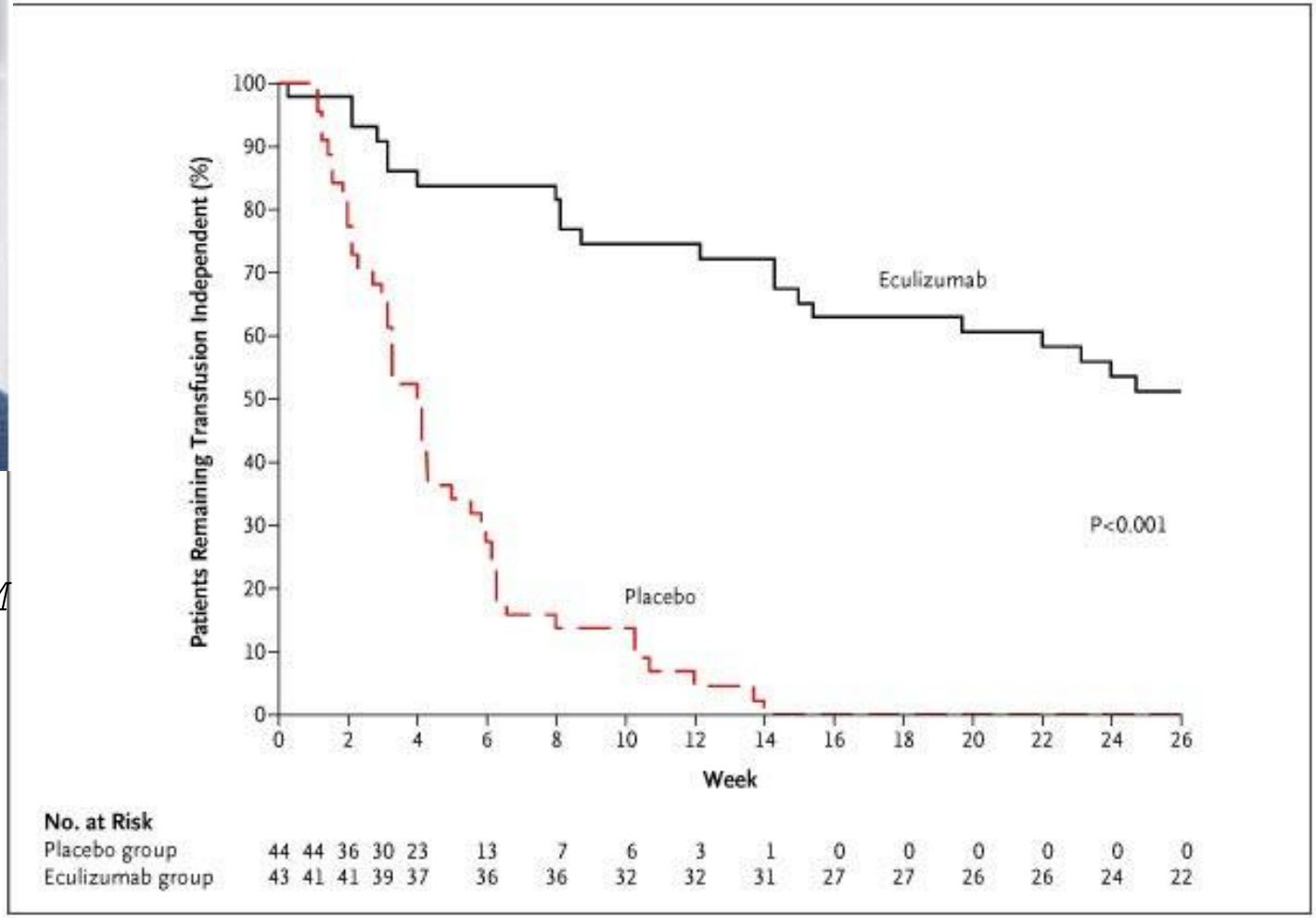
(From Gargiulo et al., *Blood* **129**:388,2017)

Micro-environment related selective advantage model of PNH

OBJECTIONS	RESPONSE
CD1d-restricted GPI-specific T cells are not *always* present in AA	**AA is heterogeneous**
In some cases of AA the target of selection seems to be MHC-restricted rather than CD1d-restricted	**AA is heterogeneous**
The number of CD1d-restricted GPI-specific T cells does not correlate with size of PNH clone	**Time-dependence of selective process**
CD8+ CD1d-restricted GPI-specific T cells may be only a fraction of selective T cells	**There may be also CD8- selective T cells**
CD1d+ GPI+ vulnerable cells are not limited to bone marrow	Selective T cells may be mainly in bone marrow

Eculizumab can abrogate the need for blood transfusion

Hillmen et al., *NEJM* **355:**1233,2006)

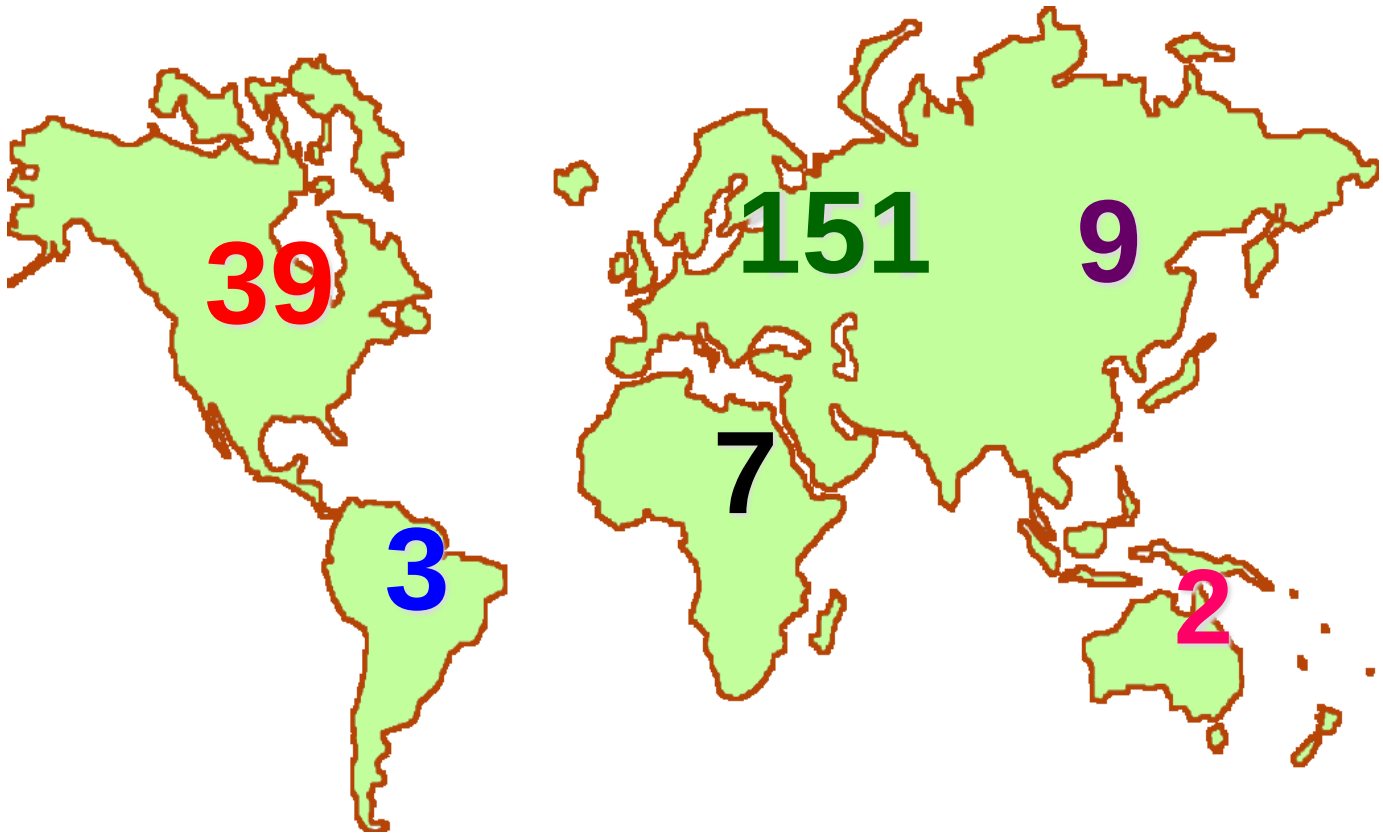


The NEW ENGLAND JOURNAL of MEDICINE

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA:
EPIDEMIOLOGY

Estimated prevalence:

between **1:100,000 and 1:1,000,000**



Age: **any**
Gender:
M/F ratio about 1

LL, 2010

PNH is rare, but seen everywhere

LIMITED INFORMATION ON EPIDEMIOLOGY OF PNH IN AFRICA

Country	*Published cases*	*Other cases*	*Est. cases in country*	*References*
Algeria	4			Afredj et al., 2015
Egypt	4			Ritz et al., 2002
Nigeria	2	4	>1000	Oni et al., 1970
South Africa	2			Manuel et al., 1969
Tanzania	4	2		Ally et al, 2019
Uganda	1			Lumora & Muyanja, 2019

The frequency of Aplastic Anaemia may be significantly higher in Africa compared to Europe

Population		Incidence cases/million/year	Confidence limits
Europe/ North America		≈2	
Thailand		≈4	
Tanzania	Observed	3.8	2.3-5.9
	"corrected'	5.9	4.0-8.3

(From Ally, Magesa & Luzzatto, *Am J Hematol* **94:**E86,2019)

LU

You

Is eculizumab available in Africa?

ChatGPT

As of my last knowledge update in January 2022, eculizumab, marketed under the brand name Soliris, may not be widely available in many parts of Africa due to factors such as regulatory approvals, he[illegible]care infrastructure, and access to specialized treatments.

PNH in India

Cureus. 2022 Aug; 14(8): e28448.
Published online 2022 Aug 26. doi: 10.7759/cureus.28448
PMCID: PMC9417682
PMID: 36046061

Classical Paroxysmal Nocturnal Hemoglobinuria Presenting With Severe Anemia and Pigmented Acute Kidney Injury

Monitoring Editor: Alexander Muacevic and John R Adler

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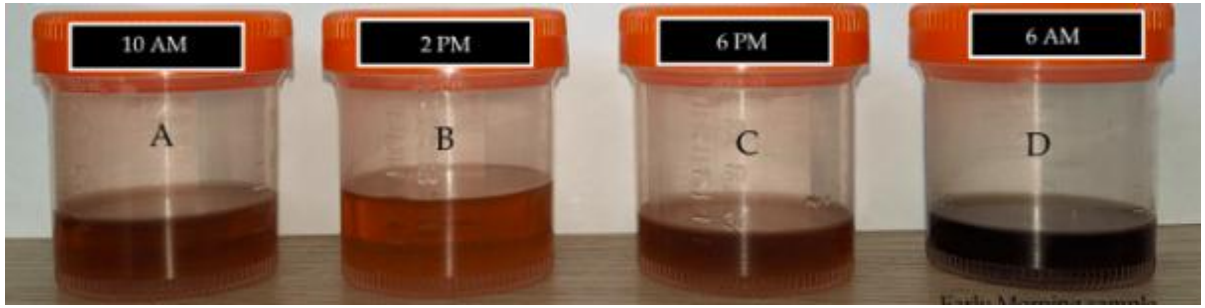

"Eculizumab is a monoclonal C5 complement inhibitor, which is used in the definite treatment of PNH, but due to its relatively recent FDA approval in 2007, its availability is limited and its cost prevents its use in South East Asian countries".

LIMITING FACTORS (*BARRIERS*) IMPEDING TREATMENT OF PNH WITH COMPLEMENT INHIBITORS

- Diagnostic facilities/diagnosis
- Drug supply and the cold chain
- Regular hospital attendance
- Monitoring therapeutic outcomes
- Confronting side effects
- Affordability

LIMITING FACTORS FOR TREATMENT OF PNH WITH COMPLEMENT INHIBITORS – IN AFRICA

- Diagnostic facilities/diagnosis
- Drug supply and the cold chain
- Regular hospital attendance
- Monitoring therapeutic outcomes
- Confronting side effects

- **Affordability**

On the long run, I think governments of indiviual countries must be responsible for the health of their citizens.

In the meantime, in my humble view contingency measures are necessary

PROPOSALS AIMING TO REDUCE THE GAP BETWEEN POTENTIAL OF TREATMENTS AND REALITY OF IMPLEMENTATION

- **Adding SCD** to the triad of conditions (HIV, tuberculosis, malaria) for which cost of treatment is born by the Global Fund.
- **BMT solidarity programme**:
  for every BMT (HSCT) procedure in Europe/US,
  0.1% of the expense could be deposited into a fund
  to support BMT in accredited centers in Africa.
- **Rare Disease treatment matching programme**:
  for every patient treated with a super-expensive drug (e.g. eculizumab) reimbursed by NHS/insurance, the manufacturer offers the drug to one patient with the same disease in Africa.

(Modified from Makani et al., *Am J Hematol* 97, **1505, 2022)**

PATHOGENESIS AND PATHOPHYSIOLOGY OF PNH: FEATURES WORTHY OF NOTE

- Complement is responsible for intravascular hemolysis, despite complement cascade intact
- Deficiency of several membrane proteins, but genes encoding those proteins intact
- Mutation in a gene of GPI biosynthesis, therefore potentially pleiotropic
- Somatic mutation will be pathogenic only if on the active X chromosome
- Disease clonal, but not neoplastic (*clonal hematopoiesis*)
- Clonal expansion consequent on *micro-environment related selective advantage* (Darwinian selection)

THANK YOU!

Ibadan
SIJI OSUNKOYA
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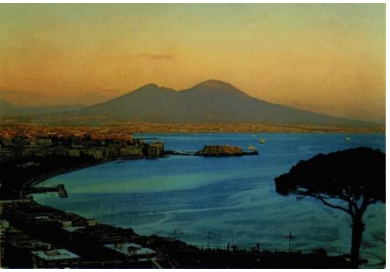
OLANIYI ONI

Dar-es-Salaam
MWASHUNGI ALLY
JULIE MAKANI
PIUS MAGESA



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Thanks to all patients with PNH
from whom I have learnt
and I continue to learn.