Paroxysmal Nocturnal Hemoglobinuria:

at the crossroads of somatic mutations, clonal expansion and immunity

Firenze, 3-4 ottobre 2024

Grand Hotel Baglioni

MaRIH
Filière de santé Maladies Rares Immuno-Hématologiques

AM
APLASIE MEDULLAIRE
centre de référence

PNH and aplastic anemia

G. SOCIE, MD, PhD

Hematology / Transplantation

Hospital St Louis, Paris

Groupe Hospitalier Universitaire
SAINT-LOUIS
LARIBOISIÈRE
FERNAND-WIDAL

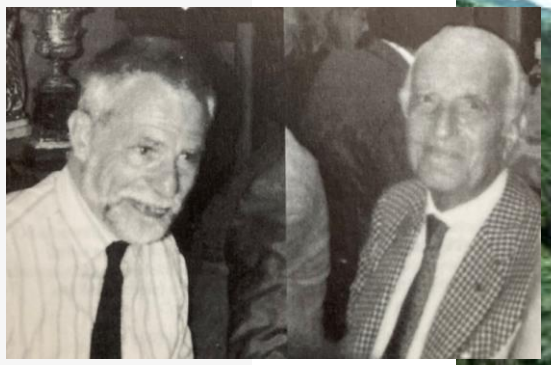
Université Paris Cité

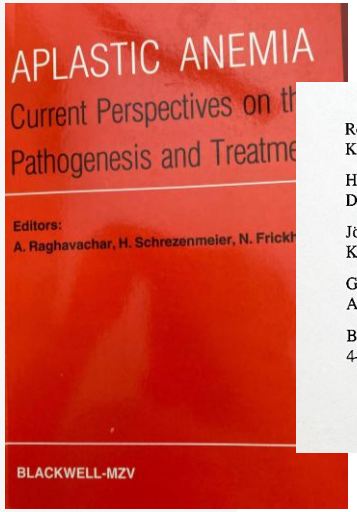
Disclosures of SOCIE GERARD

Company name	Research support	Employee	Consultant	Stockholder	Speaker bureau	Advisory board	Other

NOT ANYMORE

In memoriam





S

Reinhold E. **Schmidt,** Med. Hochschule, Abt. für Immunologie und Transfusionsmedizin, Konstanty-Gutschow-Str. 8, D-30625 Hannover, Germany

Hubert **Schrezenmeier,** University of Ulm, Dept. of Medicine III, Robert-Koch-Str. 8, D-89081 Ulm, Germany

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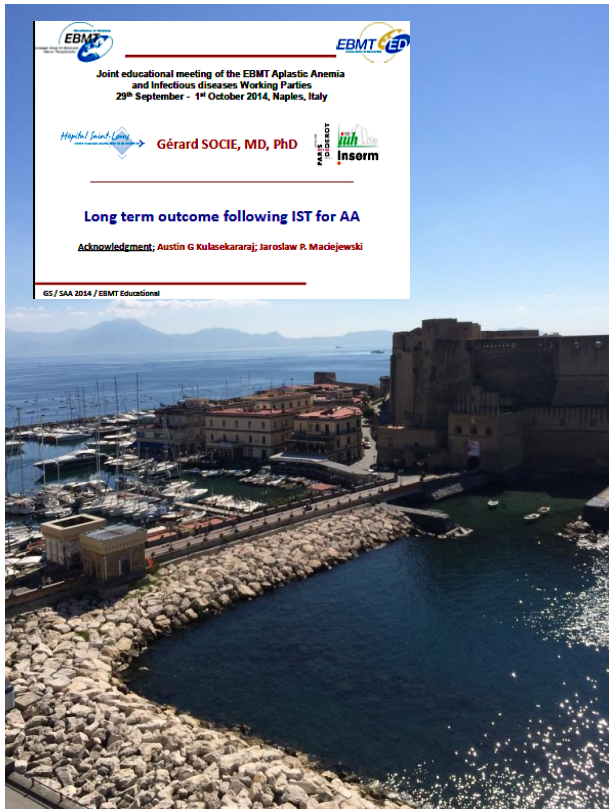
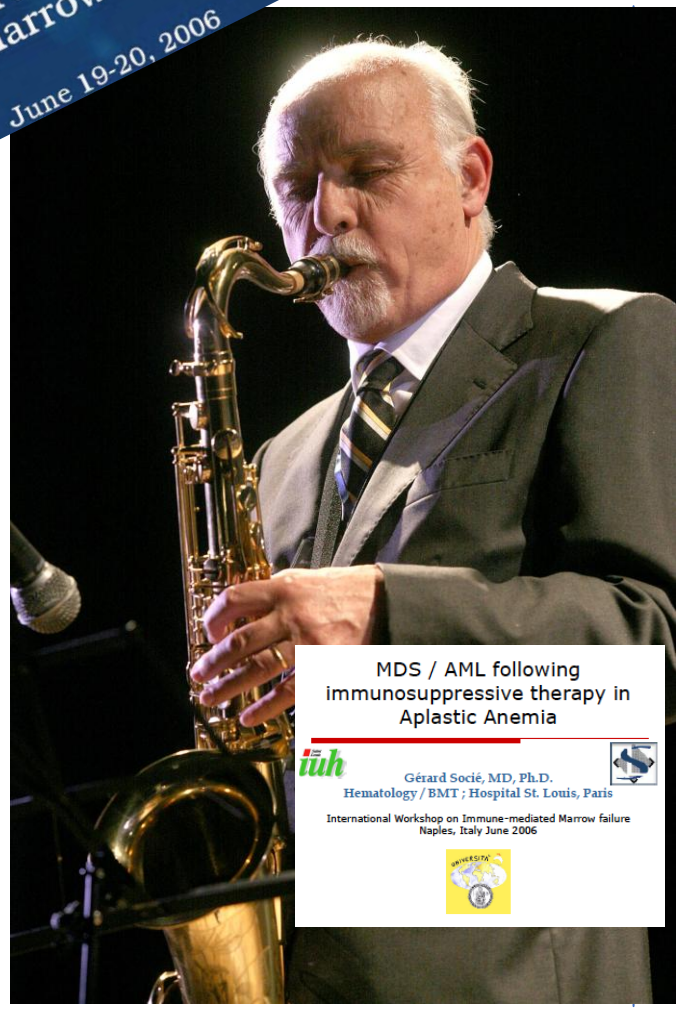
XI

Villa Vigoni

EBMT European Society for Blood and Marrow Transplantation
Napoli 2014



International Workshop on Immune-Mediated Marrow Failure
June 19-20, 2006



AA – PNH 2024

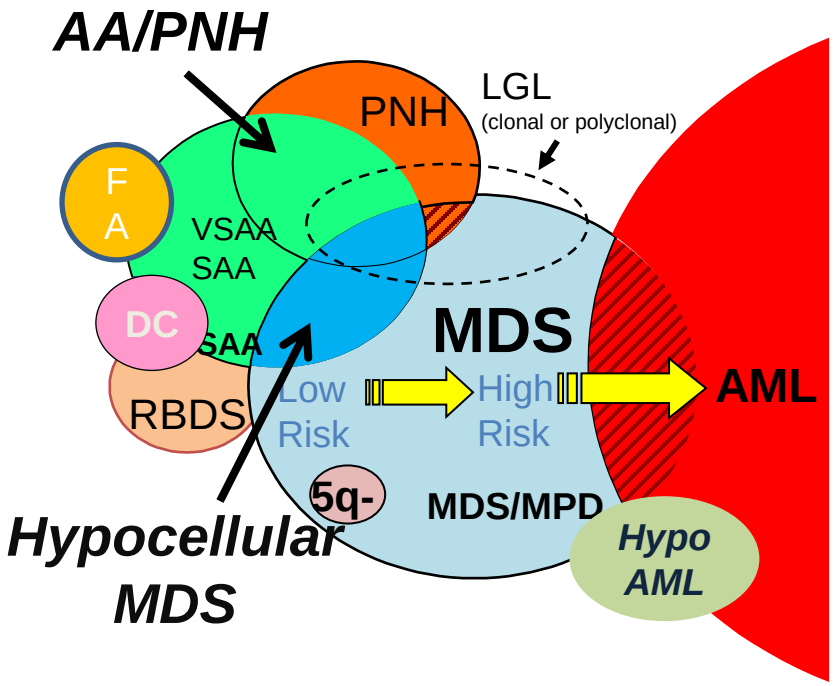
- ✓ What we know
- ✓ What we should know
- ✓ What YOU can do!

From the Bedside

To the Bench

AA – PNH 2024

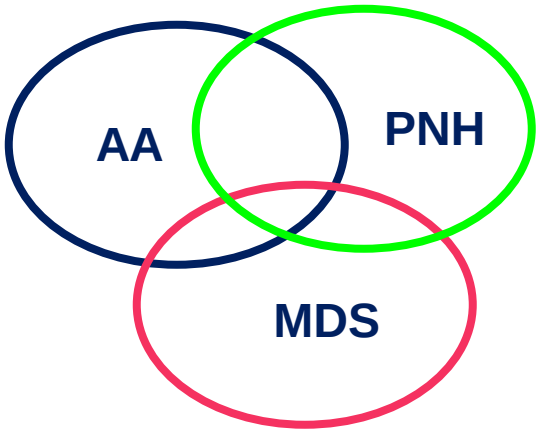
✓ What we know



Maciejewski et al. Blood 2002; 19(9):3129-35.

What I used to teach …

- ✓ **1/3 of patient with AA have GPI- cells at diagnosis**
- ✓ ***PIG-A* mutations, in AA with GPI- cells = *de novo* PNH**



What are numbers in 2024 !

AA – PNH 2024

PNH ⟹ **AA**

✓ What we know

✓ What we should know

Long-term outcome Anti C5; N = 509

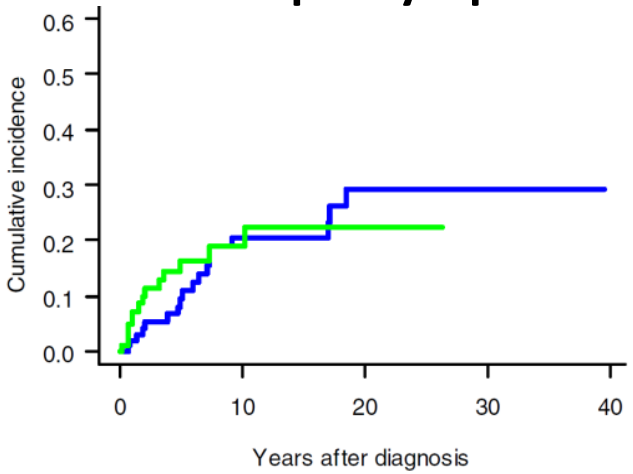
38 Transplanted for SAA
58 Treated for AA

Socié et al. *Lancet* 1996;348:573–77.

Complication	n	Median (range) time from diagnosis to complication (years)	Mean (SE) 8-year incidence rate (%)
Pancytopenia*	23	2·0 (0·3–18)	15 (3)

Peffault de Latour et al. *Blood* 2008;112:3099–106.

Bi- or pancytopenia



Kelly et al. *Blood* 2024;143:1157–66.

Cumulative incidence?

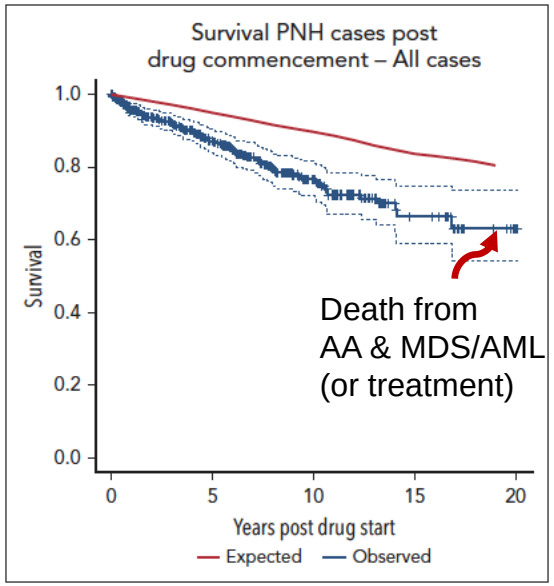


Figure 1. OS of all 509 patients compared with that of age- and sex-matched controls.

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PNH ⇒ AA

✓ What we should know

AJH 2016: 91; 366-70

RESEARCH ARTICLE

AJH

Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: a treatment versus no-treatment study

Michael Loschi,[1,2] Raphael Porcher,[3] Fiorenza Barraco,[4] Louis Terriou,[5] Mohamad Mohty,[6] Sophie de Guibert,[7] Beatrice Mahe,[8] Richard Lemal,[9] Pierre-Yves Dumas,[10] Gabriel Etienne,[10] Fabrice Jardin,[2] Bruno Royer,[11] Dominique Bordessoule,[12] Pierre Simon Rohrlich,[13] Luc Mathieu Fornecker,[14] Celia Salanoubat,[15] Sebastien Maury,[16] Jean-Yves Cahn,[17] Laure Vincent,[18] Thomas Sene,[19] Sophie Rigaudeau,[20] Stephanie Nguyen,[21] Anne-Claire Lepretre,[22] Jean-Yves Mary,[23,24] Bernadette Corront,[25] Gerard Socie,[1,24*] and Regis Peffault de Latour[1,24*]

Eculizumab vs. Control (no C' blockade)

TABLE II. Causes of Death

Cause of death	Historical controls (N = 44)	Eculizumab (N = 9)
Thrombosis	13	2
Infection	6	0
Bleeding	4	1
Aplastic anemia	3	0
Myelofibrosis	1	0
MDS	0	1
HSCT-related	0	2
Unknown	18	3

Cumulative incidence of aplastic anemia

in the treated cohort of patients was lower (**1%** [<1 to 5]) than in the historical control cohort diagnosed after 1985 (**10%** [4 to 8]) or before 1985 (**10%** [4 to 8]).

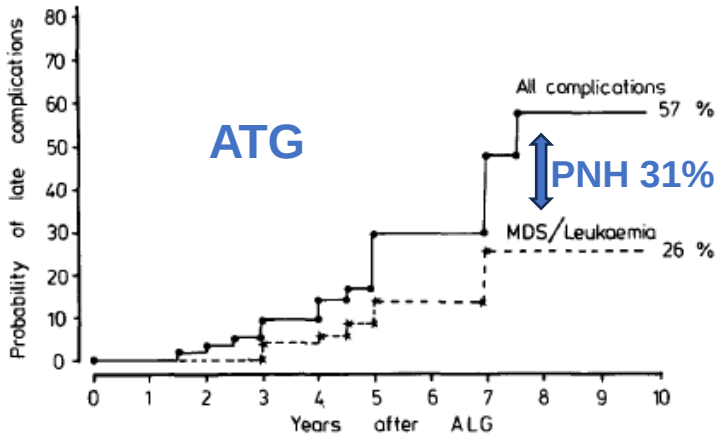
But number in patients treated with Ecu long-term follow-up was short …

AA – PNH 2024

✓ What we know

AA ⇒ PNH

Tichelli A. Br J Haematol. 1988: 69(3); 413-8



Before Flow cytometry

ATG + CSA (long-term)

PNH was diagnosed 4.3 to 9.4 years after the diagnosis of aplastic anemia (actuarial probability, **10% at 11 years**)

Frickhofen N. BLOOD. 2003: 101(4):1236-42

ATG + CSA + Eltrombopag (long-term)

??

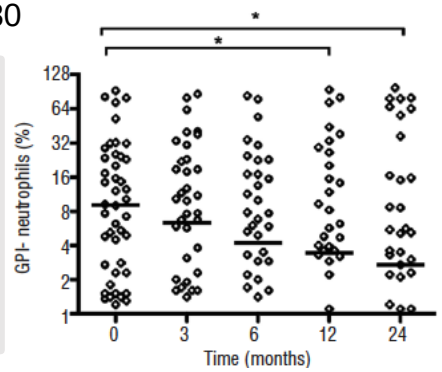
Patel BA. BLOOD. 2022: 139(1):34-43

GPI-: at diagnosis

ATG + CSA

Scheinberg P et al.
Haematologica 2010;95:1075-1080

- GPI- clone was detected in 83 (**40%**) patients pre-treatment,
- Median **clone size was ~10 %**
- In patients without a detectable clone pre-treatment, the appearance of a clone after IST was infrequent



ATG + CSA +/- Eltrombopag

Peffault deLatour R et al.
N Engl J Med 2022; 386:11- 23

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Group A: Horse ATG–Cyclosporine (N=101)	Group B: Horse ATG–Cyclosporine–Eltrombopag (N=96)	All Patients (N=197)
GPI-deficient neutrophils ≥1.0% — no./total no. (%)	44/100 (44)	33/93 (36)	77/193 (40)

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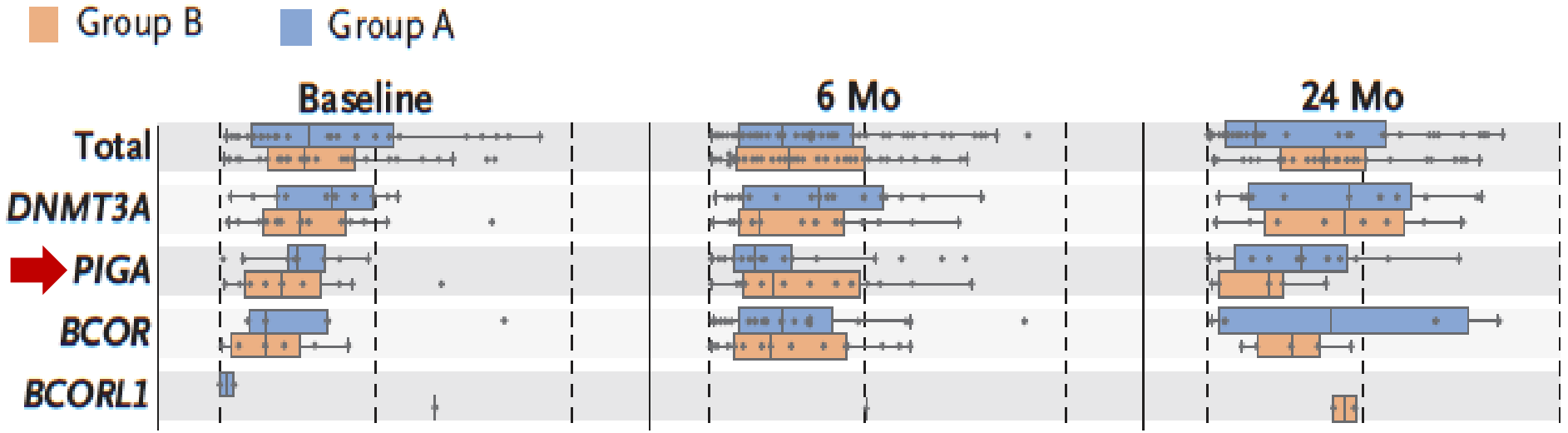
✓ What we know

AA ⇒ PNH

GPI mutation (VAF): first 24 Months

ATG + CSA +/- Eltrombopag



Peffault deLatour R et al.
N Engl J Med 2022; 386:11- 23

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✓ What we should know

AA ⇒ PNH

Eltrombopag Added to Standard IST is Superior to IST Alone as Front-line Treatment for Severe Aplastic Anemia: Final 2-year Analysis of EBMT-SAAWP RACE Study

EBMT European Society for Blood and Marrow Transplantation

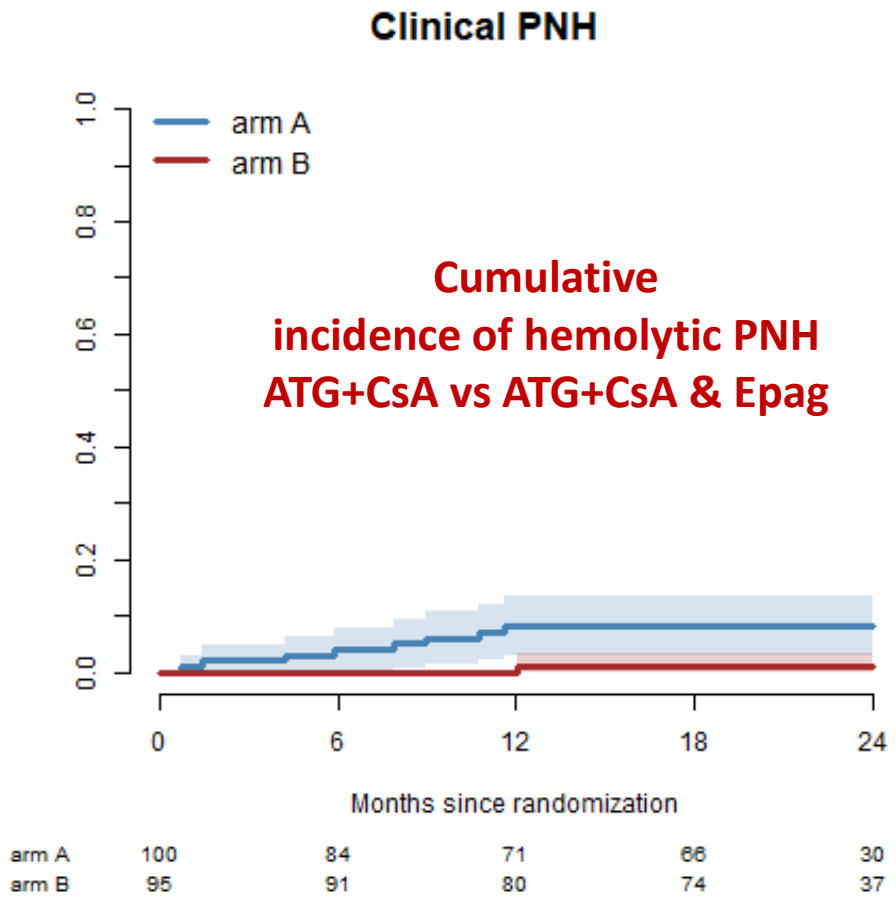
2y clinical PNH:

- ✓ Arm A (ATG + CSA) **8.1%** (95% CI, 2.7% to 13.5%)
- ✓ Arm B (ATG + CSA + Eltrombopag **1.1%** (95% CI, 0% to 3.2%)

Multivariable analysis

	HR	P-value
arm	0.12	0.000
age40	0.70	0.518
vSAA	1.14	0.265

Unpublished EBMT data; ASH 2024



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✓ What we know

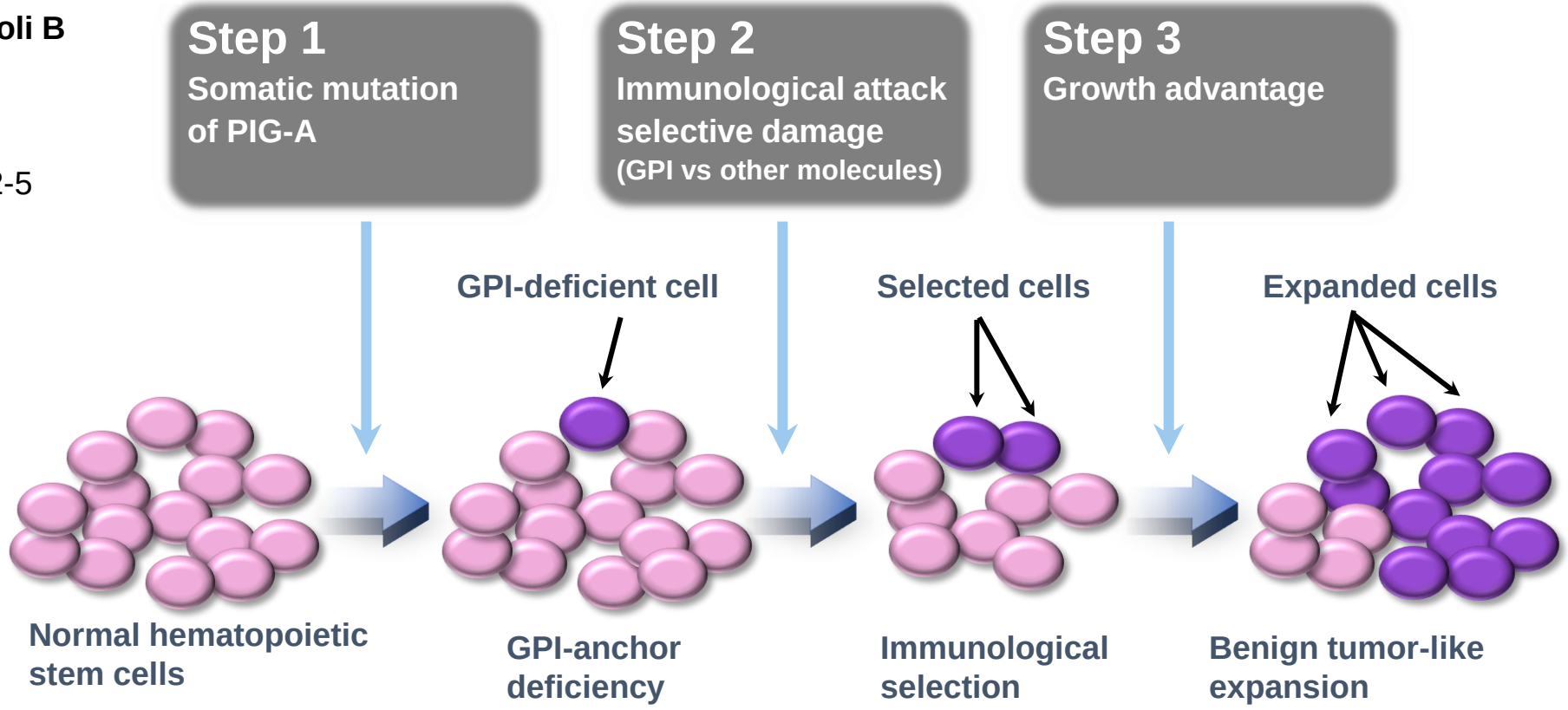
Somatic Mutations in Paroxysmal Nocturnal Hemoglobinuria: A Blessing in Disguise?

Luzzatto L, Bessler M, & Rotoli B
Cell 1997: 88; 1-4

Luzzatto L
Br J Haematol 2020: 189; 802-5

From the Bedside

To the Bench



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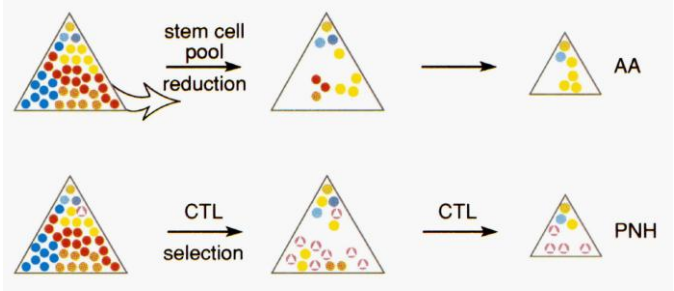
✓ What we know

EDITORIAL

***Riddle:* What Do Aplastic Anemia, Paroxysmal Nocturnal Hemoglobinuria (PNH) and "Hypoplastic" Leukemia Have in Common?**

By William Dameshek

Blood, **1967**: 30; 251- 254



BLOOD

The Journal of The American Society of Hematology

VOL 79, NO 6 MARCH 15, 1992

PERSPECTIVE

The Problem of Clonality in Aplastic Anemia: Dr Dameshek's Riddle, Restated

By Neal S. Young

Perspective

Blood, **2017**: 30; 2363- 72

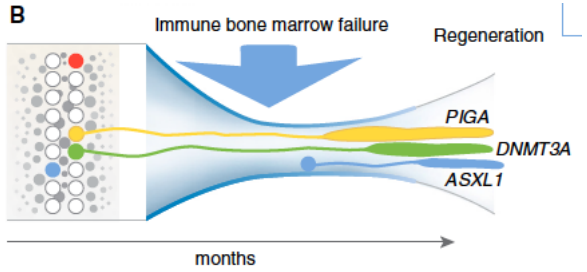
Check fo

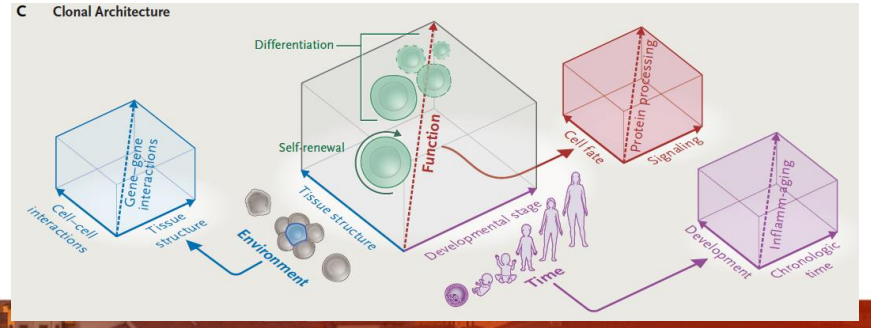
blood

Clonality in context: hematopoietic clones in their marrow environment

James N. Cooper and Neal S. Young

Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD





REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Somatic Mutations in "Benign" Disease

Satu Mustjoki, M.D., and Neal S. Young, M.D.

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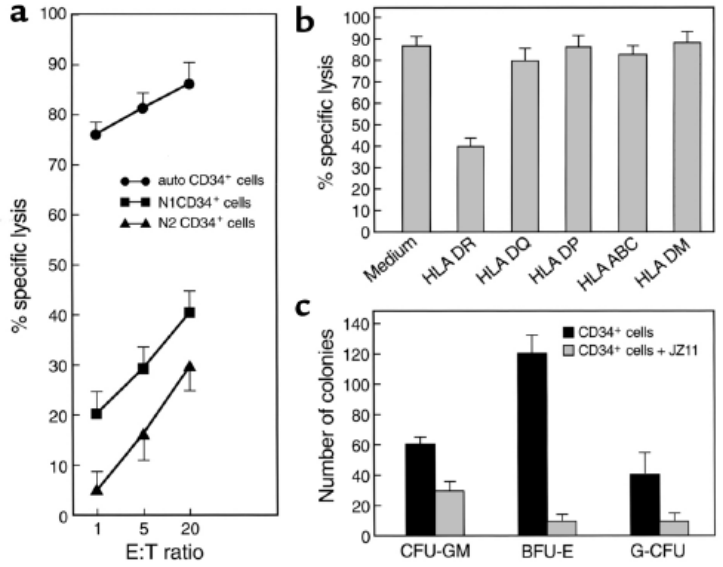
✓ What we know

Limited heterogeneity of T cell receptor BV usage in aplastic anemia

Zeng W, Maciejewski JP, Chen G, & Young NS

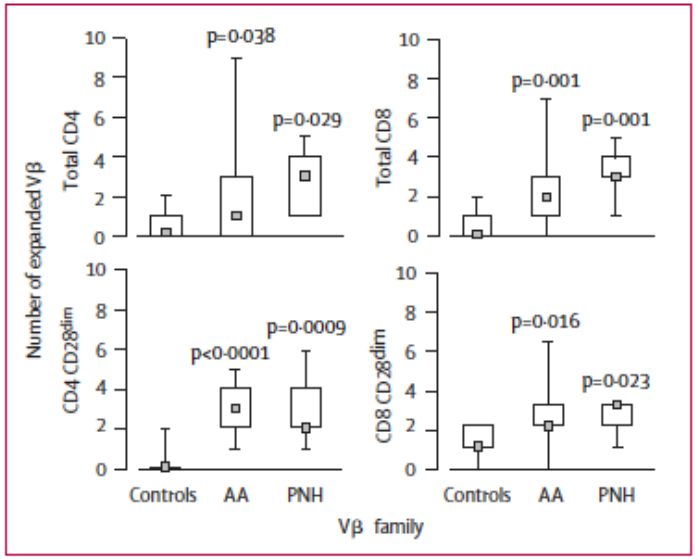
JCI 2001: 108; 765-73

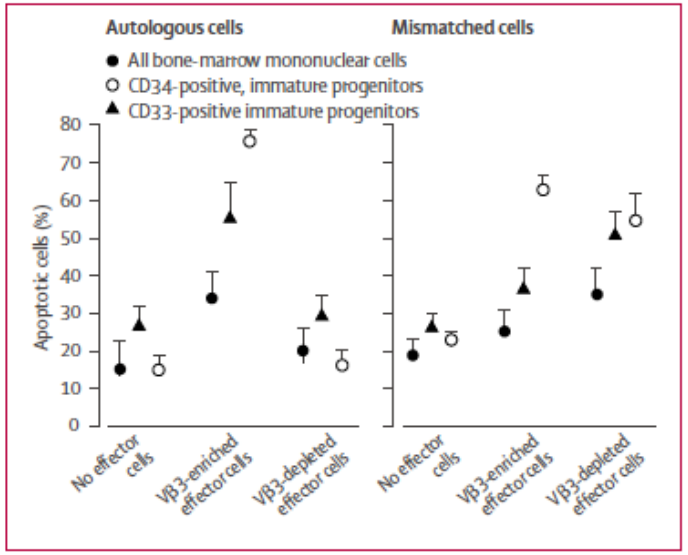


In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR β-CDR3 sequencing

Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, & Young NS

Lancet 2004; 364: 355–64





AA

Autologous T-cell mediated

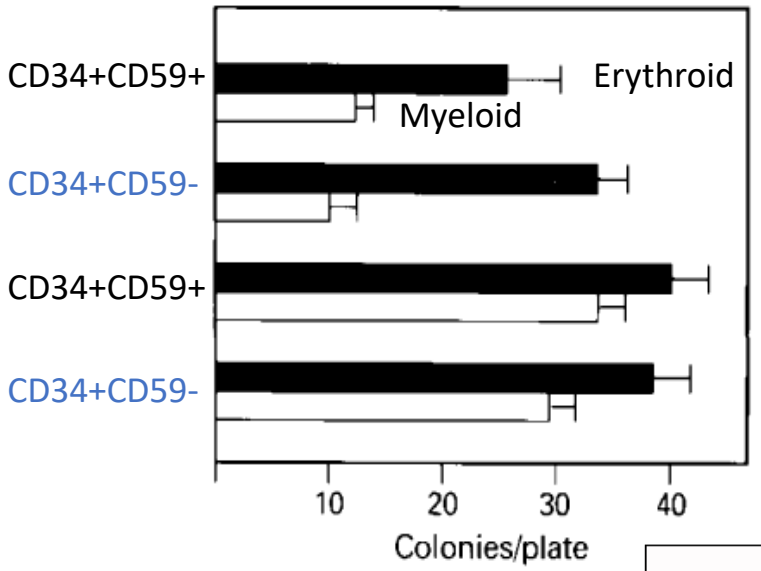
See Neal's & Lucio's Lecture

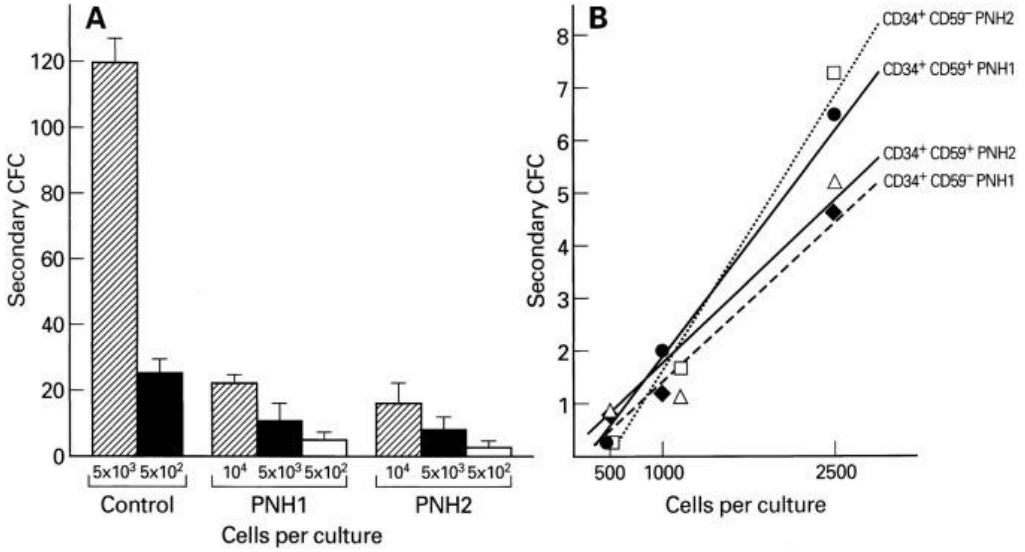
My own landmark papers

AA – PNH 2024

PNH
Functional analyses

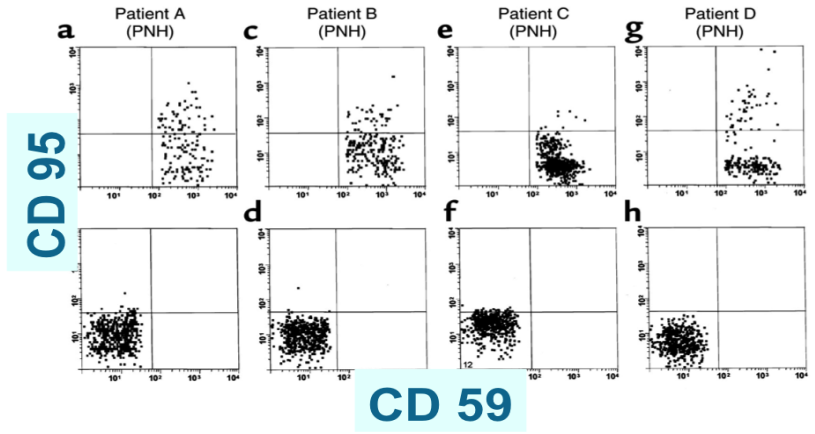
✓ What we know





Maciejewski JP et al.
BLOOD 1997; 89: 1173-81

CD34+
GPI +
GPI -



"Impaired growth and elevated Fas receptor expression in PIGA+ stem cell; in primary PNH"

J Clin Invest. 2000;106(5):689-696

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AA
Myeloid mutations

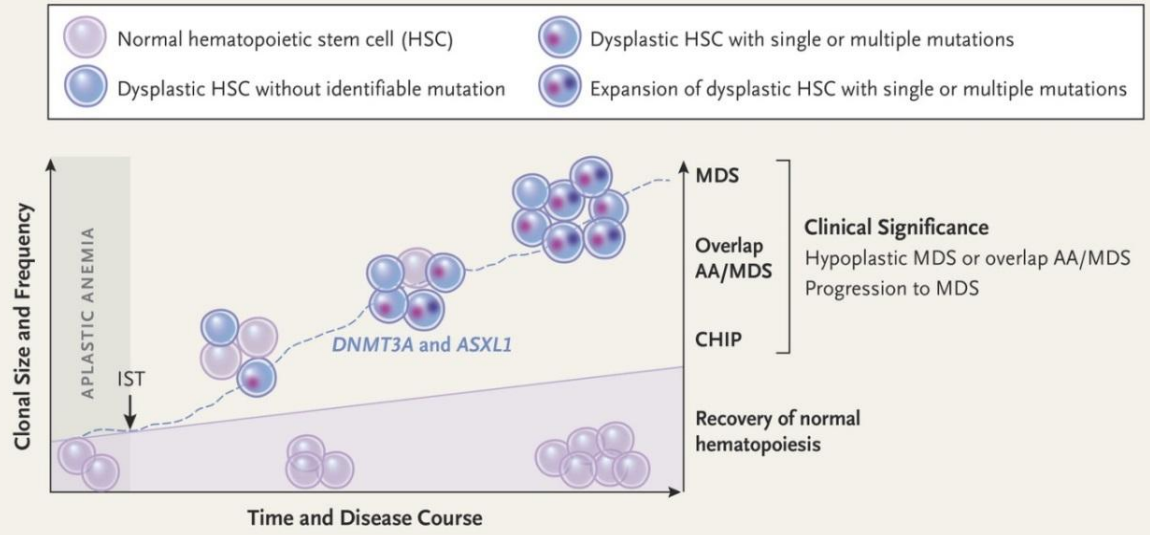
blood
124:2698-2704; 2014

The NEW ENGLAND JOURNAL of MEDICINE
373:1673-1676; 2015

KING'S College LONDON

NIH National Heart, Lung, and Blood Institute

A Aplastic Anemia and Its Evolution with and without Immunosuppressive Therapy

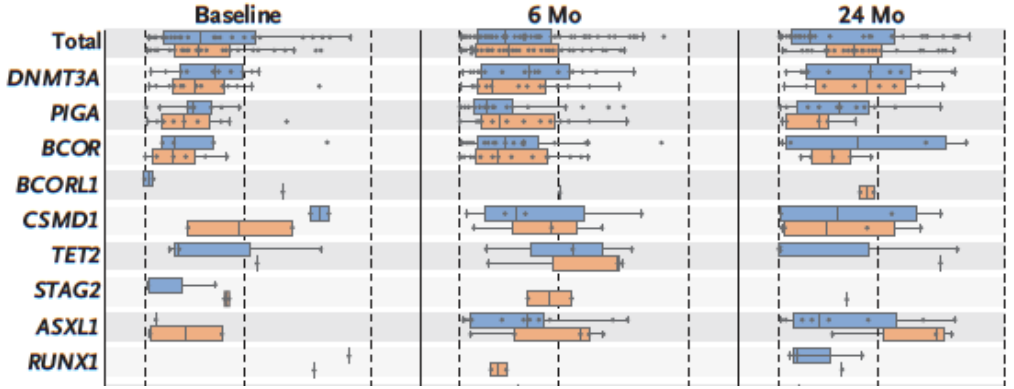


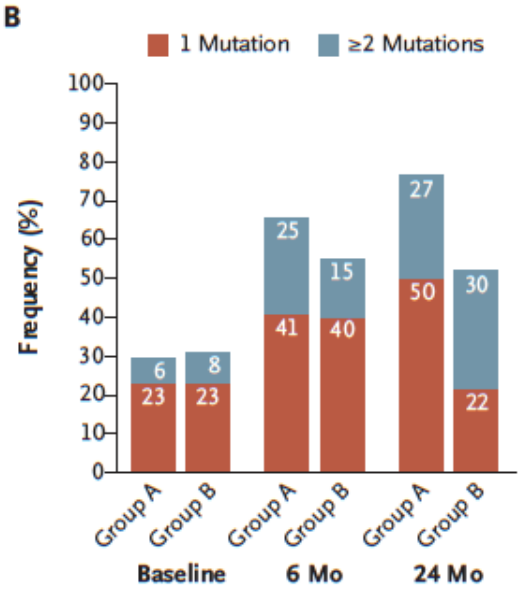
B Frequency of Four Commonly Mutated Genes in Aplastic Anemia in the Two Studies

Top 4 Genes	Kulasekararaj et al.	Yoshizato et al.
DNMT3A	8.3%	8.4%
ASXL1	8.0%	6.2%
BCOR/BCORL1	4.0%	9.3%
PIGA	NA	7.5%
Median VAF	20.0%	9.3%

The NEW ENGLAND JOURNAL of MEDICINE

✓ What we know





Peffault deLatour R et al.
N Engl J Med 2022; 386:11- 23

EBMT European Society for Blood and Marrow Transplantation

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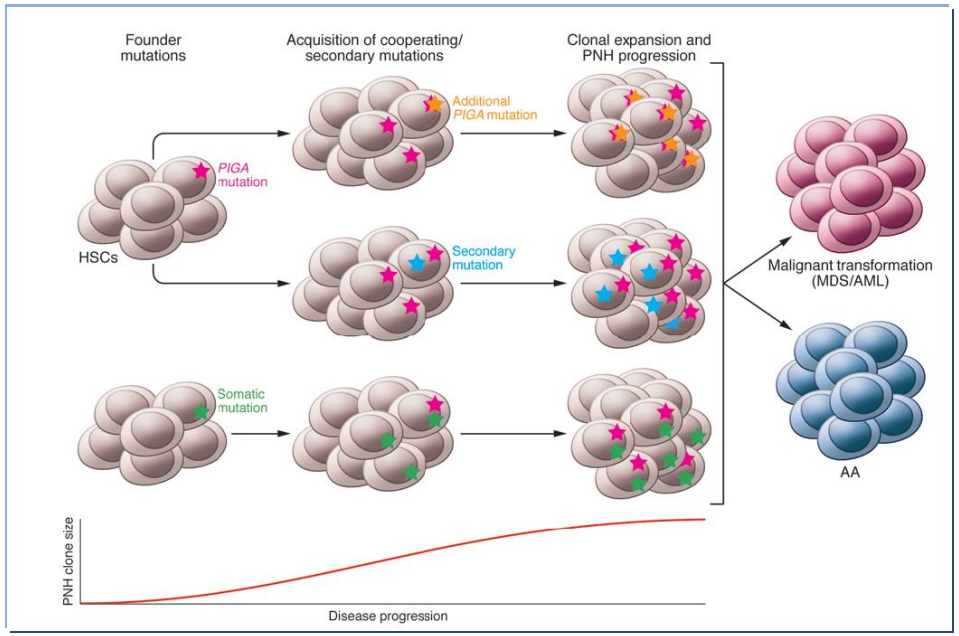
PNH
Myeloid mutations

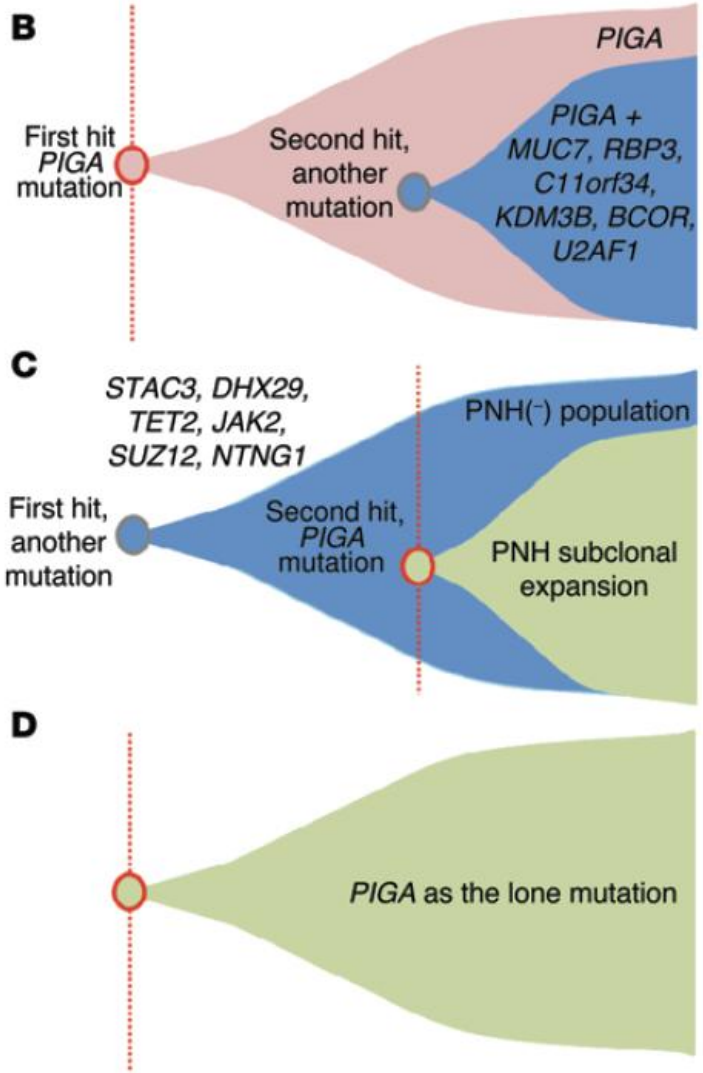
✓ What we know

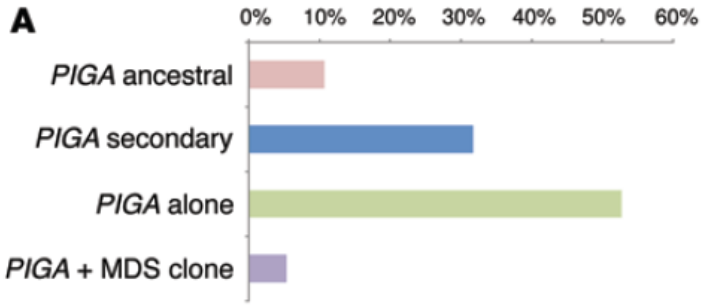
JCI The Journal of Clinical Investigation

Cleveland Clinic

124:4529-4538; 2014







AA – PNH 2024

✓ What we know

Single cell Analyses: AA

Single-cell genomics in acquired bone marrow failure syndromes

Most NON-SAA

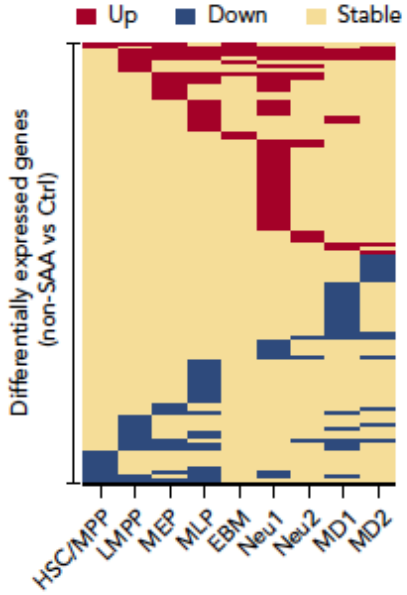
Regular Article

HEMATOPOIESIS AND STEM CELLS

Zhu C et al. BLOOD 2021; 138:23-33

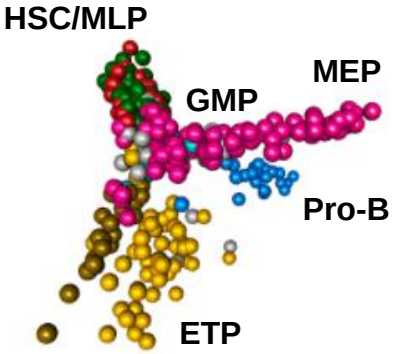
Single-cell transcriptomics dissects hematopoietic cell destruction and T-cell engagement in aplastic anemia

Wu Z & Young NS
BLOOD 2023: 142:1193- 1207



DEGs, 86.56% in 1 cell type only, indicating heterogeneous disease

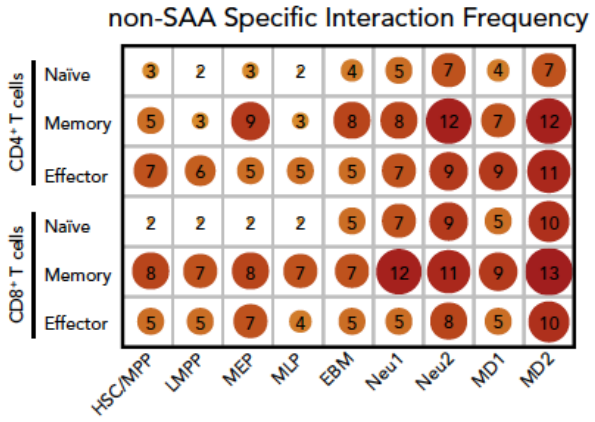
Zhao X, Gao S, et al. BMC 2020; 13:514



Normal hematopoiesis SAA ?

NIH National Heart, Lung, and Blood Institute

Only bone marrow–derived CD8 T cells enhanced expression of TGF-b and FASL: unique interactions between BM–derived T cells and HSPCs ?



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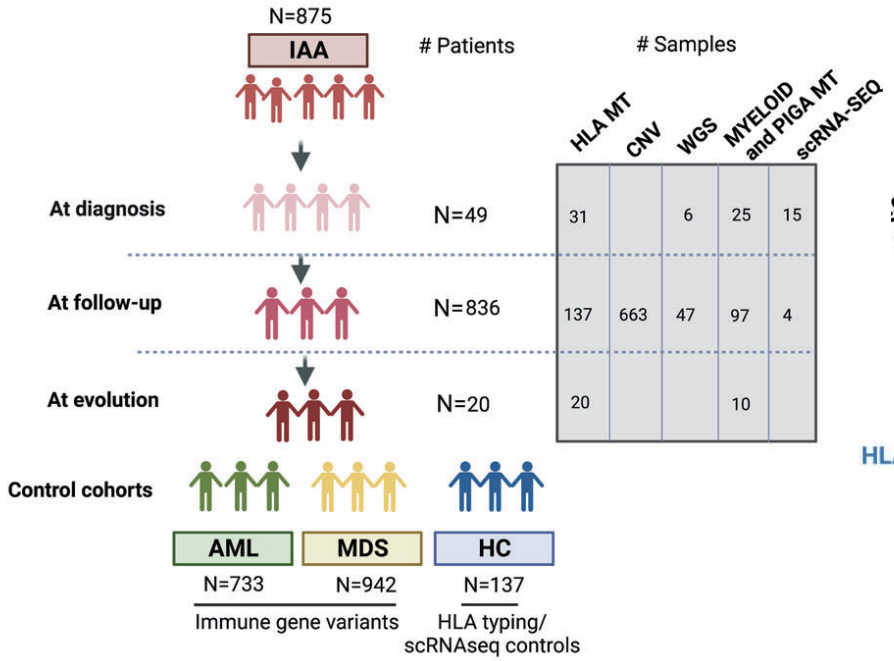
✓ What we know

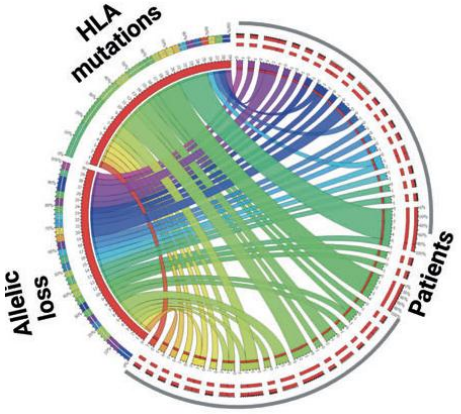
Single cell Analyses: AA / PNH HLA Loss

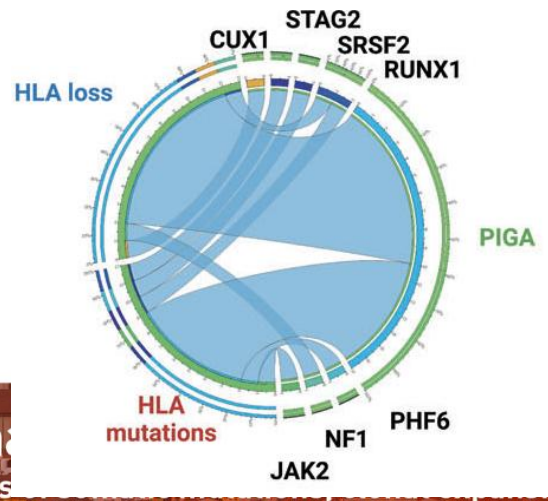
Molecular landscape of immune pressure and escape in aplastic anemia

Leukemia (2023) 37:202–211

Simona Pagliuca[1,2,11], Carmelo Gurnari[1,3,11], Colin Hercus[4], Sébastien Hergalant[5], Niroshan Nadarajah[6], Adam Wahida[6], Laila Terkawi[1], Minako Mori[1], Weiyin Zhou[7,8], Valeria Visconte[1], Stephen Spellman[9], Shahinaz M. Gadalla[7], Caiying Zhu[10], Ping Zhu[10], Torsten Haferlach[6] and Jaroslaw P. Maciejewski[1]✉







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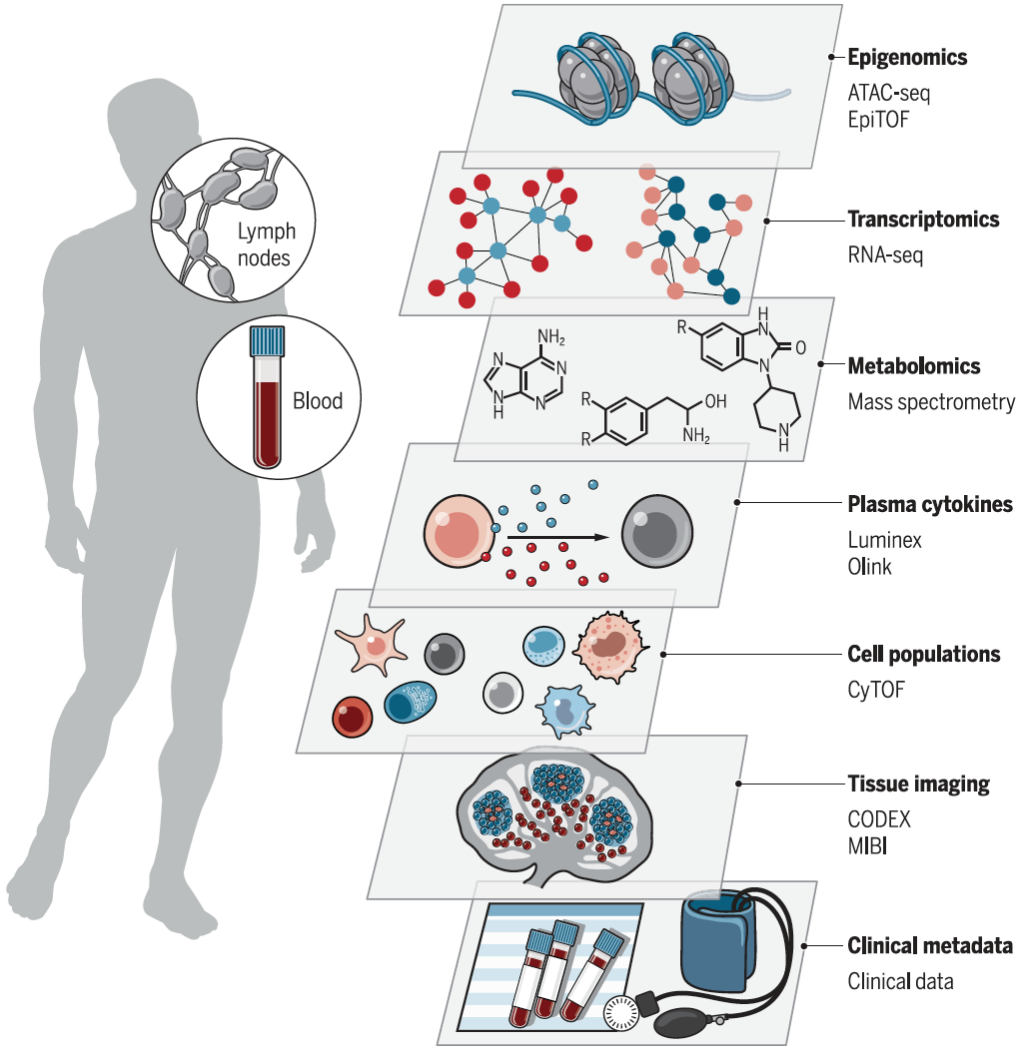

Pulendran and Davis, Science, 2020

✓ What we should know

A rare disease with few cells

- **Bloom of new (SC) technologies**
- **The advent of muti-omics in AA - PNH**

Questions (among others) now can be addressed

- AA; Disease heterogeneity
  - ✓ autoimmune vs immune mediated BMF
- PNH; Long-term (life long?) hematopoiesis sustain by 1 (few) PIG-A mutated clone (s)
- AA/PNH; Clonal (malignant?) evolution
  - ✓ Where (which cell subsets)?
  - ✓ Escape of most (mutated?) fitted clones to sustain hematopoiesis or pre-malignant state?

AA – PNH 2024

✓ What we should know

RESEARCH ARTICLE

Science AAAS

HEMATOPOIESIS

Distinct routes of lineage development reshape the human blood hierarchy across ontogeny

Faiyaz Notta,[1,2]*† Sasan Zandi,[1,2]* Naoya Takayama,[1,2] Stephanie Dobson,[1,2] Olga I. Gan,[1] Gavin Wilson,[2,4] Kerstin B. Kaufmann,[1,2] Jessica McLeod,[1] Elisa Laurenti,[6] Cyrille F. Dunant,[7] John D. McPherson,[3,4] Lincoln D. Stein,[2,4] Yigal Dror,[5] John E. Dick[1,2]‡

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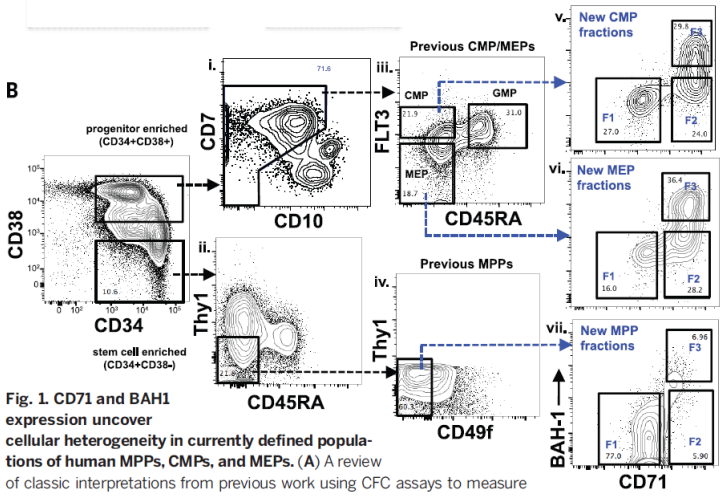
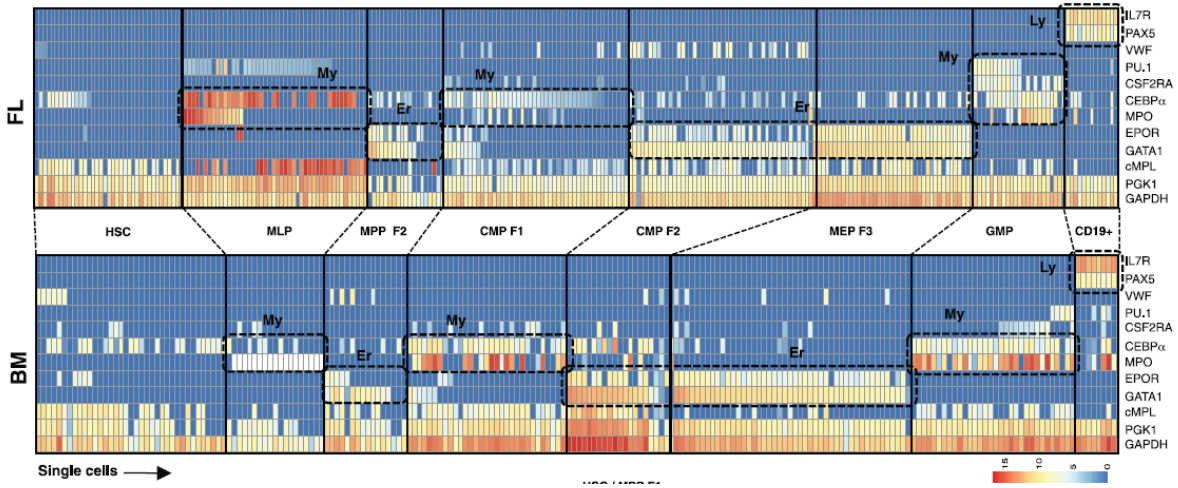
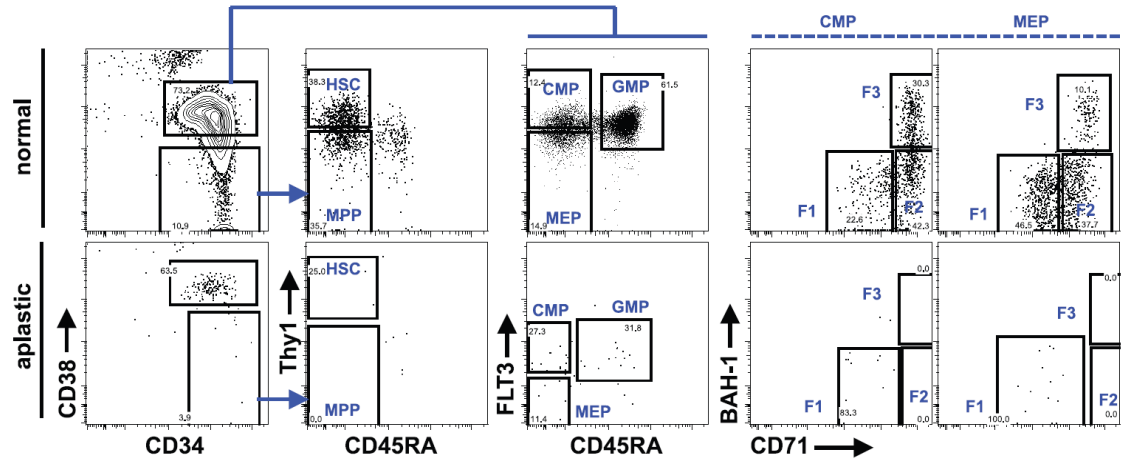
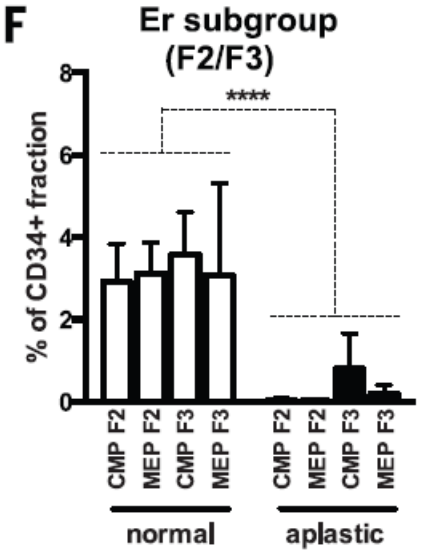


Fig. 1. CD71 and BAH1 expression uncover cellular heterogeneity in currently defined populations of human MPPs, CMPs, and MEPs. (A) A review of classic interpretations from previous work using CFC assays to measure







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✓ What we should know

RESEARCH ARTICLE

HEMATOPOIESIS

Distinct routes of lineage development reshape the human blood hierarchy across ontogeny

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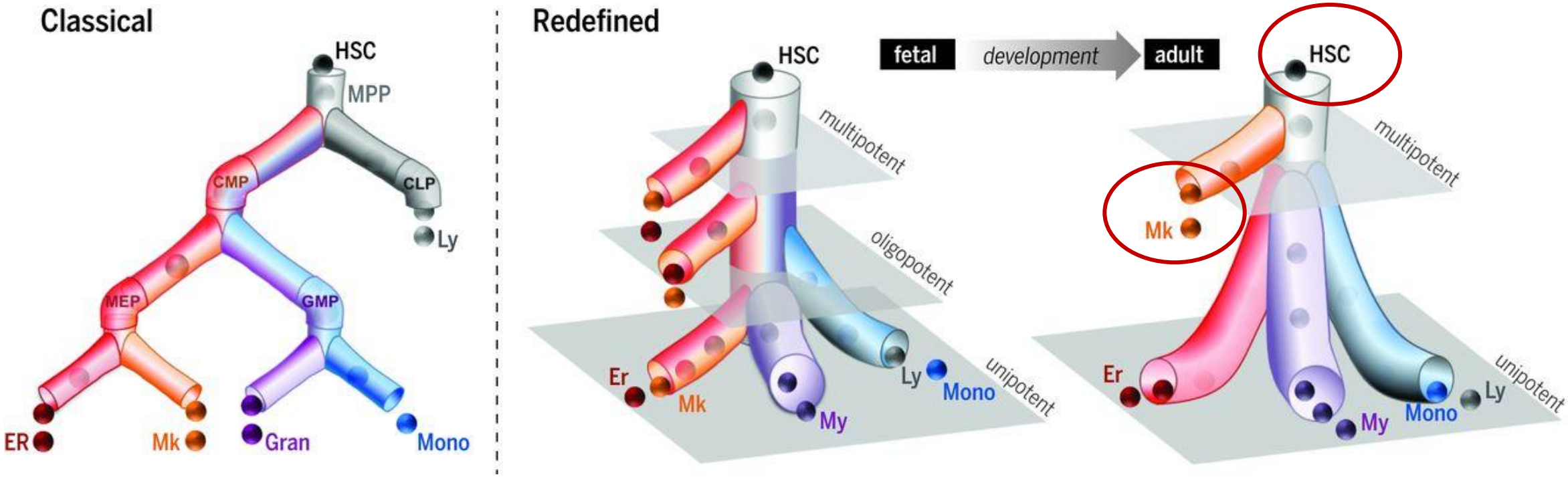

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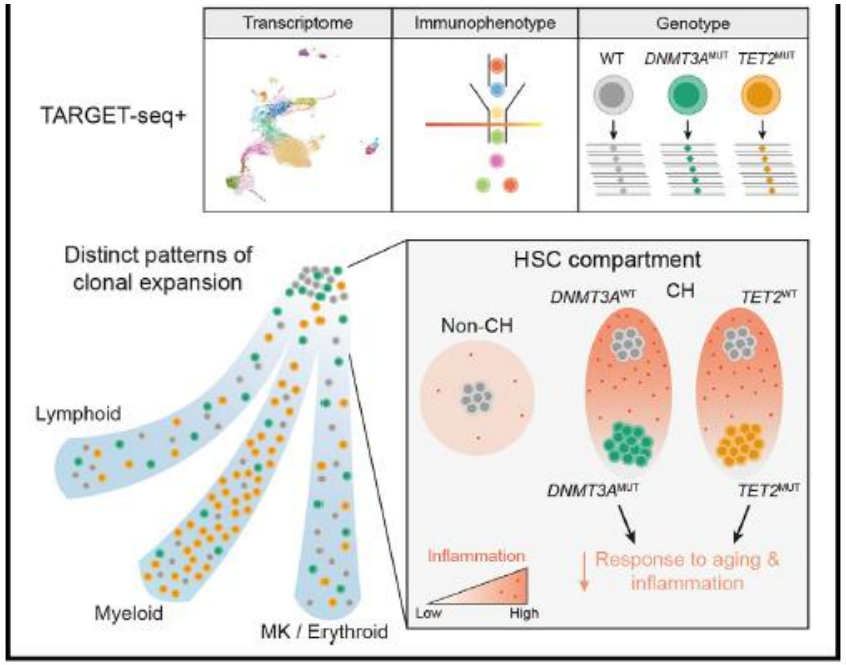
✓ What we should know

Jakobsen et al., 2024,
Cell Stem Cell 31, 1127–1144

Article

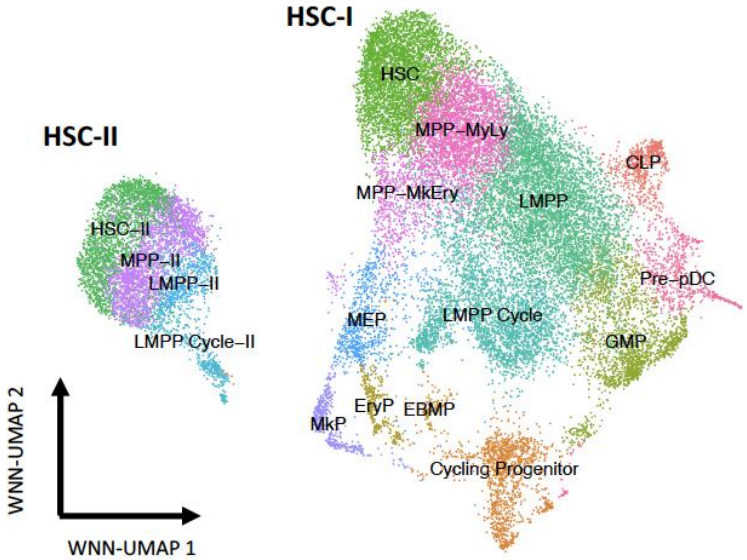
Selective advantage of mutant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging

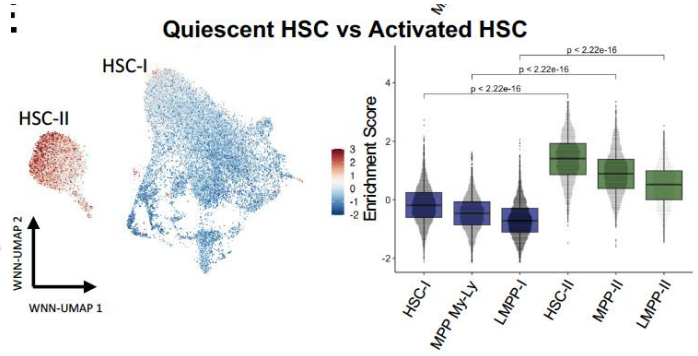


Identification of a human hematopoietic stem cell subset that retains memory of inflammatory stress

Authors: Andy G.X. Zeng[1,2]†, Murtaza S. Nagree[1]†, Niels Asger Jakobsen[3-5]†, Sayyam Shah[1], Alex Murison[1], Jin-Gyu Cheong[6-7], Sven Turkalj[3-5], Isabel N.X. Lim[1], Liqing Jin[1], Joana Araújo[1,8-11], Alicia G. Aguilar-Navarro[1,12], Darrien Parris[1], Jessica McLeod[1], Hyerin Kim[1], Ho Seok Lee[1], Lin Zhang[13-14], Mason Boulanger[1], Elvin Wagenblast[1,15], Eugenia Flores-Figueroa[1,12], Bo Wang[13,16-19], Gregory W. Schwartz[1,19], Leonard D. Shultz[20], Steven Z. Josefowicz[6-7], Paresh Vyas[3-5]‡, John E. Dick[1,2]‡, and Stephanie Z. Xie[1]‡*

bioRxiv preprint doi: https://doi.org/10.1101/2023.09.11.557271





In Revision Sept 2024

Science AAAS

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✓ What we should know

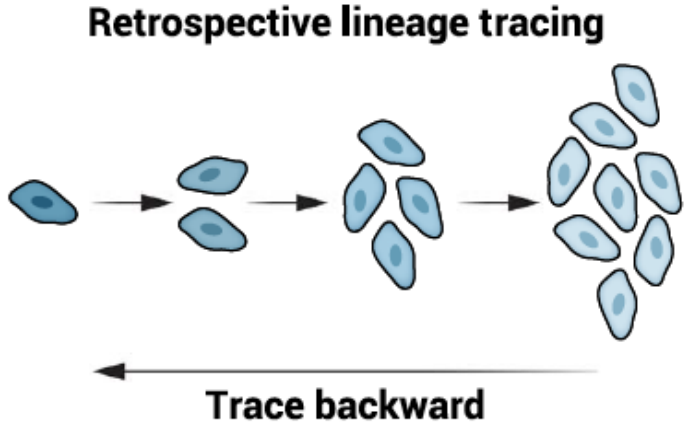
REVIEW SUMMARY

CELLULAR BARCODING

Cellular barcoding to decipher clonal dynamics in disease

Science 378, 152 (2022)

Vijay G. Sankaran*, Jonathan S. Weissman*, Leonard I. Zon*

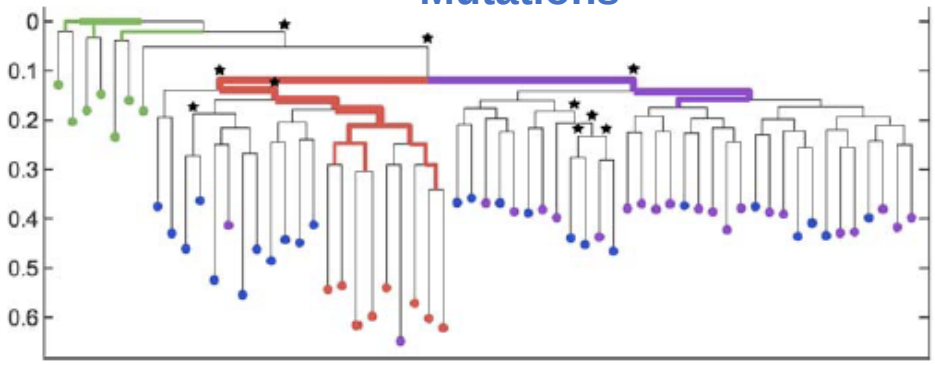


Identify sequence variation and infer ancestry

Methods

- Sequence variation = SNPs, CNVs, microsatellites

Mutations



Phylogenic tree construction

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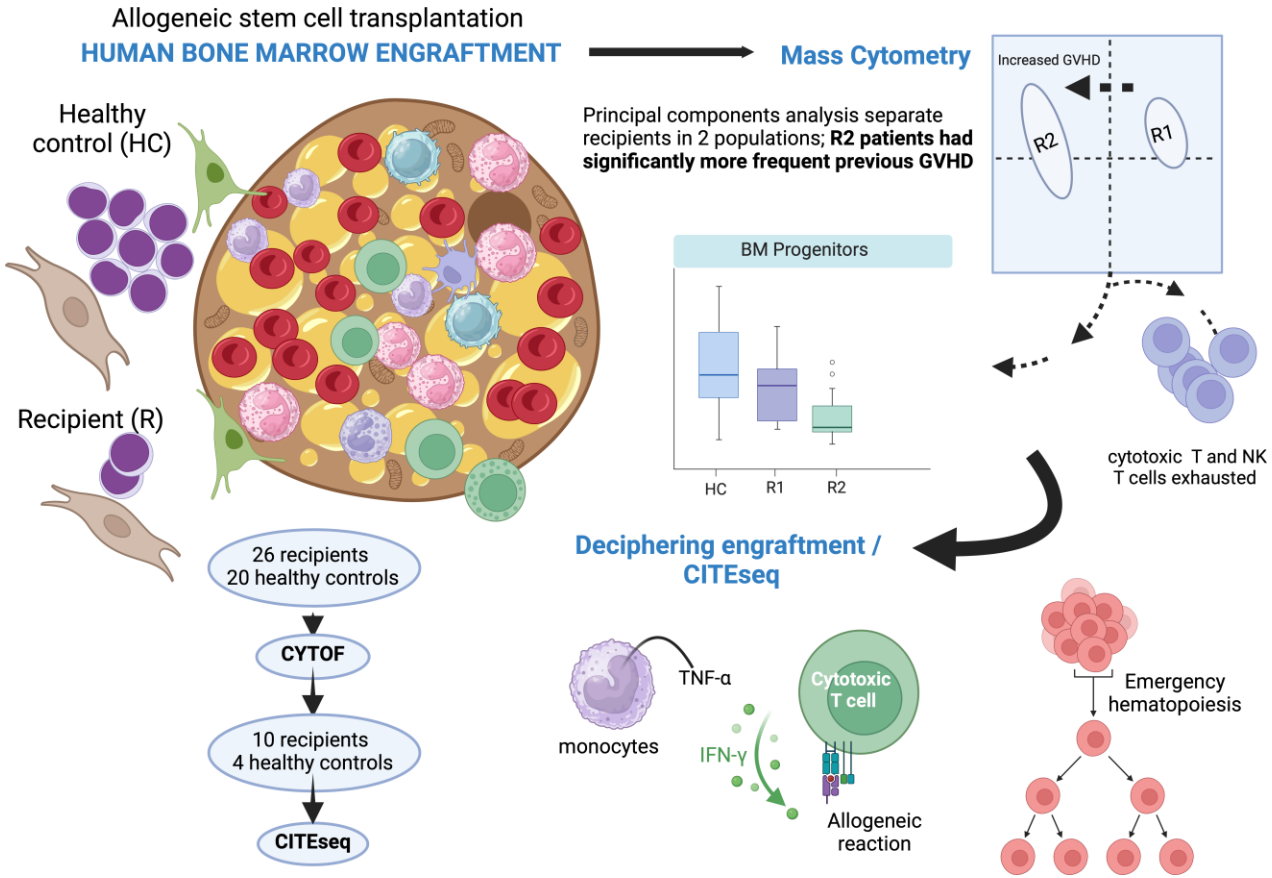
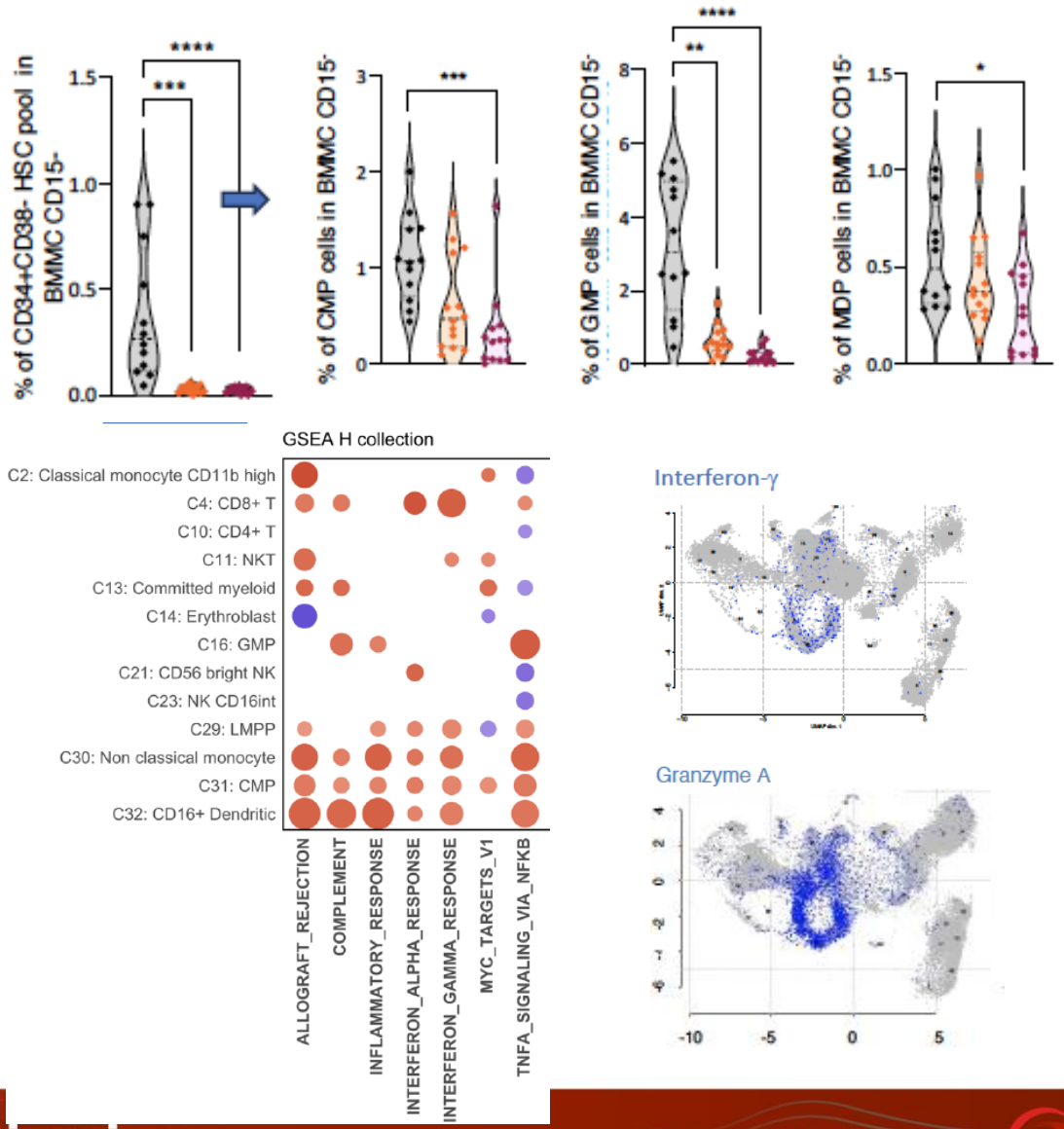
HUMAN IMMUNOLOGY PATHOPHYSIOLOGY IMMUNOTHERAPY

Inserm | Université de Paris

✓ What we should know





JCI in press preview

2024 August 29:e180331. doi: 10.1172/JCI180331

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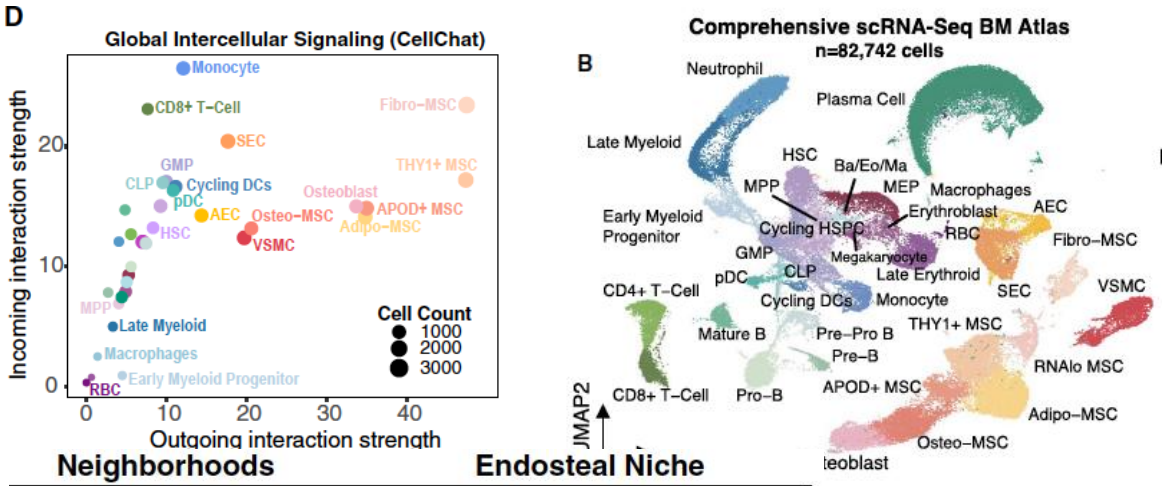
✓ What YOU can do!

A fascinating era!

Cell — Resource

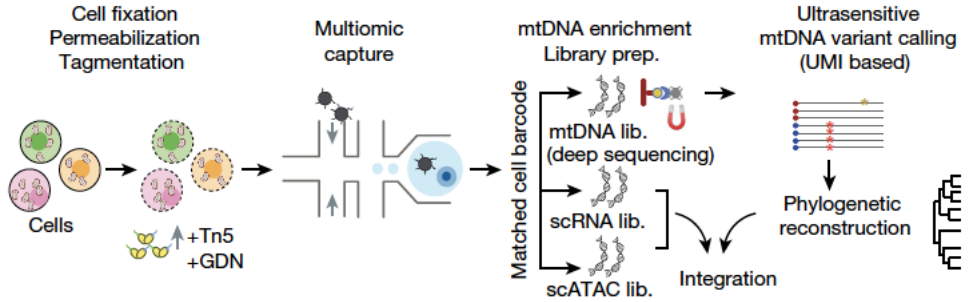
Bandyopadhyay et al., 2024, Cell 187, 1–21

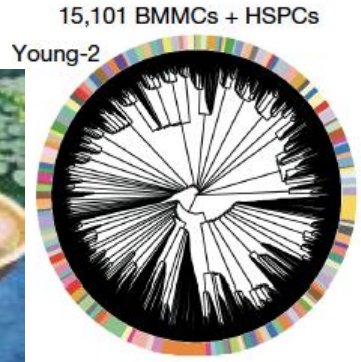
Mapping the cellular biogeography of human bone marrow niches using single-cell transcriptomics and proteomic imaging

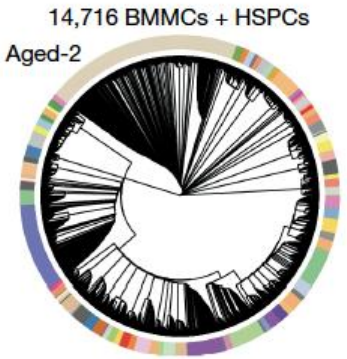


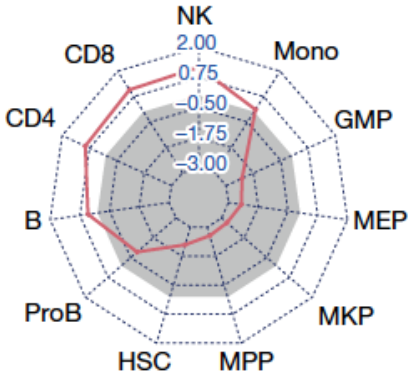
Article — **Nature**. 2024 Mar;627(8003):389-398. doi: 10.1038

Deciphering cell states and genealogies of human haematopoiesis









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✓ What YOU can do!

Habemus Papam!

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