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Filière de santé Maladies Rares Immuno-Hématologiques

Clonality & Aplastic anemia / PNH

" What is in a word" W Shakespear : Hamlet

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APLASIE MEDULLAIRE
centre de référence

Groupe Hospitalier Universitaire
SAINT-LOUIS
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FERNAND-WIDAL

PARIS DIDEROT

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EDITORIAL

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

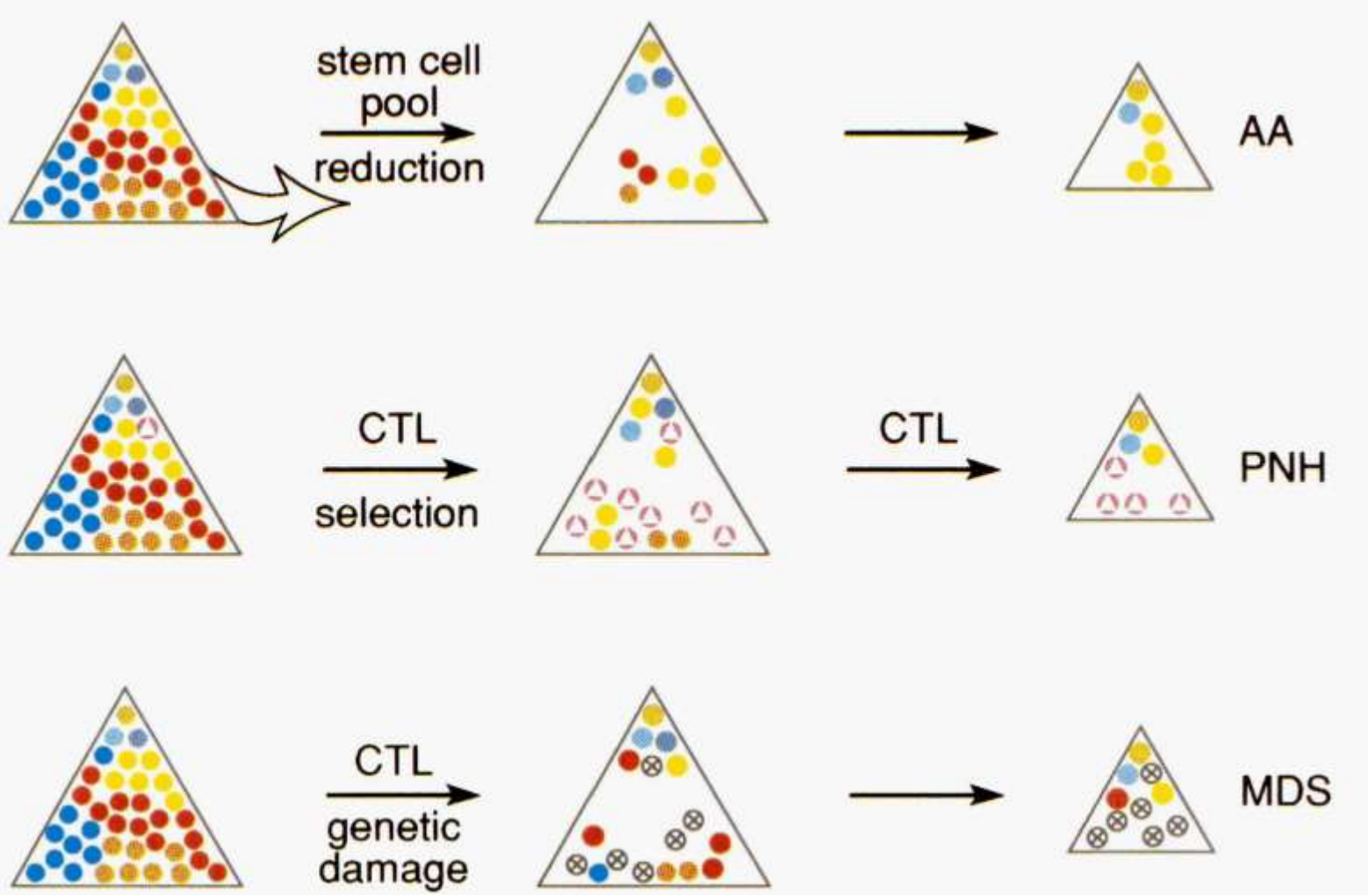
By William Dameshek

BLood, VOL. 30, No. 2 (AUGUST), 1967

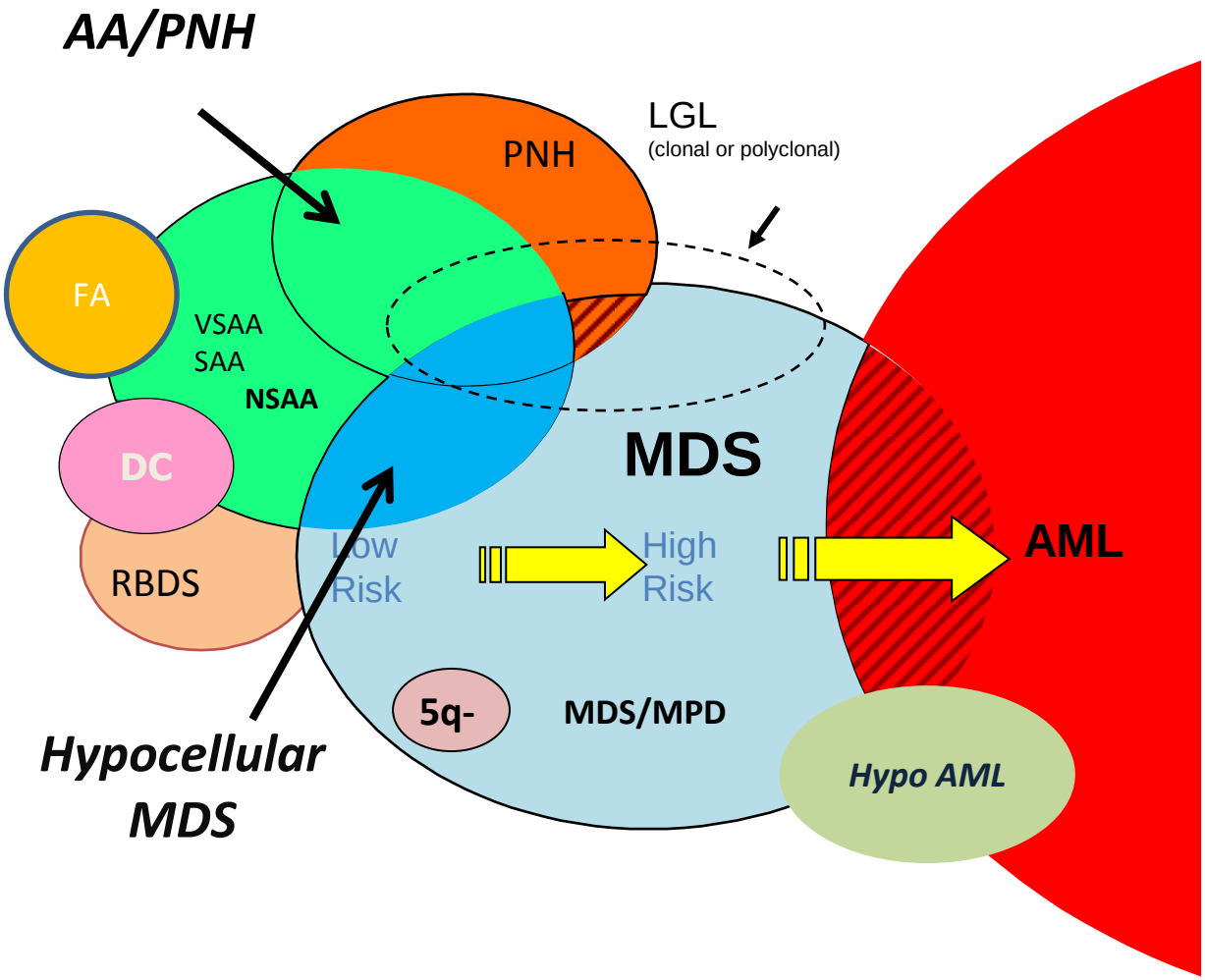
PERSPECTIVE

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

By Neal S. Young

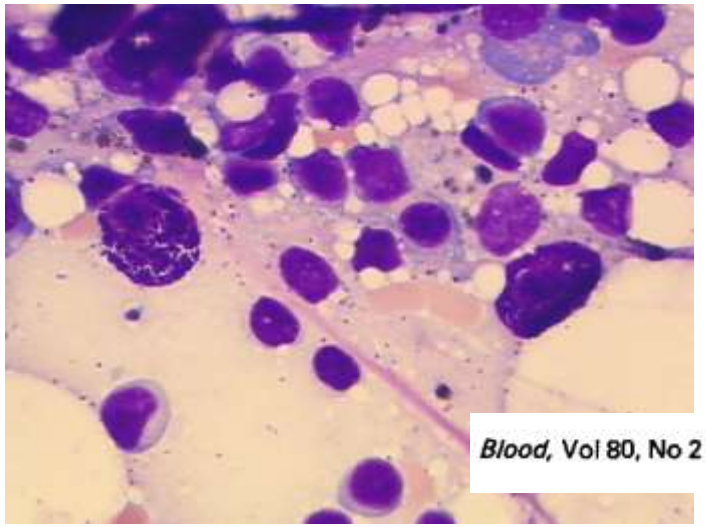


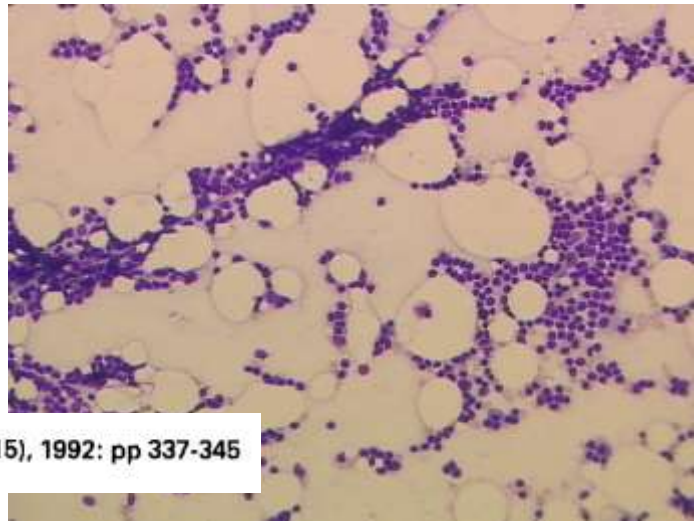
BLood, VOL. 79, No. 6 (Marsh), 1992



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

Morphology in Patients With Severe Aplastic Anemia Treated With Antilymphocyte Globulin





Blood, Vol 80, No 2 (July 15), 1992: pp 337-345

Mast cells
Plasma cells } Frequent in SAA

Increased erythroblasts } Frequent in PNH

Dyserythropoiesis } Frequent in both

Risk of over-diagnosing MDS

Tichelli et al. Blood 1992; 80 (2):337-45.

Studies	Period	N	Age (median)	Resp	Relapse	Clonal Evolution	Survival
Germany	1986-1989	84	32	65%	19%	8%	58% à 11 ans
NIH	1991-1998	122	35	61%	35%	11%	55% à 7 ans
EGMBT	1991-1998	100	16	77%	12%	11%	87% à 5 ans
Japan	1992-1997	119	9	68%	22%	6%	88% à 3 ans
Germany/Australia	1993-1997	114	9	77%	12%	6%	87% à 4 ans
Japan	1996-2000	101	54	74%	42%	8%	88% à 4 ans
NIH	1999-2003	104	30	62%	37%	9%	80% à 4 ans
NIH	2003-2005	77	26	57%	26%	10%	93% à 3 ans

Young et al. *Blood* 2006

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MALIGNANT TUMORS OCCURRING AFTER TREATMENT OF APLASTIC ANEMIA

Gérard Socié, M.D., Michel Henry-Amar, M.D., M.Sc., Andrea Bacigalupo, M.D., Jill Hows, M.D., André Tichelli, M.D., Per Ljungman, M.D., Shaun R. McCann, M.D., Norbert Frickhofen, M.D., Elizabeth Van't Veer-Korthof, M.D., and Eliane Gluckman, M.D., for the European Bone Marrow Transplantation–Severe Aplastic Anaemia Working Party*

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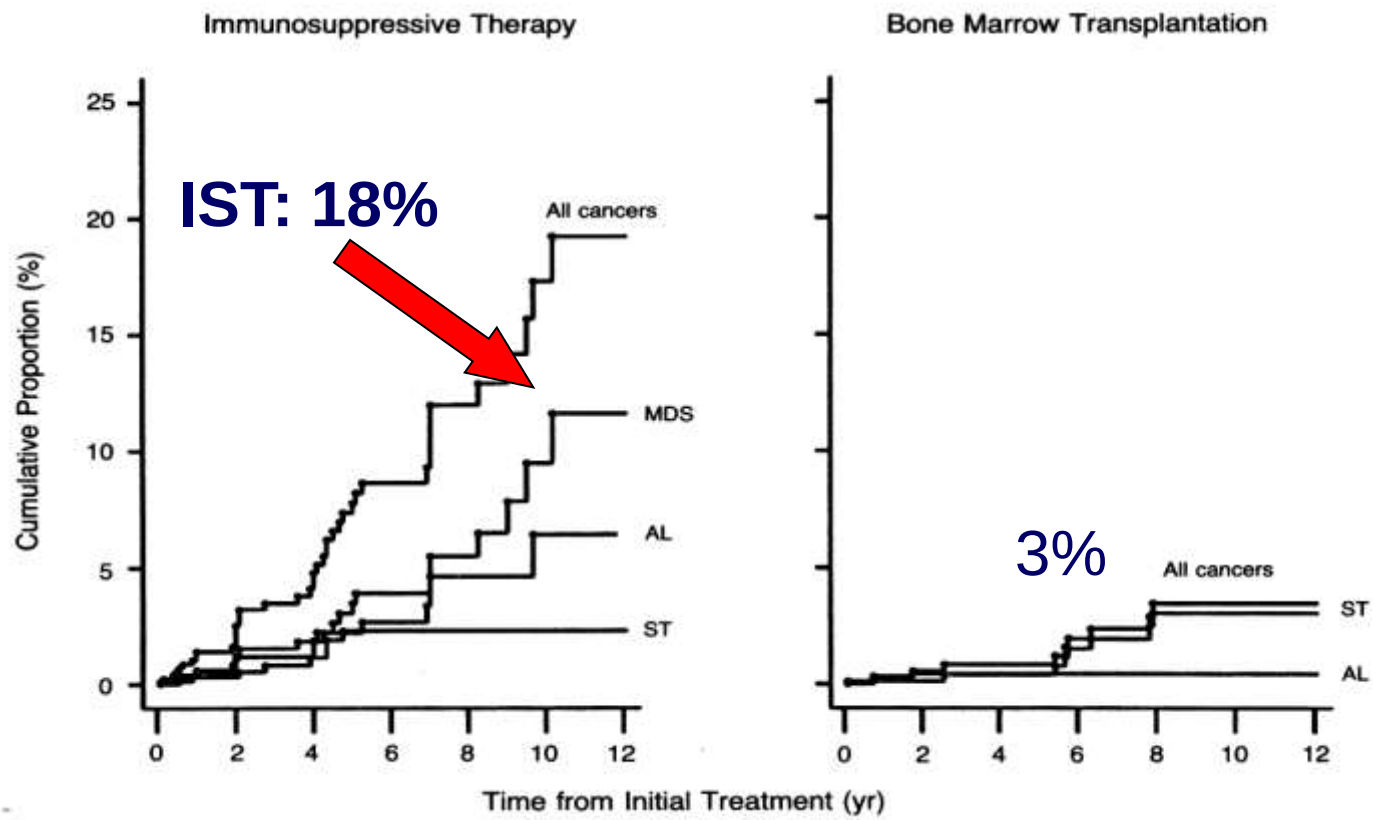
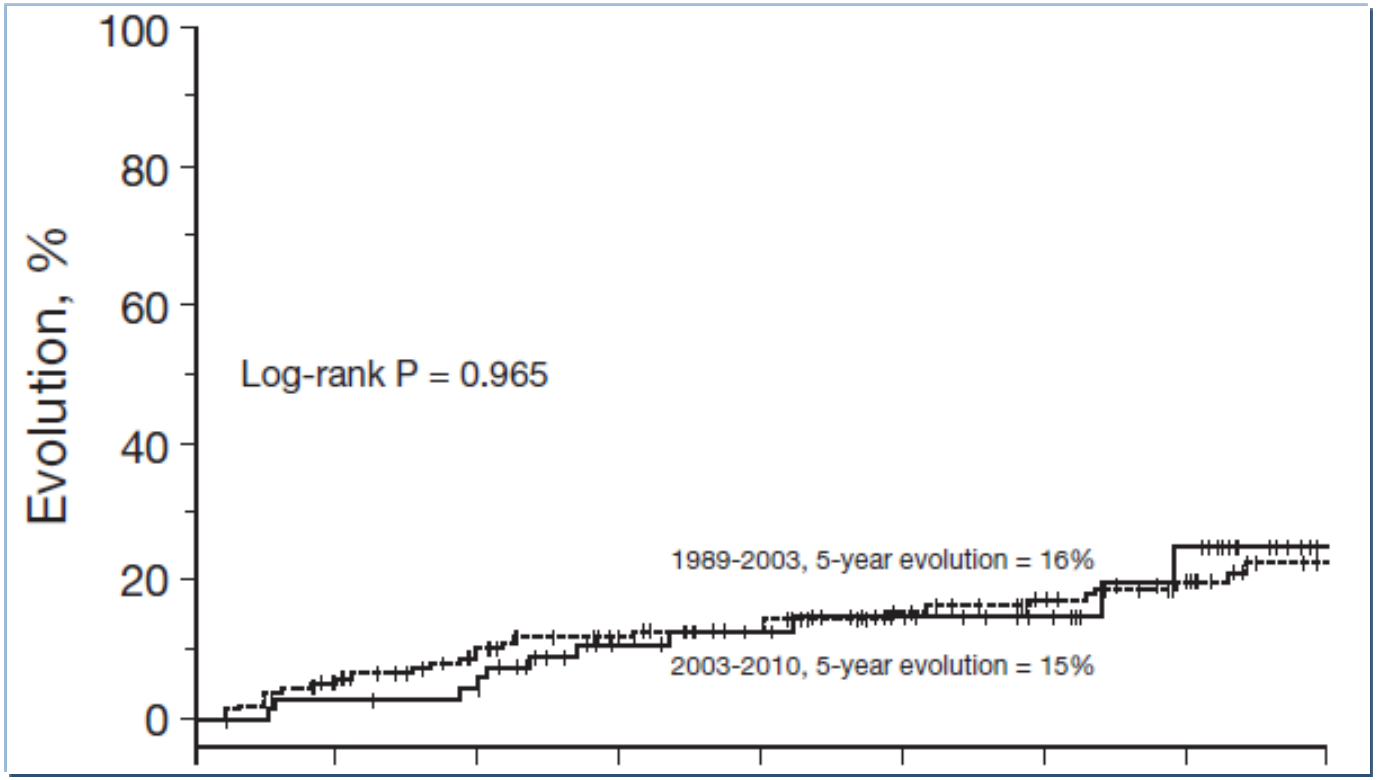


Socié G., et al. 1993; (329). 1152-1157

Clonal evolution (morphology): ~ 15%

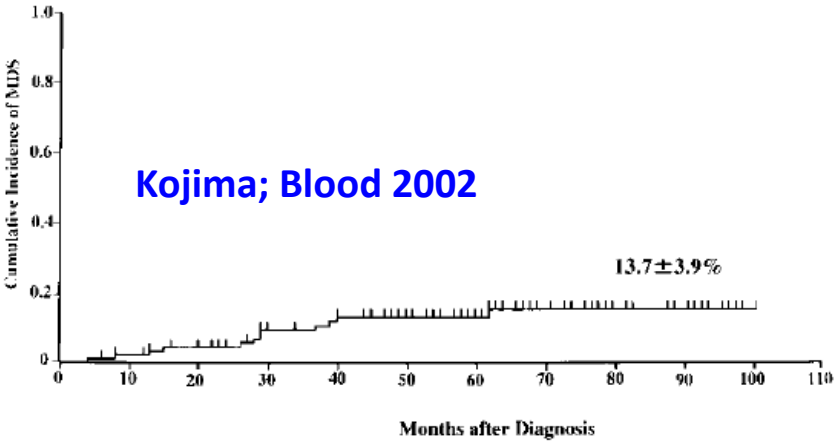


Am. J. Hematol. 89:571–574, 2014.

G-CSF / MDS-AML ?

Table 5. Relative risk of developing myelodysplastic syndrome in patients with aplastic anemia by multivariate analysis

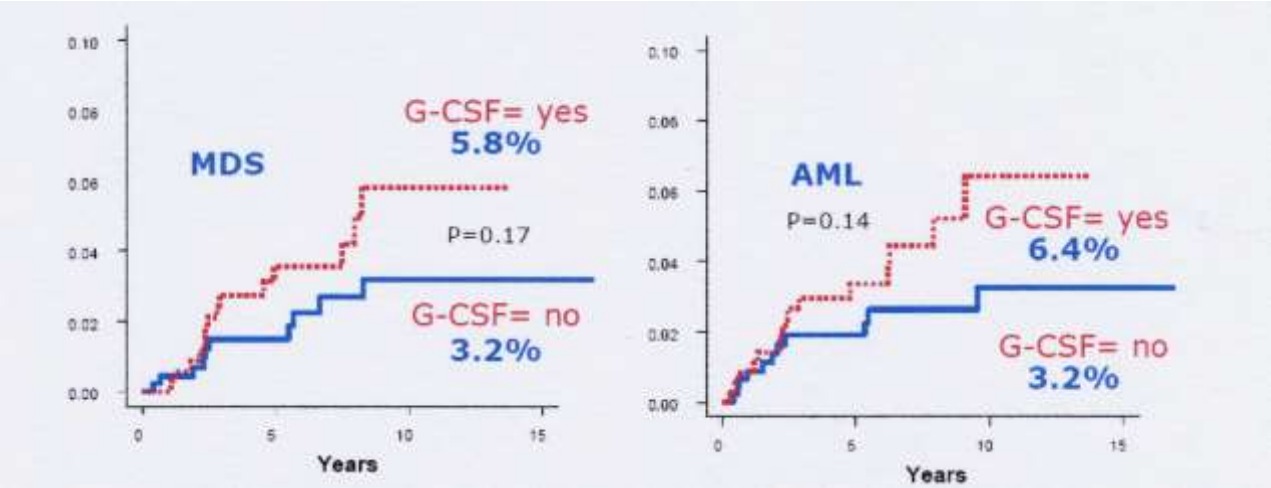
Variable	Relative risk (95% CI)	*P*
No. of days of G-CSF therapy*	1.003 (1.001-1.003)	.001
No response to therapy at 6 mo	3.46 (1.10-10.9)	.03



Kojima; Blood 2002

blood

G-CSF / MDS-AML ?



G Socié et al.
***Blood** 2007; 107:2794*

	Risk factors	HR	p
MDS	Age > 45yr	2.9	0.01
AML	Age > 45yr	4.1	0.002
	G-CSF	2.5	0.003
MDS/AML	Age > 45yr	2.9	0.001
	G-CSF	1.9	0.04

EBMT

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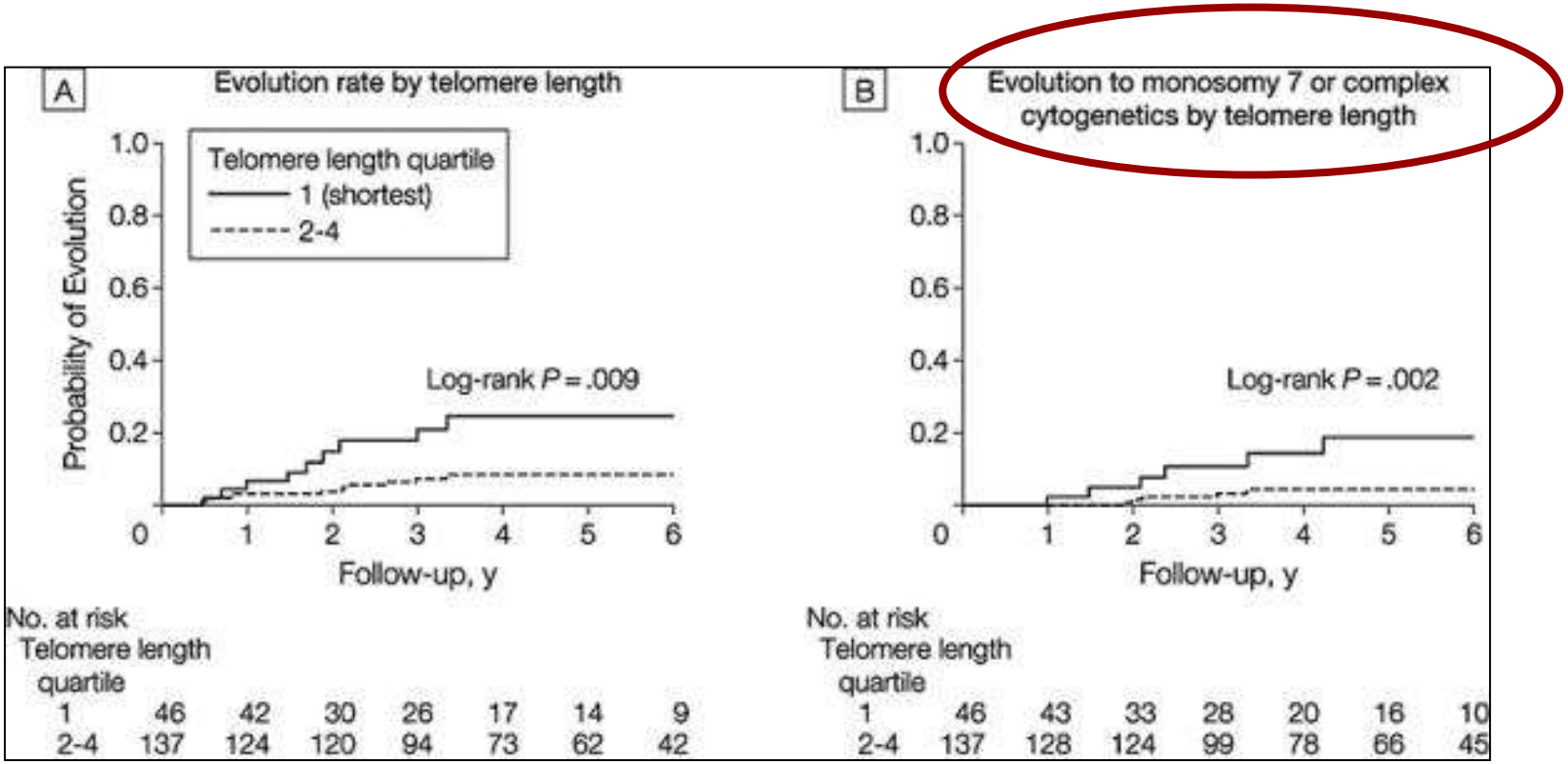
Association of Telomere Length of Peripheral Blood Leukocytes With Hematopoietic Relapse, Malignant Transformation, and Survival in Severe Aplastic Anemia.

Scheinberg, Phillip; Cooper, James; Sloand, Elaine; Wu, Colin; Calado, Rodrigo; MD, PhD; Young, Neal

JAMA. 304(12):1358-1364, September 22/29, 2010.



Skewed X-chromosome inactivation

i.e., skewing from the expected 1:1 ratio for random Lionization

- ✓ Small series from **>70%** (van Kamp et al.) to **10%**.
- ✓ Interpretation of NRXI extreme skewing (**>3:1**) in **normal females**
- ✓ Acquired during **normal aging**

Abnormal cytogenetic in AA

- ✓ **4%–11%** of AA cases
- ✓ difficulty in obtaining sufficient numbers of metaphases
- ✓ Predominant lesions; **+8, –7, & del(5q)**

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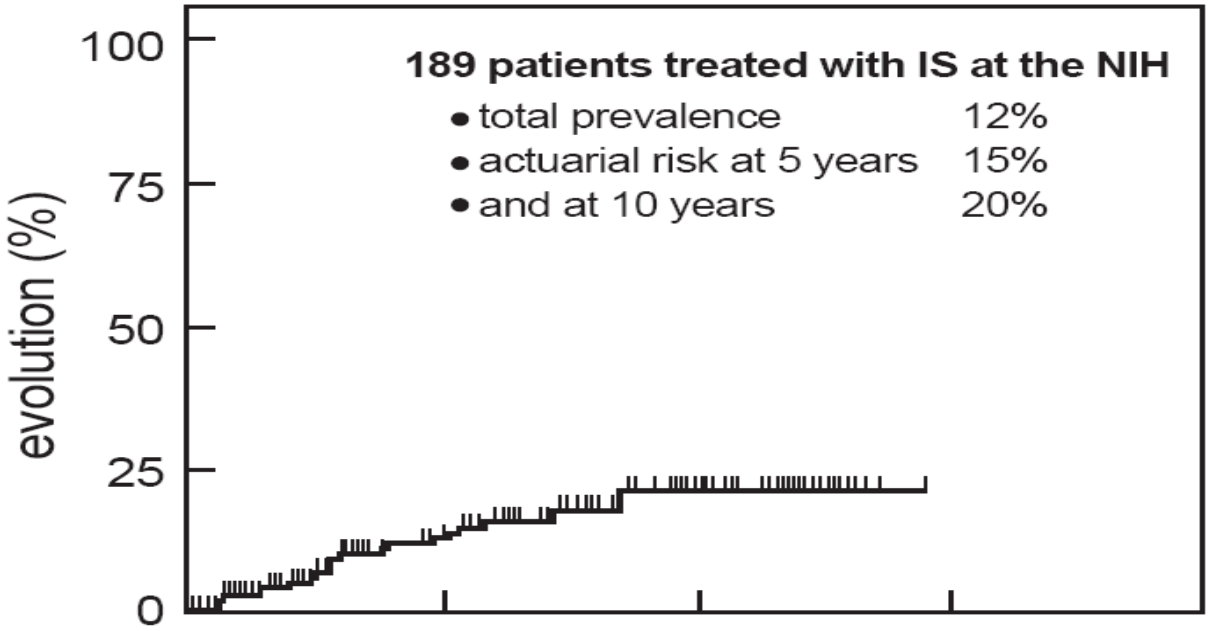
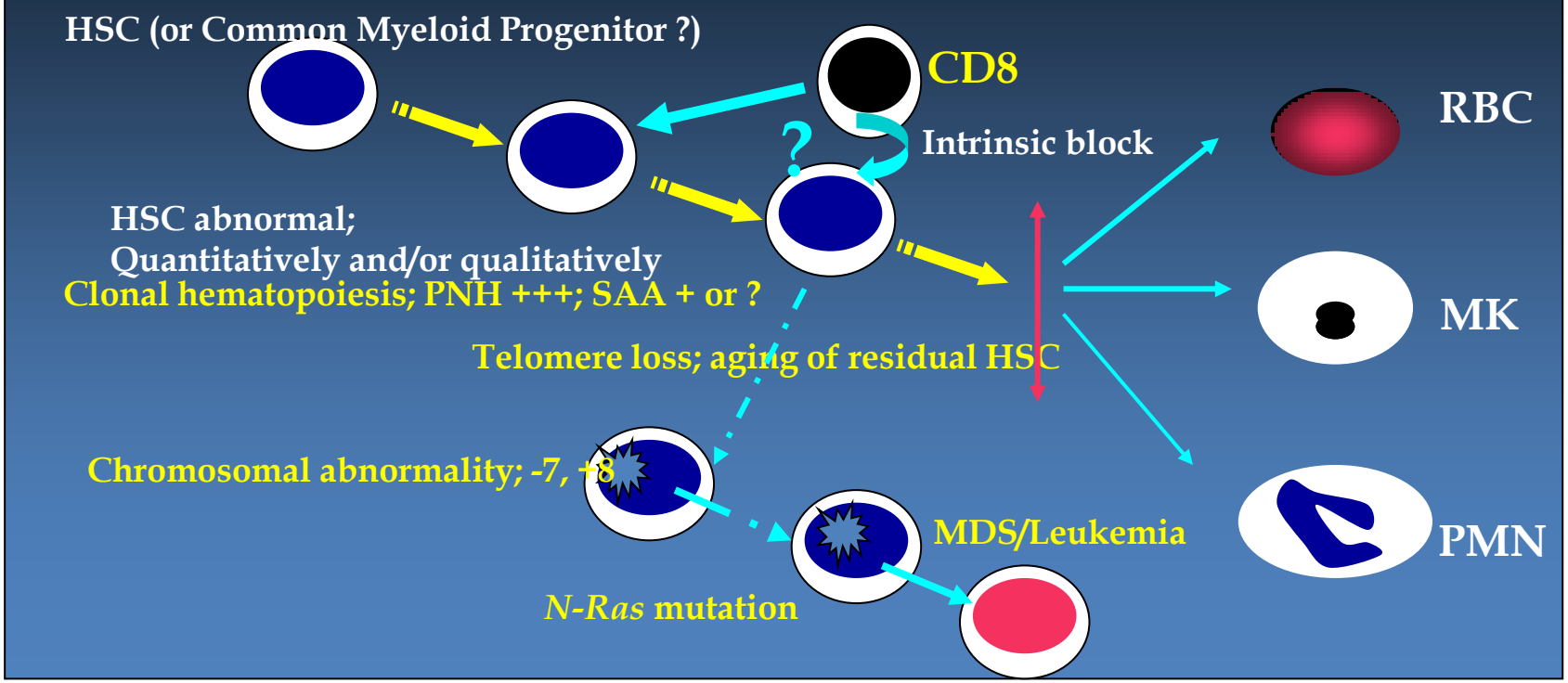
Maciejewski, Blood 2001

- No risk factors
- del7/7q, +8, 11q-, +21, +6, complex karyotypes most common



Could AA be considered a pre-pre-leukemic disorder ?

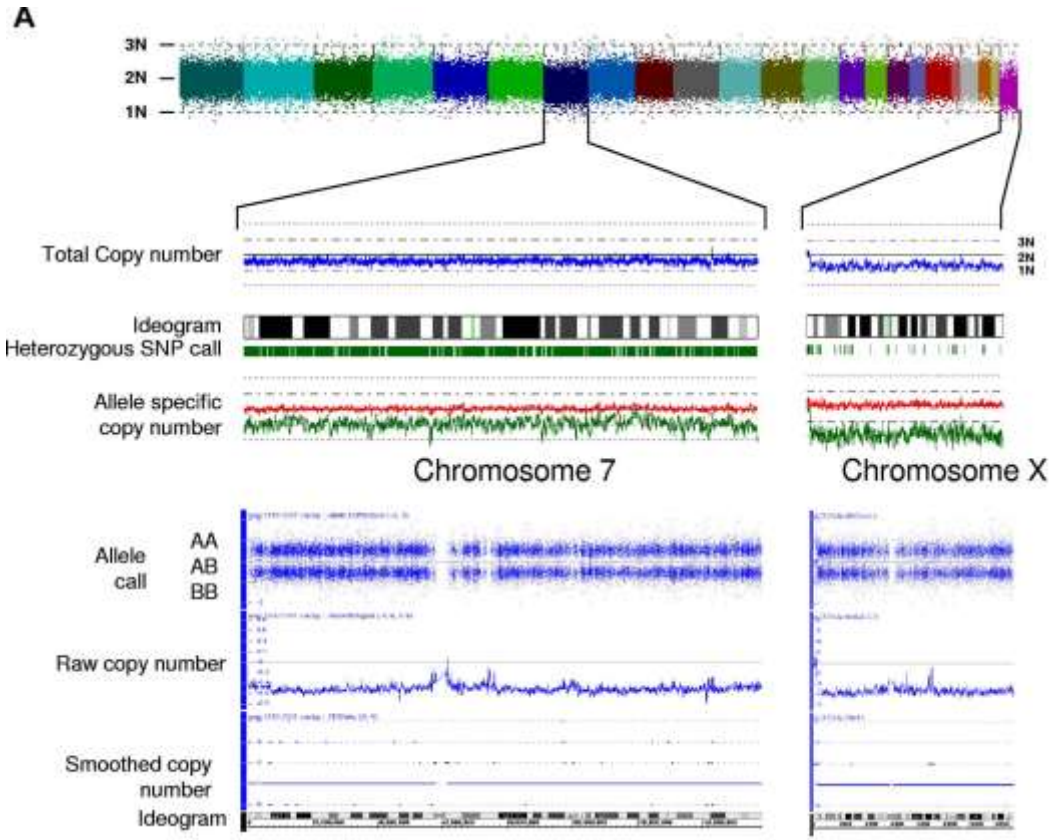
- *Eur.J. Haematol.* 1996; 60 (Suppl.): 60-63.



SNP array–based karyotyping: differences and similarities between aplastic anemia and hypo cellular MDS

Jaroslaw P. Maciejewski

AA (N = 93) and hypo cellular MDS (N = 24)



SNP-A identify cryptic clonal genomic aberrations In 19% of AA

blood

Blood. 2011;117(25): 6876-6884

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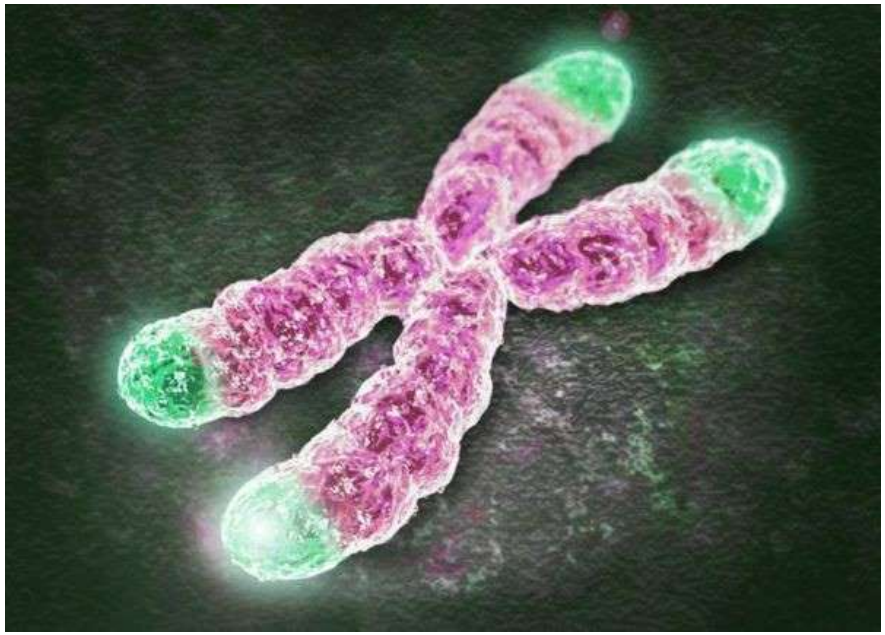

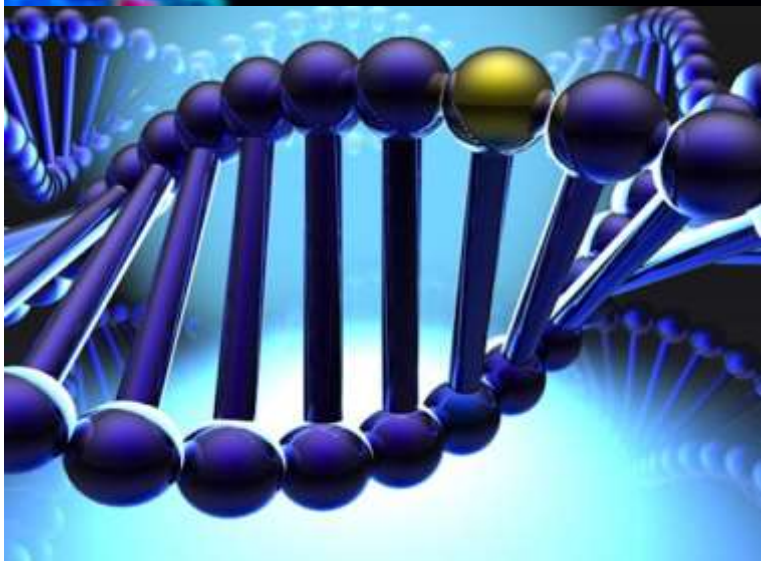

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Austin G Kulasekararaj et al. **2014**

NSAA (n=65)

SAA (n=51)

VSAA (n=23)

Others (n=11)

Aplastic Anaemia (n=150)

Evolved to MDS with somatic mutations (N=11)

- Detected pre evolution
- -7 associated with DNMT3A/ASXL1

No evolution to MDS but have mutations (N=18)

- <10% clones in 10
- Longer F/U needed

Evolution to MDS but no mutations (N=6)

- Mutated in other genes
- Undetectable low level clones

Somatic mutations identify a sub-group of aplastic anemia patients that progress to myelodysplastic syndrome

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Austin G Kulasekararaj et al. **2014**

SOMATIC MUTATIONS AT PRESENTATION ARE PREDICTIVE OF SUBSEQUENT RISK OF PROGRESSION

Longitudinal

Mutations detected by WES

Detection of somatic mutations

Initial unbiased search

Targeted deep NGS

ATG

Whole Exome Sequencing

AA presentation

MDS

Detection of mutations at presentation 8/18 (30 events)

Somatic mutations 18/18

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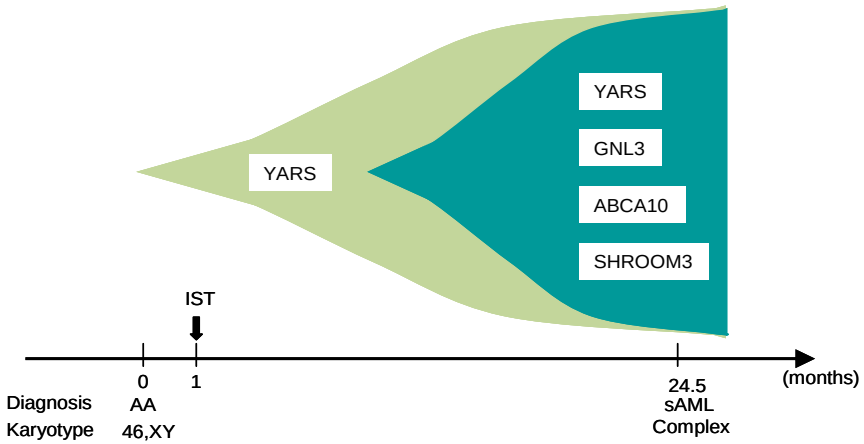
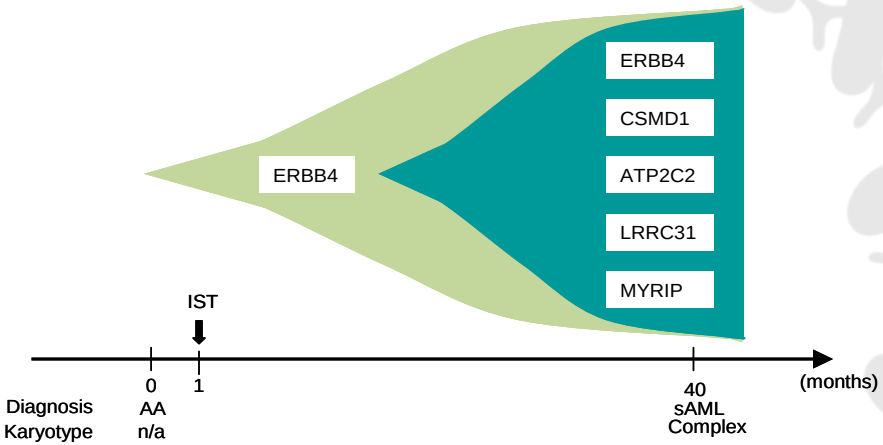
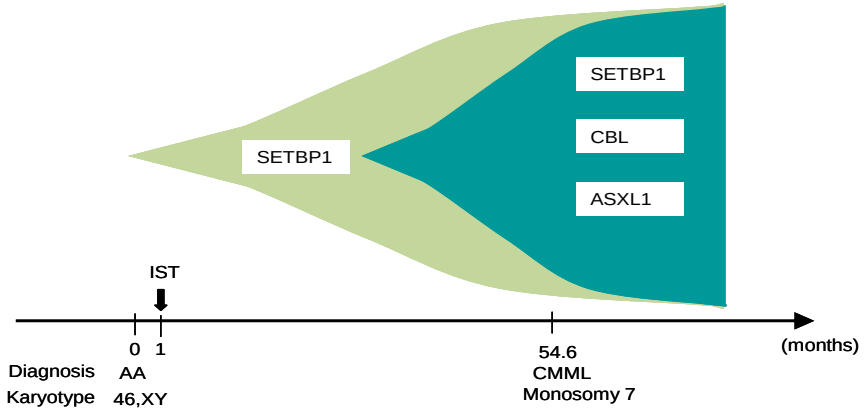
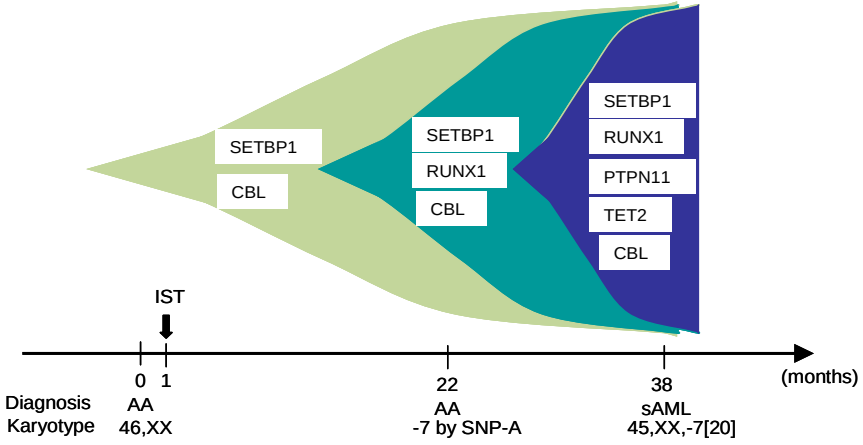
Austin G Kulasekararaj et al. **2014**









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ORIGINAL ARTICLE

Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia

T. Yoshizato, B. Dumitriu, K. Hosokawa, H. Makishima, K. Yoshida, D. Townsley, A. Sato-Otsubo, Y. Sato, D. Liu, H. Suzuki, C.O. Wu, Y. Shiraishi, M.J. Clemente, K. Kataoka, Y. Shiozawa, Y. Okuno, K. Chiba, H. Tanaka, Y. Nagata, T. Katagiri, A. Kon, M. Sanada, P. Scheinberg, S. Miyano, J.P. Maciejewski, S. Nakao, N.S. Young, and S. Ogawa

The NEW ENGLAND JOURNAL of MEDICINE

next-generation sequencing and array-based karyotyping using 668 blood samples obtained from **439 patients**

Clonal hematopoiesis; in 47%

mutations in ***DNMT3A* and *ASXL1* ;** worse outcomes.
"*However, clonal dynamics were highly variable and might not necessarily have predicted the response to therapy and long-term survival among individual patients*"

N Engl J Med
373(1):35-47; 2015

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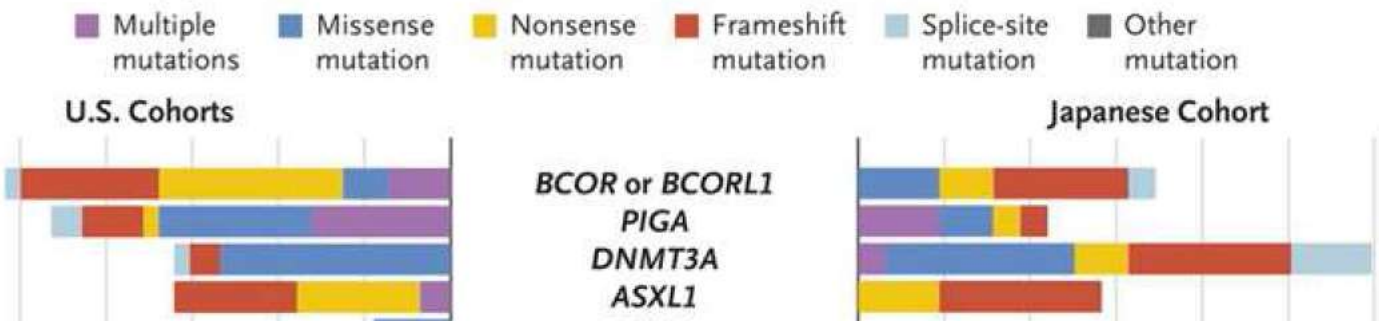

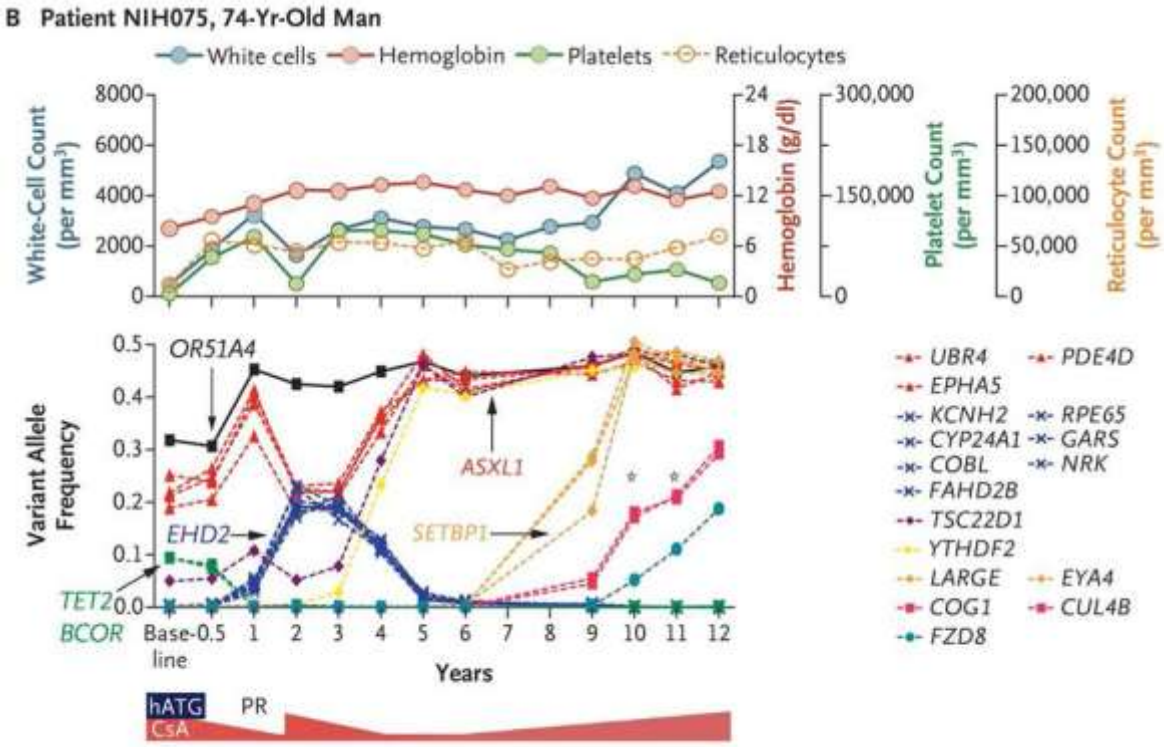

N Engl J Med
373(1):35-47; 2015

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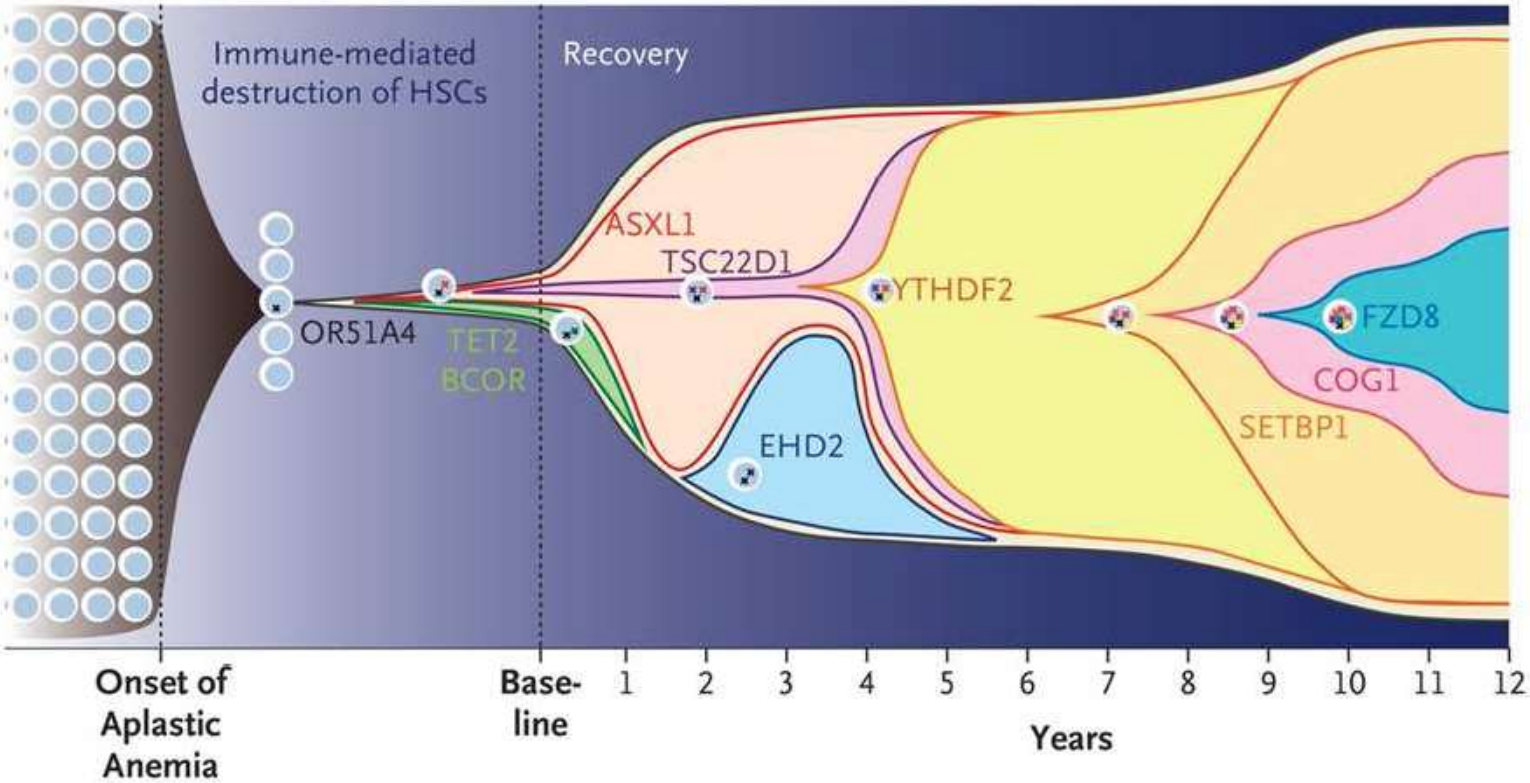
N Engl J Med
373(1):35-47; 2015



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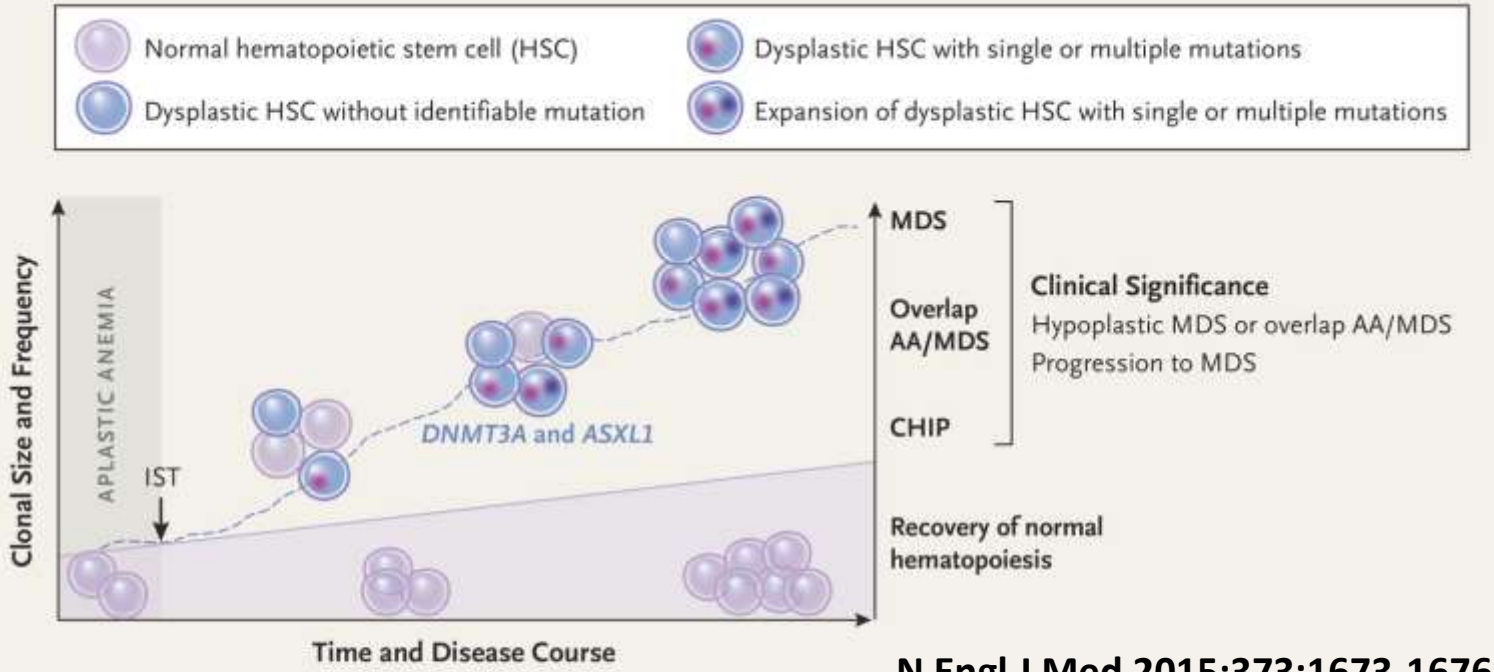
A Aplastic Anemia and Its Evolution with and without Immunosuppressive Therapy



N Engl J Med 2015;373:1673-1676.

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%

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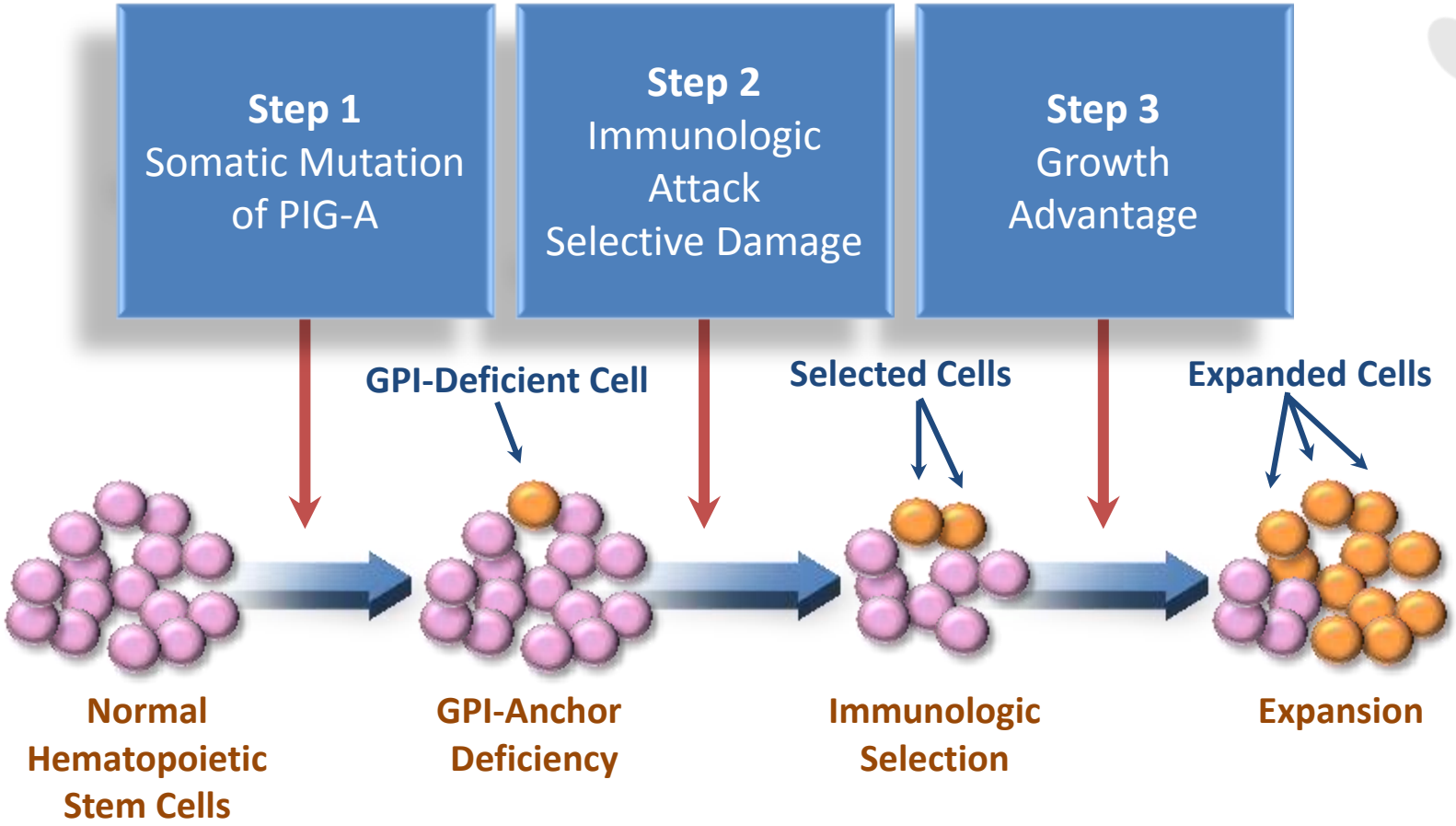
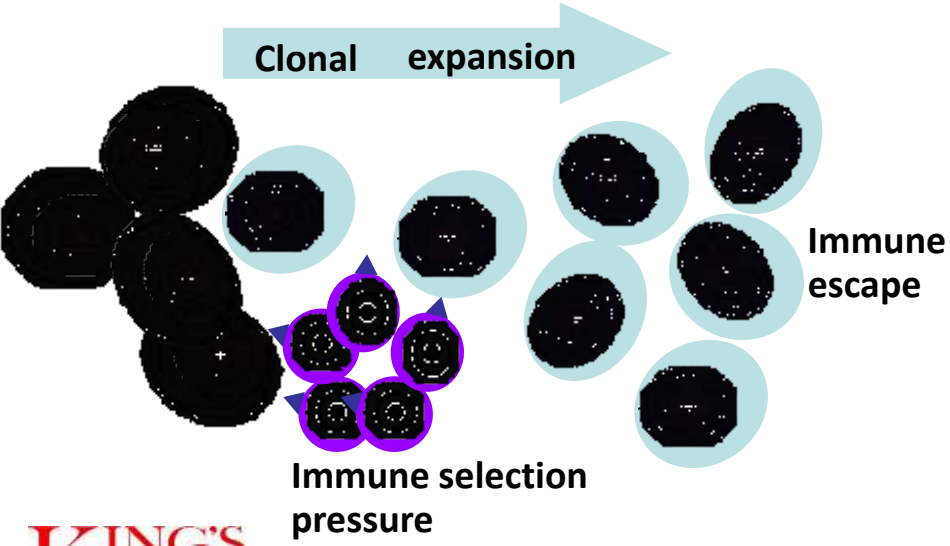


- Expansion may be due to another somatic mutation
- The need for both selection and expansion may explain the rarity of PNH

Maciejewski, **Blood 2001**

blood



-Mutations present at presentation
-Trigger immune surveillance
-Subsequent clonal evolution

VS.

-True late clonal event

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PNH clone(n=65) → PIGA sequencing (n=23)

- Clone >10% (n=17)
  - Single *PIGA* mutation (n=7)
  - Double *PIGA* mutation(n=6)
  - ***PIGA* with other somatic mutation(n=4)**
- Clone <10% (n=6)
  - No *PIG-A* mutations

large (>50%): 15
moderate (10-50%): 14
subclinical (<10%): 36

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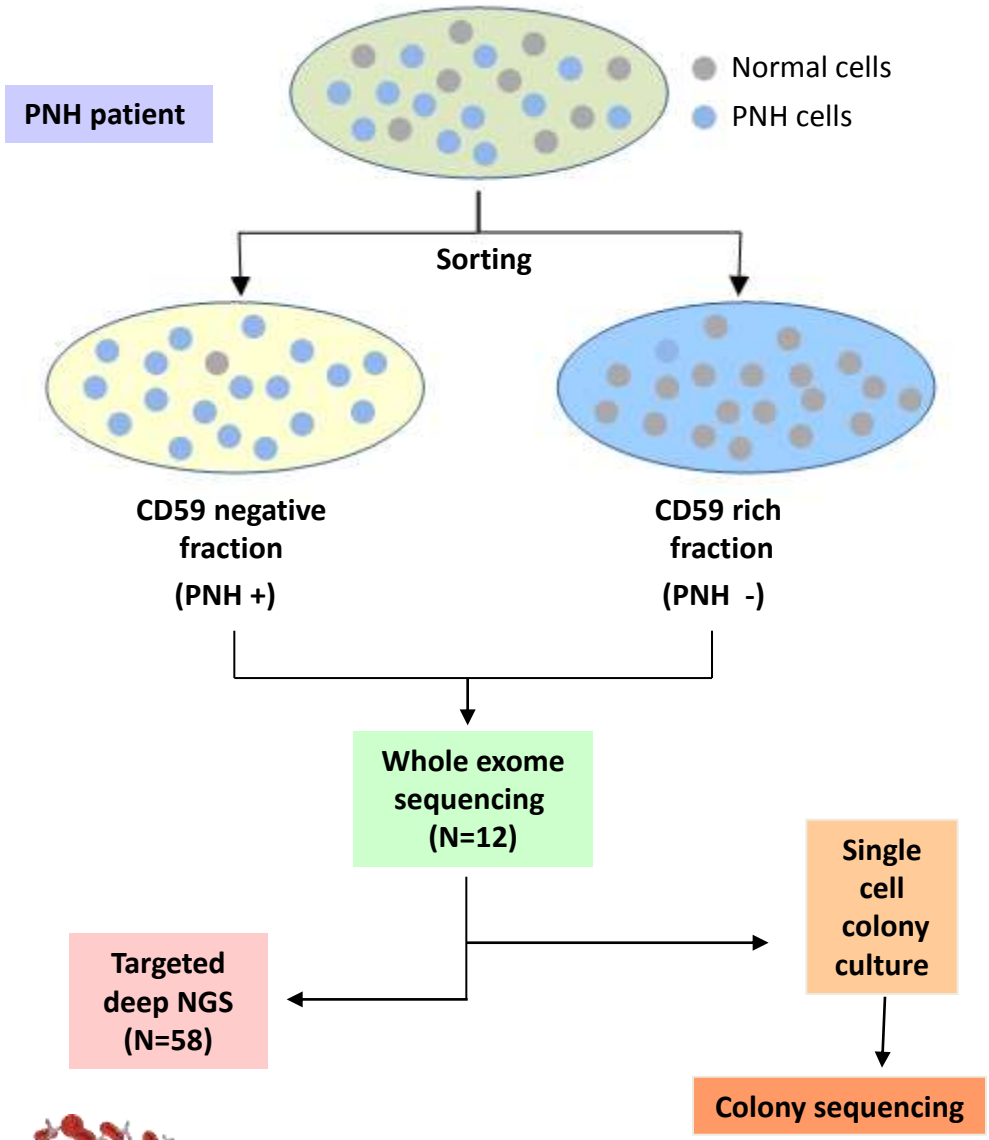
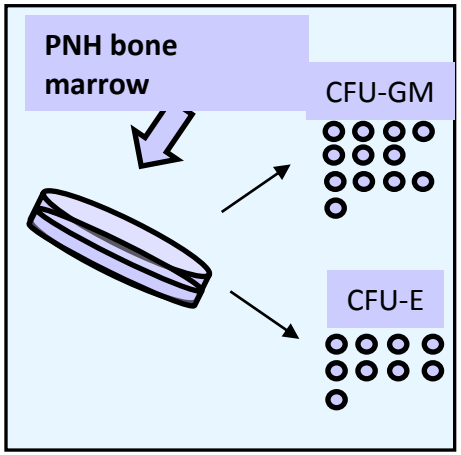
Kulasekararaj et al.
Blood 2014

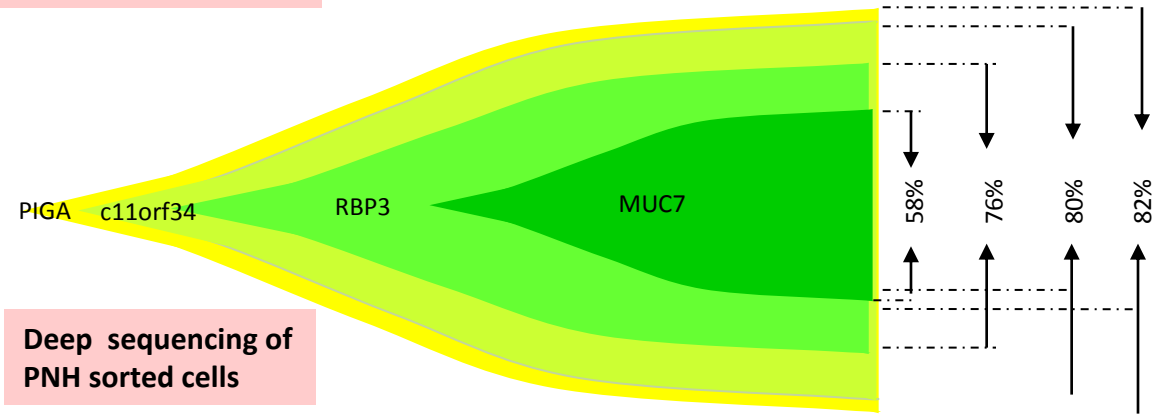
Maciejewski, JCI 2014



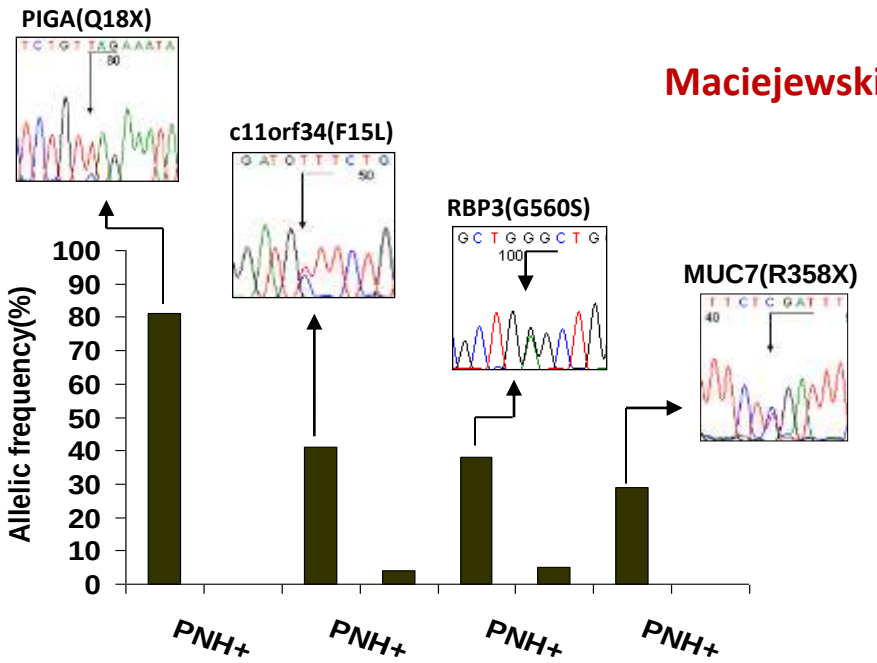
Variable	Whole cohort (N=58)	WES (n=12)
Age		
Median,y	38	43.5
PNH clone size		
Median,%	86	94
Range,%	5.0~99.8	63~99.6



Clonal architecture

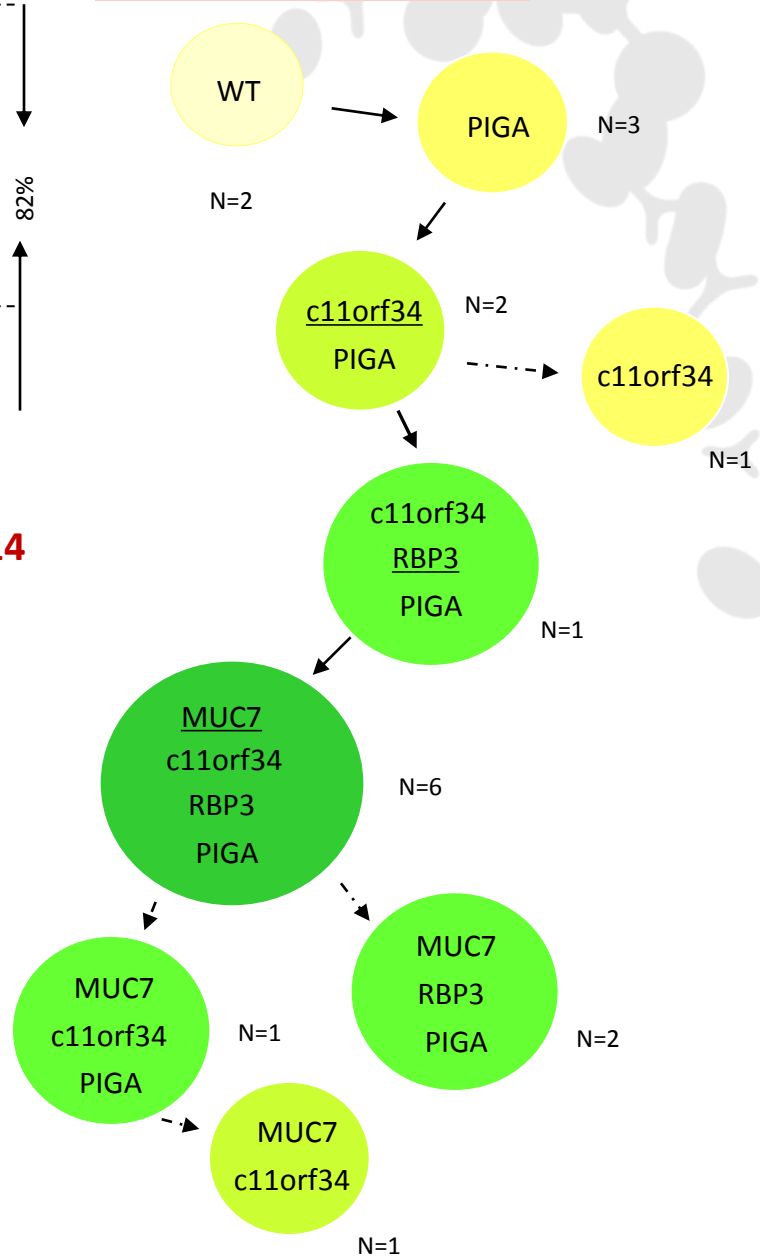


Deep sequencing of PNH sorted cells



Maciejewski, JCI 2014

Single cell colony sequencing



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Additional mutation events

Following PIG-A mutation as secondary events

CCR9, ALDH1B1, CPD, CELSR2, KDM3B, ZRSR2, MUC7, RBP3, c11orf34, PEX14, FBN1, BCOR, U2AF1, MECOM, KDM6A

Preceding *PIG-A* mutation

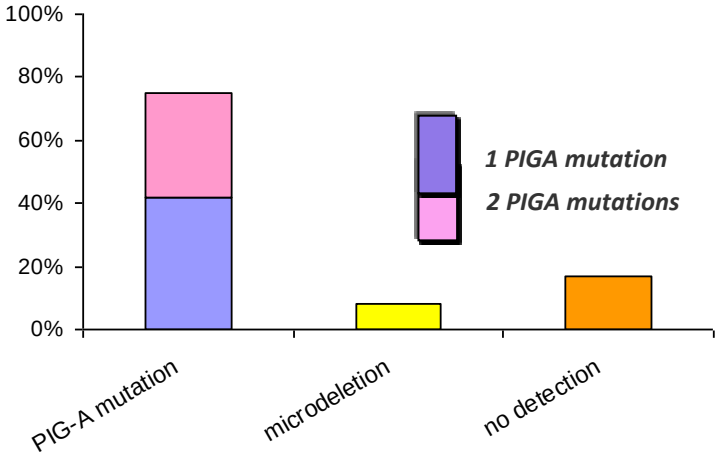
NTNG1, MAGEC1, TMC1, WDR96, NRXN3, STAC3, SLC20A1, MAN1A2, SYNE2, PEX14, DHX29

Clonogenic mutation both in PIGA mutant and wild-type cells followed by PIG-A mutation

TET2, JAK2, SUZ12

Maciejewski, JCI 2014

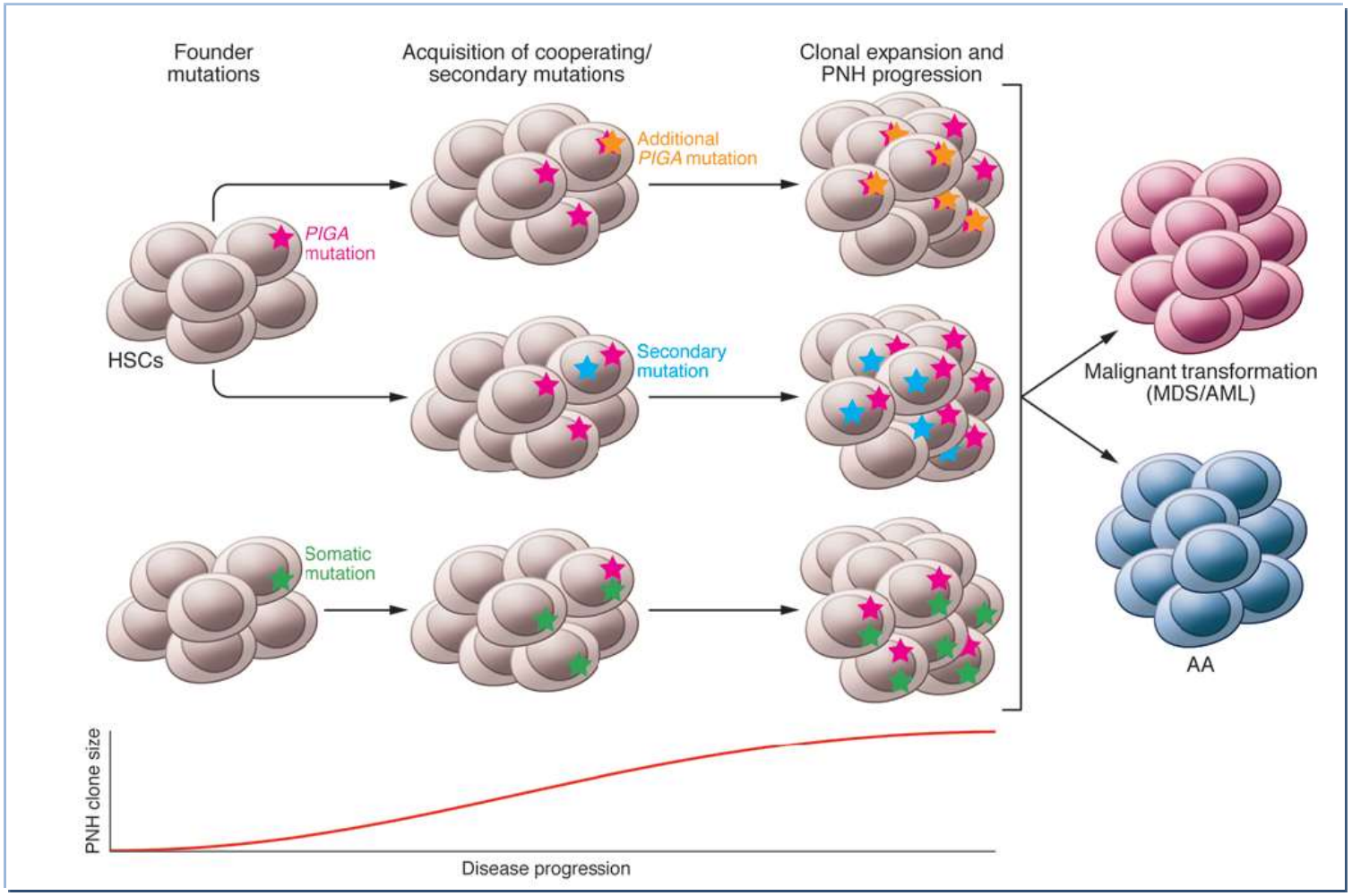
***PIG-A* mutation detection**



The Journal of Clinical Investigation

The mutational landscape of paroxysmal nocturnal hemoglobinuria revealed: new insights into clonal dominance

Stanley Chun-Wei Lee[1] and Omar Abdel-Wahab[1,2]



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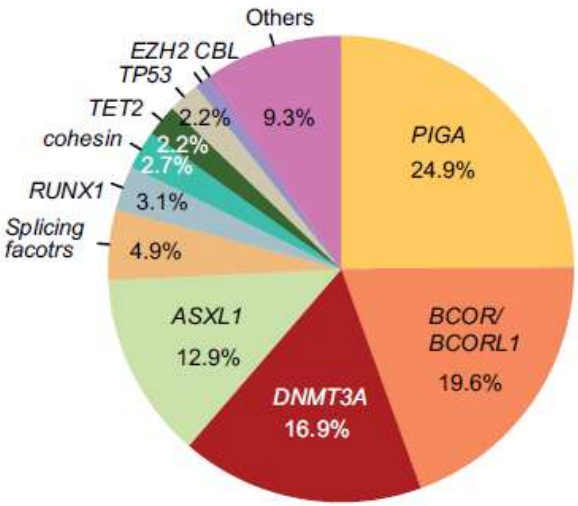
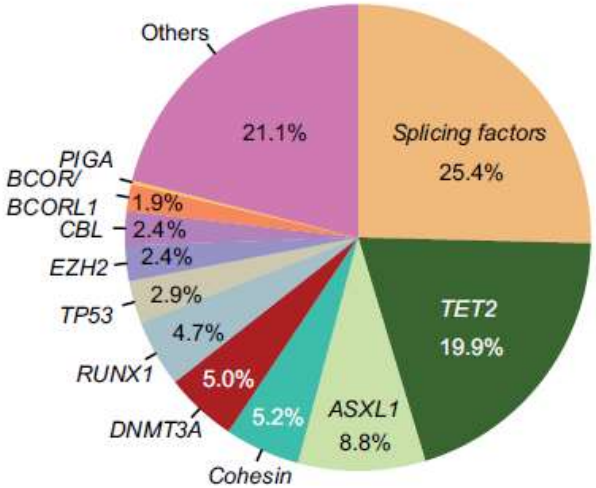
Clonal hematopoiesis in acquired aplastic anemia

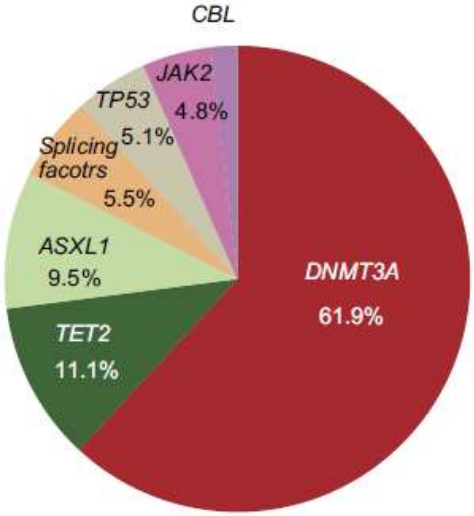
S. OGAWA; Blood 2016, in press



AA



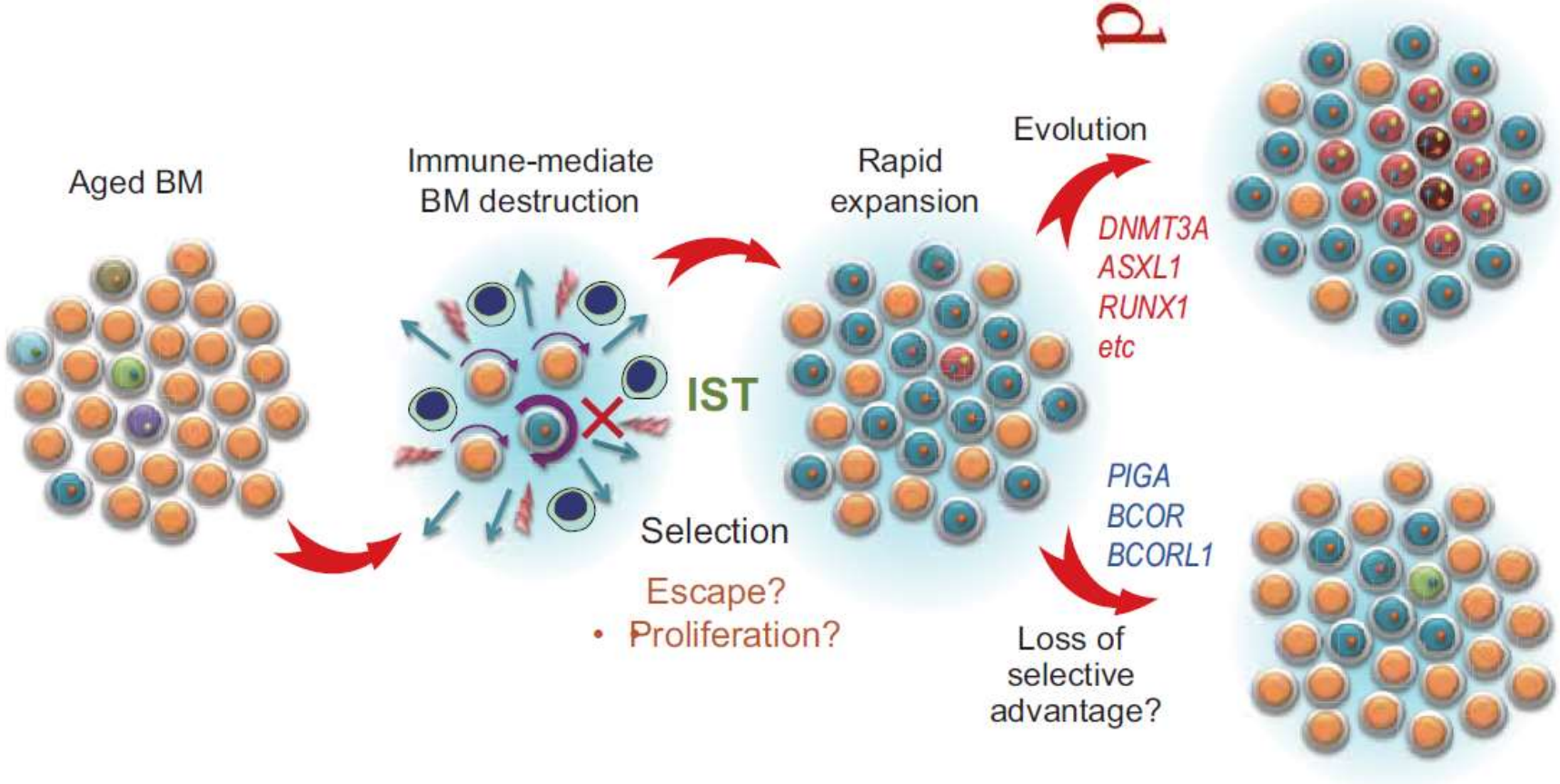
MDS



ARCH
(CHIP)

Clonal hematopoiesis in acquired aplastic anemia

S. OGAWA; Blood 2016, in press



An aging population

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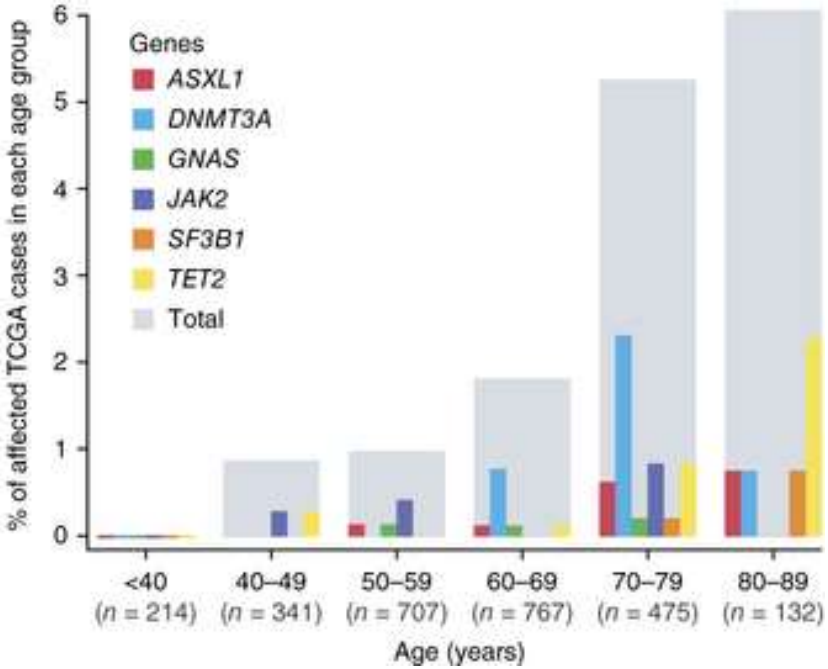
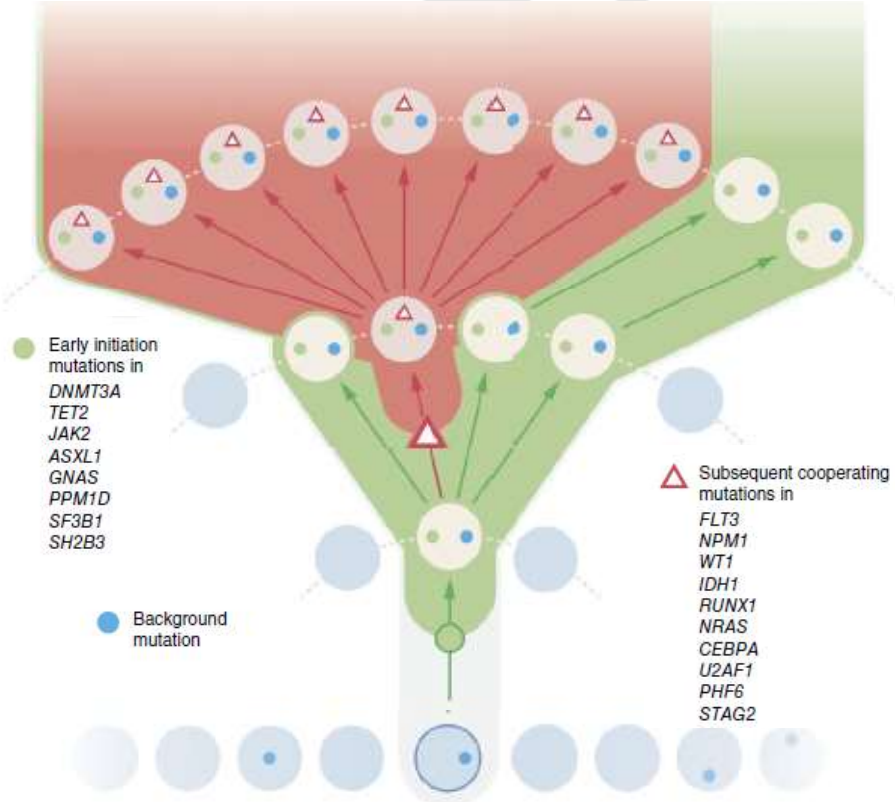
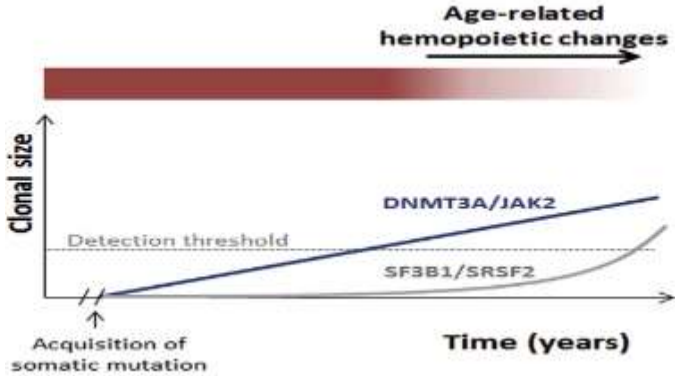
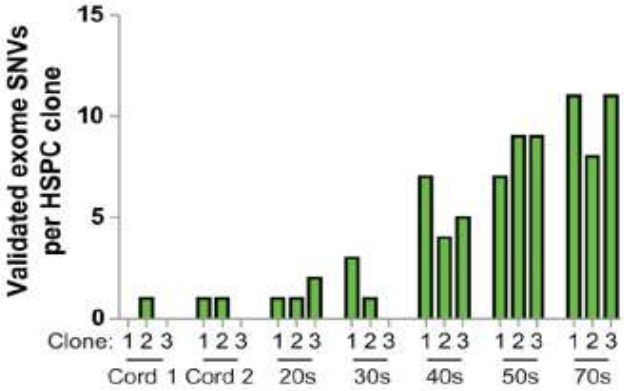








Xie, NatMed,2014,1472-1478, Genovese, NEJM, 2014,
Jaiswal, NEJM, 2014, Welch, Cell 2012
Busque, Nat Gen, 2012

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

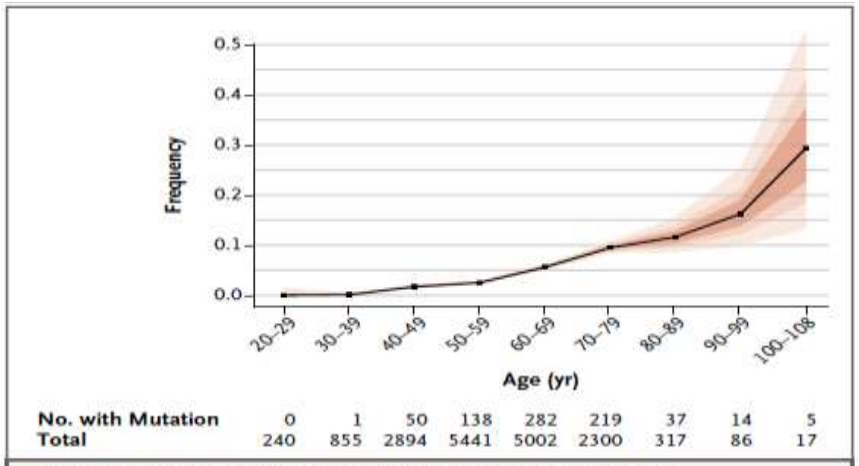
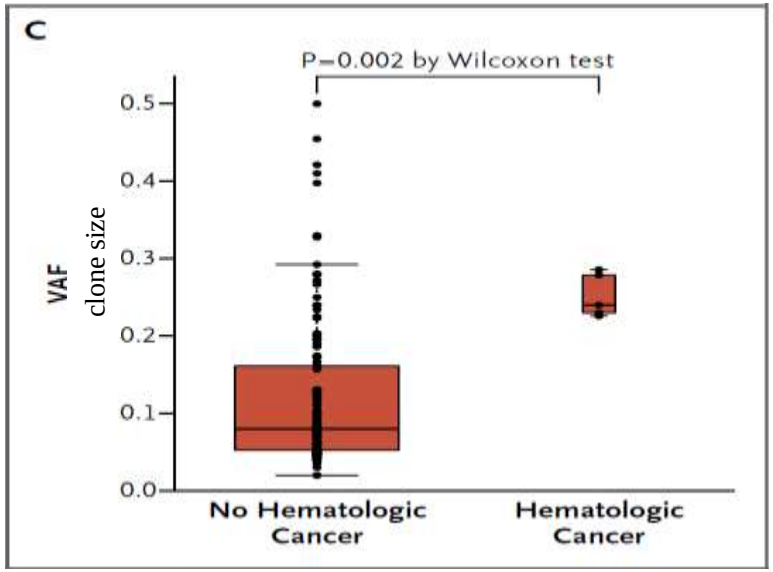


Figure 1. Prevalence of Somatic Mutations, According to Age.
Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.



Siddhartha Jaiswal *et al,* Dec 2014; Ebert lab

- **Study of 17,182 individuals unselected for hematologic phenotypes.**

- **746 persons had detectable mutation.**

- Age related (bystanders/passengers, present at/prior to diagnosis of AA); necessary to maintain hemopoiesis?
- Clonal flux
- True driver mutation(s)
- Cellular composition (myeloid vs lymphoid) and accurate determination of VAF/MAB (small clones)
- Mutational architecture, hierarchy and chronology

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Toward the CHIP's concept ?

Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes

*by David P. Steensma, Rafael Bejar, Siddhartha Jaiswal, R. Coleman Lindsley, Mikkael A. Sekeres, Robert P. Hasserjian, and **Benjamin L. Ebert***

***Blood** Volume 126(1):9-16; July 2, 2015*

blood

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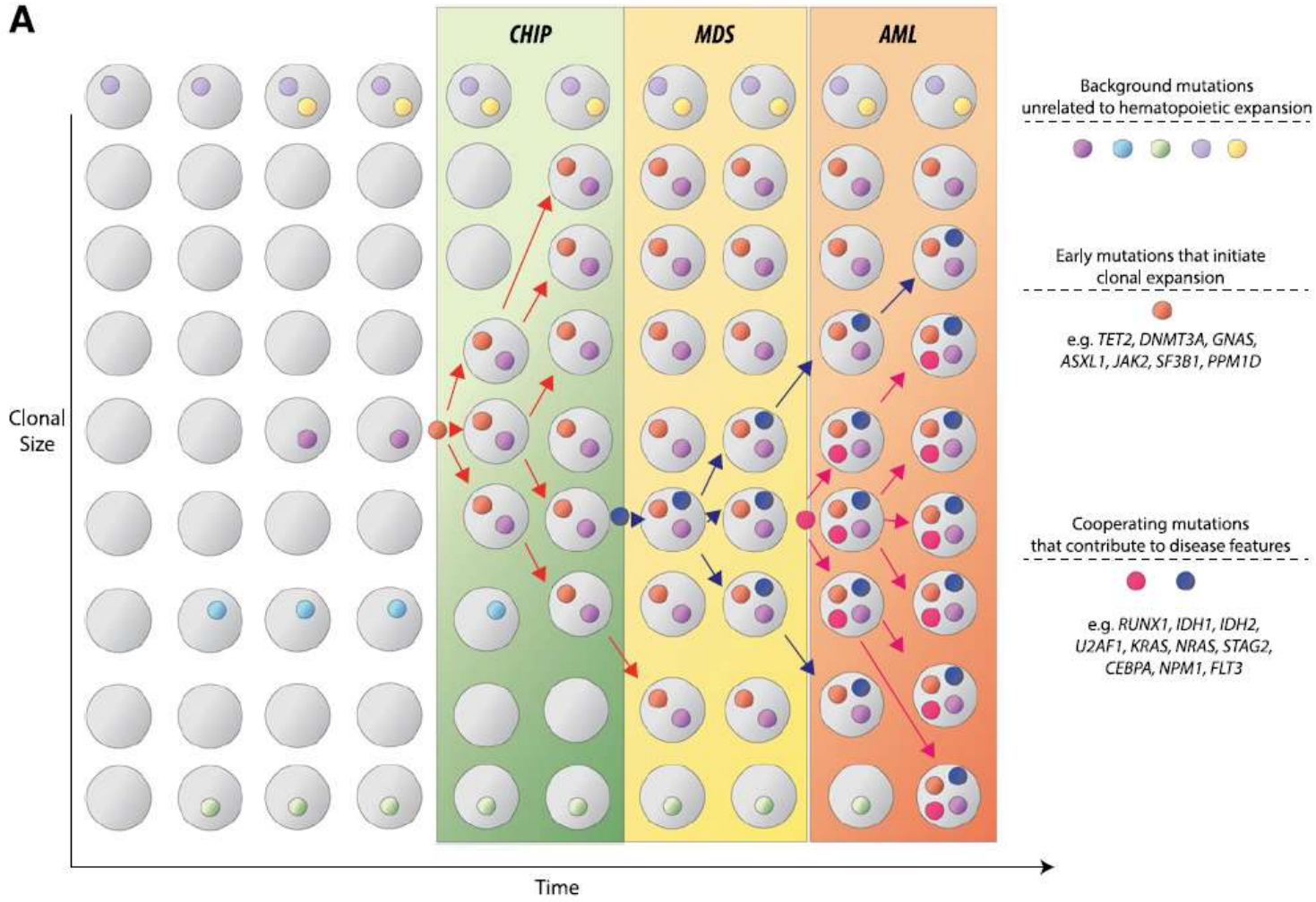
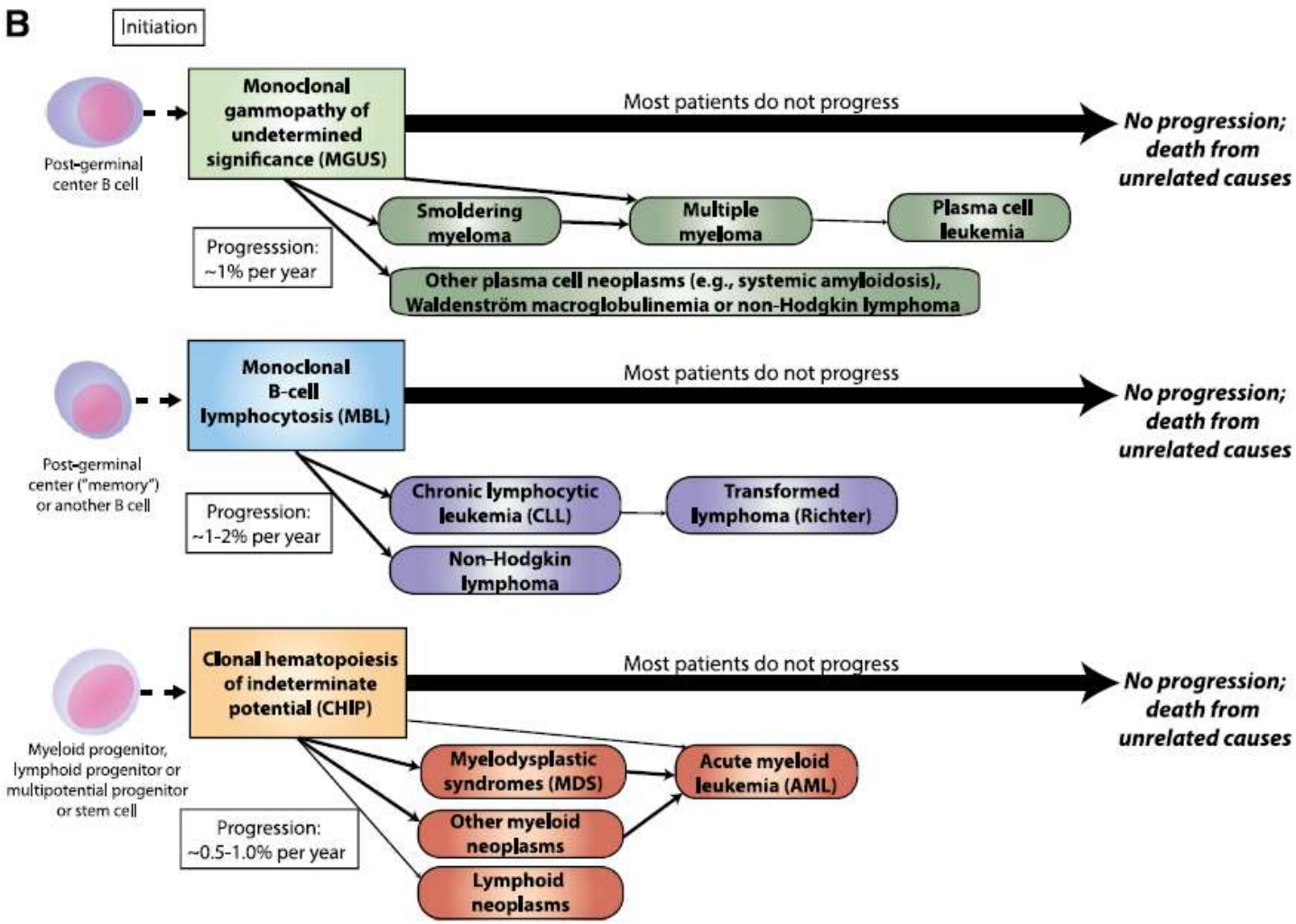
Toward the CHIP's concept ?



Toward the CHIP's concept ?



Toward the CHIP's concept ?

B

Traditional ICUS

MDS by WHO 2008

	'Non-clonal' ICUS	CHIP	CCUS	Lower Risk MDS	Higher Risk MDS
Clonality	–	+	+	+	+
Dysplasia	–	–	–	+	+
Cytopenias	+	–	+	+	+
BM Blast %	< 5%	< 5%	< 5%	< 5%	< 19%
Overall Risk	Very Low	Very Low	Low (?)	Low	High
Treatments	Obs/BSC	Observation	Obs/BSC/GF	Obs/BSC/GF IMiD/IST	HMA/HCST

Clonal Cytopenias

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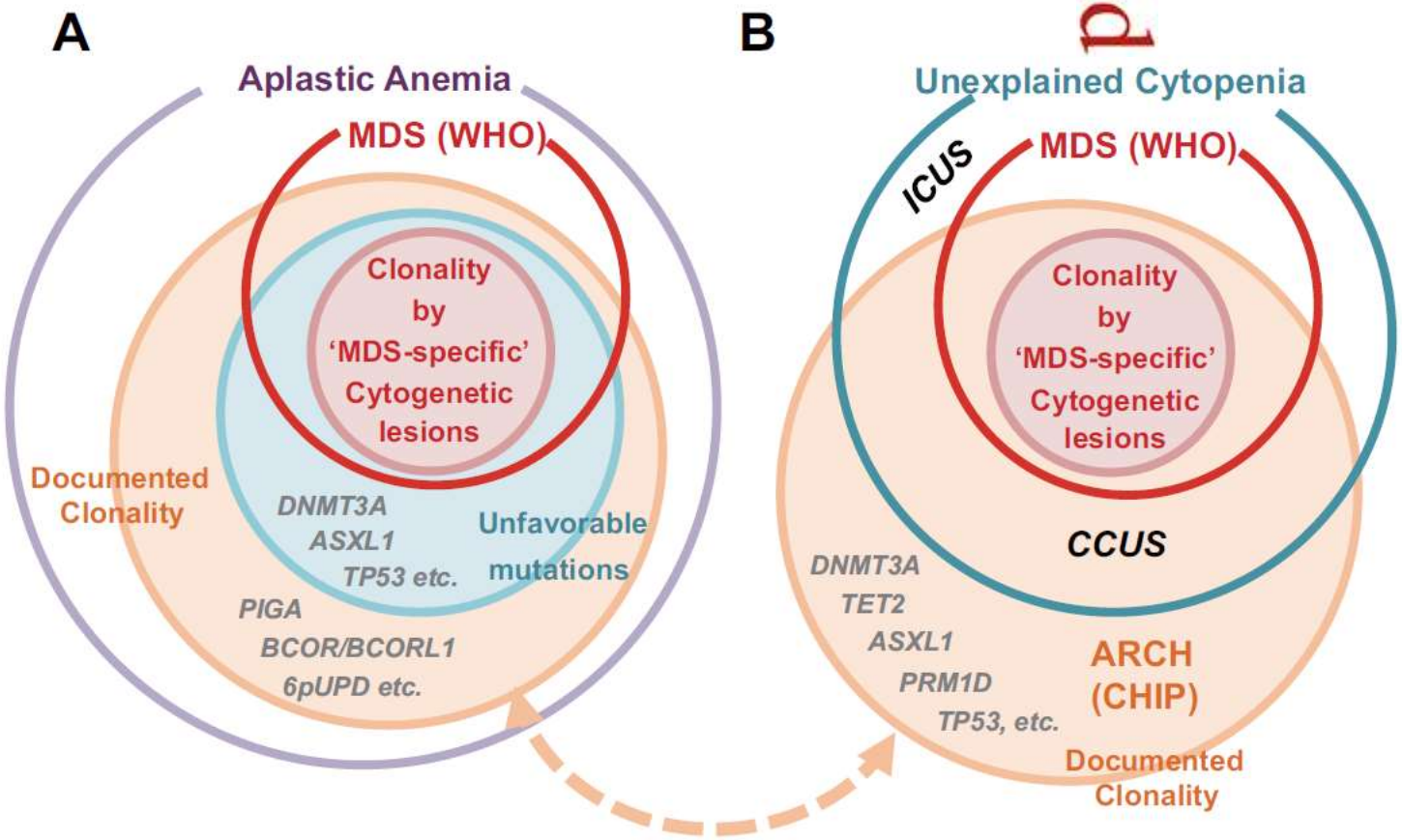
Clonal hematopoiesis in acquired aplastic anemia

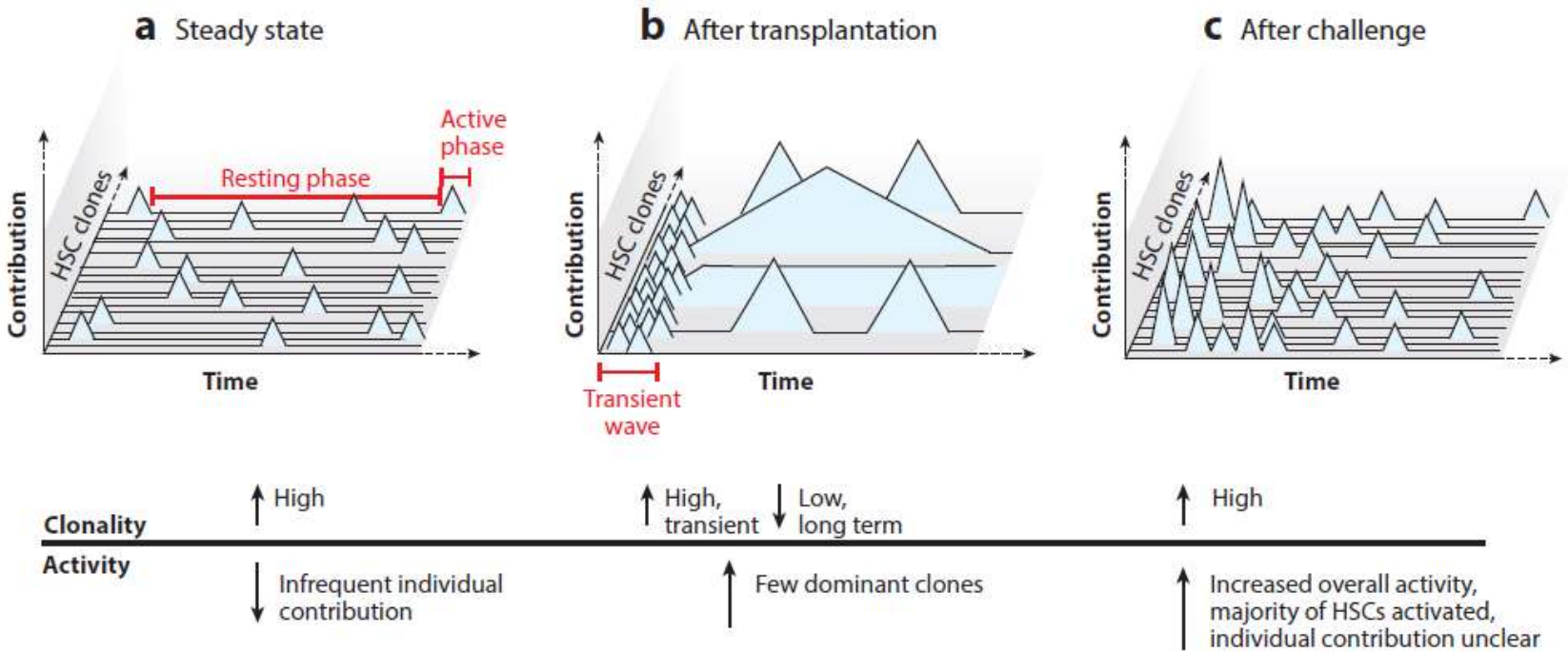
S. OGAWA; Blood 2016, in press



Fate Mapping and Quantitation of Hematopoiesis In Vivo

Thomas Höfer,[1] Katrin Busch,[2] Kay Klapproth,[2] and Hans-Reimer Rodewald[2]

Annual Review of Immunology 2016



RESEARCH ARTICLE

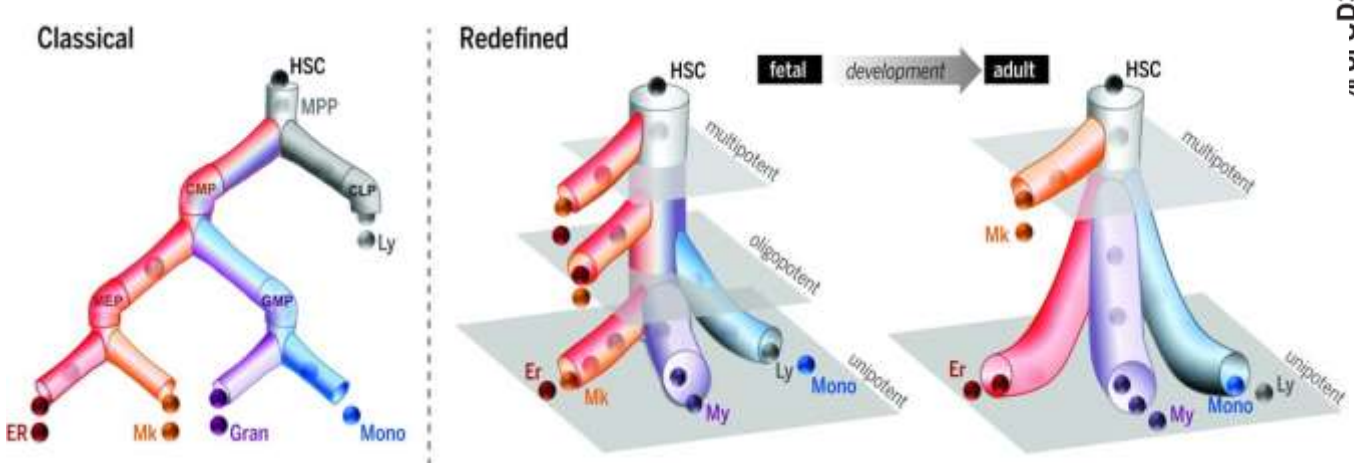
HEMATOPOIESIS

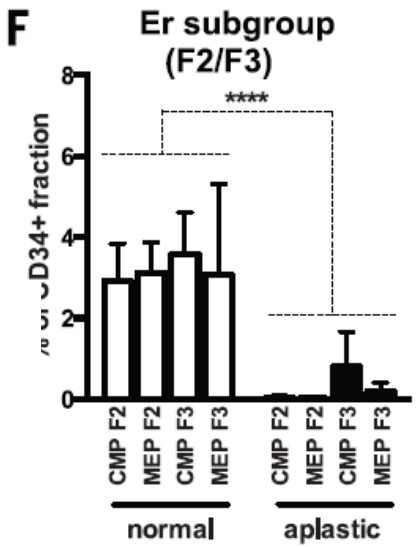
Science AAAS

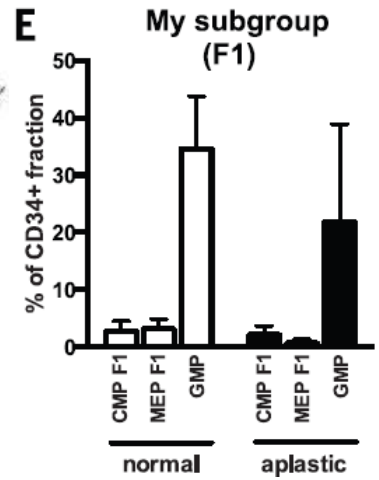
8 JANUARY 2016 • VOL 351 ISSUE 6269 139

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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ET DE LA SANTÉ

Merci pour votre attention !

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