SIGNIFICANCE OF SOMATIC MUTATIONS IN BONE MARROW FAILURE SYNDROMES

Lucio Luzzatto
Professor of Hematology
Muhimbili University Hospital,
Dar-es-Salaam, TANZANIA

AA-MDS FOUNDATION
March 17, 2016 - Rockville, MD, USA
Significance of somatic mutations in *acquired* bone marrow failure syndromes

Now we know that all 3 are clonal diseases
What’s in a word?

- Clonal
- Monoclonal
- Oligoclonal
- Clonal abundance, *relative*
- Clonal abundance, *absolute*
- Clonal evolution
CLONAL EVOLUTION IN HAEMATOLOGY

- MGUS → MM
- CML → Blast crisis
- MPD → AML
- LGL → Richter syndrome
- MDS → AML
What’s in a word?

- Clonal
- Monoclonal
- Oligoclonal
- Clonal abundance, *relative*
- Clonal abundance, *absolute*
- Clonal evolution
- Clonal dominance
- Clonal disease
- Presence of clones *versus* disease caused by a clone
- Clonal *by default*
PNH
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.
Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria

Junji Takeda*, Toshio Miyata*, Kazuyoshi Kawagoe*, Yoshiharu Iida* 1, Yuichi Endo 1, Teizo Fujita 4, Minoru Takahashi*, Tsuru Kitanishi, Teich Kinoishi*
Mutations in the PIG-A gene causing paroxysmal nocturnal hemoglobinuria are mainly of the frameshift type.

Nafa K, Mason PJ, Hillmen P, Luzzatto L, Bessler M.

Paroxysmal Nocturnal Haemoglobinuria: A Replacement of Haematopoietic Tissue?

(From Schrezenmeier et al, Acta Haematol 103:41, 2000)
DYNAMICS OF PNH CLONES IN PNH PATIENTS

(From Araten et al., Leukaemia 16:2242, 2002)
Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria

Wenyi Shen,1,2 Michael J. Clemente,1 Naoko Hosono,1 Kenichi Yoshida,1 Bartlomiej Przychodzen,1 Tetsuichi Yoshizato,1
Yuichi Shiraishi,4 Satoru Miyano,4,5 Seishi Ogawa,1 Jaroslaw R. Maciejewski,1 and Hideki Makishima1

1Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA. 2Department of Hematology, The First Affiliated Hospital of Nanyang Medical University, Nanyang, Jiangsu, China. 3Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan. 4Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
PNH: DEEP SEQUENCING IN 10 PATIENTS

- Gene mutated in 10/10  \textit{PIGA}
- Number of genes mutated, other than \textit{PIGA}, per patient  2-6
- Genes other than \textit{PIGA} recurrently mutated in more than one patient  0
- Examples of mutated genes other than \textit{PIGA}  \textit{TET2, SLC20A1}

Compiled from the data by Shen et al, \textit{JCI} \textbf{124}:4529, 2014
In PNH the expansion of the GPI-negative (PNH) clone depends on a PIGA mutation

(From Shen et al, JCI 124:4529, 2014)
Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia

## GENES RECURRENTLY MUTATED IN AA

*(From Yoshizato et al., NEJM 373:35, 2015)*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Conditions associated with germ-line mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCOR*</td>
<td>BCL6 transcription co-repressor</td>
<td>Familial MDS</td>
</tr>
<tr>
<td>PIGA*</td>
<td>GPI biosynthesis</td>
<td>Severe CNS malformations</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>DNA methylation</td>
<td>Tatton-Brown-Rahman</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Polycomb-related</td>
<td>Bohring-Opitz syndrome</td>
</tr>
<tr>
<td>JAK2</td>
<td>MAPK pathway</td>
<td>Thrombocythaemia</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Polycomb</td>
<td>Familial MDS</td>
</tr>
<tr>
<td>TP53</td>
<td>‘Patrol of the genome’; and more</td>
<td>Li-Fraumeni syndrome</td>
</tr>
<tr>
<td>BCORL1*</td>
<td>Paralogue of BCOR</td>
<td>?intellectual diversity</td>
</tr>
</tbody>
</table>

* X-linked
DYNAMICS OF HEMATOPOIETIC CLONES IN A PATIENT WITH AA

(From Yoshizato et al., *NEJM* 373:35, 2015)
The quality of clones in AA marrows influences clinical course

(From Yoshizato et al., NEJM 373:35, 2015)
MDS
Table 2. Genes with Recurrent Mutations in De Novo AML Detected in MDS and sAML Genomes.

<table>
<thead>
<tr>
<th>Gene and Mutation</th>
<th>Present in MDS Sample</th>
<th>Present in sAML Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH23 (1235insL)†</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>NPM1 (W288fs)‡</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PTPN11 (G60R)‡</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>RUNXI</td>
<td>G170R</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>del(21) (q22.11)</td>
<td>Yes</td>
</tr>
<tr>
<td>SMC3 (ex8-1 splice)†</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>STAG2 (H738fs)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TP53 (V272M)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>U2AF1 (S34F)‡</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>UMODL1†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T531P</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>V382M</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>WT1 (D436E)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>ZSWIM4 (P838A)†</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

(From Walter et al., NEJM 366:1090, 2012)
GENOME ANALYSIS INDICATES THAT MDS IS OLIGOCLONAL

(From Walter et al., NEJM 366:1090, 2012)
There is extensive overlap in the list of genes with somatic mutations in hematopoietic clones in different groups of patients.

CLONAL EVOLUTION FROM MDS TO AML

(From Walter et al., NEJM 366:1090,2012)
Population Genetics & Somatic Cell Genetics
POPULATION GENETICS IS APPLICABLE TO POPULATION OF CELLS

<table>
<thead>
<tr>
<th>Events/processes</th>
<th>In populations of organisms</th>
<th>In populations of somatic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Creates a mutant individual/family</td>
<td>Creates a mutant cell/clone</td>
</tr>
<tr>
<td>Lethal mutation</td>
<td>No offspring</td>
<td>No clonal growth</td>
</tr>
<tr>
<td>Neutral mutation</td>
<td>No visible change</td>
<td>No visible change</td>
</tr>
<tr>
<td>Mutation with absolute advantage</td>
<td>Mutant people will gradually take over</td>
<td>Clone will grow faster than other cells</td>
</tr>
<tr>
<td>Mutation with conditional advantage</td>
<td>Mutant people will increase in a certain environment</td>
<td>Clone will grow faster under certain conditions</td>
</tr>
</tbody>
</table>
Two major phenomena influence the frequency of a gene in a population of organisms or of a mutant clone in a population of somatic cells.
GENETIC DRIFT
versus DARWINIAN SELECTION
IN HUMAN POPULATIONS

**DRIFT**
- Mutant gene identical wherever it spreads
- Gene frequency may correlate with history more than with geography
- Effects magnified by small population size (bottlenecks)
- Variance analysis ($F_{ST}$) flat

**SELECTION**
- Several genes giving similar phenotype may be involved
- Gene frequency may correlate with geography more than with history
- Relatively insensitive to population size
- Variance analysis peaked ($F_{ST}$ anomalous)
Population Size of Tristan da Cunha on Dec. 31 of each year from 1816 to 1960
An example of genetic drift that can give the impression of Darwinian selection.
In any population gene frequencies are subject to drift; and the smaller the population the more dramatic will be the impact of genetic drift.
<table>
<thead>
<tr>
<th>Fitness ratio (mutant/wt)</th>
<th>Relative size of mutant population (mutant clone) after $n$ generations (cell divisions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

ADVANTAGEOUS MUTANTS versus NEUTRAL MUTANTS

Relative size of mutant population (mutant clone) after $n$ generations (cell divisions)
PATHOGENESIS OF PNH

(facts and speculations)

Time

- PIG-A plus blood cell
- PIG-A minus blood cell
- PIG-A plus damaged blood cell
- Auto-immune attack
About clones: Questions from the Chair

1. Are these clones long-lived or ephemeral?
2. Are the mutant genes that identify these clones actually expressed?
3. Can the same mutant gene be pre-leukemic in one context and not in another context?
4. PIGA versus JAK2 or BCR-ABL: which one comes first?
5. Could it be that a mutant gene is or is not picked up depending on marrow cellularity and depth of sequencing?
6. When there is BMF should we treat that or the clones that we find nevertheless?
# ACQUIRED BONE MARROW FAILURE SYNDROMES

*(pathophysiology outrageously simplified)*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Demise of ‘normal’ HSCs</th>
<th>Cause of demise</th>
<th>Somatic mutations</th>
<th>Size of clone(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNH</td>
<td>++</td>
<td>Auto-immune attack on GPI</td>
<td>PIGA</td>
<td>Large</td>
</tr>
<tr>
<td>AA</td>
<td>+++</td>
<td>Auto-immune attack on GPI or other target</td>
<td>Many</td>
<td>Small</td>
</tr>
<tr>
<td>MDS</td>
<td>+/++++</td>
<td>Telomere attrition? Age-related exhaustion?</td>
<td>Many</td>
<td>Small to large</td>
</tr>
</tbody>
</table>
THANK YOU!

Ibadan
S B ONI
B O OSUNKOYA
G J F ESAN

Napoli
BRUNO ROTOLI
FIORELLA ALFINITO

London
PETER HILLMEN
MONICA BESSLER
DAVID SWIRSKY
INDERJEET DOKAL
PHILIP MASON
MARY F MCMULLIN

Firenze
ROSARIO NOTARO
BENEDETTA PERUZZI
GIANGIACOMO GIANFALDONI
LUCIA GARGIULO

Genova
ROSARIO NOTARO
LUCIA GARGIULO
ALESSANDRO POGGI
GIANFRANCO GAETANI
CARLO FERRO
ANDREA BACIGALUPO

New York
MONICA BESSLER
ANASTASIOS KARADIMITRIS
DAVID ARATEN
ROSARIO NOTARO
KHEDOUJA NAFA
VITTORIO ROSTI
PIERPAOLO PANDOLFI