Clonal Cytopenia and Myeloid Neoplasms

Luca Malcovati, MD
Department of Molecular Medicine, University of Pavia Medical School, & Department of Hematology Oncology, IRCCS Policlinico S. Matteo Foundation, Pavia, Italy
Outline

• Pathophysiology of MDS, principles of diagnosis and classification

• The shadowlands of MDS: cytopenia of undetermined significance, hypocellular bone marrow with hematopoietic clone(s), unexplained anemia of the elderly

• Beyond cytopenia: clonal hematopoiesis of indeterminate potential

• Genetic ontogeny and natural history of age-related hematopoietic clones
Current understanding of the pathophysiology of myelodysplastic syndromes

WHO approach to the classification of tumors of hematopoietic and lymphoid tissues

Evidence of myelodysplasia

MDS

Proof of clonal hematopoiesis
Cytopenia is a *sine qua non* for any MDS diagnosis. At least 1 cytopenia must be present in order to make the diagnosis.

Dysplastic features in at least 10% of the bone marrow nucleated cells in at least one bone marrow lineage.

AND / OR

Specific chromosomal abnormality.
### Chromosomal abnormalities in primary MDS

#### MDS Unclassified
- Persistent cytopenia(s)
- BM blasts <5%
- Unequivocal dysplasia <10% in one or more myeloid lineages
- Cytogenetic abnormality considered as presumptive evidence of MDS

#### Table 4. Recurrent chromosomal abnormalities that provide presumptive evidence of primary MDS

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−5 or del(5q)</td>
<td>10-15</td>
</tr>
<tr>
<td>−7 or del(7q)</td>
<td>10</td>
</tr>
<tr>
<td>i(17q) or t(17p)</td>
<td>2-3</td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td>1-2</td>
</tr>
<tr>
<td>del(11q)</td>
<td>1-2</td>
</tr>
<tr>
<td>−13 or del(13q)</td>
<td>1-2</td>
</tr>
<tr>
<td>del(9q)</td>
<td>1</td>
</tr>
<tr>
<td>idel(X)(q13)</td>
<td>1</td>
</tr>
<tr>
<td>inv(3)(q21q26.2)</td>
<td>1</td>
</tr>
<tr>
<td>t(5;9)(p22;q34)</td>
<td>1</td>
</tr>
<tr>
<td>t(3;21)(q26.2;q22.1)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>t(1;3)(p36.3;q21.2)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>t(11;16)(p23;q13.9)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>t(2;11)(p21;q23)</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Landscape of somatic mutations in MDS

### Diagnostic value of somatic mutation analysis

#### Negative predictive value 95% CI
- No genetic lesions: 0.92 (0.88-0.95)

#### Positive predictive value 95% CI
- ≥2 mutations: 0.88 (0.84-0.92)

<table>
<thead>
<tr>
<th>VAF Cut-off</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.84</td>
<td>0.75</td>
</tr>
<tr>
<td>0.10</td>
<td>0.86</td>
<td>0.77</td>
</tr>
<tr>
<td>0.20</td>
<td>0.87</td>
<td>0.68</td>
</tr>
</tbody>
</table>

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MDS

Unexplained anemia

Cytopenia of Undetermined Significance

Genetically defined MDS

AA

Hypoplastic MDS

Bone marrow dysplasia

Normal blood count

Cytopenia

0 9 10 100
Idiopathic Cytopenia of Undetermined Significance (ICUS)

Definition criteria

- Relevant cytopenia in one or more lineage (Hb <11 g/dL, ANC<1.5x10^9/L, Plt<100x10^9/L) persistent for at least 6 months;
- Not explained by any other disease;
- No diagnostic criteria of MDS.

Valent et al. Leuk Res. 2007;31:727-736
Mutation analysis in cytopenia of undetermined significance

Clonal Cytopenia of Undetermined Significance (CCUS)

Definition criteria

• Persistent cytopenia in one or more lineage;

• Not explained by any other disease;

• No diagnostic criteria of hematological neoplasm;

• Presence of a somatic mutation associated with hematologic neoplasia (VAF ≥ 2%)
**Implications of mutation status for the diagnosis of myeloid neoplasms**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SF3B1</strong></td>
<td>0.97 (0.91-1)</td>
</tr>
<tr>
<td><strong>SRSF2</strong></td>
<td>0.93 (0.85-0.98)</td>
</tr>
<tr>
<td><strong>ZRSR2</strong></td>
<td>1 (0.95-1)</td>
</tr>
<tr>
<td><strong>U2AF1</strong></td>
<td>0.92 (0.83-0.97)</td>
</tr>
<tr>
<td><strong>RUNX1</strong></td>
<td>0.90 (0.74-0.98)</td>
</tr>
<tr>
<td><strong>EZH2</strong></td>
<td>0.97 (0.83-1.0)</td>
</tr>
<tr>
<td><strong>CUX1</strong></td>
<td>0.97 (0.83-1.0)</td>
</tr>
<tr>
<td><strong>CBL</strong></td>
<td>0.94 (0.79-0.99)</td>
</tr>
<tr>
<td><strong>BCOR</strong></td>
<td>1.0 (0.89-1.0)</td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td>0.90 (0.74-0.98)</td>
</tr>
<tr>
<td><strong>IDH1/2</strong></td>
<td>0.94 (0.89-1.0)</td>
</tr>
</tbody>
</table>

*Malcovati et al. Blood. 2017;129:3371-3378*
MDS
Unexplained anemia
Cytopenia of Undetermined Significance
Genetically defined MDS
AA
Hypoplastic MDS

Cytopenia

Normal blood count

Bone marrow dysplasia
MDS

Unexplained anemia

Genetically defined MDS

CCUS

ICUS

Unexplained anemia

AA

Hypoplastic MDS

Bone marrow dysplasia

Normal blood count

Cytopenia
Hematopoietic clones in aplastic anemia

Hypoplastic myelodysplastic syndrome: clinical, histopathological and molecular characterization

Elisa Bono, MD1*, Donal McLornan2*, Erica Travaglino3*, Shreyans Gandhi, MBBS, MD, MNAMS2*, Anna Galli3*, Alesia Abigael Khan4*, Austin G. Kulasekararaj, MBBS, MD, MRCP, FRCPath2*, Emanuela Boveri5*, Kavita Raj2*, Chiara Elena1*, Robin M. Ireland, FRCP2, Antonio Bianchessi1*, Jie Jiang2*, Gabriele Todisco1*, Luca Malcovati, MD1, Judith CW Marsh, MD6, Mario Cazzola, MD1 and Ghulam J Mufti, DM, FRCP, FRCPath2*

1Department of Molecular Medicine & Hematology Oncology, University of Pavia & IRCCS Policlinico S. Matteo Foundation, Pavia, Italy; 2Department of Haematological Medicine, King's College Hospital NHS Foundation Trust, London, United Kingdom; 3Department of Hematology Oncology, IRCCS Policlinico S. Matteo Foundation, Pavia, Italy; 4Guy’s and St Thomas’ NHS Foundation Trust, London, United Kingdom; 5Unit of Pathology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; 6Department of Haematological Medicine, Kings College Hospital, London, United Kingdom.

Integration of morphologic and genetic criteria

P<0.0001

637. Myelodysplastic Syndromes-Clinical Studies: Genes inform Genes, Morphology and Clinical Outcomes
Monday, December 11, 2017 – Time: 7.00 AM – 8.30AM
**Definition**

Peripheral blood cytopenia not attributable to causes detectable with conventional tests or to any concomitant disease

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**Malcovati L et al. Blood 2013;122:2943-2964**

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Prevalence of Clonal Hematopoiesis and Mutation Patterns in the Unexplained Anemia of Community-Dwelling Elderly Individuals: a Case-Control Study

Luca Malcovati,1,2 Anna Gallì,1 Cinzia Sala,3 Ettore Rizzo,4 Elisabetta Molteni,2 Silvia Zibellini,1 Eulalia Catamo,5 Erica Travaglino,1 Silvia Catricalà,1 Chiara Elena,1,2 Gabriele Todisco,1,2 Elisa Bono,1,2 Antonio Bianchessi,1,2 Virginia Valeria Ferretti,2 Paolo Gasparini,5 Clara Camaschella,3,6 Daniela Toniolo,3 and Mario Cazzola.1,2

1Department of Molecular Medicine, University of Pavia, Pavia, Italy; 2Department of Hematology Oncology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy; 3Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy; 4enGenome s.r.l., Pavia, Italy; 5Unit of Medical Genetics, University of Trieste & IRCCS “Burlo Garofolo”, Trieste, Italy; 6Vita Salute University, Milano, Italy.

637. Myelodysplastic Syndromes—Clinical Studies: Poster I
Saturday, December 9, 2017 - Presentation Time: 5:30 PM - 7:30 PM

Prevalence of Clonal Hematopoiesis and Mutation Patterns in the Unexplained Anemia of Community-Dwelling Elderly Individuals: a Case-Control Study

![Graph showing prevalence of clonal hematopoiesis and mutation patterns in controls and cases.](image-url)
Genetically defined MDS

CCUS

ICUS

Unexplained anemia

AA

Hypoplastic MDS
Cytopenia is a *sine qua non* for any MDS diagnosis. At least 1 cytopenia must be present in order to make the diagnosis.
Somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis

Nonrandom X-Inactivation Patterns in Normal Females: Lyonization Ratios Vary With Age
By Lambert Busque, Robert Mio, Johanne Mattioli, Elise Brais, Normand Blaise, Yves Lalonde, Marlon Maraghi, and D. Gary Gilliland

Clonal hematopoiesis without hematologic phenotype

Prevalence of clonal hematopoiesis according to age

Malcovati & Cazzola, Hematology 2015 (Data from Jaiswal et al. NEJM 2014; Genovese et al. NEJM 2014; Xie et al. Nat Med 2014)
Somatic mutations driving age-related clonal hematopoiesis

Effect of somatic mutations on the risk of hematologic cancers

Association between clonal hematopoiesis, coronary heart disease and mortality

**A. CHIP and Coronary Heart Disease**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Participants with Coronary Heart Disease/ No. at Risk</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>371,234/88,240</td>
<td>1.0 (1.0-2.3)</td>
<td>0.48</td>
</tr>
<tr>
<td>BCR</td>
<td>131/18,944</td>
<td>1.2 (1.0-1.5)</td>
<td>0.022</td>
</tr>
<tr>
<td>No mutation (reference)</td>
<td>121/16,188</td>
<td>1.0 (1.0-1.5)</td>
<td>0.98</td>
</tr>
<tr>
<td>Mutated</td>
<td>200/19,787</td>
<td>1.5 (1.1-2.2)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Fisher's exact test, p = 0.0001

**B. CHIP and Early-Onset Myocardial Infarction**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Participants with Myocardial Infarction/ No. at Risk</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF/R</td>
<td>1,366/294</td>
<td>2.0 (1.3-3.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mutation</td>
<td>1,225/27,545</td>
<td>4.4 (2.3-8.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>No mutation (reference)</td>
<td>1,225/27,545</td>
<td>1.0 (1.0-1.5)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Fisher's exact test, p < 0.0001

Hazard ratio, 1.4 (95% CI, 1.0–1.8) P = 0.03

**NEJM 2014;371:2488-2498; NEJM 2014;371:2477-2487; NEJM 2017;377:111-121**
**Definition criteria**

- Presence of a somatic mutation associated with hematological neoplasia, at a variant allele frequency of at least 2%
- Absence of persistent peripheral blood cytopenia and dysplasia
- Exclusion of other underlying conditions as primary reason for the observed mutation(s)
Distribution of mutations in clonal hematopoiesis and myeloid neoplasms

Expansion of a premalignant clone and evolution into myeloid neoplasms through subsequent mutation

**Hematopoietic stem cells** → **Driver mutation occurs** → **Expansion of hematopoietic clone** → **Development of hematopoietic cancer**

- JAK2, MPL, CALR
- SF3B1, del(5q)
- NPM1, FLT3

**MPN**
**MDS**
**AML**
Distribution of mutations in clonal hematopoiesis and myeloid neoplasms

Mutation | MDS | MPN | de novo AML
--- | --- | --- | ---
TET2 | 403 | 72 | 62
DNMT3A | 33 | 31 | 27
ASXL1 | 12 | 11 | 8
FLT3 | 31 | 31 | 31
NPM1 | 31 | 31 | 31
JAK2 | 31 | 31 | 31
CALR | 3 | 3 | 3
MPL | 1 | 1 | 1
SF3B1 | 27 | 22 | 15
SRSF2 | 24 | 22 | 15

Relationship between *SF3B1* mutation and ring sideroblasts

Quantitative enumeration of ring sideroblasts (325 MDS patients)

- 31% patients with mutation in *SF3B1*
- 97% patients with RS, 3% patients no RS

*SF3B1* mutation: positive predictive value for ring sideroblasts 97.7%

Absence of ring sideroblasts: negative predictive value for *SF3B1* mutation 97.8%

*Malcovati et al. Blood 2011;118:6239-46*
SF3B1 mutation precedes recurrent driver mutations in MDS

SF3B1 hotspot mutations induce cryptic 3’ splice site selection through use of a different branch point

### Natural history of hematopoietic clones

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CHIP</th>
<th>Unexplained Cytopenia</th>
<th>CCUS</th>
<th>MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epigenetic regulators</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Splicing Factors</strong></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Clonal Hematopoiesis of Indetermined Potential

- Genetically defined MDS
  - CCUS
  - ICUS
  - Unexplained anemia
- AA
- Hypoplastic MDS

Bone marrow dysplasia:

0 - 9 - 10 - 100

Cytopenia

Normal blood count
Clinical indication to somatic mutation analysis

- **CHIP**
- **Unexplained Cytopenia**
- **CUS**
- **MDS**

- Incidental finding in other cytopenias
- Non-myeloid cancers undergoing R/CT
- HSC donors

- Identification of subjects with CH
- Mutation patterns

- ICUS vs CCUS
- Mutation patterns

- Diagnosis (NPV; PPV)
- Classification (SF3B1)
- Prognosis
- Therapy (TP53)