Molecular abnormalities and their impact on prognosis of MDS patients

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MDS Foundation ASH symposium

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DISCLOSURE

• I have the following financial relationships:
  Consultant for: Genoptix
  Royalties from: Genoptix, Inc.

• I will not discuss off label use and/or investigational use in my presentation.
MDS: after the genomics revolution

How will we diagnose MDS?
  What is the true disease prevalence?

How will we classify disease subtypes?

How will we predict prognosis and response to therapy?

How will we develop better therapies?

Tefferi and Vardiman, NEJM 2009
MDS heterogeneity

Clonal expansion of MDS stem cell

Dysplastic hematopoietic differentiation

Peripheral cytopenias

anemia

thrombocytopenia

neutropenia
Genes mutated in MDS
Figure S1. Chemistry involved in the thymine to uracil conversion in the thymidine salvage pathway and a proposed mechanism for 5mC demethylation.

(A) Part of the thymidine salvage pathway. Thymine (T) is converted to 5-hydroxymethyl U (5hmU), 5-formyl U (5fU), and isoorotate by thymine hydroxylase (THase) in three consecutive oxidation reactions, each requiring O$_2$, α-KG, while releasing CO$_2$ and succinate. Isoorotate is then converted to uracil by isoorotate decarboxylase.

(B) Proposed mechanism of oxidative DNA demethylation initiated by TET proteins. Similar to THase, TET proteins can potentially oxidize 5mC to produce 5-hydroxymethyl C (5hmC), 5-formyl C (5fC), and 5-carboxyl C (5caC), which then may be converted to C by a decarboxylase.

Function of TET proteins

[Diagram of DNA methylation and gene expression]
Splicing factor mutations

Ebert and Bernard, *NEJM* 2011
Cohesin gene mutations

Kon et al, Nat Gen 45:1232 (2013)
MDS genomic characterization

- **Large sample set**
  - 439 samples

- **Detailed clinical annotation**
  - Cytopenias (anemia, neutropenia, thrombocytopenia)
  - Cytogenetic abnormalities
  - Percentage blasts in bone marrow
  - IPSS score and WHO/FAB classification
  - Survival
Mutations and bone marrow blasts

% of Patients with elevated blast fraction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (438)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Mut (210)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRAS (16)*</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>TP53 (33)*</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>RUNX1 (38)*</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>AXSL1 (63)</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>TET2 (90)</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>ETV6 (12)</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>EZH2 (28)</td>
<td>20%</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.006
Mutations and thrombocytopenia

![Graph showing the percentage of patients with <50k platelets/µl for different mutations](image-url)
Clinical Correlations – Mutations and Survival

- **TET2** (349<sup>wt</sup> vs. 90<sup>mut</sup>)
  - $p$-value = 0.48

- **ASXL1** (376<sup>wt</sup> vs. 63<sup>mut</sup>)
  - $p$-value = 0.003

- **RUNX1** (401<sup>wt</sup> vs. 38<sup>mut</sup>)
  - $p$-value < 0.001

- **TP53** (406<sup>wt</sup> vs. 33<sup>mut</sup>)
  - $p$-value < 0.001

- **EZH2** (411<sup>wt</sup> vs. 28<sup>mut</sup>)
  - $p$-value < 0.001

- **NRAS** (423<sup>wt</sup> vs. 16<sup>mut</sup>)
  - $p$-value = 0.006

- **ETV6** (427<sup>wt</sup> vs. 12<sup>mut</sup>)
  - $p$-value = 0.04

- **CBL** (429<sup>wt</sup> vs. 10<sup>mut</sup>)
  - $p$-value = 0.02

- **IDH2** (430<sup>wt</sup> vs. 9<sup>mut</sup>)
  - $p$-value = 0.03
**International Prognostic Scoring System (IPSS)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM blasts (%)</td>
<td>&lt;5</td>
<td>5-10</td>
<td>-</td>
<td>11-20</td>
<td>21-30</td>
</tr>
<tr>
<td>Karyotype</td>
<td>Good</td>
<td>Intermediate</td>
<td>Poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytopenias</td>
<td>0/1</td>
<td>2/3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Scores**
- Low: 0
- INT-1: 0.5 – 1
- INT-2: 1.5 – 2
- High: ≥ 2

**Cytogenetics**
- Good: normal, -Y, del(5q), del(20q)
- Poor: complex (≥ 3 abnormalities) or chromosome 7 anomalies
- Intermediate: other abnormalities

*Greenberg et al., Blood 1997*
## Risk Modeling – Multivariable Analysis IPSS

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥55 yrs vs. &lt;55 yrs</td>
<td>1.81 (1.20-2.73)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>IPSS Risk Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int1 vs. Low</td>
<td>2.29 (1.69-3.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Int2 vs. Low</td>
<td>3.45 (2.42-4.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High vs. Low</td>
<td>5.85 (3.63-9.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Mutational Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present vs. Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TP53</em> Mutation</td>
<td>2.48 (1.60-3.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>EZH2</em> Mutation</td>
<td>2.13 (1.36-3.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>ETV6</em> Mutation</td>
<td>2.04 (1.08-3.86)</td>
<td>0.029</td>
</tr>
<tr>
<td><em>RUNX1</em> Mutation</td>
<td>1.47 (1.01-2.15)</td>
<td>0.047</td>
</tr>
<tr>
<td><em>ASXL1</em> Mutation</td>
<td>1.38 (1.00-1.89)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

137/439 (31.2%) Samples carry a mutation in one or more of these genes.
Effect of mutations on IPSS

- IPSS Low (n=110)
- IPSS Int1 (n=185)
- IPSS Int2 (n=101)
- IPSS High (n=32)

- IPSS Low Mut Absent (n=87)
- IPSS Low Mut Present (n=23)

- IPSS Int1 Mut Absent
- IPSS Int1 Mut Present (n=57)

- IPSS Int2 Mut Absent (n=61)
- IPSS Int2 Mut Present (n=40)

- IPSS High (n=32)
Co-occurrence of mutations in MDS

Papaemmanuil et al., *Blood* 2013

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<table>
<thead>
<tr>
<th>Point mutations</th>
<th>Cytogenetic changes</th>
</tr>
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<tbody>
<tr>
<td>SF3B1</td>
<td></td>
</tr>
<tr>
<td>SFRS2</td>
<td></td>
</tr>
<tr>
<td>DNM1T3A</td>
<td></td>
</tr>
<tr>
<td>RUNX1</td>
<td></td>
</tr>
<tr>
<td>TET2</td>
<td></td>
</tr>
<tr>
<td>CUX1</td>
<td></td>
</tr>
<tr>
<td>IDH2</td>
<td></td>
</tr>
<tr>
<td>ASXL1</td>
<td></td>
</tr>
<tr>
<td>STAG2</td>
<td></td>
</tr>
<tr>
<td>U2AF1</td>
<td></td>
</tr>
<tr>
<td>EZH2</td>
<td></td>
</tr>
<tr>
<td>ZRSR2</td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td></td>
</tr>
<tr>
<td>BCOR</td>
<td></td>
</tr>
<tr>
<td>IDH1</td>
<td></td>
</tr>
<tr>
<td>CBL</td>
<td></td>
</tr>
<tr>
<td>TPS3</td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td></td>
</tr>
<tr>
<td>+8</td>
<td></td>
</tr>
<tr>
<td>del(5q)</td>
<td></td>
</tr>
<tr>
<td>del(5q)</td>
<td></td>
</tr>
<tr>
<td>del(7q)</td>
<td></td>
</tr>
<tr>
<td>del(12)</td>
<td></td>
</tr>
<tr>
<td>del(17p)</td>
<td></td>
</tr>
<tr>
<td>del(20q)</td>
<td></td>
</tr>
<tr>
<td>Rearr chr3</td>
<td></td>
</tr>
</tbody>
</table>

- Splicing
- DNA methylation
- Chromatin
- Signalling
- Transcription
- Other

**Co-mutated**

- q<0.1
- q<0.05
- q<0.01

**Odds ratio**

- Mutually exclusive

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Papaemmanuil et al., *Blood* 2013
TP53 mutant MDS phenotype

**TP53 mutant phenotype:**
Complex karyotype
Elevated blast count
Thrombocytopenia
Poor survival

*P < 0.0001
P = 0.83
Myelodysplasia: 5q⁻ Syndrome

Distinct haematological disorder with deletion of long arm of No. 5 chromosome


5q⁻ Syndrome

Independent WHO subtype

Phenotype:
- Refractory anemia
- Macrocytosis
- Normal/elevated platelets
- Normal/low neutrophils
- Hypolobated micromegakaryocytes
- Low rate of progression to AML
- Female predominance
Myelodysplasia: 5q− syndrome

cytokine cluster (IL3, IL4, IL5, IL13, GM-CSF)

MDS/AML

5q− syndrome

NPM1
RNA interference screen of del(5q)

## Acquired and germline ribosomal disorders

<table>
<thead>
<tr>
<th>5q- syndrome</th>
<th>Diamond Blackfan Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Acquired, somatic deletion</td>
<td>• Congenital disorder</td>
</tr>
<tr>
<td>• Refractory anemia</td>
<td>• Refractory anemia</td>
</tr>
<tr>
<td>• Macrocytosis</td>
<td>• Macrocytosis</td>
</tr>
<tr>
<td>• Predisposition to leukemia</td>
<td>• Predisposition to leukemia</td>
</tr>
<tr>
<td>• RPS14 allelic insufficiency</td>
<td>• RPS19, RPS24, RPS17, RPS7, RPL5, RPL11, RPL35A, etc. allelic insufficiency</td>
</tr>
</tbody>
</table>
Case report: del(5q) MDS

Age 5: female with severe macrocytic anemia
- Bone marrow biopsy: deficiency of erythroid progenitor cells
- Cytogenetics: 46, XX
- Diagnosis: Diamond Blackfan anemia

Age 24: continued red blood cell transfusion dependence
- Bone marrow biopsy: marked decrease in erythroid lineage, < 5% blasts

Vlachos et al., Blood 2013
<table>
<thead>
<tr>
<th>Gene</th>
<th>Inherited disorder</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPS14/RPS19</td>
<td>Diamond Blackfan anemia</td>
<td>del(5q) MDS</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Familial platelet disorder with predisposition to AML</td>
<td>MDS/AML</td>
</tr>
<tr>
<td>CEBPα</td>
<td>Inherited predisposition to AML</td>
<td>AML</td>
</tr>
<tr>
<td>GATA2</td>
<td>Familial MDS/AML</td>
<td>CML/MDS/AML</td>
</tr>
<tr>
<td>TP53</td>
<td>Li Fraumeni syndrome</td>
<td>MDS/AML</td>
</tr>
<tr>
<td>ATRX</td>
<td>α-thalassemia mental retardation syndrome</td>
<td>MDS</td>
</tr>
<tr>
<td>PTPN11, NRAS, KRAS, others</td>
<td>Noonan Syndrome</td>
<td>MDS/AML</td>
</tr>
</tbody>
</table>
Sequencing gene panels

Targeted capture of coding regions of genes known to be mutated in MDS or AML

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>DNMT3A</td>
<td>GNAS</td>
<td>LUC7L2</td>
<td>NOTCH2</td>
<td>PTPN11</td>
<td>TET2</td>
<td></td>
</tr>
<tr>
<td>ATRX</td>
<td>EED</td>
<td>IDH1</td>
<td>MAML1</td>
<td>NPM1</td>
<td>RUNX1</td>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>BCOR</td>
<td>ETV6</td>
<td>IDH2</td>
<td>MPL</td>
<td>NRAS</td>
<td>SF3A1</td>
<td>U2AF1</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>EZH2</td>
<td>JAK2</td>
<td>MYBL2</td>
<td>PHF6</td>
<td>SF3B1</td>
<td>U2AF2</td>
<td></td>
</tr>
<tr>
<td>CBL</td>
<td>FLT3</td>
<td>KIT</td>
<td>NF1</td>
<td>PRPF40B</td>
<td>SRSF2</td>
<td>WT1</td>
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<tr>
<td>CBLB</td>
<td>GATA2</td>
<td>KRAS</td>
<td>NOTCH1</td>
<td>PRPF8</td>
<td>SUZ12</td>
<td>ZRSR2</td>
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</tr>
</tbody>
</table>

Agilent SureSelect

Illumina HiSeq 2000
Clonal complexity and evolution

Walter et al., *NEJM* 2012
Summary of MDS genetics

MDS pathogenesis involves dysfunction of many cellular pathways

- Epigenetic regulators: \( TET2, ASXL1, EZH2 \)
- RNA splicing: \( SF3B1, SRSF2, U2AF1 \)
- Cohesin complex: \( STAG2, RAD21, SMC3 \)
- DNA damage response: \( TP53 \)
- Transcription factors: \( RUNX1, ETV6 \)
- Tyrosine kinase signaling: \( JAK2, NRAS, KRAS, BRAF \)
- Ribosome: haploinsufficiency for \( RPS14 \)

Mutations are powerfully associated with clinical features

- Mutations in 5 genes are independent predictors of overall survival
  - \( TP53, EZH2, ASXL1, RUNX1, ASXL1 \)
- Individual lesions associated with a specific clinical phenotype
  - Del(5q): 5q- syndrome
  - \( TP53 \): complex karyotype
  - \( SF3B1 \): RARS
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CME Post-Test

1. Mutations in which of the following genes are associated with a complex karyotype and poor prognosis in MDS?
   a. TET2
   b. TP53
   c. SF3B1

2. Multiple genes in which of the following pathways or cellular processes are recurrently mutated in MDS?
   a. RNA splicing
   b. Cohesins
   c. DNA methylation
   d. All of the above

Correct answers: 1. b  2. d
3. The hematopoietic microenvironment has **NOT** been shown to have which of the following features:
   a. Induction of myelodysplasia in humans
   b. Induction of myelodysplasia in mice
   c. Chromosomal abnormalities in patients with MDS and AML that associate with clinical outcomes

4. Mesenchymal cells of the hematopoietic microenvironment:
   a. are generally static and long-lived
   b. Are highly plastic and may undergo conversion to hematopoietic cells
   c. Are dependent on a stem cell model for cell replenishment

Correct answers: 3. a  4. c
5. Should Flow Cytometric analysis of dysplasia in MDS be part of a diagnostic approach in 2013?
   a. No, since there are no validated flow cytometric protocols available with lack of perspective clinical evaluation.
   b. Yes, in any patient with cytopenia with suspected underlying MDS.
   c. Yes, in patients with suspected MDS in the flow cytometric analysis is performed according to international guidelines.

6. Should the Results of a Flow Cytometric analysis in MDS be part of an integrated diagnostic report?
   a. No, since the flow cytometric analysis has shown independent diagnostic and prognostic value in MDS.
   b. No, since there is no relation of flow cytometric results with morphology, cytogenetic and molecular analysis.
   c. Yes, flow cytometric results add significantly in cases in which morphology and/or cytogenetics are not conclusive.

Correct answers: 5. c  6. c
7. All the following statements concerning first line treatment of MDS are true, **except one:**
   a. Erythropoietic stimulating agents (ESA) are generally the first line treatment of anemia in lower risk MDS without del 5q.
   b. Lenalidomide is generally the first line treatment of anemia in lower risk MDS with del 5q.
   c. Several drugs, when combined with hypomethylating agents (HMA), can improve survival compared to a HMA alone.
   d. About 15% of MDS patients are candidates for allogeneic stem cell transplantation.

8. All the following statements concerning second line treatments of MDS are true, **except one:**
   a. Outcome of higher risk MDS after failure/relapse from HMA treatment has recently been significantly improved by several new drugs.
   b. TP 53 mutation is frequent in lower risk MDS with del 5q, especially in patients with lenalidomide failure.
   c. The following drugs have shown some efficacy as second line treatment of lower risk MDS: HMA, Lenalidomide, antilymphocyte globulin.
   d. Most patients with lower risk MDS end up receiving regular RBC transfusions.

Correct answers: 7. c  8. a
9. Indicate the one feature which is not a critical variable related to the clinical outcome of an MDS patient:
   a. Marrow blasts
   b. Cytogenetics
   c. Depth of blood count levels
   d. Age
   e. Gender

10. Which of the following variables are also important to an MDS patient’s overall survival?
   a. Serum epo levels
   b. Specific gene mutations
   c. Lymphocytosis
   d. Number of gene mutations
   e. b and d
   f. a and c

Correct answers: 9. e  10. e
Questions/Answers/Discussion