The Molecular Genetics of Myelodysplastic Syndromes

Rafael Bejar MD, PhD

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• Shared features:
  – Ineffective differentiation and low blood counts
  – Clonal expansion of abnormal cells
  – Risk of transformation to acute leukemia

• Afflicts 15,000 – 45,000 people annually

• Incidence rises with age (mean age 71)
Myelodysplastic Syndromes

- MDS are malignancies

- But – they are different from most tumor types
  1) Can be difficult to diagnose
  2) Can be hard to classify
  3) Clinically VERY heterogeneous
     – Predicting prognosis is KEY!
Corrupted Hematopoiesis

STEM CELLS

CLP

Self-renewal

CMP

COMMITTED PROGENITORS

Pre-T cell
Pre-B cell
BFU-E
CFU-E
Meg-CFC
Mast-CFC
Eo-CFC
GM-CFC
Oc-CFC (?)

MATURE CELLS

T-Lymphocyte
B-Lymphocyte /Plasma cell
Erythrocyte
Megakaryocyte /Platelets
Basophil /Mast cell
Eosinophil
Neutrophil
Monocyte /Macrophage /Kupffer cell
Langerhans cell
Dendritic cell
Osteoclast
Splicing Factors

**SF3B1**
- Ring sideroblasts
  - RARS, RARS-T (80%+)
  - Better prognosis
  - More anemia
  - Higher MCV

**SRSF2**
- Monocytosis?
  - CMML (40%+)
  - Worse prognosis

**U2AF1**
- Linked to del(20q)?
  - Risk of AML transformation
  - Maybe worse prognosis
Clonal Evolution

Genetic Abnormalities in MDS
## Genetic Abnormalities in MDS

<table>
<thead>
<tr>
<th>Translocations/Rearrangements</th>
<th>Uniparental Disomy/Microdeletions</th>
<th>Copy Number Change</th>
<th>Point Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare in MDS</td>
<td>Rare – often at sites of point mutations</td>
<td>About 50% of cases</td>
<td>Most common</td>
</tr>
<tr>
<td>t(6;9)</td>
<td></td>
<td>del(5q)</td>
<td>Likely in all cases</td>
</tr>
<tr>
<td>i(17q)</td>
<td>4q - TET2</td>
<td>-7/del(7q)</td>
<td>~80% of cases have mutations in a known gene</td>
</tr>
<tr>
<td>t(1;7)</td>
<td>7q - EZH2</td>
<td>del(20q)</td>
<td></td>
</tr>
<tr>
<td>t(3;?)</td>
<td>11q - CBL</td>
<td>del(17p)</td>
<td></td>
</tr>
<tr>
<td>t(11;?)</td>
<td>17p - TP53</td>
<td>del(11q)</td>
<td></td>
</tr>
<tr>
<td>inv(3)</td>
<td></td>
<td>del(12p)</td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td></td>
<td>+8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Y</td>
<td></td>
</tr>
</tbody>
</table>
Point Mutations in MDS

Tyrosine Kinase Pathway
- JAK2
- KRAS
- NRAS
- BRAF
- RTK’s
- PTPN11
- CBL

Transcription Factors
- RUNX1
- ETV6
- WT1
- PHF6
- GATA2

Others
- TP53
- NPM1
- Cohesins
- RNA helicases
- GNAS
- GNB1

Epigenetic Dysregulation
- IDH 1 & 2
- DNMT3A
- EZH2
- UTX
- ASXL1
- TET2
- ATRX

Splicing Factors
- SF3B1
- U2AF1
- ZRSF2
- SF1
- SRSF2
- U2AF2
- PRPF40B
- PRPF8
- SF3A1
Myelodysplastic syndromes are diseases of the spliceosome and epigenetic regulation.

Molecular Mechanisms
Splicing factor mutations are:

- Frequent (> 50% of MDS)
- Often heterozygous
- Mutually exclusive
- Rarely mutated in AML

Mutation Frequency and Distribution

Splicing Factor Mutations

- **SF3B1**
- **SRSF2**
- **U2AF1**

Bejar et al. *JCO.* and unpublished data
Epigenetic Regulators in MDS

DNA Methylation
Methylation of CpG dinucleotides
Heritable non-coding change
Associated with gene silencing

Histone Modifications
Many types of modifications:
methylation - acetylation - phosphorylation – SUMOlation - citrullination - ribosylation
Linked to different chromatic states
Can be associated with gene silencing, priming or expression

TET2
DNMT3A
IDH 1 & 2
JAK2
SETBP1
UTX
ASXL1
EZH2
ATRX
**DNMT3A Mutations in MDS**

**De novo DNA methyltransferase**

Mutated in about 15% of cases of MDS

Mutations believed to cause loss of function

Mouse models have enhanced stem cell self-renewal and differentiation block but no dysplasia.

**TET2 Mutations in MDS**

- Cytosine → 5-methylcytosine → 5-hydroxymethylcytosine
- αKG: α-Ketoglutarate
- Fe²⁺: Iron(II) ion


**TET2 Mutations in MDS**

The most frequently mutated single gene in MDS

Mutated in about 20% of cases of MDS

Mutations believed to cause loss of function

Mouse models show hypermethylation

Myeloid differentiation bias

Enhanced stem cell self-renewal

Development of CMML like disease

**IDH1 and IDH2 Mutations in MDS**

Mutated rarely in MDS and more often in AML

Mutually exclusive with mutations of *TET2*

Mutations cause a gain of function

Mouse models have extramedullary hematopoiesis, ↑↑ progenitors, and DNA and histone hypermethylation

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**EZH2 Mutations in MDS**

Catalytic subunit of the Polycomb Repressive Complex 2 (PRC2)

Methylates lysine-27 of histone-3 (H3K27)

Mutations are believed to cause a loss of function

Mutated in over 6% of cases of MDS and 12% of CMML

Independently associated with a poorer prognosis


**ASXL1 Mutations in MDS**

Member of the polycomb gene family (like *EZH2*)

Transcriptional cofactor – associates with PRC2

Mutated in over 14% of cases of MDS and ~40% of CMML

Mutations are believed to cause a loss of function

Independently associated with a poorer prognosis

Tyrosine Kinase Signaling in MDS

Uncommon in MDS cases as a whole: ~ 10%

Activating NRAS mutations associated with:
- elevated blast proportion (> 5%)
- severe thrombocytopenia (< 50,000 per µl)
- poorer prognosis
- more common in CMML

CBL and CBLB mutations associated with:
- monocytosis
- possibly poor prognosis
- < 5% of all MDS
- more common in CMML

JAK2 V617F mutations associated with:
- no polycythemia
- no effect on overall survival
- < 5% of all MDS
- ~50% of RARS-T (same as ET)
Diagnosing MDS
Cytopenia(s):
- Hb <11 g/dL, or
- ANC <1500/μL, or
- Platelets <100 x 10⁹L

MDS “decisive” criteria:
- >10% dysplastic cells in 1 or more lineages, or
- 5-19% blasts, or
- Abnormal karyotype typical for MDS, or
- Evidence of clonality (by FISH or another test)

Other causes of cytopenias and morphological changes EXCLUDED:
- Vitamin B12/folate deficiency
- HIV or other viral infection
- Copper deficiency
- Alcohol abuse
- Medications (esp. methotrexate, azathioprine, recent chemotherapy)
- Autoimmune conditions (ITP, Felty syndrome, SLE etc.)
- Congenital syndromes (Fanconi anemia etc.)
- Other hematological disorders (aplastic anemia, LGL disorders, MPN etc.)
# Things That Aren’t Quite MDS

## Idiopathic Cytopenia(s) of Undetermined (Uncertain) Significance (*ICUS*)

<table>
<thead>
<tr>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Meaningful <strong>cytopenias</strong></td>
</tr>
<tr>
<td>2) <strong>Does not meet minimal diagnostic criteria for MDS</strong></td>
</tr>
<tr>
<td>• &gt; 10% <em>dysplastic cells</em>, or</td>
</tr>
<tr>
<td>• 5%-19% <em>blasts</em>, or</td>
</tr>
<tr>
<td>• <em>Abnormal karyotype typical for MDS</em>, or</td>
</tr>
<tr>
<td>• <em>Evidence of clonality</em></td>
</tr>
<tr>
<td>3) Other causes of cytopenias ruled out</td>
</tr>
</tbody>
</table>

## Idiopathic Dysplasia of Undetermined (Uncertain) Significance (*IDUS*)

<table>
<thead>
<tr>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal CBC or minimal cytopenias</td>
</tr>
<tr>
<td>2) <strong>Does not meet minimal diagnostic criteria for MDS</strong></td>
</tr>
<tr>
<td>3) <strong>Dysplastic changes of unknown significance</strong> present, such as</td>
</tr>
<tr>
<td>1) Pseudo–Pelger-Huët cells</td>
</tr>
<tr>
<td>2) Neutrophil hypogranularity</td>
</tr>
<tr>
<td>3) Megaloblastic changes in erythroid cells</td>
</tr>
</tbody>
</table>

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Slide borrowed from Dr. David Steensma.
Classification of MDS Subtypes
<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Blood findings</th>
<th>Bone Marrow findings</th>
</tr>
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<tbody>
<tr>
<td>Refractory cytopenia with unilineage dysplasia (RCUD)</td>
<td>RCUD</td>
<td>• Unicytopenia; occasionally bicytopenia</td>
<td>• Unilineage dysplasia (≥10% of cells in one myeloid lineage)</td>
</tr>
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<td></td>
<td></td>
<td>• No or rare blasts (&lt;1%)</td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• &lt;15% of erythroid precursors are ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts (RARS)</td>
<td>RARS</td>
<td>• Anemia</td>
<td>• ≥15% of erythroid precursors are ring sideroblasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No blasts</td>
<td>• Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• &lt;5% blasts</td>
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<td>MDS associated with isolated del(5q)</td>
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<td>• Anemia</td>
<td>• Isolated 5q31 deletion</td>
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<td>• Usually normal or increased platelet count</td>
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<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No Auer rods</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
<td>RCMD</td>
<td>• Cytopenia(s)</td>
<td>• ≥10% of cells in ≥2 myeloid lineages dysplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No or rare blasts (&lt;1%)</td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No Auer rods</td>
<td>• No Auer rods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;1 x 10⁹/L monocytes</td>
<td>• ±15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts, type 1 (RAEB-1)</td>
<td>RAEB-1</td>
<td>• Cytopenia(s)</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;5% blasts</td>
<td>• 5-9% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No Auer rods</td>
<td>• No Auer rods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;1 x 10⁹/L monocytes</td>
<td></td>
</tr>
<tr>
<td>Refractory anemia with excess blasts, type 2 (RAEB-2)</td>
<td>RAEB-2</td>
<td>• Cytopenia(s)</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 5-19% blasts</td>
<td>• 10-19% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ±Auer rods</td>
<td>• ±Auer rods</td>
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<td>• &lt;5% blasts</td>
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<tr>
<td>Refractory anemia with ring sideroblasts and thrombocytosis</td>
<td>RARS-T</td>
<td>• Anemia</td>
<td>• ≥15% of erythroid precursors are ring sideroblasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No blasts</td>
<td>• Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ≥450 x 10⁹/L platelets</td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Proliferation of large megakaryocytes</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia, type 1</td>
<td>CMML-1</td>
<td>• &gt;1 x 10⁹/L monocytes</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;5% blasts</td>
<td>• &lt;10% blasts</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia, type 2</td>
<td>CMML-2</td>
<td>• &gt;1 x 10⁹/L monocytes</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 5%-19% blasts or Auer rods</td>
<td>• 10%-19% blasts or Auer rods</td>
</tr>
<tr>
<td>Atypical chronic myeloid leukemia</td>
<td>aCML</td>
<td>• WBC &gt; 13 x 10⁹/L</td>
<td>• Hypercellular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Neutrophil precursors &gt;10%</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;20% blasts</td>
<td>• BCR-ABL1 negative</td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukemia</td>
<td>JMML</td>
<td>• &gt;1 x 10⁹/L monocytes</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;20% blasts</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• BCR-ABL1 negative</td>
</tr>
<tr>
<td>MDS/MPN – unclassified (‘Overlap Syndrome’)</td>
<td>MDS/MPN-U</td>
<td>• Dysplasia with myeloproliferative features</td>
<td>• Dysplasia with myeloproliferative features</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No prior MDS or MPN</td>
<td></td>
</tr>
</tbody>
</table>

World Health Organization
AML/MDS Classification (2008)

- Acute myeloid leukemia and related neoplasms:
  - Acute myeloid leukemia with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22); **RUNX1-RUNX1T1**
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); **CBFB-MYH11**
  - APL with t(15;17)(q22;q12); **PML-RARA**
  - AML with t(9;11)(p22;q23); **MLLT3-MLL**
  - AML with t(6;9)(p23;q34); **DEK-NUP214**
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); **RPN1-EVI1**
  - AML (megakaryoblastic) with t(1;22)(p13;q13); **RBM15-MKL1**
  - Provisional entity: AML with mutated **NPM1**
  - Provisional entity: AML with mutated **CEBPA**
  - Acute myeloid leukemia with myelodysplasia-related changes
  - Therapy-related myeloid neoplasms
  - Acute myeloid leukemia, not otherwise specified
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<td>Unilineage dysplasia (≥10% of cells in one myeloid lineage) • &lt;5% blasts • &lt;15% of erythroid precursors are ring sideroblasts</td>
</tr>
<tr>
<td></td>
<td>Refractory neutropenia (RN)</td>
<td>• Anemia • No Auer rods • &lt;1 x 10⁹/L monocytes</td>
<td>≥15% of erythroid precursors are ring sideroblasts • Erythroid dysplasia only • &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>Refractory thrombocytopenia (RT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts</td>
<td>RARS</td>
<td>Anemia • Usually normal or increased platelet count • No or rare blasts (&lt;1%)</td>
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<td>Anemia • Usually normal or increased platelet count • No or rare blasts (&lt;1%)</td>
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<td>Refractory cytopenia with multilineage dysplasia</td>
<td>RCMD</td>
<td>Cytopenia(s) • No or rare blasts (&lt;1%) • No Auer rods • &lt;1 x 10⁹/L monocytes</td>
<td>≥10% of cells in ≥2 myeloid lineages dysplastic • &lt;5% blasts • No Auer rods • ±15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts, type 1</td>
<td>RAEB-1</td>
<td>Cytopenia(s) • &lt;5% blasts • No Auer rods • &lt;1 x 10⁹/L monocytes</td>
<td>Unilineage or multilineage dysplasia • 5-9% blasts • No Auer rods</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts, type 2</td>
<td>RAEB-2</td>
<td>Cytopenia(s) • 5-19% blasts • ±Auer rods • &lt;1 x 10⁹/L monocytes</td>
<td>Unilineage or multilineage dysplasia • 10-19% blasts • ±Auer rods</td>
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<td>MDS - unclassified</td>
<td>MDS-U</td>
<td>Cytopenia(s) • ≤1% blasts</td>
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Prognostic Risk Assessment
# International Prognostic Scoring System

<table>
<thead>
<tr>
<th>Cytogenetic Risk Group</th>
<th>IPSS Karyotype Abnormalities (7 categories)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>Normal, -Y, del(5q), del(20q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>+8, any other single or double abnormality</td>
</tr>
<tr>
<td>Poor</td>
<td>Complex with ≥ 3 abnormalities, anomaly of chromosome 7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IPSS Parameter</th>
<th>Categories and Associated Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetic Risk Group</td>
<td>Good</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Bone Marrow Blast %</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Number of Cytopenias</td>
<td>0 or 1</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Definition of Cytopenias**
- Hemoglobin < 10 g/dL
- Neutrophil Count < 1.80 x 10^9/L
- Platelet Count < 100 x 10^9/L

<table>
<thead>
<tr>
<th>IPSS Risk Group</th>
<th>Points</th>
<th>% of Patients</th>
<th>Median survival, years</th>
<th>Time to 25% with AML, years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>33%</td>
<td>5.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>0.5 - 1</td>
<td>38%</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>1.5 - 2</td>
<td>22%</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>High</td>
<td>≥ 2.5</td>
<td>7%</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

International Prognostic Scoring System

LOWER Risk
- Low
- Int-1

HIGHER Risk
- Int-2
- High

Overall Survival, Years
- Patients, %
- n = 56
- n = 179
- n = 267
- n = 314

Time to AML Evolution, Years
- Patients, %
- n = 58
- n = 171
- n = 235
- n = 295
International Prognostic Scoring System

**Lower Risk**
- Observation
- EPO
- Lenalidomide
- Immune suppression
- Iron Chelation

**Higher Risk**
- Azacitidine
- Decitabine
- Allo-HSCT
- Clinical Trials
Limitations of the IPSS

• Includes 21-30% blasts – redefined as AML by WHO in 2001

• Includes few cytogenetic abnormalities

• Counts the number of cytopenias – ignores their severity

• As a consequence – the IPSS may underestimate risk

### Lower Risk Model (LR-PSS)

<table>
<thead>
<tr>
<th>Prognostic Category</th>
<th>LR-PSS Prognostic Score Value</th>
<th>Risk Category</th>
<th>Risk Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>Not normal or del(5q)</td>
<td>1</td>
<td>0-2</td>
</tr>
<tr>
<td>Age, years</td>
<td>≥ 60</td>
<td>2</td>
<td>3-4</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>&lt; 10</td>
<td>3</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Platelets, × 10⁹/L</td>
<td>50-200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM blasts, %</td>
<td>≥ 4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Category 1 (n=57)**
- **Category 2 (n=160)**
- **Category 3 (n=71)**

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Included karyotypes (19 categories)</th>
<th>Median survival, months</th>
<th>Proportion of patients in this group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>del(11q), -Y</td>
<td>60.8</td>
<td>2.9%</td>
</tr>
<tr>
<td>Good</td>
<td>Normal, del(20q), del(5q) alone or with 1 other anomaly, del(12p)</td>
<td>48.6</td>
<td>65.7%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>+8, del(7q), i(17q), +19, +21, any single or double abnormality not listed, two or more independent clones</td>
<td>26.1</td>
<td>19.2%</td>
</tr>
<tr>
<td>Poor</td>
<td>der(3q), -7, double with del(7q), complex with 3 abnormalities</td>
<td>15.8</td>
<td>5.4%</td>
</tr>
<tr>
<td>Very poor</td>
<td>Complex with &gt; 3 abnormalities</td>
<td>5.9</td>
<td>6.8%</td>
</tr>
</tbody>
</table>

Cytogenetic Abnormalities

Percentage of Patients With Normal Cytogenetics

- Normal: 58%
- Abnormal: 42%

Percentage of Patients With Normal Cytogenetics by IPSS Category

- Low/Int-1: 71%
- Int-2/High: 29%

## Scoring for the IPSS-R

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories and Associated Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetic risk group</td>
<td>Very good</td>
</tr>
<tr>
<td>Marrow blast proportion</td>
<td>≤ 2%</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>≥ 10</td>
</tr>
<tr>
<td>Platelet count (x 10⁹/L)</td>
<td>≥ 100</td>
</tr>
<tr>
<td>Abs. neutrophil count (x 10⁹/L)</td>
<td>≥ 0.8</td>
</tr>
</tbody>
</table>

Possible range of summed scores: 0-10

## Risk Groups for the IPSS-R

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Points</th>
<th>% of Patients</th>
<th>Median survival, years</th>
<th>Time until 25% of patients develop AML, years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>≤ 1.5</td>
<td>19 %</td>
<td>8.8</td>
<td>Not reached</td>
</tr>
<tr>
<td>Low</td>
<td>&gt; 1.5 – 3</td>
<td>38 %</td>
<td>5.3</td>
<td>10.8</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&gt; 3 – 4.5</td>
<td>20 %</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>High</td>
<td>&gt; 4.5 – 6</td>
<td>13 %</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Very High</td>
<td>&gt; 6</td>
<td>10 %</td>
<td>0.8</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Age Dependence of the IPSS-R

Limitations of the IPSS-R

• Less than half of patients have relevant cytogenetic abnormalities

• Heterogeneity remains within each risk category

• Excludes therapy related disease and CMML

The IPSS does not include molecular abnormalities

Challenges to Interpreting Genetic Test Results
Abnormalities often overlap!

Mutations may be very rare!

Challenges

Not all mutations are created equal!
Challenges

Mutations are not binary!

Variant Allele Frequencies by Mutated Gene
Coping with Clonality

ASXL1

Not mutated
Subclonal mutant
Clonal mutation

Leukemia-free survival

Time (months)

p=0.5
Clonal vs subclonal

RUNX1

Not mutated
Subclonal mutant
Clonal mutation

Leukemia-free survival

Time (months)

p=0.8
Clonal vs subclonal

Improved Sensitivity – Early Warning?

MDS

AML

Challenges

How to combine mutations and clinical features

- Thrombocytopenia
- Excess Blasts
- Monocytosis
- Complex Karyotype
- RUNX1
- TP53
- NRAS
- CBL
- TET2
- EZH2

Can Mutation Testing Be Useful at All?
Impact of Mutations by IPSS Group

Bejar et al. NEJM. 2011;364:2496-506.
Mutations Alone Can Be Prognostic

### Mutations Alone Can Be Prognostic

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Percent Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>10.00</td>
</tr>
<tr>
<td>ETV6</td>
<td>3.04</td>
</tr>
<tr>
<td>EZH2</td>
<td>2.04</td>
</tr>
<tr>
<td>RUNX1</td>
<td>1.85</td>
</tr>
<tr>
<td>ASXL1</td>
<td>1.84</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>1.69</td>
</tr>
<tr>
<td>NRAS/KRAS/BRAF</td>
<td>1.65</td>
</tr>
<tr>
<td>NPM1</td>
<td>1.51</td>
</tr>
<tr>
<td>SRSF2</td>
<td>1.41</td>
</tr>
<tr>
<td>U2AF1</td>
<td>1.39</td>
</tr>
<tr>
<td>TET2</td>
<td>1.37</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>1.22</td>
</tr>
<tr>
<td>JAK2</td>
<td>1.19</td>
</tr>
<tr>
<td>SF3B1</td>
<td>1.07</td>
</tr>
<tr>
<td>None of the above</td>
<td>1.00</td>
</tr>
</tbody>
</table>
## Mutation Frequency and Distribution

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>High</td>
</tr>
<tr>
<td>SF3B1</td>
<td>Moderate</td>
</tr>
<tr>
<td>SRSF2</td>
<td>Low</td>
</tr>
<tr>
<td>U2AF1</td>
<td>Very low</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>Low</td>
</tr>
<tr>
<td>TET2</td>
<td>Very low</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Low</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Low</td>
</tr>
<tr>
<td>EZH2</td>
<td>Low</td>
</tr>
<tr>
<td>Tyrosine Kinase Pathway</td>
<td>Low</td>
</tr>
</tbody>
</table>
The adverse prognostic impact of the complex karyotype is entirely driven by its frequent association with mutations of $TP53$. 

$TP53$ Mutations and Complex Karyotypes
72 MDS patients transplanted at DFCI from 2004-2009

Karyotype:
- Normal (33%)
- Complex (32%)
- -7/del(7q) (15%)
- Unknown (8%)

FAB Classification:
- RA 24%
- CMML 7%
- RAEBT 2%
- RAEB 51%
- RARS 8%
- Other 8%

Conditioning Regimen:
- Cy/TBI 31%
- Other 4%
- Bu/Flu 56%
- Other 1%

Patient/Donor Sexes:
- MM 39%
- FM 28%
- FF 23%
- MM 10%

Reduced Intensity
Ablative
Clinical Associations

Overall Survival After Transplant

Blasts < 5% (n=35)
Blasts ≥ 5% (n=37)

\[ p = 0.014 \]

Other karyotype (n=49)
Complex karyotype (n=23)

\[ p < 0.001 \]

Kristen Stevenson and Donna Neuberg
Genetic Associations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Adjusted HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 (n=14)</td>
<td>3.90 (1.85, 8.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DNMT3A (n=14)</td>
<td>3.54 (1.45, 8.64)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Overall Survival After Transplant

- **TP53 and DNMT3A Mut Absent (n=46)**
- **TP53 or DNMT3A Mut Present (n=26)**

\[ p < 0.001 \]

Survival in Complex +/- TP53 Mutation

- Non-Complex Karyotype (n=49)
- Complex and TP53 Mut Absent (n=11)
- Complex and TP53 Mut Present (n=12)
213 MDS patients Treated with Hypomethylating Agents
**ASXL1 and TET2 Mutations**

**Two Gene Analysis: ASXL1 and TET2**

<table>
<thead>
<tr>
<th>Gene Condition</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2 mutant + ASXL1 wt</td>
<td>2.37 (1.00, 5.58)</td>
<td>0.049</td>
</tr>
</tbody>
</table>
Response by Variant Abundance

<table>
<thead>
<tr>
<th>Gene (n)</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$TET2$ (50)</td>
<td>1.99 (1.05, 3.80)</td>
<td>0.036</td>
<td>1.98 (1.02, 3.85)</td>
<td>0.044</td>
</tr>
<tr>
<td>$TET2$ mut + $ASXL1$ wt (23)</td>
<td>3.65 (1.38, 9.67)</td>
<td>0.009</td>
<td>3.64 (1.35, 9.79)</td>
<td>0.011</td>
</tr>
</tbody>
</table>
### TP53 and Complex Karyotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Not Complex (n=106)</th>
<th>Complex without TP53 Mut (n=14)</th>
<th>Complex with TP53 Mut (n=26)</th>
<th>p = 0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TET2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TK Pathway</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SRSF2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SF3B1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>U2AF1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>RUNX1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DNMT3A</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BCOR</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>EZH2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IDH1&amp;2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ATRX</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PHF6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PRPF8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ZRSR2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NPM1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ETV6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>U2AF2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Karyotype</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Graph showing Kaplan-Meier curves for overall survival.*

- Not Complex
- Complex without TP53 Mut (n=14)
- Complex with TP53 Mut (n=26)

*p = 0.03*
Novel MDS subtypes based on somatic mutations:

- Splicing factor mutant MDS, or \textit{SF3B1} mutant MDS,
- \textit{TP53} mutant MDS, or ... ???

Incorporating genetics into prognostic scoring systems:

- IPSS-Revised Molecular (IPSS-RM)

Peripheral Blood Analysis

**Pros**
- Less invasive
- Less cost
- Can be more frequent
- No ‘dry taps’

**Cons**
- May not reflect marrow

---

**MYELOID NEOPLASIA**

Utility of peripheral blood for cytogenetic and mutation analysis in myelodysplastic syndrome

Azim M. Mohamedali, Heba Alkhatabi, Austin Kulasekararaj, Sneha Shinde, Syed Mian, Farooq Malik, Alexander E. Smith, Joop Gäken, and Ghulam J. Mufti

Department of Haematological Medicine, King’s College London, Rayne Institute, London, United Kingdom

“This pilot study showed a 100% SNP-A karyotype concordance and a 97% mutation concordance between the BM and PB.”

Newer genetic tests are quantitative – mutations could be used as response markers.
Screen for minimal residual disease in MDS as with APML and CML:

Opportunity for DLI, azacitidine, clinical trial, or other post-transplant intervention.

Identify adverse subclones that predict disease progression:

Sensitive Mutation Detection


Fast-COLD-PCR

Mutation Frequency Estimated by MALDI

Overall survival (%)

Mutant Allele Frequency (%)

Years

NPM1
GNAS
TET2
JAK2
KRAF
BRAF
PTPN11
NRAS

is unamplified

5′

3′

5′

5′

3′

5′

Genetically amplified
Genetics Summary

• Single gene mutations are the most common genetic abnormality in MDS
• Mutations can be challenging clinically, BUT ...
• Mutations can identify clonality and aid diagnosis
• Mutations can inform the prediction of prognosis
• Mutations may predict responses to therapy and outcomes after stem cell transplantation

Clinical access to genetic testing is now available!
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