The Molecular Basis of Myelodysplastic Syndromes

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Cecil J. Watson Lecture
University of Minnesota

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Myelodysplastic syndromes

**Incidence**
15,000 – 45,000 new cases / year

**Diagnosis**
Cellular morphology on bone marrow biopsy

**Molecular lesions**
Cytogenetic abnormalities
Mutations in individual genes
Epigenetic dysregulation

Tefferi and Vardiman, NEJM 2009
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
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)

Rescue of erythropoiesis with RPS14 overexpression

MDS patients with del (5q) vs. MDS patients without del (5q)

<table>
<thead>
<tr>
<th>GlyA/CD41</th>
<th>Empty</th>
<th>RPS14</th>
<th>Empty</th>
<th>RPS14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
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<tr>
<td>Patient 3</td>
<td></td>
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</tr>
<tr>
<td>Patient 4</td>
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</tbody>
</table>

Empty Vector vs. RPS14

<table>
<thead>
<tr>
<th>CD41</th>
<th>GlyA</th>
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<tbody>
<tr>
<td>51.5%</td>
<td>39.5%</td>
</tr>
<tr>
<td>8.62%</td>
<td>0.36%</td>
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<table>
<thead>
<tr>
<th>CD41</th>
<th>GlyA</th>
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<tbody>
<tr>
<td>29.2%</td>
<td>67.4%</td>
</tr>
<tr>
<td>3.2%</td>
<td>0.2%</td>
</tr>
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# Acquired and germline ribosomal disorders

<table>
<thead>
<tr>
<th>5q- syndrome</th>
<th>Diamond Blackfan Anemia</th>
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<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
Genetics of bone marrow failure

**Telomerase**
- Dyskeratosis congenita
- Aplastic anemia

**DNA damage repair**
- Fanconi anemia

**Ribososome**
- Diamond Blackfan anemia
- Del(5q) myelodysplastic syndrome
- Shwachman-Diamond syndrome
Ribosome biology

28S, 18S, 5.8S rRNAs (Pol I)
5S rRNA (Pol III)
80 proteins

40S subunit (RPS proteins)
60S subunit (RPL proteins)

Mature 80S ribosome

Eukaryotic ribosome
Ribosomal proteins required for pre-rRNA processing

45S

18S

5.8S

28S

32S

30S

21S

18SE

18S

18S

12S

28S

5.8S
RPS14 required for 5’ processing of 18S rRNA

Summary so far …

• Strong genetic evidence that heterozygous inactivation of ribosomal genes impairs erythropoiesis

• Ribosomal protein genes play specific roles in ribosome biogenesis

• Why does ribosomal protein gene haploinsufficiency cause an erythroid phenotype?
Activation of p53 by ribosomal haploinsufficiency

MDM2 binds and is inhibited by RPL11

Narla and Ebert, Blood 2010
p53 induction by RPS14 or RPS19 deficiency

Dutt et al., *Blood* 2010
p53 target gene induction

P21  Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
MDM2  Mdm2, transformed 3T3 cell double minute 2
DDB2  Damage-specific DNA binding protein 2, 48kDa
MAN2B1  Mannosidase, alpha, class 2B, member 1
GADD45A  Growth arrest and DNA-damage-inducible, alpha
BAX  BCL2-associated X protein
BTG2  BTG family, member 2
ADFP  Adipose differentiation-related protein
NIN1  Ninjurin 1
CES2  Carboxylesterase 2 (intestine, liver)
BCL6  B-cell CLL/lymphoma 6 (zinc finger protein 51)
DGKA  Diacylglycerol kinase, alpha 80kDa
CSPG2  Chondroitin sulfate proteoglycan 2 (versican)
FHL2  Four and a half LIM domains 2
FEZ1  Fasciculation and elongation protein zeta 1 (zygin I)
PLK3  Polo-like kinase 3 (Drosophila)

Dutt et al., Blood 2011
p53 induction is specific to the erythroid lineage

Dutt et al., *Blood* 2011
Activation of p53 in 5q- patient samples

Dutt et al., Blood 2011
Animal models of ribosomopathies
Murine model of 5q- syndrome

- Deletion including RPS14 causes macrocytic anemia
- Hematopoietic defects reversed in p53 null background

Barlow et al., Nat Med 2010
Leucine improves hemoglobinization in RPS19 deficient zebrafish

mTor pathway senses amino acid levels and regulates translation

Response of a Diamond Blackfan anemia patient to leucine (Pospisilova et al., Haematologica 2007)
Anatomy of del(5q)
Point mutations in MDS

Diagnosis

– Challenges of morphology
– Potential for diagnosis from peripheral blood

Prognosis / Prediction of therapeutic response

Biology and novel targets
MDS heterogeneity

Clonal expansion of MDS stem cell

Dysplastic hematopoietic differentiation

Peripheral cytopenias

anemia

thrombocytopenia

neutropenia
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
### Expanded Set (n = 439)

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
<td><strong>Sex</strong></td>
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</tr>
<tr>
<td>Male</td>
<td>306</td>
<td>69.7%</td>
</tr>
<tr>
<td>Female</td>
<td>133</td>
<td>30.3%</td>
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<table>
<thead>
<tr>
<th><strong>Age at time BM Sample Collected</strong></th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>&lt; 55</td>
<td>49</td>
<td>11.2%</td>
</tr>
<tr>
<td>55-65</td>
<td>88</td>
<td>20.0%</td>
</tr>
<tr>
<td>65-75</td>
<td>179</td>
<td>40.8%</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>123</td>
<td>28.0%</td>
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<table>
<thead>
<tr>
<th><strong>FAB Classification</strong></th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>RA</td>
<td>197</td>
<td>44.9%</td>
</tr>
<tr>
<td>RARS</td>
<td>47</td>
<td>10.7%</td>
</tr>
<tr>
<td>RAEB</td>
<td>160</td>
<td>36.4%</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>34</td>
<td>7.7%</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0.2%</td>
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</table>

<table>
<thead>
<tr>
<th><strong>IPSS Risk Group</strong></th>
<th>Count</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Low</td>
<td>110</td>
<td>25.1%</td>
</tr>
<tr>
<td>Int1</td>
<td>185</td>
<td>42.1%</td>
</tr>
<tr>
<td>Int2</td>
<td>101</td>
<td>23.0%</td>
</tr>
<tr>
<td>High</td>
<td>32</td>
<td>7.3%</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Karyotype</strong></th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>255</td>
<td>58.1%</td>
</tr>
<tr>
<td>Complex</td>
<td>57</td>
<td>13.0%</td>
</tr>
<tr>
<td>-5/5q</td>
<td>22</td>
<td>5.0%</td>
</tr>
<tr>
<td>-7/7q</td>
<td>10</td>
<td>2.3%</td>
</tr>
<tr>
<td>+8</td>
<td>24</td>
<td>5.5%</td>
</tr>
<tr>
<td>-13/13q</td>
<td>2</td>
<td>0.5%</td>
</tr>
<tr>
<td>-20/20q</td>
<td>18</td>
<td>4.1%</td>
</tr>
<tr>
<td>-Y</td>
<td>5</td>
<td>1.1%</td>
</tr>
<tr>
<td>Other</td>
<td>53</td>
<td>21.1%</td>
</tr>
</tbody>
</table>
## Frequency of mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
<th>%</th>
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<tbody>
<tr>
<td>TET2</td>
<td>90</td>
<td>21%</td>
</tr>
<tr>
<td>ASXL1</td>
<td>63</td>
<td>14%</td>
</tr>
<tr>
<td>RUNX1</td>
<td>38</td>
<td>9%</td>
</tr>
<tr>
<td>TP53</td>
<td>33</td>
<td>8%</td>
</tr>
<tr>
<td>EZH2</td>
<td>28</td>
<td>6%</td>
</tr>
<tr>
<td>NRAS</td>
<td>16</td>
<td>4%</td>
</tr>
<tr>
<td>JAK2</td>
<td>13</td>
<td>3%</td>
</tr>
<tr>
<td>ETV6</td>
<td>12</td>
<td>3%</td>
</tr>
<tr>
<td>CBL</td>
<td>10</td>
<td>2%</td>
</tr>
<tr>
<td>IDH2</td>
<td>9</td>
<td>2%</td>
</tr>
<tr>
<td>NPM1</td>
<td>8</td>
<td>2%</td>
</tr>
<tr>
<td>IDH1</td>
<td>6</td>
<td>1%</td>
</tr>
<tr>
<td>KRAS</td>
<td>4</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>GNAS</td>
<td>3</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>PTPN11</td>
<td>3</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>BRAF</td>
<td>2</td>
<td>&lt;1%</td>
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<tr>
<td>PTEN</td>
<td>1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>1</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

51% of MDS patients had at least one identified mutation.

52% of cases with normal karyotype had at least one mutation.

Bejar et al., *NEJM* 2011
Tyrosine kinase pathway mutations are highly exclusive of each other.

*TET2* mutations co-occur with nearly every other type of mutation.

Bejar et al., *NEJM* 2011
1. *TP53* mutations are highly associated with complex cytogenetics.

2. Complex cytogenetics cases have few mutations in genes other than *TP53*.
Mutations and bone marrow blasts

% of Patients with elevated blast fraction

- All (438)
- No Mut (210)
- NRAS (16)*
- TP53 (33)*
- RUNX1 (38)*
- AXSL1 (63)
- TET2 (90)
- ETV6 (12)
- EZH2 (28)

* p < 0.006
Mutations and thrombocytopenia

- All (430)
- No Mut (207)
- NRAS (15)*
- TP53 (33)*
- RUNX1 (37)*
- TET2 (87)
- ASXL1 (60)
- EZH2 (27)
- ETV6 (11)

* p < 0.001
Clinical Correlations – Mutations and Survival

- **TET2** (349^wt^ vs. 90^mut^)
  - *p*-value = 0.48

- **ASXL1** (376^wt^ vs. 63^mut^)
  - *p*-value = 0.003

- **RUNX1** (401^wt^ vs. 38^mut^)
  - *p*-value < 0.001

- **TP53** (406^wt^ vs. 33^mut^)
  - *p*-value < 0.001

- **EZH2** (411^wt^ vs. 28^mut^)
  - *p*-value < 0.001

- **NRAS** (423^wt^ vs. 16^mut^)
  - *p*-value = 0.006

- **ETV6** (427^wt^ vs. 12^mut^)
  - *p*-value = 0.04

- **CBL** (429^wt^ vs. 10^mut^)
  - *p*-value = 0.02

- **IDH2** (430^wt^ vs. 9^mut^)
  - *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>
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<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>
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<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

---

**International MDS Risk Classification**

**Survival**

- **Low**: 267 pts
- **Int-1**: 314 pts
- **Int-2**: 179 pts
- **High**: 56 pts

**AML Evolution**

- **Low**: 235 pts
- **Int-1**: 295 pts
- **Int-2**: 171 pts
- **High**: 58 pts

---

Greenberg et al., Blood 1997
IPSS adjusted survival

Hazard Ratio (95% Confidence Interval)

Favorable

- Univariate
- IPSS adjusted

Unfavorable

- EZH2
- TP53
- RUNX1
- ASXL1
- ETV6
- CBL
- NRAS
- IDH2
- TET2
- IDH1
- KRAS
- NPM1
- JAK2

P-values

- <0.001
- <0.001
- <0.001
- <0.001
- 0.04
- 0.05
- 0.02
- 0.08
- 0.03
- 0.50
- 0.82
- 0.53
- 0.44
- 0.99
IPSS adjusted survival

Hazard Ratio (95% Confidence Interval)

Favorable

Univariate
IPSS adjusted

Unfavorable

p-values

EZH2
<0.001
<0.001

TP53
<0.001
<0.001

RUNX1
<0.001
<0.001

ASXL1
0.004
0.006

ETV6
0.05
0.04

CBL
0.02
0.05

NRAS
0.008
0.17

IDH2
0.03
0.17

TET2
0.50
0.57

IDH1
0.82
0.52

KRAS
0.53
0.17

NPM1
0.44
0.86

JAK2
0.99
0.97
137/439 (31.2%) Samples carry a mutation in one or more of these genes.

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age</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>IPSS Risk Group</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>Mutational Status - Present vs. Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 Mutation</td>
<td>2.48 (1.60-3.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EZH2 Mutation</td>
<td>2.13 (1.36-3.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETV6 Mutation</td>
<td>2.04 (1.08-3.86)</td>
<td>0.029</td>
</tr>
<tr>
<td>RUNX1 Mutation</td>
<td>1.47 (1.01-2.15)</td>
<td>0.047</td>
</tr>
<tr>
<td>ASXL1 Mutation</td>
<td>1.38 (1.00-1.89)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

IPSS Risk Groups

- Low
- Int1
- Int2
- High
Effect of mutations on IPSS
Splicing factor mutations

Ebert and Bernard, *NEJM* 2011
Mutations present in 203 out of 288 (70%) lower IPSS risk MDS samples
Splicing factor mutations are largely mutually exclusive
Summary of MDS genetics

Mutations are present in over 70% of MDS cases

Mutations are powerfully associated with clinical features
- Mutations in 5 genes are independent predictors of overall survival
- \textit{TP53}, \textit{EZH2}, \textit{ASXL1}, \textit{RUNX1}, \textit{ASXL1}

MDS pathogenesis involves dysfunction of many cellular pathways
- Ribosome: haploinsufficiency for \textit{RPS14}
- Epigenetic regulators: mutation of \textit{TET2}, \textit{ASXL1}, \textit{EZH2}
- RNA splicing: \textit{SF3B1}, \textit{SRSF2}, \textit{U2AF1}
- DNA damage response: \textit{TP53}
- Transcription factors: \textit{RUNX1}, \textit{ETV6}
- Tyrosine kinase signaling: \textit{JAK2}, \textit{NRAS}, \textit{KRAS}, \textit{BRAF}
Development of new therapies

Challenge
- Most mutations identified are loss-of-function lesions
- Few mutations are obvious biochemical therapeutic targets

Potential
- Greater understanding of disease biology
- Improved murine models
- Development of novel screening approaches
Targeting leukemia within the hematopoietic niche

Yilmaz and Morrison, 2008
Short Hairpin RNA (shRNA) Screening Background

### Arrayed shRNA Screening

**Lentivirus** → **Recipient Cells** → **Infected Cells** → **Cells after 1 Week**

### Pooled shRNA Screening

**Lentivirus** → **Recipient Cells** → **Infected Cells** → **Cells after 1 Week**

### Determining Presence of shRNA

**Lentivirus** → **Integrate into Host Genome** → **PCR Amplify** → **Process** → **Sequence**
Pooled Screening Approach

Infected Primary LSCs → 24 Hrs → Harvest T0 → Transplant → 2 Weeks → Harvest Bones and Spleen
Pooled in vivo shRNA screen

Different Classes of Activity

- Depleting
- Enriching
- Control

shRNA Representation

- T0
- Bone Marrow
- Spleen

Replicates

$R^2 = 0.85$
Pooled in vivo shRNA screen positive controls

<table>
<thead>
<tr>
<th>Gene</th>
<th># shRNAs</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLL-AF9 Associated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mef2c</td>
<td>2/3</td>
<td>Deplete</td>
</tr>
<tr>
<td>Ccna1</td>
<td>2/3</td>
<td>Deplete</td>
</tr>
<tr>
<td><strong>Essential Genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utp18</td>
<td>2/3</td>
<td>Deplete</td>
</tr>
<tr>
<td>Ube2j2</td>
<td>2/3</td>
<td>Deplete</td>
</tr>
<tr>
<td><strong>Beta-Catenin Associated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctnnb1</td>
<td>3/3</td>
<td>Deplete</td>
</tr>
<tr>
<td>APC</td>
<td>2/3</td>
<td>Enrich</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hmgb3</td>
<td>3/3</td>
<td>Deplete</td>
</tr>
<tr>
<td>Myb</td>
<td>3/3</td>
<td>Deplete</td>
</tr>
</tbody>
</table>

![Graphs showing normalized shRNA reads for different conditions](image)
ITGB3 is essential for leukemia cells
ITGB3 loss is selectively essential for leukemia cells

- Itgb3 knockout increases the latency of MLL-AF9 leukemia
- Itgb3 knockout does not alter normal hematopoietic stem cells
Elucidation of a pathway of therapeutic targets for AML
Predictions for the future of MDS

Diagnosis: Detection of clonal mutations in the peripheral blood
Blood counts and bone marrow pathology will still be important

Classification: Molecular abnormalities define disease subtypes

Prediction: genetics will predict prognosis and response to therapy

New therapies: molecular insights and improved murine models will lead to a new generation of therapies for MDS
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