Integrating Molecular Findings into the Diagnosis and Prognosis of MDS

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Biological and Clinical Advances in MDS
San Diego, CA
December 2nd, 2016
Disclosures

Celgene: consultant, steering committee, honoraria, DSMB

Genoptix: consultant, honoraria, licensed IP

Foundation Medicine: honoraria, ad-hoc advisory board

Alexion: ad-hoc advisory board
Overview

- Genetic Heterogeneity in MDS
- Somatic Mutations as Diagnostic Markers
- Germline Predisposition Mutations
- Somatic Mutations as Prognostic Markers
MDS Genetics
Mutation Profiles in MDS
Challenges for MDS vs MPN

There are few routes to myeloproliferation but MANY routes to MDS

CML  PV  ET  PMF  SM  HES

BCR-ABL  JAK2  CALR  KIT  FIP1L1-PDGFRα

Splicing Factors

Epigenetic Regulators

TP53

Transcription Factors

60+ other genes

MDS
Diagnostic Mutations (?)
Myelodysplastic Syndromes (MDS)

- Aplastic Anemia
- Paroxysmal Nocturnal Hematuria
- Fanconi Anemia
- Myeloproliferative Neoplasms
- Vitamin Deficiency
  - Copper Deficiency
  - Iron Deficiency
- Autoimmune Disorders
- HIV
- EBV
- Hepatitis
- Medications
- Copper Deficiency
- Vitamin B12 Deficiency
- Alcohol Abuse

Diagnostic Difficulty

- Clonal
  - Acute Myeloid Leukemia (AML)
  - Myeloproliferative Neoplasms
- Non-Clonal
  - T-LGL
WHO Guidelines

Table 6. Recurring chromosomal abnormalities considered as presumptive evidence of MDS in the setting of persistent cytopenia of undetermined origin, but in the absence of definitive morphologic features of MDS

<table>
<thead>
<tr>
<th>Unbalanced abnormalities</th>
<th>Balanced abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>− 7 or del(7q)</td>
<td>t(11;16)(q23;p13.3)</td>
</tr>
<tr>
<td>− 5 or del(5q)</td>
<td>t(3;21)(q26.2;q22.1)</td>
</tr>
<tr>
<td>i(17q) or t(17p)</td>
<td>t(1;3)(p36.3;q21.1)</td>
</tr>
<tr>
<td>− 13 or del(13q)</td>
<td>t(2;11)(p21;q23)</td>
</tr>
<tr>
<td>del(11q)</td>
<td>inv(3)(q21q26.2)</td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td>t(6;9)(p23;q34)</td>
</tr>
<tr>
<td>del(9q)</td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td></td>
</tr>
</tbody>
</table>

Complex karyotype (3 or more chromosomal abnormalities) involving one or more of the above abnormalities.

≥ 15% ring sideroblasts

5-14% ring sideroblasts + SF3B1 mutation

MDS-RS


Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S.,

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

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Hazard ratio, 12.9 (95% CI, 5.8–28.7)
P<0.001

P=0.002 by Wilcoxon test
Prospective Trial

Patients with suspected MDS

At least one cytopenia:
- Hgb < 11 g/dL
- ANC < 1.8 x 10⁹/L
- Platelets < 100 x 10⁹/L

No other malignancy
B12, folate, Fe normal
Alternative cause unlikely

Blood and Bone Marrow Evaluation

Aspirate Morphology
Flow Cytometry
Cytogenetics/FISH
Molecular Genetics

Diagnosis of MDS (n=24)
Meets diagnostic criteria

Equivocal for MDS (n=21)
Some dysplasia (< 10%)
Not diagnostic of MDS

No evidence of MDS (n=99)
No dysplasia
Not diagnostic of MDS

<table>
<thead>
<tr>
<th>Pathways</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA Splicing</td>
<td>SF3B1, SRSF2, U2AF1, ZRSR2</td>
</tr>
<tr>
<td>Epigenetic Regulation</td>
<td>TET2, IDH1, IDH2, DNMT3A, EZH2, ASXL1, SETBP1</td>
</tr>
<tr>
<td>Tumor Suppression</td>
<td>TP53, PHF6</td>
</tr>
<tr>
<td>Transcription</td>
<td>RUNX1, ETV6</td>
</tr>
<tr>
<td>Activated Signaling</td>
<td>CBL, NRAS, KIT, JAK2, MPL, FLT3 (TKD)</td>
</tr>
<tr>
<td>Other</td>
<td>NPM1</td>
</tr>
</tbody>
</table>

Prospective Trial

**CLONAL**

≥ 1 Mutation Identified or Clonal Karyotype (black circles)

- 17
  - 13
    - 6
      - 2
      - 20
        - 7

**NON-CLONAL**

No Mutation Identified and Normal Karyotype

- 1
  - 6
  - 72

**MDS**

**Clonal Cytopenias of Undetermined Significance**

**ICUS**

**Idiopathic Cytopenias of Undetermined Significance**

CCUS vs. MDS Mutations

Prospective Study

Retrospective Study

ICUS (n=69)
Non-diagnostic bone marrow evaluation

RAEB/AML (n=39)
403 days

RA/RCMD (n=30)
606 days

91% had CCUS ~2 muts/patient VAF ~40%

Only 43% acquired a new mutation

Comparisons

<table>
<thead>
<tr>
<th>Mutated Genes</th>
<th>CHIP Unselected Population</th>
<th>CCUS At Diagnosis</th>
<th>CCUS Prior to MDS/AML Progression</th>
<th>MDS All Risk Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A, TET2, ASXL1, JAK2, TP53 ...</td>
<td>TET2, DNMT3A, ASXL1, SRSF2, TP53 ...</td>
<td>TET2, SRSF2, ASXL1, U2AF1, DNMT3A, ...</td>
<td>SF3B1, TET2, ASXL1, SRSF2, DNMT3A, ...</td>
<td></td>
</tr>
<tr>
<td># of Mutations</td>
<td>~1</td>
<td>~1.6</td>
<td>~2</td>
<td>~2.6</td>
</tr>
<tr>
<td>Typical VAF</td>
<td>9-12% (&gt;10% with ↑ risk)</td>
<td>30-40%</td>
<td>~40%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Mutation Rate</td>
<td>~10% of 70 year-olds</td>
<td>About 35% of ICUS</td>
<td>About 90% of ICUS</td>
<td>About 90% of MDS</td>
</tr>
</tbody>
</table>

This suggests increasing risk from CHIP through CCUS to MDS.

But we need prospective follow-up data to quantify risk!

*See ASH Oral Abstracts 298 and 299 on Sunday 12/4 at 8:15 AM*
Germline Mutations
Etiology of MDS

Familial or Congenital Predisposition

Topoisomerase II inhibitors
Ionizing radiation
DNA alkylating agents

“De novo”
(idiopathic, primary)

<2%

10-15%

85%

Often early onset and part of a larger syndrome

Peaks 1-3 or 5-7 years following exposure

Median age ~71 years; increased risk with aging

*See ASH Oral Abstract 300 on Sunday 12/4 at 8:45 AM*
1. Only younger patients are likely to have germline mutations.
   - Age of onset is variable
   - Certain mutated genes, like DDX41, can cause disease after mid-life

2. Germline mutations are not likely if there is no family history.
   - Up to 50% of cases are due to de novo, non-inherited mutations
   - Anticipation and partial penetrance can confound family history

3. Most patients will have recognizable clinical features or syndromes to suggest a germline mutation.
   - Hypomorphic mutations may cause few syndromic features but still create risk for malignancy

4. Patients who have had normal blood counts all of their lives are unlikely to have germline mutations.
   - Many mutated genes do not cause a cytopenic prodrome

5. Therapy related myeloid neoplasms are not associated with germline predisposition mutations.
   - They can be! See next talk!


Many germline predisposition genes can be somatically mutated

- Look for mutations in typical genes with VAF ~50%
- Look for bialleleic mutations in typical genes

- Consider **GERMLINE** testing in these patients and those with:
  - younger age of onset - family history - syndromic features

Classification and Prognosis
Analysis of Combined Datasets from the International Working Group for MDS-Molecular Prognosis Committee

Rafael Bejar, MD, PhD
Elli Papaemmanuil, PhD
Torsten Haferlach, MD
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Heinz Tüchler
Kristen Stevenson, MS
Donna S Neuberg, ScD
Peter Greenberg, MD
Benjamin L Ebert, MD, PhD

On behalf of the IWG for MDS investigators
MDS sample data collected from 19 centers in Europe, the United States, and Asia

**Clinical Features**
- age and sex
- blast %
- karyotype
- hemoglobin
- platelet count
- neutrophil count

**Overall Survival Data:**
- available for 3359
- 3.6 years follow-up
- 1780 deaths
- median OS 2.65 years

**Treatment Status**

**Gene Mutations**
Overall Survival by Mutation Number

17 genes sequenced in 1996 patients with OS data

- ASXL1
- CBL
- DNMT3A
- ETV6
- EZH2
- IDH1
- IDH2
- JAK2
- KRAS
- NPM1
- NRAS
- RUNX1
- SRSF2
- TET2
- TP53
- U2AF1
- SF3B1

Number of Mutated Genes

- 0 (n=377)
- 1 (n=595)
- 2 (n=460)
- 3 (n=210)
- 4 (n=125)
- 5/6/7 (n=22)
- SF3B1 only (n=207)
IPSS-R Adjusted Odds Ratios for Mutated Genes

- Hazard Ratio after adjustment for IPSS-R Risk Group

- $p = 0.05$
Division by Blast Proportion (<5%)

35%

$log_{10}(p\text{-value})$

Hazard Ratio after adjustment for IPSS-R Risk Group

$p = 0.05$
Division by Blast Proportion (5-30%)

\[-\log_{10}(p\text{-value})\]

\[\leq 4.0\]

34%

\[p = 0.05\]
Prognostic Interactions Between Mutated Genes
Molecular Clustering

Prognostic Value of Molecular Groups

331 Complex Karyotype MDS

Data Collected

Karyotype parsed for:
- # of abnormalities
- del(5q)
- del(7q), -7
- abnl chr 17, 3q, ...
- monosommal status

Clinical Features
- age and sex
- blast %
- hemoglobin
- platelet count
- neutrophil count

TP53 Mutation Status

Overall Survival

Sample data collected from centers in Europe, Asia, and the US
**TP53 Mutations and Other Genetic Features**

**TP53 Mutations and Most Frequent Somatic Mutations**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>28</td>
</tr>
<tr>
<td>RUNX1</td>
<td>12</td>
</tr>
<tr>
<td>U2AF1</td>
<td>18</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>25</td>
</tr>
<tr>
<td>TET2</td>
<td>21</td>
</tr>
<tr>
<td>TP53</td>
<td>158</td>
</tr>
</tbody>
</table>

***p < 0.001, **p = 0.001, *p = 0.014 for association with NO TP53 mutation***

**TP53 Mutations, Monosomalous Karyotype, and # of Abnormalities**

<table>
<thead>
<tr>
<th># of Abnormalities</th>
<th>3</th>
<th>4</th>
<th>5+**</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>170</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td>Monosomal Karyotype</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**p < 0.001 for association with TP53 mutation**
Overlap Between $TP53$ Mutation, Monosomal, and High Complexity

Complex Karyotype (n = 309) – excludes 22 patients with one or more unknown features

- **Monosomal Karyotype** (n = 231) 75%
  - **$TP53$ Mutated** (n = 169) 55%
  - **“Triple Negative”** 44 (14%)
  - **“Triple Positive”** 128 (41%)
  - 5+ Karyotype Abnormalities (n = 220) 71%
  - 64
  - 18

- **“Triple Positive”**
  - Monosomal Karyotype
  - 21

- **TP53 Mutated**
  - 6
  - 17
  - 18

- **Complex Karyotype** (n = 309)
Multivariable Model – Karyotype Features and $TP53$

<table>
<thead>
<tr>
<th>Three element model</th>
<th>Univariate</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% CI]</td>
<td>p-value</td>
</tr>
<tr>
<td>Monosomal Yes vs. No</td>
<td>2.01 [1.48-2.74]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.34 [0.95-1.89]</td>
<td>0.092</td>
</tr>
<tr>
<td>Number of Abnormalities 5+ vs. 3 or 4</td>
<td>2.33 [1.71-3.17]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.58 [1.11-2.25]</td>
<td>0.011</td>
</tr>
<tr>
<td>$TP53$ Mutation vs. No mutation</td>
<td>2.55 [1.93-3.35]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2.08 [1.56-2.77]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Median Overall Survival:
- $TP53$ Mutated (N=169): **8.1 months**
- $TP53$ Unmutated 5+ Abnl (N=75): **12.8 months**
- Double Negatives (N=65): **34.3 months**
<table>
<thead>
<tr>
<th>MDS Molecular Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS with SF3B1 Mutation</strong></td>
</tr>
<tr>
<td><strong>SF3B1</strong> &lt; 5% blasts</td>
</tr>
<tr>
<td><strong>MDAM</strong> &lt; 5% blasts Other splicing DNA methylation Cohesin complex RAS pathway</td>
</tr>
<tr>
<td><strong>MDSO</strong> &lt; 5% blasts No SF3B1 or MDAM mutations</td>
</tr>
<tr>
<td><strong>TP53</strong> Mostly complex karyotype</td>
</tr>
<tr>
<td><strong>MDSEB</strong> 5-19% blasts No TP53 mutation</td>
</tr>
<tr>
<td><strong>Del(5q) alone or +1</strong></td>
</tr>
<tr>
<td><strong>Del(5q)</strong> &lt; 5% blasts Deletion 5q31 ± 1 karyotype abnormality</td>
</tr>
<tr>
<td>Largely molecular system with only a few morphologic markers</td>
</tr>
<tr>
<td><strong>CCUS</strong> &lt; 5% blasts &lt;10% Dysplasia Clonal marker!</td>
</tr>
</tbody>
</table>
Summary

Broad based diagnostic sequencing can help to:

- detect clonal hematopoiesis and CCUS or MDS-RS
- indicate potential alternative diagnoses
- suggest possible germline predisposition mutations
- identify mutations that refine prognostic risk
- uncover mutations that predict response to treatment
  *See ASH Oral Abstract 69 on Saturday 12/3 at 8:00 AM*
- set a baseline for monitoring molecular progression
  *See ASH Oral Abstract 297 on Sunday 12/4 at 8:00 AM*
Acknowledgements

MDS Center of Excellence at UCSD

Elizabeth Broome  Huanyou Wang - Hematopathology
Edward Ball      Peter Curtin - BMT Group
Matthew Wieduwilt Dimitrios Tzachanis
Carolyn Mulroney Caitlin Costello
Januario Castro   Dan S. Kaufman
Sandford Shattil  John Adamson - Hematology Group
Catriona Jamieson Michael Choi
Erin Reid         Tom Kipps
Natalie Galanina  Annette Von Drygalski

Bejar Lab
Albert Perez      Sigrid Katz
Tiffany Tanaka    Brian Reilly
Emily Wheeler     Armon Azizi
Fiona Gowen-Huang

Our amazing CLINIC and INFUSION CENTER nurses and staff
And most of all – our incredible patients and families!

Evans Foundation for MDS

UC San Diego Moores Cancer Center

NIDDK