Somatic Mutations in MDS:
Insight into their Prognostic and Biological Importance

MDS Foundation Symposium
ASH Annual Meeting, December 7, 2012

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Disclosure Information
Timothy Graubert, MD

• I will not discuss off-label use and/or investigational use in my presentation.

• Financial Disclosure:
  Genoptix, Inc.  Scientific Advisory Board
Myelodysplastic Syndromes (MDS)

~15,000-25,000 new cases/yr (US)

~1/3 sAML

~2/3 BMF
<table>
<thead>
<tr>
<th>Prognostic System</th>
<th>First Author of Report</th>
<th>Place/Year of Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bournemouth</td>
<td>Mufti</td>
<td>Br J Haem 1985</td>
</tr>
<tr>
<td>Dutch</td>
<td>Kerkhofs</td>
<td>Br J Haem 1987</td>
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<tr>
<td>Spanish</td>
<td>Sanz</td>
<td>Blood 1989</td>
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<tr>
<td>Pavia</td>
<td>Cassano</td>
<td>Haematologica 1990</td>
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<tr>
<td>Rennes</td>
<td>Goasquen</td>
<td>Leuk Res 1990</td>
</tr>
<tr>
<td>Düsseldorf</td>
<td>Aul</td>
<td>Leukemia 1992</td>
</tr>
<tr>
<td>Japanese</td>
<td>Toyama</td>
<td>Leukemia 1993</td>
</tr>
<tr>
<td>Hannover Histopathology</td>
<td>Maschek</td>
<td>Eur J Haem 1994</td>
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<tr>
<td>Lille</td>
<td>Morel</td>
<td>Leukemia 1996</td>
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<tr>
<td>IPSS (original)</td>
<td>Greenberg</td>
<td>Blood 1997</td>
</tr>
<tr>
<td>WPSS, original</td>
<td>Malcovati</td>
<td>J Clin Onc 2007</td>
</tr>
<tr>
<td>MDACC Lower-Risk</td>
<td>Garcia-Manero</td>
<td>Leukemia 2008</td>
</tr>
<tr>
<td>MDACC General Risk Model</td>
<td>Kantarjian</td>
<td>Cancer 2008</td>
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<tr>
<td>WPSS, version 2 (fibrosis)</td>
<td>Della Porta</td>
<td>J Clin Onc 2009</td>
</tr>
<tr>
<td>WPSS, version 3 (Hb)</td>
<td>Malcovati</td>
<td>Haematologica 2011</td>
</tr>
<tr>
<td>IPSS-R</td>
<td>Greenberg</td>
<td>Blood 2012</td>
</tr>
</tbody>
</table>
MDS Prognosis

Overall Survival

N= 7012

Bone marrow blast %
Cytogenetics
Cytopenias

MDS Prognosis

Overall Survival

N= 7012

Table 2. Hazard Ratios for Death in a Multivariable Model.*

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥55 yr vs. &lt;55 yr</td>
<td>1.81 (1.20–2.73)</td>
<td>0.004</td>
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<tr>
<td>IPSS risk group</td>
<td></td>
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<tr>
<td>Intermediate-1 vs. low</td>
<td>2.29 (1.69–3.11)</td>
<td>&lt;0.001</td>
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<tr>
<td>Intermediate-2 vs. low</td>
<td>3.45 (2.42–4.91)</td>
<td>&lt;0.001</td>
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<tr>
<td>High vs. low</td>
<td>5.85 (3.63–9.40)</td>
<td>&lt;0.001</td>
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<tr>
<td>Mutational status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 mutation present vs. absent</td>
<td>2.48 (1.60–3.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EZH2 mutation present vs. absent</td>
<td>2.13 (1.36–3.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETV6 mutation present vs. absent</td>
<td>2.04 (1.08–3.86)</td>
<td>0.03</td>
</tr>
<tr>
<td>RUNX1 mutation present vs. absent</td>
<td>1.47 (1.01–2.15)</td>
<td>0.047</td>
</tr>
<tr>
<td>ASXL1 mutation present vs. absent</td>
<td>1.38 (1.00–1.89)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

MDS Prognosis

Overall Survival


Recurrently mutated genes in MDS


- 18 recurringly mutated genes.
- 51% (of 439) patients with at least one mutation.
- *TP53, EZH2, ETV6, RUNX1, ASXL1* independent prognostic value.
Prognostic value in low risk MDS

- 22 recurrently mutated genes tested.
- 71% (of 288) LR-PSS patients with at least one mutation.
- **EZH2** had independent (poor) prognostic value.

The spliceosome is recurrently mutated in MDS/AML
• 8 recurrently mutated genes.

• Mutated in >50% of MDS patients.

• Largely mutually exclusive.

**SF3B1: mutation spectrum**

- Mutated in 15-30% of MDS cases
- Heterozygous missense
- 50% K700E
- 98% PPV for RS
- 10-15% of CLL
- Also, AML, MPN, breast, lung, pancreatic, melanoma

**SF3B1: clinical associations**

- **Papaemmanuil, et al, NEJM, 2011**
  - Mutated SF3B1: N=72, P=0.008 (univariate)
  - Wild-type SF3B1: N=282

- **Patnaik, et al, Blood, 2012**
  - N=35 RARS SF3B1\textsuperscript{mut}, N=13 RARS SF3B1\textsuperscript{wt}, P=0.78

- **Malcovati, et al, Blood, 2011**
  - N=323, P=0.001

- **Damm, et al, Leukemia, 2012**
  - Overall survival, RARS or RCMD-RS: 
    - SF3B1 wildtype (n=17)
    - SF3B1 mutated (n=21), P=0.78
**SF3B1: biological consequences**

- Increased alternative splicing in SF3B1\textsuperscript{mut} CLL cells.

- RNA-seq SF3B1\textsuperscript{mut} (n=2) vs. normal (n=1)
  - 423/81,564 exons with differential expression.


Heterozygous mutations in founding clone (~9% of MDS).
Mutant: wildtype alleles expressed at similar levels.
**U2AF1: biological consequences**

293T cells minigene assay

MDS bulk BM cells

**U2AF1**: biological consequences

293T cells minigene assay

CD34+ cells: RNA-Seq


Graubert and Walter, unpublished
**SRSF2: mutation spectrum**

- Mutated in 47% of CMML (129/275)
- P95 hotspot

**Meggendorfer et al, Blood, 2012**
Splicing gene mutations in MDS & CLL

Ebert & Bernard, *NEJM*, 2011
Spliceosome @ ASH 2012

• *LUC7L2* (Abstract 173, Sunday, 5:30pm)

• Transgenic *U2AF1*$_{S34F}$ mice (Abstract 553, Monday, 2:45pm)

• Spliceosome modulators (Abstract 554, Monday, 3:00pm)

• *U2AF1*$_{mut}$ transcriptome (Abstract 3517, Monday, 6:00pm)

• *U2AF1* allelic heterogeneity (Abstract 3789, Monday, 6:00pm)

• *GCSFR* as a target of *U2AF1*$_{S34F}$ (Abstract 3792, Monday, 6:00pm)

• *U2AF1* clinical associations (Abstract 3804, Monday, 6:00pm)
Novel Genes/Pathways Implicated in MDS
Mutations in Epigenetic Regulators

- Genes involved in histone modification and DNA methylation are recurrently mutated in MDS and CMML.
Cohesin complex

Cohesins in MDS @ ASH 2012:

- **STAG3**
  (Abstract 173, Sunday, 5:30pm)
- **STAG1, STAG2, PDS5B**
  (Abstract 782, Monday, 6:30pm)


Novel recurrently mutated genes @ASH 2012

- **SETBP1** (Schinzel-Gledion Syndrome)
  - Abstract 2 (Plenary, Sunday, 2:25pm)
  - LBA-2 (Tuesday, 7:45am)

- **RIT1** (Ras-related GTPase)
  - Abstract 558 (Monday, 4:00pm)

- Other novel alleles
  - LBA-5 (Tuesday, 8:30am)
MDS is a mosaic of cancer genomes
Clonal Evolution from MDS to sAML

![Diagram showing clonal evolution from MDS to sAML with STAG2, PTPN11, RUNX1, and percentages at different stages.](image)

Age-dependent acquisition of mutations

Rate: \(~0.13\) exome mutations/yr

Welch, et al, Cell, 2012
Most mutations in cancer genomes are bystanders.

- **MDS initiating mutations**: (U2AF1, DNMT3A, TET2, others)
- **Progression mutations**: (TP53, others)

- **HSPC** → **MDS Founding Clone** → **Subclone 1** → **Subclone 2**

- **Passenger mutations**:
  - Pre-existing in HSPC
  - Gained between initiating and progression events
  - Gained during progression to subclones
New Questions:

1. What is the impact of recurrent mutations on MDS prognosis?

2. What is the predictive value of these mutations on response to therapy?

3. How do we make genetic testing widely available to our patients?

4. What are the biological consequences of novel recurrent mutations?

5. Do recurrent mutations provide targets for novel therapies?
Acknowledgements

The Genome Institute

- Rick Wilson
- Elaine Mardis
- Li Ding
- Dave Dooling
- Ken Chen
- Dave Larson
- Lisa Cook
- Rachel Maupin
- Bob Fulton
- Lucinda Fulton
- Sean McGrath
- Mike McLellan
- Dan Koboldt
- Joelle Veizer
- And many more

Stem Cell Biology/Siteman Cancer Center

- Tim Ley
- John DiPersio
- Matt Walter
- Jackie Payton
- John Welch
- Lukas Wartman
- Dan Link
- Michael Tomasson
- Peter Westervelt
- Sharon Heath
- Mark Watson
- Bill Shannon
- Rakesh Nagarajan
- Jack Baty
- Tamara Lamprecht

Funding: NCI, NHLBI, NHGRI, Alvin J. Siteman