Genetics of MDS

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2\textsuperscript{nd} Regional Symposium on MDS

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Overview

- MDS as a Clonal Disorder
- Landscape of Somatic Mutations
- Relationship to Other Myeloid Conditions
- Pathogenic Mechanisms of Mutations
- Clinical Implications
- Update on Plans from the IWG-PM
Welcome

HOW TO PROPERLY GREET SOMEONE DURING THE CORONAVIRUS OUTBREAK
MDS as a Clonal Disease
Clonality Alone is Not a Disease
Corrupted Hematopoiesis

Differentiation

Transformation

Normal

Early MDS

Advanced MDS

Secondary AML
Clonal Selection

## Genetic Abnormalities in MDS

<table>
<thead>
<tr>
<th>Translocations/Rearrangements</th>
<th>Rare in MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(6;9)</td>
<td></td>
</tr>
<tr>
<td>i(17q)</td>
<td></td>
</tr>
<tr>
<td>t(1;7)</td>
<td></td>
</tr>
<tr>
<td>t(3;?)</td>
<td></td>
</tr>
<tr>
<td>t(11;?)</td>
<td></td>
</tr>
<tr>
<td>inv(3)</td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td></td>
</tr>
</tbody>
</table>

**Observed Frequency in MDS**
Somatic Mutations in MDS
Most Frequently Mutated Genes


Haferlach et al. Leukemia. 2014.

DDX41
CSNK1A1
ETNK1
NFE2
...

2020
Most Frequently Mutated Genes

Tyrosine Kinase Pathway
- JAK2
- KRAS
- BRAF
- FLT3
- NRAS
- CBL
- PTPN11

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

Others
- TP53
- STAG2
- SMC3
- RAD21
- DDX41
- GNAS
- GNB1
- BCOR/L1
- NPM1

Epigenetic Regulation
- IDH 1 & 2
- DNMT3A
- EZH2
- TET2
- ATRX
- ASXL1
- UTX
- SETBP1

Splicing Factors
- SF3B1
- U2AF1
- ZRSR2
- SF3A1
- SF1
- U2AF2
- PRPF40B
- PRPF8
- BCOR/L1
Myelodysplastic syndromes are diseases of the spliceosome and epigenetic regulation.

Bejar et al. NEJM. 2011;364:2496-506.
Bejar et al. JCO. 2012;30:3376-82.
Patterns of Mutation
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

Siddhartha Jaiswal, M.D., Ph.D., Pierre Fontanillas, Ph.D., Jason Flannick, Ph.D.,
Figure 1 Circos diagrams depict the relative frequency and associations of the major mutations in MPNs (a) and MDSs (b), respectively, based on data from our work [37] on 127 classic MPNs and from Dannm's study [38] on 211 MDSs. Wild-type means no disease allele has been detected in the genes listed.


Myeloproliferative Neoplasms

Table 1. WHO classification of myeloid neoplasms and acute leukemia

<table>
<thead>
<tr>
<th>WHO myeloid neoplasm and acute leukemia classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myeloproliferative neoplasms (MPN)</strong></td>
</tr>
<tr>
<td>Chronic myeloid leukemia (CML), BCR-ABL1⁺</td>
</tr>
<tr>
<td>Chronic neutrophilic leukemia (CNL)</td>
</tr>
<tr>
<td>Polycythemia vera (PV)</td>
</tr>
<tr>
<td>Primary myelofibrosis (PMF)</td>
</tr>
<tr>
<td>PMF, prefibrotic/early stage</td>
</tr>
<tr>
<td>PMF, overt fibrotic stage</td>
</tr>
<tr>
<td>Essential thrombocythemia (ET)</td>
</tr>
<tr>
<td>Chronic eosinophilic leukemia, not otherwise specified (NOS)</td>
</tr>
<tr>
<td>MPN, unclassifiable</td>
</tr>
<tr>
<td>Mastocytosis</td>
</tr>
</tbody>
</table>

Mutation Patterns in MDS vs. AML


<table>
<thead>
<tr>
<th>MDS</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TET2</strong></td>
<td><strong>FLT3</strong></td>
</tr>
<tr>
<td><strong>SF3B1</strong></td>
<td><strong>NPM1</strong></td>
</tr>
<tr>
<td><strong>ASXL1</strong></td>
<td><strong>DNMT3A</strong></td>
</tr>
<tr>
<td><strong>SRSF2</strong></td>
<td><strong>NRAS</strong></td>
</tr>
<tr>
<td><strong>DNMT3A</strong></td>
<td><strong>TET2</strong></td>
</tr>
<tr>
<td><strong>RUNX1</strong></td>
<td><strong>IDH2</strong></td>
</tr>
<tr>
<td><strong>U2AF1</strong></td>
<td><strong>CEBPA</strong></td>
</tr>
<tr>
<td><strong>ZRSR2</strong></td>
<td><strong>RUNX1</strong></td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td><strong>IDH1</strong></td>
</tr>
<tr>
<td><strong>EZH2</strong></td>
<td><strong>TP53</strong></td>
</tr>
</tbody>
</table>

Haferlach et al. *Leukemia*. 2013
Mutation Comparisons

Malignancy Risk

No Clonal Disorder

No Clinical Disorder

ARCH

CHIP

CHIP/AA

CHIP/FP

CHIP/Tx

Conditions with Oncogenic Potential

Clonal Disease

CCUS

LR-MDS

HR-MDS

Advanced Malignancy

Mutation Number and Abundance

No Clonal Disorder

Normal

nciCUS

ARCH

CHIP

CHIP/AA

CHIP/FP

CHIP/Tx

DNMT3A

TET2

ASXL1

PPM1D

JAK2

TP53

VAF ~9-12%

1 mutation

VAF ~1-10%

1 mutation

VAF < 1%

DNMT3A

TET2

BCOR/L1

HLA locus

VAF ~2-15%

1-2 mutations

Younger

DNMT3A

TET2

ASXL1

RUNX1

TP53

VAF ~10-20%

1-2 mutations

Older

DNMT3A

PPM1D

TP53

TET2

ASXL1

VAF ~1-10%

1 mutation

DNMT3A

ASXL1

TET2

RUNX1

TP53

NRAS/JAK2/CBL

EZH2

STAG2

Abnormal karyo

VAF ~30-50%

~1-5+ mutations

EZH2

STAG2

ASXL1

VAF ~50%

>>1 mutation

Flt3

Npm1

Dnm3a

R882

t(#;#)

Idh1/2

Nras

Tet2

Cebpa

STAG2

VAF ~50%

>>1 mutation

PIGA

BCOR/L1

VAF ~2-15%

1-2 mutations

Younger

Splicing Factors

Tet2

ASXL1

DNMT3A

RUNX1

TP53

NRAS/JAK2/CBL

EZH2

STAG2

Abnormal karyo

VAF ~30-50%

~1-5+ mutations

Flt3

Npm1

Dnm3a

R882

t(#;#)

Idh1/2

Nras

Tet2

Cebpa

STAG2

VAF ~50%

>>1 mutation

Bejar R. Leukemia. 2017 Sep;31(9):1869-1871.

MOST COMMON COMMON FREQUENT
Pathogenic Mechanisms
Haploinsufficiency in 5q- Syndrome

5q-minus Syndrome: \textit{RPS14}

Splicing Factor Complexes

EXON
GU
U1 snRNP

UACUAAC
Branch Point Sequence

AG
ESE
EXON

5’ splice site

3’ splice site

Alternative Splicing Events

Skipped exon (SE)

Mutually exclusive exons (MXE)

Retained intron (RI)

 Constitutive exon
 Alternatively spliced exon

U2AF1
SRSF2
SF3B1

U2AF1
SRSF2
SF3B1
Stem vs. Progenitor Effects

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

Monocytosis
- CMML (40+%)  
- Worse prognosis

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

SF3B1

SRSF2

U2AF1
Ring Sideroblasts and SF3B1 Mutation


2016 WHO Guidelines

MDS-RS

≥ 15% ring sideroblasts

5-14% ring sideroblasts

ICUS

+ SF3B1 mutation

**Prognostic Interactions Between Mutated Genes**

*SF3B1* mutant MDS patients have fewer mutations in genes associated with greater disease risk.

*(highlighted in red)*
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
Epigenetic Regulators in MDS

**Writing**
- Acetylases, methylases, phosphorylases

**Erasing**
- Deacetylases, demethylases, phosphatases

**Reading**
- Bromodomain, chromodomain, PHD finger, WD40 repeat
DNMT3A and TET2 Mutations in MDS

DNMT3A

TET2

Cytosine

5-methylcytosine

5-hydroxymethylcytosine

5-carboxylcytosine

5-formylcytosine


**IDH1** and **IDH2** Mutations in MDS

- Mutated rarely in MDS and more often in AML
- Mutually exclusive with mutations of **TET2** and each other
- Mutations cause a *gain* of function - that drugs can target!

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**Chemical Reactions**

- Isocitrate + NADP⁺ → 2-hydroxyglutarate + NADPH
- 2-hydroxyglutarate is converted to α-ketoglutarate by IDH enzymes.

**Molecular Mechanisms**

- IDH1 and IDH2 mutations lead to a gain of function.
- These enzymes are involved in the conversion of isocitrate to α-ketoglutarate, a key step in the Krebs cycle.
- Mutations in these IDH enzymes can lead to the accumulation of 2-hydroxyglutarate, which can affect cellular metabolism and contribute to the development of cancer.

**References**

Transcription Factors and Others

RUNX1

Master regulators of differentiation

ETV6

Master regulator of stress/damage response

GATA2

TP53

Regulator of innate immune signaling?

DDX41
Clinical Implications
Analysis of Combined Datasets from the International Working Group for MDS-Molecular Prognosis Committee

Detlef Haase, MD
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Peter L. Greenberg, MD
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On behalf of the IWG for MDS investigators
MDS sample data collected from 19 centers in Europe, the United States, and Asia

**Data Summary**

**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**

From the IWG-PM Collaborative Meta-analysis
MDS sample data collected from 19 centers in Europe, the United States, and Asia

**Data Collected**

- Karyotype parsed for:
  - # of abnormalities
  - del(5q)
  - del(7q), -7
  - abnormal chr 17, 3q, 9, ...
  - monosomal status

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

**TP53 Mutation Status**

**Overall Survival**

**TP53 Co-mutation in MDS**

**Mutation Frequency by TP53 Mutation Status**

<table>
<thead>
<tr>
<th>Gene</th>
<th>No TP53 Mutation</th>
<th>TP53 Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>186</td>
<td>153</td>
</tr>
<tr>
<td>ASXL1</td>
<td>8 (4%)</td>
<td>21 (14%)</td>
</tr>
<tr>
<td>RUNX1</td>
<td>1 (1%)</td>
<td>13 (8%)</td>
</tr>
<tr>
<td>U2AF1</td>
<td>5 (3%)</td>
<td>14 (9%)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>17 (9%)</td>
<td>13 (8%)</td>
</tr>
<tr>
<td>TET2</td>
<td>19 (10%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>JAK2</td>
<td>3 (2%)</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>SF3B1</td>
<td>3 (2%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>SRSF2</td>
<td>4 (2%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>NRAS</td>
<td>5 (3%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>CBL</td>
<td>4 (2%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>EZH2</td>
<td>2 (1%)</td>
<td>6 (4%)</td>
</tr>
</tbody>
</table>

50%  40%  30%  20%  10%  0%  10%  20%  30%  40%  50%
# Multivariable Model – Karyotype Features and TP53

## Univariate

<table>
<thead>
<tr>
<th>Three element model</th>
<th>HR [95% CI]</th>
<th>p-value</th>
<th>Multivariable</th>
<th>HR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomal Yes vs. No</td>
<td>1.95 [1.46-2.62]</td>
<td>&lt;0.001</td>
<td>1.26 [0.91-1.75]</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Number Abnormalities ≥5 vs. 4 or 3</td>
<td>2.26 [1.70-3.02]</td>
<td>&lt;0.001</td>
<td>1.61 [1.16-2.24]</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>TP53 Mutation vs. No mutation</td>
<td>2.57 [1.97-3.34]</td>
<td>&lt;0.001</td>
<td>2.12 [1.61-2.79]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Median Overall Survival:

- **7.2 months**
- **14.4 months**
- **31.2 months**

Update on IWG-PM Efforts on the Impact of Somatic Mutations in MDS
International Working Group for the prognosis of MDS

13 countries | 25 centers

Elsa Bernard
Elli Papaemmanuil
International Working Group for the prognosis of MDS

**Retrospective cohorts**

- N=1,682
  - Bejar et al 2011
  - Haferlach et al 2014
  - Papaemmanuil et al 2013

**Prospective sequencing study**

- N=4,270
  - MSKCC IWG-PM Cohort
  - 13 countries 25 centers

**Validation**

- N to be determined
  - Cleveland
  - MD Anderson

**Sequencing:**

- 155 myeloid genes
- Genome-wide copy number probes
- Focal regions of LOH
- Panel information available on request
- Unmatched setting
- Coverage 600-800x
Detailed map of co-mutations: SF3B1 K700 other
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 isolated or with TET2 / DNMT3A — No shift

[Graph showing survival probability over years with different groups: SF3B1-WT, SF3B1-isolated, SF3B1 with TET2 / DNMT3A. The log-rank test indicates p = 0.3.]

[Box plot showing platelet (PLT) values with median lines and interquartile ranges for each group.]
Copy Number and LOH Detection

E-H-100358-T1-1-D1-1

E-H-118049-T1-1-D1-1

Copy ratio (log2)
**TP53 Mutation Configuration**

**Figure a:**
- Number of patients with any hit on TP53 locus
- **N=275**
- Chr17 at the TP53 locus
- cnloh
- iso17q
- normal del gain

**Figure b:**
- TP53 mutation status:
  - 1mut
  - N=5
  - 3mut
  - N=24

**Figure c:**
- TP53 subgroup:
  - 1mut
  - N=126
  - >1mut
  - N=91
  - mut+del
  - N=85
  - mut+cnloh
  - N=78

**Figure d:**
- Probability of overall survival
- Cumulative incidence of AML

**Figure e:**
- Comparison of hazard ratios for OS and AML

Elsa Bernard and Elli Papaemmanuil et al. on behalf of the IWG-PM
Summary

• MDS is a genetically heterogeneous clonal disorder caused by a wide variety of pathogenic mechanisms.
• Mutations are not specific to MDS, but certain patterns of mutation are characteristic.
• Somatic (and germline) mutations convey important clinical information.
• These variants will soon be formally incorporated into the classification and risk assessment for MDS.
MDS at UC San Diego

MDS Center of Excellence at UC San Diego

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Armon Azizi

All of our PATIENTS and INFUSION CENTER nurses and staff!

UC San Diego
MOORES CANCER CENTER