Mutational Signatures in Cytogenetic Risk Groups of De Novo AML and MDS

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Center for Personalized Diagnostics
AMP webinar: 10/4/18

Myeloid neoplasms

- Blast count
  - MDS/MPN<20%<AML
- Genetic features
  - FISH
  - Cytogenetics
  - Sequencing
- Displasia
  - (MDS)

Other myeloproliferative neoplasms (MPN)

- BCR-ABL (Ph+)
  - Chronic Myelogenous Leukemia (CML)
- Ph-
  - Polycythemia vera (PV)
  - Essential thrombocytopenia (AT)
  - Primary myelofibrosis (PMF)
Myeloid neoplasms are heterogeneous

- Cytogenetic abnormalities are associated with prognosis
- Many subtypes exist with multiple overlapping mutations
- Recurrent mutations belong to several distinct pathways
- Pre-leukemic and leukemic cells undergo clonal evolution
  - Heterogeneous cell populations with mutations conferring different functional properties

Genetic basis of myeloid neoplasms

- De novo AML
  - *NPM1*, *CBF* and *KMT2A* mutations
- MDS
  - Progression to AML associated with mutations in *TP53, RUNX1, ETV6, EZH2, ASXL1*
- sAML
  - Spliceosome complex: *SRSF2, SF3B1, U2AF1, ZRSR2*
  - Epigenetic regulators: *ASXL1, EZH2, BCOR*
  - Cohesion Complex: *STAG2*
  - Many mutations from MDS or myelofibrosis and are retained after transformation
AML risk stratification: Medical Research Council (MRC) & Southwest Oncology Group (SWOG)

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Acute Myeloid Leukemia Cytogenetic Risk Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Status</td>
<td>SWOG(1)</td>
</tr>
<tr>
<td>Favorable</td>
<td>t(15;17), inv(16)/(t;16)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal, +8, +7, −Y, del(12p)</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>+8, +7, −Y, del(12p)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4q+</td>
</tr>
</tbody>
</table>

Orozco, et al., 2012

AML risk stratification: European LeukemiaNet (ELN)

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Generic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(11;17), RUNX1-RUNX1T1</td>
</tr>
<tr>
<td></td>
<td>t(16;16), t(11;14), CEBPA, CBFB-MYH11</td>
</tr>
<tr>
<td></td>
<td>t(15;17), inv(16)</td>
</tr>
</tbody>
</table>

Intermediate |

| Wild-type NPM1 and FLT3-ITD/ATD |
| Cytogenetic abnormalities not classified as favorable or adverse |

| Wild-type NPM1 and FLT3-ITD/ATD (without adverse-risk genetic lesions)
| t(11;19), q(21), p(33), MLLT10-MLL2 |

<table>
<thead>
<tr>
<th>Adverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>8p21.1q22.1, DERE-NEU2</td>
</tr>
<tr>
<td>11q23.3, KMT2D rearranged</td>
</tr>
<tr>
<td>KIT(21)q22.2p12, BCR-ABL1</td>
</tr>
<tr>
<td>5q31(21)q22.2p12 or KIT(21)q22.2p12</td>
</tr>
<tr>
<td>Complex karyotype, 5 or more karyotype</td>
</tr>
<tr>
<td>Wild-type NPM1 and FLT3-ITD/ATD</td>
</tr>
<tr>
<td>Mutated RUNX1</td>
</tr>
<tr>
<td>Mutated NPM1</td>
</tr>
<tr>
<td>Mutated JAK2</td>
</tr>
<tr>
<td>Mutated TP53</td>
</tr>
</tbody>
</table>

Döhner et al., 2017
AML with intermediate cytogenetic risk can be modified by mutational information

Swog risk stratification

Penn’s hematological malignancies NGS panel detects mutations in the majority of AML patients

Heme Version 2: 68 genes

- ABL1, ASXL1, ATM, BCR, BCR/ABL1, BIRC3, BRAF, CALR, CBL, CDH2, CDKN2A, CEBPA, CSF1R, CSF3R, DNMT3A, DDX3X, ETV6, EZH2, FAM5C, FBXW7, FLT3, GATA2, GNAS, HNRNPK, HRAS, IDH1, IDH2, IL7R, JAK2, KLHL6, KIT, KRAS, MAPK1, MPL, MLL2, PHF6, PRPF40B, PTPN11, MAP2K1, miR-142, MYC, MYCN, MYD88, NF1, NOTCH1, NOTCH2, NPM1, NRAS, POT1, Pten, RAD21, RIT1, RUNX1, SRSF2, SETBP1, SMC1A, SF1, STAG2, SF3A1, SF3B1, TBL1XR1, TET2, TP53, TPMT, U2AF1, U2AF2, WT1, XPO1, ZRSR2, ZMYM3

Chromosome analysis

Sequence analysis

Normal 58%

Abnormal 85%

VOUS 4%

Normal 11%
Evolution of the heme panel

Heme Version 1: 33 genes
- ASXL1, ATM, BRAF, CBL, CDKN2A, DDX3X, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MAPK1, PHF6, PTEN11, MYD88, NOTCH1, NPM1, NRAS, PTEN, RUNX1, SF3B1, TET2, TP53, WT1, XPO1, ZMYM3

Heme Version 2: 68 genes
- ABL1, ASXL1, ATM, BCOR, BCORL1, BIRC3, BRAF, CALR, CBL, CDH2, CDKN2A, CEBPA, CSF1R, CSF3R, DNMT3A, DDX3X, ETV6, EZH2, FAM5C, FBXW7, FLT3, GATA2, GNAS, HNRNPK, HRAS, IDH1, IDH2, IL7R, JAK2, KIT, KRAS, MAPK1, MPL, MLL2, PHF6, PRPF40B, PTPN11, MAP2K1, miR-142, MYC, MYCN, MYD88, NF1, NOTCH1, NOTCH2, NPM1, NRAS, POT1, PTEN, RAD21, RIT1, RUNX1, SRSF2, SETBP1, SMC1A, SF1, STAG2, SF3A1, SF3B1, TBL1XR1, TET2, TP53, TPMT, U2AF1, U2AF2, WT1, XPO1, ZRSR2, ZMYM3

Overlapping Risk Stratification with Mutations from CPD

CPD 4500 cases on Heme panel

Cytogenetics 11,500 karyotypes

2190 AML or MDS

1,031 first or second karyotype for a patient

665 Overlapping patients

- Check chart to confirm AML or MDS
- Check date of karyotype for dnAML
- Check date of NGS for dnAML
- Stratify into risk categories
- No Growth/suboptimal normal karyotypes removed

124 dnAMLs
90 MDS patients
AML risk stratification: Medical Research Council (MRC) & Southwest Oncology Group (SWOG)

<table>
<thead>
<tr>
<th>Risk Status</th>
<th>SWOG(1)</th>
<th>MRC (2010)(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(15;17), t(8;21), inv(16)/t(16;16), del(1q)</td>
<td>t(15;17)(q22;q21), t(8;21)(q22;q21), t(16;16)(p13;q22)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal, +8, +6, −Y, del(12p)</td>
<td>Abnormalities not classified as favorable or unfavorable</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>abn(1q), del(5q), −5, −7/der(7q), 16q22, t(9;22), 6q, 11q23, 21q, 17p, complex (&gt;3 unrelated abnormalities)</td>
<td>abn(3q) (excluding t(3;3), q21–23;q31–35), inv(3) (q21q31) (excluding q21q31), add(5q), del(5q), −5, add(7q)/del(7q), −7, t(6;11), t(4;16) (q27;q23), t(11;11) (p11;–13q22), t(11;q23) (excluding t(11;11) (p21;–22q22) and t(11;11) (q23;p13)) (excluding t(11;11) (p21;–22q22) and t(11;19) (q23;p13)) (excluding t(11;11) (p21;–22q22) and t(11;11) (q23;p13))</td>
</tr>
<tr>
<td>Unknown</td>
<td>All other abnormalities</td>
<td>Category not recognized</td>
</tr>
</tbody>
</table>

Orozco, et al., 2012

Mutations were detected in most MRC Favorable AMLs

20 patients

These include:
- t(8;21) (n=10)
- inv(16)/t(16;16) (n=7)
- t(15;17) (n=4)

- **FLT3** is the most commonly mutated gene (4/5 of these are ITDs)
Multiple mutations were detected in most MRC Intermediate AMLs

- 82 patients
- Most common finding: normal karyotype (n=51)
- 24/33 (73%) of FLT variants are ITDs
- No negative sequencing studies

Normal karyotypes have more DNMT3A and tumor suppressor mutations compared to Trisomy 8s

Normal karyotype: (n=51 patients)
137 unique patient-gene pairs

Trisomy 8: (n=10 patients)
22 unique patient-gene pairs

Normal Karyotypes do not overlap with negative sequencing studies
TP53 is the most commonly mutated gene in MRC Unfavorable AMLs

29 patients
Complex Karyotypes (defined as ≥4 abnormalities)

Negative sequencing studies (n=2) overlap with either a complex karyotype or del(5q)

Functional Categorization of Variants

*WT1 is a tumor suppressor even though mutant WT1 is associated with DNA hypermethylation of PRC1 targets in AML (Sinha et.al. Blood 2015)

Priya Velu, MD, PhD
Molecular Genetic Pathology Fellow
Functional categorization of mutations by MRC in AML

Increased signaling pathway mutations in favorable and tumor suppressors in unfavorable

Differences between functional categorization of mutations in normal and trisomy 8 karyotypes (Intermediate)

- DNA methylation (DNMT3A)
- Tumor suppressors (WT1)
- Numbers are low for trisomy 8
Main conclusions from AML (MRC)

- “Negative” sequencing studies exist in both favorable and unfavorable cytogenetic categories. (Limitations of a targeted panel)
- Signaling pathway genes are the most commonly mutated in favorable
  - More variants in favorable than other karyotypes
- No DNA methylation gene mutations in favorable karyotypes
- Tumor Suppressors are mainly mutated in unfavorable karyotypes
  - Almost exclusively TP53
- NPM1 mutations only occur in intermediate karyotypes
- WT1 mutations are common in favorable and intermediate, do not occur in unfavorable
- Within intermediate karyotypes:
  - Tumor suppressors, signaling pathway and DNA methylation genes mutated in more normal karyotypes than trisomy 8s
  - Chromatin regulatory, spliceosome complex and transcription factor genes mutated more in trisomy 8 than normal karyotypes.

AML risk stratification: European LeukemiaNet (ELN)
There are significant differences between MRC and ELN categorization schemes

<table>
<thead>
<tr>
<th>MRC (2010)[6]</th>
<th>ELN Favored AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(15;17)(q22;q21), t(8;21) (q22;q22), inv(16) (p13q22)</td>
<td>25 patients</td>
</tr>
<tr>
<td>Abnormalities not classified as favorable or unfavorable</td>
<td>These include:</td>
</tr>
<tr>
<td>abn(3q) (excluding t(3;3)) (q21–25)x3–35), inv(3) (q21q26)/t(5;32)x2q26), a(16)x5q), del(5q), –5, add(7q)del(7q), –7, 13q111</td>
<td>• t(8;21) (n=10)</td>
</tr>
<tr>
<td>q7t(8;21), t(19;11) (p11–13p223), t(11q23) (excluding t(9;11) (p21–22)x20 and t(11;19) (q23;p13); t(9;22)x2q34q11), –17/Δ17(1q21)complex</td>
<td>• inv(16)/t(16;16) (n=7)</td>
</tr>
<tr>
<td>Category not recognized</td>
<td>• NPM1 mutant with FLIT3 ITD VAF &lt;50%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(15;17)(q22;q22), RUNX1-RUNXI</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13;q22) or t(16;16)(p13;q22)</td>
</tr>
<tr>
<td></td>
<td>Mutated NPM1 without PLT3-ITD or with PLT3-ITD&lt;50%</td>
</tr>
<tr>
<td>Baseline mutated CEBPA</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Mutated NPM1 and PLT3-ITD&lt;50%</td>
</tr>
<tr>
<td>Wild-type NPM1 without PLT3-ITD or with PLT3-ITD&lt;50% (without adverse-risk genetic lesions)</td>
<td>Cytogenetic abnormalities not classified as favorable or adverse</td>
</tr>
<tr>
<td>Adverse</td>
<td>t(8;21)(q22;q22), DDX5-REX1</td>
</tr>
<tr>
<td></td>
<td>t(11;19) (q22;q13), ETV6 rearranged</td>
</tr>
<tr>
<td></td>
<td>t(13;14) (p33;q12), BCOR-ABL1</td>
</tr>
<tr>
<td></td>
<td>t(13;17) (p33;q21) or t(3;33) (q21), s.2 6 20, G(1)G(2) A(1)E(1)OM(1)E(1)</td>
</tr>
<tr>
<td></td>
<td>❌ or del(15q); –7, –17(17p)</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype:</td>
</tr>
<tr>
<td></td>
<td>3'unconventional karyotype:</td>
</tr>
</tbody>
</table>

*These or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or fusions, that is, t(8;21), inv(16) or t(16;16), t(6;11), t(11;19) (q23.3), t(6;9), inv(3) or t(3:3); AML with BCOR-RE11
**ELN Intermediate AML**

- 56 patients
- 4 added with t(15;17)
- 35 removed
  - 3 v. 4 abnormalities
  - *NPM1* wild type with *FLT3* ITD high VAF
  - *NPM1* mutant with *FLT3* ITD low VAF or no *FLT3*
  - *RUNX1*
  - *ASXL1*
  - *TP53*

127 unique patient-gene pairs*

**MRC Intermediate AML**

- 82 patients
- Most common finding: normal karyotype (n=51)
- 24/33 (73%) of *FLT* variants are ITDs
- 8 of these have VAF <50% with *NPM1* mutations (ELN favorable)
- *ASXL1, RUNX1* and *TP53* mutant AMLs are all ELN unfavorable (n=17 from this group)
- *NPM1* wild type with *FLT3* ITD high (>50%) ELN unfavorable (n=7)
  - 2 with t(6;9)
ELN unfavorable AML

50 patients
- Complex Karyotypes (>3 abnormalities)
- *NPM1* wild type with FLT3 ITD high
- *RUNX1*
- *ASXL1*
- *TP53*
- *t(6;9)*

107 unique patient-gene pairs*

Shift of patients from MRC vs ELN

MRC

Favorable (n=20)
Intermediate (n=82)
Unfavorable (n=29)

ELN

Favorable (n=25)
Intermediate (n=56)
Unfavorable (n=50)

*Shift of patients from MRC vs ELN*
### Functional Categorization of Variants

#### Table 1

**Functional Categorization of Genes**

<table>
<thead>
<tr>
<th>Chromatin Regulation</th>
<th>DNA Methylation</th>
<th>Signaling Pathway</th>
<th>Transcription Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>BRAF</td>
<td>ATM</td>
<td>CSF3R</td>
</tr>
<tr>
<td>BCOR</td>
<td>CBL</td>
<td>CSF1R</td>
<td>GATA2</td>
</tr>
<tr>
<td>BCORL1</td>
<td>CSF1R</td>
<td>FLT3</td>
<td>MYC</td>
</tr>
<tr>
<td>EZH2</td>
<td>GNAS</td>
<td>IL1R</td>
<td>MYCN</td>
</tr>
<tr>
<td>ZMYM3</td>
<td>IKBG</td>
<td>KLH3</td>
<td>RUNX1</td>
</tr>
<tr>
<td>MLL2</td>
<td>JAK2</td>
<td>KRAS</td>
<td>TBL1XR1</td>
</tr>
<tr>
<td>COHESIN</td>
<td>KIT</td>
<td>MPP2</td>
<td>TUMOR</td>
</tr>
<tr>
<td>COMPLEX</td>
<td>MAP2K1</td>
<td>MKK1</td>
<td>SUPPRESSOR</td>
</tr>
<tr>
<td>RAD21</td>
<td>MEK2</td>
<td>MYD88</td>
<td>ETV6</td>
</tr>
<tr>
<td>SMC1A</td>
<td>NFKB</td>
<td>NOTCH1</td>
<td>HNRNPK</td>
</tr>
<tr>
<td>STAT2</td>
<td>NUP214</td>
<td>NTRK1</td>
<td>PTEN</td>
</tr>
<tr>
<td>NPM1</td>
<td>NUT1</td>
<td>PDGFRB</td>
<td>TRRAP</td>
</tr>
<tr>
<td>NPM1</td>
<td>RAS</td>
<td>PTPN11</td>
<td>WTI</td>
</tr>
<tr>
<td>PHARMACOGENETIC</td>
<td>RAS</td>
<td>TET1</td>
<td>PHF6</td>
</tr>
<tr>
<td>TMPT</td>
<td>RAS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other/Unknown

1. **Functional Categories by ELN in AML**

- 1 unfavorable with NPM1 because this patient also had an ASXL1 mutation
- Negative in all 3
- Signaling pathway mutations common in favorable
- Tumor suppressor mutations common in unfavorable
Trends in functional categories are unchanged

Differences between MRC, ELN and SWOG

- MRC and SWOG are very similar. Two intermediate cases would be changed (1 to SWOG favorable, 1 to SWOG unfavorable)
  - Therefore no additional analysis with SWOG
- ELN incorporated t(6;9) into unfavorable which moved two cases from intermediate to unfavorable (also had unfavorable mutations)
- ELN favorable does not include t(15;17) in criteria
- ELN includes mutation detection: all ASXL1, RUNX1 and TP53 mutant AMLs are unfavorable
  - NPM1 wild type or mutant with FLT3 ITD
    - FLT3 ITD high = >50% VAF. Need to consider % blasts in the sample
- ELN has more unfavorable AMLs from our cohort
MDS risk stratification by International Prognostic Scoring System (IPSS-R)

<table>
<thead>
<tr>
<th>Cytogenetic prognostic subgroups</th>
<th>Cytogenetic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>No Cases with matching sequencing</td>
</tr>
<tr>
<td>Good</td>
<td>Normal, del(5q), del(12p), del(20q), double including del(5q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>del(7q), +8, +19, i(17q), any other single or double independent clones</td>
</tr>
<tr>
<td>Poor</td>
<td>-7, inv(3)(t(3q);del(3q), double including -7/del(7q), Complex: 3 abnormalities</td>
</tr>
<tr>
<td>Very poor</td>
<td>Complex: &gt;3 abnormalities</td>
</tr>
</tbody>
</table>

**Good MDS**

11 unique patient-gene pairs

7 patients
Intermediate MDS

63 patients

182 unique patient-gene pairs*

Poor MDS

7 patients

13 unique patient-gene pairs*
Very Poor MDS

13 patients

17 unique patient-gene pairs*

Functional Categories by IPSS-R in MDS
Main conclusions from MDS

• “Negative” sequencing studies exist in 3/4 cytogenetic categories. (Limitations of a targeted panel)
• Spliceosome complex genes are the most commonly mutated in good MDS
  • More variants in good than other karyotypes
• Cohesion complex gene mutations are exclusive to intermediate MDS
• Tumor suppressors are mainly mutated in very poor karyotypes
  • Almost exclusively TP53
• Very few NPM1 mutations in MDS
• The n is low for all risk categories except intermediate

Overall considerations for study design

• Clean data set with dnAML and new MDS.
  • Could include sAMLs
  • Older MDS
    • For both, karyotypes could be different than the current data set but you could still link risk/prognosis from karyotype to mutational signatures
• WT1 is a tumor suppressor
  • Mutant WT1 can cause a hyper-methylated phenotype in AML
    • This is causal and not a direct function by WT1
• Mutations by functional category trends do not differ between MRC and ELN AML
  • Survival data may show one to be superior to the other
• Use type of alteration as a way to stratify cytogenetics
  • Trisomys, monosomys, translocations, deletions, etc.
Thank you key players

• Priya Velu, Penn MGP fellow
• Jennifer Morrissette, Director of Cytogenetics and Clinical Director of CPD
• Dan Ackerman, Staff Scientist
• Ashkan Bigdeli, Bioinformatics Specialist

• Beckman Coulter Life Sciences (Genomic Reagents)
  • We have transitioned the majority of our extractions onto FormaPure Total for FFPE, cytology, fresh tissue and are validating for bone cores