Molecular Characterization of MDS & Predisposition

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Genomic Molecular Profiling

- Gene expression microarrays
- miRNA gene expression array
- Single-nucleotide polymorphism array (copy number alterations)
- Next generation whole genome & exonic pyrosequencing
- Mass spectrometry-based genotyping
- RNA transcriptome sequencing
Acquired Uniparental Disomy

• Loss of heterozygosity without change in DNA copy number
• Described in diverse haematological and non-haematological malignancies
• Associated with driver mutations in specific oncogenes or tumour suppressor genes, e.g.
  – 9p: V617F JAK2 in MPN
  – 13q: FLT3 ITD in AML

Fitzgibbon et al., Cancer Res. 2005;65:9152-4.
Acquired Uniparental Disomy (aUPD) As A Consequence Of Mitotic Recombination
Regions of Likely aUPD in MDS

- SNP 6.0 analysis; n=148
- 63 tracts of copy neutral runs of homozygosity >20Mb in 46 (31%) cases
- 17 different chromosomes; 7 recurrent regions

Ernst et al., Nat Genet. 2010;42:722-6
Somatic Mutations are Independent Covariates for Disease Behavior

- Relevance of somatic mutations investigated in 630 pts
- 111 cancer related genes surveyed by mass spec genotyping (OncoMap), pyro- and Sanger sequencing
- Mutations in ≥1 genes in 51%; 52% of pts with normal karyotype
- Most commonly mutated genes: TET2 (21%), ASXL1 (14%), RUNX1 (9%), TP53 (8%), and EZH2 (6%)
- Mutations of RUNX1, NRAS, and TP53 associated with severe thrombocytopenia & increased myeloblasts (p<0.001).

• Multivariable analysis, mutations of TP53 (HR, 2.48), EZH2 (HR, 2.13), ETV6 (HR, 2.04), RUNX1 (HR, 1.47), and ASXL1 (HR, 1.38) were independent predictors of ↓OS vs. age, sex, IPSS.

**Five Mutations Identify Unfavorable Prognosis Independent of IPSS**

**MDS n=439, 18 genes**

<table>
<thead>
<tr>
<th>Mutational Status</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<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**

Overall Survival According to IPSS Risk Category & Mutation Status

Low Risk
- Low risk, mutation absent (N=87)
- Low risk, mutation present (N=23) [P=0.001]
- Intermediate-1 risk (N=185)

Int-1
- Intermediate-1 risk, mutation absent (N=128)
- Intermediate-1 risk, mutation present (N=57) [P<0.001]
- Intermediate-2 risk (N=101)

Int-2
- Intermediate-2 risk, mutation absent (N=61)
- Intermediate-2 risk, mutation present (N=40) [P=0.02]
- High risk (N=32)

High
- High risk, mutation absent (N=15)
- High risk, mutation present (N=17) [P=0.26]
- Intermediate-2 risk (N=101)

Figure 1. Mutations and Cytogenetic Abnormalities in 223 Samples with at Least One Mutation.
Mutations in the 11 most frequently mutated gene groups are shown by colored bars. Each column represents 1 of the 223 samples with a mutation in one or more of the genes listed. Darker bars indicate samples with two or more distinct mutations in that gene group. The karyotype of each of the 223 samples is also shown.
Figure 1. Mutations and Cytogenetic Abnormalities in 223 Samples with at Least One Mutation.
Mutations in the 11 most frequently mutated gene groups are shown by colored bars. Each column represents 1 of the 223 samples with a mutation in one or more of the genes listed. Darker bars indicate samples with two or more distinct mutations in that gene group. The karyotype of each of the 223 samples is also shown.
Genetic Pathways & Somatic Mutations

Figure 1. Mutations and Cytogenetic Abnormalities in 223 Samples with at Least One Mutation.

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*methylcytosine dioxygenase at 4q24 catalyzing mC conversion to hmC. Bejar et al. N Engl J Med. 2011;364:2496-506
Inhibition of TET2 Mediated Conversion of 5mC Disturbs Hematopoiesis

- αKG-dependent dioxygenase converting 5-mC to 5-hmC → 5-carboxyl(ca)C excision
- Conditional TET2 knockout & TET2 +/- increases stem cell self-renewal leading to myeloid malignancy (MDS, MPN, CMML).
  \(^{^\text{^\textsuperscript{\texttrademark}}}\) Moran-Crusio K, et. al. Cancer Cell 2011; 20: 11.
- Level of 5-hmC decreased in TET2\(_{\text{mu}}\) MPN PMN DNA & in TET2 knockdown CB-CD34+ cells.
- TET2 RNAi in CB-CD34+ cells skews progenitor commitment to granulo-monocytic vs. erythroid.
- The high frequency of overlap mutations suggests cooperative tumorigenesis.
Somatic Mutation Driven Methylator Phenotypes in MDS & AML

**Gene**
- *TET2*

**Activity**
- $mC \rightarrow hmC$

**Mutation**
- Inactivation

**Consequence**
- $mC$ Retention

**IDH1/2**
- $\alpha$KG $\rightarrow$ 2HG
- Gain of Fn
- $\uparrow 2HG$

**Promoter Hypermethylation**
- Maturation Impairment

*IDH1*, isocitrate dehydrogenase-1; *IDH2*, IDH mitochondrial homologue; *TET2*, tet oncogene family member 2; $\alpha$KG, $\alpha$ketoglutarate; 2HG, 2-hydroxyglutarate; $mC$, methylcytosine; $hmC$, hydroxy-$mC$.  

Figueroa ME. Cancer Cell 2010;18: 553.  
Jankowska A. ASH 2010; 1a.
Impact of *DNMT3A* Mutations on Outcome

- 13 somatic mutations in 12/150 MDS patients (8%).
- 2 truncating, 11 missense in MTase domain, all heterozygous.

**Overall Survival**

- **DNMT3A wild type**
- **DNMT3A mutant**

- *P*=0.02

**Event-free Survival**

- **DNMT3A wild type**
- **DNMT3A mutant**

- *P*=0.04

**LFS**

- **DNMT3A mutant**

- *P*=0.06

*DNMT3A* mutations occur in all morphologic and IPSS categories, and associated with inferior OS, EFS and LFS.

Epigenetic Program Mutations In MDS

IDH1/2

2hG

TET2

CH$_2$OH

CH$_2$OH

DNMT3a

EVI-1

TET2

IDH1/2

EZH2

ASXL1

MLL

JAK2

isocitrate
Epigenetic Program Mutations In MDS

- **IDH1/2***
- **TET2**
- **DNMT3a**
- **EVI-1**
- **IDH1/2**
- **EZH2**
- **ASXL1**
- **MLL**
- **JAK2**
Epigenetic Program Mutations In MDS

- **IDH1/2**
- **TET2**
- **EZH2**
- **ASXL1**
- **PRC2**
- **MLL**
- **MLL**
- **JAK2**

Genes and Mutations:
- IDH1/2
- TET2
- EZH2
- ASXL1
- PRC2
- MLL
- JAK2

Chemical Reactions:
- Isocitrate
- 2-HG

Methylations:
- H3K27me3
- H3K4me3
Epigenetic Program Mutations In MDS

- DNMT3a
- EVI-1
- IDH1/2
- TET2
- EZH2
- ASXL1
- MLL
- JAK2

**Key Pathways:**
- **Δ2-HG Metabolism**
  - IDH1/2
  - TET2

**Epigenetic Markers:**
- H3K27me3
- H3K4me3
- H2A/H4-R
- H3Y41-P

**Key Proteins:**
- PRC2
- EZH2
- ASXL1
- PRDM5
- JAK2 V617F
## Response to Azanucleoside Treatment by Mutation Status

<table>
<thead>
<tr>
<th>Institution</th>
<th>No. Pts.</th>
<th>Gene(s)</th>
<th>Overall Mutant</th>
<th>Response WT (%)</th>
<th>P value</th>
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<tbody>
<tr>
<td>GFM</td>
<td>86</td>
<td><em>TET2</em></td>
<td>11/13 (85)*</td>
<td>34/73 (47)</td>
<td>0.01</td>
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<tr>
<td>Taussig (#3461a)</td>
<td>88</td>
<td><em>DNMT3A, TET2, IDH1/2</em></td>
<td>12/28 (64)</td>
<td>21/60 (35)</td>
<td>0.01</td>
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<tr>
<td></td>
<td></td>
<td><em>DNMT3A</em></td>
<td>6/7 (86)</td>
<td>33/81 (41)</td>
<td>0.02</td>
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<tr>
<td></td>
<td></td>
<td><em>TET2</em></td>
<td>12/18 (67)</td>
<td>27/70 (39)</td>
<td>0.03</td>
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<tr>
<td></td>
<td></td>
<td><em>ASXL1</em></td>
<td>11/13 (85)</td>
<td>14/37 (38)</td>
<td>0.003</td>
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<tr>
<td>OSU^ (#944a)</td>
<td>46</td>
<td><em>DNMT3A</em></td>
<td>6/8 (75)</td>
<td>13/38 (34)</td>
<td>0.05</td>
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</tbody>
</table>

^AML pts treated with decitabine.  
*includes mCR in ORR.

Exome Sequencing Of Refractory Anemia With Ringed Sideroblasts

- Exome sequencing; RARS (n=8)
- \textbf{SF3B1} mutations in 6 cases (core component of pre-RNA splicing machinery)
- Mutations predicted to retain structural integrity of protein
- \textbf{SF3B1} \textsubscript{mu} CD34 cells underexpress genes involved in mitochondrial function

### SF3B1 Mutations In MDS And Other Malignancies

#### Subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Total</th>
<th>Variants</th>
<th>%</th>
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<tbody>
<tr>
<td>RA</td>
<td>91</td>
<td>9</td>
<td>9.9%</td>
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<tr>
<td>RARS</td>
<td>59</td>
<td>40</td>
<td>67.8%</td>
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<tr>
<td>RCMD</td>
<td>53</td>
<td>3</td>
<td>5.7%</td>
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<tr>
<td>RCMD-RS</td>
<td>23</td>
<td>13</td>
<td>56.5%</td>
</tr>
<tr>
<td>RAEB-1, RAEB 2</td>
<td>110</td>
<td>6</td>
<td>5.5%</td>
</tr>
<tr>
<td>Other</td>
<td>18</td>
<td>1</td>
<td>5.6%</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>354</td>
<td>20.3%</td>
</tr>
</tbody>
</table>

#### Histology

<table>
<thead>
<tr>
<th>Histology</th>
<th>Total</th>
<th>Variants</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>AML</td>
<td>57</td>
<td>3</td>
<td>5.3%</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>172</td>
<td>2</td>
<td>1.2%</td>
</tr>
<tr>
<td>Renal cancer</td>
<td>30</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td>CLL</td>
<td>40</td>
<td>2</td>
<td>5.0%</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>32</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Salivary</td>
<td>27</td>
<td>1</td>
<td>3.7%</td>
</tr>
<tr>
<td>Cancer cell lines</td>
<td>746</td>
<td>8</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

Clinical Significance of SF3B1 Mutations

Prolonged EFS independent of age, gender and karyotype.

PAPAEMMANOUIL E, ET AL. NEJM 2011; 365: 1384.
Frequent Mutations In Components Of Splicing Machinery In MDS

- Exome sequencing (n=29)
- Targeted sequencing of 8 genes (5 snRNPs, 3 accessory proteins) in the spliceosome complex (n=582)


*small nuclear ribonuclear proteins.

Affected genes involved in 3'-splice site recognition
Mutations in genes encoding major components of splicing machinery are common in MDS.

Spliceosomal gene mutations largely mutually exclusive.

Non-Syndromic Familial MDS
Transcription Factor Germline Mutation or SNP

- Transcription Factor (TF) Gene Mutations with Hematologic Prodrome
  - RUNX1 (thrombocytopenia)
  - CEBPA (eosinophilia)
- TF Gene Mutation without Prodrome
  - GATA2 zinc finger-2 (ZF2) mutation
- TP53 codon 72 polymorphism
Familial Platelet Disorder with Propensity for Myeloid Malignancy (FPD/AML)

- Autosomal dominant
- Moderate thrombocytopenia with normal size platelets
- Variable platelet dysfunction & bleeding history
- Nonsense or missense RUNX1 (AML1) heterozygous mutation or chromosome 21q22 intragenic microdeletion involving RHD (DNA binding & CBFβ heterodimerization)
- Mutational haplodeficiency most common; higher MDS/AML risk in dominant negative mutants
- Variable latency with incomplete MDS/AML penetrance
- Lifetime MDS/AML risk >35% (age 30-75 years)
- Either homozygous somatic mutations (75%) or cytogenetic abnormalities acquired with MDS/AML transformation

Familial CCAAT Enhancer Binding Protein Alpha (C/EBPα) Mutation

- Autosomal dominant
- **Hematologic prodrome:** eosinophilia
- **Transformation phenotype:** normal karyotype, M1 or M2 AML, aberrant CD7 expression, favorable prognosis
- Mutations restricted to transactivating DNA-binding domain (TAD) abolishing the 42kd full length protein; 30kd isoform binds & neutralizes the wt42-kd protein in a dominant negative fashion
- Complete penetrance in all carriers (age 4-39 years)
- Somatic deletions or insertions in the basic leucine zipper (bZIP) region acquired at transformation disrupting dimerization analogous to sporatic mu-C/EBPα AML
- 11% of suspected sporatic C/EBPα AML harbor germline mutations

Familial MDS with GATA2 Gene Mutation

1. Syndromic (accessory somatic features)
   - Emberger syndrome – primary lymphedema, deafness
   - MonoMAC – monocytopenia with mycobacterial infection
   - DCML – dendritic, monocyte & lymphoid (B, NK) cell defects

2. Non-Syndromic
   - Familial MDS/AML

Familial $GATA2_{\text{mu}}$ MDS/AML

$GATA2$ zinc finger-2 (ZF2) mutation
- Heterozygous missense mutation (C>T: p.Thr354Met) or 3bp deletion (p.Thr355del)
- Reduced transactivation ($RUNX1, PU.1, FOG1$)
- 4 families with trans-generational MDS or MDS/AML
- Autosomal dominant with high penetrance
- No hematologic prodrome in carriers
- Rapid onset (teens-40s), poor outcome without SCT
- Mutation absent in sporadic MDS/AML ($n=268$) & normal population ($n=695$)

Del5q MDS & TP53 Codon 72 Genotype
McGraw KR, et. al. ASH 2010; 612a

P53 in the Hypoplastic Anemia of Del5q MDS

• *RPS14* haplodeficiency disrupts ribosome assembly, accelerates MDM2 degradation & stabilizes p53 (Dutt. Blood 2010)
• *TP53* inactivation is sufficient to rescue the hematologic phenotype in a del(5q) MDS murine model (Barlow. Nat Med 2010)
• A SNP in *TP53* exon 4 (codon 72) is linked to cancer and mutagen susceptibility, and treatment outcome.
• The homozygous CC allele encodes a proline residue (vs. GG: arginine) with diminished apoptotic potential
Genotype Frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control [n=89]</th>
<th>Non-Del(5q) [n=92]</th>
<th>Del(5q) [n=93]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>9.0</td>
<td>9.8</td>
<td>19.4</td>
</tr>
<tr>
<td>CG</td>
<td>33.7</td>
<td>59.8</td>
<td>49.5</td>
</tr>
<tr>
<td>GG</td>
<td>57.3</td>
<td>30.4</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Odds ratio for CC vs GG:
- Del5(q) vs Control: (OR) = 4.1, 95% CI: 1.45 - 11.76
- Non-Del5(q) vs Control: (OR) = 1.8, 95% CI: 0.514 – 6.46

## Allele Frequencies

### Allele Frequency (%)

<table>
<thead>
<tr>
<th></th>
<th>Controls [n=89]</th>
<th>Non-Del(5q) [n=92]</th>
<th>Del(5q) [n=93]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td>25.8</td>
<td>39.7</td>
<td>44.1</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>74.2</td>
<td>60.3</td>
<td>55.9</td>
</tr>
</tbody>
</table>

- [Del5(q) vs Non-Del5(q); p=0.40]
- [Del5q) vs Control; p<0.001]
- [Non-Del5(q) vs Control; p=0.005]  
  (Fisher’s exact test)

Progression Free Survival (PFS) by Genotype

Non-Del(5q)

Del(5q)

CC, n=7
CG, n=50
GG, n=22

CC, n=16
CG, n=43
GG, n=24

P=0.01

P=0.30

(Log rank test)

Summary

Cryptic somatic genomic abnormalities are demonstrable in most MDS patients that refine prognostic discrimination & account for heterogeneity within IPSS categories.

Mutations involving major components of the RNA splicing complex & chromatin modifying genes predominate in MDS and may have a key role in disease pathogenesis.

Patients with a family history of MDS/AML should be screened for germline TF mutations and carriers excluded from allogeneic donor selection.

*TP53* codon 72 genotype may confer susceptibility to MDS (Del5q>other) and influence natural history of disease.