Molecular Landscape of MDS and its clinical applications

Papaemmanuil E, PhD
Assistant Attending
Computational Oncology
MSKCC
Disclosure

I have no relevant financial relationships to disclose.
The post cancer gene discovery promise

• Biology;
• Molecular oncology;
• Patient tailored medicine;
• Development of rational clinical and therapeutic protocols;
Well – documented gene mutations, frequencies and prevalence amongst the key MDS subtypes;
Increasingly understood patterns of co-mutation;
Recent insights into the biological mechanisms of spliceosome deregulation and downstream effectors;
Incorporation into diagnostic and clinical practices is not yet clear;
1. >200 recurrently mutated genes;
2. Mostly infrequently mutated genes <5%;
3. Most patients have more than one mutation;
4. Clonal heterogeneity;
5. Systematic – pairwise interactions;
6. Increasing complexity in variables to be considered in clinical practice;
Our goal...

1. Insights into the molecular mechanisms of disease biology;

1. Diagnostic biomarkers – genotype, phenotype correlations;

2. Prognostic biomarkers – clinical outcome relationships;

3. Predictive biomarkers – response to therapeutic intervention;

4. Molecular tools to monitor disease progression and response;

5. Therapies tailored to the individual genetic and clonal profiles;
Myelodysplastic syndromes
Splicing factors are disease defining, and much of the focus of biomarker research in MDS
# 2008 WHO Classification of MDS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Blood findings</th>
<th>BM findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia with unilineage dysplasia</td>
<td>1-2 cytopenias</td>
<td>&gt;10% of the cells in one myeloid lineage dysplasia;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;5% blasts</td>
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<tr>
<td>Refractory anemia with ringed sideroblasts (RARS)</td>
<td>Anemia</td>
<td>≥15% ringed sideroblasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;5% blasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia (RCMD)</td>
<td>Cytopenias</td>
<td>Dysplasia, &lt;5% blasts</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts 1 (RAEB-1)</td>
<td>Cytopenias</td>
<td>5-9% blasts</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts 2 (RAEB-2)</td>
<td>Cytopenias</td>
<td>10-19% blasts</td>
</tr>
<tr>
<td>MDS with isolated del 5q</td>
<td>Anemia with normal to High plt count</td>
<td>Isolated deletion 5q</td>
</tr>
</tbody>
</table>
Mutations in SF3B1 define a distinct molecular and clinical subgroup in MDS

- **Lower blast counts**;
- **Higher presence of ringed sideroblasts**;
- Lower incidence of multi lineage dysplasia (46.3% vs 82.6%, $P < .001$);
- Lower proportion of dysplastic myeloid cells and megakaryocytes;
- Higher absolute neutrophil and platelet counts ($P < .001$);
- **Better overall survival ($P= 0.003$) and lower cumulative incidence of disease progression**
1. Re-evaluation of bone marrow morphology in patients with \textit{SF3B1} mutations but not diagnosed as an MDS with ringed sideroblasts, identifies that SF3B1 predicts for the presence of RS.

\textbf{*Sf3B1* mutation} $\rightarrow$ 97% positive predictive value for ringed sideroblasts
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*Sf3B1 mutation*  
97% positive predictive value for ringed sideroblasts

2. We show the % of RS is correlated with % VAF of *SF3B1* mutations

![Box plot showing correlation between SF3B1 mutation burden and percentage of bone marrow ring sideroblasts.](Malcovati_Blood_2012)
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Patterns of co-mutation

- SF3B1 mutations define a distinct molecular subgroup that is mutually exclusive to mutations in TP53, and / or cytogenetic abnormalities or complex karyotype.

- Co-mutation with DNMT3A (20%) :
  - No effect on overall survival or event free survival;
  - Increased involvement of multi lineage dysplasia
$P = 2.69 \times 10^{-7}$
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- Co-mutation with \( ASXL1 \) (7%), \( RUNX1 \) (6%), \( EZH2 \) (5%):
  - Increased transfusion dependency
  - Worse prognosis (\( P = 0.004 \)), disease progression (\( P=0.002 \))

Larger numbers are warranted to study the effects of co-mutation in MDS prognosis
• Defines a distinct clinical and pathologic entity, one characterised by the presence of ringed sideroblasts;

• Molecular testing for \textit{SF3B1}, more accurately identifies patients in this group, compared to $\%$blast / $\%$ ringed sideroblast thresholds;

• \textit{SF3B1} is an independent prognostic predictor of clinical outcome associated with favorable prognosis;

• Attention should be given on the pattern of co-mutation: ASXL1 : RUNX1 : EZH2 and potentially others..

\textit{SF3B1} mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts

Luca Malcovati$^{1,2}$, Mohsen Karimi$^{3}$, Elli Papaemmanuil$^{4}$, Ilaria Ambaglio$^{2,5}$, Martin Jädersten$^{3}$, Monika Jansson$^{3}$, Chiara Elena$^{1,2}$, Anna Galli$^{2}$, Gunilla Walldin$^{3}$, Matteo G. Della Porta$^{2,5}$, Klas Raaschou-Jensen$^{6}$, Erica Travaglino$^{2}$, Klaus Kallenbach$^{7}$, Daniela Pietra$^{2}$, Viktor Ljungström$^{8}$, Simona Conte$^{3}$, Emanuela Boveri$^{9}$, Rosangela Invernizzi$^{5,10}$, Richard Rosenquist$^{8}$, Peter J. Campbell$^{6}$, Mario Cazzola$^{1,2}$, and Eva Hellström Lindberg$^{3}$
• **Hotspot mutations, P95, Challenging molecular region, low NGS coverage sometimes misses mutations.**
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![Diagram showing protein structure with Pro95 highlighted]

• **High risk MDS & CMML & sAML**

![Graph showing gene expression and disease progression]

• Associated with granulocytic disease, poor outcome and increased risk of leukemic transformation.

• Poor prognosis irrespective of estimates clonal status.

• Is frequently co-mutated with other adverse prognosis genes: ASXL1, STAG2, RUNX1, resulting in further deterioration in overall survival.
• **Hotspot mutations, P95, Challenging molecular region, low NGS coverage sometimes misses mutations.**

![Hotspot mutations diagram](image)

• **High risk MDS & CMML & sAML**

![High risk MDS chart](image)

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• **Hotspot mutations, P95, Challenging molecular region, low NGS coverage sometimes misses mutations.**

![Diagram showing protein structure with amino acid positions and hotspot mutations.](image)

- **High risk MDS & CMML & sAML**

  ![Diagram showing genetic variations in different diseases.](image)

- **Associated with granulocytic disease, poor outcome and increased risk of leukemic transformation.**

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- **Is frequently co-mutated with other adverse prognosis genes: ASXL1, STAG2, RUNX1, resulting in further deterioration in overall survival.**
• **Hotspot mutations**,  

![Diagram of U2AF1](image)

• ~7%, mostly high risk MDS & CMML & AML

• Associated with young age, and high-risk of leukemia transformation.

• **Adverse prognosis**:  

![Graph of event-free survival](image)

Wu et al Am J Hematology 2014; Thol et al Blood 2012
• Whilst SF3B1, associated with favourable prognosis;
• SRSF2; U2AF1, associated with inferior outcomes;
• ZRSR2 – unclear:
Clinical presentation and clinical outcomes

*SF3B1*

*SF1 PRPF40B*

*ZRSR2*

*SRSF2 : U2AF1*
Positive testing for splicing factor mutations is not unique to MDS
SRSF2 has shared genomic landscape between MDS and AML
SF3B1 mutations occur in distinct genetic background in AML
Testing positive for mutations in splicing factor genes.
Busque et al 2012; Jaiswal et al NEJM 2014; Genovese et al NEJM 2014; Xie et al Nat Medicine 2014; McKerrel et al Cell reports
Busque et al 2012; Jaiswal et al NEJM 2014; Genovese et al NEJM 2014, Xie et al Nat Medicine 2014; McKerrel et al Cell reports
• Presentation of splicing factor mutations in myeloid neoplasms
Clinical presentation

- Presentation of splicing factor mutations in myeloid neoplasms

2175 myeloid neoplasms

Data presented from panel sequencing

% of patients with mutation in panel
MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance

Brian Kwok¹, Jeff M. Hall¹, John S. Witte², Yin Xu¹, Prashanti Reddy¹, Keming Lin¹, Rachel Flamholz¹, Bashar Dabbas¹, Aine Yung¹, Jenan Al-Hafidh¹, Emily Balmert¹, Christine Vaupel¹, Carlos El Hader¹, Matthew J. McGinniss¹, Shareef A. Nahas¹, Julie Kines¹, and Rafael Bejar³

Author Affiliations
Formally modeled genomic structure to account for:
Gene mutations + *genetic interactions* + diagnostic variables + demographic variables.

and build personalised prediction models
26 centers: 2600 cases committed

Meta-analysis ~5000-6000 well annotated MDS cases
Invitation to Join Project

Begin receiving clinical data

Finish receiving clinical data

Send out sample queries and MTAs

Begin processing MTA agreements

Begin sending sample tubes

QC and library Construction

Begin receiving samples at MSKCC

2015
Acknowledgements

Peter Campbell  Wellcome Trust Sanger Institute
  •  Moritz Gerstung

AML- SG
  •  Hartmut Dohner
  •  Richard Schlenk
  •  Lars Bullinger
  •  Konny Dohner

Papaemmanuil Lab / MSKCC Leukemia Genomics
  •  Gunes Gundem
  •  Matahi Moarii
  •  Franck Rappaport
  •  Juan Medina
  •  Komal Rathi
  •  Noushin Farnhoud
  •  Minal Patel
  •  Kristina Knapp
  •  Irene Phillip
  •  Yesenia Werner
  •  Marc Robert de Massy

MSKCC iGO
  •  Agnes Viale
  •  Kety Huberman