Mutational Impact on Diagnostic and Prognostic Evaluation of MDS

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Disclosure

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The good prognosis associated with SF3B1 is shifted with co-mutators in which of the following genes?

a. TET2  
b. DNMT3A  
c. RUNX1  
d. All of the above
Clinical management of MDS

Clinical, Morphology and Cytogenetics

MDS/MPN
NF1, CBL, KRAS, PTPN11, JMML
SETBP1 & ETVN1 - sCML

MDS
SF3B1 – MDS RA

MPN
JAK2, MPL, CALR, SH2B3 CSF3R - CNL

Molecular

AML
FLT3-ITD & FLT3-TKD
NPM1
CEBPA
KIT
KMT2A-PTD

MPN
DNMT3A
ASXL1
EZH2
RUNX1
SETBP1
TP53

TET2
CBL
NRAS/KRAS
SF3B1
SRSF2
U2AF1
ZRSR2
SETBP1

Figure from:
Patel et al Clinical Lymphoma Myeloma and Leukemia 2017
Evolution of MDS stratification

Diagnosis
- 1982: FAB
- 1997: IPPS
- 2008: WHO
- 2012: IPPS-R
- 2016: WHO

Prognosis
- 1982
- 1997: IPPS
- 2008
- 2012: IPPS-R
- 2016: WHO

Cytopenias Dysplasia

Cytogenetics

Gene mutations

References:
- Arber et al Blood 2016
- Greenberg et al Blood 2012
- Vardiman et al Blood 2008
- Bennett Semin Oncol 2005
- Greenberg et al Blood 1997
Integration of recent molecular discoveries

- Diagnostic biomarkers
  - Genes level
  - Mutation hotspots
  - Genetic Interactions

- Treatment
  - Optimal treatment decisions
  - Hypo-methylating agents
  - Transplant

- Genomic Architecture

- MDS Classification
  - Biologically defined
  - Molecular subgroups
  - Clinical presentation

- Treatment

- IPSS Molecular
  - Integrative risk models
  - Patient tailored risk estimates

- Prognostication

- Clinical
MDS mutations across pathways

- **Splicing factors**: SF3B1, SRSF2, U2AF1, ZRSR2
- **DNA methylation**: TET2, DNMT3A, IDH1/2
- **Histone modification**: ASXL1, EZH2, BCOR, EP300
- **Cohesin components**: STAG2, RAD21, SMC1A, SMC3
- **Transcription factors**: RUNX1, ETV6, CUX1, GATA2
- **Signal transduction**: CBL, JAK2, NRAS, KRAS, MPL, NF1, PTPN11, KIT, FLT3
- **p53 pathway**: TP53, PPM1D

Figure from Kennedy and Ebert JCO 2017

Kennedy and Ebert JCO 2017
Sperling et al Nature Rev 2017
Saez et al Blood 2017
Haferlach et al Leukemia 2014
Yoshida et al Nature 2011
Papaemmanuil et al NEJM 2011
Splicing factor deregulation central to MDS biology

- Splicing factors: SF3B1, SRSF2, U2AF1, ZRSR2
- DNA methylation
- Histone modification
- Cohesin components
- Transcription factors
- Signal transduction
- p53 pathway

Mutation Frequency (%)
Genomic population studies identify molecular prognostic markers

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Authors</th>
<th>Year</th>
<th>Prognostic genes in multivariate model</th>
</tr>
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<tbody>
<tr>
<td>439</td>
<td>Bejar et al.</td>
<td>2011</td>
<td>ASXL1, ETV6, EZH2, RUNX1, TP53</td>
</tr>
<tr>
<td>738</td>
<td>Papaemmanuil et al.</td>
<td>2013</td>
<td>EZH2, DNMT3A, SF3B1, SRSF2, RUNX1, TET2, TP53</td>
</tr>
<tr>
<td>944</td>
<td>Haferlach et al.</td>
<td>2014</td>
<td>ASXL1, KRAS, LAMB4, NPM1, PRPF8, RUNX1, TP53</td>
</tr>
<tr>
<td>508</td>
<td>Nazha et al.</td>
<td>2017</td>
<td>CBL, NRAS, TP53</td>
</tr>
<tr>
<td>685</td>
<td>Tefferi et al.</td>
<td>2018</td>
<td>ASXL1, SF3B1, RUNX1</td>
</tr>
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→ Need to develop a robust consensus prognostic model
1. Incorporate gene mutations into formal classification i.e. **WHO classification** for MDS?

2. Improve prognostic accuracy and treatment decisions by consideration of gene mutations in formal prognostic i.e. **IPSS molecular model**?
International Working Group for the prognosis of MDS

13 countries | 25 centers
# International Working Group for the prognosis of MDS

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<thead>
<tr>
<th>Retrospective cohorts</th>
<th>Prospective sequencing study</th>
<th>Validation</th>
</tr>
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<td>N=1,682</td>
<td>N=4,270</td>
<td>N to be determined</td>
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<td>Bejar et al 2011</td>
<td>MSKCC IWG-PM Cohort</td>
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International Working Group for the prognosis of MDS

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Study Entry Criteria:

Diagnostic, pre-treatment samples
Complete clinical annotation
  Diagnostic
  Morphology | CBC
  Treatment
Long-term follow-up data
International Working Group for the prognosis of MDS

Retrospective cohorts
- N=1,682
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Validation
- N to be determined
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- 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
Integration of clinical and molecular data

Demographics
- age, sex

Clinical Parameters
- blood counts, blasts%

Karyotype
- cytogenetics

Molecular

Treatment
- hma, transplant

AML transformation
- transformation in 20% of patients

Overall survival
- median follow-up 3.5 years
Outline

Cohort overview
  Clinical
  Molecular
  Correlative analysis

Splicing factors
  Disease defining
  Mutual exclusivity
  Co-mutations effect

Molecular classes
  Ab-initio clustering
  Diagnostic value
  Prognostic value
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IWG-MDS MSKCC cohort
Clinical cohort characteristics
Genomic analysis backbone

1) Comprehensive annotation of putative oncogenic mutations
   - Mutation distribution among patients
   - Gene-level annotation
   - Hotspot-level annotation
   - Patterns of co-mutations
   - Associations with clinical presentation and outcome

2) Integration of cytogenetics data and NGS copy-number events
   - Cytogenetics score and complex karyotype
   - Focal LOH

3) Unmatched sequencing data
   - Control for germline infiltration
Molecular cohort characteristics

Combination of 4 variant callers
Sequencing metrics

Population based metrics
Somatic | Germline

Background mutation rate
Statistical recurrence

Mutation signature analysis

Identification of new putative drivers
Correlative analysis

5,002,459 raw calls
82,773 filtered
13,063 oncogenic
2,322 new

data-driven cutoffs

statistical recurrence
manual curation
Most mutations are not recurrent

5,002,459 raw calls
82,773 filtered
13,063 oncogenic
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data-driven cutoffs

statistical recurrence
manual curation

Coding substitutions

in_cosmic_or_oncoKB
no
yes

Count

Recurrence of mutations

1 2 3 4 5 >5
Annotation of new likely oncogenic variants

RUNX1 example
Annotation of new likely oncogenic variants

RUNX1 example

- RUNX1 WT (N=1516)
- RUNX1 putative (N=49)
- RUNX1 Cosmic I OncoKb (N=270)
95% of patients with driver alteration(s)
Median of 4 oncogenic events per patient
Clinical features trend with number of drivers
Disease subtypes with number of drivers

- WHO criteria not met
- NA
- other
- 5q-
- RCU
- RARS
- RCMD-RS
- RCMD
- RAEB
- MDS-AML
- CMML

Number of driver alterations per patient:
- 0
- 1-2
- 3-4
- 5-6
- 7 or more

Proportion of patients:
- 0.00
- 0.25
- 0.50
- 0.75
- 1.00
Risk groups with number of drivers

IPSS Good MDS

Substratify by number of oncogenic events

Evaluate prognostic significance
Risk groups with number of drivers

- IPSS Good MDS
- Substratify by number of oncogenic events
- Evaluate prognostic significance

![Graph showing survival probability over years for different risk groups with varying number of oncogenic events.]
Detailed map of co-mutations
Detailed map of co-mutations

Molecular classification

Disease modelling

Drug development
Main points

1. Sequencing analysis of 4,270 MDS patients with molecular and clinical annotation
2. Developed methodology to characterize rare non-recurrent variants
3. >13,000 oncogenic events, characterize at least 1 mutation in 95% of MDS
4. Mutation negative cases, not satisfying standard diagnostic criteria
5. Number of mutations, associated with severity of clinical presentation and outcome
6. Strong and novel patterns of co-mutation: framework for genotype-clinical studies
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Splicing factors as candidate biomarkers

55% of MDS patients have mutations in splicing factor genes

Disease defining | Associate with diagnostic MDS features | Mutually Exclusive | Early clonal events
Splicing factors as candidate biomarkers

Altered in 1718 (55.96%) of 3070 samples.

- Sf3b1
- Srsf2
- U2af1
- Zrsr2
- Prpf8

Legend:
- Missense_Mutation
- Frame_Shift_Del
- Multi_Hit
- Nonsense_Mutation
- In_Frame_Del
- Splice_Site
- Frame_Shift_Ins
- In_Frame_Ins
**Incorporation of SF3B1 status in WHO 2016**

**WHO 2016 MDS-RS:**
more than 15% ringed sideroblasts or
more than 5% of ringed sideroblasts and SF3B1 mutation

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**SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts**

Lucia Malcovati,1,2 Mohamad Karimi,3 Ellii Papaemmanuil,4 Ilaria Ambaglia,2,8 Martin Jüdenson,3 Monika Jansson,3 Chiara Elena,1,2 Anna Galli,2 Gunilla Waldin,4 Matteo G. Dela Porta,5,6 Klas Raschou-Jensen,5 Erica Travaglini,6 Klaus Kallenbach,7 Daniela Pietra,2 Viktor Ljungström,6 Simona Conte,3 Emanuela Boveri,9 Rosangela Invernizzi,5,10 Richard Rosenquist,9 Peter J. Campbell,4 Mario Cazzola,1,2 and Eva Hellström Lindberg9

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**Figure 2. Relationship between SF3B1 mutant allele burden and proportion of ring sideroblasts.** Values for percentage of ring sideroblasts are grouped here in 3 arbitrary categories: < 15% (n = 183), 15% to 50% (n = 85), and > 50% (n = 57). Data are shown in a box plot depicting the smallest and largest observation (lowest and highest horizontal line, respectively), lower and upper quartile with median value (box), and outliers (dots).

Malcovati et al Blood 2011
Papaemmanuil et al NEJM 2011
Detailed map of co-mutations: SF3B1 K700 other
Mutation hotspots with SF3B1

p<0.001
SF3B1 cases have a median of 2 other alterations

From 0 to many other alterations with median of 2

8% of SF3B1 isolated cases

Co-occurrence occurs with various genes or copy-number events
SF3B1 cases have a median of 2 other alterations

From 0 to many other alterations with median of 2

8% of SF3B1 isolated cases

Co-occurrence occurs with various genes or copy-number events
SF3B1 cases have a median of 2 other alterations

**Effect of co-mutators on the SF3B1 phenotype and outcome?**

Is it clinically relevant?
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 isolated or with TET2 / DNMT3A: No shift
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 isolated or with TET2 / DNMT3A  No shift
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 isolated or with TET2 / DNMT3A — No shift

Log-rank: p = 0.3

Survival probability

years

0 % 25 % 50 % 75 % 100 %
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 with ASXL1 or RUNX1 or TP53  

Shift
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 with ASXL1 or RUNX1 or TP53

Shift

- **PLT**
- **BM_BLAST**
- **RINGED_SIDEROBLASTS**

Legend:
- SF3B1–WT
- SF3B1 isolated or with TET2 I DNMT3A
- SF3B1 with ASXL1
- SF3B1 with ASXL1
- SF3B1 with RUNX1
- SF3B1 with TP53
SF3B1 phenotype and outcome shift with co-mutators

SF3B1 with ASXL1 or RUNX1 or TP53

Shift
Formalize approach across MDS genes in cohort

The genetic context in which mutations occur shape clinical trajectories

Global analysis of MDS landscape

Genotype- Clinical

Genotype- Outcome
Integrative genotype-clinical analyses

Many mutations

SF3B1
DNMT3A
ASXL1
BCOR

Integration of molecular and morphological features

Clinical relevance
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Frequent gene mutations

Patients
Comprehensively evaluate **patterns of co-mutations** and mutual exclusivity.
Comprehensively evaluate patterns of co-mutations and mutual exclusivity

Identify non-overlapping molecular subgroups
Molecular clustering identifies patient classes

Patient molecular profiles

n=192 recurrent genetic alterations

n=12,970 patient alterations

Minimum class numbers explained by data
Molecular clustering identifies patient classes

- Patient molecular profiles
- Minimum class numbers explained by data
- Class assignment of each individual

$n=192$ recurrent genetic alterations
$n=12,970$ patient alterations

Ab-initio
Probabilistic | Bayesian
Consider only molecular features
Each gene can be present in more than one class
Each class can be defined by more than one gene
Each patient is assigned probability to each class
Comprehensively evaluate patterns of co-mutations and mutual exclusivity

Identify non-overlapping molecular subgroups
Comprehensively evaluate **patterns of co-mutations** and mutual exclusivity

Identify **non-overlapping molecular subgroups**
Comprehensively evaluate **patterns of co-mutations** and mutual exclusivity

Identify **non-overlapping molecular subgroups**

Test for specific **associations** between clinical presentation & outcomes
Molecular groups are associated with clinical features

Platelet variations across molecular subgroups
Molecular groups are associated with clinical features

Bone marrow blast% variations across molecular subgroups
Molecular groups are associated with demographics

Male | Female occurrence variations across molecular subgroups
Molecular groups are associated with outcome

Rate of AML transformation vary across molecular subgroups
Morphology subtypes are split across molecular groups

Different molecular profile groups lead to a given phenotype

Complementary approaches
Prognostic significance of molecular subgroups
Molecular subgroups hold independent prognostic value

IPSS-R across molecular subgroups

- IPSSR_good_comp1 (n = 295)
- IPSSR_good_comp3 (n = 122)
- IPSSR_good_comp4 (n = 365)
- IPSSR_good_comp6 (n = 143)
- IPSSR_int (n = 450)

IPSS-R good patients from one molecular subgroup (n=122) behave as IPSS-R int
Take home messages

Deliver molecular blueprint of MDS

95% of patients with at least one driver alteration

*SF3B1* phenotype and good prognosis can shift with specific co-players

Molecular clustering identifies classes of patients that are associated with demographics, clinical features and outcome

Findings from this study will inform future clinical decision support in MDS
Molecular sub-classification | IPSS-molecular
**Oversight Steering Committee:**

**Classification Committee**

**Treatment decisions HMA Transplant**
Valeria Santini, Aziz Nazha, De Witte T, Cazzola M, Bejar R, Hellstrom-Lindberg E, Cazzola M, Malcovati L
Thank you

**IWG collaborators**

**Clinical Data Curation**

Maria Creignou - Lindberg Lab  
Luca Malcovati  
Kelly Bolton

**WHO Classification**

Robert Hasserjian

**IPSS-R**

Heinz Tuechler

**Cytogenetics**

Detlef Hasse  
Raf Bejar

**Papaemmanuil Lab | MSKCC**

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Sean Devlin, Pierre Guilmin

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Juan Medina, Juan Arango Ossa,  
Joe Zhou, Max Levine, Gunes Gundem, Teja Yellapantula,  
Teng Gao, Noushin Farnoud, Matthieu Cornet, Dominik Glodzik
See you in Copenhagen
The good prognosis associated with SF3B1 is shifted with co-mutators in which of the following genes?

a. TET2  
b. DNMT3A  
c. RUNX1  
d. All of the above