Diagnosis and Prognosis in Acute Myeloid Leukemia
— The Art of Distinction —

“The discovery of acquired mutations … in AML has not only changed the role of the microscope in diagnosing leukemia but also influenced the management … and how we think about their causes”

Bob Löwenberg NEJM 2008
**Introduction**

Gene mutations play an important role in the pathogenesis of MDS.

Can we learn from examples in AML? In AML, mutations are practice changing

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prognosis</th>
<th>Treatment</th>
<th>Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>favorable</td>
<td>ATRA? (Schlenk et al NEJM 2008)</td>
<td>no</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>unfavorable</td>
<td>FLT3 inhibitors? (eg. Midostaurin)</td>
<td>yes</td>
</tr>
</tbody>
</table>

**Cytogenetic changes in MDS**

-20 q-
-7/7q-
complex
Del (5q)

ASXL1
RUNX1
TET2
EZH2
TP53
e.tc…

normal
Targets of mutations in MDS
- Results of research at the beginning of 2011 -

Epigenetic modification
- TET2 (20%)
- ASXL1 (15%)
- EZH2 (6%)
- DNMT3A (6%)
- IDH1/IDH2 (3%)

Differentiation
- RUNX1 (9%)

Tumor suppressor
- p53 (8%)

Bejar et al JCO/NEJM 2011

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Targets of mutations in MDS
- Results of research at the end of 2011 -

Novel Mutations identified in patients with RARS

Mutations in RNA splicing factor 3B, subunit 1 (SF3B1)
Identified in 65% (53/82) of patients with RARS

Papaemmanuil et al NEJM 2011
Targets of mutations in MDS
- Results of research at the end of 2011 -

Incidence in MDS
- **SF3B1** 6.5%
  (75.3% in RARS/RCMD-RS)
- **SRSF2** 11.6%
- **U2AF1** 11.6%
- **ZRSR2** 7.7%
  etc..

**Novel mutations in the splicing machinery**

Yoshida et al Nature 2011

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**Mutations of multiple components of the splicing machinery**

**Splicing Mutations**
- Majority are heterozygous point mutations
- Some genes have mutational hotspots

Yoshida et al Nature 2011
Frequencies of spliceosome pathway mutations among 582 cases with various myeloid neoplasms

Mutations in splicing genes:
- rare in de novo AML
- frequent in MDS
- SF3B1 mutations most frequent in RARS/RCMD-RS
- SRSF2 mutations very frequent in CMML

Markers in MDS

We need to ask ourselves whether the markers are:

- Diagnostic
- Prognostic
- Predictive
- Targetable
Diagnostic?

To this day, no single known mutation is 100% specific for MDS or a subgroup of MDS.

However, in conjunction with morphology, molecular markers can help to make the diagnosis (mutations, cytogenetic changes).

Several mutations are more suggestive of certain MDS subtypes
- SF3B1 and RARS/RCMD-RS
- SRSF2 for CMML

Prognostic impact?

Splicing genes:
- U2AF1 (Gaubert et al Nat Genetics 2011)
- SRSF2 (Thol et al Blood 2012)

Others:
- ASXL1 (Thol et al JCO 2011)
- EZH2 (Ernst et al Nat Genetics 2010, Nikoloski et al Nat Genetics 2010)
- DNMT3A (Walter et al Leukemia 2011, Thol et al Haematologica 2011)

Prognostic impact sometimes controversial or unknown
Prognostic effect in MDS - SF3B1

- SF3B1 mutated
- SF3B1 WT

533 MDS patients

Overall Survival

Leukemia-free survival

P=0.009

P=0.032

Malcovati et al Blood 2011

Event-free survival

Prognostic effect in MDS with ring sideroblast - SF3B1

- SF3B1 mutated
- SF3B1 WT

48 MDS-RARS patients

P=0.06

Patnaik et al Blood 2011

43 MDS-RCMD-RS patients
Prognostic effects in MDS - U2AF1

150 MDS patients

Prognostic effects in MDS - SRSF2

154 MDS patients

Graubert et al Nat Genet. 2011

Thol et al. Blood 2012
Prognostic impact of TET2 – initial study

Overall Survival

- 88 MDS patients
- TET2 mutated
- TET2 WT

Leukemia-free survival

Event-free survival

Kosmider et al Blood 2009

Prognostic impact of TET2 - subsequent studies

- 320 MDS patients
- Allelic burden?
- Next generation sequencing:
  - OS between patients with a high (> 25% RMA) or low (≤ 25%) level mutation was not different (p=0.45)

Smith et al Blood 2010

- Similar results by Bejar et al NEJM 2011
Prognostic effect - ASXL1

154 MDS patients

Multivariate analysis:

- Overall survival
  - p=0.04

- Time to AML
  - p=0.024

Thol et al. JCO 2011

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Prognostic effects in MDS/MPN - EZH2

Ernst et al Nat Genet. 2010
Prognostic effects in MDS- **EZH2**

119 MDS patients

Nikoloski et al Nat Genet. 2010

Prognostic effects in MDS- **DNMT3A**

8% out of 150 MDS (incl. RAEB-T) patients mutated

Walter et al Leukemia 2011

3% out of 193 MDS patients mutated

**DNMT3A** mutations associated with AML transformation (P=0.043)

Thol et al Haematologica 2011
Summary prognostic impact of common mutations in MDS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2</td>
<td>20</td>
</tr>
<tr>
<td>ASXL1</td>
<td>10</td>
</tr>
<tr>
<td>SRSF2</td>
<td>10</td>
</tr>
<tr>
<td>U2AF1</td>
<td>10</td>
</tr>
<tr>
<td>RUNX1</td>
<td>10</td>
</tr>
<tr>
<td>TP53</td>
<td>10</td>
</tr>
<tr>
<td>SF3B1</td>
<td>10</td>
</tr>
<tr>
<td>EZH2</td>
<td>5</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>5</td>
</tr>
<tr>
<td>RAS</td>
<td>5</td>
</tr>
<tr>
<td>ETV6</td>
<td>5</td>
</tr>
</tbody>
</table>

Predictive and targetable?

Multiple cooperating mutations in each patient

- Which are more relevant?
- Does the allelic burden matter?

Thol et al. Blood 2012
Treatment algorithms

MDS

IPSS

low/int-1

int-2/high

Del(5q) Epo < 500 Epo > 500

< 60 y > 60 y

donor no donor

Lenalidomide Epo +/- G-CSF trials

Chelation therapy

ATG/Alemtuzumab?

Azacitidine

Allogeneic stem cell transplantation

and trials ....

Lenalidomide and del(5q)

- Del(5q) predicts response to Lenalidomide

List et al NEJM 2006

- In patients with del(5q), mutated TP53 predicts lower response to lenalidomide

Jädersten et al JCO 2011
Lenalidomide and MDS without del(5q)

**Study cohort**
- 42 patients
- 31 MDS (13 RARS)
- 7 MDS/MPN
- 2 PFM
- 2sAML

--> small heterogenous patient group

- Cytogenetic predictors of response:
  - Normal karyotyp, Trisomy 8

- Molecular markers studied in 21 patients
  - *(TET2, CBL, EZH2, ASXL1, TP53, RAS, IDH1/2, DNMT3A, UTX)*:
    - no predictors for response

Sugimoto et al J Hematol Oncol. 2012

Hypomethylating agents and epigenetic regulators

Genes involved in epigenetic modification are frequently mutated in MDS: *TET2, EZH2, ASXL1, IDH, DNMT3A*

→ Do demethylating agents help here?

Azacitidine
Decitabine

**TET2 mutations and hypomethylating agents**

- Response to Azacitidine improved in mutated TET2 but no difference in OS
  
  Itzykson et al, Leukemia 2011

- Similar results in a cohort of 93 MDS patients
  
  Traina et al, Blood (ASH Annual Meeting Abstracts 2011); 118: 461.

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**DNMT3A mutations and hypomethylating agents**

In AML:

- Similar results in a cohort of 93 MDS patients
  
  Traina et al, Blood (ASH Annual Meeting Abstracts 2011); 118: 461.
Other mutations and hypomethylating agents

- Impact of mutations affecting ASXL1, EZH2, IDH on response to hypomethylating agents unknown.

- To date, data is very limited about predictive value of molecular markers for response to therapy (e.g., splicing genes)

--> no data on prospective trials

Splicing genes and CLL

In CLL, SF3B1 mutations are associated with fludarabine-refractoriness

Rossi et al Blood 2011
Who and when to transplant?

Answers:
- Only curative approach
- Mainly offered to younger patients (< 60 years)
- Disease-free survival: 30-50% Chen et al Blood 2007

Questions:
- Who benefits from transplantation?
- When is the best timing for transplantation?
- Are there any molecular markers predicting patients’ outcome?

Who and when to transplant?

Many patients are undergoing allogeneic transplant after transformation to AML.

After AML transformation
- outcome less favorable,
- more intense induction therapy needed $\rightarrow$ more treatment related morbidity

Molecular markers might identify patients and time points for transplant…
Molecular markers for outcome after allogeneic hematopoietic stem cell transplantation in MDS

Hannover study

Aim: To investigate the prognostic impact of ASXL1 mutations in a cohort of patients with high risk MDS or secondary AML following MDS (sAML) undergoing allogeneic HSCT.

Methods: 105 patients evaluated for mutations in ASXL1 by direct sequencing.

Overall survival according to characteristics of HSCT patients.

Könecke*, Thol* Blood (ASH Annual Meeting Abstracts 2011);118: 1709

Multivariate analysis: Overall survival for ASXL1 p=0.008
Treatment algorithms

MDS

IPSS

low/int-1

int-2/high

Molecular markers?

Del(5q)       Epo <500       Epo > 500

<60y                        > 60 y

Donor                     no donor

Lenalidomide             Epo +/- G-CSF

Chelation therapy

Allogeneic stem cell transplantation

ASXL1… ?

TP53

TET2

DNMT3A

Should molecular genetics guide individualized therapy in MDS?

I am leaving you with questions and not with answers...

But we do have the tools to answer our questions:

- Clinical trials
- Molecular diagnostic including
Next Generation Sequencing

I am leaving you with questions and not with answers…
Summary

Diagnosis and Prognosis in Myelodysplastic syndrome — The Art of Distinction —

We need to ask ourselves whether the markers are:

- Diagnostic (helpful e.g. SF3B1 and RARS)
- Prognostic (yes)
- Predictive (yes, but prospective trials needed)
- Targetable (work to be done)

Is this future of MDS?

Novel/others: Treatment F

TET2 → Treatment A

RUNX1 → Treatment B

EZH2 → Treatment E

ASXL1 → Treatment C

SF3B1 → Treatment D

The answer can come from trials
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TRANSLATIONAL RESEARCH TRAINING IN HEMATOLOGY