MRD in acute myeloid leukemia

Meeting VAKB
8 februari 2011
Nancy Boeckx, MD, PhD

Introduction AML

› What is it?
  ◦ clonal expansion of myeloid precursor cells with reduced capacity to differentiate
  ◦ as opposed to ALL/CLL, it is limited to the myeloid cell line

› Incidence
  ◦ 2.7 per 100,000
  ◦ occurs at any age, but incidence increase with age
  ◦ vast majority occurs in adults, especially at >60 years

› Diagnosis (FAB – WHO 2002/2008)
  ◦ morphology
  ◦ flow cytometric immunophenotyping
  ◦ cytogenetics
  ◦ molecular biology
  ◦ clinical history
AML and related precursor neoplasms (WHO 2008)

- **Acute myeloid leukemia with recurrent genetic abnormalities**
  - AML with t(8;21)(q22;q22); (AML1/ETO)
  - AML with abnormal eosinophils inv (16)(p13q22) or t(16;16)(p13;q22); (CBFB-MYH11)
  - Acute promyelocytic leukemia (AML with t(15;17)(q22;q12)(PML-RARα) & variants
  - AML with t(9;11)(p22;q23); MLLT3-MLL
  - AML with t(6;9)(p23;q34); DEK-NUP214
  - AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2); RPN1–EV1
  - AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15–MKL1
  - AML with mutated NPM1
  - AML with mutated CEBPA

- **Acute myeloid leukemia with myelodysplasia–related changes**

- **Therapy–related myeloid neoplasms**

- **Acute myeloid leukemia, NOS (not otherwise categorized)**
  - Acute myeloid leukemia minimally differentiated
  - Acute myeloid leukemia without maturation
  - Acute myeloid leukemia with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic and monocytic leukemia
  - Acute erythroid leukemias
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis

- **Myeloid sarcoma**

- **Myeloid proliferations related to Down syndrome**

- **Blastic plasmacytoid dendritic cell neoplasm**

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Pretreatment prognostic factors for AML outcome

- age
- type of AML (*de novo* / secondary)
- presenting WBC count
- diagnostic karyotype
- molecular abnormality

• favourable
• intermediate
• adverse
Pretreatment cytogenetic entities

<table>
<thead>
<tr>
<th>Cytogenetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
</tr>
<tr>
<td>t(15;17)(q22;q12~21)</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
</tr>
<tr>
<td>inv(16)(p13;q22)/t(16;16)(p13;q22)</td>
</tr>
<tr>
<td>Intermediate</td>
</tr>
<tr>
<td>entities not classified as favorable or adverse</td>
</tr>
<tr>
<td>Adverse</td>
</tr>
<tr>
<td>abn(3q) [excluding t(3;5)(q21<del>25;q31</del>35)]</td>
</tr>
<tr>
<td>inv(3)(q21;q26)/t(3;3)(q21;q26)</td>
</tr>
<tr>
<td>add(5q), del(5q), −5</td>
</tr>
<tr>
<td>−7, add(7q)</td>
</tr>
<tr>
<td>t(6;11)(q27;q23)</td>
</tr>
<tr>
<td>t(10;11)(p11~13;q23)</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
</tr>
<tr>
<td>−17, abn(17p) with other changes</td>
</tr>
<tr>
<td>complex (&gt;3 unrelated abnormalities)</td>
</tr>
</tbody>
</table>

Impact of cytogenetic entities on overall survival (adults AML)

Treatment of AML

- **Induction therapy**
  - 1 or 2 cycles
  - intensive chemotherapy (anthracyclines and cytarabine)
  - goal: complete remission
    - reduction malignant BM blast (<5%)
    - restoration of normal hematopoiesis (PLT >100x10^9/L, neutro >1.0 x10^9/L)

- **Post-remission therapy**
  - killing of leukemia cells that may remain in BM/PB, but are undetectable by microscopy (= minimal residual disease (MRD))
  - prevention of relapse (improve leukemia-free and overall survival)
    - consolidation therapy: 2 or 3 courses chemotherapy, autologous or allogeneic stemcell transplantation

Rational for MRD analysis in AML

- most important prognostic factors for AML in upfront risk stratification in current treatment schedules
  - molecular aberrancies
  - karyotype

- HOWEVER, treatment outcome within as such defined risk groups is still quite heterogeneous
Intra- and post-treatment prognostic factors

- pre-treatment factors cannot capture prognostically relevant variations in efficacy of antileukemic therapy (patient-related differences in drug metabolism)
- THEREFORE, need for therapy-dependent prognostic factors which can be implemented into risk-adapted treatment strategies

⇒ MRD assessment
  - after induction
  - during consolidation
  - pre-/postTx
  - during follow-up

Assessment of treatment response

- morphological assessment (% blasts in BM)
  - insensitive, subjective & interobserver variation

- international recommendations for reporting response criteria
  - more objective laboratory methods
  - providing a deeper measurement of remission
    - multiparameter flow cytometry (MFC)
      ⇒ based on detection of leukemia-associated immunophenotypes (LAIPs)
    - real-time quantitative PCR (RQ-PCR)
      ⇒ based on detection of fusion genes, gene mutations, overexpressed genes
In **healthy individuals**: normal maturation / differentiation of hematopoietic cells is associated with reproducible, sequentially occurring antigen expression patterns.

Intensities of various myeloid antigens by stage of **myeloid maturation** corresponds to morphologic cytology stages as follows:
- I myeloblast
- II promyelocytes
- III myelocytes
- IV metamyelocytes/bands
- V neutrophils


In **healthy individuals**: normal maturation / differentiation of hematopoietic cells is associated with reproducible, sequentially occurring antigen expression patterns.

Intensities of various myeloid antigens by stage of **monocytic maturation** corresponds to morphologic cytology stages as follows:
- Monoblast
- Promonocytes
- Monocytes

In healthy individuals: normal maturation / differentiation of hematopoietic cells is associated with reproducible, sequentially occurring antigen expression patterns

AML blasts show distinct cell surface antigen patterns, which are either not detectable or found on only small numbers of normal bone marrow cells

### LAIP classification

<table>
<thead>
<tr>
<th>LAIP classification</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asynchronous expression of antigens</td>
<td>CD15+CD13+CD34+</td>
</tr>
<tr>
<td></td>
<td>CD11b+CD33+CD34+</td>
</tr>
<tr>
<td></td>
<td>CD36+CD117+CD34+</td>
</tr>
<tr>
<td>Cross-lineage expression of lymphoid antigens</td>
<td>CD19+CD34+CD13+</td>
</tr>
<tr>
<td></td>
<td>CD2+CD34+CD33+</td>
</tr>
<tr>
<td></td>
<td>CD7+CD34+CD33+</td>
</tr>
<tr>
<td>Overexpression of antigens</td>
<td>CD34+CD13+CD33++</td>
</tr>
<tr>
<td></td>
<td>CD34+CD33+CD13++</td>
</tr>
<tr>
<td>Lack of antigen expression</td>
<td>CD34+CD13+CD33–</td>
</tr>
<tr>
<td></td>
<td>CD34+CD33+HLADR–</td>
</tr>
</tbody>
</table>
Identification of LAIP (example of HOVON 42a)

<table>
<thead>
<tr>
<th>Tube</th>
<th>Fluorochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FITC PBS</td>
</tr>
<tr>
<td>2</td>
<td>CD34 CD22</td>
</tr>
<tr>
<td>3</td>
<td>CD15 CD96</td>
</tr>
<tr>
<td>4</td>
<td>CD2 CD36</td>
</tr>
<tr>
<td>5</td>
<td>CD3 CD13</td>
</tr>
<tr>
<td>6</td>
<td>CD36 CD133</td>
</tr>
</tbody>
</table>

**LAIP**
- CD45: WBC marker
- CD34: primitive marker
- CD33: pan myeloid marker
- CD15: mature myeloid marker

asynchronous expression of antigens: CD15+CD13+CD34+CD45+

Example of MRD detection (4 color analysis) ⇒ LAIP cross–lineage expression of CD7

Diagnosis AML 22%  BM after 1st cycle 1.27%  BM after 2nd cycle 0.36%  Relapse 27%

[Diagram showing asynchronous expression of antigens and example of MRD detection with CD33 and CD7 markers]
Example of MRD detection (4 color analysis) 
⇒ LAIP lack of antigen expression

Diagnosis AML  BM after 1st cycle  BM after 2nd cycle  BM after 3rd cycle

Aberrant immunophenotypes | regenerating BM | normal BM
--- | --- | ---
| median | range | median | range |

**Cross-lineage antigen expression**
- CD7
  - CD34+ CD13+ CD7+ 0.07 0.07-0.08 0.04 <0.01-0.07
- CD56
  - CD34+ CD117+ CD56+ 0.02 0.01-0.07 0.01 <0.01-0.09
- CD22
  - CD34+ CD13+ CD22+ 0.02 0.01-0.17
- CD19
  - CD34+ CD13+ CD19+ 0.02 <0.01-0.04

**Asynchronous antigen expression**
- CD15
  - CD34+ CD13+ CD15+ 0.06 0.01-0.07 0.02 0.01-0.08

**Lack of antigen expression**
- CD33neg
  - CD34+ CD13+ CD33neg 0.01 <0.01-0.01
- HLA-DR3neg
  - CD34+ CD117+ HLA-DR3neg 0.01 <0.01-0.09

Background of LAIPs in normal /regenerating BM
Specific RQ-PCR MRD markers in AML

<table>
<thead>
<tr>
<th>Fusion genes</th>
<th>Chromosomes</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML1-ETO</td>
<td>t(8;21)</td>
<td>10-15%</td>
<td>6-8%</td>
</tr>
<tr>
<td>PML-RARα</td>
<td>t(15;17)</td>
<td>8-10%</td>
<td>2-5%</td>
</tr>
<tr>
<td>CBFβ-MYH11</td>
<td>inv(16) and t(16;16)</td>
<td>5-8%</td>
<td>5-7%</td>
</tr>
<tr>
<td>MLL fusion</td>
<td>11q23 aberrations</td>
<td>15-20%</td>
<td>3-7%</td>
</tr>
<tr>
<td>DEK-CAN</td>
<td>t(6;9)</td>
<td>~1%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutations of genes</th>
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<th></th>
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<tr>
<td>FLT3-ITD mutations</td>
<td>13q12</td>
<td>5-25%</td>
<td>20-25%</td>
</tr>
<tr>
<td>dupMLL</td>
<td>11q23</td>
<td></td>
<td>6%</td>
</tr>
<tr>
<td>NPM1 mutations</td>
<td>5q35</td>
<td>6.5%</td>
<td>35%</td>
</tr>
</tbody>
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<table>
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<tr>
<th>Abnormal expression of genes</th>
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<tbody>
<tr>
<td>WT1 overexpression</td>
<td>11p13</td>
<td>80-85%</td>
<td>80-85%</td>
</tr>
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**MRD analysis in clinical practice?**
RQ-PCR MRD monitoring and pre-emptive therapy in \textit{PML-RARα}+ APL

- **MRC AML12 trial**: no routinely MRD monitoring, no pre-emptive therapy
- **MRC AML15 trial**: routinely MRD monitoring and pre-emptive therapy

=> Lower CIR (5%) in AML15 trial compared to AML12 trial (CIR = 12%)

Grimwade D et al. JCO 2009;27:3650-58

**Cumulative incidence of clinical relapse in APL treated with extended all-trans-retinoic acid (ATRA) and anthracycline-based chemotherapy**

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**Childhood AML (trials in USA)**

- Outcome of childhood AML trials \textbf{WITHOUT} MRD assessment and MRD-directed therapy

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<th>Study</th>
<th>3 year EFS</th>
<th>OS</th>
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<td>St. Jude AML97 (Leukemia 2009)</td>
<td>44%</td>
<td>50%</td>
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<tr>
<td>Pediatric Oncology Group Study 9421 (Blood 2006)</td>
<td>36%</td>
<td>54%</td>
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<tr>
<td>Children's Cancer Group 2961 (Blood 2008)</td>
<td>42%</td>
<td>52%</td>
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- Outcome for patients in AML trial \textbf{WITH} flow cytometric MRD assessment and MRD-directed therapy
MRD-directed therapy for childhood AML (AML02 multicentric trial)

**DIAGNOSIS:** LAIP identification

**INDUCTION 1** (Daunorubicine, Etoposide, Cytarabine (HD/LD))

- **Day 22:** MRD assessment
  - **MRD <1%**
  - **INDUCTION 2** Start immediately (Dauno, Eto, Cyta + GO)
  - MRD assessment
  - **CONSOLIDATION**

- **MRD ≥1%**
  - **INDUCTION 2** Start after haematopoietic recovery (Dauno, Eto, Cyta)
  - MRD assessment
  - **CONSOLIDATION**


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- **Outcome of childhood AML trials WITHOUT MRD assessment and MRD-directed therapy**
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- **Outcome for patients in AML trial WITH flow cytometric MRD assessment and MRD-directed therapy**
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<tr>
<td>AML02 trial (Lancet Oncology 2010)</td>
<td>63%</td>
<td>71.1%</td>
</tr>
</tbody>
</table>
Clinical significance of flowcytometric MRD detection in adult AML (HOVON/SAKK 42a study)

- flow cytometric MDR analysis at predefined time points based on diagnostic LAIP’s
- no feedback to clinic: no therapeutic interventions

<table>
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<tr>
<th>MRD frequency after 1st cycle of chemotherapy</th>
<th>MRD frequency after 2nd cycle of chemotherapy</th>
<th>MRD frequency after consolidation therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Graph" /> <strong>MRD &lt; 0.8% (n=147)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>MRD &lt; 0.06% (n=133)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>MRD &lt; 0.6% (n=110)</strong></td>
</tr>
<tr>
<td><img src="image.png" alt="Graph" /> <strong>MRD &gt; 0.8% (n=17)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>MRD &gt; 0.06% (n=49)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>MRD &gt; 0.6% (n=11)</strong></td>
</tr>
<tr>
<td><img src="image.png" alt="Graph" /> <strong>Survival fraction</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>Survival fraction</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>Survival fraction</strong></td>
</tr>
<tr>
<td><img src="image.png" alt="Graph" /> <strong>RFS (months)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>RFS (months)</strong></td>
<td><img src="image.png" alt="Graph" /> <strong>RFS (months)</strong></td>
</tr>
</tbody>
</table>

*17 MRD+: median RFS 8.6 mo
147 MRD−: median RFS >47 mo

*49 MRD+: median RFS 7 mo
133 MRD−: median RFS >47 mo

*11 MRD+: median RFS 7.3 mo
110 MRD−: median RFS >47 mo

**Issues when performing MRD**

- Standardization of assays
  - 
  - 
  - 
  - 

Terwijn M. et al. Abstract ASH 2010
Initiatives for standardisation

- RQ–PCR
  - EAC (Europe Against Cancer)
  - ELN (European LeukemiaNet)

- MFC
  - HOVON AML–MRD working group
  - Euroflow

Issues when performing MRD

- Standardization of assays
- Techniques: MFC *versus* RQ–PCR?
MRD by MFC: some considerations

- requires detailed knowledge of normal BM phenotypes to identify LAIPs
- application of comprehensive antibody panels at diagnosis => suggestions by (inter-)national consensus groups
- use of 4 or more colors in MFC
- sensitivity ranges from 0.1% to 0.01%: depending on background in normal or regenerative BM
  (a theoretical sensitivity of ≤0.1% or 0.01% requires that at least 100,000 or 1,000,000 cells respectively can be acquired (100 events are required for a clear recognition of a population))

- applicable in the vast majority of AML patients
- LAIPs may change between diagnosis and relapse (~80%)
  - immature markers (CD34, CD117, HLA-DR) are more frequently gained
  - differentiation markers (CD14, CD15, CD11b) are more frequently lost at relapse

van der Velden VHJ. Leukemia, 2010, 24: 1599–1606
MRD by RQ–PCR: some considerations

- **fusion genes**
  - high sensitivity (>10^{-4}/10^{-6})
  - only applicable in a minority of patients
  - extremely stable between diagnosis and relapse

- **gene mutations**
  - $MLL-PTD, NPM1$: stable between diagnosis and relapse

- **overexpressed genes**
  - relatively low sensitivity
  - considered for use in cases that lack fusion genes or specific mutations
  - $WT1, EVI1$: stable between diagnosis and relapse

Comparison of MFC and RQ–PCR

- highly significant correlations are found in quantitative comparisons (~70% concordance rate in tandem MRD analysis by MFC and RQ–PCR)
  
Perea G. Leukemia, 2006; 20: 87–94

- MRD levels of both methods correlate with prognosis

- RQ–PCR is superior in most cases of CBF–leukemias
Issues when performing MRD

- Standardization of assays
- Techniques: MFC *versus* RQ-PCR?
- Type of sample: PB *versus* BM?

Comparison between flowcytometric MRD levels in 43 paired BM/PB samples from 18 pediatric AMLs.
- 1 BM MRD levels correlate with 1 PB MRD levels
- MRD levels in BM > MRD levels in PB
- ratio: 1 to more than 100-fold

Comparison between RQ-PCR (CBFB-MYH11) MRD levels in 62 paired PB/BM samples from 10 AML patients (children and adults).
- BM MRD levels: generally higher than PB MRD levels
- variable ratio between BM MRD levels an the corresponding PB (0.8 to almost 100)

Van der Velden VHJ et al. Leukemia, 2010;24:1599–606
WT1 transcript expression level (ELN assay) in PB and BM from normal controls and diagnostic AMLs

Issues when performing MRD

- Standardization of assays
- Techniques: MFC versus RQ-PCR?
- Type of sample: PB versus BM?
- Frequency of MRD sampling?
Kinetics of relapse of APL. Rate of rise of normalized \( \text{PML-RAR}\alpha \) levels in successive samples before relapse. Median increment was 1.1 log/month (0.5 to 2.5 logs/month).

- in vast majority of patients: molecular relapse is followed by hematological relapse
- window of opportunity to act upon
- kinetics of relapse: depending on molecular aberration:
  - \( \text{PML-RAR}\alpha < \text{RUNX1-RUNX1T1} \)<\( \text{CBF}\beta-\text{MYH11} \)
  - fast relapse: FLT3-ITD +

Grimwade D et al. JCO 2009;27:3650-3658

Frequency of molecular MRD monitoring for AML in CR: example \( \text{RUNX1-RUNX1T1 / AML1-ETO} \)

- correlation between relapse detection frequency and sampling interval
- median time from molecular relapse to hematological relapse in days depending on sampling frequency

Hokland P, Ommen HB. Blood. 2010 Nov 19, prepublished online.
Frequency of MRD sampling for AML in CR

<table>
<thead>
<tr>
<th>Sampling Interval</th>
<th>RDF</th>
<th>tR (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBFB-MYH11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>6 months*</td>
<td>50%</td>
</tr>
<tr>
<td>BM</td>
<td>avoid</td>
<td></td>
</tr>
<tr>
<td>RUNX1-RUNX1T1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>3 months</td>
<td>85%</td>
</tr>
<tr>
<td>BM</td>
<td>4 months</td>
<td>95%</td>
</tr>
<tr>
<td>PML-RAF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>1 month</td>
<td>75%</td>
</tr>
<tr>
<td>BM</td>
<td>2 months</td>
<td>100%</td>
</tr>
<tr>
<td>NPM1-FLT3-ITD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>4 months</td>
<td>50%</td>
</tr>
<tr>
<td>BM</td>
<td>6 months</td>
<td>50%</td>
</tr>
<tr>
<td>NPM1-FLT3-ITD+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>3 months</td>
<td>100%</td>
</tr>
<tr>
<td>BM</td>
<td>4 months</td>
<td>100%</td>
</tr>
<tr>
<td>WT1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>2 months</td>
<td>85%</td>
</tr>
<tr>
<td>BM</td>
<td>4 months</td>
<td>95%</td>
</tr>
</tbody>
</table>

Table 2: Proposed guidelines for MRD follow-up using CBFB-MYH11, RUNX1-RUNX1T1, PML-RAF, NPM1 or WT1 as molecular markers. RDF = relapse detection frequency. tR = median time from molecular to hematological relapse. *One additional MRD sampling recommended 3 months after end of chemotherapy to detect early relapses.

When to stop MRD monitoring?

=> stop MRD surveillance after 3 years

most relapses occur prior to the 2 year point after D/ allowing a few extra MRD determinations

Conclusions

- the use of standardized MFC and RQ-PCR assays are recommended for MRD assessment

- clinical importance of detecting and measuring MRD
  - it can be a guide to **DETERMINING PROGNOSIS** and **RELAPSE RISK**
  - **PREDICT RECURRENCE** of leukemia
  - enable **INDIVIDUALIZATION OF TREATMENT**

- it is to be expected that implementation of MRD assessment and MRD-directed therapy in future clinical trials will influence patient outcome