Clinical Significance of Minimal Residual Disease in Leukemia and Lymphoma I

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Minimal Residual Disease - Rationale

Recovering bone marrow

Leukemia

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Blood 100:52, 2002
MRD Assays in Acute Leukemia – The First 2 Decades

1989  PCR amplification of TCRγ in ALL  d’Auriol et al. Leukemia 3: 155
1990  Comparison of PCR and phenotype  Campana et al. Leukemia 4: 609
1990  MRD in adult ALL and AML  Campana et al. Blood 76: 163
1994  MRD in childhood ALL  Brisco et al. Lancet 343: 196
1998  MRD in childhood ALL  van Dongen et al, Lancet 352: 1731
1999  PCR and flow cytometry in tandem  Neale et al. Leukemia 13: 1221
2002  Significance of MRD in blood in ALL  Couslan-Smith et al. Blood 100: 2399
<table>
<thead>
<tr>
<th>Method</th>
<th>ALL</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry</td>
<td>98%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>$(10^{-4})$</td>
<td>$(10^{-3})$</td>
</tr>
<tr>
<td>PCR - Ig/TCR genes</td>
<td>90%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td></td>
<td>$(10^{-5})$</td>
<td></td>
</tr>
<tr>
<td>PCR - fusion transcripts*</td>
<td>&lt;50%</td>
<td>&lt;30%</td>
</tr>
<tr>
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<td>$(10^{-3}-10^{-5})$</td>
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*Additional targets: WT1, NPM1
Detection of MRD by PCR Amplification of Fusion Transcripts

- **Advantages**
  - High sensitivity
  - A set of primers can be applied to multiple patients
  - Stability of the targets during the disease course
  - Relatively rapid

- **Limitations**
  - Applicable to a minority of cases
  - False-negative results due to RNA degradation
  - Difficult quantitation of MRD due to unknown number of transcripts per cell
Detection of MRD by PCR Amplification of Ig or TCR Genes

- **Advantages**
  - High sensitivity
  - It can be applied to most patients with ALL
  - Fixed copy number of targets per cell
  - Good quantitation of MRD

- **Limitations**
  - False-negative results due to oligoclonality and clonal evolution
  - Laborious preparation of reagents and PCR conditions for individual patients
Detection of MRD by Flow Cytometry

- **Advantages**
  - Accurate quantitation of MRD
  - It can be applied to most patients with ALL and AML
  - It can be used to sort leukemic cells for molecular and functional studies
  - It can distinguish viable from apoptotic cells
  - It provides an overview of normal hematopoiesis
  - Continuous progress in antibodies, fluorochromes (e.g. nanocrystals), instrumentation (e.g. multiple lasers, sorting) and analytic computers and software

- **Limitations**
  - False-negative results due to immunophenotypic shifts
## MRD Assays in Acute Leukemia

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*Additional targets: WT1, NPM1

**TXV:** 481/482 (99.8%) pts could be monitored by flow and/or PCR of Ig/TCR

**AML02:** 200 of 215 (95%) pts could be monitored by flow
# Flow Cytometry vs. PCR

<table>
<thead>
<tr>
<th></th>
<th>PCR-</th>
<th>PCR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow+</td>
<td>16*</td>
<td>94</td>
</tr>
<tr>
<td>Flow-</td>
<td>1005</td>
<td>6†</td>
</tr>
</tbody>
</table>

*13/16 PCR-neg had signals at <0.01%
† 2 of 6 flow-neg had cells at <0.01%

Discordant results: 22/1121 (2.0%)

---

% MRD by Flow

% MRD by PCR

Discordant: 28/736 (3.8%)

Kerst et al., Br J Haematol, 2005
MRD in ALL – Flow and PCR

A

Remission BM from ALL pt.

CD10

MRD
0.29%

CD58

10^4

10^3

10^2

10^1

10^0

CD66c

10^4

10^3

10^2

10^1

10^0

BM from healthy donor

CD10

CD58

10^4

10^3

10^2

10^1

10^0

CD66c

10^4

10^3

10^2

10^1

10^0

B

MRD
0.20%

ΔRn

10^1

10^0

10^-1

10^-2

10^-3

0 10 20 30 40 50 Cycle
Leukemia-associated Immunophenotypes

- Marker combinations normally confined to the thymus (e.g., CD3/TdT)

- Molecules not expressed in normal cells (e.g., E2A-PBX1)

- Marker combinations expressed differently in normal and leukemic cells

Definition of normal phenotypes
Acute Lymphoblastic Leukemia - Response to Treatment

Leukemic Cells

Weeks after Diagnosis

0 2 6 22

Leukemic Cell Counts (Log Scale)

- 10^12
- 10^10
- 10^8

Morphologic Remission

44% 34% 15% 7%

MRD
Prognostic Significance of MRD in ALL

Childhood ALL
- Coustan-Smith E. et al. *Blood* 2000, 96, 2691-2696 *(St Jude)*
- Flohr T. et al. *Leukemia* 2008, 22, 771-782 *(iBFM)*

Adult ALL
- Bruggemann M et al. *Blood* 2006, 107, 1116-1123 *(GMALL)*

MRD during early phases of therapy is a strong and independent prognostic factor
Prognostic Significance of MRD (cont.)

Relapsed ALL

- Coustan-Smith E et al. *Leukemia* 2004; **18**, 499-504
- Paganin et al. *Leukemia* 2008; **22**, 2193-2200

“Isolated” extramedullary relapse


Allogeneic transplant

- Krejci et al. *Bone Marrow Transplant* 2003; **32**, 849-51
# Outcome According to MRD on Day 46 in TXV

<table>
<thead>
<tr>
<th>MRD on Day 46</th>
<th>No. Patients</th>
<th>% 5-yr EFS (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>290</td>
<td>86 (4)</td>
</tr>
<tr>
<td>Positive</td>
<td>64</td>
<td>73 (11)*</td>
</tr>
</tbody>
</table>

*56% EFS for MRD-positive pts in TXIII*
### Outcome According to MRD on Day 46 in TXV

<table>
<thead>
<tr>
<th>MRD level</th>
<th>No. Patients</th>
<th>5-year EFS (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01% to &lt;1%</td>
<td>57</td>
<td>83 (9)</td>
</tr>
<tr>
<td>1% to 5%</td>
<td>10</td>
<td>45 (24)</td>
</tr>
<tr>
<td>&gt;5%</td>
<td>6</td>
<td>50 (35)</td>
</tr>
</tbody>
</table>
MRD Strategy in TXVI

- Leukemia-associated phenotype
  - Yes
    - Monitor MRD by flow
    - Ambiguous result?
      - Day 15, Day 43
        - MRD pos at day 43
          - Continue MRD monitoring
        - MRD neg at day 43
          - T-ALL (PB)
    - T-ALL (PB)
  - No
    - Develop PCR assay
      - MRD pos at day 43
        - Continue MRD monitoring
      - MRD neg at day 43
        - Stop MRD monitoring (but resume if necessary)

- Store DNA

- B-lineage ALL
Use of MRD in Total XVI

- **Day 15:** patients with MRD $\geq 1\%$ receive intensified remission induction therapy; further intensification for those with $\geq 5\%$

- **Day 43:**
  - Patients with standard-risk ALL become high-risk if MRD $\geq 0.01\%$
  - All patients become very high-risk if MRD $\geq 1\%$

- **Continuation**
  - Patients with MRD $\geq 0.1\%$ become very high-risk
MRD “Lite” Concept

- Normal CD19+ cells expressing CD10 and/or CD34 in bone marrow are extremely sensitive to corticosteroids
  ➔ After 2 weeks of remission induction therapy, they are <0.01%

- Leukemic cells in patients with B-lineage ALL are CD19+ CD10+ and/or CD34+
  ➔ In these patients, CD19+ CD10+ and/or CD34+ cells at day 15-26 indicate MRD
  ➔ their absence indicates good response

- Advantages:
  - Much reduced antibody panel
  - Can be performed with a one-laser cytometer
  - Easy interpretation
Normal B cell differentiation

Early Pre-B/Pro B

Pre-B

Mature B

Bone Marrow

Peripheral Blood
Corticosteroid therapy (early induction chemotherapy)
MRD “Lite” Studies

Coustan-Smith et al., Blood 2006
## MRD Lite vs MRD Full

<table>
<thead>
<tr>
<th></th>
<th>MRD LITE</th>
<th>MRD FULL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can be used in presence of normal regeneration?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient-specific markers</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Stage of chemotherapy</td>
<td>Day 15-26 of induction only</td>
<td>All stages</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1 in 10,000 (0.01%)</td>
<td>1 in 10,000 (0.01%)</td>
</tr>
<tr>
<td>Fluorochromes</td>
<td>3 or 4</td>
<td>4+</td>
</tr>
<tr>
<td>Lasers</td>
<td>1 or 2</td>
<td>2</td>
</tr>
<tr>
<td>Costs</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Easy</td>
<td>Complex</td>
</tr>
</tbody>
</table>
### Recife Pilot Study #1

<table>
<thead>
<tr>
<th>Traditional risk features</th>
<th>Day 19 MRD</th>
<th>Risk Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>&lt;0.01%</td>
<td>Low</td>
</tr>
<tr>
<td>Good</td>
<td>&gt;0.01%</td>
<td>Standard</td>
</tr>
<tr>
<td>Poor</td>
<td>Any</td>
<td>High</td>
</tr>
</tbody>
</table>

Poor = T-lineage ALL, or B-lineage ALL with WBC $\geq 50K$, age 10-15 yrs, testicular/CNS 3 leukemia, and/or adverse genotypes ($BCR-ABL$, $MLL$ rearrangements, hypodiploidy $<45$ chromosomes)
Application of MRD Results

- **Optimize intensity of therapy**
  - Identify patients who require more intensive therapy
  - Identify patients who may be cured with less intensive therapy

- **MRD as a measure of drug resistance in vivo**
  - Identify molecular determinants of treatment response
    - Gene expression at diagnosis and MRD during induction → new prognostic factors
    - SNPs in drug-metabolizing genes and MRD
  - Identify novel drug-resistant subtypes of leukemia
MRD in AML
## Molecular Studies of Residual Disease in AML

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Target</th>
<th>No. of patients</th>
<th>Informative timepoint</th>
<th>Informative MRD level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(15;17)</td>
<td>RARA-PML PML-RARA</td>
<td>105</td>
<td>After 3 courses of chemotherapy</td>
<td>0.01%</td>
<td>Burnett et al, 1999</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>PML-RARA</td>
<td>163</td>
<td>Post-consolidation</td>
<td>0.01%</td>
<td>Diverio et al, 1998</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>AML1-MTG8</td>
<td>25</td>
<td>Post-consolidation / intensification</td>
<td>1000 mol./µg of RNA</td>
<td>Tobal et al, 2000</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>AML1-MTG8</td>
<td>51</td>
<td>Post-first consolidation</td>
<td>0.01%</td>
<td>Morschhauser et al, 2000</td>
</tr>
<tr>
<td>inv(16)</td>
<td>CBFB-MYH11</td>
<td>16</td>
<td>Post-completion of chemotherapy</td>
<td>10 transcript copies</td>
<td>Marcucci et al, 2001</td>
</tr>
</tbody>
</table>
Clinical Significance of MRD in Adult AML

San Miguel et al. Blood 2001; 98: 1746-1751

Maurilio et al. JCO 2008; 26: 1-8
Immunophenotypic Differences between ALL and AML
Sensitivity of Detection of MRD in AML
Specificity of Flow Cytometric Detection of MRD
Clinical Significance of MRD in Childhood AML OS According to MRD Post-Induction 1 – Morphology-neg. Patients

MRD-neg (n = 27) 63% ± 10%
MRD-pos (n = 14) 36% ± 14%

P = 0.043

Br J Haematol, 2003
The AML02 Trial
The first AML Clinical Trial to Use MRD to Guide Treatment

Children's Hospital
Seattle, WA

Stanford University Medical Center
Palo Alto, CA

Cook Children's Medical Center
Forth Worth, TX

Texas Children's Cancer Center
Houston, TX

Children's Hospital
Michigan-Detroit

Dana Farber Cancer Institute
Boston, MA

St. Jude Children's Research Hospital
Memphis-Tennessee
Induction I
LD-AraC
Daunomycin
Etoposide
vs
HD-AraC
Daunomycin
Etoposide

Induction II
ADE+GO

Consolidation
SCT

MRD Studies in AML02

Risk-based consolidation
HR → SCT
SR with MSD → SCT
All others → chemotherapy

MRD test
200 of 210 (95%) patients had aberrant phenotypes at diagnosis.

1313 follow-up BM samples, 1296 (99%) were adequate.

83 of 200 (42%) studied on day 22 had ≥ 0.1% MRD
  - SJCRH: 37/94 (39%)
  - Non-SJCRH: 46/106 (43%)

17 of 44 (39%) had ≥ 0.1% MRD after Induction I in AML97.
### AML

**MRD on Day 22 According to Genetic Subtype**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th># Pts. Studied</th>
<th># MRD pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>inv(16)</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>t(9;11)</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Other 11q23</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>71</td>
<td>37</td>
</tr>
<tr>
<td>FLT3 ITD</td>
<td>29</td>
<td>25</td>
</tr>
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</table>

**MRD:** $\geq 0.1\%$ of bone marrow mononuclear cells with leukemia phenotypes

$P<0.001$
AML02 - Survival According to MRD

80% ± 6% MRD-negative (n=115)
51% ± 9% MRD-positive (n=78)
AML02 - Survival According to MRD Level

Years on Study

<table>
<thead>
<tr>
<th>Probability</th>
<th>80% ± 6%</th>
<th>MRD &lt; 0.1% (n=115)</th>
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<tbody>
<tr>
<td>76% ± 15%</td>
<td>MRD 0.1 –&lt;1% (n=26)</td>
<td></td>
</tr>
<tr>
<td>42% ± 10%</td>
<td>MRD ≥ 1% (n=52)</td>
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### Detection of MRD in Bone Marrow vs Peripheral Blood in AML at Day 22

<table>
<thead>
<tr>
<th></th>
<th>PB-</th>
<th>PB+</th>
</tr>
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<tbody>
<tr>
<td>BM-</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>BM+</td>
<td>26*</td>
<td>41</td>
</tr>
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MRD+ ≥0.1%

*In 17 of the 26 PB samples, cells with AML phenotypes were detected (<0.1%)
## MRD Studies – Applications

<table>
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<tr>
<th>Application</th>
<th>Action</th>
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<tbody>
<tr>
<td>Detect poor early treatment response</td>
<td>Treatment intensification</td>
</tr>
<tr>
<td>Detect good early treatment response</td>
<td>Treatment reduction</td>
</tr>
<tr>
<td>Detect relapse</td>
<td>Treatment intensification; prepare for HSCT</td>
</tr>
<tr>
<td>Measure MRD before HSCT</td>
<td>Optimize timing of HSCT</td>
</tr>
<tr>
<td>Measure MRD after HSCT</td>
<td>Modulate immunosuppression, DLI, etc</td>
</tr>
<tr>
<td>Measure MRD after novel drug combinations or novel agents</td>
<td>Apply MRD-based stopping rule; design MRD-based Ph. II studies</td>
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