Minimal Residual Disease Evaluation in Hematologic Malignancies

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My Disclosures

• None
- CML
- **AML**
  - **APL**
  - *CBF leukemia*
  - *Non-CBF leukemia*
- ALL
- Plasma cell neoplasia
- B-cell lymphoma`s
- Background
- Methodologies
- Clinical significance
- Conclusions and perspectives
Complete hematological remission ≠ cure
• Multiparameter Flow Cytometry (MFC)
• PCR
  – Fusion transcripts
  – Gene mutations
  – Gene overexpression

CS Hourigan and JE Karp Nature reviews, Clinical Oncology 2013 (review)
Proportion of AML patients informative for MRD detection by RT-qPCR for leukemia-specific targets according to age

D Grimwade and S Freeman. Blood 2014 (prepublished July, 21)
Multiparameter Flow cytometry

- Leukemia-associated immunophenotypes (LAPs)
- Different from normal
- Leukemia stem cells

CD117+ subsets

CD33 PC5.5 LOGICAL

CD65 FITC LOGICAL

CD33 PC5.5 LOGICAL

CD34+ subsets

SS INT LIN EXP-SSC L

CD45 KO LOGICAL

CD34 PC7 LOGICAL

CD117 APC LOGICAL

CD14 ECD LOGICAL

CD65 FITC LOGICAL

CD15+

CD13 PE LOGICAL

CD11b APC750 LOGICAL
Identification of aberrant marker expression on AML blasts

NPM1+ AML case 1: Diagnostic sample

Core markers

IP changes:
1. CD13 bright expression
2. CD7 coexpression

CD45 KO LOGICAL
CD34 LOGICAL
CD33 PC5.5 LOGICAL
CD117 APC LOGICAL
CD117+/CD34- cells
CD34+ cells
NPM1 AML case 1: post induction sample

CD34+ cells

CD117+/CD34- cells
Identification of immunophenotypic profiles of mature and immature subpopulations that are ‘different from normal’

Normal profiles

Different from normal
NPM1+ AML / Flt3 ITD (77%) case 2: post induction sample

CD117+/CD34- cells with normal phenotype/ abnormal phenotype

CD34+ cells
Leukemia stem cells (CD34+/CD38-)

Leukemic stem cells:
12.5 % of leukemic blasts
3.5% of total nucleated cells

Aberrant phenotype:
HLA-DR negative
CD7 positive
CD123 positive
Remaining issues

- Evolving antibody panels
- Quantitation of MFC-MRD
- Definition of MRD positivity / negativity

JM Jaso, SA Wang, JL Jorgensen and P Lin. Bone Marrow Transplantation 2014 (review)
Principle of real time quantitative polymerase chain reaction (RQ-PCR)

J Gabert et al. Leukemia 2003
Determining the sensitivity of MRD detection and variation of leukemic transcripts

J Gabert et al. Leukemia 2003
Monitoring PML-RARA fusion transcripts: standard of care in APL

* MRD is the most powerful predictor of disease relapse ($p < 0.0001$; 406 patients included in MRC AML 15 trial)
* Initiation of pre-emptive therapy on reappearance of PCR positivity

* Bone marrow is recommended sample
* Required assay sensitivity: 10-4
* Low-risk disease (WBC < 10 X 10e9/L): MRD monitoring until end of treatment
* High-risk disease (WBC > 10 X 10e9/L and slow kinetics): 3 monthly MRD monitoring
* MRD monitoring in clinical trials to assess safety and efficacy of de-intensified treatment (ongoing study: I-BFM group / childhood APL)

Minimal residual disease can identify CBF leukemia patients at risk for relapse

<table>
<thead>
<tr>
<th>Study</th>
<th>No of patients</th>
<th>Time points</th>
<th>Cut-off levels Transcripts</th>
<th>MRD response and Clinical relevance</th>
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<tbody>
<tr>
<td>J Liu Yin et al. Blood 2012</td>
<td>163 pts with t(8;21) 115 pts with inv(16)</td>
<td>After induction course 1 After therapy Serial monitoring</td>
<td>RUNX1-RUNX1T1: &gt; 3-log reduction (BM samples) RUNX1-RUNX1T1: &gt; 500 copies (BM) and 100 copies (PBL) CBF beta-MYH11: &lt; 10 copies (PB samples) CBF beta-MYH11: &gt; 50 copies (BM) and &gt; 10 copies (PBL)</td>
<td>47% of pts: CIR of 4% 51% of pts: CIR of 21% Predicts Relapse Predicts Relapse</td>
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<td>E Jourdan et al. Blood 2013</td>
<td>96 pts with t(8;21) 102 pts with inv(16)</td>
<td>After 2nd course (MRD2)</td>
<td>&gt; 3-log reduction of RUNX1-RUNX1T1 and CBFbeta-MYH11 transcripts (BM samples)</td>
<td>71/91 pts (78%) with t(8;21) and 53/85 pts (62%) with inv(16) Associated with lower hazard of relapse (SHR=0.27), longer RFS (HR=0.34) and trend for longer OS (HR= 0.46) MRD is independent risk factor by multivariate analysis (not c-Kit and/ or Flt3 ITD mutation)</td>
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<tr>
<td>HH Zhu et al. Blood 2013</td>
<td>116 MRD eligible pts with t(8;21)</td>
<td>After 2nd consolidation</td>
<td>&gt; 3-log reduction of RUNX1-RUNX1T transcripts = major molecular response (MMR) (BM samples)</td>
<td>Non- MMR (HR)= 69/116 pts (59%) MMR (LR)= 47/116 pts (40%) Non-MMR: 40 pts received HSCT and 29 pts CT: CIR of 22% versus 78.9% MRD, treatment strategy and c-KIT mutation status: Independent risk factors by multivariate analysis</td>
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NPM1 mutated transcript level is associated with relapse risk and prognosis

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<tr>
<td>J Kronke et al. J Clin Oncol 2011 German-Austrian AML study group</td>
<td>245 pts</td>
<td>After double induction</td>
<td>No NPM1 mutant transcripts</td>
<td>CIR of 6.5%, OS of 90% (at 4 years)</td>
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<td>After completion of therapy</td>
<td>No NPM1 mutant transcripts</td>
<td>CIR of 53%; OS of 51% (at 4 years)</td>
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<td>Serial monitoring post treatment</td>
<td>&gt; 200 NPM1mut/10e4 ABL copies</td>
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<td>Early detection of relapse</td>
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<td>N Shayegi et al. Blood 2013 Study Alliance Leukemia</td>
<td>184 pts</td>
<td>After cessation of therapy</td>
<td>&gt; 1% NPM1 mut /ABL1</td>
<td>HR for relapse: 13.2</td>
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<td>≤ 1% NPM1 mut / ABL1</td>
<td>HR for relapse: 3.4</td>
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<td>&gt;10% NPM1 mut/ABL1</td>
<td>HR for relapse: 61.4</td>
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<td>≤10% NPM1 mut/ABL1</td>
<td>HR for relapse: 1.8</td>
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<td>After BMT</td>
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<td>Median time to relapse was not reached</td>
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Kinetics of Wilms` Tumor gene (WT-1) expression post induction provides prognostic information

* D. Gilloni at al. (JCO 2009) ELN study

Standardized WT-1 assay including 91 Pts with pretreatment WT-1 level of
> 2x10^4 WT1 copies/ 10^4 ABL copies

* WT-1 is not leukemia specific: 50 and 250 WT-1 copies/10e4 ABL copies are the cut-off levels for normal Blood and Bone Marrow, respectively
* Assay sensitivity: 2- log reduction in ca 45% of patients
Prognostic relevance of treatment response measured by FCM-MRD

*S Freeman et al. J Clin Oncol 2013: MRC AML-16 study of 892 pts of > 60 years, 427 pts in CR after one or two courses*

# MRD negativity after course 1 (sensitivity of LAIP: 0.05% -0.2%) in pts in CR confers a better OS at 3 years
# MRD status independently predicted survival in intermediate-risk pts
# Cytogenetics remained the most significant prognostic indicator for survival (HR: 2.3) followed by MRD status (HR: 1.98) and secondary disease (HR: 2.2)
Prognostic relevance of treatment response measured by FCM-MRD

M Terwijn et al. J Clin Oncol 2013: HOVON/SAKK study of 517 pts < 60 years; 389 pts available for analysis

# MRD > 0.1% after course 2 (23% of pts) was associated with Relapse of 72% at 4 years (HR: 2.97 (univariate analysis) and HR: 2.6 (multivariate analysis)
Conclusions

- MRD is of prognostic significance and can be used for risk stratification
- MRD (persistent high levels- rising levels) after front line therapy predicts relapse
- Many patients without MRD relapse
Perspectives

- Studies are ongoing to investigate whether MRD assessment may be useful to help make decisions concerning
  1. Pre-emptive therapy after completion of therapy/post transplant
  2. Bone marrow transplantation in 1st remission
  3. Treatment de-intensification

- Better understanding of the clonal architecture of AML is needed by targeted sequencing of the diagnostic sample
- Implementation of newer methodologies such as next generation sequencing/digital PCR
- Standardization and Further development of flow cytometry assays: mass cytometry and automated analysis algorithms