Minimal Residual Disease as a Surrogate Endpoint in Acute Myeloid Leukemia Clinical Trials

Adriano Venditti
Hematology, University “Tor Vergata”, Rome, Italy
Minimal Residual Disease

MRD = AML chemoresistance
Methods for detecting MRD in AML

1. PCR on
   - chimeric fusion genes
   - mutations
   - gene over-expression

2. Flow cytometry
   - “Leukemia-associated phenotypes”, that are absent or very infrequent in NBM
   - “Empty spaces” that are not usually occupied during normal myeloid maturation
<table>
<thead>
<tr>
<th>Fusion genes</th>
<th>Mutations</th>
<th>Overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>PML-RARA</em></td>
<td><em>NPM1</em></td>
<td><em>WT1</em></td>
</tr>
<tr>
<td><em>CBFB-MYH11</em></td>
<td><em>FLT3</em></td>
<td><em>BAALC</em></td>
</tr>
<tr>
<td><em>RUNX1-RUNX1T1</em></td>
<td><em>CEBPA</em></td>
<td><em>ERG</em></td>
</tr>
<tr>
<td>MLL-fusion partner</td>
<td><em>MLL-PTD</em></td>
<td><em>MN1</em></td>
</tr>
<tr>
<td><em>DEK-NUP214</em></td>
<td><em>RUNX1</em></td>
<td>~ 30% of AMLs</td>
</tr>
</tbody>
</table>

25-30% of AMLs stable at relapse

~ 75% of CN-AML

~ 30% of AMLs sensitivity
RUNX1-RUNX1T1 & CBFB-MYH11 in MRD analysis

patients with >500 RUNX1-RUNX1T1 copies in BM

patients with >50 CBFB-MYH11 copies in BM

Liu Yin JA et al., Blood 2012
NPM1 mutations for MRD detection

Post-Induction

A

b

C

D

P < .001

Overall Survival (%)

Overall Survival (%)

P < .001

Krönke J et al., JCO 2011
Impact of NPM1 transcript levels during follow-up

$NPM1^{\text{mut}}$ transcript level > 200

relapse rate (%)

time (months)

Courtesy of K. Döhner
Designing LAIP-based MRD studies in AL

- Minimum 6-colour technology
- Aberrant LAIP identified at diagnosis in >90% of AMLs
- Average sensitivity 0.01% (10^-4)

56 pts with *de novo* AML enrolled in the EORTC/GIMEMA protocols

MRD levels $\geq 3.5 \times 10^{-4}$ (0.035%) cells at the end of consolidation predicts relapse

*Venditti et al, Blood 2000*
ROC analysis for MRD threshold identification

Al Mawali et al, Cytometry 2008
Maximally selected log-rank statistics analysis

Buccisano et al., 2006; Maurillo et al., JCO 2008
MRD by Flow in adult AML

- Tor Vergata University Hospital (TVUH)
- 142 adults with AML in CR (median age 52y, range 18-75; 50 ≥ 60y)
- EORTC-GIMEMA AML-10, AML-12, AML-13
- MRD negativity: < 3.5 x 10^{-4} (0.035%)

Maurillo et al, JCO 2008
Is outcome prediction improved by MRD assessment?

1. Also patients belonging to the most favorable categories do have relapse

2. Normal karyotype is molecularly heterogeneous

3. Cytogenetic/genetic risk-stratification inadequate for predicting relapse in individual patients

4. Presence of MRD at morphologic CR anticipates relapse

5. MRD captures differences in treatment response due to
   - molecular heterogeneity
   - inter-patient variability
Comprehensive risk-assessment

1. Integration of baseline prognosticators (cytogenetics, genetics) and parameters inherent the quality of response (MRD)

2. Optimization of post-remission therapy
   - development of RISK-ADAPTED strategies
   - more proper use of ASCT
Improving risk-stratification in AML

Buccisano F et al, Blood 2010
### Integrated risk-score

<table>
<thead>
<tr>
<th>Low-Risk</th>
<th>High-Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good K / MRD-</td>
<td>Adverse K</td>
</tr>
<tr>
<td>Int K / MRD-</td>
<td>FLT3-ITD+</td>
</tr>
<tr>
<td>4 yrs. CIR = 15%</td>
<td>Good K / MRD+</td>
</tr>
<tr>
<td></td>
<td>Int K / MRD+</td>
</tr>
<tr>
<td></td>
<td>4 yrs. CIR = 77%</td>
</tr>
</tbody>
</table>

---

*Buccisano et. al. Blood 2010; Vol 116. pages 2295-2303*
### AlloSCT vs AutoSCT for High-Risk AML

<table>
<thead>
<tr>
<th>Low-Risk</th>
<th>High-Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good K / MRD-</td>
<td>Adverse K</td>
</tr>
<tr>
<td>Int K / MRD-</td>
<td>FLT3-ITD+</td>
</tr>
<tr>
<td>4 yrs. CIR = 15%</td>
<td>Good K / MRD+</td>
</tr>
<tr>
<td></td>
<td>Int K / MRD+</td>
</tr>
<tr>
<td></td>
<td>4 yrs. CIR = 77%</td>
</tr>
</tbody>
</table>

- **NO AlloSCT**
  - Koreth, JAMA 2009
  - Cornelisson, Nat Rev 2013

- **With AlloSCT**

  ![Graph showing survival probability over time](image)

  - Allograft
  - Autograft

  - Time (years)

  - p<0.03

- Buccisano F et al Blood 2010
1. Donor versus no donor approach no more applicable

2. Transplant versus no transplant approach based on the accurate evaluation of the individual
   - risk of relapse
   - risk of non-relapse mortality
High-Risk AML: Retrospective vs prospective

Retrospective (77pts)
- AlloSCT: 21%
- AuSCT: 43%
- No SCT: 14%
- Rel before SCT: 22%

Prospective (34 pts)
- AlloSCT: 74%
- AuSCT: 0%
- No SCT: 0%
- Rel before SCT: 26%
High-Risk AML: Retrospective vs prospective

Overall Survival

Cumulative Proportion Surviving

Time (days)

P = 0.006
High-Risk AML: Retrospective vs prospective

Disease Free Survival

Cumulative Proportion Surviving

- Prospective High Risk
- Retrospective High Risk
- Retrospective Low Risk

P = 0.013
GIMEMA AML1310: a study of risk-adapted and MRD-directed therapy for adult AML

EudraCT number 2010-023809-36
ClinicalTrials.gov Identifier NCT01452646

Low-risk: CBF/Kit^wt; NPM1+/FLT3-
Int-risk: all others
High-risk: Adverse K; FLT3+

alloSCT: MRD, MUD, UCB, HRD
Issues still pending

1. Who is the candidate for MRD testing?
2. Source
   - BM versus PB?
3. Time-points and thresholds
   - therapy dependents?
4. Failure to predict relapse in 25% of MRD negative
   - sensitivity/specificity (technical reasons)
   - LSC (biologic explanation)
Profiling the candidate for MRD testing

<table>
<thead>
<tr>
<th>Gen/Cytogen Category</th>
<th>YES</th>
<th>NO</th>
<th>? YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>Intermediate</td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td></td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>
## MPFC: 4 vs > 4 color assay

<table>
<thead>
<tr>
<th></th>
<th>MRD POS</th>
<th>MRD NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 colors</td>
<td>126</td>
<td>46</td>
</tr>
<tr>
<td>&gt;4 colors</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>All groups</td>
<td>156</td>
<td>50</td>
</tr>
</tbody>
</table>

- **4 colors**: 73% vs 27%
- **>4 colors**: 88% vs 12%
- **All groups**: 76% vs 24%

*P*=0.062
Polychromatic Assay

Subset 3
CD34+CD19+CD13+CD33+
CD38^{dim}CD117-
FISH t(6;12) 80%

Subset 2
CD34+CD19+CD13+CD33+
CD38^{high} CD117+
FISH t(6;12) 20%

Subset 1
CD34+CD19+CD13-CD33-
FISH t(6;12) negativa
132 patients (52 HDAC, 80 SDAC)
Median follow-up: 6.8 yrs (0.2-15.1)
Survival analysis: ARA-C schedule + MRD

Overall Survival

Disease Free Survival

Survival Probability

p=0.007

p=0.0005
Conclusions

1. Measurement of MRD contributes to refine risk assessment

2. A comprehensive risk stratification (cytogenetic/genetic plus MRD), helps allocate patients into a more realistic category of risk

3. Patient’s assignment to optimal treatment modality on the basis of their adjusted risk of relapse

4. Technical and biological issues still remain to be addressed
Acknowledgments

Unità di Onco-Ematologia Clinico-Sperimentale
Centro di Rierimento Oncologico, Aviano

Pietro Bulian
Valter Gattei

Ematologia
Fondazione Policlinico
Tor Vergata, Roma

Adriano Venditti
Luca Maurillo
Maria Ilaria Del Principe
Giovanni Del Poeta
Francesco Lo Coco
William Arcese
Sergio Amadori

GIMEMA Group
Roma

Marco Vignetti
Paola Fazi
Giulio D’Alfonso
Alfonso Piciocchi

Laboratorio di Neuro-Immunologia,
Fondazione Santa Lucia
Roma

Daniela F. Angelini
Adamo Diamantini
Luca Battistini

Stem Cell Laboratory,
Department of Hematology
VU University Medical Center
Amsterdam, The Netherlands

Gerritt Schuurhuis