MRD detection in acute leukemia

Novel concepts and developments for flow cytometric MRD detection

Jacques J.M. van Dongen
on behalf of

Dept. of Immunology, Erasmus MC, Rotterdam

Detection of minimal residual disease (MRD) in ALL

Follow-up in years

Detection limit of immunophenotyping and PCR techniques

Monitoring of a T-ALL patient

1986

Course of disease

% CD5+/TdT+ cells

PCR analysis of Ig/TCR genes

Germline IGH gene

Rearranged gene

V-D-J recombination

Relapse free survival in I-BFM-SG study according to the combined MRD information at time points 1 and 2 (n=129)

Europe

European Study Group on MRD detection

Chairman: J.J.M. van Dongen

Supported by Leukemia & Lymphoma Research, LeukemiaNet, and EuroClonality
Current MRD technique in lymphoid malignancies

Disadvantages of Ig/TCR-based MRD-PCR techniques:
- labor intensive (junctional regions per patient);
- require specialized laboratories;
- time consuming (target identification: 4 to 6 weeks)

Faster technique needed: 8-color flow cytometry?

Comparison between molecular techniques and flow cytometry in hematological malignancies

<table>
<thead>
<tr>
<th>Molecular techniques</th>
<th>Flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>fast: 1-2 hours</td>
</tr>
<tr>
<td>Target</td>
<td>protein/cells</td>
</tr>
<tr>
<td>Applicability</td>
<td>broad</td>
</tr>
<tr>
<td>Multiplexing</td>
<td>relatively easy</td>
</tr>
<tr>
<td>Accuracy</td>
<td>quantitative</td>
</tr>
<tr>
<td>Focus</td>
<td>any subpopulation</td>
</tr>
<tr>
<td>Facilities</td>
<td>standard lab needed</td>
</tr>
</tbody>
</table>

EFS of MRD-based risk groups (FCM at day 29) in COG protocol

MRD window, time points, MRD techniques and QR & sensitivity

Therapy-induced immunophenotypic shifts
Standardization in diagnostic flow cytometry

Standardization according to literature generally refers to:
- lists of CD codes and markers per disease
- rarely a specific antibody is recommended
- reproducible settings (e.g. based on standard beads)
- care for the selection of optimal antibody clones per marker/CD code
- design of combinations of multiple 8-color tubes

However, standardization according to GLP guidelines demands for much higher levels of standardization, e.g.
- Careful selection of optimal antibody clones per marker/CD code
- Synchronized light scatter experiments
- Careful selection of optimal antibody clones per marker/CD code
- Design of combinations of multiple 8-color tubes

Automatic identification of populations

Multidimensional analysis:
Automated separation among different cell populations (APS view)

Maturation of neutrophil precursors in normal BM

Standardization is essential, e.g.
Synchronized light scatter experiments

“Local” settings
EuroFlow settings

7 different normal PB samples acquired in 7 different centers
Normal PB samples processed according to the standardized EuroFlow sample preparation protocol

Full standardization and novel software (fast and easy)
Maturation stage of neutrophil precursors in normal BM

N-dimensional neutrophil maturation in normal BM versus AML

Aberrant phenotype of AML blasts

Dissection of normal precursor-B-cell differentiation

Four BCP-ALL cases vs normal precursor B-cells in EuroFlow immunostaining 1
New concept for flow cytometric MRD detection

Dissection of normal precursor-B-cell differentiation

EuroFlow immunostaining 2

B-cell regeneration: time-point dependent

Regenerating B-cells: identical to normal B-cells
(in EuroFlow immunostaining 2)

Fixed (supervised) APS plots based on normal B-cell differentiation

BCP-ALL versus normal B-cell differentiation
(in EuroFlow immunostaining 2)

Fixed (supervised) APS plots based on normal B-cell differentiation

New concept for flow cytometric MRD detection

Discrimination between normal regenerating precursor B-cells and aberrant ALL blast cells via flow cytometric PCA (automated population separation)

Dissection of normal BM

Normal vs Regenerating BM

ALL blast cells

Responsible scientists: V.H.J. van der Velden and E. Majdikova
Conclusions

1. PCR-based MRD diagnostics (IG/TCR genes or fusion genes) is currently the gold standard in most European ALL protocols.

2. Differences in MRD value between ALL protocols are mainly caused by application of different non-standardized MRD techniques, which also differ in sensitivity (e.g., current flow cytometry does not reach $\leq 10^{-3}$).

3. Novel concepts in ≥8-color flow cytometry can potentially replace PCR-based MRD diagnostics, based on discrimination of normal-regenerating precursor cells from aberrant blasts cells via PCA (see APS tool developments by EuroFlow).

4. Full standardization, regular Quality Control, and guidelines for data interpretation and data reporting are essential for international comparability of MRD results (within and between treatment protocols).

Collaborative international networks are essential for innovation.