Overview of hot topics in flow cytometry including MRD in acute leukemia

ISLH
Milan, May 2016
MC Béné
• No conflicts of interest
Morphology & Flow cytometry

- Settled technologies
- Somehow taken for granted…
- Yet, getting more and more automated
  - Digitalized imaging
  - HIV follow-up
  - CBC instruments
Flow cytometry
still a multifaceted versatile tool

- **Instruments**: 4 to 12 parameters and growing
- **Antibody combinations**
  - Lab-based
    - Many consensus-based proposals
    - Financial limitations
    - Recruitment-based limitations
  - Commercial solutions
    - Custom-made panels
    - Ready-to-use mixes
      - Liquid (vials)
      - « Dry » tubes
- **Analysis software**
  - Imagination rules!
Bone marrow cartography
Arnoulet et al. Cytometry B 2009

- Identification of mature cells
- Boolean definition of progenitors:
  - « NOT lymphocytes AND NOT monocytes AND NOT granulocytes »
- GTLLLF « bermudes »
The CD45 scattergram
STANDARDIZATION
HARMONIZATION
Euroflow: the idea

- Standardize instruments
- Find the best antibodies
- Fix panels

- Overall a good idea
- A lot of work
- A lot of money

Van Dongen et al, 2012, Leukemia
Pros and cons

- Comprehensive of most hematological malignancies
- Some good display ideas
- Files merge for comparison

- Complex backbones
- Complex displays
- Blackbox of the PCA analysis and APS presentation
- Expensive to apply
Comprehensive Berlin handout 2009 Final version 2012
A lot of markers
an interesting display
Confusing representations
HARMONEMIA

• A universal strategy for comparing flow cytometry immunophenotyping data obtained from instruments of different make

• Lysed unstained blood: no more than 15-20% cells in the first channel for each color → determination of PMT voltages

Lacombe et al, 2016, Leukemia
Unlabeled cells
Normalized target channel for beads on 23 instruments
Merging example

Next step: atlas of 8/10 colors normal bone marrow
Routine samples in 22 configurations

**PMN CD11b**

A

Navios 10C

Navios 8C

Canto II 8C

**Mo CD11b**

B

Navios 10C

Navios 8C

Canto II 8C

**PMN CD16**

C

Navios 10C

Navios 8C

Canto II 8C

**Mo CD33**

D

Navios 10C

Navios 8C

Canto II 8C
ACUTE LEUKEMIA AND MINIMAL RESIDUAL DISEASE

Pubmed ~2900 papers
+Flow cytometry ~600 papers
Flow cytometry and acute leukemia

- Essential for diagnosis/classification
  - Lineage assignment and blockade stage
  - AML with minimal differentiation
  - Myelomonocytic lineage
  - MPAL (mixed phenotype acute leukemia)

- Morphology and flow cytometry for rapid early orientation of patients’ management

- Follow-up: MRD
How to find the « bad » cells?

The candy jar concept

- Morphology
- Cytogenetics
- Flow cytometry
- Molecular probes

Clinical relevance

- Size
- Shape
- Colour
- Smell
- Taste
General principles

• Specificities of acute leukemia
  – Constant medullary involvement
  – Frequent peripheral involvement
  – Fast evolution
    • Of the disease
    • Of answer to therapy
    • Of relapses

• Cellular specificities
  – Molecular (DNA, RNA)
  – Immunophenotypic : leukemia associated immunophenotype LAIP
Immunophenotypic detection of MRD

- Immunophenotypic anomalies of blasts
  - Aberrant expression on leukemic cells
    - Decreased expression
    - Hyperexpression
    - Asynchronism
    - Lineage infidelity
      ➔ Notion of Leukemia Associated ImmunoPhenotype (LAIP)
  - Immunophenotypes absent on normal cells
    ➔ Notion of « empty gate»
Recent american survey
Keeney et al. 2016, Arch Pathol Lab Med

- Questionnaire to 549 laboratories
- 500 answers (91%)
Threshold?

• In this survey: $10^{-3}$ to $10^{-5}$
  – ???
  – Number of cells counted?
  – Number of parameters?
  – Expected population of abnormal cells?
    • 10? 20? 50?
    • None!?
Limits of MRD detection

- Appropriate diagnostic panels
- Fresh cells
- Number of cells available
  - Necessary limited in aplastic patients
  - Conditions sensitivity (clusters)
  - Conditions the number of informative combinations
- Remain pragmatic!
  - $10^{-4} = 1/10000$ cells = 10/100000
The phases of MRD

The graph illustrates the number of leukemic cells over time since the start of therapy. The phases include Induction, Consolidation/Transplantation, Maintenance, Relapse, CR, MRD, and Cure. The red line represents the number of leukemic cells decreasing during Induction and Maintenance, while the green line shows a subsequent increase during Relapse before decreasing again towards cure.
Application to peripheral blood in AML induction

Lacombe et al, 2009, Leukemia
Sequential daily assessment

1 log decrease : 90% BDR
Prognostic value
Also stratifies intermediate risk cytogenetics

Karyotype I

Karyotype N
Diagnosis D0

Applicable to automated flow

Hematoflow®
Chemotherapy D1
Chemotherapy D2
Chemotherapy D3
**Chemotherapy D4**

![Flow cytometry scatter plot and table data]

<table>
<thead>
<tr>
<th>Color</th>
<th>Name</th>
<th>% Gated</th>
<th>Number</th>
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<tbody>
<tr>
<td>Cyan</td>
<td>Lymph. B</td>
<td>51.67</td>
<td>10307</td>
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<tr>
<td>Blue</td>
<td>T-</td>
<td>19.11</td>
<td>3012</td>
</tr>
<tr>
<td>Green</td>
<td>T+</td>
<td>2.28</td>
<td>454</td>
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<tr>
<td>M-</td>
<td>0.60</td>
<td>119</td>
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<tr>
<td>M+</td>
<td>0.40</td>
<td>79</td>
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<tr>
<td>Lymph. T&amp;NK</td>
<td>21.39</td>
<td>4268</td>
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<tr>
<td>Lymph. Total</td>
<td>73.06</td>
<td>14573</td>
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<tr>
<td>Mono. Total</td>
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<tr>
<td>Imm. Gran</td>
<td>0.30</td>
<td>69</td>
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<td>Eosino. Total</td>
<td>14.36</td>
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<tr>
<td>Neutro. Mature</td>
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<tr>
<td>Neutro. Total</td>
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<tr>
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<td>Xi</td>
<td>0.01</td>
<td>1</td>
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<tr>
<td>XM</td>
<td>0.03</td>
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<td>Xn</td>
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<tr>
<td>Beso. Total</td>
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<td>3</td>
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<tr>
<td>Total Diff</td>
<td>100.00</td>
<td>19947</td>
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MRD IN ACUTE LYMPHOBLASTIC LEUKEMIA
First studies in the early 1990s

Empty spaces of normal bone marrow
Gaipa G et al. 2013, Cyometry B
Fossat et al. 2015, Cytometry B
Karawajew et al. 2015 Haematologica

<table>
<thead>
<tr>
<th>CD58</th>
<th>CD10</th>
<th>CD19</th>
<th>CD34</th>
<th>CD22</th>
<th>CD20</th>
<th>Syto41</th>
<th>CD45</th>
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<tr>
<td>CD38</td>
<td>CD10</td>
<td>CD19</td>
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<td>CD22</td>
<td>CD20</td>
<td>Syto41</td>
<td>CD45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Correlation with PCR</th>
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<tbody>
<tr>
<td>CD58 $10^{-4}$</td>
<td>80.0</td>
<td>94.4</td>
<td>88.1%</td>
</tr>
<tr>
<td>CD38 $10^{-4}$</td>
<td>88.1</td>
<td>95.1</td>
<td>91.3%</td>
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</table>
Regenerating bone marrow: normal spaces, changed proportions

Karawajew et al, 2015, Haematologica

Van Dongen et al, 2015, Blood
Incidence on outcome

**Basso et al, 2009, JCO**

- 815 Patients Day 15

**Eveillard et al, 2015, Hem Oncol**

- 123 Patients Day 21

- pos-pos: p=0.0001
- pos-neg: p=0.0001
- neg-neg: p<0.0001
Pui et al. 2015
MRD driven therapy D19
MRD IN ACUTE MYELOBLASTIC LEUKEMIA
MRD and prognosis of MRD in AML

Kern et al. *Blood* 2004

Perea et al. *Leukemia* 2006

**Figure 2** Prognostic value of a 0.1% cutoff value of MRD assessed by FC in AML with t(8;21) and inv(16). Cumulative incidence of relapse according to MRD detected by FC at the end of chemotherapy treatment.
HOVON experience
Terwijn M et al. JCO, 2013

Screening panel

<table>
<thead>
<tr>
<th>CD34</th>
<th>CD22</th>
<th>CD45</th>
<th>CD117</th>
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<tr>
<td>CD15</td>
<td>CD13</td>
<td>CD45</td>
<td>CD14</td>
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<tr>
<td>DR</td>
<td>CD33</td>
<td>CD45</td>
<td>CD11b</td>
</tr>
<tr>
<td>CD2</td>
<td>CD56</td>
<td>CD45</td>
<td>CD7</td>
</tr>
<tr>
<td>CD36</td>
<td>CD133</td>
<td>CD45</td>
<td>CD19</td>
</tr>
</tbody>
</table>

Mature blasts: CD117
Overexpression: CD14
Lack: CD11b
Cross-lineage: CD7
Asynchronous: CD19
Molecular MRD status provides the most powerful prognostic factor in NPM1 mutant AML

In multivariate analysis MRD status was only significant independent prognostic factor, considering:
- Age
- WBC
- Mutational profile (51 gene panel including \(FLT3\)-ITD, \(DNMT3A\), \(WT1\))

Immunophenotypic complexity

CD34
CD117
CD33
CD13
MRD a new strategy for AML

Boolean equation established at diagnosis

\[
\text{MRD} = \text{NOT A} \land \text{NOT B} \land \text{NOT C} \land \text{NOT D} \land \text{NOT E} \land \text{NOT F}
\]
Diagnosis
Importance of the notion of cluster

Diagnosis

MRD1 5%
Diagnosis

MRD1 $5 \times 10^{-3}$
Diagnosis

Negative MRD1
Incidence on outcome

C

### Disease free survival

<table>
<thead>
<tr>
<th>MRD level</th>
<th>Median (95%CI) months</th>
<th>% ±SE at 36 months</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5x10^5</td>
<td>Not reached</td>
<td>75.7 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>≥5x10^5-5x10^4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>≥5x10^4-5x10^3</td>
<td>18 (8-35)</td>
<td>16.7 ± 8.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥5x10^3-5x10^2</td>
<td>11 (7-15)</td>
<td>11.2 ± 34</td>
<td></td>
</tr>
<tr>
<td>≥5x10^2</td>
<td>4 (0-6)</td>
<td>0</td>
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</tr>
</tbody>
</table>

### Overall survival

<table>
<thead>
<tr>
<th>MRD level</th>
<th>Median (95%CI) months</th>
<th>% ±SE at 36 months</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5x10^5</td>
<td>Not reached</td>
<td>81.4 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>≥5x10^5-5x10^4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>≥5x10^4-5x10^3</td>
<td>26 (18-40)</td>
<td>42.3 ± 5.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥5x10^3-5x10^2</td>
<td>16 (13-20)</td>
<td>16.5 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>≥5x10^2</td>
<td>16 (13-20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• Three 10 colors tubes. Any detectable level considered positive.
• Median positives: 1% (range 0.004-7.6%)
Take home messages

- MRD feasible in flow cytometry
- Rapid and available at bedside
- Current significant threshold <10^{-4}
- \( \Rightarrow \text{lower than } 10^{-5} \) easily accessible when no event is detected
- If events detected 10 to 20
- Early response is the key!
Acknowlegements

- GEIL
- EGIL
- ELN WP10
- EHA SWG Diagnostics