ANÁLISIS AUTOMÁTICO.
Leucemia aguda linfoblástica: diagnóstico y EMR

CANCER RESEARCH CENTER IBSAL, UNIVERSITY
& UNIVERSITY HOSPITAL OF SALAMANCA

3er Curso Práctico de Citometría de Flujo
Valencia, 5 de marzo de 2020

FLOW CYTOMETRY DIAGNOSTICS IN
HEMATO-ONCOLOGY

1. Making the diagnosis
   Normal ↔ reactive/regenerating ↔ malignant
2. Classification of hematopoietic malignancies
   - relation with prognosis
   - relevance of risk-group definition in treatment protocols
3. Disease extension
   (e.g. in case of CSF)
4. Identification of therapeutic targets
   (e.g. for antibody or CAR-T cell therapy)
5. Evaluation of treatment effectiveness
   Detection of minimal residual disease (MRD)

ALOT: WHO vs DATA BASE
CLASSIFICATION OF ACUTE LEUKEMIA

<table>
<thead>
<tr>
<th>Compass result</th>
<th>Disease category (initial diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-ALL</td>
<td>BCP-ALL</td>
</tr>
<tr>
<td>Typical T &amp; B, Myeloid</td>
<td>516</td>
</tr>
<tr>
<td>T, B or Myeloid with mixed populations</td>
<td>5</td>
</tr>
<tr>
<td>T, B or Myeloid with mixed populations</td>
<td>5</td>
</tr>
<tr>
<td>AML</td>
<td>5</td>
</tr>
</tbody>
</table>

99.3% Correlation with WHO diagnosis
Summary:

Immunophenotyping for diagnosis, classification and monitoring of acute lymphoblastic leukemia

- Immunophenotyping provides essential information for the diagnosis of AML vs BCP-ALL vs T-ALL vs MPAL.
- Innovative software and data base tools introduced by the EuroFlow Consortium, provide a basis for cost-effective and standardized (automated) diagnostic screening of AML vs. BCP-ALL vs T-ALL and MPAL.

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Algorithm for EuroFlow antibody panels in hemato-oncology

Multi-tube EuroFlow classification panel for B-cell precursor ALL (BCP-ALL)*

Tube Pacific
Blue
Orange FITC PE PerCP-
Cy5.5 PE-Cy7 APC APC-
H7 Aim**

1
2
3
4

CD20
Smlg


CD9
CD21

CD45
CD45
CD45
CD45

CD58
Cylg


NuTdT
CD15
and
CDw65

CD66c
CD33
CD13
NG2

CD34
CD34
CD34
CD34

CD19
CD19
CD19
CD19

CD10
Smlg

and
CD117
CD22
CD123

CD38
Smlg


CD24
CD81

Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers

Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Backbone markers are indicated in bold; Cy= cytoplasmic; Sm= surface membrane; Nu= nuclear.

** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics).

Responsible scientist: L Lhermitte

Bene et al, Leukemia 1995

Multi-tube EuroFlow classification panel for B-cell precursor ALL (BCP-ALL)*

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Bene et al, Leukemia 1995
### Diagnostic Genetic Aberrant Immunophenotype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Genetic Lesion</th>
<th>Aberrant Immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null/BI</td>
<td>11q23</td>
<td>CD34+; CD10-; 7.1+; CD15-</td>
</tr>
<tr>
<td>Common/BII</td>
<td>t(9;22)*</td>
<td>CD34+; CD10-, CD38+, CD10+</td>
</tr>
<tr>
<td>Common/BII</td>
<td>t(12;21)</td>
<td>CD34+, CD10-, CD20-, CD13+</td>
</tr>
<tr>
<td>Pre-B/BIII</td>
<td>t(1;19)</td>
<td>CD34-, Cy5+; CD20+, CD10+, CD9+</td>
</tr>
<tr>
<td>Common/BII</td>
<td>t(5;14)</td>
<td>CD10+ with associated eosinophilia</td>
</tr>
</tbody>
</table>

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### BCP-ALL: Genotypic-Phenotypic Associations

**EuroFlow Report, Van Dongen et al., Leukemia 2012; 26: 1908-1975**

*Protein expression profiles for normal maturing thymocytes*:
- **CD34- CD10- CD45RA- CD99- CD7lo**
- **NuTdT- CyCD3lo CD1a+ CD4+ CD8+ CD2hi CD45hi**
- **CD1a- sCD3hi sTCRαβ+ CD44+ CD45RA+ CD5hi**

**IM (sCD3- cTCRβ-)**
- **T-cell acute lymphoblastic leukemia**
  - Expression of T-cell-specific markers:
    - CD3
  - Expression of T-cell associated antigens:
    - CD2; CD6; CD4+*
  - Absence of multiple T-cell-associated markers:
    - CD5- CD8
  - Expression of ≥1 stem cell-associated antigens:
    - CD34; CD17; HLA-DR, CD13; CD33; CD11b, and CD65
  - Presence of myeloid-associated gene mutations:
    - FLT3, NRAS/KRAS, DNMT3A, IDH1, IDH2
  - Infrequent T-ALL-associated gene mutations:
    - NOTCH1, CEB1/G2
WHO 2016 CLASSIFICATION OF LYMPHOID NEOPLASMS

- B-lymphoblastic leukemia/lymphoma
  - With recurrent genetic abnormalities:
    - t(9;22)(q34.1;q11.2); BCR-ABL1
    - t(1;19)(q23;p13.3); TCF3-PBX1
    - Hyperdiploidy
    - t(5;14)(q31.1;q32.3); IL3-IGH
    - t(1;19)(q23;p13.3); TCF3-PBX1
    - iAMP21 (provisional entity)
  - Not otherwise specified (NOS)

- T-lymphoblastic leukemia/lymphoma:
  - Early T-cell precursor ALL (provisional)
  - NK-cell lymphoblastic leukemia/lymphoma (provisional)

B CELL PRECURSOR ALL WITH SWITCH TO MONOCYTIC LINEAGE LEUKEMIC CELLS

- NLL and BCR-ABL1 ALL subtypes
  - DUX4 rearranged and other swBCP-ALL
  - Unique expression of CD371, CD140a, CD146
  - Distinct phenotype from normal mature monocytes based on CD45RA, CD98, CD135 expression

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FCM vs CC for detection of ALL blasts in CSF samples

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- Immunophenotyping provides information for further objective subclassification of BCP-ALL and T-ALL. Automation of subclassification of ALL is under development.

Flow cytometry is significantly more sensitive than conventional cytology as regards the detection of leptomeningeal involvement in ALL, EuroFlow databases for automated CSF analysis being currently under construction.
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CLINICAL UTILITY OF MRD in ALL

- To evaluate the depth of response at the systemic level (e.g. BM and PB): new CR criteria
- To predict outcome: risk stratification of ALL patients during and after therapy.
- To personalize ALL patient therapy: MRD as a surrogate endpoint for progression-free survival.

PROGNOSTIC IMPACT OF MRD IN CHILDHOOD ALL

- To evaluate the depth of response at the systemic level (e.g. BM and PB): new CR criteria
- To predict outcome: risk stratification of ALL patients during and after therapy.
- To personalize ALL patient therapy: MRD as a surrogate endpoint for progression-free survival.

NEXT GENERATION FLOW (NGF):
EuroFlow NGF-MRD Approach

Optimized two-tube antibody panels for improved MRD consistency and precision and evaluation of sample quality.
Standardized sample processing & acquisition of ≥10^7 for high-sensitivity
Innovative multivariate and automated data analysis software tools for higher reproducibility

MRD DETECTION IN ADULT ALL BY NEXT GENERATION FLOW (EuroFlow method)

1. Sample preparation & staining
2. Data acquisition and analysis
3. Data interpretation

BCP-ALL: impact of the number of cells evaluated in the sensitivity of Flow-MRD

AIM: to evaluate >10^7 cells (2 x 5 x 10^6 cells/tube)
Automated gating and labeling of cell populations

How does it work?

**Clustering phase**

**Classification phase**

Groups of events

Cell populations

Identifying the pathways that link individual events in an (N)-dimensional space

A software tool similar to Compass based on a Reference Database

**Automated gating for MRD detection in BCP-ALL BM**

**Novel multi-colour high-throughput flow cytometry for MRD detection in BCP-ALL**

**EuroFlow T-ALL MRD tubes: % aberrant phenotypes**

<table>
<thead>
<tr>
<th></th>
<th>T1 only</th>
<th>T2 only</th>
<th>Neither T1 nor T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001% MRD</td>
<td>32/73</td>
<td>4/73</td>
<td>1/73</td>
</tr>
<tr>
<td>0.08% MRD</td>
<td>38/73</td>
<td>5/73</td>
<td>1/73</td>
</tr>
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</table>

Overall Performance of EuroFlow tubes

T1 informative in 95%
T2 informative in 54%
PETHEMA ADULT ALL HR-11: What can we learn from the previous PETHEMA HIGH-RISK NON-BCR1-ABL1 ADULT ALL PROTOCOL

**ALL HR-11**

**Induction-1**

Centralized MRD

| <0.1% | C1+C2+C3 |
| <0.01% | C1+C2+C3 |

**Maintenance**

| ≥0.1% | No morphologic CR |
| I-2 (FLAG-IDA) | C1 |

Centralized MRD

| <0.1% | Centralized MRD |
| ≥0.1% | Centralized MRD |

Allo-HSCT

Failure

Off study

| ≥0.01% | Morphologic CR |
| Ph-neg ALL (18–60 yr.) | SR & HR I-1* |
| I-2 |

Genetic study

Pre-Phase CR

MRD1

<0.01%

MRD1

<0.01% & Genetics

MRD1

<0.01% & Genetics + C1+C2+C3

MRD2

<0.001%

MRD2

≥0.001%

Early Allo-HSCT

Delayed Allo-HSCT

Re-Induction

MRD2

<0.01%

MRD2

≥0.01%

Early pre-T FLAG-ida

| Any MRD level | Genetics |
| No CR | CR |

MRD post I2

<0.01%

Regardless of genetics

MRD post I2

≥0.01%

Regardless of genetics

Clinical trial

RESIDUAL CD19- BCP-ALL BLASTS AFTER BLINATUMUMAB THERAPY

Simultaneous MRD, CARCD19 T-cell and Immune Monitoring in B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL)

Slide prepared by L. Martin and S. Gutierrez
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- Flow cytometry is significantly more sensitive than conventional cytology as regards the detection of leptomeningeal involvement in ALL. EuroFlow databases for automated CSF analysis are currently under construction.
- EuroFlow panels and software tools have been built for MRD analysis of BCP-ALL and T-ALL, which represent a robust and standardized tool to obtain clinically relevant information for adequate patient management. Adaptations are currently ongoing for monitoring of novel immune therapies.

ACKNOWLEDGEMENTS

EuroFlow is an independent scientific consortium, which aims at innovation in flow cytometry for improvement of diagnostic patient care

EuroFlow is a working group of EHA (European Hematology Association)

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THE CIC/USAL-IBSAL TEAM

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