Leucemia linfoblástica aguda
Diagnóstico y seguimiento

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ICO-Hospital Universitari Germans Trias i Pujol. Badalona
Grupo PETHEMA
Diagnostic work-up in ALL

- Anamnesis, physical examination
- Complete blood count, coagulation status, serum biochemical study
- EKG, LVEF (advanced age or history of cardiac disease)
- Chest X-ray film
- Bone marrow smear (morphology, cytochemistry)
- Bone marrow biopsy (only if dry tap)
- Immunophenotypic study (BM, PB)
- Cytogenetics
- FISH
- Study of molecular rearrangements (PCR)
- CSF study
- Storage: cells, DNA, RNA.
ALL. WHO Classification, 2008

• **B precursor ALL**
  - t(9;22); BCR/ABL
  - 11q23; MLL
  - t(1;19); E2A/PBX1
  - t(12;21); ETV/CBF alpha
  - Hyperdiploid ALL
  - Hypodiploid ALL

• **T-ALL**

• **Burkitt-like ALL (mature B-ALL)**
  - t(8;14), t(2;8), t(8;22); C-MYC
LAL. Morphology

Sensitivity: $10^{-2}$
Phenotypic study

Sensitivity: $10^{-4}$ (4 colors), $\geq 10^{-5}$ (>4 colors)
## Phenotypic classification. Precursor B-ALL

<table>
<thead>
<tr>
<th>Pro-B</th>
<th>+</th>
<th>±</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>±</th>
<th>-</th>
<th>++</th>
<th>±</th>
<th>-</th>
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<tbody>
<tr>
<td>Common</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>-</td>
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<tr>
<td>Pre-B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Mature B</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>cyCD3</td>
<td>SCD3</td>
<td>CD7</td>
<td>CD1a</td>
<td>TdT</td>
<td>CD2</td>
<td>CD5</td>
<td>CD4/CD8</td>
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<tr>
<td>Pro-T</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/±</td>
<td>-</td>
<td>-</td>
<td>-/-</td>
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<tr>
<td>Pre-T</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>+/±</td>
<td>+</td>
<td>+</td>
<td>+/- or +/+</td>
<td></td>
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<tr>
<td>Cortical</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±/±</td>
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</tr>
<tr>
<td>Mature</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>±/-</td>
<td>+</td>
<td>+</td>
<td>+/- or -/+</td>
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</table>
Flow Cytometry

CD20+ ALL with My: CD33+; CD66C++
# Biphenotypic leukemias

<table>
<thead>
<tr>
<th>Score</th>
<th>B lin</th>
<th>T lin</th>
<th>Myeloid lin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CD79a</td>
<td>CytCD3/CD3s</td>
<td>Anti-MPO</td>
</tr>
<tr>
<td></td>
<td>Cyt IgM</td>
<td>anti-TcR α/β</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyt CD22</td>
<td>anti TcR γ/δ</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CD19</td>
<td>CD2</td>
<td>CD117</td>
</tr>
<tr>
<td></td>
<td>CD10</td>
<td>CD5</td>
<td>CD13</td>
</tr>
<tr>
<td></td>
<td>CD20</td>
<td>CD8</td>
<td>CD33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD10</td>
<td>CD65</td>
</tr>
<tr>
<td>0,5</td>
<td>TdT</td>
<td>TdT</td>
<td>CD14</td>
</tr>
<tr>
<td></td>
<td>CD24</td>
<td>CD7</td>
<td>CD15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD1a</td>
<td>CD64</td>
</tr>
</tbody>
</table>

Score $\geq 2$ for lineage assignment (myeloid, B or T)
Cytogenetics

**Hiperdiploidy**
52,XY,+X,+6,+14,+17,+21,+mar

**Hipodiploidy**
41,XX,-4,-9,add(9)(p21),-15,-20,-22

*Sensitivity: $10^{-2}$*
Pseudodiploidy

46,XX,t(4;11)(q21;q23)

46,XX,+8,-12,der(19)t(1;19)(q23;p13.3),
+der(19)t(1;19)(q23;p13.3),-20

Sensitivity: $10^{-2}$
Pseudodiploidy

Burkitt’s leukemia

46, XY, t(9;22)(q34.1;q11.2)
Main cytogenetic differences between ALL in children and adults

<table>
<thead>
<tr>
<th></th>
<th>Incidence (%)</th>
<th>Cure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
<td>Adults</td>
</tr>
<tr>
<td>Philadelphia</td>
<td>&lt; 5</td>
<td>20-30</td>
</tr>
<tr>
<td>chromosome</td>
<td></td>
<td>&lt; 5 (adults)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25 (children)</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>28</td>
<td>&lt; 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-80</td>
</tr>
<tr>
<td>TEL-AML1</td>
<td>25</td>
<td>&lt; 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80-90</td>
</tr>
</tbody>
</table>

Faderl et al Cancer 2003
ALL. FISH

Sensitivity: $5 \times 10^{-2}$

nuc ish(ABL1x3), (BCRx3), (ABL1con BCRx2)[90/100]
Ig & TCR rearrangements

IgH clonal

TCR clonal

Sensitivity: $10^{-4} - 10^{-5}$ (RQ-PCR)
Quantification of the amount of mRNA transcripts

BCR/ABL - t(9;22)(q34.1;q11.2)

1 & 2: Patient 1 (positive p190)
3 & 4: Patient 2 (negative p190)
5: Positive control  p190
6: Negative control
7: Marker of molecular weight
RQ-PCR

Standard curve

Linear dynamic range (5 Logs)

Sensitivity: $10^{-5}$ - $10^{-6}$
Molecular follow-up (RQ-PCR)
molecular subgroups in childhood ALL

(Pui et al., 2004)
Genetic Heterogeneity in Adult ALL

- BCR-ABL (t(9;22)) 25%
- HOX11 (10q24) 8%
- TAL1 (lp32) 11.5%
- LYL1 (19p13) 3%
- HOXT11L2 (5q35) 1%
- MLL rearrangements (t(4;11), t(11;19), t(9;11)) 10%
- Others 23%
- Hypodiploidy (<45 chromosomes) 2%
- Hyperdiploidy (>50 chromosomes) 7%
- TEL-AML1 (t(12;21)) 2%
- MYC (t(8;14), t(2;8), t(8;22)) 4%
- E2A-PBX1 (t(1;19)) 3%
- MLL-ENL 0.5%
Usefulness of diagnostic work-up

- Diagnosis
- **Prognosis**
- MRD evaluation and follow-up
- Early detection of relapses
Prognostic impact of genetic and molecular classification of childhood ALL

Pui C-H. Lancet 2008;371:1030
Genetics and prognosis in adult ALL.
(MRC UKALLXII/ECOG 2993, n= 1522)

Outcome by CD20 expression and therapy according to age subgroups

Protocol

Young

Elderly

T-ALL: prognostic value of differentiation stage/phenotype

GMALL protocols

Baak U et al, Leukemia 2008
Prognostic impact of HOX1/TLX1 in adult T-ALL

Impact of BAALC expression on survival in adult T-ALL

Effect of NOTCH1 mutation status on long-term prognosis in childhood T-ALL

EFS impact of \textit{NOTCH1-FBXW7} mutations within \textit{ERG/BAALC} expression groups

![Graph A](image1)

- \textit{NOTCH1 - FBXW7} mutated (n=13)
- \textit{NOTCH1 - FBXW7} wild-type (n=7)

\textit{Low ERG/BAALC}

\textit{p}=0.076

![Graph B](image2)

- \textit{NOTCH1 - FBXW7} mutated (n=30)
- \textit{NOTCH1 - FBXW7} wild-type (n=13)

\textit{High ERG/BAALC}

\textit{p}=0.6

Genetic Alterations of IKZF1, EBF1, and BTLA/CD200 and the Cumulative Incidence of Relapse in the Original Cohort

CIR according to IKZF1 deletion in BCR-ABL+ ALL

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Usefulness of diagnostic work-up

- Diagnosis
- Prognosis
- MRD evaluation and follow-up
- Early detection of relapses
MRD analysis in ALL – How?

- t(9;22)(q34;q11)
- CD19
- CD34
- TCRB Locus 7q34
- TCRB-Gene-rearrangement
MRD analysis in ALL – How?

MRD level

Detection limit light microscopy

10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0}

Cytogenetics  FISH  Flow  PCR

- Immune Genes
- Fusion Genes
Study of Fusion Transcripts

• Advantages
  – High sensitivity: $1 \times 10^{-5} - 10^{-6}$
  – Leukemia-specific
  – Stable target during evolution
  – Standardized: useful in cooperative multicenter clinical trials

• Pitfalls
  – Not patient-specific
  – Useful in a minority of patients
  – Risk of contamination
  – Poor reproducibility when small numbers of transcripts are present
Comparison of PCR and Flow Cytometry for MRD Study in Ph-negative ALL

<table>
<thead>
<tr>
<th></th>
<th>PCR analysis of Ig and TCR gene rearrangements</th>
<th>Multiparameter flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>RQ-PCR: $10^{-4} - 10^{-5}$</td>
<td>3- to 4-color: $10^{-3} - 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6- to 9-color: $10^{-4} - 10^{-5}$</td>
</tr>
<tr>
<td><strong>Applicability</strong></td>
<td>Precursor B-ALL: 90-95% T-ALL: 90-95%</td>
<td>Precursor B-ALL: 80-95% T-ALL: 90-95%</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>- High sensitivity</td>
<td>- Applicable for almost all cases</td>
</tr>
<tr>
<td></td>
<td>- High degree of standardization</td>
<td>- Rapid</td>
</tr>
<tr>
<td></td>
<td>- Applicable for almost all cases</td>
<td>- Information on benign cells</td>
</tr>
<tr>
<td></td>
<td>- DNA stability (multicenter setting)</td>
<td>- Information on malignant cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Increasing standardization</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>- Time consuming</td>
<td>- Immunophenotypic shifts in precursor-B-cells during regeneration</td>
</tr>
<tr>
<td></td>
<td>- Potential instability of targets (clonal evolution phenomena), two independent targets recommended</td>
<td>- Modulation of antigen expression during induction therapy</td>
</tr>
<tr>
<td></td>
<td>- Extensive knowledge/experience needed</td>
<td>- Low cellularity during/after induction</td>
</tr>
<tr>
<td></td>
<td>- Relatively expensive</td>
<td>- Limited sensitivity/applicability using 3- to 4-color flow cytometry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Extensive experience needed in ≥6-color flow cytometry</td>
</tr>
</tbody>
</table>

Modified from Bruggemann et al. Leukemia 2010; 24. 521-535
Implications of MRD as a Prognostic Factor

- Assessment of response to therapy
- Assignment of risk group to modulate treatment
  - Intensity
    - High-risk group: intensification of therapy, SCT
    - Low-risk group: deintensification (balance toxicities – including TRM- and risk of relapse)
  - Duration
  - Timing of stem cell transplantation
  - Treatment post-SCT
  - New drugs in low MRD status (i.e.: alemtuzumab (CALGB), blinatumomab (EWALL))
- Detection of early relapse
Predictive value of MRD
- Childhood ALL -

„MRD after induction is most important independent prognostic factor“

I-BFM-SG

EORTC trial 58881
CIR among 379 children with B-lineage ALL whose MRD levels were less than 0.01% on day 46

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Group</th>
<th>Method</th>
<th>N</th>
<th>Prognostic Model</th>
<th>DFS</th>
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<tr>
<td>Brüggemann</td>
<td>2006</td>
<td>GMALL</td>
<td>PCR</td>
<td>105 SR</td>
<td>$&lt;10^{-4} \text{d}<em>{11} + &lt;10^{-4} \text{d}</em>{24}$</td>
<td>100%</td>
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<td></td>
<td></td>
<td>$&gt;10^{-4} \text{d}<em>{24} + &gt;10^{-4} \text{w}</em>{16}$</td>
<td>6%</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>All others</td>
<td>53%</td>
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<tr>
<td>Bassan</td>
<td>2009</td>
<td>NILG</td>
<td>PCR</td>
<td>142 SR &amp; HR</td>
<td>$&lt;10^{-4} \text{wk}<em>{16}$, $&lt;10^{-4} \text{wk}</em>{22}$</td>
<td>72%</td>
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<td>All others</td>
<td>14%</td>
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<td>Ribera</td>
<td>2009</td>
<td>PETHEMA</td>
<td>Flow</td>
<td>202 HR</td>
<td>$&lt;10^{-3}(\text{wk}_{5})$ &amp; $&lt;5\times10^{-4}$ (wk16)</td>
<td>77%</td>
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<td></td>
<td>$\geq10^{-3}(\text{wk}_{5})$ &amp; $\geq5\times10^{-4}$ (wk16)</td>
<td>31%</td>
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</table>
Prognostic significance of MRD in adult ALL

- Standard risk ALL: MRD based risk stratification -

**DFS**

P < 0.001

**Overall Survival**

P < 0.001

M Brüggemann et al. Blood 2006; 107: 1116
Prognostic significance of MRD in adult ALL

JM Ribera et al, ASH 2009
Usefulness of diagnostic work-up

- Diagnosis
- Prognosis
- MRD evaluation and follow-up
- Early detection of relapses
MRD as a Predictor of Relapse in Adults with Standard-Risk, Ph-negative ALL

## Prognostic Significance of MRD before SCT

<table>
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<tr>
<th>Author</th>
<th>Year</th>
<th>N</th>
<th>Relapse rate (%)</th>
<th>MRD-pos</th>
<th>MRD-neg</th>
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<td>Patel</td>
<td>2010</td>
<td>25 (A)</td>
<td>75%</td>
<td>23%*</td>
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<tr>
<td>Giebel</td>
<td>2009</td>
<td>123 (A)</td>
<td>57%*</td>
<td>17%*</td>
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<td></td>
<td></td>
<td></td>
<td>62%**</td>
<td>8%**</td>
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<tr>
<td>Bader</td>
<td>2009</td>
<td>91 (P)</td>
<td>57%</td>
<td>13%</td>
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<td>Spinelli</td>
<td>2007</td>
<td>37 (A)</td>
<td>46%</td>
<td>0%</td>
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<tr>
<td>Sramkova</td>
<td>2007</td>
<td>25 (P)</td>
<td>75%</td>
<td>6%</td>
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<tr>
<td>Sanchez</td>
<td>2001</td>
<td>40 (A/P)</td>
<td>67%</td>
<td>17%</td>
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<tr>
<td>Van der</td>
<td>2001</td>
<td>17 (P)</td>
<td>67%</td>
<td>20%</td>
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<tr>
<td>Velden</td>
<td></td>
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</table>

* B-lin ALL, ** T-ALL
White blood cells from a patient with acute lymphoblastic leukaemia

Thank you!

Lancet Oncology 2009