Acute Leukemias: Morphology and Beyond

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NABL Assessor

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Not all cases diagnosed morphologically
Genetic markers: Diagnosis and Prognostification
Progress is ongoing

Cost
Availability
Training

Small and Medium size labs
Practicing Pathologists
Referral labs
Institutional practice

FAB, WHO

Ground realities

MRD

Important Marker for prognostification

Diagnostic Dilemmas: Morphology, IPT, Genetics
LEUKEMIAS:

- Malignant clonal populations
- Derived from bone marrow stem cells

MYELOID / LYMPHOID

ACUTE / CHRONIC

- Present quickly
- Rapidly fatal if untreated
- FAB: Proposed by Bennett et al 1976

- Reviewed with criticism 1985-90

- WHO: Project undertaken in 1995
  Classification published in 2001

- Update of WHO classification: Published 2008
Problems with FAB

Critisisms received: Ref. Leukemia Diagnosis A guide to the FAB classification: Dr. Barbara Bain 1990

1) Not all cases of acute leukemia will be diagnosed with requirement of 30% blasts.

2) Not all cases of AML will be diagnosed with requirement of 3% MPO/SBB positive blasts.

3) Not all cases of AML can be classified by the criteria of FAB classification.

4) The FAB categories are not reproducible.

5) The FAB classification is illogical.
Problems with FAB

Critisisms received: Ref. Leukemia Diagnosis A guide to the FAB classification: Dr. Barbara Bain 1990

6) The FAB classification is arbitrary.

7) The FAB classification does not allow for bilineage leukemia.

8) The FAB classification excludes important information about cases which may be more biologically relevant than morphology and cytochemistry, for example cytogenetic and immunological characteristics and evidence of multilineage involvement.
- FAB: Proposed by Bennett et al, 1976
- Reviewed with criticism, 1985-90
- WHO: Project undertaken in 1995
  Classification published in 2001
  Combined Morphology, Immunophenotype, Genetic Features, Clinical syndromes
- Update of WHO classification: Published 2008
  Rapid technological progress
WHO

- Primary stratification according to lineage
- Definition of distinct diseases in each category
  - Morpho, IPT, Genetics, Clinical
- Cell of origin postulated

Stage of differentiation, Gene rearrangements, Mutations

Prognostic impact, Response to Rx, Targeted Rx
Revised WHO Classification 2008

- Specimen Requirements
- Assessment of Blasts
  - Blast Lineage
  - Genetic Features
- Correlation / Reporting of Data

Guidelines for using Revised WHO Classification 2008 for AML *Blood July'09*
Revised WHO Classification 2008

- Specimen requirements
  - PB and BM collected before starting Rx
  - PB and BM aspirate smears/touch imprints stained with Wright-Giemsa
  - BM Biopsy 1.5 cm, At right angles to the cortical bone, recommended for all cases
  - BM specimens for complete cytogenetic analysis, flow cytometry and molecular genetic studies preserved. Processed based on initial karyotype, clinical, morphologic and immunophenotypic findings.
Assessment of Blasts

- Blasts %: Visual Determination

- Myeloblasts, Monoblasts, Promonocytes, megakaryoblasts counted as Blasts

- Abnormal Promyelocytes: Blasts equivalent in APL

- Proerythroblasts: counted as blasts only in Erythroleukemia

- Flow cytometric assessment of CD34+ cells not recommended as substitute for visual assessment of Blasts.

- Poor aspirate/Fibrosis: CD34 by IHC may be informative
Revised WHO Classification 2008

Assessment of Blast Lineage

- Multiparameter Flow: At least 3 color: To determine lineage and aberrant antigen profile

- Cytochemistry may be helpful but not essential

- IHC on biopsy may be helpful
Revised WHO Classification 2008

- **Assessment of Genetic Features**
  - Complete cytogenetic analysis from BM at Diagnosis
  - FISH, RT-PCR, Mutational status: As guided by Clinical, Lab and Morphological information

- **Correlation / Reporting of Data**
  - All data should be assimilated into one report that states the WHO diagnosis.
Lab Issues:

- History / Clinical signs and symptoms
- Collection of blood and bone marrow
- Preparation of smears
- Morphology
- Special stains
- IPT / IHC, Genetic Studies
- Classifications / Terminology
- MRD
Lab Issues in Acute Leukemias

- History / Clinical signs and symptoms ??
- Collection of blood and bone marrow
- Preparation of smears
- Morphology
- Special stains
- IPT / IHC
- Terminology
- MRD

Short history < 3 months
Symptoms due to BM failure: Anaemia/Bleeding / Infection
DIC (?AML M3)
Sweating / Fever / General malaise
LNpathy, HSmegaly (?ALL)
Infiltration of Meninges / Testes (?ALL)
Skin / Bones / Gum hypertrophy (?AML M4/M5)
- **Collection of blood and bone marrow**
  - Procedure - Technique
  - Preparation of fresh smears
  - PBS reporting
  - Use of vacutainers
  - Good quality CBC – platelet count

- **Preparation of smears**
  - Preparation of adequate number of good quality smears
  - Training of the doctors

- **Morphology**
  - Training of Technical staff and Doctors

*Your eyes don’t see what your brain doesn’t know!*
- **Special stains**
  - Helpful in many instances
  - Training of the technical staff
  - Interpretation

- **IPT / IHC, Genetic Analysis**
  - Available at referral institutes / Speciality laboratories
  - Cost
  - Time factor?

- **Classifications / Terminology**
  - FAB / WHO / Revised WHO 2008

- **MRD**
  - At research level??
- **Special stains**
  - Helpful in many instances
  - Training of the technical staff
  - Interpretation

- **IPT / IHC, Genetic Analysis**
  - Available at referral institutes / Speciality laboratories
  - Cost
  - Time factor?

- **Classifications / Terminology**
  - FAB / WHO / Revised WHO 2008

- **MRD**
  - At research level??
Morphological diagnosis?

Cytogenetics?

Immunophenotype?

FAB?

WHO?
Significant Changes in Diagnosis and Classification of AML

- AML with recurrent genetic abnormalities
  - t(8;21) / t(16;16), APL: Acute leukemia regardless of the blast count
  - t(9;11) / other 11q23: ≥ 20% blasts

- APL: PML-RARA and variant RARA translocations: Recognized separately

- MLL abnormalities: Separately categorized

- t(6;9), inv3, t(1;22): Added newly

- Provisional entities: Mutated NPM1, Mutated CEPBA

- FLT3 strongly recommended in cytogenetically normal AML
Significant Changes in Diagnosis and Classification of AML

- AML with recurrent genetic abnormalities
  - t(8;21) / t(16;16), APL : Acute leukemia regardless of the blast count
  - t(9;11) / other 11q23 : >/= 20% blasts

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- MLL abnormalities

- t(6;9), inv3, t(1;22)

- Provisional entities : Mutated NPM1, Mutated CEPBA

- FLT3 strongly recommended in cytogenetically normal AML
AML M4 Eo:
AML with
inv(16)(p13.1;q22);C
BFB-MYH11
AML M2: Acute Myeloid Leukaemia with t(8;21)(q22;q22); RUNX1-RUNX1T1
AML M5: Acute Myeloid Leukaemia with t(9;11)(q22;q23); MLLT3-MLL
Significant Changes in Diagnosis and Classification of AML

- AML with Myelodysplasia related changes
  - Name changed and expanded
    - History of MDS
    - Cytogenetic abnormality
    - 50% cells dysplastic

- Therapy related myeloid neoplasms
  - No subcatagorization
Acute Myeloid Leukaemia with inv(3) or t(3;3)(q21;q26.2); RPN1-EVI1
Significant Changes in Diagnosis and Classification of AML

- AML Not Otherwise Specified
  - Acute Erythroid and Acute Megakaryoblastic: Reclassified

- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome

- Blastic Plasmacytic Dendritic cell neoplasm
  - New category to include those derived from precursor of plamacytoid dendritic cells
Significant Changes in Diagnosis and Classification of AML

- AML Not Otherwise Specified
  - Acute Erythroid and Acute Megakaryoblastic: Reclassified

- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
ALL

- The most common malignant neoplasm of childhood
- The first disseminated cancer shown to be curable.
- Dramatic improvement in the prognosis which was once very poor.
- Represents the model malignancy for the principles of cancer diagnosis, prognosis and treatment.
Significant Changes in Diagnosis and Classification of ALL

- **Change in Nomenclature**
  - B lymphoblastic leukaemia / lymphoma NOS
  - B lymphoblastic leukaemia / lymphoma with recurrent genetic abnormalities
    - (t(9;21), MLL rearranged, t(12;21), Hyperdiploidy, Hypodiploidy, t(5;14), (1;19))
  - T lymphoblastic leukaemia / lymphoma
Need for prognostication

- Only high-risk or very-high-risk patients are treated with intensive therapy.

- Almost all adult patients and high risk children are candidates for intensive therapy.

- Less toxic treatments (usually antimetabolites) reserved for low-risk or standard-risk patients.
# Prognostic factors at a glance

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Favorable</th>
<th>Unfavorable</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count</td>
<td>$&lt;10 \times 10^{10}/L$</td>
<td>$&gt;200 \times 10^{10}/L$</td>
</tr>
<tr>
<td>Age</td>
<td>3-7 years</td>
<td>$&lt;1 \text{ yr, } &gt;10 \text{ yrs}$</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White</td>
<td>Black</td>
</tr>
<tr>
<td>Node, liver, spleen enlargement</td>
<td>Absent</td>
<td>Massive</td>
</tr>
<tr>
<td>Testicular enlargement</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>CNS leukemia</td>
<td>Absent</td>
<td>Overt(Blasts+Pleocytosis)</td>
</tr>
<tr>
<td>FAB morphology</td>
<td>L1</td>
<td>L2</td>
</tr>
<tr>
<td>Ploidy</td>
<td>Hyperdiploidy</td>
<td>Hypodiploidy $&lt;45$</td>
</tr>
<tr>
<td>Cytogenetic markers</td>
<td>Trisomies 4,10,17</td>
<td>t(9;22)(bcr-abl)</td>
</tr>
<tr>
<td></td>
<td>t(12;21)(TEL-AML1)</td>
<td>t(4;11)(MLL-AF4)</td>
</tr>
<tr>
<td>Time to remission</td>
<td>$&lt;14$ days</td>
<td>$&gt;28$ days</td>
</tr>
<tr>
<td>Minimal Residual Disease</td>
<td>$&lt;10^{-4}$</td>
<td>$&gt;10^{-3}$</td>
</tr>
</tbody>
</table>
MRD

MINIMAL RESIDUAL DISEASE
IN ACUTE LEUKEMIA
Basic aspects of detection of MRD in acute leukaemia

MRD detection by immunology and Flowcytometry: ALL, AML

MRD detection by PCR
- Antigen receptor genes
- Clinical value
- False negative and false positive MRD tests

Chromosomal translocations as MRD targets: ALL, AML

FLT3, cKit, WT1
Minimal Residual Disease

- Minimal residual disease (MRD) is defined as the lowest level of disease detectable in patients in clinical remission by the methods available.

- Independent predictive value

- Flowcytometry and Molecular techniques are the methods of choice
The goal is…

- To adjust patients’ therapy in order to reduce the risk of relapse and of over-treatment

- To determine the quality of stem cell harvests for autologous SCT

- To predict early relapse in patients following allogeneic SCT
The patients of acute leukemia carry a burden of up to $10^{12}$ malignant blasts at presentation.

They may carry up to $10^{10}$ blasts even if in complete clinical remission.

**DFS**: 30-40% Adult ALL, 85% Childhood ALL
Morphologic emission
< 05 % blasts in the bone marrow

Disease undetectable by light microscopy

Does not mean that the leukaemia cells are totally eradicated from the body

Their level at this stage is beyond the sensitivity of classical cytomorphologic methods

Courtesy: Dr. Letizia Foroni
Evaluation of Technique

- Detection of leukaemia cell specific marker: ‘distinguish blasts from normal marrow cells’
  - Sensitive - one in $10^4$ malignant cells
  - Specific - detect malignant cells in the background of normal cells
  - Reproducible
  - Quantitative

- Molecular techniques and Flow cytometry satisfy the criteria
Currently used markers:

- Immunophenotypic markers:
  - Flow Cytometry

- Genetic markers:
  - Molecular techniques - PCR
Flow Cytometry

Requires the identification of a leukaemic-specific pattern of antigen expression at presentation…
…which is then sought at follow up

- Advantages:
  - Rapid
  - Widely available
  - Sensitive: 1 in 10^-4 / -5
  - Useful in over 90%

- Sample has to be fresh
- Technical skills
<table>
<thead>
<tr>
<th>Cell lineage</th>
<th>Marker combination</th>
<th>Applicability (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-lineage ALL</td>
<td>TdT/CD5/CD3</td>
<td>90-95</td>
</tr>
<tr>
<td></td>
<td>CD34/CD5/CD3</td>
<td>20-25</td>
</tr>
<tr>
<td>B-lineage ALL</td>
<td>CD19/CD34/CD10/CD38</td>
<td>40-60</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/CD58</td>
<td>40-60</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/CD45</td>
<td>40-60</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/TdT</td>
<td>40-50</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/CD66c</td>
<td>30-40</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/TdT/IgM</td>
<td>10-20</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/CD22</td>
<td>10-15</td>
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<tr>
<td></td>
<td>CD19/CD34/CD10/CD13</td>
<td>10-15</td>
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<tr>
<td></td>
<td>CD19/CD34/CD10/CD15</td>
<td>10-15</td>
</tr>
<tr>
<td></td>
<td>CD19/CD34/CD10/NG-2</td>
<td>5-10</td>
</tr>
<tr>
<td>AML</td>
<td>CD33/CD34/CD117/CD15</td>
<td>20-40</td>
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<tr>
<td>--------------</td>
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<td>--------</td>
</tr>
<tr>
<td></td>
<td>CD33/CD34/CD117/CD13</td>
<td>20-40</td>
</tr>
<tr>
<td></td>
<td>CD13/CD33/CD34/CD56</td>
<td>20-30</td>
</tr>
<tr>
<td></td>
<td>CD13/CD33/CD34/CD133</td>
<td>20-30</td>
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<tr>
<td></td>
<td>CD13/CD33/CD34/CD7</td>
<td>20-30</td>
</tr>
<tr>
<td></td>
<td>CD13/CD33/CD34/CD38</td>
<td>15-20</td>
</tr>
<tr>
<td></td>
<td>CD33/CD34/CD117/HLA-Dr</td>
<td>15-20</td>
</tr>
<tr>
<td></td>
<td>CD13/CD33/CD34/CD15</td>
<td>15-20</td>
</tr>
<tr>
<td></td>
<td>CD33/CD34/CD117/CD11b</td>
<td>10-15</td>
</tr>
<tr>
<td></td>
<td>CD13/CD33/CD34/CD19</td>
<td>5-10</td>
</tr>
</tbody>
</table>
Molecular Techniques - PCR

Types of Molecular Targets (markers)

- **Patient Specific** -
  - Allele Specific Oligonucleotides
  - Concept: Leukemic clone evolves from a single cell.

- **Disease specific** -
  - Fusion gene transcripts
PCR based MRD detection using antigen receptor genes

- IG and TCR rearrangement process gives rise to a unique rearrangement in each B cell and T cell.

- Because of its unique size and base pair combination, CDR 3 segment distinguishes the rearranged IGH gene.

- All the cells derived from a single cell precursor carry the same rearrangement
PCR strategy for the identification of clonal IGH gene rearrangement in B-precursor ALL
Patients leukemic clone identified and sequenced

CDR 3 segment is identified on presentation sample

Specific primers are designed for the unique marker

Subsequent follow up samples are tested using this specific primer sequence for detection of residual abnormal clone
Family primers homologous to the FR1 or FR2 or FR3 (framework) regions are used in conjunction with a JH consensus primer. Different size fragments can be identified depending on the set of primers used.
Example of a VH3 clonal population in a B-precursor ALL patient
Methodology for patient specific markers

- Diagnostic sample: DNA extraction
- Identification of marker
- Heteroduplex analysis / Gene scan
- Sequencing
- Identification of unique sequence to design the primer
- RQ assay to establish the ‘quantitative range’ and ‘sensitivity’ using the primer
- Testing of the follow up samples to document the level of MRD

VHJ van der Velden et al. Leukemia 2007
RQ-PCR: rapid, reproducible and quantitative results for both Ig/TCR and gene fusion RT-PCR targets

- The amount of target molecules in the sample are calculated
- A housekeeping gene or control gene is amplified in the same sample to be able to correct for DNA/RNA quality and input
- Quantification of MRD is performed
## Frequency of IG and TCR gene rearrangements in precursor B and T lineage ALL

<table>
<thead>
<tr>
<th>Gene rearrangement</th>
<th>Precursor B - ALL</th>
<th>T-ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Childhood</td>
</tr>
<tr>
<td>IgH</td>
<td>75-80%</td>
<td>90-95%</td>
</tr>
<tr>
<td>DH-JH</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>VH-DH-JH</td>
<td>&gt;95%</td>
<td></td>
</tr>
<tr>
<td>Igk</td>
<td>40-50%</td>
<td></td>
</tr>
<tr>
<td>Vk-Jk</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Vk-Kde</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Igλ</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>TCRB</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>TCRG</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>TCRD</td>
<td>50%</td>
<td></td>
</tr>
</tbody>
</table>
Chromosomal translocations as molecular targets for MRD analysis

- Fusion gene transcripts

- Prognostic value: risk stratification
  - \( t(9;22), t(4;11) \): Poor prognosis
  - \( t(12;21), t(15;17), t(8;21), \text{inv}(16) \): Good prognosis

- cDNA template

- Reproducible, sensitive, widely applicable

- Stable during the course of the disease
# Fusion genes suitable for MRD analysis in adult and childhood ALL

<table>
<thead>
<tr>
<th>Chromosomal Translocation</th>
<th>Fusion gene</th>
<th>Relative frequency per type of leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Precursor-B-ALL</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
</tr>
<tr>
<td>t(1;19)(q23;p13)</td>
<td>E2A-PBX1</td>
<td>5-8%</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>MLL-AF4</td>
<td>3-5%***</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABL p190</td>
<td>3-5%</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABL p210</td>
<td>1-2%</td>
</tr>
<tr>
<td>t(12;21)(p13;q22)</td>
<td>ETV6-AML1</td>
<td>25-30%</td>
</tr>
<tr>
<td>Del(1)(p32;p32)</td>
<td>SIL-TAL1</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>40-45%</strong></td>
</tr>
</tbody>
</table>

*** In infant ALL, the frequency of t(4;11) can be as high as 70%
## Fusion genes suitable for MRD analysis in AML

<table>
<thead>
<tr>
<th>Chromosomal Translocation</th>
<th>Subtype</th>
<th>Fusion gene</th>
<th>Relative frequency</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>M2</td>
<td>AML1-ETO</td>
<td>10-14%</td>
<td>6-8%</td>
<td></td>
</tr>
<tr>
<td>t(15;17)(q22;q21)</td>
<td>M3</td>
<td>PML-RARα</td>
<td>8-10%</td>
<td>5-15%</td>
<td></td>
</tr>
<tr>
<td>inv(16)(p13;q22)</td>
<td>M4Eo</td>
<td>CBFβ-MYH11</td>
<td>5-7%</td>
<td>5-6%</td>
<td></td>
</tr>
<tr>
<td>t(6;11)(q27;q23)</td>
<td>M4, M5a/b</td>
<td>MLL-AF6, MLL-AF9, MLL-AF10</td>
<td>22%</td>
<td>2-3%</td>
<td></td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(10;11)(p11;q23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>M1, M2, M4, M7</td>
<td>DEK-CAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>M0, M1</td>
<td>BCR-ABL</td>
<td>Rare</td>
<td></td>
<td>Rare</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>25-30%</td>
<td>20-25%</td>
<td></td>
</tr>
</tbody>
</table>
## Childhood ALL

<table>
<thead>
<tr>
<th>Group</th>
<th>MRD level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>(&lt; 10^{-4})</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>Between (10^{-3}) and (10^{-4})</td>
</tr>
<tr>
<td>High risk</td>
<td>(&gt;10^{-2})</td>
</tr>
</tbody>
</table>
Childhood ALL

- Patients with no detectable disease or levels \(<10^{-4}\) at day 28 represent a low risk group and have the best outcome.

- Sequential quantitative measurements of residual disease (at 12 and 20 weeks) identify patients with a fast decline in disease to undetectable levels that strongly correlates with durable CCR.

- Any level of detectable disease beyond three months identifies patients at high risk.

Borowitz et al Blood 2008
MRD quantification in childhood ALL

DFS: day 28

DFS: 2-5 months

DFS: 6-9 months

DFS: 10-24 months

Gamerio et al 2002
Adult ALL

**B ALL:**
- MRD-PCR positivity at early time-points and level of MRD are important predictors of outcome
- Over 50% of patients have detectable residual disease post induction
- Residual disease decreases with time in patients destined to remain in CCR
- In patients who will eventually relapse, the disease is never fully eradicated

**T ALL:**
MRD tests during maintenance are best at predicting outcome.

Bruggemann et al Blood 2006
MRD quantification in adult ALL

**DFS: 0-2 months**
- ≤ 10^4 (N=11 pts)
- > 10^4 - 10^6 (N=17 pts)

p=0.01

**DFS: 3-6 months**
- > 10^7 - 10^8 (N=16 pts)
- ≤ 10^7 (N=11 pts)

p=0.0006

**DFS: 6-9 months**
- > 10^7 - 10^8 (N=10 pts)
- ≤ 10^7 (N=13 pts)

p=0.0001
Prognostic value in AML

Four groups of patients are identified

<table>
<thead>
<tr>
<th>MRD level</th>
<th>Relapse rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 : 10^4</td>
<td>No relapse</td>
</tr>
<tr>
<td>1 : 10^3 - 10^4</td>
<td>14 %</td>
</tr>
<tr>
<td>1 : 10^2 - 10^3</td>
<td>50 %</td>
</tr>
<tr>
<td>&gt; 1 : 10^2</td>
<td>84 %</td>
</tr>
</tbody>
</table>
To avoid false negative results during MRD-PCR monitoring in ALL:

- At least two molecular targets are used

and

- one of them, if possible, should be a gene fusion transcript.
Conclusions

- Need for Training: In routine labs
  - Procedure
  - Preparation of slides
  - Morphology
  - Special Stains

- Specialized tests: Genetic Studies, IPT, IHC
  - Need for more centralized facilities
  - Cost
  - Availability
  - Trained personnel
Conclusions

- FAB: Morphological diagnosis

- WHO and Revised WHO: Genetic diagnosis and prognostic indicators

- Minimal Residual Disease: Important prognostic marker. Need for standardisation of techniques: Flow / PCR

- Keeping updated is need of the time: Conglomerated efforts on the laboratory front to keep up with the rest of the world
Acute Leukemia of Ambiguous Lineage
Mixed phenotype acute leukemia B cell, with t(9;22) (q34;q11.2); BCR-ABL
Recent advances

Role of micro-RNA in ALL:

- MicroRNAs (miRNAs) are non-coding RNAs (ncRNAs) that regulate gene expression.

- The full spectrum of miRNAs in a specific cell type (the miRNome) varies between normal and pathologic tissues.

- The miRNAs can serve as molecular biomarkers of cancer with prognostic implications.

- New miRNA-based diagnostic, prognostic and predictive kits will be available.
## Role of micro-RNAs in ALL

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression</th>
<th>Biomarker</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-128b</td>
<td>High</td>
<td>Diagnostic</td>
<td>-</td>
</tr>
<tr>
<td>miR-128a and miR-128b</td>
<td>High</td>
<td>Diagnostic</td>
<td>Differentiate ALL from AML</td>
</tr>
<tr>
<td>miR-7, miR-198, miR-663</td>
<td>High</td>
<td>Prognostic</td>
<td>Higher risk of CNS relapse</td>
</tr>
<tr>
<td>miR-126, miR-345, miR-222, miR-551a</td>
<td>Low</td>
<td>Prognostic</td>
<td>Higher risk of CNS relapse</td>
</tr>
<tr>
<td>miR-17-92 cluster</td>
<td>High</td>
<td>Diagnostic</td>
<td>-</td>
</tr>
<tr>
<td>miR-16</td>
<td>Low</td>
<td>Prognostic</td>
<td>Better prognosis in childhood ALL</td>
</tr>
</tbody>
</table>
Genetic profiling

- **Microarray** –
  - Uses gene chips to sequence genes or portions of genes
  - Enables the identification of novel genetic alterations

- **Next generation sequencing** –
  - Uses PCR under oil immersion
  - Allows over 1 million PCR reactions at once
Newly discovered genetic abnormalities

- Deletions in IKZF1:
  - IKAROS gene – regulator of hematopoietic system
  - Ikaros acts as a highly clinically-relevant tumor suppressor in B-cell ALL and particularly in high-risk B-cell ALL
  - The modest decrease in Ikaros activity is sufficient to contribute to leukemogenesis
  - Genetic alterations of Ikzf1 might serve as a prognostic marker for B-cell ALL outcome.
Newly discovered genetic abnormalities

<table>
<thead>
<tr>
<th>Genetic abnormality</th>
<th>Function</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRLF2 overexpression</td>
<td>Tumor suppressor gene</td>
<td>Poor prognosis</td>
</tr>
<tr>
<td>PAX5 mutation</td>
<td>B-cell activator protein</td>
<td>-</td>
</tr>
<tr>
<td>iAMP21</td>
<td>Three copies of RUNX1</td>
<td>Poor prognosis and higher risk of relapse</td>
</tr>
</tbody>
</table>