Acute lymphoblastic leukemia (ALL) is a malignant disease which arises from the clonal uncontrolled proliferation of immature lymphoid cells. This uncontrolled proliferation of leukemic cells will crowd out the normal hematopoietic cells in the bone marrow, typically resulting in too many immature white cells and not enough functioning red cells or platelets in the peripheral blood. It is a heterogeneous disease in terms of its pathology and the populations it affects.

**Immunophenotype:**

ALL may be classified as B-cell precursor or T-cell lineage depending on the expression of lineage markers. B-ALL represents approximately 75-80% of adult cases of ALL. T-ALL represents 10–15% of pediatric and approximately 25% of adult cases of ALL.

Based on American Cancer Society estimates, there will be about 6,590 new cases of ALL in the US in 2016 in both children and adults.

**Minimal Residual Disease**

**Why do we assess it?**

The treatment of ALL has evolved significantly over the past three decades, resulting in about 85–90% of patients achieving complete remission (CR). Relapses can be attributed to minimal residual disease (MRD) that is undetectable by standard diagnostic techniques. Despite the patient being in CR, up to 10% malignant leukemic cells can still be present in the bone marrow. Studies in adults with ALL have also shown the strong correlation between MRD and risk for relapse, and the prognostic significance of MRD measurements during and after initial induction therapy.

**Response Categories Defined by Molecular Assessment**

Assessment of molecular response is conducted only after the patients attain complete cytologic remission with ≥ 1 marker for MRD, and requires availability of samples at various time points.

**Responses are then categorized as follows:**

- **Molecular CR / complete MRD response/MRD negativity:** Defined as MRD at a specific time point with polymerase chain reaction (PCR) assay sensitivity of ≥ 10⁻¹⁴
- **Molecular failure / MRD persistence:** Defined as the persistent quantifiable presence of MRD with a PCR assay sensitivity of ≥ 10⁻¹⁴
- **Molecular relapse / MRD reappearance:** Defined as reappearance of MRD within the quantitative range (≥ 10⁻⁶) after prior achievement of molecular CR

**Figures andTables:**

- Figure 1: Hypothetical Correlation of MRD Levels and Risk of Relapse
- Table: Specific Phenotypes and Prognostic Factors

The Three Main Methods for Detection of MRD

Morphologic assessment is not a very sensitive technique for measuring MRD, and has largely been replaced by more sensitive methodologies. The three main methods have been summarized here.

**Immunophenotyping using flow cytometer**

Immunophenotyping is the detection of cell surface antigens on lymphocytes using a flow cytometer. Flow cytometry analysis distinguishes leukemic cells from normal lymphocytes and progenitors. Malignant cells may be identified by their leukemia-associated immunophenotypes, which are usually defined at diagnosis. These cellular properties can be evaluated at a single cell level.15, 19

Additionally, RQ-PCR can be used to analyze fusion transcripts such as BCR-ABL, or MLL gene fusions.18 Gene fusions tend to occur in specific regions, not requiring primers to be generated for each patient.15

**Next-generation sequencing (NGS)**

NGS amplifies all gene segments and identifies all clonal gene rearrangements. This approach is very sensitive and has a limit of detection of 10^-6.18, 20

It provides greater flexibility compared to PCR since it does not require sequence-specific probe. The same “off-the-shelf” assay can be used to evaluate different patients. It could potentially be used for monitoring the clonal evolution of the disease.19

**Polymerase chain reaction (PCR)**

Real-time quantitative PCR (RQ-PCR) is used to detect and quantify gene rearrangements in the variable region of the immunoglobulin (lg) gene, as well as the T cell receptor (TCR) in patient blast cells.19 Individual patient rearrangements have to be characterized and primers generated.15

Additionally, RQ-PCR can be used to analyze fusion transcripts such as BCR-ABL, or MLL gene fusions.18 Gene fusions tend to occur in specific regions, not requiring primers to be generated for each patient.15

### Features of MRD Detection Methods in ALL

<table>
<thead>
<tr>
<th>Method</th>
<th>Applicability</th>
<th>Sensitivity</th>
<th>Important Considerations</th>
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</thead>
<tbody>
<tr>
<td>MFC 19, 21, 22, 23</td>
<td>ALL: &gt;90%</td>
<td>3- to 4-color: 10^-4 to 10^-6</td>
<td>Widely applicable and available</td>
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<td></td>
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<td>Also depends on cell input.</td>
<td>Does not require baseline sample</td>
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<td>6- to 9-color: 10^-4^-6</td>
<td>Clonal heterogeneity undetectable</td>
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<td></td>
<td></td>
<td>10^-4^-10^-6</td>
<td>Requires bone marrow aspiration</td>
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<td>Fresh sample necessary</td>
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<tr>
<td>RQ (real-time quantitative)</td>
<td>ALL: 90-95%</td>
<td>10^-6-10^-4</td>
<td>More expensive than MFC</td>
</tr>
<tr>
<td>PCR</td>
<td></td>
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<td>Requires bone marrow aspirate</td>
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<tr>
<td></td>
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<td></td>
<td>Requires baseline sample</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Time consuming</td>
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<tr>
<td>Fusion transcript</td>
<td>BCP-ALL: 25-40%</td>
<td>10^-3-10^-5</td>
<td>Possible differences in expression levels</td>
</tr>
<tr>
<td>PCR 19, 21, 22, 23</td>
<td>T-ALL: 10-15%</td>
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<td>– tumor response</td>
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<td></td>
<td></td>
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<td>– Require target throughout therapy</td>
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<tr>
<td>NGS 19, 21, 22, 23</td>
<td>&gt;95% all lymphoid malignancies</td>
<td>10^-6</td>
<td>Requires one week or more</td>
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<td></td>
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<td>Limited clonal heterogeneity detected</td>
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<td>Bone marrow aspirate or peripheral</td>
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<td>blood sample acceptable</td>
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<td>Fresh Sample not necessary</td>
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<td>Expensive, but costs decreasing</td>
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<td>Requires baseline sample or stored sample</td>
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<td>from a time point with detectable disease</td>
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</tbody>
</table>

**MRD Summary**

Morphological assessment cannot detect very low numbers of cells representing minimal residual disease in patient samples. Consequently, techniques using flow cytometry, RQ-PCR and NGS have been developed, which allow for more sensitive detection as well as the quantification of these residual leukemic cells. These methods are highly sensitive and can detect 1 cell in 10^6 to as low as 10^-6.2, 14, 19, 24, 25

A number of studies have shown that the detection of MRD in patients with ALL, both children and adults, is an independent risk parameter of high clinical relevance.2 This applies to de novo patients with ALL who may also undergo stem cell transplant.14

MRD testing may help to predict relapse and overall outcome, or may be employed to stratify patients based on risk of relapse.2, 26

The assessment of MRD has been incorporated as stratification in many protocols.27 A consensus on the timing of assessment and the definitions of common MRD terminology is becoming increasingly important when evaluating patients. Also, the standardization of MRD methodologies is important to ensure comparability within an MRD treatment protocol, as well as to provide a solid basis for the comparison of MRD data between different treatment protocols.

**References**