

Acute Lymphoblastic Leukemia and Minimal Residual Disease



Acute Lymphoblastic Leukemia – Pathophysiology and Epidemiology

Acute lymphoblastic leukemia (ALL) is a malignant disease which arises from the clonal uncontrolled proliferation of immature lymphoid cells.¹⁻²

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.³

It is a heterogeneous disease in terms of its pathology and the populations it affects.⁴

Immunophenotype:

ALL may be classified as B-cell precursor or T-cell lineage depending on the expression of lineage markers.¹

B-ALL represents approximately 75-80% of adult cases of ALL.⁵⁻⁶

T-ALL represents 10–15% of pediatric and approximately 25% of adult cases of ALL.^{1, 5}

Based on American Cancer Society estimates, there will be about 6,590 new cases of ALL and about 1,430 deaths due to ALL in the US in 2016 in both children and adults.³

Acute lymphoblastic leukemia is the most common childhood cancer. Current 5-year overall survival exceeds 90%.^{5, 7}

ALL is less common in adults and the treatment outcomes are significantly lower than in children with ALL, especially those with relapsed ALL.⁸

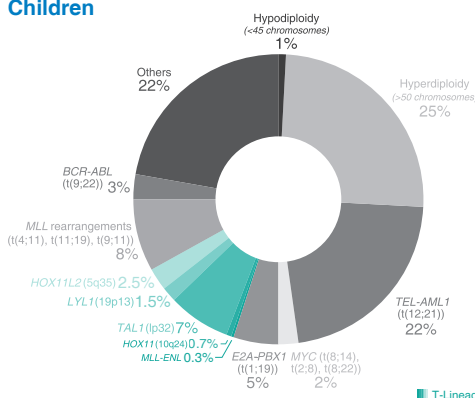
Some reasons for this difference include the higher incidence of poor prognostic cytogenetics and a lack of favorable cytogenetics in adults.⁹

Specific Phenotypes	Children	Adults*
Poor Prognostic Factors		
Ph+	3% ¹⁰	20–30% ¹¹
** <i>MLL</i> rearrangements	2-8% ^{10, 12}	5–10% ^{10, 12}
Favorable Prognostic Factors		
<i>TEL/AML1</i>	50% ¹²	10% ¹²
Hyperdiploid	25–30% ¹³	7% ¹⁰

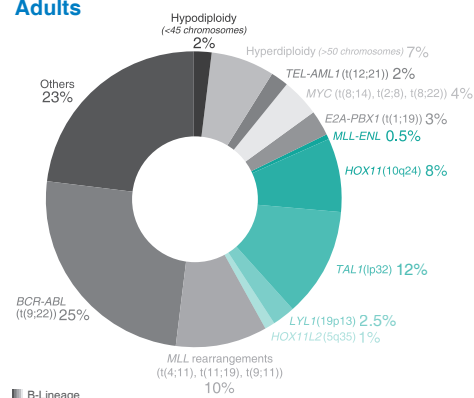
*Adults have higher incidence of poor prognostic cytogenetics and lack favorable risk cytogenetics

**50-75% of ALL in infants^{12, 13}

Children



Adults



Adapted from Pui CH et al, *N Engl J Med*. 2004.

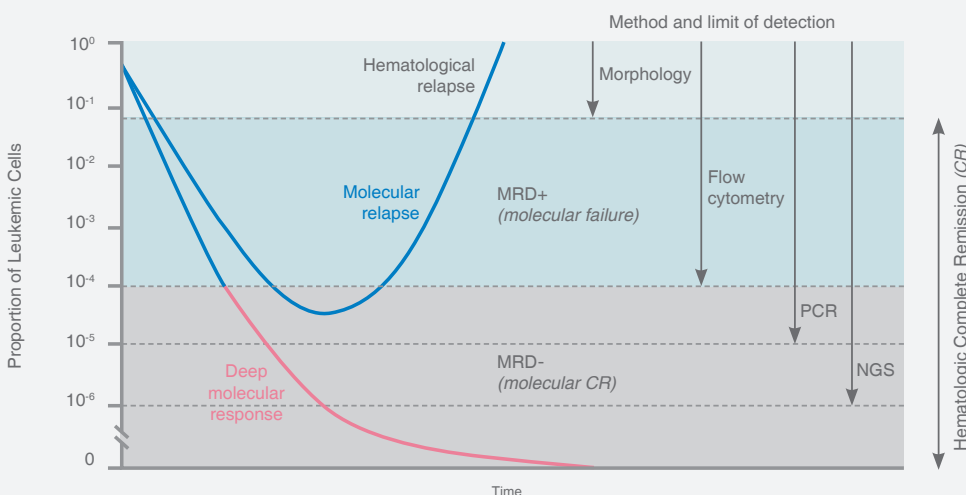
Minimal Residual Disease Why do we assess it?

The treatment of ALL has evolved significantly over the past three decades, resulting in about 85–90% of patients achieving complete remission (CR).¹⁴

Relapses can be attributed to minimal residual disease (MRD) that is undetectable by standard diagnostic techniques. Despite the patient being in CR, up to 10¹⁰ malignant leukemic cells can still be present in the bone marrow.^{14–16}

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.^{14–15}

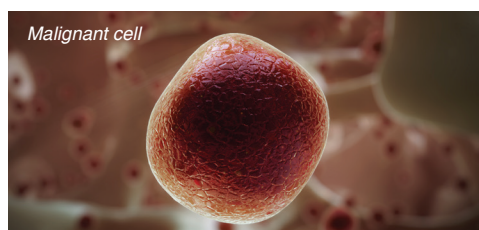
Figure 1 Hypothetical Correlation of MRD Levels and Risk of Relapse^{15, 17}



Adapted from Bruggemann M et al, *Blood*. 2012; Hoelzer D et al, *Hematology Am Soc Hematol Educ Program*. 2002.

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.^{14, 18}

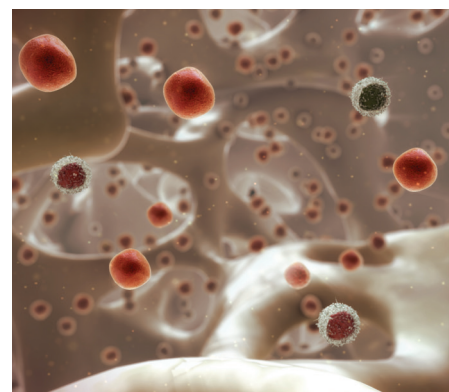


Responses are then categorized as follows:

Molecular CR / complete MRD response/MRD negativity: Defined as MRD at a specific time point with polymerase chain reaction (PCR) assay sensitivity of $\geq 10^{-4}$ ¹⁴

Molecular failure / MRD persistence: Defined as the persistent quantifiable presence of MRD with a PCR assay sensitivity of $\geq 10^{-4}$ ¹⁴

Molecular relapse / MRD reappearance: Defined as reappearance of MRD within the quantitative range ($\geq 10^{-4}$) after prior achievement of molecular CR¹⁴

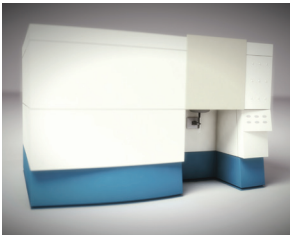


The Three Main Methods for Detection of MRD

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

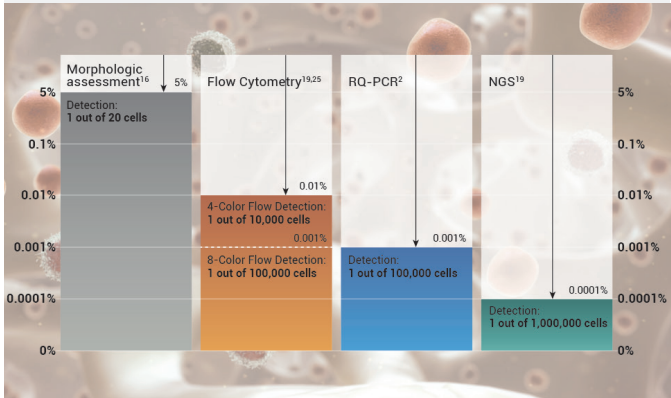
Immunophenotyping is the detection of cell surface antigens on lymphocytes using a flow cytometer. Flow cytometry analysis distinguishes leukemic cells from normal lymphocytes and progenitors. Malignant cells may be identified by their leukemia-associated immunophenotypes, which are usually defined at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



Next-generation sequencing (NGS)

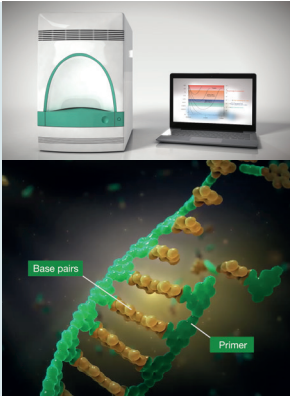
NGS amplifies all gene segments and identifies all clonal gene rearrangements. This approach is very sensitive and has a limit of detection of 10⁻⁶.^{18, 20}

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 (Ig) 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.¹⁹ 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

Method	Applicability	Sensitivity	Important Considerations	
MFC ^{19, 21, 22, 23}	ALL: >90%	3- to 4-color: 10 ⁻³ –10 ⁻⁴ 6- to 9-color: 10 ⁻⁴ –10 ⁻⁶ Also depends on cell input	<ul style="list-style-type: none">Widely applicable and availableTurnaround in hoursRelatively inexpensiveDoes not require baseline sample	<ul style="list-style-type: none">Clonal heterogeneity undetectableStandardization ongoing (EuroFlow/IMF)Requires bone marrow aspirateFresh sample necessary
RQ (real-time quantitative) – PCR ^{19, 21, 22, 23}	ALL: 90–95%	10 ⁻⁵ –10 ⁻⁶	<ul style="list-style-type: none">Standardized (EuroMRD)Fresh sample not necessaryClonal heterogeneity undetectablePatient-specific primers necessary	<ul style="list-style-type: none">More expensive than MFCRequires bone marrow aspirateRequires baseline sampleTime consuming
Fusion transcript PCR ^{2, 19}	BCP-ALL: 25–40% T-ALL: 10–15%	10 ⁻⁴ –10 ⁻⁵	<ul style="list-style-type: none">RapidUnequivocal link with leukemic/preleukemic cloneStable target throughout therapy	<ul style="list-style-type: none">Possible differences in expression levels (transcripts/cells) during the course of treatmentRNA instability → false negativeRisk of cross contamination → false positive
NGS ^{19, 21, 22, 23}	>95% all lymphoid malignancies	10 ⁻⁶	<ul style="list-style-type: none">Limited clonal heterogeneity detectedNot yet standardizedBone marrow aspirate or peripheral blood sample acceptableFresh Sample not necessary	<ul style="list-style-type: none">Requires one week or moreLimited availabilityExpensive, but costs decreasingRequires baseline sample or stored sample from a time point with detectable disease

MFC, multiparameter flow cytometry; PCR, polymerase chain reaction; NGS, next-generation sequencing; IMF, International Myeloma Foundation

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 10⁻⁴ 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

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