

# MEASURABLE RESIDUAL DISEASE AND ACUTE LYMPHOBLASTIC LEUKEMIA

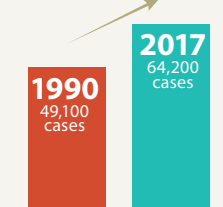
## ALL Pathophysiology

- Acute lymphoblastic leukemia (ALL) is a malignant disease that is characterized by abnormal proliferation and differentiation of a clonal population of lymphoid cells.<sup>1</sup>
- ALL is a heterogenous disease in terms of its pathology and the population it affects.<sup>2</sup> It is characterized by a nonspecific presentation consisting of a combination of constitutional symptoms and signs of bone marrow failure.<sup>1</sup>

## ALL Epidemiology and Prognosis

- ALL is the most common form of leukemia in children and adolescents, accounting for up to 80% of cases.<sup>3</sup>
- There will be an estimated 5,690 new cases of ALL and 1,580 deaths due to ALL in the USA in 2021.<sup>4</sup>
- The current 5-year survival rate is approximately 90% in high-income countries, and children who are more than 1 year old at diagnosis tend to have the best outcomes.<sup>3</sup>
- Although most cases of ALL occur in children, most deaths from ALL (about 4 out of 5) occur in adults.<sup>4</sup> Adults have higher relapse rates and poorer outcomes compared with children, with overall survival rates of approximately 20–40%.<sup>5</sup>

## Global Number of ALL Cases Has Increased<sup>6</sup>



## Immunophenotypes



- ALL is classified as B-cell or T-cell lineage, depending on the expression of lineage markers.<sup>7</sup>
- B-ALL is the most common type of ALL in children and adults, representing approximately 88% and 76% of cases, respectively.<sup>3</sup>
- T-ALL represents 12% of pediatric and 24% of adult cases of ALL.<sup>8</sup>

## High-Risk Factors for Adult ALL<sup>9,10</sup>

### Patient-related:

- Age: > 40/55/65 years
- ECOG score: > 1

### Disease-related:

- WBC ( $\times 10^9/l$ ): > 30 (B-ALL) / > 100 (T-ALL)
- Immunophenotype: pro-B/early or mature-T
- Extramedullary disease: central nervous system involvement

### Cytogenetics:

- Hypodiploidy
- *KMT2A* rearranged t(4;11) or others
- t(v;14q32)/IgH
- t(9;22)(q34;q11.2): *BCR-ABL1*
- Complex karyotype ( $\geq 5$  chromosomal abnormalities)
- *BCR-ABL1*-like (Ph-like) ALL
- *iAMP21*
- t(17;19): *TCF3-HLF* fusion
- Alterations of *IKZF1*

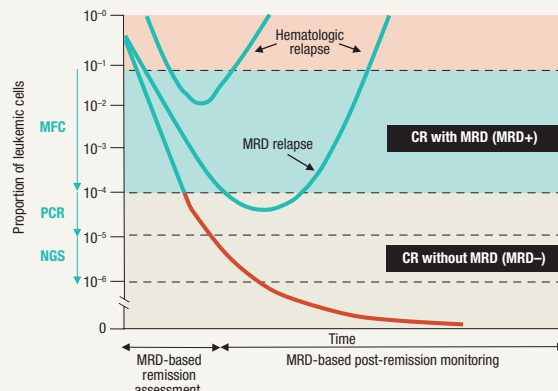
### Response dynamics:

- Corticosteroid sensitivity (blast count after pre-phase)
- Early blast cell response (bone marrow morphology)
- Time to CR (number of courses)
- MRD+ post-induction

## Estimated Frequency of Specific Genotypes of B-ALL in Children and Adults<sup>8</sup>

Genotype	Children	Adults
<i>BCR-ABL1</i> t(9;22)	3%	25%
<i>E2A-PBX1</i> t(1;19)	5%	3%
Hypodiploidy < 45 chromosomes	1%	2%
Hyperdiploidy > 50 chromosomes	25%	7%
<i>MLL</i> rearrangements (eg, t[4;11], t[11;19], t[9;11])	8%	10%
<i>MYC</i> t(8;14), t(2;8), t(8;22)	2%	4%
<i>TEL-AML1</i> t(12;21)	22%	2%
Others	22%	23%

## MRD is a Strong Prognostic Indicator in ALL<sup>1</sup>

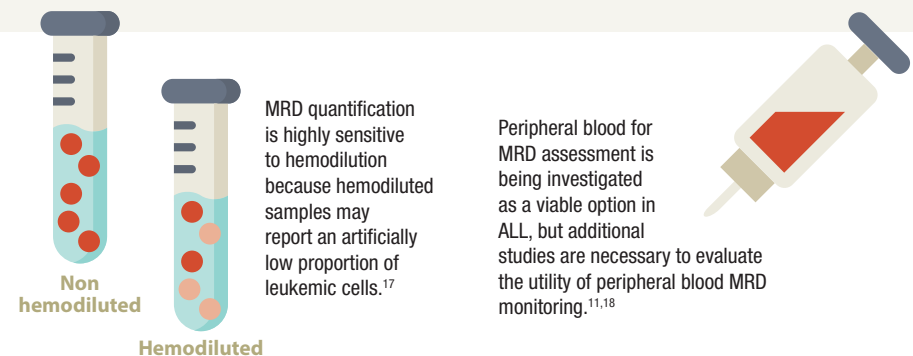


## Measurable/Minimal Residual Disease (MRD)

- MRD is defined as the presence of detectable leukemic cells (generally  $> 10^{-4}$  or 0.01% [meaning 1 leukemic cell in 10,000 normal cells]) within the bone marrow during remission.<sup>12,13</sup>
- Studies collectively show the high prognostic value of MRD (both during and after initial induction therapy) in assessing relapse risk for patients with ALL.<sup>14</sup>
- Approximately 30–40% of patients with ALL may still harbor MRD, despite achieving CR ( $< 5\%$  blasts in the bone marrow) with induction and consolidation chemotherapy.<sup>13,15</sup>
- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) state that MRD is an essential component of patient evaluation over the course of sequential therapy and recommend that MRD be assessed upon completion of initial induction therapy, with additional time points guided by the regimen used.<sup>10</sup> Additionally, serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden.<sup>10</sup>
- MRD assessment helps guide risk stratification and treatment planning in B-ALL.<sup>12,14</sup>

## Importance of the “First Pull” of Bone Marrow Aspirate for MRD Assessment

- The level of MRD found in the aspirate is dependent on which sample is used.<sup>16</sup> Even a second small-volume pull from the same aspiration site reduces the number of leukemic cells by up to 50% due to hemodilution.<sup>16</sup>
- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend an initial pull of  $\leq 3$  mL of bone marrow aspirate as optimal for MRD quantification to avoid hemodilution of the specimen.<sup>10</sup>



## Methods for MRD Detection in ALL

### Flow Cytometry

- Flow cytometry detects abnormal surface markers on leukemic cells.<sup>19</sup>
- While turnaround is rapid, interpretation of flow cytometry is challenging and can be difficult to standardize.<sup>20</sup>
- Immunostaining protocols, antibody panels, and gating strategies differ significantly between centers, leading to variability in results.<sup>21</sup>



### Polymerase Chain Reaction (PCR)

- PCR detects genetic abnormalities, such as fusion transcripts (eg, *BCR-ABL*), *Ig/TCR* gene rearrangements, or *MLL* gene rearrangements, using target sequences.<sup>19</sup>
- PCR for *Ig/TCR* is highly standardized and widely used in clinical trials in Europe.<sup>20</sup>
- However, PCR is laborious and expensive, and individual rearrangements may be unstable over time.<sup>22</sup>

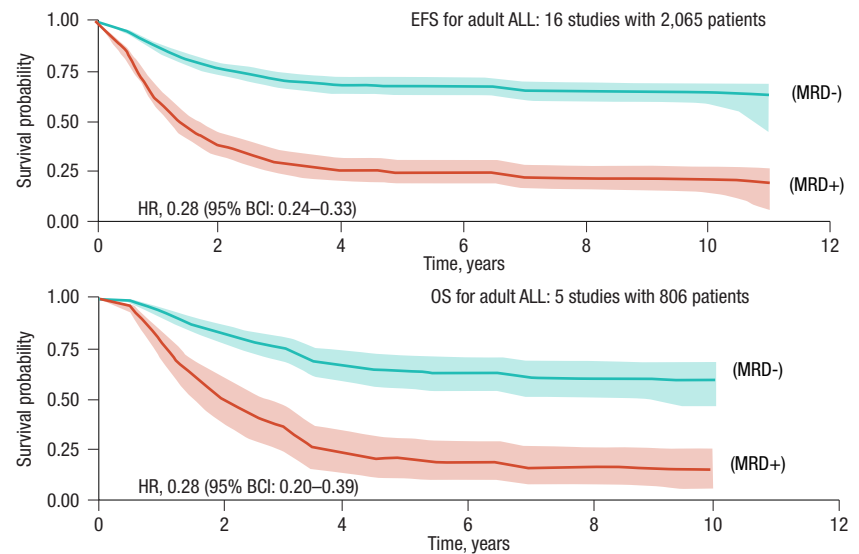


### Next-Generation Sequencing (NGS)

- NGS detects leukemia-specific *Ig/TCR* sequences.<sup>20</sup>
- NGS uses the same principle as PCR for *Ig/TCR*; however because of its high multiplexing capability, it does not require consensus PCR primers and can directly read the clonal sequence for detection.<sup>20</sup>
- NGS techniques may have sensitivity to below  $10^{-6}$  to quantify ALL MRD in bone marrow or peripheral blood samples.<sup>23</sup>



## In a Meta-analysis, Presence of MRD Tripled the Risk of Hematological Relapse or Death Over 10 Years<sup>14</sup>



This information is presented for the purpose of demonstrating the utility of MRD testing as a prognostic indicator in ALL. This analysis is treatment agnostic.

### Molecular Assessment in ALL

Assessment of the molecular response is conducted only after patients attain complete cytologic remission with  $\geq 1$  marker for MRD, and requires the availability of samples at various time points. Responses are categorized as follows:<sup>13</sup>

#### Molecular CR/complete MRD response/MRD negativity:

- Defined as the absence of MRD at a specific time point with an assay sensitivity of  $\geq 10^{-4}$

#### Molecular failure/MRD persistence:

- Defined as the persistent quantifiable presence of MRD with an assay sensitivity of  $\geq 10^{-4}$

#### Molecular relapse/MRD reappearance:

- Defined as reappearance of MRD within the quantitative range ( $> 10^{-4}$ ) after prior achievement of molecular CR

Method	Target	Sensitivity	Some potential benefits	Some potential limitations
Flow cytometry <sup>20,21,24</sup>	Leukemia-associated immunophenotypes	3–4 color: $10^{-3}$ to $10^{-4}$ (0.1–0.01%) 6–9 color: $10^{-4}$ to $10^{-5}$ (0.01–0.001%)	<ul style="list-style-type: none"> <li>Rapid</li> </ul>	<ul style="list-style-type: none"> <li>Limited sensitivity/standardization</li> <li>Difficult to interpret</li> </ul>
PCR <sup>20,21,24</sup>	<b>RT-qPCR:</b> Abnormal gene fusions (eg, <i>BCR-ABL</i> )	$10^{-4}$ to $10^{-5}$ (0.01–0.001%)	<ul style="list-style-type: none"> <li>High sensitivity</li> <li>Specific</li> </ul>	<ul style="list-style-type: none"> <li>Only possible in leukemias that harbor fusion transcripts</li> <li>Risk of cross contamination</li> </ul>
	<b>ASO-PCR:</b> Ig and TCR gene rearrangements		<ul style="list-style-type: none"> <li>High sensitivity</li> <li>Standardized</li> </ul>	<ul style="list-style-type: none"> <li>Time consuming</li> <li>Patient-specific primers needed</li> </ul>
NGS <sup>20,25</sup>	Ig and TCR gene rearrangements	$10^{-6}$ (0.0001%)	<ul style="list-style-type: none"> <li>High sensitivity</li> <li>No patient-specific primers required</li> <li>Available via reference lab</li> <li>Some are FDA-cleared<sup>26</sup></li> </ul>	<ul style="list-style-type: none"> <li>Turnaround time (~ 7 days)</li> </ul>

## MRD Summary and Clinical Implications

- MRD is a strong prognostic indicator in B-ALL and may help guide risk stratification and treatment planning.<sup>12,14</sup>
- NCCN Guidelines<sup>®</sup> recommend MRD assessment at the completion of induction therapy and at other time points, guided by the regimen used, which may be important to detect emergence of resistant proliferating clones early, facilitating clinical intervention prior to relapse.<sup>10</sup> Additionally, serial monitoring may be increased in patients with molecular relapse or persistent low-level disease burden.<sup>10</sup> NCCN Guidelines<sup>®</sup> recommend an initial small-volume ( $\leq 3$  mL) pull of bone marrow aspirate for MRD assessment to prevent hemodilution of the specimen.<sup>10</sup>
- Flow-based and NGS-based MRD detection methods have high correlation for  $\geq 10^{-4}$  leukemia burden, and providers may choose which works best in their practice.<sup>24</sup>
- The widespread adoption of MRD as a meaningful endpoint may be improved with further understanding of outcomes data across heterogeneous studies, treatments, and patients.<sup>14</sup> Improved understanding of the context-dependent prognostic power of MRD, with different implications for time point, prior therapy, and biologic risk group, may aid more universal incorporation of MRD into clinical practice.<sup>27</sup>

ALL, acute lymphoblastic leukemia; ASO-PCR, allele specific oligonucleotide PCR; B-ALL, B-cell acute lymphoblastic leukemia; BCI, Bayesian Credible Interval; *BCR-ABL*, breakpoint cluster region-Abelson gene fusion; CI, confidence interval; CR, complete remission; ECOG, Eastern Cooperative Oncology Group; EFS, event-free survival; FDA, Food and Drug Administration; HR, hazard ratio; IAMP21, intrachromosomal amplification of chromosome 21; Ig, immunoglobulin; *IKZF1*, IKAROS family zinc finger 1; *KMT2A*, lysine methyltransferase 2A; MFC, multiparameter flow cytometry; *MLL*, mixed-lineage leukemia; MRD, measurable/minimal residual disease; NGS, next-generation sequencing; OS, overall survival; PCR, polymerase chain reaction; Ph, Philadelphia chromosome; RT-qPCR, real-time quantitative PCR; TCR, T-cell receptor; WBC, white blood cell

References: 1. Terwilliger T, Abdul-Hay M. *Blood Cancer J*. 2017;7:e577. 2. Stock W. *Hematology Am Soc Hematol Educ Program*. 2010;2010:21–29. 3. Bonaventura A, et al. *Lancet Hematol*. 2017;4:e202–e217. 4. American Cancer Society. Key Statistics for Acute Lymphoblastic Leukemia (ALL). <https://www.cancer.org/cancer/acute-lymphocytic-leukemia/about/key-statistics.html>. Accessed June 16, 2021. 5. Jabbour E, et al. *Cancer*. 2015;121:2517–2528. 6. Dong Y, et al. *Exp Hematol Oncol*. 2020;19:4. 7. Chiarini F, et al. *Biochimica et Biophysica Acta*. 2016;1863:449–463. 8. Pui CH, et al. *N Engl J Med*. 2004;350:1535–1548. 9. Hoelzer D, et al. *Ann Oncol*. 2016;27:e69–e82. 10. NCCN makes no warranties of any kind whatsoever regarding its content, use or application and disclaims any responsibility for their application or use in any way. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines<sup>®</sup>) for Acute Lymphoblastic Leukemia V.1.2021. ©National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed June 17, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org. 11. Short NJ, et al. *Am J Hematol*. 2019;94:257–265. 12. Bassan R, et al. *Haematologica*. 2019;104:2029–2039. 13. Gökbüget N, et al. *Blood*. 2012;120:1868–1876. 14. Berry DA, et al. *JAMA Oncol*. 2017;3:e170580. 15. Bekjord K, et al. *Blood*. 2014;123:3739–3749. 16. Paietta E, et al. *Clin Lymphoma Myeloma Leuk*. 2015;15:52–56. 17. Mayfield E. Best Practices in MRD Quantification: The Importance of the First Bone Marrow Pull. <https://nucleus.asic.org/Full-Article/best-practices-in-mrd-quantification-the-importance-of-the-first-bone-marrow-pull>. Accessed June 17, 2021. 18. Caustan-Smith E, et al. *Blood*. 2002;100:2399–2402. 19. Sriyagaman M, et al. *Blood*. 2012;120:4470–4481. 20. Chen X, Wood B. *Best Pract Res Clin Haematol*. 2017;30:237–248. 21. van Dongen JJ, et al. *Blood*. 2015;125:3996–4009. 22. Kotrova M, et al. *Blood*. 2015;126:1045–1047. 23. Logan AC, et al. *Biol Blood Marrow Transplant*. 2014;20:1307–1313. 24. Schrappe M. *Hematology Am Soc Hematol Educ Program*. 2012;2012:137–142. 25. Thoi F, et al. *Genes Chromosomes Cancer*. 2012;51:689–695. 26. FDA Decision Summary for ClonSEQ. [https://www.accessdata.fda.gov/cdrh\\_docs\\_reviews/DEN170080.pdf](https://www.accessdata.fda.gov/cdrh_docs_reviews/DEN170080.pdf). Accessed June 17, 2021. 27. Brüggemann M, Kotrova M. *Blood Adv*. 2017;1:2456–2466.