MEASURABLE RESIDUAL DISEASE AND ACUTE LYMPHOBLASTIC LEUKEMIA

AMGEN® Oncology

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.[1]
- ALL is a heterogenous disease in terms of its pathology and the population it affects.[2] It is characterized by a nonspecific presentation consisting of a combination of constitutional symptoms and signs of bone marrow failure.[1]

ALL Epidemiology and Prognosis

- ALL is the most common form of leukemia in children and adolescents, accounting for up to 80% of cases.[3]
- ALL constituted 12.4% of all leukemia cases in 2017, with 64,200 cases recorded.[4]
- 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.[3]
- Although most cases of ALL occur in children, most deaths from ALL (about 4 out of 5) occur in adults.[5] Adults have higher relapse rates and poorer outcomes compared with children, with overall survival rates of approximately 20–40%.[6]

Global Number of ALL Cases Has Increased[4]

Immunophenotypes

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

High-Risk Factors for Adult ALL[9]

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

Disease-related:
- WBC (×10⁹/l): > 30 (B-ALL) / > 100 (T-ALL)
- Immunophenotype: pro-B/early or mature-T
- Extramedullary disease: central nervous system involvement

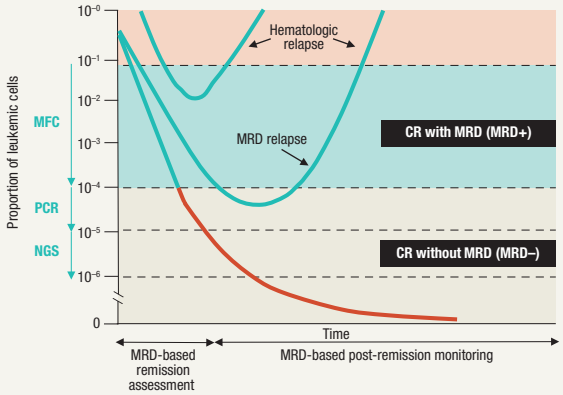
Cytogenetics and genetics:
- Ph+/t(4;11)+/other adverse
- *BCR-ABL1+*
- *MLL+*
- *PBX-E2A+*
- Ph-like
- *IKZF1* del
- *ETP*
- unmutated NOTCH1

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[8]

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

MRD is a Strong Prognostic Indicator in ALL[10]

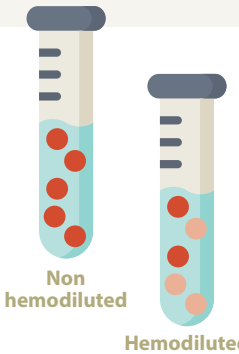


Measurable/Minimal Residual Disease (MRD)

- 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.[11,12]
- Studies collectively show the high prognostic value of MRD (both during and after initial induction therapy) in assessing relapse risk for patients with ALL.[13]
- 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.[12,14]
- ESMO Guidelines state the MRD is the most important prognostic factor in ALL and mandate MRD assessment by immunophenotype or molecular probe at diagnosis.[9] Following the diagnosis and induction, MRD should be monitored every 3 months using bone marrow aspiration.[9] MRD-positivity post-induction is considered to be a high-risk factor in adult ALL.[9]
- MRD assessment helps guide risk stratification and treatment planning in B-ALL.[11,13]

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.[15] Even a second small-volume pull from the same aspiration site reduces the number of leukemic cells by up to 50% due to hemodilution.[15]
- An initial pull of ≤ 3 mL of bone marrow aspirate is recommended as optimal for MRD quantification to avoid hemodilution of the specimen.[16]



MRD quantification is highly sensitive to hemodilution because hemodiluted samples may report an artificially low proportion of leukemic cells.[15]

Peripheral blood for MRD assessment is being investigated as a viable option in ALL, but additional studies are necessary to evaluate the utility of peripheral blood MRD monitoring.[10,17]

Methods for MRD Detection in ALL

Flow Cytometry

- Flow cytometry detects abnormal surface markers on leukemic cells.[18]
- While turnaround is rapid, interpretation of flow cytometry is challenging and can be difficult to standardize.[19]
- Immunostaining protocols, antibody panels, and gating strategies differ significantly between centers, leading to variability in results.[20]

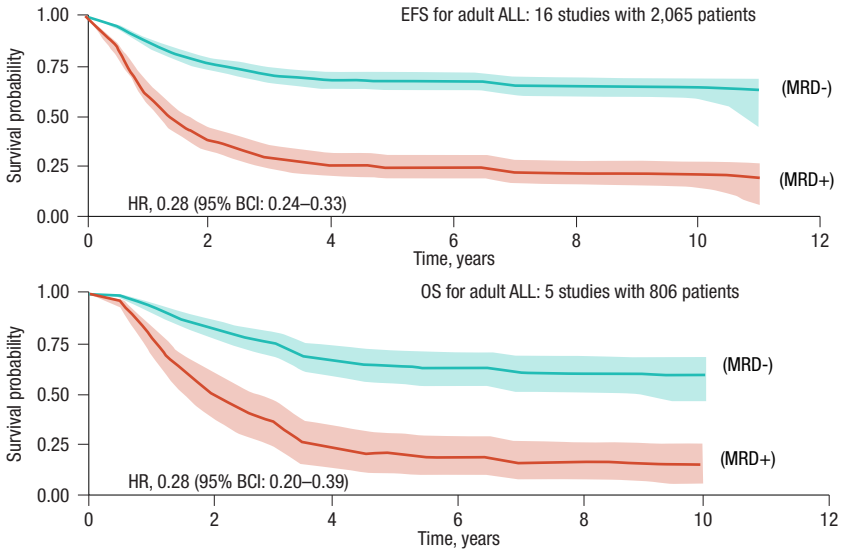
Polymerase Chain Reaction (PCR)

- PCR detects genetic abnormalities, such as fusion transcripts (eg, *BCR-ABL*), *Ig/TCR* gene rearrangements, or *MLL* gene rearrangements, using target sequences.[18]
- PCR for *Ig/TCR* is highly standardized and widely used in clinical trials in Europe.[19]
- However, PCR is laborious and expensive, and individual. rearrangements may be unstable over time.[21]

Next-Generation Sequencing (NGS)

- NGS detects leukemia-specific *IG/TCR* sequences.[19]
- 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.[19]
- NGS techniques may have sensitivity to below 10^{-6} to quantify ALL MRD in bone marrow or peripheral blood samples.[22]

In a Meta-analysis, Presence of MRD Tripled the Risk of Hematological Relapse or Death Over 10 Years[13]



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 ≥ 1 marker for MRD, and requires the availability of samples at various time points. Responses are categorized as follows:[12]

Molecular CR/complete MRD response/MRD negativity:

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

Molecular failure/MRD persistence:

- Defined as the persistent quantifiable presence of MRD with an assay sensitivity of ≥ 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[19,20,23]	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%*)	• Rapid	• Limited sensitivity/standardization • Difficult to interpret
PCR[19,20,23]	**RT-qPCR:** Abnormal gene fusions (eg, *BCR-ABL*)	10^{-4} to 10^{-5} (*0.01–0.001%*)	• High sensitivity • Specific	• Only possible in leukemias that harbor fusion transcripts • Risk of cross contamination
	ASO-PCR: Ig and TCR gene rearrangements		• High sensitivity • Standardized	• Time consuming • Patient-specific primers needed
NGS[19,24]	Ig and TCR gene rearrangements	10^{-6} (*0.0001%*)	• High sensitivity • No patient-specific primers required • Available via reference lab • Some are FDA-cleared[25]	• Turnaround time (~ 7 days)

MRD Summary and Clinical Implications

- MRD is a strong prognostic indicator in B-ALL and may help guide risk stratification and treatment planning.[11,13]
- MRD is the most important prognostic factor in ALL.[9] MRD-positivity post-induction is considered to be a high-risk factor in adult ALL.[9] An initial pull of ≤ 3 mL of bone marrow aspirate is recommended as optimal for MRD quantification to avoid hemodilution of the specimen.[16]
- Flow-based and NGS-based MRD detection methods have high correlation for ≥ 10^{-4} leukemia burden, and providers may choose which works best in their practice.[23]
- The widespread adoption of MRD as a meaningful endpoint may be improved with further understanding of outcomes data across heterogenous studies, treatments, and patients.[13] 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.[26]

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. Bonaventure A, et al. *Lancet Hematol.* 2017;4:e202–e217. 4. Dong Y, et al. *Exp Hematol Oncol.* 2020;19:4. 5. American Cancer Society. Key Statistics for Acute Lymphoblastic Leukemia (ALL). https://www.cancer.org/cancer/acute-lymphocytic-leukemia/about/key-statistics.html. Accessed 16 June 2021. 6. Jabbour E, et al. *Cancer.* 2015;121:2517–2528. 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:v69–v82. 10. Short NJ, et al. *Am J Hematol.* 2019;94:257–265. 11. Bassan R, et al. *Haematologica.* 2019;104:2028–2039. 12. Gökbuget N, et al. *Blood.* 2012;120:1868–1876. 13. Berry DA, et al. *JAMA Oncol.* 2017;3:e170580. 14. Beldjord K, et al. *Blood.* 2014;123:3739–3749. 15. Paietta E, et al. *Clin Lymphoma Myeloma Leuk.* 2015;15:S2–S6. 16. Helgestad J, et al. *Pediatr Blood Cancer.* 2011;57:224–226. 17. Coustan-Smith E, et al. *Blood.* 2002;100:2399–2402. 18. Brüggeman M, et al. *Blood.* 2012;120:4470–4481. 19. Chen X, Wood B. *Best Pract Res Clin Haematol.* 2017;30:237–248. 20. van Dongen JJ, et al. *Blood.* 2015;125:3996–4009. 21. Kotrova M, et al. *Blood.* 2015;126:1045–1047. 22. Logan AC, et al. *Biol Blood Marrow Transplant.* 2014;20:1307–1313. 23. Schrappe M. *Hematology Am Soc Hematol Educ Program.* 2012;2012:137–142. 24. Thol F, et al. *Genes Chromosomes Cancer.* 2012;51:689–695. 25. FDA Decision Summary for ClonoSEQ. https://www.accessdata.fda.gov/cdrh_docs_reviews/DEN170080.pdf. Accessed June 17, 2021. 26. Brüggemann M, Kotrova M. *Blood Adv.* 2017;1:2456–2466.

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