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Measurable residual disease monitoring after hematopoietic stem cell transplantation

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To the Editor:

Measurable residual disease (MRD) monitoring has become integral to post-transplant surveillance in hematologic malignancies, providing critical guidance for risk stratification and therapeutic decision-making following allogeneic hematopoietic stem cell transplantation (HSCT). Persistent MRD post-HSCT has a strong prognostic value, and early detection can inform timely therapeutic interventions [1]. Recent advances in technology and quality assurance have enhanced the precision and clinical applicability of MRD assessment.

MRD refers to the presence of residual leukemic cells below the detection threshold of conventional morphologic evaluation using microscopy, and its assessment is now standard in the management of hematologic malignancies including acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). MRD monitoring after allogeneic HSCT, however, differs in several important aspects from MRD assessment during conventional chemotherapy or pre-transplant evaluation. In the post-transplant setting, MRD must be interpreted in the context of donor–recipient chimerism, immune reconstitution, and the dynamic balance between graft-versus-leukemia and graft-versus-host disease, and the kinetics of MRD around engraftment and during immunosuppression tapering have distinct prognostic implications compared with non-transplant settings [2].

Optimal techniques for MRD monitoring should possess a sensitivity of at least 10^{-3}, meaning the ability to detect a single cancer cell among 1,000 normal cells. However, methods with higher sensitivity, ranging from 10^{-4} to 10^{-6}, are preferred in clinical practice to ensure reliable identification of residual disease and early recurrence. The ideal approach should be widely applicable across diverse patient populations, reproducible among different laboratories, and amenable to straightforward standardization protocols. Furthermore, rapid turnaround times for results are essential for timely clinical decision-making and intervention. Quantitative MRD assessment is preferred over qualitative methods.

MRD can be detected using several advanced technologies, each with distinct advantages and limitations (Table 1). Multiparameter flow cytometry (MFC) employs two main principal approaches for MRD detection: leukemia-associated aberrant immunophenotype (LAIP) and the different-from-normal (DfN) method [3]. MFC is widely applicable across various leukemia subtypes and rapidly provides results with sensitivity typically ranging from 10^{-3} to 10^{-4}; next-generation flow cytometry can further increase sensitivity up to 10^{-6}, although interpretative variability remains a challenge. In ALL, the EuroFlow Consortium has established a standardized operating procedure for flow cytometric MRD assessment in B-cell precursor ALL (BCP-ALL), employing two 8-color antibody tubes for comprehensive detection [4]. Their protocol enables staining and acquisition of large numbers of cells—exceeding 4 million per sample—achieving a sensitivity of 10^{-5} (0.001%) and ensuring applicability in over 98% of patients. This approach has set a benchmark for harmonized, high-sensitivity MRD detection in BCP-ALL.

Quantitative real-time PCR (qPCR) and digital PCR (dPCR) achieve high sensitivity—up to 10^{-5} or 10^{-6}—but are restricted to patients who have appropriate

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Table 1 Comparative characteristics of MRD technologies

Technology	Major target	Sensitivity	Advantages	Limitations
MFC	LAIP or DfN	$10^{-3} \sim 10^{-6}$	Universal, fast, wide applicability	Needs expertise, interpretative variability
qPCR/dPCR	Fusion transcripts or gene-specific mutations	$10^{-4} \sim 10^{-6}$	High sensitivity, fast	Limited applicability
NGS	SNV, fusion genes, or Ig/TCR gene rearrangement	$10^{-4} \sim 10^{-6}$	Wide applicability, scalability	Higher cost, longer TAT

Abbreviations: *MFC* multicolor flow cytometry, *LAIP* leukemia-associated aberrant immunophenotype, *DfN* different-from-normal, *qPCR* quantitative real-time polymerase chain reaction, *dPCR* digital polymerase chain reaction, *SNV* single nucleotide variant, *NGS* next-generation sequencing, *Ig* immunoglobulin, *TCR* T-cell receptor, *TAT* turnaround time

genetic markers detectable with these assays. In AML, reliable molecular MRD monitoring methods have been established using qPCR or dPCR assays targeting *PML::RARA*, core-binding factor (CBF) fusions—specifically, *RUNX1::RUNX1T1* and *CBFB::MYH11*—and *NPM1* mutations [3]. However, a significant limitation is that only 30–40% of AML patients are positive for these specific genetic markers. In contrast, for ALL, a patient-specific strategy is feasible, involving identification of the patient-specific immunoglobulin (Ig) or T-cell receptor (TCR) gene clonotype followed by the creation of unique primers for each patient to monitor MRD. This approach has the advantage of broad applicability, since most ALL patients harbor clonal Ig/TCR rearrangements. Nevertheless, it requires substantial time and labor to generate individualized assays and presents challenges for standardization.

As a result, next-generation sequencing (NGS)-based Ig/TCR assays—using universal standardized primers—are now more commonly employed for MRD monitoring in ALL, enabling streamlined workflows and robust reproducibility across different laboratories. The clonoSEQ assay (Adaptive Biotechnologies Inc, Seattle, WA, USA) has been FDA-cleared as an in vitro diagnostic test for detecting MRD in bone marrow samples from patients with B-ALL, providing a highly standardized and clinically validated method for MRD assessment in ALL [5]. In Korea, the NGS-based Ig/TCR gene clonality test was approved as a new medical technology in August 2020, and has been selectively reimbursed since January 2023. Currently, the test is being performed in the form of a laboratory-developed test (LDT) using LymphoTrack assay (InVivoScribe Technologies, San Diego, CA, USA) at hospital laboratories and reference centers [6].

NGS-based MRD monitoring in AML is highly versatile, enabling simultaneous analysis of gene mutations, fusion genes, and SNPs—making it valuable for cases with missing diagnostic samples and for tracking clonal evolution throughout disease course. This scalability supports both patient-specific panels, which target mutations detected at diagnosis but require custom assay development, and agnostic panel approaches, which use standardized gene panels applicable to all patients and facilitate rapid, cost-effective standardization [3]. Despite the genetic heterogeneity of AML, approximately 80–90% of patients have at least one mutation among 30–50 principal leukemogenic driver genes, supporting the broad applicability of agnostic panel-based monitoring approaches [7]. Moreover, even widely used MRD markers such as *NPM1* can be lost in a subset of relapsed patients, leading to false-negative MRD; therefore, multigene monitoring is increasingly favored to capture clonal changes [8]. Despite these technical strengths, standalone NGS-based MRD analysis in AML remains limited by a lack of consensus and robust clinical evidence regarding interpretation, cutoff thresholds, and selection of marker genes most predictive for relapse [3]. Challenges include distinguishing clonal hematopoiesis from true residual disease and contextualizing persistent mutations after therapy, requiring integrated diagnostic approaches and further standardization [9].

Discrepancies between MRD testing modalities frequently occur in the clinical monitoring of leukemias. These differences can arise from methodological sensitivity, sample quality, or from the distinct cellular or molecular phenomena being measured. For example, persistent *BCR::ABL1* RNA in Ph-positive ALL patients who are Ig/TCR MRD-negative may reflect clonal hematopoiesis rather than residual leukemic disease [10]. Studies have reported substantial discordance rates between MFC and NGS [11, 12]. These findings underscore the importance of combined and complementary MRD monitoring strategies, and highlight the need for careful interpretation—especially when results differ between methods—to enhance relapse prediction and optimize post-transplant management.

MRD monitoring after HSCT is essential for relapse prediction and guiding post-transplant interventions. In clinical practice, MRD is commonly assessed at predefined time points after allogeneic HSCT, such as around

neutrophil engraftment, at approximately day 30 and day 60–90, and at regular intervals thereafter, with additional testing triggered by clinical suspicion of relapse [13]. Serial measurements allow evaluation of MRD kinetics rather than single time-point results, and rising or reappearing MRD after initial post-transplant clearance is consistently associated with an increased risk of impending hematologic relapse [14]. Persistent or increasing MRD in this setting can guide risk-adapted preemptive strategies, including accelerated tapering or discontinuation of immunosuppression, donor lymphocyte infusion, and the use of targeted agents or hypomethylating agents as maintenance or preemptive therapy [15]. Multiparametric technologies—particularly when combined—now enable robust, sensitive, and widely applicable surveillance. Korean experience highlights the utility of NGS for both broad coverage and interlaboratory standardization, supporting the ongoing evolution of precision MRD monitoring and harmonized practice.

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SS wrote the main manuscript text.

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