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Review Article

Next-Generation Sequencing for Minimal Residual Disease Detection in AML: Current Technologies and Clinical Implications

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Abstract:
Minimal residual disease (MRD) has become a significant predictor of relapse and survival in acute myeloid leukemia (AML), indicating the extent of remission beyond traditional morphological evaluation. Although multicolor flow cytometry and quantitative PCR are essential methodologies in minimal residual disease identification, both are constrained by immunophenotypic variability, the necessity for stable molecular targets, and limited sensitivity. Advancements in next-generation sequencing (NGS) have revolutionized the minimal residual disease (MRD) field by enabling highly sensitive, mutation-driven identification of leukemic clones across a broad genomic landscape. Contemporary error-suppressed next-generation sequencing techniques—such as unique molecular identifiers, duplex sequencing, and single-molecule molecular inversion probes—have enhanced analytical sensitivity to the 10^{-5} to 10^{-6} range, enabling the detection of ultra-low-frequency variations with greater specificity. These techniques improve clinical risk classification, refine prognostication within genetically defined AML subtypes, and guide therapeutic options, including post-remission therapy, targeted inhibition, and the timing and intensity of allogeneic stem cell transplantation. Innovative applications, such as single-cell sequencing, cell-free DNA studies, and integrative multi-omic MRD evaluation, enhance the capabilities of genomics-based monitoring. Nonetheless, obstacles remain, such as differentiating cancer mutations from clonal hematopoiesis, standardizing analytical pipelines, establishing clinically relevant thresholds, and incorporating NGS MRD into standardized treatment protocols. This review encapsulates contemporary NGS methods for AML MRD diagnosis, assesses their clinical ramifications and constraints, and suggests future pathways necessary for comprehensive clinical integration. With advancements in the area, NGS-based MRD is set to become a pivotal element of precision-guided AML control.

Keywords: Minimal Residual Disease (MRD); Acute Myeloid Leukemia (AML); Next-Generation Sequencing (NGS); Error-Suppressed Sequencing; Clonal Hematopoiesis

Introduction

Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous hematologic malignancy characterized by the clonal expansion of aberrant myeloid progenitors. Its genomic landscape includes recurrent driver mutations, epigenetic alterations, and chromosomal abnormalities that shape disease phenotype, therapeutic response, and prognosis. Large-scale sequencing efforts have shown that AML evolves through a hierarchical architecture of founder and subclonal mutations, contributing to substantial inter- and intra-patient variability. This complexity underscores the need for refined biomarkers that can guide risk-adapted treatment strategies beyond conventional morphologic assessment [1,2].

Minimal residual disease (MRD)—the persistence of leukemic cells below the threshold of microscopic detection—has emerged as one of the strongest predictors of relapse and survival in AML. MRD positivity after induction or consolidation therapy correlates with significantly higher recurrence rates, inferior overall survival, and adverse post-transplant outcomes. Consequently, MRD assessment is increasingly incorporated into clinical trials, risk stratification systems, and transplant decision-making frameworks. Despite its importance, the optimal timing, methodology, and clinical interpretation of MRD detection remain subjects of active investigation [3,4].

Historically, MRD monitoring has relied on multiparameter flow cytometry (MFC) and quantitative PCR (qPCR). While both methods remain valuable, they have inherent limitations: MFC is affected by immunophenotypic variability and operator-dependent interpretation, and qPCR is restricted to a narrow set of stable, mutation-specific targets such as NPM1 or core-binding factor fusions. These constraints limit their ability to capture the full mutational diversity and clonal complexity that characterize modern AML biology [5].

The advent of next-generation sequencing (NGS) has transformed MRD assessment by enabling sensitive, mutation-informed detection across a broad range of genetic lesions. Error-corrected NGS technologies—including unique molecular identifiers, duplex sequencing, and single-molecule inversion probe methods—can identify ultra-low–frequency variants with high specificity, helping overcome the challenges associated with conventional techniques. As a result, NGS-based MRD has the potential to improve prognostication, refine post-remission risk assessment, and support more individualized therapeutic decision-making [6].

In this review, we summarize the molecular basis of MRD in AML, outline contemporary NGS platforms and analytical approaches, evaluate their clinical applications and limitations, and discuss emerging technologies poised to shape next-generation MRD monitoring. Together, these advances highlight the expanding role of NGS MRD as a cornerstone of precision AML management [7].

Molecular Basis of MRD in AML

Genetic Landscape of AML Relevant to MRD

The molecular heterogeneity of acute myeloid leukemia (AML) significantly affects disease persistence and relapse patterns, rendering genomic context crucial for interpreting minimal residual disease (MRD) [8]. Acute Myeloid Leukemia (AML) is propelled by recurrent somatic mutations that can be classified into several functional categories, including transcription factor fusions (e.g., RUNX1-RUNX1T1, CBFB-MYH11), mutations in signaling pathways (FLT3-ITD/TKD, KIT, RAS), alterations in epigenetic regulators (DNMT3A, TET2, IDH1/2, ASXL1), mutations in NPM1 exon-12, and genes that influence hematopoietic differentiation (CEBPA, RUNX1)[9]. Certain mutations among them serve as very informative MRD markers due to their stability and specificity to leukemia. NPM1 mutations occur in around one-third of AML cases and generally signify early clonal occurrences, rendering them suitable molecular anchors for MRD surveillance [10].

The mutational architecture of AML generally consists of two primary categories of lesions: initiating (founder) mutations and collaborating (progression) mutations. Initiating lesions, frequently associated with epigenetic modifiers like DNMT3A or TET2, create preleukemic clones that may endure despite the effective elimination of leukemic blasts [11]. Conversely, collaborating lesions like NPM1, FLT3-ITD, or IDH1/2 frequently propel leukemogenesis and delineate the predominant leukemic clone at the time of diagnosis. Due to their heightened specificity for leukemia and stronger correlation with disease activity, these cooperative mutations frequently represent the most clinically significant targets for MRD detection. Comprehending this hierarchy is crucial for understanding residual variations and distinguishing between persistent malignant clones and background clonal hematopoiesis [12].

Clonal Evolution and Implications for MRD Detection

The course and relapse of AML are influenced by dynamic clonal evolution, as selective forces from chemotherapy, targeted therapy, and the immunological microenvironment alter the composition of leukemic subclones. Founder mutations, usually associated with genes that govern epigenetic regulation or self-renewal, frequently endure despite treatment owing to their functional significance in initial clonal growth and their existence in preleukemic hematopoietic stem cells. The detection of these lesions in long-lived nonmalignant hematopoietic cells indicates that their persistence does not inherently denote active leukemia, presenting a significant challenge for MRD interpretation [13]. Acute myeloid leukemia (AML) is marked by dynamic clonal evolution in response to treatment pressure, where founder and subclonal mutations affect the probability of relapse (Figure 1).

Conversely, subclonal mutations that arise during leukemic transformation or illness development may serve as more sensitive indications of residual disease. This includes mutations obtained during clonal diversification—such as FLT3-ITD, RAS-pathway mutations, or other cooperative events—that may be eliminated with effective treatment or may re-emerge under therapeutic pressure. Relapse often arises from tiny subclonal populations that are present at diagnosis but undetectable by standard testing. High-sensitivity NGS technologies facilitate the identification of low-frequency subclones, enhancing relapse prediction and guiding therapy approaches[14].

A significant obstacle in NGS-based MRD evaluation is differentiating malignant clones from age-associated clonal hematopoiesis (CHIP). CHIP-related mutations, predominantly in DNMT3A, TET2, and ASXL1, may endure or even proliferate with chemotherapy; however frequently do not indicate actual leukemic burden. Their existence affects the interpretation of MRD data, especially when identified at low variant allele frequencies in remission samples. Advanced analytical methodologies, encompassing the integration of variant allele frequency dynamics, identification of co-occurring leukemic-specific mutations, and incorporation of immunophenotypic or clinical context, are crucial for distinguishing CHIP from MRD and preventing overtreatment [15].

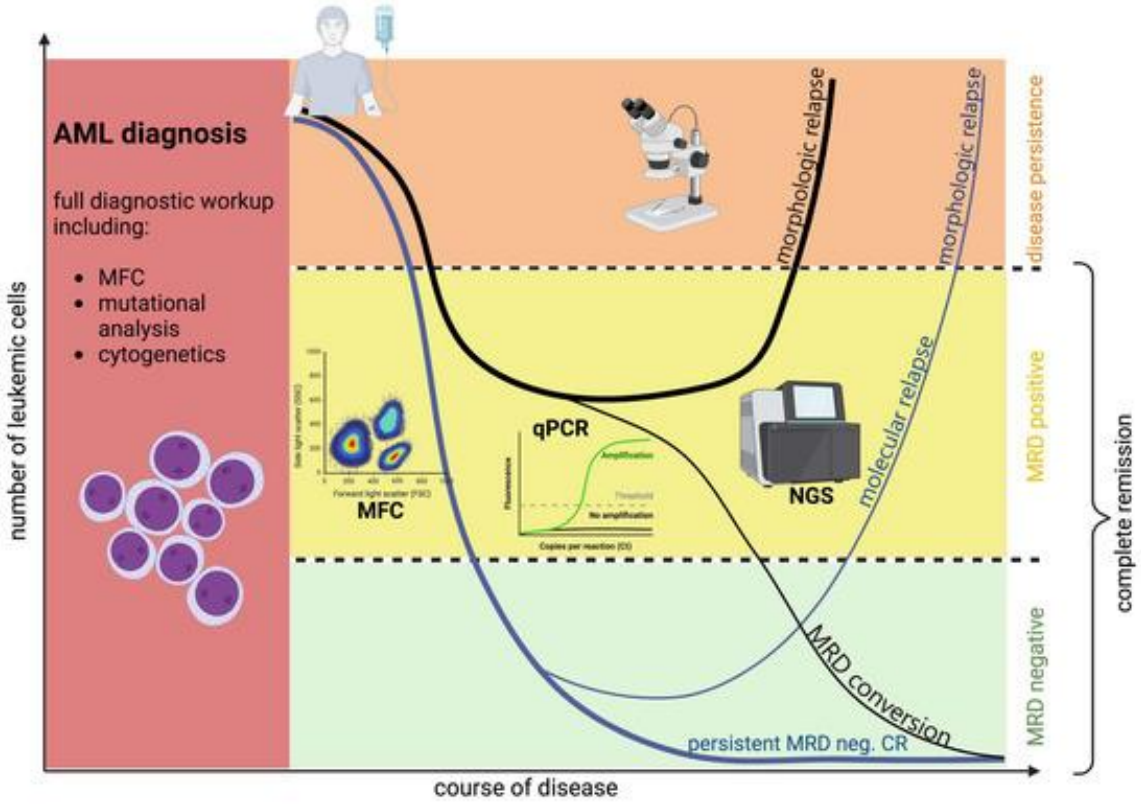

Figure 1. Overview of MRD detection in AML. (A) Clonal architecture showing founder and subclonal mutations and therapy-induced selection. (B) Sensitivity of MRD detection methods: MFC, qPCR/dPCR, targeted NGS, and error-corrected NGS. (C) Clinical integration: post-induction/consolidation, pre-/post-HSCT, targeted therapy monitoring, and relapse prediction. Adapted from Moritz J. et al., Biomedicines 2024;12(3):599.

Next-Generation Sequencing Platforms and Approaches for MRD Detection

Next-generation sequencing (NGS) has revolutionized MRD detection in acute myeloid leukemia (AML) by enabling sensitive, mutation-informed monitoring beyond the capabilities of conventional assays. Multiple NGS platforms and methodological approaches have been developed, each with distinct advantages, limitations, and clinical applications.

Amplicon-Based Targeted NGS

Amplicon-based NGS utilizes PCR amplification of specific genomic areas to identify mutations with high depth of coverage. Its principal advantages are rapid turnaround, substantial sequencing depth, and economic efficiency, facilitating the sensitive identification of low-frequency variations, generally ranging from 0.1% to 1% variant allele frequency (VAF). Amplicon methodologies are extensively employed for the identification of prevalent recurrent mutations, including NPM1, FLT3, and IDH1/2. Limitations encompass constrained genome coverage, vulnerability to PCR amplification bias, and diminished accuracy in GC-rich or intricate areas. Notwithstanding these limitations, amplicon-based NGS is exceptionally appropriate for clinical MRD surveillance in AML cases with well-defined mutations [16].

Hybrid Capture–Based Targeted NGS Panels

Hybrid capture panels employ probes to enhance genomic regions of interest before sequencing, thereby offering extensive coverage of mutation hotspots and structural variants. In contrast to amplicon-based techniques, hybrid capture allows for the concurrent analysis of several genes, hence enhancing the identification of coexisting subclonal mutations and structural variations. Performance characteristics generally attain sensitivities between 0.1% and 0.5% VAF in the absence of specialist error suppression. Although hybrid capture is slower and more expensive than amplicon methods, it provides greater flexibility and is especially advantageous for intricate AML genomes or research-focused MRD evaluation [17].

Error-Suppressed Sequencing Technologies

- Error-suppressed NGS techniques have developed to address technical constraints and attain ultra-sensitive MRD detection, achieving limits of 10^{-5}–10^{-6}. Essential tactics encompass:
- Unique Molecular Identifiers (UMIs): Labeling individual DNA molecules facilitates the distinction between genuine variations and sequencing mistakes.
- Duplex Sequencing (DS): Both strands of DNA are sequenced, facilitating error correction and nearly full eradication of false positives.
- Single-Molecule Molecular Inversion Probes (smMIPs) provide precise and efficient targeted sequencing of certain genomic regions.

These techniques are especially beneficial for tracking low-burden subclones and post-transplant minimal residual disease (MRD), when conventional next-generation sequencing (NGS) fails to provide adequate sensitivity [18,19].

Whole-Exome and Whole-Genome Sequencing

Whole-exome (WES) and whole-genome sequencing (WGS) provide comprehensive mutation detection across the coding genome or entire genome, respectively. While invaluable for research into clonal evolution and the discovery of novel driver mutations, their clinical utility for MRD is limited. The primary constraints are lower depth of coverage, high cost, extended turnaround times, and insufficient sensitivity for low-frequency variants (<1%). Consequently, WES/WGS are currently complementary to targeted NGS in MRD studies rather than routine clinical tools [20].

RNA-Based Sequencing Methods

RNA sequencing facilitates the identification of fusion transcripts, alternative splicing events, and variations in expression levels linked to leukemic clones. It is especially pertinent for the surveillance of NPM1-mutated acute myeloid leukemia, KMT2A fusions, and other transcript-based anomalies. RNA-based methodologies provide the benefit of identifying disease-specific transcripts in the absence of genomic alterations; nevertheless, RNA degradation and fluctuating expression levels may constrain sensitivity and repeatability [21].

3.6 Bioinformatic Pipelines for Ultra-Low–Frequency Variant Detection

Precise MRD evaluation depends on advanced computational techniques to differentiate genuine variants from sequencing artifacts and clonal hematopoiesis. Essential factors to consider are:

- Error Modeling: Quantitative frameworks to address sequencing and PCR inaccuracies.
- Variant Filtering: Approaches to eliminate technological artifacts while preserving authentic low-frequency mutations.
- CHIP Discrimination: Incorporation of longitudinal VAF trends and co-mutation

patterns to differentiate pre-leukemic clones from malignant minimal residual disease (MRD).

- Standardization Challenges: Variations in pipelines, variant calling thresholds, and reporting formats hinder cross-study comparability, underscoring the necessity for unified bioinformatic standards.

This collection of NGS platforms and methodologies offers a range of choices for MRD detection, encompassing targeted, clinically relevant tests and extensive research-oriented analysis. The selection of a platform is contingent upon the type of mutation, required sensitivity, sample type, and clinical context, underscoring the necessity of incorporating both technical and biological factors in NGS-based AML MRD monitoring [18,22].

Several NGS platforms are available for MRD monitoring, each with distinct sensitivity and coverage characteristics (Table 1)."

Taken together, these NGS approaches form a graduated spectrum of depth, breadth, and sensitivity. Amplicon sequencing and hybrid-capture panels are the most clinically mature and widely implemented modalities, whereas UMI- and duplex-based error-suppressed methods provide the greatest analytical sensitivity but currently face practical challenges related to cost, turnaround time, and workflow complexity. In contrast, whole-exome and whole-genome sequencing remain primarily research tools due to insufficient depth for low-level MRD. Recognizing how these approaches complement one another helps contextualize their role in clinical MRD monitoring.

Table 1. Comparison of MRD Detection Modalities in AML

Modality	Sensitivity	Coverage	Turnaround	Strengths	Limitations	Typical Clinical Use
NGS (amplicon-based)	0.1–1% VAF	Selected recurrent genes	Fast	Cost-effective, high depth	Limited gene panel, PCR bias	Routine MRD for NPM1, FLT3, IDH1/2
NGS (hybrid capture)	0.1–0.5% VAF	Broad panel (50–500 genes)	Moderate	Captures subclones & structural variants	Higher cost, longer prep	Complex AML genomes, research
Error-suppressed NGS (UMI/DS/smMIP)	10^{-5}–10^{-6}	Targeted	Slow	Ultra-sensitive, tracks rare subclones	Expensive, complex workflow	Post-transplant MRD, early relapse prediction
Flow Cytometry (MFC)	0.01–0.1%	Immunophenotype	Fast	Widely available, real-time	Immunophenotypic drift, operator-dependent	Routine clinical MRD
qPCR / Digital PCR	10^{-5}–10^{-6}	Specific mutations/fusions	Fast	High sensitivity, quantitative	Limited to defined targets	NPM1, RUNX1-RUNX1T1, CBFB-MYH11

Analytical Performance and Standardization Challenges

Despite the transformative potential of next-generation sequencing (NGS) for MRD detection in AML, several analytical and standardization considerations must be addressed to ensure clinical reliability and reproducibility. These factors determine assay sensitivity, interpretability, and comparability across laboratories, which are essential for integrating NGS MRD into routine clinical practice.

Sensitivity, Specificity, and Reproducibility

NGS-based MRD detection exhibits exceptional analytical sensitivity, especially when integrated with error-suppression techniques, frequently exceeding 0.01% variant allele frequency (VAF) in optimized

procedures. In contrast to multiparameter flow cytometry (MFC), which generally identifies leukemic cells at 0.1–0.01%, next-generation sequencing (NGS) offers extensive coverage of mutational targets and can find subclonal variants overlooked by immunophenotypic methods. Quantitative PCR (qPCR) is the benchmark for detecting highly recurring mutations like NPM1, with sensitivities reaching as low as 10^{-5}–10^{-6}; yet, qPCR is restricted to particular targets. NGS integrates the extensive capabilities of MFC with near-qPCR sensitivity for specific mutations, providing consistent detection across longitudinal samples. Inter-assay repeatability may fluctuate based on library preparation, sequencing depth, error-correction techniques, and bioinformatic workflows [19].

Further strengthening of NGS MRD will require coordinated multi-institutional validation efforts to ensure reproducibility across platforms and laboratories. The development of standardized reference materials and clinically calibrated control samples is especially important for evaluating assay sensitivity and error-suppression performance. Large, prospective studies comparing NGS MRD against established clinical outcomes will be essential to define the analytical robustness required for routine implementation.

Limit of Detection and Clinical Threshold Settings

The clinical interpretation of NGS MRD necessitates the establishment of thresholds that forecast relapse risk. Numerous studies demonstrate that persistent mutations exceeding 0.1–1% VAF following induction or consolidation correlate with markedly elevated recurrence rates, while the thresholds are individual to each mutation and affected by the illness setting. Ultra-sensitive detection at levels of 10^{-5}–10^{-6} can identify developing subclones months prior to clinical relapse, providing a potential opportunity for preventative intervention. Nonetheless, establishing clinically actionable thresholds is difficult due to the persistence of pre-leukemic clones or clonal hematopoiesis, which may not lead to manifest illness [23].

Across contemporary clinical trials, MRD thresholds vary by mutation type and treatment phase, but several patterns have become consistently recognized. For NPM1-mutated AML, many ELN- and trial-based protocols define a threshold of approximately 10^{-3} (0.1% NPM1 copies/ABL1 or <1% VAF) after induction as clinically significant, with deeper thresholds of 10^{-4}–10^{-5} associated with improved relapse-free survival following consolidation [10,26]. In contrast, FLT3-ITD is typically monitored using a VAF-based cutoff of *0.01–0.05%* in post-remission settings, with persistent or rising FLT3-mutant clones above these ranges correlating with early relapse and poorer transplant outcomes [14,23]. For fusion transcripts such as RUNX1-RUNX1T1 and CBFB-MYH11, qPCR-anchored trial frameworks often use ≥1-log increase from prior nadir or transcript levels >10^{-3} as high-risk markers [3,26]. Consolidating these widely referenced cutoffs highlights the ongoing need for standardized thresholds but also provides practical guidance for interpreting MRD dynamics across commonly monitored AML genotypes.

Pre-Analytical Variables

The type of sample and the timing of collection substantially affect MRD detection. Bone marrow aspirates offer superior sensitivity due to the concentration of leukemic cells compared to peripheral blood; however, serial blood-based monitoring is less intrusive and may detect circulating subclones. The ideal timing for MRD evaluation differs according to treatment phase: post-induction, post-consolidation, and pre-transplant assessments are pivotal points for prognostication and therapy decisions. Inconsistent or delayed sampling may result in the underestimation of residual disease and jeopardize clinical interpretation [24].

Inter-Laboratory Variability and Need for Harmonization

Standardization among laboratories is crucial for the integration of NGS MRD into clinical practice. Variability stems from discrepancies in sequencing platforms, library preparation techniques, bioinformatic workflows, and reporting standards. The implementation of reporting systems, including defined variant allele frequency thresholds and minimal residual disease positive criteria, is essential for comparison. The utilization of reference standards, such as synthetic DNA controls and thoroughly defined clinical samples, enhances test validation and external quality assessment. Collaborative efforts among multiple institutions are in progress to develop standardized protocols, enhance quality assurance, and minimize discrepancies in MRD reporting, which will be essential for regulatory endorsement and clinical implementation [25].

Synopsis: The analytical performance and standardization are pivotal to the clinical applicability of NGS MRD. Although high sensitivity, specificity, and repeatability establish NGS as an exceptional method for AML MRD diagnosis, meticulous attention to clinical thresholds, sample management, and standardized processes is essential to guarantee precise, interpretable, and similar outcomes across institutions.

Overall, much of the perceived complexity surrounding NGS MRD reflects practical barriers such

as laboratory workflow variability, cost, and turnaround time rather than theoretical limitations of the sequencing technologies themselves. Continued harmonization efforts, supported by standardized bioinformatic pipelines and reference materials, will be essential to ensure that clinically mature NGS approaches can be deployed consistently across institutions."

Clinical Utility of NGS-Based MRD in AML

NGS-based minimal residual disease (MRD) assessment has rapidly emerged as a transformative tool in acute myeloid leukemia (AML), offering prognostic precision and guiding therapeutic decision-making. Its clinical utility spans risk stratification, post-remission management, monitoring of targeted therapy response, and early relapse prediction.

Prognostic Value Across AML Subtypes

The prognostic significance of NGS MRD differs among genetically categorized AML subsets:

- NPM1-mutated acute myeloid leukemia (AML): The retention of NPM1-mutated clones during induction or consolidation significantly forecasts relapse and decreased survival outcomes. Next-generation sequencing facilitates the precise identification of remaining NPM1 mutations, even at ultra-low frequencies ($<10^{-4}$), enhancing post-remission risk assessment [7].
- Core-binding factor acute myeloid leukemia (CBF-AML): Minimal residual disease (MRD) detection with next-generation sequencing (NGS) targeting RUNX1-RUNX1T1 or CBFB-MYH11 fusions is associated with recurrence risk, enhancing quantitative polymerase chain reaction (qPCR)-based tests and offering insights into subclonal evolution.
- FLT3-mutated AML: Due to the significant recurrence tendency of FLT3-ITD–positive AML, NGS MRD can identify persistent or developing FLT3 subclones that may require preemptive intervention or intensification of therapy.
- Secondary and therapy-related AML: Monitoring minimal residual disease (MRD) in these genetically intricate subtypes poses challenges; however, next-generation sequencing (NGS) for detecting cooperating mutations or enduring founder clones has shown prognostic significance, identifying patients at heightened risk of relapse despite morphologic remission [26].

Common driver and cooperating mutations vary in their suitability for MRD monitoring (Table 2)."

Table 2. Mutation-Specific MRD Relevance in AML

Mutation / Alteration	MRD Suitability	Prognostic Value	Recommended MRD Method	Notes
NPM1	High	Strong predictor of relapse	NGS, qPCR	Stable founder mutation; widely used in trials
FLT3-ITD / FLT3-TKD	Moderate	High relapse risk	NGS, dPCR	Subclonal dynamics are important; they may guide targeted therapy
DNMT3A	Low	Often, CHIP has limited predictive value	NGS	Pre-leukemic clone; persistent post-therapy
RUNX1-RUNX1T1	High	Predicts relapse in CBF-AML	qPCR, RNA-seq, NGS	Fusion transcript allows sensitive monitoring
CBFB-MYH11	High	Prognostic in CBF-AML	qPCR, RNA-seq, NGS	Strong MRD marker post-consolidation
IDH1/2	Moderate	Guides targeted therapy	NGS	Useful for therapy monitoring; may emerge as resistance
TET2 / ASXL1	Low	CHIP; limited MRD utility	NGS	Not recommended as sole MRD marker

Guiding Treatment Decisions

NGS MRD informs multiple therapeutic decisions:

- Risk stratification following induction and consolidation: MRD-positive status indicates patients at high risk of relapse despite achieving morphologic remission,

informing intensification methods or the consideration of early allogeneic hematopoietic stem cell transplantation (HSCT).

- **Optimization of HSCT:**
- Pre-transplant minimal residual disease (MRD) forecasts relapse and post-transplant survival. Patients exhibiting detectable minimal residual disease frequently derive advantages from intensified conditioning or supplementary bridging therapy.
- Post-transplant minimal residual disease (MRD) facilitates early management by donor lymphocyte infusions (DLI), immunomodulation, or targeted treatments, potentially averting overt recurrence and enhancing long-term outcomes [19,27].

An additional scenario in which NGS MRD is increasingly relevant is the management of older or unfit patients treated with hypomethylating agents combined with venetoclax. Early studies suggest that persistent MRD—particularly NPM1 or FLT3-mutant clones—after several treatment cycles correlates with early relapse, while deep remissions may help identify patients who can transition to lower-intensity maintenance strategies. As HMA/ven regimens become standard of care in this population, NGS MRD may aid in treatment tailoring and in determining duration of therapy.

Monitoring Response to Targeted Therapies

NGS MRD facilitates dynamic monitoring of disease burden during targeted therapy:

- **FLT3 inhibitors:** Detection of emerging FLT3-mutant subclones can indicate resistance or incomplete response, informing dose adjustment or combination strategies.
- **IDH1/2 inhibitors:** MRD tracking can guide treatment continuation or escalation, particularly in patients with low-burden residual disease.
- **Menin inhibitors:** For MLL-rearranged or NPM1-mutated AML, NGS MRD allows early identification of therapeutic escape and supports adaptive treatment strategies.

Early Relapse Detection and Prediction

NGS MRD offers a significant lead-time advantage over conventional flow cytometry by detecting ultra-low–frequency leukemic clones months before clinical relapse. Integration of NGS MRD into longitudinal surveillance enables:

- Timely preemptive interventions to prevent hematologic relapse.
- Identification of clonal evolution and emergent resistance mutations.
- Personalized monitoring schedules based on mutational profile, treatment intensity, and transplant status [28].

Summary: NGS MRD provides robust prognostic information, informs treatment decisions across AML subtypes, guides post-transplant management, and enables early detection of relapse. Its integration into routine clinical practice promises a precision-guided approach to AML therapy, improving risk-adapted outcomes and optimizing the use of targeted interventions.

Comparisons with Other MRD Modalities

Multiple modalities are employed for minimal residual disease (MRD) detection in AML, each with distinct advantages, limitations, and clinical applicability. Understanding these differences is essential for selecting the optimal method or integrating complementary approaches.

Multicolor Flow Cytometry (MFC)

MFC identifies anomalous immunophenotypic patterns in leukemic blasts, facilitating MRD evaluation in most AML patients. Its advantages encompass swift execution, extensive applicability, and the capacity to identify phenotypically characterized leukemic populations irrespective of particular mutations. MFC generally attains sensitivities of 0.01–0.1%, comparable to conventional NGS for high-burden diseases. Nonetheless, it possesses significant limitations compared to NGS: immunophenotypic plasticity and therapy-induced antigen modulation can obscure leukemic cells, inter-laboratory reproducibility is problematic, and it exhibits reduced sensitivity for identifying rare subclones or differentiating pre-leukemic from malignant populations [29].

Quantitative PCR (qPCR) and Digital PCR (dPCR)

qPCR and dPCR have exceptional sensitivity for minimal residual disease (MRD) identification, achieving limits of 10^{-5} to 10^{-6} for recurrent, well-defined targets, including NPM1 mutations and fusion transcripts (RUNX1-RUNX1T1, CBFB-MYH11). These methodologies provide accurate quantification and defined prognostic thresholds, although they are limited to patients possessing particular genetic mutations. Furthermore, qPCR necessitates standard curves and meticulous calibration, whereas dPCR provides absolute quantification with enhanced repeatability and less technical variability. In

comparison to NGS, these PCR-based techniques exhibit great sensitivity but are limited in their application for heterogeneous or subclonal AML [3,30].

Integrated MRD Assessment

The integration of NGS with MFC or PCR harnesses the synergistic advantages of each technique, facilitating a more thorough assessment of MRD. Integrated methodologies can identify residual disease overlooked by a singular technique, resolve inconsistencies between molecular and immunophenotypic minimal residual disease, and offer comprehensive risk classification. Algorithms integrating genetic and immunophenotypic data are progressively utilized in clinical trials to inform therapy intensification, transplantation decisions, and post-remission surveillance. This multi-modal MRD assessment improves sensitivity, specificity, and prognosis accuracy, establishing a basis for tailored, MRD-focused AML therapy.

Synopsis: Although MFC and PCR-based techniques are important for MRD detection, NGS provides extensive genome coverage, enhanced sensitivity for subclonal populations, and the capability to track dynamic clonal evolution. The integration of various MRD modalities offers a synergistic strategy, enhancing detection accuracy and guiding precision-targeted treatment actions [31].

Emerging Technologies and Evolving Concepts

Advances in molecular profiling and computational biology are expanding the landscape of minimal residual disease (MRD) monitoring in AML. Novel technologies promise to enhance sensitivity, capture clonal complexity, and enable predictive modeling that may transform personalized disease management.

Single-Cell NGS for Clonal Mapping

Single-cell sequencing facilitates high-resolution characterization of individual leukemic cells, permitting the discovery of rare subclones that may resist treatment. Single-cell NGS elucidates the hierarchical and temporal evolution of AML clones by reconstructing clonal architecture, thereby identifying which subclones are predisposed to induce relapse. This method enables accurate MRD tracking, guiding focused intervention tactics and providing the capability to observe therapy-induced clonal selection with exceptional resolution [32].

Cell-Free DNA for MRD Monitoring

Circulating tumor DNA (ctDNA) in peripheral blood represents a non-invasive biomarker for MRD. Early studies demonstrate that ctDNA can detect residual leukemic clones, including those undetectable by bone marrow sampling, and may provide a lead-time advantage for relapse prediction. While challenges remain—including assay sensitivity, standardization, and distinguishing malignant DNA from clonal hematopoiesis—cell-free DNA offers a promising avenue for serial, minimally invasive MRD surveillance, particularly in patients unsuitable for frequent bone marrow assessments [33].

Multi-Omic MRD Strategies

Integrative multi-omic approaches combine genomic, epigenomic, transcriptomic, and proteomic data to enhance MRD detection and biological interpretation. Epigenetic MRD assessment, such as methylation profiling, can detect residual leukemic signatures that persist even in the absence of detectable mutations. Proteogenomic strategies leverage aberrant protein expression alongside mutational data, providing functional context to MRD and potentially identifying therapy-responsive or resistant subclones. Such multi-dimensional analyses have the potential to capture residual disease comprehensively and improve relapse risk stratification [34].

Although these emerging approaches offer unprecedented resolution and biological insight, each will require rigorous clinical-grade validation before adoption. Benchmarking single-cell sequencing, ctDNA-based MRD, and multi-omic assays against existing NGS and PCR modalities will be crucial to determine their sensitivity, reproducibility, and real-world clinical value. Current barriers, including cost, limited input material for ctDNA, and computational complexity, must be addressed for these technologies to advance toward standardized MRD testing.

AI and Machine Learning in MRD Prediction

Artificial intelligence (AI) and machine learning (ML) are progressively utilized in MRD data to improve predicted precision and assimilate intricate longitudinal information. Algorithms can simulate variation dynamics, identify early indicators of recurrence, and categorize patients according to individualized risk profiles. AI-driven analysis of serial NGS data may differentiate benign clonal hematopoiesis from clinically significant minimal residual disease and enhance the timing of treatment therapies. These computational tools are prepared to enhance precision-guided AML management and inform adaptive treatment regimens [35].

Synopsis: Emerging technologies, such as single-cell NGS, cell-free DNA assays, multi-omic integration, and AI-driven analytics, present unparalleled prospects to enhance MRD diagnosis, comprehend clonal dynamics, and predict relapse. As

these methodologies advance, they are expected to enhance and, in certain scenarios, exceed traditional MRD monitoring, propelling the subsequent evolution of precision AML treatment.

Regulatory, Economic, and Implementation Considerations

The clinical integration of next-generation sequencing (NGS) for minimal residual disease (MRD) monitoring in AML requires consideration of regulatory frameworks, cost-effectiveness, and operational feasibility. These factors are critical to ensure that NGS MRD assays can be reliably adopted in routine clinical practice and produce actionable results for patient management.

Clinical Adoption and Regulatory Landscape

Notwithstanding the increasing evidence endorsing NGS MRD as a predictive and therapeutic instrument, regulatory approvals are still constrained. Current clinical guidelines from organizations like the European LeukemiaNet (ELN) and the National Comprehensive Cancer Network (NCCN) recognize minimal residual disease (MRD) as a vital biomarker; however, they have yet to offer standardized recommendations for next-generation sequencing (NGS)-based MRD assessment. Most commercial and laboratory-developed tests function under CLIA/CAP accreditation, with continuous initiatives to standardize reporting, establish clinically relevant thresholds, and provide reference materials. Regulatory deficiencies encompass the standardization of test sensitivity, repeatability criteria, and explicit directives for incorporating NGS MRD outcomes into therapeutic protocols [22].

Cost-Effectiveness and Health Economics

The adoption of NGS MRD incurs more initial expenses compared to traditional flow cytometry or PCR-based techniques, attributed to sequencing, bioinformatic analysis, and staffing needs. Nevertheless, numerous studies indicate that the capacity of NGS MRD to stratify patients, avert recurrence, and direct tailored therapy may counterbalance these expenses by decreasing hospitalizations, optimizing transplant timing, and circumventing inefficient medications. Cost-effectiveness assessments are increasing, especially in high-risk AML or pre-transplant contexts involving NGS MRD; nonetheless, thorough health economic evaluations are scarce and crucial for widespread implementation [36].

Laboratory Workflow Integration

Operational factors are crucial for the effective clinical deployment of NGS MRD. The turnaround time must correspond with therapeutic decision milestones, specifically during induction, after consolidation, and before transplantation. Efficient sample processing, library preparation, and sequencing workflows are essential, combined with a robust bioinformatic infrastructure capable of detecting ultra-low-frequency variants. Staff must be proficient in molecular methodologies, quality assurance, and data analysis. The standardization of reporting, encompassing variant allele frequencies, MRD positive thresholds, and longitudinal comparisons, is essential for maintaining consistency among institutions. Incorporating MRD results into clinical processes necessitates collaboration with treating physicians to guarantee that these results facilitate prompt therapeutic decisions [37].

Summary: Although NGS MRD possesses transformative potential for AML control, regulatory clarity, cost-effectiveness, and optimized laboratory procedures are essential for its wider implementation. Addressing these problems would guarantee the reliable deployment of NGS MRD to inform tailored treatment regimens and enhance patient outcomes.

Future Directions

Next-generation sequencing (NGS)–based minimal residual disease (MRD) monitoring has demonstrated transformative potential in acute myeloid leukemia (AML), yet several critical areas require further development to realize its full clinical impact.

Harmonized Guidelines and Standardization

The lack of standardized methodologies and reporting frameworks remains a major barrier to clinical adoption. Future efforts must focus on developing harmonized guidelines that define assay performance, limit of detection, and criteria for MRD positivity. Consensus standards will facilitate inter-laboratory comparability, enhance regulatory oversight, and allow reliable integration of NGS MRD into treatment algorithms.

Standardized MRD Thresholds for Intervention

Clinically actionable MRD thresholds are essential to guide therapeutic decisions, including intensification, transplant timing, or preemptive targeted therapy. Large-scale studies are needed to establish mutation-specific and treatment-phase–specific thresholds that reliably predict relapse while minimizing unnecessary interventions [38].

Prospective Clinical Trials Incorporating NGS MRD

Prospective, multi-center trials that integrate NGS MRD as a stratification and response biomarker are critical. Such studies will evaluate the predictive power of NGS MRD for relapse, validate its utility in guiding therapy, and determine the impact of MRD-directed interventions on overall survival and quality of life.

Personalized MRD-Driven Treatment Algorithms

Future clinical practice will increasingly rely on MRD-driven precision strategies. Integration of NGS MRD with genomic, immunophenotypic, and clinical data will enable adaptive treatment algorithms tailored to individual patient risk and clonal dynamics. This approach holds the potential to optimize therapy, minimize toxicity, and improve long-term outcomes in AML.

Summary: The next frontier in AML management involves translating NGS MRD from a prognostic biomarker into a cornerstone of personalized therapy. Achieving this goal requires standardized guidelines, validated thresholds, prospective clinical evidence, and incorporation of MRD into precision-driven treatment strategies.

Conclusions

Next-generation sequencing (NGS) has become an effective instrument for identifying minimal residual disease (MRD) in acute myeloid leukemia (AML), providing exceptional sensitivity, specificity, and the capacity to detect subclonal and changing leukemic populations. Increasing data indicate that NGS MRD offers substantial prognostic insights across many AML subtypes, informs risk-adjusted treatment choices, directs transplant approaches, and facilitates early identification of potential relapse.

In contrast to conventional methods like flow cytometry and PCR, NGS provides extensive genome coverage, improved identification of rare subclones, and the ability to monitor clonal change over time. Emerging technologies, such as single-cell sequencing, cell-free DNA assays, multi-omic profiling, and AI-driven analytics, are poised to enhance MRD monitoring and incorporate dynamic molecular data into precision-guided therapy.

Notwithstanding these advancements, obstacles persist, including assay standardization, identification of clinically meaningful thresholds, harmonization of reporting frameworks, and incorporation into regular clinical processes. Future clinical trials and consensus recommendations will be crucial to fully harness the potential of NGS MRD.

In summary, NGS-based MRD evaluation is set to become fundamental in AML management, facilitating individualized, risk-adjusted approaches that refine relapse prediction, direct focused interventions, and ultimately improve patient outcomes. Its incorporation into regular practice signifies a pivotal advancement toward precision treatment in AML. As these technologies evolve, comprehensive clinical validation, harmonized analytical criteria, and standardized reporting frameworks will be necessary to support their transition from high-resolution research tools to routine components of MRD-guided AML therapy.

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References

1. Vakiti A, Reynolds SB, Mewawalla P. Acute Myeloid Leukemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2025 Nov 14]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507875/
2. Naji NS, Sathish M, Karantanos T. Inflammation and Related Signaling Pathways in Acute Myeloid Leukemia. Cancers

[Internet]. 2024 Nov 26 [cited 2025 Nov 14];16(23):3974. Available from: https://doi.org/10.3390/cancers16233974

3. Moritz J, Schwab A, Reinisch A, Zebisch A, Sill H, Wölfler A, et al. Measurable Residual Disease Detection in Acute Myeloid Leukemia: Current Challenges and Future Directions. Biomedicines [Internet]. 2024 Mar 6 [cited 2025 Nov 14];12(3):599. Available from: https://doi.org/10.3390/biomedicines12030599
4. Song M, Pan W, Yu X, Ren J, Tang C, Chen Z, et al. Minimal Residual Disease Detection: Implications for Clinical Diagnosis and Cancer Patient Treatment. MedComm (2020) [Internet]. 2025 May 15 [cited 2025 Nov 14];6(6):e70193. Available from: https://doi.org/10.1002/mco2.70193
5. Thakur A, Weis T. Measurable residual disease monitoring in acute myeloid leukaemia: Techniques, timing and therapeutic implications. Semin Hematol [Internet]. 2025 Jun 1 [cited 2025 Nov 14];62(3):167–76. Available from: https://doi.org/10.1053/j.seminhematol.2024.12.001
6. Ștefan AI, Radu LE, Jardan D, Coliță A. The emerging role of next-generation sequencing in minimal residual disease assessment in acute lymphoblastic leukemia: a systematic review of current literature. Front Med (Lausanne) [Internet]. 2025 [cited 2025 Nov 14];12:1570041. Available from: https://doi.org/10.3389/fmed.2025.1570041
7. Liu Y, Cheng H, Sun M, Gong T, Ma J. The clinical utility and prognostic value of next-generation sequencing for measurable residual disease assessment in acute myeloid leukemia. Ther Adv Hematol [Internet]. 2025 Jun 26 [cited 2025 Nov 14];16:20406207251349261. Available from: https://doi.org/10.1177/20406207251349261
8. Shen Q, Gong X, Feng Y, Hu Y, Wang T, Yan W, et al. Measurable residual disease (MRD)-testing in haematological cancers: A giant leap forward or sideways? Blood Rev [Internet]. 2024 Nov 1 [cited 2025 Nov 14];68:101226. Available from: https://doi.org/10.1016/j.blre.2024.101226
9. Álvarez N, Martín A, Dorado S, Colmenares R, Rufián L, Rodríguez M, et al. Detection of minimal residual disease in acute myeloid leukemia: evaluating utility and challenges. Front Immunol [Internet]. 2024 Jun 13 [cited 2025 Nov 14];15:1252258. Available from: https://doi.org/10.3389/fimmu.2024.1252258
10. Falini B, Dillon R. Criteria for Diagnosis and Molecular Monitoring of NPM1-Mutated AML. Blood Cancer Discov [Internet]. 2024 Jan 8 [cited 2025 Nov 14];5(1):8–20. Available from: https://doi.org/10.1158/2643-3230.BCD-23-0182
11. Makkar H, Majhi RK, Goel H, Gupta AK, Chopra A, Tanwar P, et al. Acute myeloid leukemia: novel mutations and their clinical implications. Am J Blood Res [Internet]. 2023 Feb 15 [cited 2025 Nov 14];13(1):12–27. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10013618/
12. Lai A, Liu W, Zhou C, Li Y, Wei S, Liu K, et al. Prognostic impact of co-mutations in adults with IDH1/2-mutated acute myeloid leukemia. Blood Sci [Internet]. 2025 Mar 28 [cited 2025 Nov 14];7(2):e00231. Available from: https://doi.org/10.1097/BS9.0000000000000231
13. Kim N, Hahn S, Choi YJ, Cho H, Chung H, Jang JE, et al. Comprehensive insights into AML relapse: genetic mutations, clonal evolution, and clinical outcomes. Cancer Cell Int [Internet]. 2024 May 19 [cited 2025 Nov 14];24:174. Available from: https://doi.org/10.1186/s12935-024-03368-4
14. Fedorov K, Maiti A, Konopleva M. Targeting FLT3 Mutation in Acute Myeloid Leukemia: Current Strategies and Future Directions. Cancers (Basel) [Internet]. 2023 Apr 15 [cited 2025 Nov 14];15(8):2312. Available from: https://doi.org/10.3390/cancers15082312
15. Fan WL, Yeh JK, Hsieh LC, Tsai ML, Ho MY, Huang YC, et al. Prognostic significance of clonal hematopoiesis in STEMI: a 10-year follow-up reveals high-risk gene mutations. Hum Genomics [Internet]. 2025 May 12 [cited

2025 Nov 14];19(1):51. Available from: https://doi.org/10.1186/s40246-025-00730-1

16. Pei XM, Yeung MHY, Wong ANN, Tsang HF, Yu ACS, Yim AKY, et al. Targeted Sequencing Approach and Its Clinical Applications for the Molecular Diagnosis of Human Diseases. Cells [Internet]. 2023 Feb 1 [cited 2025 Nov 14];12(3):493. Available from: https://doi.org/10.3390/cells12030493
17. Mao W, Wang J, Li T, Wu J, Wang J, Wen S, et al. Hybrid Capture-Based Sequencing Enables Highly Sensitive Zoonotic Virus Detection Within the One Health Framework. Pathogens [Internet]. 2025 Mar 7 [cited 2025 Nov 14];14(3):264. Available from: https://doi.org/10.3390/pathogens14030264
18. Yoest JM, Shirai CL, Duncavage EJ. Sequencing-Based Measurable Residual Disease Testing in Acute Myeloid Leukemia. Front Cell Dev Biol [Internet]. 2020 May 8 [cited 2025 Nov 14];8:249. Available from: https://doi.org/10.3389/fcell.2020.00249
19. Li X, Liu T, Bacchiocchi A, Li M, Cheng W, Wittkop T, et al. Ultra-sensitive molecular residual disease detection through whole genome sequencing with single-read error correction. EMBO Mol Med [Internet]. 2024 Sep [cited 2025 Nov 14];16(9):2188–209. Available from: https://doi.org/10.1038/s44321-024-00139-6
20. Isaic A, Motofelea N, Hoinoiu T, Motofelea AC, Leancu IC, Stan E, et al. Next-Generation Sequencing: A Review of Its Transformative Impact on Cancer Diagnosis, Treatment, and Resistance Management. Diagnostics (Basel) [Internet]. 2025 Sep 23 [cited 2025 Nov 14];15(19):2425. Available from: https://doi.org/10.3390/diagnostics15192425
21. Ahmed F, Zhong J. Advances in DNA/RNA Sequencing and Their Applications in Acute Myeloid Leukemia (AML). Int J Mol Sci [Internet]. 2024 Dec 24 [cited 2025 Nov 14];26(1):71. Available from: https://doi.org/10.3390/ijms26010071
22. Pratiwi L, Mashudi FH, Ningtyas MC, Sutanto H, Romadhon PZ. Genetic Profiling of Acute and Chronic Leukemia via Next-Generation Sequencing: Current Insights and Future Perspectives. Hematol Rep [Internet]. 2025 Mar 28 [cited 2025 Nov 14];17(2):18. Available from: https://doi.org/10.3390/hematolrep17020018
23. Li Y, Solis-Ruiz J, Yang F, Long N, Tong CH, Lacbawan FL, et al. NGS-defined measurable residual disease (MRD) after initial chemotherapy as a prognostic biomarker for acute myeloid leukemia. Blood Cancer J [Internet]. 2023 Apr 24 [cited 2025 Nov 14];13(1):59. Available from: https://doi.org/10.1038/s41408-023-00832-8
24. Leonardos D, Benetatos L, Apostolidou E, Koumpis E, Dova L, Kapsali E, et al. Applications of Multiparameter Flow Cytometry in the Diagnosis, Prognosis, and Monitoring of Multiple Myeloma Patients. Diseases [Internet]. 2025 Sep 30 [cited 2025 Nov 14];13(10):320. Available from: https://doi.org/10.3390/diseases13100320
25. Lavrichenko K, Engdal ES, Marvig RL, Jemt A, Vignes JM, Almusa H, et al. Recommendations for bioinformatics in clinical practice. Genome Med [Internet]. 2025 Oct 17 [cited 2025 Nov 14];17(1):124. Available from: https://doi.org/10.1186/s13073-025-01592-7
26. Krönke J, Schlenk RF, Jensen KO, Tschürtz F, Corbacioglu A, Gaidzik VI, et al. Monitoring of Minimal Residual Disease in NPM1-Mutated Acute Myeloid Leukemia: A Study From the German-Austrian Acute Myeloid Leukemia Study Group. J Clin Oncol [Internet]. 2011 Jul [cited 2025 Nov 14];29(19):2709–16. Available from: https://doi.org/10.1200/JCO.2011.35.0371
27. Zhao Y, Guo H, Chang Y. MRD-directed and risk-adapted individualized stratified treatment of AML. Chin J Cancer Res [Internet]. 2023 Oct 30 [cited 2025 Nov 14];35(5):451–69. Available from: https://doi.org/10.21147/j.issn.1000-9604.2023.05.02
28. Crisà E, Dogliotti I, Lia G, Cerrano M, Audisio E, Lanzarone G, et al. Integration of Next-Generation Sequencing in Measurable Residual Disease Monitoring in Acute Myeloid Leukemia and Myelodysplastic

Neoplasm. Cancers (Basel) [Internet]. 2025 Aug 31 [cited 2025 Nov 14];17(17):2874. Available from: https://doi.org/10.3390/cancers17172874

29. Ally F, Chen X. Acute Myeloid Leukemia: Diagnosis and Evaluation by Flow Cytometry. Cancers (Basel) [Internet]. 2024 Nov 16 [cited 2025 Nov 14];16(22):3855. Available from: https://doi.org/10.3390/cancers16223855
30. Di J, Sheng T, Arora R, Stocks-Candelaria J, Wei S, Lutz C, et al. The Validation of Digital PCR-Based Minimal Residual Disease Detection for the Common Mutations in IDH1 and IDH2 Genes in Patients with Acute Myeloid Leukemia. J Mol Diagn [Internet]. 2025 Feb [cited 2025 Nov 14];27(2):100–8. Available from: https://doi.org/10.1016/j.jmoldx.2024.10.005
31. Xie D, An B, Yang M, Wang L, Guo M, Luo H, et al. Application and research progress of single-cell sequencing technology in leukemia. Front Oncol [Internet]. 2024 Aug 29 [cited 2025 Nov 14];14:1389468. Available from: https://doi.org/10.3389/fonc.2024.1389468
32. Liang JH, Hua W, Yin H, Li Y, Zhang XY, Liang JH, et al. Clinical implications of ctDNA-based minimal residual disease detection in newly diagnosed peripheral T-cell lymphoma: a single-center cohort study. Cell Commun Signal [Internet]. 2025 Oct 15 [cited 2025 Nov 14];23(1):441. Available from: https://doi.org/10.1186/s12964-025-02038-8
33. Fan BL, Chen LH, Chen LL, Guo H, Fan BL, Chen LH, et al. Integrative Multi-Omics Approaches for Identifying and Characterizing Biological Elements in Crop Traits: Current Progress and Future Prospects. Int J Mol Sci [Internet]. 2025 Feb 9 [cited 2025 Nov 14];26(4):1466. Available from: https://doi.org/10.3390/ijms26041466
34. Lawal OP, Igwe EP, Olosunde A, Chisom EP, Okeh DU, Olowookere AK, et al. Integrating Real-Time Data and Machine Learning in Predicting Infectious Disease Outbreaks: Enhancing Response Strategies in Sub-Saharan Africa. Asian J Microbiol Biotechnol [Internet]. 2025 May [cited 2025 Nov 14];10(1):147–63. Available from: https://doi.org/10.37284/ajmb.10.1.2526
35. Enabulele ABO, Eleweke CC, Okechukwu O, Akanbi OO, Majesty C. A Strategic Project Management Framework for Implementing Patient-Centered Digital Health Record Systems to Improve Chronic Disease Outcomes in the United States. J Sustain Res Dev [Internet]. 2025 Nov 13 [cited 2025 Nov 14];1(2):55–67. Available from: https://doi.org/10.69784/jsrd.v1i2.75
36. Lawal O, Babatunde ET, Muhammed I, Okiki QN, Chibueze ES, David VO, et al. Comparative Genomics of Zoonotic Pathogens: Genetic Determinants of Host Switching and Cross-Species Transmission. J Clin Med Kaz [Internet]. 2025 Sep 14 [cited 2025 Oct 7]; Available from: https://doi.org/10.23950/jcmk/15332