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Review Article

Pathology Section

Measurable Residual Disease in Acute Leukaemias: A Narrative Review on Specimen Adequacy, Assay Selection, Interpretive Pitfalls and Challenges for the Practicing Pathologist

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ABSTRACT

Measurable Residual Disease (MRD) is a key prognostic marker in acute leukaemias, guiding therapy and risk stratification. Even in morphologic remission, residual clones drive relapse, highlighting the need for sensitive, standardised detection. This narrative review synthesises evidence from PubMed, Embase, and Scopus (2000–2025) together with international guidelines from the European LeukaemiaNet (ELN), the National Comprehensive Cancer Network (NCCN), the World Health Organisation (WHO), and the EuroFlow Consortium (EuroFlow). Multiparameter Flow Cytometry (MFC) remains the frontline tool for its accessibility and speed, though challenged by haematogone overlap, immunophenotypic shifts, and CD19-negative relapse. Molecular assays {Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR), Next-Generation Sequencing (NGS)} provide higher sensitivity but require caution in clonal haematopoiesis; DNMT3A, TET2, and ASXL1 mutations are excluded from Acute Myeloid Leukaemia (AML) Minimal Residual Disease (MRD) reporting. Standardised Breakpoint Cluster Region (BCR)::ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase (ABL1) monitoring exemplifies harmonised molecular metrics, though broader consensus is needed. Comparative insights across B-precursor Acute Lymphoblastic Leukaemia (B-ALL), T-cell Acute Lymphoblastic Leukaemia (T-ALL) , AML, and related neoplasms reveal disease-specific thresholds. Emerging technologies, namely, digital PCR, error-corrected NGS, single-cell profiling, promise unprecedented resolution, positioning MRD as a cornerstone of precision therapy.

Keywords: Clonal haematopoiesis, Flow cytometry, Neoplasm, Precision therapy

INTRODUCTION

The MRD detection has transformed acute leukaemia management and is now a major prognostic marker for risk stratification and therapeutic decision-making [1-3]. Even in morphologic remission (<5% blasts), residual leukaemic cells may persist below microscopic detection and strongly correlate with relapse and survival outcomes [1,3]. The prognostic significance of MRD at post-induction, post-consolidation, and pretransplant stages has led the ELN to incorporate Complete Remission without Measurable Residual Disease (CRMRD) into routine response assessment [2].

For haematopathologists, MRD evaluation extends beyond technical assay performance and requires attention to specimen adequacy, assay sensitivity, and interpretive pitfalls [3,4]. Therapy-related immunophenotypic shifts, haemodilution, regenerating precursor populations, and antigen modulation following targeted therapies may complicate interpretation [4]. Emerging immunotherapies such as CD19-directed CAR-T therapy have further introduced challenges including antigen-negative relapse [5], while highly sensitive Next-Generation Sequencing (NGS) techniques complicate distinction between true residual leukaemia and clonal haematopoiesis [6,7].

The present narrative review summarises current MRD detection platforms, including MFC, molecular methods, and NGS, along with their interpretive pitfalls and contemporary guideline recommendations relevant to routine haematopathology practice [3,4,7].

Pre-analytical Pitfalls in MRD Assessment

Reliable MRD detection in acute leukaemias depends heavily on preanalytical factors that influence sensitivity and reproducibility [3,4]. The first-pull marrow aspirate is the most informative, as subsequent pulls are diluted with peripheral blood, lowering leukaemic cell yield and increasing false-negative risk [3].

Specimen handling is equally critical. Delayed transport or processing compromises labile antigens such as CD10 and CD34 [4,8]. Anticoagulant choice affects staining quality: Ethylenediaminetetraacetic Acid (EDTA) is generally preferred, whereas heparin may cause antigen shedding and reduced staining reliability [4]. Cryopreservation can alter antigen expression and reduce cell viability [4]. Robust flow cytometric analysis requires high cell viability, ideally ≥85-90% [4]. For molecular assays, Ribonucleic Acid (RNA) integrity is essential, as degradation during transport or delayed processing reduces RT-qPCR reproducibility and sensitivity [3,8].

Therapy-induced hypocellularity further limits event acquisition, thereby diminishing assay sensitivity [4]. To achieve the conventional MRD detection threshold of 10^{-4}, acquisition of at least 1-2 million events is recommended for flow cytometry, with even higher counts required for next-generation flow approaches [4].

These pitfalls underscore the importance of standardised protocols for specimen collection, transport, processing, and reporting [3,4,8]. Optimisation of each preanalytical step preserves antigen stability, cell viability, and nucleic acid integrity, thereby ensuring technically valid and clinically meaningful MRD results [3,4,8]. Ultimately, meticulous attention to preanalytical variables safeguards diagnostic accuracy and strengthens the role of MRD in risk stratification and therapeutic planning.

Specimen Adequacy

Accurate MRD assessment in acute leukaemias begins with appropriate specimen selection [3,4]. Bone marrow aspirates

remain the gold standard because they provide enriched leukaemic populations and superior sensitivity for both flow cytometric and molecular assays [3]. Peripheral blood is less sensitive for most applications but may be useful for selected molecular techniques such as RT-qPCR and NGS [3,8].

Timing of MRD assessment is also critical. Evaluation at post-induction, post-consolidation, and pretransplant time points provides important prognostic information, while persistent pretransplant MRD strongly predicts relapse risk [3,9]. Longitudinal monitoring of MRD kinetics further refines assessment of disease trajectory and treatment response [9].

Quality-control measures are essential to ensure assay validity [4]. Hypocellular specimens reduce sensitivity, whereas haemodilution from peripheral blood contamination may lead to false-negative interpretation [4]. Prompt specimen handling and standardised processing reduce technical artefacts and improve reproducibility [4]. For flow cytometry, acquisition of at least 1-2 million events is generally required to achieve 10^{-4} sensitivity, with higher event counts necessary for next-generation approaches [4].

Clinically, inadequate specimens may underestimate residual disease burden and adversely affect therapeutic decisions [3]. Standardised MRD reports should therefore document specimen quality, cellularity, haemodilution, and any technical limitations that may influence interpretation [3,4]. Emphasis on specimen adequacy ensures MRD results remain both technically reliable and clinically meaningful, reinforcing its central role in modern leukaemia management.

Liquid Biopsy in MRD Assessment

When bone marrow sampling is inadequate or impractical, liquid biopsy provides a valuable alternative for MRD detection [7,10,11]. Circulating tumour Deoxyribonucleic Acid (ctDNA) and cell-free DNA (cfDNA) assays identify leukaemic genomic alterations directly from peripheral blood, allowing MRD assessment without invasive marrow procedures [7,10]. This approach is particularly useful in patients with poor marrow yield, haemodilution, or contraindications to repeated aspirations [10].

Although generally less sensitive than marrow-based assays, liquid biopsy is minimally invasive, easily repeatable, and well-suited for longitudinal monitoring of disease kinetics, relapse risk, and therapeutic response [7,10,11]. Integration with NGS and digital PCR improves detection of low-frequency variants and clonal evolution while helping distinguish residual leukaemia from clonal haematopoiesis [6,7,11]. In addition, liquid biopsy may better capture systemic disease heterogeneity than localised marrow sampling [10,11].

Multiparameter Flow Cytometry (MFC): Methodology and Interpretive Challenges

Technical principles and sensitivity: The MFC is a cornerstone of MRD detection in acute leukaemias because it enables rapid, multiparametric immunophenotypic analysis applicable to most patients [3,4]. Two complementary strategies are commonly employed: the Leukaemia-Associated Immunophenotype (LAIP) approach, which tracks aberrant antigen patterns identified at diagnosis, and the Different-from-Normal (DfN) approach, which identifies deviations from normal hematopoietic maturation [4]. The ELN recommends combining both approaches to improve diagnostic accuracy [2].

Sensitivity depends on antibody panel design, event acquisition, and operator expertise [3,4]. Conventional 8-10-colour panels analysing 1-2 million events typically achieve sensitivities of approximately 10^{-4}, whereas next-generation flow approaches using 12-15-colour panels may extend sensitivity to 10^{-5}-10^{-6} [3,4,12]. Advanced gating strategies incorporating markers such as CD22, CD24, CD81, CD73, CD86, and CD304 improve detection of rare leukaemic populations and enhance prognostic precision [12-14].

Haematogone-blast Discrimination

Distinguishing leukaemic blasts from benign haematogones remains a major interpretive challenge in B-precursor Acute Lymphoblastic Leukaemia (B-ALL) [4,13]. Haematogones follow an orderly maturation pattern characterised by progressive loss of CD34 and CD10 with gain of CD20 and CD45 expression [13,14]. Post-chemotherapy haematogone expansion is common and may mimic residual leukaemia, especially when immature subsets overlap phenotypically with blasts [Table/Fig-1] [13].

Cell type	Markers	Pattern
Haematogones (Type I–III)	CD34, CD10, CD45, CD20	Gradual maturation, CD34/CD10 decline, CD45/CD20 rise
Leukaemic blasts	Aberrant CD10/CD34 persistence and abnormal CD45/CD20	Arrested maturation and aberrant antigen expression

[Table/Fig-1]: Haematogone vs. leukaemic blast features [13].

To improve discrimination, modern MRD panels incorporate markers such as CD81, CD73, CD86, and CD304 [12-14]. CD81 underexpression is observed in a large proportion of B-ALL cases and remains relatively stable following therapy, while CD86 demonstrates high LAIP retention [12,13]. CD304 (neuropilin-1) is particularly valuable in CD10-dim or CD10-negative leukaemias and improves sensitivity in high-resolution assays [14]. Collectively, these refinements improve differentiation between regenerative haematogones and true residual leukaemia, thereby reducing false-positive MRD interpretation [Table/Fig-2] [12-14].

CD19 Loss and Post-Immunotherapy Challenges

The advent of CD19-targeted immunotherapies, including Chimeric Antigen Receptor T-cell (CAR-T) therapy and bi-specific T-cell engagers, has transformed the treatment of relapsed or refractory B-ALL but introduced new diagnostic challenges in MRD assessment [4,5,15]. CD19 antigen loss or downregulation, reported in approximately 10-20% of post-therapy relapses, compromises

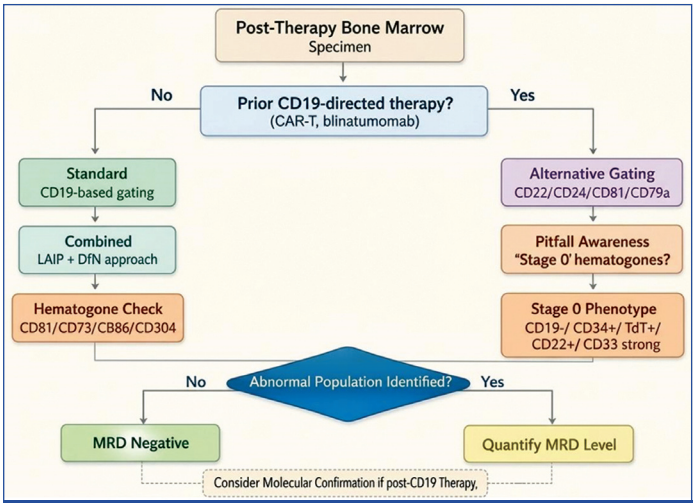
Marker	Expression on blasts	Expression on haematogones	LAIP stability (%)	Clinical utility
CD81	Aberrantly decreased (82%)	Uniformly bright	91	• Highly sensitive • Stable post-therapy • Useful in haematogone-rich specimens
CD73	Variable and often aberrant	Distinct pattern	91	• High stability • Complements CD81
CD86	Aberrant expression	Normal pattern	100	• Highest stability • No LAIP loss post-induction
CD58	Overexpressed	Lower expression	≈80	• Well-validated • Included in EuroFlow panels
CD304	Variable aberrant expression	Distinct pattern	High	• Stable marker • Useful in CD10-dim/negative cases
CD123	Often overexpressed	Lower/absent	Moderate	• Useful in combination panels

[Table/Fig-2]: Novel markers for haematogone-blast discrimination in B-ALL MRD [12-14].

conventional flow-cytometric gating strategies that depend on CD19 as a primary B-lineage marker [5,15].

To address this limitation, alternative gating strategies incorporating CD22, CD24, CD81, and CD79a are increasingly recommended because these markers usually remain stable despite CD19 modulation [4,14,16]. However, recognition of stage 0 haematogones characterised by $CD19^+/CD34^+/TdT^+/CD22^+/CD38^+$ phenotypes is essential, as these benign precursors may mimic residual leukaemic populations [17]. Integration of MFC with molecular techniques such as RT-qPCR and NGS improves diagnostic confidence and reduces false-negative interpretation in post-immunotherapy specimens [3,15].

The MFC-MRD interpretation algorithm [Table/Fig-3] summarises this workflow. Initial assessment includes documentation of prior CD19-directed therapy followed by selection of appropriate gating strategies-standard CD19-based panels in untreated patients and alternative CD22/CD24/CD81/CD79a-based panels in post-therapy settings [4,14,16]. Haematogone discrimination using markers such as CD81, CD73, CD86, and CD304 further improves specificity before final MRD quantification [14,16,17].



[Table/Fig-3]: MFC-MRD interpretation algorithm in the post-immunotherapy era [4,14,16,17].

Clinical Utility

Awareness of false-positive and false-negative sources in MRD assessment is essential for accurate interpretation and appropriate clinical decision-making [3,4,16]. Pitfalls including haematogone expansion, CD19 antigen loss, clonal haematopoiesis, haemodilution, and hypocellular marrows may significantly affect assay sensitivity and specificity [3,6,15,16]. Misclassification can lead to inappropriate therapeutic escalation or de-escalation, incorrect transplant timing, and inaccurate clinical trial endpoints [3,6]. Standardised reporting that incorporates recognition of these pitfalls strengthens the role of MRD as a reliable prognostic and therapeutic biomarker [Table/Fig-4] [3,4,6,15-18].

RT-qPCR in MRD Detection

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) remains the gold standard for molecular MRD monitoring in leukaemias with recurrent fusion transcripts [3,18,19]. Its high sensitivity, reproducibility, and quantitative precision make it indispensable, particularly in BCR::ABL1-positive leukaemias, where transcript levels directly correlate with therapeutic response and relapse risk [18,19]. RT-qPCR typically achieves sensitivities of 10^{-4} to 10^{-5}, enabling detection of Deep Molecular Responses (DMR) and longitudinal disease monitoring [18].

A major advance has been the introduction of the International Scale (IS) for BCR::ABL1 quantification, which standardises reporting across laboratories and reduces inter-laboratory variability [19]. This harmonised framework defines clinically relevant milestones such as Major Molecular Response (MMR) and DMR, thereby facilitating uniform patient monitoring and evidence-based treatment decisions worldwide [18,19].

Source	Mechanism	Interpretive impact
Haematogones	Benign B-cell precursors overlap immunophenotypically with leukaemic blasts (CD10/ CD34 expression)	False-positive MRD In B-ALL remission samples
CD19 Loss	Antigen modulation after CAR-T or bi-specific therapy; residual leukaemic cells escape CD19-based gating	False-negative MRD if alternative markers not used
Clonal Haematopoiesis (CHIP)	Persistence of DNMT3A, TET2, ASXL1 mutations in remission without relapse correlation	False-positive molecular MRD in AML
Hypocellular marrow	Low event counts post-therapy reduce sensitivity below 10^{-4}	False-negative MRD due to insufficient sampling
Delayed transport	Antigen degradation (CD10, CD34) and RNA instability	False-negative MRD from compromised assay reproducibility
Stage 0 haematogones	$CD19^-/CD34^+/TdT^+$ precursors mimic leukaemic blasts post-immunotherapy	False-positive MRD in CD19-negative contexts
Cryopreservation effects	Antigen shedding and reduced viability (<85%)	False-negative MR' from poor flow cytometry performance

[Table/Fig-4]: Common false-positive and false-negative sources in MRD assessment [3,4,6,15-18].

Despite these advantages, reproducibility for non-BCR::ABL1 molecular targets remains less standardised because validated international reference scales are lacking [3,18]. Although NGS offers broader genomic assessment and improved sensitivity, RT-qPCR continues to serve as the benchmark for fusion transcript monitoring owing to its established harmonisation and clinical validation [3,18,19].

NGS-based MRD Detection

Sensitivity and broad mutation detection: The NGS has emerged as a transformative modality for MRD assessment, with sensitivities approaching 10^{-6} [7,10,20]. Unlike targeted molecular assays, NGS enables broad mutational profiling across the leukaemic genome, allowing detection of clonal evolution and rare subclones that may evade conventional monitoring [7,20]. This capability is particularly valuable in Acute Myeloid Leukaemia (AML), where marked genetic heterogeneity complicates MRD detection and prognostication [7,10]. By identifying low-frequency variants and tracking mutational dynamics, NGS improves risk stratification and supports individualised therapeutic strategies [7,10,20].

The CHIP Challenge

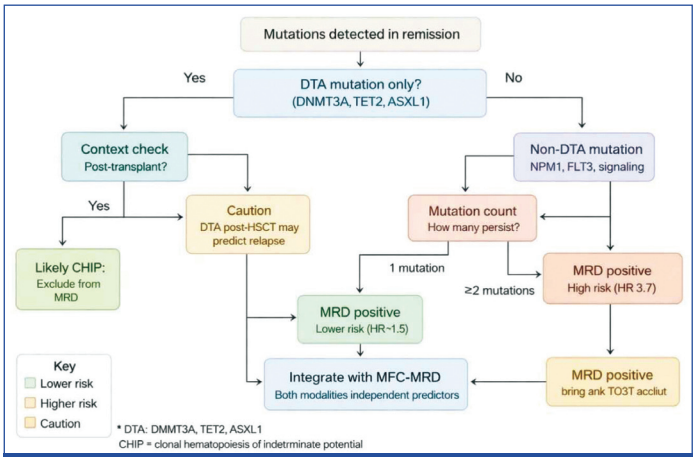
A major interpretive challenge in NGS-based MRD assessment is distinguishing residual leukaemia from Clonal Haematopoiesis of Indeterminate Potential (CHIP), which becomes increasingly common with aging [6,21]. Mutations involving DNMT3A, TET2, and ASXL1 (DTA mutations) may persist during remission without reflecting active leukaemia or increased relapse risk [6,21]. Consequently, ELN and NCCN guidelines recommend excluding isolated DTA mutations from MRD interpretation, whereas persistence of non-DTA mutations such as NPM1 or FLT3 is associated with significantly increased relapse risk [21-22].

Decision Algorithms for NGS-MRD

Current interpretive algorithms emphasise exclusion of isolated DTA mutations, contextual evaluation of post-transplant findings, and risk stratification based on persistence of non-DTA mutations [10,21,22]. Integration of serial monitoring with clinical and transplant data further improves prognostic precision and therapeutic decision-making [Table/Fig-5,6] [7,10,23,24].

Scenario	Recommendation	Rationale
Isolated DTA mutation in remission	Exclude from MRD	Reflects CHIP and relapse risk is similar to MRD-negative
DTA + non-DTA mutation	Report as MRD-positive	Co-occurrence increases relapse risk
DTA mutation post-transplant	Interpret with caution	May predict relapse in HSCT setting
≥2 non-DTA mutations	High-risk MRD positive	Strong predictor of relapse (HR 3.7)
Single non-DTA mutation	MRD-positive, lower risk	Prognostically significant but less severe
High stable VAF (40-50%)	Investigate germline predisposition	May indicate inherited variants (RUNX1, GATA2, DDX41)

[Table/Fig-5]: Guidance for DTA and CHIP-associated Mutations in MRD Assessment [6,21,22].



[Table/Fig-6]: Conceptual schematic on NGS-based MRD interpretation: Navigating the CHIP challenge [7,23,24].

Complementarity with Flow Cytometry

The NGS and MFC provide complementary information in MRD assessment [4,7,11]. NGS excels in detecting persistent molecular alterations and clonal evolution, whereas MFC evaluates immunophenotypic abnormalities and regenerative marrow patterns [4]. Patients negative by both NGS and MFC demonstrate the most favourable relapse-free survival, highlighting the prognostic value of combined modality assessment [7,10].

Clinical Integration

The NGS is increasingly integrated into clinical trials and transplant protocols for risk stratification and therapeutic planning [7,10]. Ongoing efforts aim to harmonise reporting standards and reduce inter-laboratory variability, similar to the International Scale used in BCR::ABL1 RT-qPCR monitoring [Table/Fig-7] [3,4,7,10,18-20].

Digital PCR and Emerging Technologies in MRD Detection

Digital PCR: Precision at the lowest levels: Digital PCR (dPCR) enables highly precise MRD quantification by partitioning samples into thousands of independent amplification reactions [8]. Unlike conventional RT-qPCR, dPCR provides absolute quantification without reliance on amplification curves, thereby improving reproducibility and sensitivity [25]. This approach is particularly valuable in leukaemias with recurrent fusion transcripts or hotspot mutations, especially in deep remission states where residual transcript levels approach the limits of conventional assays [25,26].

Error-corrected NGS: Ultra-sensitive mutation profiling: Error-corrected ecNGS incorporates Unique Molecular Identifiers (UMIs) and computational correction algorithms to distinguish true low-frequency variants from sequencing artefacts [20,27]. These refinements allow sensitivities approaching 10^{-6} while improving detection of rare therapy-resistant subclones [20,27]. Beyond MRD detection, ecNGS provides insight into clonal evolution and treatment resistance, thereby supporting adaptive therapeutic strategies [25].

Single-cell MRD: Mapping clonal architecture: Single-cell MRD technologies represent an emerging frontier in leukaemia monitoring [28]. Unlike bulk sequencing methods, single-cell platforms simultaneously evaluate genetic alterations and immunophenotypic characteristics at the individual-cell level, thereby revealing clonal heterogeneity, lineage relationships, and rare resistant populations [28]. These approaches improve understanding of disease persistence, immune escape, and therapeutic resistance while potentially enhancing prognostic precision [28].

Guideline Integration in MRD Assessment

International guidelines have standardised MRD assessment and incorporated it into routine leukaemia management [4,10,22,29]. The ELN established a clinically significant threshold of 0.1% for AML MRD detection by MFC and introduced the remission category CRMRD [9]. This framework elevated MRD from a research tool to a validated clinical endpoint influencing transplant decisions and therapeutic escalation [10,22].

The NCCN further refined molecular MRD interpretation by recommending exclusion of isolated DTA mutations from MRD reporting to avoid false-positive interpretation related to clonal haematopoiesis [21,22].

The EuroFlow Consortium contributed substantially to standardisation through validated antibody panels and Standardised Operating Procedures (SOP) that improved inter-laboratory reproducibility and clinical outcome prediction, particularly in paediatric B-ALL [4,29].

The WHO incorporated MRD into contemporary disease classification frameworks, reinforcing its prognostic significance and role in therapeutic decision-making [28]. Together, these harmonised frameworks have established MRD assessment as a cornerstone of modern leukaemia management [Table/Fig-8] [2,10,21,22,27,28].

Standardisation and Reporting

The clinical utility of MRD depends on reproducibility and harmonisation across laboratories [3,12,29]. The EuroFlow Consortium has significantly improved standardisation through validated antibody panels and SOPs, with EuroFlow-based MRD monitoring associated with improved outcomes in paediatric B-ALL

Modality	Sensitivity range	Detection basis	Strengths	Limitations	Clinical utility
RT-qPCR	10^{-4} – 10^{-5}	Fusion transcript quantification (e.g., BCR::ABL1)	High precision, standardised International Scale, reproducible	Limited to known targets and inter-lab variability for non-standard genes	Benchmark for molecular MRD in CML and select AML subtypes
Multiparameter Flow-Cytometry (MFC)	10^{-3} – 10^{-5}	Immunophenotypic aberrancies	Rapid, widely available, captures cellular context	Subjective gating, haematogone overlap, lower sensitivity	Core method for MRD in ALL and AML and complements molecular assays
Next-Generation Sequencing (NGS)	Up to 10^{-6}	Mutation profiling across genome	Ultra-high sensitivity and detects clonal evolution with broad applicability	Requires CHIP exclusion, cost and bioinformatic complexity	Emerging gold standard and integrates with MFC for comprehensive risk stratification

[Table/Fig-7]: Comparative overview of MRD detection modalities [3,4,7,10,18-20].

Guideline body	Key contributions	Thresholds/ Recommendations	Clinical impact
ELN	Landmark consensus for AML MRD; introduced CRMRD remission category	0.1% cut-off (AML MFC); CRMRD definition	Standardised remission definitions; improved risk stratification
NCCN	Addressed interpretive pitfalls of clonal hematopoiesis	Excludes DNMT3A, TET2, ASXL1 from MRD	Prevents false-positives; aligns with ELN consensus
EuroFlow	Developed harmonised SOPs and validated panels	Standardised flow cytometry protocols	Improved reproducibility; Better paediatric B-ALL outcomes
WHO	Incorporated MRD into disease classification	Embeds MRD into diagnostic systems	Reinforces MRD as cornerstone; Ensures global consistency

[Table/Fig-8]: Comparison of International Guidelines for MRD assessment [2,10,21,22,27,28].

[12,29]. A threshold of 10^{-4} (0.01%) is widely accepted for MRD detection, although the ELN recommends 0.1% for AML flow cytometric MRD while acknowledging that lower thresholds may further refine risk stratification [2]. Terminology has evolved from "minimal" to "measurable" residual disease to emphasise that MRD negativity reflects disease below assay detection limits rather than complete absence of leukaemia [3]. Standardised reporting frameworks remain essential for inter-laboratory comparability and clinical decision-making [Table/Fig-9] [2,3,12,27].

Element	Content requirements
Method	MFC, RT-qPCR, NGS, digital PCR, or combination; include panel/target details
Sensitivity	Limit of detection achieved (e.g.,"10^{-4}, 0.01%); note if target sensitivity not reached
Quantitative result	MRD level with units (%, NCN, IS); include trend comparison with prior results
Interpretation	MRD-positive, MRD-negative, or MRD not evaluable; clinical timepoint context
Limitations	Specimen adequacy issues, haemodilution, post-immunotherapy antigen loss, excluded targets

[Table/Fig-9]: Essential elements for standardised MRD reporting [2,3,12,27].

AML vs ALL: Differences in MRD Assessment

Technical challenges in AML: The MRD assessment in AML remains technically challenging because of clonal heterogeneity and overlap with CHIP [6,21]. Flow cytometric sensitivity in AML is generally lower than in lymphoid malignancies, commonly ranging from 10^{-3} to 10^{-4} [2,4]. Interpretation of NGS requires exclusion of CHIP-associated mutations such as DNMT3A, TET2, and ASXL1, which may persist during remission without conferring relapse risk [6,21]. These challenges have limited universal integration of MRD as a definitive therapeutic endpoint in AML [21].

Higher Sensitivity in ALL

In contrast, ALL benefits from highly standardised and sensitive MRD methodologies [1,3,12]. PCR-based detection of immunoglobulin/T-cell receptor rearrangements and MFC routinely achieve sensitivities of 10^{-4} to 10^{-5} with excellent reproducibility [1,3]. Standardised EuroFlow panels and harmonised molecular assays have enabled MRD to become a robust prognostic marker directly influencing therapeutic intensity, transplant eligibility, and clinical trial stratification in ALL [1,12,29].

Immunotherapy in ALL

In B-ALL, MRD has become a therapeutic trigger [1,3,30]. The BLAST trial established blinatumomab as an effective MRD-directed therapy in adults with B-cell precursor ALL, demonstrating MRD clearance and improved survival outcomes. CD19-directed CAR-T therapy has also shown durable activity in relapsed/refractory ALL and supports MRD-guided immunotherapeutic approaches [5].

Prognostic Integration

The MRD positivity in AML strongly predicts relapse and influences transplant timing and maintenance strategies, although standardised treatment algorithms remain less consistent across Institutions [31]. The QUAZAR AML-001 trial demonstrated the value of oral azacitidine maintenance therapy in AML patients in remission, highlighting the emerging integration of MRD-guided post-remission strategies [31].

In ALL, MRD status is a major therapeutic determinant [1,3,32]. Persistent MRD identifies patients who may benefit from treatment intensification, allogeneic transplantation, or immunotherapies. The BLAST trial established blinatumomab as an effective therapy in MRD-positive B-ALL, while CD19-directed CAR-T therapy has demonstrated efficacy in eradicating residual disease [5,32].

Therapeutic Applications

In AML, MRD assessment primarily guides transplant decisions, post-remission maintenance, and risk stratification [31]. In ALL, MRD is directly actionable and functions as a gateway to therapies such as blinatumomab, inotuzumab, ozogamicin, and CAR-T cells [5,32]. Thus, ALL currently represents the most mature model of MRD-driven therapeutic decision-making [1,3].

Consensus and Standardisation

The AML MRD assessment remains limited by variability in assay methodology and threshold interpretation across Institutions [21]. Conversely, ALL MRD assessment benefits from harmonised EuroFlow protocols and standardised molecular monitoring frameworks such as the International Scale for BCR::ABL1 quantification [12,18,29]. These consensus-driven approaches have enabled global reproducibility and strengthened MRD as a cornerstone of ALL management [3,12,18].

Together, these differences illustrate that AML MRD assessment continues to face technical and interpretive challenges, whereas ALL has successfully operationalised MRD as a clinically actionable biomarker guiding therapy intensity, transplant eligibility, and immunotherapy deployment [1,3].

MRD-guided Therapy

Therapy escalation and De-escalation: Persistent MRD identifies patients at high risk of relapse and often supports therapy escalation, including intensified chemotherapy, targeted agents, immunotherapy, or additional consolidation [1,3,33]. Conversely, sustained deep molecular remission may support treatment de-escalation in selected settings to reduce toxicity while maintaining disease control [3].

Transplant Planning

The MRD status is pivotal in allogeneic Hematopoietic Stem Cell Transplantation (HSCT) [9]. Pre-transplant MRD positivity predicts inferior outcomes, including lower leukaemia-free survival, reduced overall survival, and higher relapse incidence [9]. Therefore, MRD kinetics can help refine transplant timing, conditioning intensity, and post-transplant maintenance or monitoring strategies [9].

Maintenance Strategies

Maintenance therapy is increasingly shaped by MRD status [31]. In AML, the QUAZAR AML-001 trial showed that oral azacitidine maintenance prolonged overall and relapse-free survival in patients in first remission who were not candidates for transplantation [31]. In ALL, serial MRD surveillance helps guide the duration and intensity of maintenance therapy [1,3].

MRD as a Surrogate Endpoint

The MRD is increasingly used as a surrogate endpoint in clinical trials because MRD clearance correlates strongly with relapse-free

and overall survival [3,4,6,8]. Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) studies in adult ALL further support the prognostic value of MRD response and its role in treatment stratification [8]. Thus, MRD has evolved from a prognostic marker into a clinically actionable endpoint for trial design and precision-guided therapy [1,3,32-34].

Role of MRD in Clinical Trials

The MRD has emerged as an important surrogate endpoint in leukaemia clinical trials, enabling earlier assessment of therapeutic efficacy and accelerating drug development [1,3,33]. As a biomarker of treatment response, MRD allows evaluation of therapeutic impact before overt clinical relapse, thereby supporting adaptive trial design and facilitating regulatory approval of novel therapies [1,33].

Landmark studies illustrate this role. The Blinatumomab as a Strategy to Treat Minimal Residual Disease (BLAST) trial demonstrated that blinatumomab effectively eradicated MRD in B-cell precursor ALL, translating into improved relapse-free and overall survival [32]. Similarly, the QUAZAR AML-001 trial highlighted the value of MRD-guided maintenance strategies by demonstrating prolonged remission and survival with oral azacitidine in AML patients in first remission [31]. These studies underscore how MRD status informs therapeutic escalation, maintenance therapy, and transplant planning [29,30].

The GIMEMA MRD studies further validated MRD as a clinically meaningful endpoint by demonstrating strong correlations between MRD clearance and survival outcomes in adult ALL [34]. Incorporation of MRD into trial endpoints has therefore refined risk stratification, strengthened evaluation of novel therapies, and supported precision-guided treatment strategies [1,34].

Collectively, these trials demonstrate the evolution of MRD from a prognostic marker into a validated clinical endpoint that streamlines drug evaluation and individualises leukaemia therapy [1,3,31-34].

Clinical Significance

The MRD status has become a critical determinant of prognosis and therapeutic decision-making in leukaemia, particularly in allogeneic HSCT [3,9]. A meta-analysis of 19 studies demonstrated that pre-HSCT MRD positivity was associated with significantly inferior leukaemia-free survival, reduced overall survival, and markedly increased relapse incidence [9]. These findings emphasise the prognostic importance of MRD in transplant timing, conditioning intensity, and post-transplant monitoring strategies [9].

Beyond single assessments, MRD kinetics provide additional insight into disease biology and relapse risk [1,3]. Serial monitoring captures clonal dynamics over time, distinguishing transient positivity from persistent residual disease [3]. Rising MRD levels may predict impending relapse, whereas sustained MRD negativity is associated with durable remission and favourable long-term outcomes [1,3]. Thus, longitudinal MRD assessment improves risk stratification, guides adaptive therapy, and enhances communication between laboratory and clinician [3].

Together, MRD status and MRD kinetics have become central components of modern leukaemia management, shaping transplant decisions, therapeutic escalation, and long-term disease monitoring [1,3,9].

Bias and Limitations in MRD Assessment

Despite major advances in MRD detection, several methodological limitations and systemic biases continue to affect interpretation and clinical application [1-3,33]. These challenges underscore the need for cautious interpretation and greater harmonisation across Institutions and assay platforms [3].

Heterogeneity across Studies

One of the major limitations is variability in definitions of MRD positivity, assay sensitivity thresholds, and reporting standards [3,33]. While many flow cytometric studies use thresholds around 10^{-4} (0.01%), others apply 0.1% or lower cut-offs depending on methodology and disease context [2,3]. This heterogeneity complicates meta-analyses, limits inter-study comparability, and creates uncertainty in translating MRD findings into uniform clinical decisions [3,33].

Publication Bias

Publication bias also affects the MRD literature [1,33]. Studies demonstrating strong prognostic significance of MRD positivity are more likely to be published than negative or inconclusive studies, potentially exaggerating the perceived predictive value of MRD [33]. Consequently, limitations related to assay variability, disease-specific biology, and borderline MRD results may be underrepresented in the published evidence base [1,33].

Translation Gap between Sensitivity and Clinical Actionability

Another major challenge is the disconnect between ultra-sensitive detection technologies and clinically actionable disease thresholds [20,27]. Techniques such as ecNGS and digital PCR can detect extremely low levels of residual disease approaching 10^{-6} sensitivity [20,27]. However, the biological and clinical significance of these ultra-low-level findings remains uncertain, particularly when CHIP-associated mutations such as DNMT3A, TET2, or ASXL1 are detected [3,6]. Excessive sensitivity may therefore increase the risk of overtreatment or inappropriate therapeutic escalation without clear survival benefit [3].

Bias and Variability

To address these limitations, harmonisation of assay methodologies, thresholds, and reporting standards remains essential [3,29]. Transparent reporting of both positive and negative findings may reduce publication bias and improve evidence quality [33]. Prospective validation of emerging ultra-sensitive technologies is also necessary to define clinically meaningful MRD thresholds [20,25]. Collaborative initiatives such as ELN consensus recommendations and EuroFlow standardisation protocols represent important advances toward improving reproducibility and inter-laboratory comparability [3,29].

Future Directions in MRD Assessment

Single-cell MRD and error-corrected NGS: Emerging technologies such as single-cell MRD analysis and ecNGS are transforming MRD assessment [20,27,28]. Single-cell sequencing enables characterisation of individual leukaemic cells, thereby revealing clonal heterogeneity and rare therapy-resistant subclones that may contribute to relapse [28]. By integrating genomic and immunophenotypic profiling, single-cell approaches provide a multidimensional understanding of disease persistence and clonal evolution [20,28]. ecNGS, using UMIs and computational correction algorithms, improves distinction between true low-frequency variants and sequencing artefacts, achieving sensitivities approaching 10^{-6} [20,27]. These advances enhance prognostic precision and support adaptive therapeutic strategies by detecting residual disease below conventional assay thresholds [20,27].

AI-Driven Interpretation

Artificial Intelligence (AI) and machine learning approaches offer significant potential in MRD interpretation [4,35]. Current MRD assessment, particularly MFC, remains operator-dependent and subject to inter-laboratory variability [4]. AI-driven platforms may

automate gating strategies, integrate multimodal data from flow cytometry, RT-qPCR, and NGS, and improve reproducibility [4,35]. Predictive modelling based on MRD kinetics further enables estimation of relapse risk and supports individualised therapeutic decision-making [35].

Global Harmonisation

Despite technological progress, lack of universal harmonisation remains a major challenge [18,29]. Standardisation initiatives such as the International Scale for BCR::ABL1 monitoring and EuroFlow SOPs have substantially improved inter-laboratory reproducibility [29]. However, broader alignment among organisations including the ELN, NCCN, WHO, and EuroFlow Consortium is still required to establish universally accepted MRD frameworks [18,29]. Such harmonisation would improve comparability across studies, strengthen meta-analyses, and reinforce MRD as a validated clinical endpoint [2,29].

Future advances in MRD assessment will therefore depend on both technological innovation and international standardisation [4,18,20,27-29,35]. Single-cell MRD analysis and ecNGS promise unprecedented sensitivity and resolution, while AI-driven interpretation may reduce variability and improve reproducibility [4,35]. Together with global harmonisation efforts, these developments are expected to strengthen MRD as a reliable and indispensable component of precision leukaemia management [18,29].

CONCLUSION(S)

The MRD has become a cornerstone of acute leukaemia management, guiding treatment decisions, transplant planning, and relapse prediction. Meta-analyses show that pre-transplant MRD positivity is associated with inferior survival, while longitudinal MRD kinetics provide greater prognostic value than single assessments. Despite major advances, interpretive challenges remain, including haematogone overlap, antigen-negative relapse after CD19-directed therapies, and false-positive findings related to clonal haematopoiesis. MFC, RT-qPCR, and NGS provide complementary strengths, with the International Scale for BCR::ABL1 representing a landmark in assay harmonisation. Future progress will depend on standardised reporting, close laboratory-clinician collaboration, and integration of emerging technologies such as digital PCR, error-corrected sequencing, and single-cell profiling, which promise to further refine MRD assessment and strengthen its role in precision leukaemia care.

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