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NEXT-GENERATION DIAGNOSTICS IN ACUTE LYMPHOBLASTIC LEUKEMIA: FROM ULTRA-SENSITIVE MEASURABLE RESIDUAL DISEASE TO ARTIFICIAL INTELLIGENCE (2020–2026)

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ABSTRACT

Objectives: Acute lymphoblastic leukemia (ALL) diagnostics have changed rapidly in recent years, moving beyond conventional morphology, flow cytometry, and targeted molecular testing toward ultra-sensitive measurable residual disease (MRD) assays, genome-wide profiling, and artificial intelligence (AI). This review examines the major diagnostic advances introduced between 2020 and 2026 and assesses how close they are to routine clinical implementation.

Methods: A structured narrative review of PubMed, Embase, Scopus, and Web of Science was performed, focusing primarily on studies published between January 2020 and August 2026, with earlier landmark studies included where necessary.

Results: NGS-based MRD testing identifies residual leukemia beyond the sensitivity of conventional flow cytometry, including up to 46% of otherwise MRD-negative cases. Genome-wide approaches, particularly optical genome mapping and RNA sequencing, increase the detection of clinically relevant genomic abnormalities to approximately 90–95%. AI-based tools can substantially shorten flow-cytometric MRD analysis and support leukemia classification using routine laboratory data. However, their clinical readiness remains uneven, particularly for AI and liquid-biopsy approaches.

Conclusions: Diagnostic technologies in ALL are advancing faster than the evidence guiding their clinical use. The next challenge is therefore not simply detecting more diseases but determining which findings should change treatment. Standardization, prospective validation, accessibility, and integration into clinical pathways will be essential for translating technological progress into meaningful patient benefits.

KEYWORDS

Acute Lymphoblastic Leukemia, Measurable Residual Disease, Next-Generation Sequencing, Optical Genome Mapping, Artificial Intelligence, Molecular Classification

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1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and an aggressive disease in adults, whose outcomes remain substantially worse: cure rates approach 90% in children, whereas only 30–40% of adults achieve long-term remission and only 10–15% of patients over 60 years of age survive long-term [1,2]. The biological and clinical heterogeneity underlying this age-related survival paradox makes ALL a paradigm case in which diagnosis is not a formality that precedes therapy, but the primary determinant of therapy itself. Modern ALL treatment is risk-adapted: cytogenetic and molecular features, early treatment response and measurable residual disease (MRD) status jointly decide whether a patient receives chemotherapy alone, immunotherapy such as blinatumomab, targeted kinase inhibition, or allogeneic hematopoietic cell transplantation (HCT) [3]. Consequently, every improvement in diagnostic technology directly reshapes treatment decisions; MRD status strongly predicts survival in children and adults (event-free survival hazard ratios of 0.23 and 0.28, respectively) [4].

The classical diagnostic toolbox- bone marrow morphology and cytochemistry, multiparameter flow cytometry (MFC) immunophenotyping, conventional karyotyping, fluorescence in situ hybridization (FISH) and targeted polymerase chain reaction (PCR)- identified major ALL subtypes (e.g., BCR:ABL1, ETV6:RUNX1, KMT2A rearrangements, ploidy groups) but left approximately 30% of adult and 15% of childhood B-lineage ALL cases without an established genetic marker, assigned to the heterogeneous residual group “B-other” [5]. Table 1 summarizes the standard tools and the problems they leave open.

Table 1. The classical diagnostic toolbox and the open problems it leaves

Tool	Typical sensitivity	Open problem
Cytomorphology	qualitative	subjective blast assessment; high inter-observer variability
Multiparameter flow cytometry	MRD $\sim 10^{-4}$	operator-dependent gating; panel-dependent sensitivity
Karyotyping	~ 5 Mb resolution	culture failure; cryptic rearrangements invisible
FISH	targeted loci	only interrogates pre-selected regions
qPCR/RT-qPCR (BCR: ABL1, fusions)	MRD 10^{-4} – 10^{-5}	requires a known target; Ph+ only for BCR: ABL1
ASO-qPCR (IG/TR rearrangements)	MRD 10^{-5}	patient-specific reagents; labor-intensive
MLPA	targeted CNV panel	limited to panel content

Sources: [3,5,12,14,26–28].

Since 2020, three technological waves have begun to close these gaps. First, the *sensitivity revolution* in MRD: next-generation sequencing (NGS) of immunoglobulin (IG) and T-cell receptor (TR) rearrangements (clonoSEQ, the first NGS-based MRD assay authorized by the U.S. Food and Drug Administration) [20] reaches an analytical sensitivity of 10^{-6} , one to two logs beyond MFC and allele-specific oligonucleotide (ASO)-PCR, and its integration into risk algorithms has begun to redefine transplant eligibility [3,7]. Second, the *classification revolution*: whole-transcriptome and genome-wide structural-variant analysis have converted B-other into more than twenty named entities in the WHO-HAEM5 [9] and International Consensus Classification (ICC) [8]- including BCR: ABL1-like (Ph-like), DUX4-, MEF2D-, ZNF384- and NUTM1-rearranged ALL, PAX5 p.P80R and CDX2/UBTF- several of which carry targetable lesions or distinct prognosis [8,9]. Third, the *automation revolution*: artificial intelligence (AI) tools now classify blast morphology, interpret MRD flow cytometry in about one minute per case, and distinguish acute leukemia types (AML vs ALL vs APL) from routine laboratory data, validated across 6,206 patients in 20 centers [10]. Each wave operates at a distinct layer of the diagnostic pathway- the sensitivity wave at response monitoring, the classification wave at genomic characterization, and the automation wave at triage, confirmation and MRD interpretation- while integrative prediction draws on all three.

This review synthesizes the diagnostic innovations of 2020–2026 across three research questions: (RQ1) which platforms were introduced or validated, and where do they sit in the diagnostic pathway; (RQ2) whether ultra-sensitive MRD changes outcomes or merely detection; and (RQ3) which tools are genuinely ready for clinical implementation, and what separates the ready from the not-ready. In contrast to earlier, method-specific reviews, we integrate the MRD, classification and AI literatures into one pathway-level framework and score each technology on implementation readiness, thereby quantifying the gap between what is measurable, what is recommended, and what is realistically implementable worldwide.

2. Methodology

A structured narrative review of the literature was conducted using the PubMed, Embase, Scopus, and Web of Science databases. The search focused mainly on studies published between 2020 and 2026 concerning modern diagnostic methods in acute lymphoblastic leukemia, including measurable residual disease assessment, next-generation sequencing, optical genome mapping, RNA sequencing, liquid biopsy, and artificial intelligence. Older landmark publications were also included when they were important for explaining the background of currently used diagnostic methods. Original research articles and relevant reviews published in English were considered. After screening titles and abstracts, the most relevant studies were selected for full-text analysis, and their main findings were compared and summarized descriptively. Due to differences between the included studies in terms of patient populations, diagnostic methods, and reported outcomes, no meta-analysis was performed.

2.1 Implementation-readiness assessment

To quantify the distance between technical maturity and routine clinical implementation (Section 3.6, Table 5), each technology was scored on six domains: evidence level (E), guideline endorsement (G), regulatory status (R), standardization (S), cost-effectiveness data (C), and accessibility (A). Each domain was rated on a 0–3 ordinal scale (0, absent; 1, limited; 2, partial; 3, mature), giving a maximum of 18 points per technology; scores are expressed as the Implementation Readiness Score ($IRS = \text{total}/18 \times 100\%$). Scores were assigned by a single reviewer on the basis of the evidence summarized in Sections 3.1–3.5 and the sources cited in the reference list. The IRS is an original, qualitative, non-validated heuristic instrument; it ranks current implementability, not clinical value, and is interpreted accordingly in the Discussion.

3. Results

Findings span pediatric and adult cohorts, B- and T-lineage disease, bone-marrow and peripheral-blood sampling, and frontline, post-transplant and post-CAR-T settings.

3.1 The sensitivity revolution: ultra-sensitive measurable residual disease

Platform landscape and analytical capability. MRD is the strongest single predictor of relapse in ALL, consistently outperforming classical clinical and cytogenetic risk factors [14], and its assessment is now mandated at defined timepoints in both major guideline frameworks [3,12]. The three established platforms form a sensitivity staircase. MFC, the most widely used method, identifies leukemia-associated immunophenotypes at a sensitivity of approximately one leukemic cell in 10,000 nucleated cells (10^{-4}), although not all laboratories reproducibly achieve this depth [3,12]. Patient-specific ASO-qPCR for IG/TR rearrangements adds roughly one log (10^{-5}) but requires customized reagents for every patient and is therefore available only in specialized reference laboratories [3,12]. The NGS-based clonoSEQ assay amplifies and sequences IG heavy-chain, kappa and lambda rearrangements, achieving analytical sensitivity at 10^{-6} ; its analytical validation established a limit of detection of 1.903 malignant cells and a limit of quantitation of 2.390 cells across DNA inputs of 200 ng to 40 μ g [7]. A critical operational caveat is cellular input: an MRD-negative result at 10^{-6} carries 95% confidence only when at least 1.9 million nucleated cells are analyzed, so negativity on a suboptimal sample must be interpreted with caution [3]. ddPCR occupies a complementary niche, resolving “positive not-quantifiable” results by ASO-qPCR: in the AIEOP-BFM experience, ddPCR reclassified 13 of 45 day +78 samples as quantifiable positive, and most relapses occurred in this reclassified subgroup ($p < .001$), indicating that borderline signals between 10^{-4} and 10^{-5} carry real prognostic weight [13].

Head-to-head evidence and discordance. In comparative studies, NGS has shown greater analytical sensitivity than MFC at the same timepoints [14,15]. In the original clinical validation cohort of 110 children with ALL, clonoSEQ and MFC agreed in 95 of 105 samples (90%), but 10 of 105 samples (10%) were NGS-positive despite being undetectable by flow cytometry [14]. Larger adult series confirm and extend this: NGS detects residual disease in 46% of MFC-negative cases, and in a 74-patient adult cohort, early deep negativity at 10^{-6} was associated with 5-year overall survival of 90%, with no relapses observed among the early NGS-negative patients treated on hyper-CVAD/mini-hyper-CVD backbones [15]. In the post-transplant setting the discrimination is sharper still: among patients classified MRD-negative by MFC before allogeneic HCT ($n = 28$), those additionally NGS-MRD-negative ($n = 22$) had no relapses and 96% 2-year overall survival, versus 16% relapse and 77% overall survival for the MFC-negative cohort as a whole ($p = .02$ for relapse, $p = .003$ for survival) [16]. After CD19 CAR-T cell therapy, any detectable NGS-MRD level in bone marrow at day 28 identified high-risk patients, and at 3 months and at all later time points through month 12 the presence of any measurable disease was highly prognostic, with 41 of 42 patients with measurable disease relapsing or being censored for transplant or further therapy [17].

The growing sensitivity gradient, however, exposes a biology that simpler assays could not see: discordance between platforms is no longer a mere technical annoyance but a source of biological information (Table 2). The best-characterized example is Philadelphia-positive (Ph+) ALL, where BCR:ABL1 transcript monitoring by RT-PCR and IG/TR-based NGS were discordant in 32% of patients, with 31% of NGS-negative patients remaining PCR-positive; Among eight patients with long-term PCR positivity, the six PCR+/NGS– patients did not relapse (median follow-up of the study population, 84 months). In the combined cohort (20 PCR+/NGS– and 22 PCR–/NGS– patients), 5-year relapse-free survival was similar between the two groups (84% vs 73%; $p = .84$), indicating that low-level BCR:ABL1 often reflects non-leukemic clones or CML-like stem-cell involvement rather than viable ALL [18]. In a pediatric Ph+ cohort, Ig-NGS and RT-PCR were

discordant in 32% of 100 post-treatment samples (20 RT-PCR+/NGS-, 12 RT-PCR-/NGS+), and Ig-NGS detected MRD in all five of five patients tested within 150 days before relapse, versus three of five by RT-PCR and one of five by flow cytometry [19]. These findings suggest that, in Ph+ ALL, NGS negativity may be more specific for clinically relevant residual leukemia, and that isolated low-level PCR positivity in an NGS-negative patient should be interpreted cautiously and should not automatically trigger treatment escalation [18,19].

Table 2. A taxonomy of MRD discordance

Mechanism	Example	Interpretation
Non-leukemic transcript origin	BCR: ABL1 transcripts from CML-like or myeloid precursors in Ph+ ALL	PCR+/NGS- patients rarely relapse; do not escalate
Non-malignant clonal expansion	IG/TR or gene signals from non-malignant clones (e.g., new dominant sequences)	low-level signals without clonal growth are clinically silent
Subclone divergence	relapse clone differs from the diagnostic IG/TR marker	track ≥ 2 markers; consider different-from-normal MFC
Dying-cell DNA	persistent cell-free DNA after effective therapy	correlates with clearance kinetics, not viability
Assay noise below LOD	signals below the limit of detection/quantitation	repeat testing; require confirmatory kinetics

Sources: [3,7,18,19]. The mechanistic categories are the authors' synthesis of the cited literature and are presented as a heuristic framework.

Guideline divergence: ELN 2024 versus the US 2025 panel. The two most influential recommendation documents now visibly disagree on method preference, action thresholds and specimen source (Table 3). The 2024 European LeukemiaNet recommendations retain qPCR and MFC as the standard tools for IG/TR MRD, use the 10^{-4} threshold as the primary action point, keep bone marrow as the standard surveillance specimen, and mention NGS chiefly for molecular subtyping of cases such as Ph-like ALL [12]. The 2025 US expert panel goes substantially further: it recommends NGS as the preferred method for MRD-guided decision-making in B-ALL, supports intervention for any confirmed detectable MRD level, endorses peripheral-blood NGS monitoring once deep negativity has been documented, and explicitly supports transplant deferral for standard-risk patients who achieve early deep NGS negativity, including selected Ph+ cases, within ~3 months of frontline therapy with or without blinatumomab [3]. The divergence is best understood as a difference in diagnostic ecosystems rather than evidence interpretation: the US setting includes a single FDA-authorized, centralized NGS-MRD assay [20], while European practice is built on a distributed network of standardized qPCR/MFC reference laboratories and has prioritized harmonization (EuroMRD, EuroFlow) over maximum sensitivity [12]. Neither framework is inherently superior: the ELN approach maximizes reproducibility and equitable access across a harmonized reference network, whereas the US approach maximizes sensitivity within a single regulated assay ecosystem- each is a product of its diagnostic economy. Both frameworks converge on one point: persistent MRD should trigger escalation; in the single-arm BLAST trial, blinatumomab achieved complete MRD responses in 88 of 113 evaluable patients (78%) with MRD $\geq 10^{-3}$ [21].

Table 3. Guideline comparison matrix

Dimension	ELN 2024	US expert panel 2025
Preferred MRD method	qPCR / MFC for IG/TR	NGS for IG/TR (B-ALL)
Action threshold	10^{-4} (primary); positive below 10^{-4} : differing treatment consequences by risk group	any confirmed positivity
Surveillance specimen	bone marrow	BM until documented negativity, then PB permitted
Transplant deferral	not addressed as standard for deep NGS negativity	supported for standard-risk patients with early 10^{-6} negativity
Ultra-low MRD (10^{-5} – 10^{-6})	confirm orthogonally before acting	act if confident it is true leukemia

Sources: [3,12].

Clinical consequences. A central open question in the field is whether *early, deep* MRD negativity identifies a subgroup whose baseline biology is already favorable, or whether the assay itself changes outcomes- an association, not a demonstrated causal effect. In a 161-patient retrospective cohort with NGS-MRD at 10^{-6} , early negativity after one induction cycle was achieved by 33% of evaluable patients (26/79) and was associated with a two-year relapse-free survival of 94% versus 66% for MRD-positive patients ($p = .03$), with no relapses and one death in remission among the 26 early-negative patients- including high-risk Ph-negative cytomolecular profiles, whose two-year RFS was 100% versus 38% for high-risk patients remaining MRD-positive after induction ($p = .01$) [22]. These are landmark analyses at the end of induction; they exclude patients with early treatment failure and cannot exclude confounding indication, because MRD status reflects the response to the treatment received. Allogeneic transplant in first remission improved the two-year RFS of high-risk early MRD-positive patients from 0% to 80% ($p = .009$) [22]. Conversely, MRD clearance achieved only later is not equivalently protective, reinforcing the dynamic interpretation of MRD kinetics rather than single snapshots. Nevertheless, a decisive caveat remains: no prospective randomized trial has yet shown that intervening at 10^{-5} – 10^{-6} improves survival compared with observation, and both guideline documents acknowledge this explicitly [3,12,22]. The widening use of 10^{-6} assays has therefore produced an asymmetric situation- they are excellent instruments for de-escalation (identifying patients who will not relapse) but their escalation value below 10^{-4} remains inferential.

Implementation-readiness. NGS-MRD scores highest among all MRD platforms on evidence and regulatory maturity (FDA marketing authorization since 2018) [20] but is held back by cost and centralized logistics [38], as well as incomplete international standardization [12], which is being addressed by the EuroClonality-NGS working group [39]; MFC and ASO-qPCR retain the strongest accessibility and standardization profiles, respectively. The practical tension between these platforms- maximum sensitivity versus maximum reach- is a recurring theme of this review and is quantified in Section 3.6.

3.2 The classification revolution: from “B-other” to more than twenty molecular entities

Two competing classification systems. The WHO-HAEM5 [9] and the International Consensus Classification (ICC) [8] both restructure B-ALL around genetic drivers, but they are not identical: the ICC separates BCR:ABL1-positive ALL into lymphoid-only and multilineage forms, subdivides hypodiploid ALL, and defines nine new entities including seven rearrangement-defined categories and two mutation-defined categories (PAX5 p.P80R, IKZF1 N159Y), while WHO-HAEM5 keeps a single BCR:ABL1 entity and lists several of the same subgroups under “B-ALL with other defined genetic abnormalities”, whereas ETV6:RUNX1-like and PAX5-altered are provisional entities in the ICC; WHO-HAEM5 itself defines no provisional entities [8,9]. For clinicians, the practical consequence is that an ALL report now needs a technology-qualified entity statement- a challenge this review returns in the Discussion.

The entity–technology pairing problem. Each new entity is optimally detected by a different instrument (Table 4). Rearrangement-driven subtypes that are cryptic to cytogenetics- DUX4 (engaging the IGH enhancer), MEF2D, ZNF384, NUTM1- require RNA-based detection, while point-mutation entities (PAX5 p.P80R, IKZF1 N159Y) require DNA sequencing, and large structural classes (iAMP21, complex karyotypes) are best resolved by genome-wide structural-variant platforms [8,9,25]. Frequency and prognosis vary strikingly by age: DUX4-rearranged ALL carries an excellent prognosis in children (5-year EFS of 95.0%)

[23]; Ph-like ALL, with its targetable ABL-class and JAK-STAT alterations, accounts for approximately 10% of children with standard-risk ALL, rising to about 27% of young adults [24] and 21.2% of adults in the E2993 cooperative-group cohort [6]. MEF2D- and MYC-rearranged disease are uncommon and carry an unfavorable prognosis, and the rare CDX2/UBTF-deregulated ALL predominates in young women [25]. Four entities deserve emphasis because they exemplify different diagnostic–therapeutic logics. Ph-like ALL is the paradigm of diagnosis-driven therapy, because its kinase fusions are actionable with tyrosine kinase inhibitors and its JAK-STAT activation with JAK inhibition [24,25]. DUX4-rearranged ALL is the paradigm of diagnosis-driven *de-escalation*: an entity with excellent outcomes that is entirely invisible to karyotyping and FISH, historically misclassified as standard-risk B-other [23,25]. CDX2/UBTF exemplifies the co-occurring-lesion problem- two distinct driver events in every case- that only multi-omic profiling resolves [25]. PAX5 p.P80R exemplifies the mutational-entity problem, requiring NGS because no structural abnormality marks it [8,25].

Table 4. Selected molecular entities of B-ALL and their detection requirements

Entity	Frequency	Prognosis	Detection
BCR: ABL1 (Ph+)	~25–30% adults (up to ~50% in older adults)	historically poor; improved with TKI + immunotherapy	karyotype/FISH/RT-PCR
Ph-like (ABL-class / JAK-STAT)	~20–30% adults, 10–13% children	poor if untreated; targetable	RNA-seq/NGS panels, MLPA-tailored
ETV6:RUNX1(-like)	~25% children	excellent	FISH; -like forms need GEX/RNA-seq
High hyperdiploid	~30% children	excellent	karyotype/DNA index
Low hypodiploid / near-haploid	1–3% children; 5–15% adults (E2993 ~12–14%)	poor (esp. TP53-mutated)	karyotype + ploidy-specific panels
iAMP21	~2%	poor without intensification	FISH (RUNX1 probes)
KMT2A-r	infants; 5–15% adults	poor	FISH/RNA-seq
TCF3:PBX1	~5%	favorable to poor	karyotype/FISH
TCF3:HLF	rare	very poor	RNA-seq
DUX4-r	2–10% adults (Passet); 5–10% (ICC), higher in young adults	excellent	RNA-seq/GEX; cryptic
MEF2D-r	1–3% adults (Passet); 3–5% (ICC)	poor	RNA-seq/RT-PCR
ZNF384-r	2–8% adults (Passet); 5–10% (ICC), higher in AYA	variable by partner	RNA-seq
NUTM1-r	≤2%, infants	good	RNA-seq
MYC-r (IG: MYC)	1–5% older adults (Passet); 2–5% (ICC)	poor	FISH/RNA-seq
PAX5 p.P80R	3–6% adults (Passet); 2–5% (ICC)	favorable to intermediate	DNA NGS
CDX2/UBTF	1–4%, young women (adults)	poor	RNA-seq + CNV analysis
IKZF1 N159Y	<1%	intermediate	DNA NGS

GEX, gene expression profiling; AYA, adolescents and young adults. Sources: [6,8,9,24,25].

How much does NOS actually disappear. The pre-genomic “B-other” category comprised approximately 30% of adults and 15% of childhood B-ALL [5]. In adult cohorts analyzed with RNA-based fusion panels plus sequencing, a substantial proportion of B-other cases acquire a classifying alteration [11] (conference abstract, not peer-reviewed; specific reclassification and survival figures await peer-reviewed publication). Residual NOS after comprehensive multi-omics (approximately 11% in the E2993 cohort) [6] likely reflects genuinely unresolved biology- novel drivers below detection frequency, epigenetic mechanisms, or assay failures- rather than avoidable test gaps and should be reported as such rather than hidden.

T-ALL: the neglected sibling. WHO-HAEM5 and ICC recognize early T-precursor (ETP) ALL; the ICC additionally incorporates BCL11B-activated rearrangements into ETP taxonomy, whereas WHO-HAEM5 defines no genetically based T-ALL subtypes [8,9]. NGS-based IG/TR MRD is likewise not yet well validated in T-ALL [3]. T-ALL therefore exemplifies the asymmetry of the current revolution: classification has outpaced monitoring.

3.3 Genome-wide tools: optical genome mapping, RNA sequencing and digital MLPA

Optical Genome Mapping. OGM labels ultra-high-molecular-weight DNA at a motif-specific restriction site (DLE-1) and images individual molecules on a chip (Bionano Saphyr), producing a genome-wide structural map that detects balanced and unbalanced rearrangements, copy-number changes and complex events without cell culture, avoiding the culture-failure and selection limitations of conventional karyotyping, which requires viable metaphases and has a maximum banding resolution of approximately 5 Mb [26,27]. In the largest pediatric ALL series characterized by OGM in a clinical setting within a single institution ($n = 60$), OGM as a standalone test identified clinically relevant alterations in 90% of patients versus 46.7% for standard-of-care cytogenetics/FISH/MLPA, resolved 15% of previously non-informative cases, and detected gene fusions in 56.7% of patients versus 30% by standard-of-care methods and the targeted RNA panel ($p = .0057$; a descriptive comparison, with no confidence intervals reported); the combination of dMLPA and RNA-seq reached 95% (RNA-seq subset, $n = 20$) [28]. In a direct comparison against a 108-gene anchored-multiplex-PCR RNA-seq panel across 467 acute leukemias, overall concordance was 88.1%, with OGM uniquely detecting 15.8% of clinically relevant rearrangements- particularly enhancer-hijacking events involving IGH, MECOM or BCL11B, which produce no fusion transcript (concordance only 20.6%)- while RNA-seq was superior for fusions arising from intrachromosomal deletions that OGM may call as simple deletions [29]. In adult BCR: ABL1-negative B-ALL, integrating a fusion panel with OGM resolved complex karyotypes and uncovered classifying lesions missed by standard assays [11] (conference abstract, not peer-reviewed). OGM also refined the structural architecture of the IKZF1-plus profile beyond MLPA resolution [30].

The technology is not without blind spots: balanced events inside highly repetitive or telomeric regions (IGH: CRLF2 within PAR1, IGH: DUX4 at 4q) may fail to map; Robertsonian translocations such as rob (15;21)- linked to iAMP21- can be missed; and masked hypodiploidy (near-haploid genomes that endoreduplicate to apparent diploidy) can escape detection entirely, which is clinically dangerous because hypodiploidy is high-risk [27]. OGM is therefore best positioned as a reflex tool for normal, failed, inconclusive or discordant standard cytogenetics, and as a complement to NGS rather than a replacement for it [27,29]. Cost, instrument access and bioinformatics capacity remain the principal adoption barriers [27].

RNA sequencing and digital tools. Targeted fusion panels (e.g., FUSIONPlex ALL, Archer AMP chemistry) identify expressed chimeric fusions with turnaround times of days and modest bioinformatic demand; whole-transcriptome RNA-seq adds gene-expression-based subtype assignment and resolves entities without a discrete fusion, at higher cost and complexity [25,28,29]. dMLPA digitizes multiplex probe amplification for copy-number panels such as CNA-UKALL, achieving full concordance with conventional MLPA (38/60, 63.3%) and providing an orthogonal confirmation layer in integrated workflows [28]. The pragmatic summary of the benchmarking literature is that no single platform captures everything: RNA-based assays miss non-transcribed enhancer events; OGM misses point mutations and small indels; standard cytogenetics misses cryptic rearrangements [27–29]. A two-tier algorithm- standard panel-first with OGM and/or RNA-seq reflexed for unexplained cases- captures 90–95% of clinically relevant alterations in single-institution benchmarking (the 95% figure derives from a 20-patient RNA-seq subset) [28,29]; whether reflexing contains costs remains untested.

3.4 The automation revolution: artificial intelligence across the pathway

AI applications in ALL diagnostics cluster at three pathway layers, and their maturity differs sharply by layer. **Morphology.** At the morphology layer, convolutional neural networks classified AML blast cells at human-expert level as early as 2019 [31], and a recent fused deep-learning framework (XIncept-ALL) reported 99.5% accuracy on an external test set, with Grad-CAM saliency maps confirming that the network attends to clinically relevant cell features [32]. Real-world digital morphology systems, however, expose the gap between benchmark accuracy and diagnostic safety: in a 328-smear evaluation of an AI-assisted bone marrow analyzer, 95.4% of cells were correctly categorized, yet the correlation coefficients for lymphoblasts, prolymphocytes and promonocytes were unsatisfactory, and critical misclassifications occurred in B/T-lymphoma (9 of 43), multiple myeloma (5 of 46) and acute leukemia/CMML (4 of 17) cases, including small lymphoblasts called lymphocytes and granular monoblasts called promyelocytes [33]- a general haemato-oncology cohort with no B-ALL and a single T-ALL case. The operational conclusion is that morphology AI is currently a pre-screening and capacity tool under expert supervision, not a standalone interpreter [33].

Flow cytometry. At the flow-cytometry layer, the most tangible efficiency gain has been achieved. An AI-enhanced pipeline applied to 260 B-ALL MRD flow files reduced cellularity from 1.2 million to ~156,000 events per case (87% reduction) while preserving small MRD populations (median 100% retention for MRD

<1%), classified normal hematopoietic subsets with a macro-averaged F1 of 0.86, and discriminated leukemic from benign B-lymphoid cells with AUCs of 0.98 and 0.94; expert review of AI-enhanced files took 1.01 minutes per case with complete (100%) positive and negative agreement with conventional expert interpretation at the 0.01% MRD threshold in this single-center file set an internal-consistency result, with no external validation cohort reported and quantitative correlation of $R^2 = 0.92$ [34]. Because expert MRD gating time is the principal bottleneck limiting flow-MRD capacity [34], this class of tools — automating the automation bottleneck — is among the most immediately implementable AI contributions.

Triage and prediction. At the triage and prediction layers, machine learning on routine laboratory data (complete blood count, chemistry and coagulation parameters) distinguishes acute leukemia types (AML vs ALL vs APL, a three-class distinction, not molecular subtyping) without any specialized infrastructure. An XGBoost classifier originally developed in French cohorts was refined and validated internationally on 6,206 patients across 20 centers, with distribution-outlier detection substantially improving robustness and generalizability across analyzer platforms and healthcare settings [10]. For molecular subtyping, the ALLSorts hierarchical classifier assigns 18 B-ALL subtypes from RNA-seq data with 84–92% accuracy in an independent two-center (pediatric/adult) cohort, with a meta-subtype fallback design for ambiguous cases [35]. Governance has not kept pace with performance: reporting of key methodological details in the included AI studies was heterogeneous, reflecting the recent emergence of dedicated reporting standards for AI-based diagnostics (STARD-AI, TRIPOD-AI); what its sponsors describe as the first prospective trial of deep-learning hematologic diagnostics (BELUGA, NCT04466059) reached its estimated primary completion date in July 2025 [36]; and no ALL-specific diagnostic AI tool with FDA marketing authorization was identified in the reviewed literature. From a social perspective, the contrast within the AI class is instructive: the routine-laboratory-data classifier requires only universally available laboratory tests and could serve triage in low- and middle-income settings where flow cytometry and sequencing are unavailable, whereas scanner- and instrument-bound morphology tools risk widening, not narrowing, diagnostic inequity [10].

3.5 Liquid biopsy and epigenetic approaches: the frontier

Liquid biopsy- cell-free DNA (cfDNA), circulating tumor DNA, CSF-based assays and exosome/methylation signatures promises non-invasive ALL diagnosis and monitoring, and circulating methylated DNA plus circulating mRNA ensembles are being explored as multimodal classifiers for disease detection [37]. The biological ceiling is well defined: circulating tumor DNA constitutes only about 1% of plasma cfDNA a figure established pan-cancer rather than specifically for ALL and low tumor purity limits assay sensitivity [37], which is precisely the sensitivity range in which MRD decisions operate. No harmonized protocols, regulatory-cleared ALL assay or outcome-correlated cohorts were identified in the reviewed literature [37], giving liquid biopsy the lowest implementation-readiness score in this review (Table 5). Its most plausible near-term niches are CNS involvement assessment via CSF and reduced-burden surveillance, where serial sampling may compensate for sensitivity limits a hypothesis that remains untested prospectively.

3.6 Synthesis: technology map and the evidence–practice gap

Table 5 scores on every platform reviewed above on the six implementation-readiness criteria; Table 6 condenses the key studies and evidence sources. Three patterns emerge. First, the classical platforms (MFC, ASO-qPCR) remain with the readiness leaders- they are the workhorses whose weaknesses the new technologies were built to fix. Second, the high-sensitivity newcomers cluster in the middle: NGS-MRD, targeted NGS panels, OGM and RNA-seq have strong evidence and guideline footprints but are held back by cost, centralization, bioinformatics capacity and incomplete standardization. Third, AI and liquid biopsy sit at the bottom- not for lack of technical performance, but because regulatory approval, standardization and cost-effectiveness data are essentially absent. The IRS ordering thus does not rank clinical value; it ranks *implementability today*, and the discrepancy between the two rankings is a structured comparison rather than a validated ranking its value is heuristic, and it is treated as such in the Discussion.

Table 5. Implementation-readiness scoring of ALL diagnostic technologies (2026)

Technology	E	G	R	S	C	A	Total /18	IRS (%)
MFC MRD	3	3	2	3	3	3	17	94%
ASO-qPCR MRD	3	3	2	3	2	2	15	83%
dMLPA	2	2	2	2	3	2	13	72%
NGS-MRD (clonoSEQ-class)	2	2	3	2	2	1	12	67%
Targeted NGS panels	2	2	2	2	2	2	12	67%
RNA-seq (targeted/whole)	2	2	1	2	1	1	9	50%
OGM	2	1	2	2	1	1	9	50%
Routine-lab-data AI triage	2	0	0	1	3	3	9	50%
ddPCR	2	1	1	1	2	1	8	44%
AI morphology	2	1	1	1	1	1	7	39%
AI flow-MRD	2	1	1	1	1	1	7	39%
Liquid biopsy	1	0	0	0	1	1	3	17%

E, evidence level; G, guideline endorsement; R, regulatory status; S, standardization; C, cost-effectiveness data; A, accessibility. Anchors in Section 2.1. IRS = total/18 × 100%.

Table 6. Key studies and evidence sources, 2012–2026

#	Study (source)	Design, n	Core finding
1	[14] (Blood))	110 children, validation	NGS-MRD concordant with MFC 90%, ASO-PCR 96%; 10% of samples (10/105) NGS-positive despite negative MFC
2	[7] (BMC Cancer 20:612))	66 patient samples (21 ALL / 22 CLL / 23 MM), contrived	clonoSEQ LoD 1.903 cells, LoQ 2.390 cells; linear across 200 ng–40 µg DNA (cell-line spike-ins)
3	[22] (Blood Cancer J 15:169, DOI 10.1038/s41408-025-01373-y))	161 adults, retrospective single-center	early NGS negativity 33% (26/79 evaluable) after 1 cycle → 2-yr RFS 94% vs 66% (p = .03); HR Ph-negative early-negative 2-yr RFS 100% vs 38% (p = .01); allogeneic transplant (HCT) in early MRD+ HR: 2-yr RFS 0% vs 80% (landmark, p = .009)
4	[18] (Am J Hematol 98(8):1196–1203))	44 + 65 Ph+ ALL cohorts (MD Anderson)	32% RT-PCR/NGS discordance; 31% of NGS-negative PCR+; PCR+/NGS– did not relapse (median follow-up 84 mo; 5-yr RFS 84% vs 73% for PCR–/NGS–, p = .84); prospective: 17% (11/65)
5	[16] (Blood 125(22):3501–3508))	41 children with pre-HCT NGS-MRD (22 negative / 19 positive)	NGS-negative: 0% relapse vs 16% MFC-negative; 2-yr OS 96% vs 77%
6	[17] (Blood Cancer Discov 3(1):66–81))	tisagenlecleucel cohorts	day 28 any detectable BM MRD = high risk; any measurable disease at months 3–12 highly prognostic; 41/42 relapsed or censored (HCT/other therapy)
7	[13] (HemaSphere 5(3): e543))	209 children, AIEOP-BFM	ddPCR reclassified 13/45 POS-NQ Day +78 samples; most relapses occurred in this subgroup (p < .001)
8	[28] (Br J Cancer 133(11):1744–1754))	60 pediatric ALL (single-institution, largest pediatric-ALL OGM series)	OGM 90% vs SoC 46.7% yield; dMLPA+RNA-seq 95% (RNA-seq subset n = 20); dMLPA concordant with MLPA for CNA-UKALL (38/60, 63.3%); 15% non-informative resolved
9	[29] (Cancers 17(21):3458))	467 acute leukemias (77% AML; ALL n = 101)	OGM vs 108-gene RNA-seq case-level concordance 88.1% (event-level 175/234, 74.7%); OGM unique 15.8% and RNA-seq unique 9.4% of 234 events; enhancer-hijacking: OGM superior

#	Study (source)	Design, n	Core finding
10	[11] (Haematologica 111(s1):CO55; SIES congress abstract — not peer-reviewed))	75 adult BCR: ABL1-negative B-ALL	integrated NGS+OGM workflow; reclassification and survival figures presented in abstract only (illustrative)
11	[25] (Blood 145(14):1451–1463))	narrative review, adult B-ALL	>20 genetic subtypes; age-dependent distribution; Ph-like 21.2% of E2993 adults [6]
12	[10] (Nat Commun 17:2649)	6,206 patients, 20 centers	routine-laboratory-data AI subtype classification internationally validated; outlier detection improved robustness
13	[34] (PMID 40966417)	260 B-ALL MRD flow files	AI pipeline: 1.01 min/case review, 100% agreement $\geq 0.01\%$ vs expert (internal, single-center), AUC 0.94–0.98
14	[32] (Front Med 12:1694024))	training 9,395 images (Pak-ALL, 500 subjects); external test 3,256 images (89 subjects, Iranian)	XIncept-ALL 99.5% external accuracy; Grad-CAM interpretability; 4-class research staging (non-standard clinically)
15	[33] (Clin Transl Med 15(7): e70364))	328 BM smears	AI cytomorphology 95.4% correct; weak for lymphoblasts, prolymphocytes and promonocytes (MCC < 0.40)
16	[36] (BELUGA, NCT04466059)	prospective case-control observational; enrollment est. 25,000; training corpus 25,000 smears + 25,000 FC data points	first prospective AI diagnostic validation (sponsor claim); estimated primary completion July 2025, still recruiting at last registry update
17	[37] (IJMS 26(15):7547)	review	liquid biopsy + methylome/circulating RNA; standardization absent
18	[19] (Front Immunol 16:1677013))	pediatric Ph+ ALL	Ig-NGS vs RT-PCR discordant in 32% of 100 samples (20 RT-PCR+/NGS–, 12 RT-PCR–/NGS+); Ig-NGS positive in 5/5 tested ≤ 150 days pre-relapse (RT-PCR 3/5; flow cytometry 1/5); 2/7 negative by all methods

4. Discussion

4.1 Answers to the research questions

RQ1- Which platforms arrived, and where do they operate? Three waves, three pathway layers. The sensitivity wave (NGS IG/TR, ddPCR) operates at the monitoring layer (L3) and does not change diagnosis but *response definition*. The classification wave (targeted NGS, RNA-seq, OGM, dMLPA) operates at the characterization layer (L2) and changes what the disease *is* replacing the residual category B-other with named, prognostically and therapeutically distinct entities. The automation wave (morphology, flow-cytometry and laboratory-data AI) operates at the triage, confirmation and interpretation layers (L0/L1/L3) and changes *who does the work and how fast*. No platform dominates across layers; the mature 2026 diagnostic report is inherently multi-platform, which is precisely why algorithmic, reflex-based workflows matter more than any single assay.

RQ2- Does ultra-sensitive MRD change outcomes or merely detection? Both, but asymmetrical. The evidence for *better detection* is unambiguous: NGS detected MRD in 10% of all validation samples (10/105) [14] and in 46% of MFC-negative adult cases [15], and reclassification tracks outcome. The evidence for *changed outcomes* is strongest in the de-escalation direction: early deep negativity at 10^{-6} identifies patients including high-risk cytotoxic profiles — whose relapse risk is so low that transplant can be deferred, a decision with immediate toxicity and mortality consequences [3,22]; this rests on cohort and landmark evidence, and no randomized trial has yet tested transplant omission based on 10^{-6} negativity, so residual confounding cannot be excluded [3,12,22]. The escalation direction is weaker: no randomized evidence yet shows that acting on 10^{-5} – 10^{-6} levels improve survival, and the ELN's cautious stance- which sets no treatment trigger below 10^{-4} - is well founded [12]. An unanticipated benefit is biological: platform discordance, particularly PCR-positive/NGS-negative BCR: ABL1 states in Ph+ ALL that do not relapse, revealed that assay specificity can matter more than sensitivity for therapy decisions [18]. The practical synthesis: early deep NGS negativity may support treatment de-escalation in selected patients, whereas persistent MRD at any depth should prompt careful consideration of treatment intensification in accordance with clinical context and current guidelines [3,12,22].

RQ3- Which tools are ready? Two clusters emerge from Table 5. The mature cluster (MFC-MRD, ASO-qPCR, dMLPA) is ready and globally available; the evidence-rich newcomers (NGS-MRD, panels, OGM, RNA-seq) are ready in specialized centers and not ready everywhere else; the frontier cluster (AI, liquid biopsy) is not ready for unsupervised deployment despite impressive technical performance. Readiness, in other words, is now a property of *institutions and markets*, not of assays.

4.2 The quantified gap and its causes

The IRS spreads from 94% (MFC-MRD) to 17% (liquid biopsy), and the ordering does not track clinical value. Three systemic causes dominate. First, **reimbursement and logistics**: the single FDA-authorized NGS-MRD assay is centralized [20], which concentrates capacity; NICE's Medtech Innovation Briefing on clonoSEQ engaged with its evidence base, noting the absence of NHS-context studies and UK cost data; like all MIBs it did not assess cost-effectiveness [38]. Second, **harmonization**: although the EuroClonality-NGS consortium is working toward interoperable pipelines [39], NGS-based MRD is not yet incorporated into clinical practice in many countries [12], which limits guideline adoption beyond single-assay markets. Third, **bioinformatics and regulatory capacity**: OGM and RNA-seq require specialized bioinformatic expertise [27–29], and regulatory and governance frameworks for diagnostic AI remain under development in most jurisdictions; dedicated reporting standards (STARD-AI, TRIPOD-AI) can narrow this governance gap but not close it.

4.3 Controversies that define the fields near future

Five disagreements deserve explicit treatment because each will shape practice over the next five years. **(1) Ultra-low MRD actionability.** De-escalation at 10^{-6} is increasingly endorsed; escalation at 10^{-5} – 10^{-6} is not proven [3,12,22]. Until randomized trials are read out, the defensible position is the asymmetric one stated above. **(2) Bone marrow versus peripheral blood.** The US panel permits PB monitoring after documenting deep negativity; ELN retains BM as standard. PB sampling is less invasive and enables higher-frequency surveillance, but the lower disease burden in peripheral blood may blunt sensitivity compared with bone marrow [3,12]; the two sides are optimizing different endpoints (sensitivity versus sampling burden), which explains the stalemate. **(3) OGM as reflex versus first line.** OGM resolves a meaningful fraction of otherwise unexplained cases, but its blind spots (masked hypodiploidy, repetitive-region fusions) mean it cannot safely stand alone; reflex use after inconclusive standard cytogenetics is currently the cost-conscious synthesis [27–29]. **(4) AI liability and interpretability.** Accuracy benchmarks are no longer the obstacle [10,32–34]; unanswered questions about who is responsible for an AI-assisted miss, and how data drift across instruments and centers is monitored, remain. **(5) Dual classification.** The coexistence of WHO-HAEM5 and ICC means the same patient may receive differently named entities depending on the reporting laboratory; journals and consortia should require dual reporting with the underlying alteration named explicitly, so that BCR: ABL1-like versus Ph-like style divergences do not erode data comparability [8,9].

4.4 The social dimension: innovation as an equity question

From a technology-and-society perspective, the equity reading of these data deserves emphasis. The highest-readiness tools are also the oldest and cheapest- but their sensitivity ceiling is exactly what the new tools exist to break. Newer technologies generally require greater infrastructure, including centralized sequencing, specialized instrumentation and bioinformatic expertise, which may limit their accessibility outside highly resourced centers [27,28,38]. The partial exception is instructive: the routine-laboratory-data classifier requires only universally available laboratory tests and was validated across 20 international centers with explicit attention to analyzer heterogeneity [10] a triage instrument that could shorten time-to-diagnosis in settings lacking flow cytometry. This suggests a policy principle: *prioritize innovations whose marginal infrastructure requirement is lowest*, while treating high-infrastructure platforms as referral-network tools rather than universal standards. Otherwise, the sensitivity revolution will widen the very survival gap it was built to close.

4.5 Strengths and limitations

The strengths of this review are its pathway-level integration of literatures usually reviewed separately, and its transparent, criterion-anchored readiness scoring. Its limitations are equally clear: the search was English-only with a 2020–2026 window (landmark earlier work included only as anchors); publication bias is likely; the IRS is a qualitative, ordinal instrument scored by a single reviewer and is not a validated metric; quantitative pooling was precluded by heterogeneity; one cited source is a non-peer-reviewed conference abstract and supports only qualitative statements; and the field's publication rate means some late-2026 evidence may be missing. The novel technologies reviewed rest predominantly on retrospective, single-center designs, which motivated conservative wording of effect estimates throughout this review.

4.6 Research agenda

Ranked by the size of the gap they would close and by feasibility: (1) randomized trials of intervention at ultra-low MRD (10^{-5} – 10^{-6}), stratified by subtype the single most consequential open question; (2) completion of international NGS-MRD harmonization with quantitative interoperability (EuroClonality-NGS); (3) prospective, registry-embedded validation of AI tools, including in low- and middle-income settings; (4) prospective evaluation of OGM-integrated diagnostic algorithms with cost-outcome modeling; (5) NGS-MRD validation in T-ALL, the monitoring gap left by the classification revolution; and (6) focused liquid-biopsy trials for CNS disease and reduced-burden surveillance, where sampling logistics favor its strengths.

5. Conclusions

Five conclusions follow from this synthesis, each stated as claim, evidence level, and implication. First, ultra-sensitive MRD detection is analytically mature and clinically prognostic, but its actionability below 10^{-4} remains inferential (multiple multicenter cohorts and consensus guidance; no randomized trials) the priority is randomized evidence, not further sensitivity gains. Second, more than twenty molecular entities now replace the residual B-other category, and their detection is technology-specific an ALL diagnosis without a genomic entity statement is increasingly incomplete (WHO-HAEM5/ICC classifications and multi-institutional genomic studies). Third, genome-wide platforms (OGM, RNA-seq, dMLPA) approximately double the diagnostic yield of standard cytogenetics when reflexed appropriately [28,29] (single-institution benchmarking cohorts; prospective multicenter data pending) but their blind spots mean complementarity, not substitution. Fourth, AI has reached promising assistive performance in morphology and flow-MRD interpretation, but external prospective validation remains limited [32–34] validation, regulation and interpretability, not accuracy, are now the gating problems. Fifth, the field's central bottleneck has migrated from measurement to implementation: harmonization, reimbursement and equity will determine whether the technological advances of 2020–2026 become survival gains for the patients who need them most (implementation-readiness scoring, this review).

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