

Short Review

The Application of Next-Generation Sequencing in LeukemiaKonstantinos Agiannitopoulos ¹, Elisavet Kouvidi ^{2,*}1. Faculty of Biology, University of Athens, Athens, Greece; E-Mail: kagiannitopoulos@yahoo.gr2. Phenotypos Lab, Katechaki 40A, Athens, Greece; E-Mail: ekouvidi@gmail.com* **Correspondence:** Elisavet Kouvidi; E-Mail: ekouvidi@gmail.com**Academic Editor:** Alexandr Sember**Collection:** [Genetic Testing](#)*OBM Genetics*

2025, volume 9, issue 4

doi:10.21926/obm.genet.2504321

Received: May 02, 2025**Accepted:** December 17, 2025**Published:** December 25, 2025**Abstract**

Leukemia is a heterogeneous group of hematologic malignancies characterized by the dysfunctional proliferation of white blood cells in the bone marrow. Genetic alterations are important risk factors for the development and progression of leukemia, and their detection is crucial. Although many genetic techniques, including karyotyping, fluorescence *in situ* hybridization (FISH), and polymerase chain reaction (PCR), have provided valuable information, they all have the limitation of incomplete genomic coverage. The evolution of genomic technologies, including Next Generation Sequencing (NGS) and Third Generation Sequencing (TGS), has enabled a more comprehensive and detailed characterization of the genetic landscape of leukemia. NGS technology has revolutionized leukemia diagnosis, treatment, and minimal residual disease monitoring, and its integration into routine leukemia care will enhance patient outcomes and pave the way for truly personalized medicine.

Keywords

Hematologic malignancies; karyotyping; FISH; PCR; NGS; diagnosis; treatment; minimal residual disease



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1. Introduction

Leukemia is a diverse and complex group of hematological malignancies that originate in the bone marrow and affect blood cell production. It is characterized by the uncontrolled proliferation of abnormal white blood cells, which interfere with normal hematopoiesis and immune function [1, 2]. The development and progression of leukemia are driven by a variety of genetic mutations, chromosomal abnormalities, and epigenetic changes, all of which contribute to disease heterogeneity. These genetic alterations can influence leukemia classification, prognosis, and treatment response, underscoring the importance of molecular diagnostics in disease management [3].

1.1 Traditional Diagnostics

Historically, leukemia diagnosis has relied on a combination of morphological assessment, immunophenotyping, cytogenetics, and molecular techniques [4]. Conventional cytogenetics, or karyotyping after G-banding, provides a whole-genome view and enables the detection of large-scale structural and numerical chromosomal rearrangements, including translocations, deletions, duplications, inversions, and aneuploidies, at a single-cell level [4]. Cytogenetic analysis has been instrumental in identifying hallmark leukemia-associated chromosomal rearrangements, such as the Philadelphia chromosome, originating from the translocation t(9;22)(q34;q11) in more than 90% cases of t(9;22) in chronic myeloid leukemia (CML) cases and some acute lymphoblastic leukemias (ALL) [2, 4]. Moreover, it can reveal clonal diversity in leukemia by identifying multiple clones with distinct chromosomal abnormalities. Cytogenetic analysis is a crucial tool for assessing prognosis, diagnosing, risk-stratifying, assessing relapse risk, and guiding treatment in various leukemias. However, this method is limited by the need to divide cells, its resolution, and its inability to detect cryptic chromosomal rearrangements smaller than 5 Mb [5].

Fluorescence *In Situ* Hybridization (FISH) is a molecular cytogenetic technique that uses fluorescent probes to detect specific chromosomal abnormalities, including translocations, deletions, and amplifications, in both dividing and non-dividing cells. It is handy for identifying submicroscopic, recurrent leukemia-associated genetic alterations that result in the formation of chimeric fusion genes, such as the t(8;21)(q22;q22) translocation in AML, between *RUNX1* (*Runt-related transcription factor 1*) and *RUNX1T1* (*RUNX1 translocation partner 1*) genes, creating RUNX1::RUNX1T1 fusion gene, or the t(9;22)(q34;q11) translocation in CML, between *BCR* (*breakpoint cluster region, serine/threonine kinase activity*) and *ABL1* (*ABL proto-oncogene 1, non-receptor tyrosine kinase*) genes, resulting in BCR::ABL1 fusion gene. While FISH is more sensitive and faster than karyotyping, it still detects only the targeted abnormalities for which probes are designed [6]. Polymerase Chain Reaction (PCR)-based techniques enable the targeted amplification and detection of specific genetic mutations or fusion transcripts, such as *NPM1* (*nucleophosmin 1*) and *FLT3* (*FMS-like tyrosine kinase 3*) mutations, or the PML::RARA fusion gene in AML, resulting from the t(15;17)(q24;q21) translocation between the *PML* (*promyelocytic leukemia*) and *RARA* (*retinoic acid receptor alpha*) genes. Quantitative PCR (qPCR) is often used to monitor minimal residual disease (MRD) in patients with leukemia. However, PCR-based methods are limited in scope as they can only analyze predefined genetic targets [7].

1.2 NGS in Leukemia

While traditional diagnostic methods provide valuable information, they often fail to capture the full genetic complexity of leukemia. Next-generation sequencing (NGS) overcomes these limitations by enabling comprehensive, high-throughput genomic analysis. The term NGS encompasses several short-read and long-read sequencing technologies based on massively parallel sequencing. NGS has fundamentally transformed the fields of genomics and molecular diagnostics by providing rapid and cost-effective genetic analysis through methodologies, such as targeted gene panels, Whole Exome Sequencing (WES), and Whole Genome Sequencing (WGS) [8, 9]. Unlike traditional sequencing methods, which are labor-intensive and limited in scope, NGS-based approaches enable the simultaneous sequencing of millions of DNA fragments and the comprehensive examination of the genetic landscape of leukemia.

NGS can detect a wide range of genetic alterations, including single-nucleotide variants, insertions and deletions (indels), copy number variations (CNVs), and structural rearrangements [5]. SNVs are point mutations in specific genes that can impact leukemia development, progression, and treatment response. For example, mutations in *TP53* (*tumor protein p53*), *ASXL1* (*additional sex combs-like 1*), and *RUNX1* are associated with poor prognosis in AML, while mutations in *NOTCH1* (*notch receptor 1*) and *SF3B1* (*splicing factor 3b subunit 1*) play a role in chronic lymphocytic leukemia (CLL) [10]. Indels are small insertions and deletions of nucleotides that can disrupt gene function and contribute to leukemogenesis. For instance, *NPM1* mutations, which involve small insertions, are commonly seen in AML and have important prognostic implications [11]. CNVs are changes in gene dosage, such as amplifications or deletions, which are crucial in leukemia classification. Deletions of genes like *IKZF1* (*IKAROS family zinc finger 1*) in ALL are associated with a high risk of relapse [12]. Structural chromosomal rearrangements, including translocations, deletions, or inversions, can lead to the formation of fusion genes, which represent recurrent genetic events commonly observed in leukemia [13]. Epigenetic modifications such as DNA methylation changes and aberrant alternative splicing can contribute to leukemogenesis and therapy resistance [14].

NGS technology has been particularly impactful in the study and management of hematological malignancies such as leukemia. One of the significant advantages of NGS in leukemia diagnostics is its ability to uncover driver mutations and clonal evolution patterns, which provide insights into molecular disease mechanisms and can help predict disease progression and treatment resistance [15, 16]. By identifying specific genetic alterations associated with different leukemia subtypes, NGS facilitates more precise classification, refines risk stratification and guides targeted therapy selection [17, 18].

Additionally, NGS plays a critical role in the development and implementation of precision medicine approaches for leukemia treatment. By identifying actionable mutations, clinicians can tailor treatments to individual patients, improving therapeutic outcomes and minimizing unnecessary toxicity [19, 20].

Beyond diagnosis and treatment selection, NGS is increasingly used for MRD detection, early relapse detection, and tracking clonal evolution during therapy. This real-time genetic monitoring enables dynamic treatment adjustments, improving long-term patient outcomes [21, 22].

1.3 Risk Stratification

NGS has significantly improved risk stratification in leukemia by providing detailed insights into the genetic alterations that influence disease progression, define disease subtypes, predict outcomes and relapse risk, and guide therapeutic decision-making, including the choice between intensive chemotherapy and allogeneic stem cell transplantation. Traditionally, risk assessment in leukemia has relied on a combination of clinical and laboratory parameters, including age, white blood cell count, and results from cytogenetic and molecular analyses [23].

NGS offers a complementary perspective by enabling parallel analysis of hundreds of genes and high-resolution detection of point mutations, small insertions/deletions, and copy number changes. Importantly, NGS does not replace classical cytogenetics or FISH but rather broadens the analytical spectrum, bridging the gap between cytogenetic architecture and molecular detail. The integration of NGS with cytogenetic and molecular data thus provides a more complete and precise framework for leukemia risk stratification [24]. AML is a highly heterogeneous disease with variable survival outcomes influenced by distinct genetic and cytogenetic abnormalities. Advances in NGS have refined risk stratification by identifying mutations that classify patients into favorable, intermediate, or adverse prognostic groups. Among these, *NPM1* mutations in the absence of *FLT3-ITD* (*FMS-like tyrosine kinase 3 – internal tandem duplication*) are associated with a favorable prognosis, particularly in younger patients [25, 26]. *CEBPA* biallelic mutations are linked to improved survival and lower relapse rates [27]. *FLT3-ITD* low allelic ratio could confer intermediate risk. Still, targeted therapy with FLT3 inhibitors (e.g., midostaurin, gilteritinib) improves outcomes, while a high allelic ratio is associated with aggressive disease and a high relapse rate. *TP53* mutations are strongly linked to chemotherapy resistance and poor survival, often requiring alternative treatment approaches [28]. *ASXL1*, *RUNX1*, and *DNMT3A* mutations contribute to adverse prognosis and influence decisions regarding stem cell transplantation [29].

By incorporating these molecular markers into risk models, NGS enables clinicians to make personalized treatment decisions, such as identifying patients who would benefit from intensive chemotherapy, targeted therapies, or allogeneic stem cell transplantation.

In ALL, genetic alterations play a crucial role in predicting treatment response and relapse risk. NGS has helped classify ALL into molecular subtypes, many of which have distinct prognostic implications. *ETV6-RUNX1* fusion is common in pediatric ALL and is associated with a high cure rate. Hyperdiploidy, defined as the presence of more than the normal diploid number of chromosomes (typically 47–57 per cell), is a common chromosomal abnormality in ALL and is associated with a favorable prognosis, indicating a good response to standard chemotherapy [30]. *IKZF1* deletions are associated with high relapse rates, particularly in B-cell ALL [31]. The Philadelphia chromosome gives rise to the *BCR-ABL1* fusion gene, which encodes a constitutively active tyrosine kinase that promotes uncontrolled cellular proliferation. Historically associated with high-risk leukemia, the prognosis of BCR–ABL1-positive disease has been dramatically improved by the introduction of tyrosine kinase inhibitors (TKIs), such as imatinib and dasatinib, transforming it into a targetable and manageable subtype [32]. Rearrangements involving *CRLF2* (*cytokine receptor-like factor 2*) in B-cell ALL lead to overexpression of the CRLF2 receptor and activation of downstream signaling pathways, thus contributing to the development and maintenance of leukemia. These alterations are associated with poor prognosis and are observed at a higher frequency in specific patient

populations, including Hispanic patients and individuals with Down syndrome, which may contribute to their relatively adverse outcomes [33].

Beyond risk stratification, NGS is essential for detecting mutations associated with therapy resistance. Resistance mechanisms can be broadly categorized as primary (present at diagnosis, conferring an inherent lack of response) or secondary (acquired during treatment, driving relapse after an initial response). Primary resistance includes mutations such as *FLT3-ITD* in AML, which reduce the efficacy of conventional chemotherapy, and *TP53* mutations across AML, ALL, and CLL, which are strongly linked to chemotherapy resistance and poor prognosis [34, 35].

Secondary resistance arises when mutations develop under therapeutic pressure. For example, *BTK* (Bruton's tyrosine kinase) and *PLCG2* mutations in CLL emerge during treatment with BTK inhibitors like ibrutinib, necessitating alternative therapies such as venetoclax. Similarly, additional mutations in *FLT3* or other signaling pathways can drive relapse despite targeted therapy [36, 37]. By distinguishing between primary and secondary resistance, NGS provides critical insights into why patients fail standard regimens and helps guide the selection of next-line targeted or experimental therapies.

1.4 Advantages of NGS in Targeted Therapy

The integration of molecular and cytogenetic analyses has been pivotal in the development of targeted therapies for leukemia. NGS enables the precise identification of genetic mutations and molecular abnormalities, while conventional cytogenetics provides critical information on chromosomal alterations, together guiding risk stratification and personalized treatment approaches [38]. Unlike traditional chemotherapy, which broadly attacks rapidly dividing cells and often causes significant toxicity, targeted therapies focus on specific genetic alterations within leukemia cells, leading to more effective treatment with fewer side effects. By analyzing the genetic landscape of leukemia at high resolution, NGS has allowed researchers and clinicians to identify actionable mutations and design targeted drugs that specifically block mutated proteins, inhibit abnormal signaling pathways, and reprogram the leukemia cell environment to promote disease control [39] (Table 1).

Table 1 Detection of key genetic alterations in different leukemias using Next-Generation Sequencing (NGS) and their clinical significance.

Leukemia Type	Key Mutations/Alterations Detected by NGS	Clinical Significance
Acute Myeloid Leukemia (AML)	<i>NPM1</i> , <i>FLT3-ITD</i> , <i>CEBPA</i> , <i>IDH1/2</i> , <i>TP53</i> , <i>ASXL1</i> , <i>RUNX1</i> , <i>DNMT3A</i>	Refines risk stratification; guides targeted therapies (e.g., <i>FLT3</i> and <i>IDH</i> inhibitors); prognostic implications (favorable vs. adverse risk)
Acute Lymphoblastic Leukemia (ALL)	<i>ETV6-RUNX1</i> fusion, <i>IKZF1</i> deletions, <i>CRLF2</i> rearrangements, <i>BCR-ABL1</i> (Philadelphia chromosome), hyperdiploidy	Defines molecular subtypes; predicts relapse risk; guides targeted therapy (e.g., TKIs for <i>BCR-ABL1</i> -positive ALL)

Chronic Lymphocytic Leukemia (CLL)	<i>NOTCH1, SF3B1, TP53, BTK, PLCG2</i> mutations	Predicts prognosis and therapy resistance; identifies resistance to <i>BTK</i> inhibitors (ibrutinib) and supports switching to venetoclax or other targeted therapy
Acute Promyelocytic Leukemia (APL)	<i>PML-RARA</i> fusion	Diagnostic hallmark; predicts sensitivity to ATRA/arsenic trioxide; enables MRD monitoring

Traditional bulk NGS provides an average representation of all genetic alterations in a leukemia sample, thereby greatly advancing our understanding of the mutational landscape. However, bulk sequencing cannot resolve tumor heterogeneity or distinguish between different subclones, thereby limiting insights into clonal architecture and treatment resistance [40]. In contrast, cytogenetic analysis, although less comprehensive at the nucleotide level, retains the advantage of examining cells individually. This cell-by-cell resolution can reveal structural and numerical chromosomal abnormalities as well as clonal diversity that bulk NGS may obscure. Nevertheless, cytogenetics lacks the sensitivity to detect smaller genetic lesions and subclonal mutations that are readily captured by sequencing-based approaches [41]. Single-cell sequencing (scRNA-seq and scDNA-seq) has recently emerged as a powerful tool that combines the strengths of both approaches. It enables the dissection of tumor heterogeneity, the tracking of clonal evolution over time, and the identification of rare resistant subclones with potential clinical significance. Such findings highlight the potential of single-cell technologies to refine risk stratification and guide precision medicine strategies in leukemia [15].

As summarized in Table 2, each genomic method offers unique strengths and constraints that together contribute to a comprehensive understanding of leukemia genetics. Conventional cytogenetics remains a cornerstone of hematologic diagnostics, not only for its ability to identify large chromosomal abnormalities but also because it uniquely allows direct visualization and analysis of individual cells, thereby revealing clonal diversity within a sample, an advantage lost in most sequencing-based approaches [42]. FISH extends this capability to both dividing and interphase cells, enabling targeted detection of specific rearrangements. However, because FISH probes hybridize only to predetermined genomic regions, it cannot provide a comprehensive overview of the genome [43]. Methods such as comparative genomic hybridization (CGH) and its array-based derivative (array-CGH) share hybridization principles with FISH and allow genome-wide copy-number assessment, yet they lack the nucleotide-level precision achieved by sequencing technologies [44].

Table 2 Advantages and limitations of genetic techniques used in leukemia analysis.

Technique	Advantages	Limitations
Conventional Cytogenetics (Karyotyping)	Provides a whole-genome view; Detects large chromosomal abnormalities (translocations, deletions, duplications) at a single cell level; reveals clonal heterogeneity; well-established in leukemia classification (e.g., Philadelphia chromosome in CML) well established in leukemia classification (e.g., t(9;22) Philadelphia chromosome in CML)	Requires actively dividing cells; low resolution (>5 Mb); unable to detect cryptic or submicroscopic rearrangements
Fluorescence <i>In Situ</i> Hybridization (FISH)	Applicable to both dividing and non-dividing cells; sensitive, rapid, and allows cell-by-cell analysis of specific translocations or copy number changes (e.g., <i>BCR-ABL</i> , <i>RUNX1-RUNX1T1</i>); adaptable to multiplex or locus-specific probes	Provides targeted rather than genome-wide information, because probes hybridize only to selected loci; although comparative genomic hybridization (CGH) and array-CGH share conceptual similarity with FISH and offer genome-wide copy number assessment, they still lack the base-pair resolution of sequencing.
PCR-based Methods (qPCR, RT-PCR)	Highly sensitive; detects specific mutations or fusion genes (e.g., <i>NPM1</i> , <i>FLT3</i> , <i>PML-RARA</i>); useful for MRD monitoring	Restricted to known targets; unable to detect novel or unexpected alterations
Next-Generation Sequencing (NGS)	High-throughput and comprehensive; detects SNVs, small indels, and CNVs; supports risk stratification, targeted therapy design, and MRD detection; Illumina-based short-read sequencing is now cost-efficient and widely accessible due to rapid price reductions	Short-read platforms (e.g., Illumina) have limited capability to detect large or complex structural variants and repetitive regions; interpretation of large data sets requires robust bioinformatics pipelines
Third-Generation Sequencing (Long-Read) (TGS)	Long reads (PacBio, Oxford Nanopore) accurately detect large SVs, complex rearrangements, repetitive sequences, and full-length transcript isoforms; provide near-complete genome assemblies	Higher per-base error rates (though improving); somewhat higher cost and lower throughput compared with short-read platforms
Optical Mapping	Captures ultra-long DNA molecules (hundreds of kb); provides genome-wide visualization of large structural variants and rearrangements; excellent complement to sequencing	Cannot detect single-nucleotide variants or small indels; specialized equipment required

Array-CGH and MLPA	Genome-wide or targeted detection of copy number alterations and gene dosage changes; cost-effective and routinely used in diagnostic laboratories	Unable to detect balanced translocations, inversions, or low-level mosaicism; lower resolution compared with sequencing
Single-Cell Sequencing (scRNA-seq, scDNA-seq)	Resolves tumor heterogeneity and clonal structure; identifies rare resistant subclones; tracks clonal evolution dynamically	Technically demanding, costly, and not yet widely adopted in clinical diagnostics
Epigenetic Profiling (e.g., WGBS, methylation arrays, ATAC-seq, ChIP-seq)	Reveals methylation patterns, chromatin accessibility, and histone modifications; elucidates gene regulation and epigenetic drivers of leukemogenesis	Requires high-quality DNA or chromatin; complex data analysis; currently limited to research settings

Regarding NGS, it should be noted that the term encompasses both short-read and long-read platforms, which differ substantially in their capabilities. Standard Illumina short-read sequencing has become increasingly affordable and routine, offering high-throughput detection of SNVs, indels, and CNVs. Nevertheless, its ability to resolve large or complex structural variants remains limited. In contrast, third-generation long-read sequencing (TGS) methods, such as PacBio SMRT and Oxford Nanopore Technologies (ONT), can span kilobase-scale genomic regions, providing accurate structural variant detection and phasing information [45]. Moreover, optical mapping currently represents the most powerful complement to sequencing for genome-wide structural variant characterization. Finally, techniques like array-CGH, MLPA, and epigenetic profiling (WGBS, ATAC-seq, ChIP-seq) provide essential layers of copy-number, dosage, and regulatory landscape data. The integration of these complementary approaches offers the most complete molecular view of leukemia pathogenesis and supports the refinement of precision diagnostics and therapy selection [46].

1.5 Challenges of NGS

Despite its transformative potential, implementing NGS in leukemia management faces several practical and clinical challenges. The cost of NGS remains a significant barrier, particularly in resource-limited healthcare systems, where routine use may not be feasible without demonstrated cost-effectiveness. Several studies have evaluated the health-economic impact of NGS-based panels compared with conventional testing, with mixed conclusions depending on assay design, disease setting, and healthcare system reimbursement policies [47].

A significant challenge in genomic profiling is the interpretation of large datasets, particularly those generated by NGS, which creates vast amounts of information, including variants of uncertain significance (VUS), and can complicate clinical decisions. This complexity often requires bioinformatics expertise, multidisciplinary molecular tumor boards, and continual updates to variant databases. However, complementary approaches, including Optical Genome Mapping (OGM), TGS, such as ONT and PacBio, and High-throughput Chromosome Conformation Capture (Hi-C), can provide additional structural and functional genomic insights. Some platforms, like OGM, offer more automated analysis, reducing the need for extensive bioinformatics support [48].

Equally important is the lack of standardization across laboratories and institutions. International initiatives, such as those led by the European Leukemia Net (ELN) [49] and the Association for Molecular Pathology (AMP) [50], have emphasized the need for harmonized pipelines covering sample preparation, sequencing, data processing, and reporting standards. Without standardized protocols, reproducibility and comparability of results across centers remain limited, slowing the integration of NGS into global clinical guidelines. Addressing these challenges will be critical for ensuring that NGS not only provides deep biological insights but also translates into consistent, equitable, and cost-effective improvements in leukemia patient care.

1.6 MRD & Precision Medicine

MRD refers to the small number of leukemia cells that remain in the body after treatment and may cause relapse. While flow cytometry and PCR are widely used for MRD detection, they have important limitations. Flow cytometry can miss rare malignant subclones if they do not display the expected immunophenotypic markers, while PCR is restricted to predefined targets and cannot

track newly emerging mutations. In contrast, NGS-based MRD assays provide much greater sensitivity by detecting mutant alleles present at frequencies as low as 10^{-5} to 10^{-6} , and they allow simultaneous tracking of multiple mutations across the genome. This enables the identification of rare resistant subclones that might otherwise be undetected and supports personalized monitoring of clonal evolution over time. NGS-based MRD detection is now increasingly applied in both AML and ALL, guiding treatment decisions such as whether to intensify therapy or proceed with stem cell transplantation [21, 51].

1.7 Emerging Directions

Artificial intelligence (AI) and machine learning (ML) are increasingly being integrated with next-generation sequencing (NGS) to enhance the accuracy, efficiency, and clinical utility of genomic data analysis. The vast amount of information generated by NGS presents considerable challenges for data interpretation, reproducibility, and the timely extraction of clinically actionable insights. AI and ML algorithms offer significant advantages in this context, as they can identify complex, non-linear patterns in high-dimensional datasets that may not be discernible through conventional bioinformatics pipelines. These technologies facilitate the prediction of therapeutic responses, the discovery of previously unrecognized or rare genomic alterations, and the automation of routine interpretive processes, thereby minimizing human error and expediting clinical decision-making [52].

In oncology, the application of AI-driven approaches is particularly noteworthy. For instance, in CML, advanced computational models are being developed to analyze sequential NGS datasets and monitor clonal evolution in real time. Such platforms enable early detection of resistance-associated mutations, providing predictive insights into TKIs resistance. The ability to identify molecular signatures of resistance at an incipient stage holds significant clinical value, as it supports timely therapeutic modifications that may improve patient outcomes and delay disease progression [53]. Beyond CML, AI-enhanced NGS frameworks are being explored across a spectrum of hematological malignancies and solid tumors, where they hold promise for refining patient stratification, optimizing precision oncology strategies, and informing prognostic assessments. Collectively, the integration of AI and ML with NGS represents a transformative advance in translational genomics and personalized medicine [54].

2. Conclusions

In summary, NGS has emerged as a transformative tool in leukemia research and clinical management. By enabling comprehensive genomic profiling, NGS enhances diagnostic precision, refines risk stratification, and informs the use of targeted and individualized treatment strategies. Its application to minimal residual disease monitoring and clonal evolution tracking has further established NGS as an indispensable component of precision oncology.

Nonetheless, challenges such as high implementation costs, complex data interpretation, and the lack of global standardization continue to be barriers to its universal adoption in clinical practice. Addressing these limitations will be crucial for achieving equitable access and consistent integration of NGS into leukemia care worldwide.

Looking forward, future studies should focus on expanding the use of single-cell sequencing to resolve heterogeneity, integrating ultra-sensitive NGS for real-time MRD monitoring, and harnessing artificial intelligence and machine learning to enable more accurate, automated

interpretation of genomic data. The combination of these emerging technologies will not only improve early detection of relapse and resistance but also accelerate the transition toward fully personalized therapeutic strategies. Ultimately, the continued evolution of NGS and its allied technologies is expected to improve leukemia outcomes further and serve as a model for precision medicine in other malignancies.

Abbreviations

ABL1	<i>ABL proto-oncogene 1, non-receptor tyrosine kinase</i>
AI	Artificial intelligence
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AMP	Association for Molecular Pathology
APL	Acute promyelocytic leukemia
ASXL1	<i>Additional sex combs-like 1</i>
ATAC-seq	Assay for transposase-accessible chromatin using sequencing
BCR	<i>Breakpoint cluster region</i>
BTk	<i>Bruton's tyrosine kinase</i>
CAR-T	Chimeric antigen receptor T-cell therapy
CEBPA	<i>CCAAT/enhancer-binding protein alpha</i>
CGH	Comparative genomic hybridization
ChIP-seq	Chromatin immunoprecipitation sequencing
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CNV(s)	Copy number variation(s)
CRLF2	<i>Cytokine receptor-like factor 2</i>
DNMT3A	<i>DNA (cytosine-5)-methyltransferase 3 alpha</i>
ELN	European Leukemia Net
ETV6	<i>ETS variant transcription factor 6</i>
FISH	Fluorescence <i>in situ</i> hybridization
FLT3	<i>FMS-like tyrosine kinase 3</i>
FLT3-ITD	<i>FMS-like tyrosine kinase 3 – internal tandem duplication</i>
Hi-C	High-throughput chromosome conformation capture
IDH1/2	<i>Isocitrate dehydrogenase 1/2</i>
IKZF1	<i>IKAROS family zinc finger 1</i>
Indels	Insertions and deletions
ML	Machine learning
MLPA	Multiplex ligation-dependent probe amplification
MRD	Minimal residual disease
NGS	Next-generation sequencing
NOTCH1	<i>Notch receptor 1</i>
NPM1	<i>Nucleophosmin 1</i>
OGM	Optical genome mapping
ONT	Oxford Nanopore Technologies

PacBio	Pacific Biosciences
PCR	Polymerase chain reaction
PLCG2	<i>Phospholipase C gamma 2</i>
PML	<i>Promyelocytic leukemia</i>
qPCR	Quantitative polymerase chain reaction
RARA	<i>Retinoic acid receptor alpha</i>
RUNX1	<i>Runt-related transcription factor 1</i>
RUNX1T1	<i>RUNX1 translocation partner 1</i>
scDNA-seq	Single-cell DNA sequencing
scRNA-seq	Single-cell RNA sequencing
SF3B1	<i>Splicing factor 3B subunit 1</i>
SMRT	Single-molecule real-time sequencing
SNV(s)	Single nucleotide variant(s)
SV(s)	Structural variant(s)
TGS	Third-generation sequencing
TKI(s)	Tyrosine kinase inhibitor(s)
TP53	<i>Tumor protein p53</i>
WGBS	Whole-genome bisulfite sequencing
WES	Whole-exome sequencing
WGS	Whole-genome sequencing

Author Contributions

Both authors contributed to the conception and design of the study. KA wrote the manuscript; EK critically revised the manuscript. Both authors read and approved the final version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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