Review

A Review of Emerging Cytogenetic and Molecular Approaches in Identifying Abnormalities in Acute Lymphoblastic Leukemia

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Review

A Review of Emerging Cytogenetic and Molecular Approaches in Identifying Abnormalities in Acute Lymphoblastic Leukemia

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Abstract: Acute Lymphoblastic Leukemia (ALL) is a diverse hematologic malignancy characterized by the uncontrolled proliferation of lymphoid precursors. A comprehensive understanding of the genetic alterations associated with ALL is crucial for accurate diagnosis and the development of effective treatment strategies. This review explores recent advancements in cytogenetic and molecular techniques, including updates to the World Health Organization's classification of both B-cell and T-cell ALL, with a focus on new and emerging methodologies. Recent cytogenetic analyses have revealed a variety of chromosomal abnormalities, including Philadelphia chromosome-like (Ph-like) abnormalities, which are associated with distinct genomic alterations often linked to poor prognosis. These abnormalities involve complex rearrangements and mutations impacting multiple signaling pathways, particularly those involving key oncogenes such as ABL1 and CRLF2. In addition, molecular studies have identified critical mutations beyond traditional markers, highlighting new entities such as DUX4 gene rearrangements and alterations in the TP53 pathway. Emerging techniques, including next-generation sequencing (NGS) and optical genome mapping (OGM), are providing deeper insights into the intricate landscape of genetic alterations in ALL. OGM, as an alternative to NGS, offers unique advantages in detecting structural variations and providing a more comprehensive genomic overview. These advancements not only enhance our understanding of leukemogenesis but also reveal potential therapeutic targets. Grasping these developments is essential for refining prognostic assessments and guiding personalized treatment approaches in both B-cell and T-cell ALL.

Keywords: Acute Lymphoblastic Leukemia (ALL); cytogenetic abnormalities; molecular abnormalities

1. Introduction

Acute Lymphoblastic Leukemia (ALL) is a significant entity in hematologic malignancies, marked by the uncontrolled proliferation of lymphoid precursors in both the bone marrow and peripheral blood. While it is the most prevalent type of leukemia among children, it also occurs in a considerable number of adults. This dual prevalence underscores the importance of understanding ALL across different age groups, particularly in those younger than 15 years [1]. The etiology of ALL is complex and multifactorial, with genetic alterations playing a fundamental role in its pathogenesis.

Cytogenetic and molecular studies have emerged as indispensable tools in unraveling the genomic landscape of ALL. Cytogenetic abnormalities, involving structural and numerical alterations in chromosomes, provide critical insights into the genetic drivers of leukemogenesis [2].

The 5th edition of the WHO classification of hematolymphoid tumors has introduced several newly recognized entities, particularly within the context of acute lymphoblastic leukemia (ALL). This update reflects the increasing understanding of ALL's complexity, driven by advancements in sequencing technologies that allow for the identification of novel genetic fusions and mutations [3]
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Subtypes such as B-ALL with BCR::ABL1-like features and ETV6::RUNX1-like characteristics, which have distinct clinical and prognostic implications [3][4].

The genomics of acute lymphoblastic leukemia (ALL) has been thoroughly explored, leading to the identification of several distinct subtypes characterized by their cytogenetic and molecular features. Each subtype exhibits unique clinical and prognostic implications.

This overview focuses on the genomic alterations associated with B-ALL, T-ALL, and mixed phenotype acute leukemia (MPAL), highlighting their specific molecular characteristics and their impact on disease outcomes as well as the detection techniques used in this matter.

We will explore various methodologies—such as FISH, PCR, and NGS—that enhance the detection of genetic abnormalities in ALL. These techniques are essential for refining prognostic evaluations and facilitating personalized treatment strategies.

However, challenges persist, including high costs, technical complexities, and the need for specialized expertise, which can impede widespread adoption. Addressing these issues is crucial for improving accessibility to advanced diagnostic tools and ultimately enhancing patient outcomes in ALL.

2. Genetic Classification and Subtypes of B-Acute Lymphoblastic Leukemia

The classification of B-acute lymphoblastic leukemia (B-ALL) has traditionally focused on recurrent genetic abnormalities, such as the BCR::ABL1 fusion (Philadelphia chromosome; Ph+), ETV6::RUNX1, TCF3::PBX1, IGH::IL3, and MLL rearrangements, as well as variations in ploidy, including hyperdiploidy and hypodiploidy [5]. The 5th edition classification of hematolymphoid tumors has further refined the categorization of B-lymphoblastic leukemia/lymphoma, defining several distinct subtypes. These include B-lymphoblastic leukemia/lymphoma not otherwise specified (NOS), high hyperdiploidy, and iAMP21. It also identifies subtypes characterized by specific genetic alterations, such as those with BCR::ABL1 fusion, BCR::ABL1-like features, KMT2A rearrangement, ETV6::RUNX1 fusion, ETV6::RUNX1-like features, TCF3::PBX1 fusion, IGH::IL3 fusion, TCF3::HLF fusion, and other defined genetic abnormalities. Each of these subtypes carries its own clinical and prognostic implications, contributing to the overall understanding and treatment of B-ALL [2] Table 1.

B-ALL is characterized by various other genetic abnormalities, including mutations in genes such as IKZF1, which is associated with poor prognosis [6]. Changes in gene copy numbers, including amplifications and deletions, are common and can influence disease progression [7]. Such as B-ALL/LBL with intrachromosomal amplification of chromosome 21 (iAMP21) [8]

Characteristic mutations, such as those in MYD88 can also be observed across different B-cell malignancies, underscoring their shared genetic landscape [9].

The category of B-ALL with other defined genetic abnormalities includes potential novel entities (table 2), including B-ALL with DUX4, MEF2D, ZNF384 or NUTM1 rearrangements; B-ALL with IG::MYC fusions; and B-ALL with PAX5alt or PAX5 p.P80R abnormalities, B-ALL with UBTF::ATXN7L3/PAN3,CDX2 (“CDX2/UBTF”),B-ALL with IKZF1 N159Y [10]

Table 1. Genetic Alterations and Prognostic Factors in B-ALL.

Genetic Abnormality	Prognosis	Age Group	Percentage of Cases	Immunophenotyping Biomarkers
High-hyperdiploidy	Very favorable prognosis (> 90% long-term survival)	Most frequent in children	25–35% of B-ALL cases	Typically positive for CD10, CD19, CD22
iAMP21	High relapse risk; intensive therapy improves outcomes	Older children (median age: 9 years)	~2% of pediatric cases	Positive for CD10, CD19
BCR::ABL1	Historically poor prognosis, improved with TKIs; measurable residual	<15 years: 2–4%, 15-39 years: 10%, 40-49 years: 25%, >50 years: 20-40%	Increases with age	Positive for CD34, CD19, BCR::ABL1 fusion

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	disease (MRD) is a strong predictor			
BCR::ABL1-like features	High-risk; worse overall survival, high MRD likelihood	Varies (higher in older adults)	10–15% in children, 25–30% in young adults	Similar to BCR::ABL1, may lack IKZF1 alterations
KMT2A rearrangement	Generally poor prognosis	Infants <1 year, increases with age	70–80% in infants	Positive for CD10, CD19
ETV6::RUNX1	Very favorable prognosis; often better outcomes than other types	Most common in children (ages 2–10)	~25% of childhood cases	Positive for CD10, CD19
TCF3::PBX1	Intermediate to favorable prognosis with modern therapy; increased CNS relapse risk	More frequent in children	~5% of pediatric cases	Positive for CD10, CD19
TCF3::HLF	Dismal outcomes; historically considered incurable	Mostly children, rare in adults	<1% of childhood cases	Positive for CD19
ETV6::RUNX1-like features	Undefined outcomes; small case series indicate potential for late relapses	More common in childhood	1–3% of childhood cases	Variable

Table 2. B-cell lymphoblastic leukaemia/lymphoma NOS.

Genetic Alteration	Prognosis	Age Group	Percentage of Cases	Immunophenotyping Biomarkers	Therapy and Treatment	Detection Techniques
DUX4 rearrangement	Best outcome; 5-year event-free survival: 95% (children), 80% (adults)	All ages, better in children	Variable	CD2+ (70%), CD13++, CD34++, CD38++, CD371+	Standard chemotherapy; tailored based on response	Next-generation sequencing (RNA/DNA)
MEF2D rearrangement	Intermediate to poor outcome; 5-year overall survival: ~70% (children), ~30% (adults)	All ages	Rare	CD10−, CD5, CD38+, cMu+	Intensive chemotherapy; potential targeted therapies	RNA sequencing or RT-PCR
ZNF384 rearrangement	Prognosis varies; monocytic differentiation may influence outcomes	All ages	Rare	CD10− (73%), CD13+, CD33+, CD65−, CD15−, CD25+ (25%), myeloperoxidase− (+ in MPAL)	Standard chemotherapy; consideration of lineage switch	Break-apart FISH or next-generation sequencing (RNA/DNA)
PAX5alt	Prognosis varies; can be associated with poorer outcomes	All ages	~7.5% of B-ALL cases	Not specifically defined	Standard chemotherapy; depends on specific alterations	Next-generation sequencing (RNA/DNA)
PAX5 p.P80R	Poorer prognosis associated with additional PAX5 alterations	All ages	Rare	CD2+, CD33+, CD65−, CD15−	Standard chemotherapy; may involve additional therapies	DNA sequencing methods

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NUTM1 rearrangement	Favourable prognosis; seen in infant cases with germline KMT2A variants	Most frequent in infants	Up to 1/3 in infants	Not specifically defined	Sensitive to histone deacetylase inhibitors	Break-apart FISH or RNA/DNA sequencing
MYC rearrangement	Poor prognosis in adults (<20% 5-year overall survival); better in children with Burkitt-like therapy	More common in adults	0.1% in children, 4.3% in adults	Not specifically defined	Burkitt lymphoma therapy for children; intensive chemotherapy for adults	Karyotype or FISH analysis

3. Genetic Classification and Subtypes of T-Acute Lymphoblastic Leukemia

T-cell acute lymphoblastic leukemia (T-ALL) is biologically different from B lymphoblastic leukemia (B-ALL) and exhibits distinct patterns in how the disease responds over time [11]. T-cell acute lymphoblastic leukemia (T-ALL) constitutes roughly 12% to 15% of all cases diagnosed [12], accounting for only 10% to 15% of pediatric and up to 25% of adult ALL cases [13].

Many translocations may not be identifiable through standard karyotyping and instead necessitate molecular genetic analyses for accurate detection.

For example, the TAL1 locus is deregulated in approximately 20% to 30% of T-ALL cases; however, the specific t(1;14)(p33;q11.2) translocation is only detectable by karyotyping in about 3% of instances. More frequently, cryptic insertions or deletions that occur upstream of TAL1 are responsible for its deregulation [14].

In terms of cytogenetic abnormalities, an abnormal karyotype is found in 50% to 70% of T-ALL cases. The most common recurrent abnormalities involve rearrangements of the TRA and TRD genes at 14q11.2, TRB at 7q34, and TRG at 7p14.1, often linked to a variety of partner genes. These translocations typically result in the transcriptional dysregulation of the partner gene by placing it near the regulatory regions of one of the T-cell receptor loci [15].

Key genes frequently involved in these rearrangements include T-lineage transcription factors suggested by ICC such as TLX1, TLX3, TAL1, TAL2, LMO1, LMO2, LYL1, and various NKX2 family members, as well as OLIG2 and several HOXA genes (Table 3) [16]. Additionally, transcription factors like MYC and MYB, along with the cytoplasmic tyrosine kinase gene LCK, may also play a role in these cytogenetic changes [17,18].

Other significant rearrangements associated with T-ALL include alterations involving MLLT10, KMT2A, ABL1, and NUP98, which contribute to the complexity of the disease's genetic landscape [19]

Table 3. Genetic Abnormalities Associated with T-ALL/LBL: Prognosis, Age Group, Percentage of Cases, and Pathway.

Genetic Abnormality	Prognosis	Age Group	Percentage of Cases	Pathway
NOTCH1 mutations	Better outcomes associated	All age groups	>75% activation	NOTCH signaling
FBXW7 mutations	Better outcomes associated	All age groups	30% (loss-of-function)	NOTCH signaling
EZH2 mutations	Poor prognosis	All age groups	Rare	Epigenetic regulation

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SUZ12 mutations	Poor prognosis	All age groups	Rare	Epigenetic regulation
EED mutations	Poor prognosis	All age groups	Rare	Epigenetic regulation
PHF6 mutations	Poor prognosis	All age groups	Rare	Chromatin modification
KDM6A mutations	Poor prognosis	All age groups	Rare	Chromatin modification
IL7R mutations	Poor prognosis if mutated	All age groups	Common in T-ALL	JAK/STAT
JAK1 mutations	Poor prognosis with activating mutations	All age groups	Common in T-ALL	JAK/STAT
JAK3 mutations	Poor prognosis if mutated	All age groups	Rare	JAK/STAT
CDKN2A deletions	Poor prognosis	All age groups	~30% (deletion)	Cell-cycle regulation
TAL1 rearrangements	Poor prognosis	All age groups	20-30%	Various pathways
TLX1 rearrangements	Generally favorable prognosis	All age groups	Common in translocations	Various pathways
TLX3 rearrangements	Poor prognosis	All age groups	Common in translocations	Various pathways
HOXA gene rearrangements	Poor prognosis	All age groups	Common in translocations	Various pathways
BCL11B deletions	Poor prognosis	All age groups	Rare	Tumor suppressor
ETV6 mutations	Associated with ETP-ALL phenotype	Typically younger patients	Rare	Tumor suppressor
KMT2A rearrangements	Poor prognosis	All age groups	Rare	Various pathways
NUP98 rearrangements	Poor prognosis	All age groups	Rare	Various pathways

4. Cytogenetic and Molecular Techniques in the Diagnosis of Acute Lymphoblastic Leukemia (ALL)

Dr. Janet D. Rowley's identification of the t(9;22) translocation in chronic myeloid leukemia and Dr. Lore Zech's discovery of t(8;14) in Burkitt's lympho`ma during the 1970s marked significant advancements in understanding hematologic malignancies. Since then, a variety of recurring chromosomal abnormalities—such as translocations, inversions, deletions, and both gains and losses—have been identified in these cancers [20].

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These genetic alterations not only serve as important diagnostic indicators for various subtypes of leukemia and lymphoma, but they are also associated with different prognoses [21].

More recently, many of these abnormalities and gene mutations have been established as key criteria for classifying leukemia and lymphoma in authoritative guidelines, including the WHO 5th edition, the International Consensus Classification (ICC) 2022, and the European Leukemia Network (ELN) 2022, playing a crucial role in diagnostic and prognostic assessments.

Many of the chromosomal abnormalities and gene mutations found in leukemia and lymphoma can be identified and analyzed through various techniques, including chromosome banding analysis, fluorescence in situ hybridization (FISH), genomic microarrays, and next-generation sequencing (NGS). The advancement of innovative genomic technologies, such as optical genome mapping (OGM), whole genome sequencing (WGS), and whole transcriptome sequencing (RNA-seq), is paving the way for the discovery of additional recurrent genetic alterations in clinical diagnostics [21].

5. What's New in Cytogenetics and Hematology?

5.1. Optical Genome Mapping (OGM) and ALL

Optical genome mapping (OGM) is a cytogenomic technology that can be used to detect structural variants (SVs) in the genome of patients with hematological malignancies [22].

OGM aligns with the diagnostic scope of traditional cytogenomic clinical testing while also providing valuable new insights in specific situations. By combining the diagnostic advantages of various complex and expensive tests, such as karyotyping, fluorescence in situ hybridization, and chromosomal microarrays, into a single, cost-effective assay, many clinical laboratories are increasingly considering the adoption of OGM [23].

This technology enables the generation of images of molecules with an average N50 exceeding 250 kb and can achieve approximately 300× genome coverage per flow cell (utilizing three flow cells per chip and two chips per instrument run). By fluorescently labeling ultra-long high-molecular-weight (UHMW) DNA molecules with a specific 6-mer single-stranded DNA motif (currently direct labeling enzyme-1 [DLE-1]: CTTAAG), we achieve an average label density of 15 labels per 100 kb [24].

This approach allows us to examine exceptionally long stretches of DNA, reducing the number of fragments required to map entire chromosomes, thus speeding up the process and minimizing errors. Moreover, unlike traditional methods, there is no need for pre-processing or manipulation of the DNA, enabling us to analyze the DNA in its natural state. In essence, this technology offers a true representation of the genomic landscape [24].

In previous studies, 100% sensitivity was achieved in detecting previously identified clinically relevant aberrations, supported by a thorough technical comparison for analytical validity. Specifically, the evaluation of OGM against FISH demonstrated both 100% sensitivity and specificity. When assessing OGM's effectiveness in detecting translocations through karyotyping, 100% sensitivity and a positive predictive value (PPV) of up to 82% were observed. Additionally, comparisons with CNV microarrays revealed a sensitivity of 100% for both structural variants (SV) and copy number variations (CNV), with PPVs of 96% for SV calls and up to 81% for CNV calls. These findings indicate that OGM consistently maintains 100% sensitivity across all comparisons, with a PPV exceeding 80%. However, to further decrease the already minimal false positive rates, particularly in CNV detection, additional enhancements are required [25].

In hematology, the capability to detect balanced and unbalanced events in one assay can be among the greatest benefits of OGM, and it was proven by a recent study [26]. While analyzing 37 ALL, the comparatif results of the current cytogenetic techniques with OGM were concordant (table 4).

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Table 4. Comparison of previous diagnostic findings with OGM.

Number of ALL cases	Karyotype results	FISH results	CNV-microarray results [aberrant cell fraction]	Optical mapping results (SV tool and/or CNV tool)	Aberrations beyond scope of optical mapping	Result
37	45,XY,der(18;22)(q10;q10)[2]/45,X,-Y,der(18;22)(q10;q10),+22[6]/46,XY[2]	BCR-ABL1/t(9;22) (q34;q11.2): wt KMT2A (11q23): wt BCR (22q11) gain [96/100]	9p21.3(21976766_22009308)x1[0.4] 9p13.2(36915132_37070373)x3[0.9] 11q23.3(118358115_118470528)x1[0.75] 18pterp11.21(136226_15148589)x1[0.9] 22q11.1qter(16888900_51197839)x3[0.75] (Y)x0[0.6], [Loss of chrY]	9p21.3 loss: concordant (SV) 9p13.2 gain: concordant (SV) 11q23.3 loss: concordant (SV/CNVe) 18pterp11.21 loss: concordant (CNV) 22q11.1qter gain: concordant (CNVe) ChrY loss: concordant (CNV)	centromeric breakpoints: der(18;22)(q10;q10)	concordant

6. What's New in Molecular and Hematology?

6.1. Next-Generation Sequencing (NGS)

Advancements in molecular diagnostics have largely been driven by the exploration of hematologic cancers. Key developments include the early identification of the Philadelphia chromosome through cytogenetic techniques in the 1970s, the introduction of polymerase chain reaction for highly sensitive mutation detection and monitoring, and, most recently, the application of targeted next-generation sequencing to enhance the prognostic and treatment strategies for leukemia [27].

Future progress in molecular hematopathology is expected to come from: enhancements in the efficiency and scope of next-generation sequencing (NGS) technologies; innovative library chemistry and sequencing methods, including long-read and long-range sequencing; advancements in bioinformatics, particularly in error correction; ongoing efforts to unify various diagnostic and monitoring approaches; and significant developments in our understanding of the reference genome.

6.2. Genome Reference Overview

The human reference genome has served as a cornerstone of genomic research since its initial draft was published over two decades ago. The latest version, GRCh38, offers a composite view that reflects various individual haplotypes, providing a scaffold for each chromosome. However, this version still contains approximately 210 Mb of gaps or uncharacterized regions—151 Mb of which are entirely missing and 59 Mb represented by computational simulations—accounting for around 6.7% of the overall chromosome scaffolds. These missing sequences introduce an observational bias, often described as the "streetlamp effect," which confines studies to the limits set by the reference genome.

The introduction of GRCh37 in 2009 marked a significant step in clinical applications, but it also had its limitations, such as the absence of certain structural variations and the challenge of mapping non-reference sequences [28].

By 2013, GRCh38 was released, enhancing the reference with more accurate annotations and increased structural variation detection. Nevertheless, it still faced issues with limited clinical adoption due to its complexity and the slow validation process in laboratories [29].

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In 2022, the Telomere-to-Telomere (T2T) consortium completed the T2T-CHM13, representing the first fully assembled haploid human genome. This groundbreaking sequence provides a contiguous depiction of all autosomes and chromosome X, aside from unresolved ribosomal DNA arrays. The use of T2T-CHM13 enhances genomic studies by revealing 3.7 million additional single-nucleotide polymorphisms (SNPs) in regions not aligned with GRCh38, along with a more accurate representation of copy number variants (CNVs) from the 1000 Genomes Project. Despite its advantages, such as comprehensive representation and improved analysis capabilities, T2T-CHM13 is currently primarily utilized for research rather than clinical practice [30] (Table 5).

Table 5. Overview of Genome References and Their Adoption Status.

Genome Reference	Year Released	Organization	Adoption Status
GRCh37/hg19	2009	Genome Reference Consortium	Widely adopted in clinical settings
GRCh38/hg38	2013	Genome Reference Consortium	Limited clinical uptake
T2T-CHM13	2022	T2T Consortium	Primarily for research use

6.3. Pangenome Information

In recent years, there has been a significant push toward adopting a pangenomic reference to mitigate reference bias. The rapid evolution of pangenomic techniques has made it increasingly viable to advocate for the integration of a pangenome into routine genomic analyses (Table 6) [31].

Table 6. Status of the Human Pangenome Reference.

Pangenome Status	Organization	Adoption Status
Ongoing	Human Pangenome Reference Consortium	Research use
Advantages	High-quality assemblies from diverse populations; Collaboration with T2T Consortium	

A "pangenome" is defined as the complete set of genomic information for a species, a concept that originated in the study of highly variable bacterial genomes. The development of pangenome data infrastructure is rooted in the high-throughput generation of high-quality phased haplotypes—segments of chromosomes that are identified based on maternal or paternal inheritance. This approach aims to enhance the current human reference genome by incorporating individuals from a variety of genomic and biogeographic backgrounds, targeting at least 350 diploid genomes that provide reference-quality haplotypes, totaling 700 haplotypes [32].

It is essential to consider the ethical, legal, and social implications (ELSI) while creating policies and protocols for inclusion, data acquisition, and stewardship throughout the research process, from participant recruitment to the dissemination of findings. To achieve the best possible phased genomes, priority should be given to long-read and long-range sequencing technologies and haplotype-aware algorithms [33].

Efforts must also be made to fill gaps in diploid genomes, particularly in complex regions, ensuring that challenging variants are accurately identified. Building a robust ecosystem of tools for pangenome reference will help in annotating genes and other genomic features.

An iterative process involving design, development, and community engagement is vital for addressing user needs effectively. Clear communication strategies will enhance understanding of the pangenome reference resource, empowering the community to report and rectify any errors. Controlled access to data will be facilitated through established genomic platforms such as the

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International Nucleotide Sequence Database Collaboration (INSDC), the National Center for Biotechnology Information (NCBI), UCSC Genome Browser, Ensembl, the WashU Epigenome Browser, and NHGRI's cloud-based analysis platform, AnVIL [34].

Pangenomes can be used in hematology to improve the diagnosis and treatment of blood diseases by identifying unique variants that can be targeted with gene-based therapies, no deep studies have been currently made in this specific area.

7. Conclusions

In conclusion, the integration of cytogenetic and molecular techniques such as FISH, PCR, and NGS has revolutionized the diagnostic landscape of Acute Lymphoblastic Leukemia (ALL). These methodologies have significantly enhanced our ability to detect and characterize genetic abnormalities, leading to more precise prognostic assessments and tailored therapeutic strategies.

However, there remains a pressing need to explore further abnormalities and incorporate innovative approaches like Optical Genome Mapping (OGM) and pangenomic methodologies. These advancements could enhance our understanding of the disease's genetic complexities, ultimately leading to improved prognostic capabilities and more effective treatment strategies. Embracing these new technologies will be crucial for refining our approaches to diagnosis and therapy in ALL.

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