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Cytogenetics, Molecular Genetics and Epigenetics and Their Impact on The Management of Acute Lymphoblastic Leukemia

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Abstract

The recent improvements in the outcomes of patients with acute lymphoblastic leukemia reflect the progress in the field of diagnostics and the achievements in therapeutic interventions. In particular, the availability of more advanced technology in the cytogenetic and molecular laboratories has resulted in the stratification of patients into specific risk groups and thus several novel agents as well as targeted therapies have been introduced. Additionally, precision medicine will be applicable in the near future and thus the recent developments will facilitate the provision of safer modalities of therapy that may ultimately yield not only higher responses but also improved survival rates.

This review represents a recent update on the role of various cytogenetic assays and molecular techniques in patients with acute lymphoblastic leukemia. It is composed of a number of sections including: history of cytogenetics, disease etiology and pathophysiology, utilization of various cytogenetic assays diagnostically, disease-specific cytogenetic and molecular abnormalities, common disease genetic subtypes, prognosis and risk stratification, refractory and relapsed disease, novel therapies, precision medicine and drug repurposing.

Keywords: Acute lymphoblastic leukemia; Cytogenetic techniques; Molecular assays; Next generation sequencing; Minimal residual disease; Novel therapies; Precision medicine

Introduction

Genetics is the study of heredity or genes and factors related to all aspects of genes [1]. The field of cytogenetics focuses on studying the number, structure, function and origin of chromosomal abnormalities as well as the evolution of chromosomes [2]. Genetics is a young science with the broadest principles known for approximately 100 years while the application of genetic analyses in the diagnostics of cancer has been evolving over the last 40 years [3].

Throughout the 1960s and 1970s, specific chromosomal abnormalities were identified, and their presence has recently been correlated with outcome [4]. Thus, cytogenetics has determined not only the incidence but also the significance of specific chromosomal anomalies in acute lymphoblastic leukemia (ALL) [4]. ALL results from a process of malignant transformation of a progenitor lymphocytic cell in the B or T lineage [5]. Thereafter, multistep sequential alterations develop in several oncogenes in tumor suppressor genes or in microRNA genes in carcinogen cells [5]. However, the effect of prior malignancy on the risk of development of ALL or on the prognosis of this type of leukemia is unknown [6,7]. Environmental exposures and genetic factors predispose to ALL [8-11]. Multiple hits may be required for disease progression [12]. Environmental exposures can modify DNA methylation which is dramatically altered in malignant diseases including ALL [12].

Landmarks in the History of Cytogenetics

In 1595, Janssen invented the single-lens optical microscope and in 1665, "cell" was first described by Robert Hooke [1]. In 1859, Charles Darwin published "on the origin of species by means of natural selection" [1]. In 1879, the German pathologist Arnold examined carcinoma and sarcoma cells and found that voluminous nuclei of cancer cells facilitated the analysis [2]. Fleming and Hansemann were the first to examine human mitotic chromosomes in 1882 and 1890 [2,3]. In 1888, Wadeyer made the first description of chromosome as a colored body and this description was derived from Greek: "chroma: color and soma: body" [2]. Boveri proposed the relationship between cancer and chromosomes in the year 1902 [1,3]. Eight years later, Thomas Hunt Morgan showed that genes are located on chromosomes [1]. Blakslee and Avery in 1937 and Levan in 1938 used colchicine for chromosome preparation and the first implementation was in plant cytogenetics [2]. In 1949, Matthy found that unspread and tangled chromosomes make it difficult to count the number of mammalian chromosomes in a preparation [2]. Four years later, the structure of DNA was described by James Watson and Francis Crick [1].

In 1955, Eagle developed specific culture media and established that the cytogenetic analysis of chromosomes depends on spontaneously dividing cells [1,2]. One year later, Tjio and Levan used cultured embryonic cells and they were that first researchers to report the correct number of human chromosomes as 46 [1-4]. In 1959, Lejeune was the first to observe the extra-copy of chromosome 21 and in 1960, Moorhead established *in vitro* culture method for the accumulation of dividing cells using colchicine to arrest cells at metaphase [2,3]. In the same year, Nowell discovered the mitogenic property of phytohemagglutinin resulting in further technical improvements particularly the use of peripheral blood cells and also he described the first acquired chromosomal anomaly (Philadelphia chromosome) in patients with chronic myeloid leukemia (CML) [2,3]. In 1966, Steele and Breg Jr successfully cultured amniotic fluid cells to karyotype fetal

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chromosomes [1]. Towards the end of 1960s, phytohemagglutinin was used and banding techniques were introduced in the cytogenetic laboratories [2]. In 1974, Hahnemann developed "in vitro" culture technique for chorionic villi, then Niazi in 1981 and Brambati and Simoni in 1983 made further improvements in this culture technique [2].

In 1977, Sanger developed the first sequencing method or technology and three years later, Maxam-Gilbert made further developments in the sequencing methods [1]. In the year 1982, fluorescence *in situ* hydribization (FISH) was developed and ten years later the initial comparative genomic hybridization (CGH) technology was developed [1-4]. In 1985, Kerry Mullis invented polymerase chain reaction (PCR) and in 1993, Mullis and Smith shared Nobel Prize for their invention and development of the PCR technique [1,3]. In 1987, Applied Biosystems marketed the first automated sequencing machine in the United States [1,3]. The year witnessed 1990 launching of human genome project [1]. In 2001, draft of human genome project was reported and two years later, the human genome project was officially completed [1,2,4]. In the year 2000, Lynx Therapeutics developed the massively parallel sequencing and three years later the second and third generation sequencing platforms were launched by: Applied Biosystems, ILLUMINA, Roche Company, Pacific Biosciences, Oxford Technologies, Solexa and Helicos Biosciences [1]. In 2006, array CGH was developed and the year 2009 witnessed development of next generation sequencing (NGS) and sequencing of complete acute myeloid leukemia genome by Mardis [1-3].

Etiology and Pathophysiology

ALL is produced as a result of a process of malignant transformation of a progenitor lymphocytic cell in the B and T lineages [5]. ALL can be de novo, secondary or therapy-related [6]. The main predisposing or etiological factors are: (1) hereditary, familial or genetic factors, and (2) environmental exposures [5-8]. Mutation in one gene which is critical for proliferation, differentiation and survival of progenitor cells may provoke or initiate the process of leukemogenesis [5]. Both B-cell and T-cell ALL types comprise multiple subtypes that harbor distinct constellation of somatic structural DNA rearrangements and sequence mutations that commonly perturb: lymphoid development, cytokine receptors, kinase and RAS signaling, tumor suppression and chromatin modification [9]. However, there is evidence that evolution of leukemia is a result of a sequential multistep process that involves alterations in several oncogenes in tumor suppressor genes or micro-RNA genes in carcinogen cells [5].

The following are risk factors for the development of ALL: (1) ionizing radiation, (2) cytotoxic chemotherapy, (3) infectious agents such as Epstein–Barr virus, (4) HLA (human leucocyte antigen)-DPB1 alleles coding for specific amino acid polymorphisms, (5) low dietary folate and alterations in folate metabolism as a result of polymorphisms in genes encoding methylenetetrahydrofolate reductase enzyme, and (6) genetic factors with low or high penetrant mutations or polymorphisms [8,10,11]. Studies have shown the following: (1) negative correlation with the consumption of antioxidants and diabetes mellitus; (2) controversial association with tobacco smoking; (3) suggestive but not proven associations with: paints, solvents, benzene and other petroleum products, pesticides and metals; (4) association with advanced or increased maternal age; and (5) that DNA methylation can be modified or altered by a number of environmental exposures [8,10,12-14]. However, identification of environmental factors that are associated with the risk of leukemia is difficult due to several reasons that include: (1) inability to confirm and quantify exposures, (2) lack of prospective cohorts, (3) presence of different variants of leukemia, and (4) inadequate understanding of the pathophysiology of leukemia such as gene-environment interaction or infection-environment interaction [10].

ALL has genetic background as several studies have confirmed the existence of the following genetic abnormalities and polymorphisms in patients with ALL: (1) IKZF1 (7p12.2); (2) ARID5B (10q21.2); (3) CEBPE (14q11.2); (4) GATA3 (10p14); (5) CDKN2A and CDKN2B (9p21); and (6) PIP4K2A (10p12.2) [9,15-25]. ARID5B and PIP4K2A predispose to ALL with hyperdiploid cytogenetic abnormality while GATA3 predisposes to Philadelphia-like (Ph-like) ALL [9]. Novel germline genetic risk factors independently influence ALL prognosis and strongly modify host susceptibility to adverse effects of cytotoxic drugs such as vincristine, L-asparaginase and corticosteroids [25]. Techniques that have led to establishing risk factors for the development of ALL include: (1) whole genome or next generation sequencing (NGS), (2) single nucleotide polymorphism (SNP) assays, and (3) genome-wide association studies (GWAS) [7,9,17-20,23-26]. Sequencing technology helps in: improving the diagnosis, monitoring minimal residual disease (MRD), detection of early relapse and guiding precise therapies [9].

Secondary or therapy-related ALL (t-ALL) is a distinct clinical entity [6]. It occurs after specific malignancies such as hematological malignancies (HMs) and solid tumors or autoimmune disorders (AIDs) such as psoriasis [6,27]. t-ALL is associated with the use of immunosuppressive therapies, cytotoxic chemotherapy and radiotherapy [6,27]. The median times from the diagnosis of the primary malignancy or AIDs till the evolution of t-ALL are: 67,69 and 70 months for HMs, solid tumors and AIDs respectively [6,27]. t-ALL carries poor prognosis and has a higher risk of death compared to de novo disease particularly in patients who are younger than 40 years [6].

Cytogenetic Techniques and Cytogenetic Abnormalities

The various cytogenetic tests used in the diagnosis of ALL

There are 2 types of chromosomal aberrations: (1) those involving changes in the number of chromosomes (gains or losses), and (2) those giving rise to structural abnormalities including: translocations, deletions, insertions and inversions [3,4,28]. Chromosomal abnormalities may be balanced or unbalanced in form, with unbalanced rearrangements leading to changes in copy numbers [4]. Chromosomal abnormalities give useful information on: disease biology, diagnosis, prognosis, predictive biomarkers, sub-classification of HMs into different disease subtypes, prediction of outcome and response to treatment and finally development of novel and targeted therapies [28,29]. The various types of cytogenetic techniques are shown in Table 1 [30-33]. The indications, limitations, resolution and advantages of each category of tests are shown in Table 2 [3,34-36].

Conventional cytogenetics has played a pivotal role in the detection of recurrent chromosomal rearrangements in patients with HMs thus aiding: diagnosis, prognosis and identification of the involved genes [37]. Conventional cytogenetics, which is also called chromosomal banding analysis or karyotyping, examines the patient's chromosomes in a sample of cells obtained from: peripheral blood, bone marrow (BM) aspirate or other body tissues [3]. The technique requires a sufficient number of dividing cells, mitoses of acceptable quality, and cells that are arrested at the metaphase stage of the cell cycle using the compound colcemide [3,28]. Thereafter, the metaphase spread cells are further analyzed using a banding technique [28]. However, conventional cytogenetic analysis of chromosomal abnormalities in patients with HMs is hampered by low mitotic index and poor quality of metaphases [37,38]. A minimum of 20 metaphase cells are usually

(1)	**Conventional cytogenetics; G-banding:**
	Karyotyping on cells derived from cell cultures using banding analysis.
(2)	**Molecular cytogenetics, FISH techniques:**
	Centromere-specific probes.
	Whole chromosome painting probes
	Local-specific FISH; single copy genomic probes
	Multicolor FISH (M-FISH)
	Multiplex metaphase FISH
	SKY: spectral karyotyping
	SCAN: spectral color banding
	Translocation fusion probes
(3)	**Molecular techniques to analyze DNA, RNA on proteins directly:**
	PCR (polymerase chain reaction):
	- Reverse transcriptase (RT) PCR
	- Quantitative, real-time (Q-RT) PCR.
	Array comparative genome hybridization (CGH)
	Microarray analysis such as SNP (single nucleotide polymorphism)
	NGS (next generation sequencing):
	- Targeted sequencing
	- Exome sequencing
	- Transcriptome sequencing
	- Whole genome sequencing
FISH: Fluorescence *In Situ* Hybridization	

Table 1: Various tests performed in cytogenetic laboratories.

Techniques	Resolution	Advantages	Limitations	Indications
Conventional cytogenetics	Low	Whole genome overview	Requires mitosis (dividing cells)	Diagnostic work up of several hematological malignancies (HMs) such as acute leukemias, myelodysplastic syndrome and chronic myeloid leukemia (CML)
		Detects balanced and unbalanced abnormalities		
		Sensitive and specific	Requires fresh tissue Inability to detect small or subtle abnormalities	
		Provides direction for molecular studies		Detection of chromosomal abnormalities in HMs
FISH	Intermediate	Simple setup	Limited number of probes	Diagnostic work up and reevaluation at progression of several HMs such as acute leukemias, non-Hodgkin lymphoma, multiple myeloma (MM) and chronic lymphocytic leukemia (CLL)
				Chromosome enumeration and identification
		Does not require cell division		
		Fast results	Small spectrum of analysis needs prior knowledge of affected chromosomal locus or gene	
		Detects balanced and unbalanced abnormalities		
M FISH and M banding	Intermediate	Can unravel complex abnormalities	Labor intensive	Selected cases with complex karyotype or complex metaphase FISH
			Not routinely performed	
MLPA	Intermediate high	High throughout	Not available in all centers	In some centers for CLL and other lymphoproliferative disorders
			Limited number of probes	
			Detects only unbalanced abnormalities	
PCR	High	Detects balanced and unbalanced abnormalities such as mutations	Limited number of	Diagnostic work up and follow up, by MRD monitoring, of several hematological disorders such as acute leukemias and CML
		Sensitive and specific		
		Rapid turnaround time	abnormalities can be investigated Targeted approach (not a screening tool)	
		Can be performed on fresh, frozen and paraffin embeded samples		Detection of early relapse of HMs
CGH and SNP arrays	High	Whole genome overview SNP arrays can detect copy number neutral loss of heterogygosity	Expensive Detects unbalanced abnormalities	Diagnostic work up of several hematological disorders such as CLL and MM
			Requires significant malignant infiltration ≥ 20%	
NGS	Very high	Detects balanced and unbalanced abnormalities	Expensive	For research purposes
		Whole genome overview		Comprehensive identification of all clinically relevant genomic alterations
PCR: Polymerase Chain Reaction FISH: Fluorescent In Situ Hybridization CGH: Comparative Genomic Hybridization SNP: Single Nucleotide Polymorphism NGS: Next Generation Sequencing				

Table 2: Comparison between the various genetic techniques.

required to determine whether the cells obtained from a BM sample are cytogenetically normal [28]. Monosomies require 3 metaphase cells, while trisomies require 2 metaphase cells [28].

Currently, detection of cytogenetic abnormalities in the clinical arena is primarily based on: karyotyping, FISH or both [37]. However, the increasing range of cytogenetic technologies provides detection of different types of chromosomal and genetic defects [4]. FISH, other molecular techniques and more recently array CGH have been used in a complementary fashion to accurately locate the breakpoints involved in chromosomal rearrangements. Nevertheless, conventional cytogenetics remains the gold standard methodology for the detection of established chromosomal abnormalities within the routine clinical environment [4].

FISH is a technique that uses fluorescently labelled probes to locate the positions of specific DNA sequences on chromosomes [39]. FISH has expanded cytogenetics by means of: (1) identifying cryptic genetic lesions that are not shown by routine karyotyping but can affect the response to treatment, and (2) enhancing the identification of non-random translocations and deletions pinpointing regions which contain genes that are involved in leukemogenesis [37,38]. Currently, FISH and other molecular approaches are used to screen for the significant abnormalities in patients with ALL [4].

The application of multicolor FISH techniques has highlighted a number of novel chromosomal abnormalities and elucidated complex karyotypes [4]. Multicolor FISH combines the screening potential of cytogenetics with the accuracy of molecular genetics allowing the visualization of the entire human genome [38]. One of the main advantages of FISH is its ability to use non-dividing interphase cells as DNA targets, enabling the screening of large numbers of cells and providing access to a variety of cells with different hematopoietic activity [38].

There are 2 genome wide techniques that can be used to detect copy number alterations (CNAs) and these are: NGS and array-based CGH [40]. CGH, which provides alternative means of genome-wide screening for CNAs, was first developed to detect CNAs in solid tumors [39,40]. It uses 2 genomes: a test and a control, which are differentially labelled and competitively hybridized to metaphase chromosomes [39,40]. Array CGH has greatly increased genomic resolution over classical cytogenetic techniques, but its resolution is relatively limited compared to the more advanced molecular techniques [37,40]. The fluorescent signal intensity of the labelled test DNA relative to that of the reference DNA can then be linearly plotted across each chromosome in order to allow the identification of CNAs [39,40]. CGH can be used to quickly scan the entire genome for imbalance and it does not require cells that are undergoing division [39,40]. Thus, array CGH is a mature robust technology of lower cost and more accessibility than NGS [40]. SNP arrays have been used to demonstrate CNAs whilst concurrently showing loss of heterozygosity [4]. There is no doubt that the evolving field of cytogenetics continues to be instrumental in understanding the genetics of HMs including ALL [4].

PCR applications in patients with malignant hematological disorders include: detection of fusion genes, detection of genetic mutations, post-hematopoietic stem cell transplantation (HSCT) chimeric analysis and determination of clonality of lymphoid cells [41]. Specimens that are suitable for PCR analysis include: fine needle aspiration samples, small tissue biopsies, BM aspirates and decalcified biopsies, cells scraped from histological or cytological slides and cells that are microdissected from tissue specimens [42]. Table 3 shows a comparison between the three PCR techniques that are used in molecular laboratories [41].

Variables	Reverse transcriptase PCR	Real time quantitative PCR	Digital PCR
Qualitative assessment of a genetic sequence	Yes	Yes	Yes
Quantitative assessment of a genetic sequence	- No - Can be semi-quantitative	Yes	Yes
Technical complexity	++	+++	+++
Principle	Amplification of target gene and then its detection with gel electrophoresis against a standard	Amplification of target gene and real time quantification using SYBR green dye	Direct counting of target genetic sequence and quantification using difference in negative and positive dye labeled nucleotide sequences
Need for gel electrophoresis	Required	Not required	Not required
Uses	Identification of presence or absence of a particular nucleic acid	Quantification of a particular nucleic acid sequence	- Quantification of a particular nucleic acid sequence - Rarely allele detection

Table 3: Comparison between various PCR techniques.

For decades, Sanger sequencing, based on a chain-terminator method, was the primary means for determining the sequence of DNA [43]. NGS refers to deep, high throughout sequencing technologies that use DNA, RNA or methylation sequencing and they are valuable in revealing genomic, transcriptomic and epigenetic landscapes of individual cancers [43-47]. NGS has revolutionized research in patients with HMs and has recently led to a number of significant discoveries related to: early disease diagnosis, clonal evolution, risk stratification and selection of the most favorable and personalized therapeutic intervention [48]. The recent application of NGS in patients with HMs has provided us with novel insights into disease initiation, disease progression and response to treatment [42]. The new sequencing technologies including NGS have a number of advantages in comparison with Sanger sequencing, that first emerged in 1977 and dominated the field for 3 decades, including: (1) higher levels of precision and sensitivity, (2) higher resolution, and (3) shorter time to obtain results [43-45]. Thus, nowadays, it is possible to obtain the sequence of the entire human genome within days to weeks using only a single sequencing instrument [43].

NGS has shed some light on the genetic abnormalities in hematopoietic stem or progenitor cells as the precursor to ALL pathogenesis [19]. Based on these newly described genetic abnormalities, ALL is now being reclassified into newly identified subgroups [19,49]. Detection of measurable residual disease (mRD) is an important marker of increased relapse risk in pediatric and adult ALL [19,49]. Detection of mRD by gene sequencing is an extremely sensitive technique that may identify patients who are likely to benefit from HSCT [49,50]. However, NGS is superior to flow cytometry in detecting mRD in ALL [49]. Also, whole genome sequencing (WGS) studies in ALL patients and ethnically matched controls have identified inherited genetic variations in lymphoid neoplasm-related genes which are likely to increase susceptibility to ALL [19].

Considering the heterogeneity of leukemias and the widening spectrum of therapeutic strategies, novel diagnostic techniques are needed for patients with HMs [51]. Thus, the ultimate goal in the post-genomic era will be to extend the successful transition from gene discovery to therapeutic interventions achieved in CML to other HMs

[48]. However, the majority of genetically defined leukemia subtypes are accurately predictable by gene expression profiling (GEP) [51]. Treatment-specific sensitivity assays are being developed for targeted therapies such as imatinib in BCR-ABL positive ALL [51]. GEP studies help in identification of a long list of genes expressed in ALL that have been associated with drug resistance or sensitivity to several drugs and these include: 33 genes for prednisolone, 40 genes for vincristine, 35 genes for asparaginase and 20 genes for daunorubicin [52-54].

Epigenetics is a study of all heritable alterations in gene expression and chromatin structure due to chemical modifications that do not involve changes in the primary gene nucleotide sequence [55,56]. Epigenetic inactivation of pivotal genes involved in correct cell growth is a hallmark of human pathologies, particularly cancer [55]. The following 3 epigenetic mechanisms affect gene expression and are associated with disease progression in acute leukemia: (1) DNA methylation and hypermethylation, (2) histone modification, and (3) non-coding RNAs or alteration in miRNA expression [37,55,56]. Disruption of any of these 3 distinct and mutually reinforcing epigenetic mechanisms leads to inappropriate gene expression, thus resulting in the evolution of cancer and the development of other epigenetic diseases [55]. Acute leukemias, including ALL, are heavily influenced by epigenetics [55-57]. Epigenetic targeted therapies might be particularly appealing as a prolonged therapy in the post-remission setting where they could target specific sub-clones once disease debulking has been achieved by the standard cytotoxic chemotherapy administered to induce remission of acute leukemia [57]. The plethora of genetic lesions in epigenetic regulators offers many possible targets for drug discovery and will definitely attract the attention of the pharmaceutical industry, although the drug ability of enzymes has already biased this choice [37].

Molecular and cytogenetic abnormalities in ALL patients

The recurrent genetic abnormalities that are encountered in patients with B-cell ALL, the common genes that are implicated as well as the prognostic significance of these genetic abnormalities are shown in Table 4 [33,58,59]. Various studies have shown that the following molecular abnormalities occur in patients with ALL: BCR-ABL1, ATM, MLL, TCRα, TCRβ, HOX11, HOX11L2, CDKN2A, CDKN2B, TAL1, TAL2, C-MYC, CRLF2, IKZF1, JAK2, CASP8AP2, IgH/BCL11B, NUP214-ABL, miR15, miR16, TCF3-PBX1, E2A-HLF, ETV6-RUNX1, EBF1, RB1, CREBBP1, SETD2, FOXO3A, KMT2A (MLL), EPHA7, GRIK2, BLIMP1 and FYN [60-62]. The types of gene rearrangements that are seen in patients with T-cell ALL, their subtypes as well as examples of each subtypes are shown in Table 5 [63]. The signaling pathways as well as the various genetic mutations that are found in patients with B-cell ALL and T-cell type of ALL are shown in Tables 6 and 7 respectively [63-69].

In patients with precursor B ALL, the following cytogenetic alterations are usually encountered: (1) ploidy alterations (hyperdiploidy > 50 chromosomes and hypodiploidy < 45 chromosomes) with gains of the following 8 chromosomes accounting for 80% of all gains in ALL: 4,6,10,14,17,18,21 and x; (2) E2A-PBX1 fusion: t1,19 (q23;p13); (3) BCL-ABL fusion: t9,22 (q34;q11); (4) MLL rearrangement (11q23); and (5) ETV6-RUNX1: t12,21 (p13;q22) [63,69].

In patients with mature B-cell (Burkitt type) ALL, the following cytogenetic alterations have been described: (1) MYC-Ig: t 8,14; t 2,8 and t 8,22; and (2) additional recurrent chromosomal abnormalities involving chromosomes 1,6,7,12,13,17 and 22: [a] gains of long arm of chromosomes: 1, +19; 7, +7q and 12, +12q; [b] deletion 17p13; and [c] abnormalities of band 13q34 [63]. The following signaling pathways

Recurrent genetic abnormality	Examples	Common genes implicated	Prognosis
Aneuploidy	Hyperdiploidy: > 50 chromosomes	---	Good
	Hypodiploidy: < 45 chromosomes	---	Poor
Recurrent translocations	t 9,22 (q34;q11)	BCR-ABL1	Intermediate
	t 12,21 (p13;q22)	ETV6-RUNX1	Good
	t 10,14 (q24;q11)	HOX11	-----
	t 5,15 (q35;q32)	HOX11L2	-----
	t 1,19 (q23;p13)	E2A-PBX1	Intermediate
	t 1,14 (p32;q11)	TAL1	----
	t v,q233 (v;q23)	MLL 11q23 rearrangement	Poor
	t 2,8 ; t 8,14; t 8,22	C-MYC	-----
	t 5,14 (q31;q32)	IL3-JGH	-----
Additional genetic alterations	BCR-ABL1-like JAK mutations including (9p24)	IKZF1; CRLF2; JAK mutations	Poor
		RUNX1; CDKN2A/B (p16); CRLF2	Poor
	iAMP21	RUNX1; P2RY8-CRLF2	
	IgH @ 11q32 rearrangement	IgH with multiple fusion partners	Poor
			Poor
	FLT 3 (13q12) mutations	FLT 3	Poor
	PAX 5 (9p13) rearrangemens/deletions	PAX 5 with multiple fusion partner	Unknown
Relapsed ALL	-------	CDKN2A/B, ETV6, IKZF1, CREBBP and NT5C2	Poor

Table 4: Recurrent genetic abnormalities in B-cell ALL.

Type of gene rearrangement	Subtype	Examples
Rearrangements involving TCR genes	Deregulation of homebox genes	(a) TLX1 (HOX11) t 10,14 (q24; q11 and its variant t (7,11) (q35;q24)
		(b) TLX 3 (HOX11L2): t 5,14 (q35;q32)
		(c) HOXA @ cluster (inv7) (p15;q34)
	Deregulation of TAL1-related genes	(a) TAL1(SCL,TCL5) t 1,14 (p32;q11); t 1,17 (p32;q34); t1,14 (p34;q11)
		(b) TAL2 t7,9 (q34;q32)
		(c) LYL1 t 7,19 (q34;p13)
	Deregulation of LIM-domain only genes	LMO1 and LMO2
	Deregulation of family of tyrosine kinase	LCK gene t 1,7 (p34;q34)
	Deregulation of MYB gene	Duplication and t 6,7 (q23;q34)
Fusion gene rearrangements	PICALM-MLL10 (CALM-AF10)	t 10,11 (p13;q14)
	MLL fusions	t 11,19 (q23;p13). MLL-MLLT1 (ENL) gene fusion is the most common MLL translocation partner in T- cell ALL
	ABL1 fusions	ABL1 fusions are rare except: NUP214- ABL fusion (t9,9) (q34;q34) which is identified in 6% of T- cell ALL

Table 5: Cytogenetic alterations in T-cell ALL.

are present in patients with T-cell ALL: NOTCH1-MYC signaling axis, PI3K/AKT/m-TOR pathway, IL7/JAK/STAT pathway, RAS pathway, ABL kinase pathway, NF-kB pathway, Hedgehog pathway, calcineurin-NFAT pathway, Wnt signaling pathway and altered metabolic homeostasis pathway [70].

Signaling pathway	Examples
RAS signaling	NRAS
	KRAS
	FLT3
	PTPN11
	BRAF
	NF1
B-cell differentiation and development pathway	PAX1
	IKZF1 (IKAROS)
JAK/STAT signaling	JAK1
	JAK2
	Mutations in JAK regulators: CRLF1 and IL7R
TP53/RB1 pathway	TP53
	RB1
	CDKN2A/CDKN2B
Other signaling pathways	BCR/ABL1
	BCR/ABL1-like kinase fusions
	ETV6-RUNX1
	IL3-IgH
	MYC(MYC-Igh, MYC-IgK, MYC-IgL)
	CREBBP
	BCL2
	TEL-AML1
	Unknown genes
	E2A-PBX1
	KMT2A rearrangement
	ERG deletion
	TCF-HLF
	iAMP21
	TBL1 XR1

Table 6: Genetic mutations in B-cell ALL.

Signaling pathway	Examples
Cell cycle defects	CDKN2A/ CDKN2B
	TLX1/HOX11
	TAL1 plus LMO1/2
	TLX3/HOX11L2
	LYL plus LMO2
	TP53-RB-P27
Differentiation impairment	HOXA10/11
	MLL fusion/MLL-ENL
	PICALM-MLLT10
	TAL2
Proliferation and survival	ABL1 fusions
	FLT3
	NRAS
	ETV6-PBL2
	LCK
	ETV6-JAK2
	PTEN
	Unknown genes
Cell renewal capacity	NOTCH1
	Unknown genes
Other signaling pathways	WT1
	MYC rearrangement
	BXW7 KMT2A rearrangement
	AK1
	10- TCR beta (7q35)
	IKZF3
	TCR gamma (7p14-15)
	BRAF
	TCR alpha/delta (14q11.2)
	CALM-AF10
	Mutated genes in early thymic progenitors (PTP)-ALL
	NUP2
	ABL1
	p16 (del 9, p 21)

Table 7: Genetic mutations in T-cell ALL.

Specific Genetic Subtypes of ALL

Philadelphia chromosome positive ALL

ALL is the most common cancer in children. Two thirds of cases of ALL occur in children, while one third of cases occur in adults [71]. Treatment of ALL has produced remarkable improvements in outcome for children but the outlook for adults remains poor [71]. Philadelphia chromosome positive (Ph+) ALL is the most common cytogenetic abnormality found in adults with ALL as it accounts for 25% of ALL cases in adulthood [71-73]. However, the prevalence of Ph+ ALL increases with age as follows: < 5% in children, 12%-30% in patients between 18 and 35 years, 40%-44% in patients between 35 and 50 years, and > 50% in patients > 60 years of age [71,74-76].

Treatment of patients with Ph+ ALL is composed of several stages that may span over 3 years and these stages include: (1) induction phase during which cytotoxic chemotherapy and tyrosine kinase inhibitors (TKIs) are administered; (2) consolidation phase that includes either administration of consolidation chemotherapy combined with TKIs for patients not eligible for HSCT or allogeneic HSCT for transplantation candidates; and (3) maintenance phase that can be either in the form of maintenance chemotherapy (composed of 6-mercaptopurine, methotrexate, vincristine, prednisolone or dexamethasone alone) for non-transplantation candidates or maintenance therapy with TKIs after allogeneic HSCT for transplantation eligible patients [75,77,78]. Induction therapy for patients with Ph+ ALL is composed of: (1) cytotoxic chemotherapy with one of several chemotherapeutic regimens such as hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine), CCG regimens, GRAAPH 2003, GRALL AFRO7 or GRALL AFRO9; and (2) TKIs with either dasatinib or imatinib [75,77]. However, the choice of TKI is usually influenced by several factors including: toxicity profiles, administration schedules, comorbid medical conditions, cost and patient preference [77]. Patients who have a disease that is refractory to imatinib can be treated with dasatinib, nilotinib or ponatinib [75,79-81]. Nevertheless, monitoring of minimal residual disease (MRD) by PCR is essential at all stages of therapy in Ph+ ALL patients [75,77,78].

Prior to the era of TKIs, with conventional chemotherapy alone, Ph+ ALL was associated with: high relapse rate, disease-free survival (DFS) not exceeding 10% and for < 12 months, and poor overall survival (OS) [71,80]. When managed with multiagent chemotherapeutic regimens alone, patients with Ph+ ALL have inferior outcomes in terms of remission duration and OS compared with their Ph- ALL patients [73]. Until recently, the outcome of adults with Ph+ ALL has

been poor particularly if treatment includes chemotherapy alone and not allogeneic HSCT [71,80]. Treatment with chemotherapy alone or chemotherapy and allogeneic HSCT in adult patients with Ph+ ALL has resulted in 5-year OS between: 45%-53% in patients <60 years of age and <10% in patients ≥ 60 years of age [71,75].

The introduction of TKIs in the treatment of Ph+ ALL has significantly improved the depth and duration of complete remission (CR), thus allowing more patients to proceed to allogeneic HSCT [71]. TKIs have become an integral part of front-line therapy for Ph+ ALL [75,79]. Studies have shown that TKIs given alone or in combination with cytotoxic chemotherapy in the front-line treatment in patients with Ph+ ALL can produce CR rates exceeding 90% [75,79]. Several clinical trials with adequate follow-up have shown that cytotoxic chemotherapy combined with TKIs, particularly imatinib, is the treatment of choice for newly diagnosed patients with Ph+ ALL [73]. However, the use of second generation TKIs, particularly dasatinib, in patients with Ph+ ALL may further improve the outcome of these patients [73,79]. The recent improvements in the outcomes of patients with Ph+ ALL are not merely due to TKIs, but due to the implementation of an integrated strategy that incorporates: cytotoxic chemotherapy, allogeneic HSCT, second generation TKIs and molecular monitoring to guide therapeutic decisions [79].

Treatment of patients having Ph+ ALL includes: cytotoxic chemotherapy like other ALL subtypes, TKIs such as imatinib and dasatinib, and allogeneic HSCT particularly in adults [72,75,76]. Although allogeneic HSCT is a potentially curative therapeutic modality, it has its own limitations and it requires availability of a healthy human leukocyte antigen (HLA) matching donor and it is associated with relatively high rates of relapse and non-relapse mortality [76]. Haploidentical allogeneic HSCT represents an alternative option for patients with Ph+ ALL who lack an HLA matching donor and its use has been associated with lower relapse rate [82].

In children with Ph+ ALL, only 20%-30% of patients are expected to be cured with chemotherapy alone, while allogeneic HSCT can cure up to 60% of patients who have an HLA matching donor [74]. In children having Ph+ ALL, cytotoxic chemotherapy combined with TKIs are the initial treatment of choice as this combination has been shown to be well tolerated and efficacious [74,83]. TKIs administered in the early phases of treatment can dramatically reduce MRD and improve the outcome of children with Ph+ ALL [84]. Unlike the situation in adults, allogeneic HSCT is still controversial in children with Ph+ ALL [74].

In adults with Ph+ ALL, allogeneic HSCT is recommended for transplantation eligible patients who have suitable donors as it has been shown to be the only proven curative therapeutic intervention [71,73,85]. The administration of TKIs before allogeneic HSCT has significantly improved the long-term outcome of allogeneic HSCT in patients with Ph+ ALL [76]. However, international multicenter studies with long-term follow-up are needed to determine whether consolidation followed by maintenance chemotherapy plus TKIs are equivalent to allogeneic HSCT from related or unrelated donors [74]. In elderly patients with Ph+ ALL, combination of imatinib and low-dose chemotherapy composed of vincristine and dexamethasone has produced: excellent initial efficacy, less morbidity and mortality compared to intensive chemotherapy, but frequent relapses [85]. In patients with Ph+ ALL, the mechanisms underlying resistance to TKIs, particularly imatinib, are multifactorial and include mutations in the bcr-abl tyrosine kinase domain such as T315I mutation [75,80,81]. In patients with relapsed Ph+ ALL, the combination of hyper-CVAD chemotherapy and dasatinib has been shown to be an effective therapeutic option [86].

Philadelphia chromosome-like ALL

Philadelphia chromosome-like (Ph-like) B-lineage ALL is one of the new high-risk (HR) subtypes characterized by genetic alterations that activate various signaling pathways including: tyrosine kinases, cytokine receptors and epigenetic modifiers [19]. The recently introduced advanced genetic analysis methods such as WGS and transcriptome sequencing have led to the discovery of novel genetic subtypes of ALL such as Ph-like ALL [87]. Ph-like ALL shares a similar GEP with Ph+ ALL (with t9,22 and BCR-ABL1 fusion) [88]. However, it lacks the BCR-ABL fusion or t9,22 by routine cytogenetics, FISH or molecular analysis [87,88]. Recently, the world health organization (WHO) classification of myeloid neoplasms and acute leukemia was adapted to include Ph-like subtype of B-cell ALL as a provisional entity [89].

Ph-like ALL can be detected in approximately: 10%-15% of children, 20%-25% of adolescents and 20%-23% of adults with B-cell ALL [88,90-92]. It is encountered more frequently in male adults and has associations with: Hispanic ethnicity, Down's syndrome and inherited genetic variants in GATA3 (rs3824662) [91,92]. When compared to other pre-B ALL subtypes, Ph-like ALL has the following characteristic features: (1) higher median leukocyte count at presentation, (2) higher median levels of MRD at the end of induction therapy, (3) inferior estimated event-free survival (EFS) at 5 years [58% versus (vs.) 84% in children, 41% vs. 83% in adolescents and 24% vs 63% in young adults], and (4) inferior estimated OS at 5 years [73% vs. 92% in children, 66% vs. 93% in adolescents and 26% vs 75% in young adults [88]. The various genetic abnormalities that are encountered in patients with Ph-like ALL and their targeted therapies are shown in Table 8 [19,93-97] The investigational targeted therapies in Ph-like ALL are shown in Table 9 [89,98].

Unfortunately, Ph-like ALL is essentially heterogeneous and a HR subtype of ALL [19,92-95,98]. It has characteristic and distinct genetic mutations and rearrangements that are activated by a diverse array of cytokine receptor and tyrosine kinase signaling [87,89,92-94,96]. The genetic lesions in adults with Ph-like ALL resembles that in children with Ph-like ALL but differs from the profile in the remaining patients with pre-B ALL [99]. Deletion mutations involving IKZF1 gene, that codes for the transcription factor IKAROS, underlie many cases as a key factor inducing aggressive phenotype and poor responses to treatment [19]. The high frequency of IKZF1 gene alterations and the presence of CRLF2 gene rearrangements are associated with poor long-term

Class	Examples	Percentage	Targeted therapies
ABL fusions or rearrangements	ABL1, ABL2, CSF1R1, PDGFRB	22%	Imatinib, dasatinib, nilotinib, poatinib
EPOR or JAK2 rearrangements	EPOR, JAK2	18%	JAK2 inhibitors
CRLF2 rearrangements	CRLF2	20%	JAK2 inhibitors
JAK-STAT pathway alterations	JAK1, JAK3, FLT3, IL2RB, IL7R, TYK2, SH2B3	20%	JAK1 inhibitors, JAK3 inhibitors, FLT3 inhibitors, TYK2 inhibitors
RAS pathway mutations or alterations	NRAS, KRAS, NF1, PTPN1	10%	Future drugs
Other kinase alterations	NTRK3, PTK2B	9%	Crizotinib, FAK inhibitors

Table 8: Genetic abnormalities and their targeted therapies in Philadelphia-like acute lypmhoblastic leukemia ALL.

Drug	Target	Mode of action
Givinostat	CRLF2	Histone deacetylase inhibitor class 1/2
JQ1	CRLF2	BET inhibition
Luminespib	CRLF2	Heat shock protein (HSP90) inhibitor
Selumetinib	CRLF2	MEK 1/2 inhibitor + ATP competitive JAK2 inhibitor
Rapamycin	m-TOR activated pathway	m-TOR inhibition
Gedatolisib	PI3K + m-TOR activated pathway	Dual inhibitor of PI3K-α, PI3K-γ and m-TOR
Birinapant	Tumor necrosis factor-α-dependent	SMAC mimetic

Table 9: Investigational targeted therapies in Ph-like leukemia.

outcome in patients with Ph-like ALL [91,92]. Studies have shown that: (1) BCR-ABL1-like B-cell ALL is mutually exclusive of the well-defined recurring chromosomal abnormalities in B-cell ALL such as: BCR-ABL1, TCF3-PBX1, t12,21 (ETV6-RUNX1), and 11q23 (KMT2A) and that (2) deletions or mutations in IKZF1 gene are the hallmark of not only Ph-like ALL but also BCR-ABL1-positive ALL [87,88].

Ph-like ALL is associated: with resistance to cytotoxic chemotherapy, very high risk of disease relapse and poor OS [88,90,92,93,95,96,98,99]. The identification of a wide range of genetic alterations that activate certain signaling pathways makes such gene alterations amenable to inhibition by a variety of TKIs [97]. Also, the combination of TKIs and cytotoxic chemotherapy may improve the outcome of patients with Ph-like ALL [92,95]. Thus, Ph-like ALL illustrates the modern treatment paradigm of precision medicine and presents a unique opportunity for harnessing international collaboration to further improve the outcome of ALL patients in general [92]. Therefore, prospective studies are needed to determine if incorporation of TKIs targeting kinase alterations into the intensive chemotherapeutic regimens will improve the outcome of patients with Ph-like ALL [91]. However, in a group of patients with B-cell ALL treated with frontline hyper-CVAD-based chemotherapy, the outcome of Ph-like ALL was inferior to the other B-cell ALL subgroups [90].

Although most patients with Ph-like ALL have MRD after induction chemotherapy and poor EFS, approximately 40% of pediatric patients with Ph-like ALL respond well to chemotherapy and can be cured with relatively low-intensity therapies [91]. Nevertheless, novel therapies are needed to improve the outcome of patients with Ph-like ALL as this this subtype of ALL represents a disease where precision medicine testing, and treatment approach should be implemented [90,94,99]. Newly discovered genetic alterations that drive HR Ph-like ALL can now be targeted with TKIs and JAK inhibitors leading to clinical trials for this particular HR subset [100]. Birinapant is a small molecule mimetic of the apoptotic regulator SMAC. It targets tumor necrosis factor (TNF)-α-dependent signaling [98]. One study published in 2016 showed that: (1) birinapant exhibited profound anti-leukemic activity as a single agent or in combination with cytotoxic chemotherapy in pediatric patients with Ph-like ALL, and (2) the activity of birinapant was superior in pre-B ALL compared to T-cell ALL [98].

IKAROS protein and IKZH1 gene

IKAROS is a DNA-binding zinc finger protein [101-103]. It functions as a regulator for gene expression and chromatin remodeling [101,103]. IKAROS is required for development of all lymphoid lineages [101,102]. It is required for lymphoid development since its depletion abrogates fetal T and B lymphocytes and perturbs myeloid development [104]. The biological roles of IKAROS protein include: (1) regulating the development and function of the immune system, and (2) acting as a master regulator of hematopoietic differentiation and B-cell development [103,104].

The number of IKAROS family members is large, thus the functional forms of IKAROS proteins are very diverse due to protein-protein interaction [102]. IKAROS family members are critical for a functional immune system that confers protection against invading organisms while minimizing the risk of: leukemic transformation, immune pathology and autoimmunity [104]. There is emerging evidence that targeting IKAROS family proteins is feasible, mainly through post-translation modification, and is effective in therapeutic applications [102]. IKAROS has shown tumor suppressor activity in the lymphoid lineage in mouse models [103,105]. The mechanisms by which IKAROS functions as tumor suppressor in pre-B ALL remain poorly understood [105]. Also, the mechanism by which deletion of IKZF1 gene influences the pathogenesis of pre-B ALL is still unclear [106]. Inactivation of the tumor suppressor gene IKZF1 encoding the transcriptional regulator IKAROS is the hallmark of BCR-ABL1 positive pre-B ALL [105]. Genetic depletion of different IKAROS targets including CTNND1 and the early hematopoietic cell surface marker CD34 has resulted in reduced leukemic growth [105]. Elevated CTNND1 expression contributes to maintenance of murine B-ALL cells with compromised IKAROS function [105].

Genomic profiling studies have identified IKZF1 as a clinically important tumor suppressor in ALL, particularly HR-ALL that is associated with poor prognosis [103]. The identification of IKAROS target genes may guide therapeutic strategies in patients with ALL [104]. IKZF1 mutations occur in approximately 5% of patients with T-cell ALL and in > 70% of Ph+ ALL and Ph-like disease subtype [107]. Aberrant NOTCH activity in T-cell ALL compromises IKAROS function in mouse and human T-cell ALL and may explain the relative infrequency of IKAROS gene mutations in human T-cell ALL [107].

IKZF1 gene is located on chromosome 7 p12.2 sub-band [108]. It was first identified in the year 1992 as an important transcription factor for the maturation of T-lymphocytes in mice [102,108]. Thus, IKZF1 gene is an essential transcription factor which is involved primarily in lymphoid differentiation [108]. It is expressed in: hematopoietic stem cells (HSCs), all lymphoid cells and some myeloid cells [104]. IKZF1 gene codes for IKAROS zinc finger protein [101,103,108]. Molecular pathways, specifically controlled by IKZF1, that indicate the role of this gene in the pathogenesis of B-cell ALL have been identified recently [106]. IKZF1 gene deletions have been associated with: increased risk of relapse and poor outcome in pre-B ALL [101,108]. IKZF1 gene deletion has been found to be an independent predictor of treatment outcome and a strong candidate marker for integration in future treatment stratification strategies as an independent risk factor when MRD data are taken into account [101,108]. Thus, IKZF1 status may contribute to a molecular-based stratification algorithm aiming at improving outcome of patients with ALL [101].

The characteristic features of IKZF1 mutated ALL include: (1) HR-ALL except for ERG-deleted cases, (2) occurrence in 84% of Ph+ALL at diagnosis, (3) occurrence in 68% of Ph-like ALL, (4) frequently encountered in the progression of CML to lymphoid blast cell crisis, (5) high adhesion potential to HSC niche through integrins, and (6) possible response to focal adhesion kinase inhibitors and retinoid receptor agonists [19]. Deletion or mutation of IKZF1 gene correlates with poor clinical outcome [104]. Studies in humans have shown that: (1) IKZF1 knockdown alone does not impart intrinsic chemotherapy resistance suggesting that the association with poor prognosis may be

due to: additional lesions, micro-environmental interactions with the BM niche or other reasons, and that (2) correlation between IKZF1 gene and the incidence of ALL, as shown by SNP studies, suggesting variation in IKAROS protein or its regulatory elements as a genetic risk factor for ALL [104,109].

Recently, experimental studies using mouse models of pre-B ALL have shown that IKZF1 alterations have the following adverse effects: (1) arresting normal differentiation of HSCs, (2) skewing lineage of leukemia from myeloid to lymphoid, and (3) in Ph+ leukemia, conferring resistance to TKI therapy without abrogating ABL1 inhibition, thus strategies aimed at directly restoring normal IKZF1 expression are promising therapeutic approaches [110]. Also, recent studies have shown: (1) deletions or mutations of IKZF1 gene correlate with poor prognosis in other subtypes of pre-B ALL, thus providing evidence that IKAROS protein is an important tumor suppressor in pre-B ALL, (2) IKZF1 alterations have been reported in pre-B ALL carrying BCR-ABL1 rearrangement and have been associated with poor treatment outcome, (3) occurrence of IKZF1 aberrations in association with other genetic alterations such as JAK mutations and translocations involving chemokine receptor like factor 2 (CRLF2) in childhood ALL, and (4) IKZF1 alterations in B-cell ALL lead to induction of multiple genes associated with proliferation and treatment resistance suggesting identification of new therapeutic targets for HR disease [101,105,107]. Recently, there have been controversial reports on the benefit of intensifying conventional maintenance chemotherapy by giving vincristine and glucocorticoid pulse therapies in patients with IKZF1 deleted ALL [111,112].

Prognosis and Risk Stratification in ALL

Factors that are associated with prognosis and risk stratification in patients with ALL include: (1) age, (2) white blood count (WBC) count at presentation and percentage of blasts in the peripheral blood, (3) presence or absence of extramedullary disease, particularly central nervous system (CNS) involvement, (4) immunophenotyping profile and ALL subtype, (5) cytogenetic abnormalities, (6) genetic mutations as well as molecular and GEP, (7) rapidity and degree of cytoreduction, and (8) presence or absence of mRD at any stage of treatment [60,63,113-115]. Recently, the correlation between the following old risk factors and prognosis in ALL is no longer significant: (1) gender, (2) race, (3) French-American and British (FAB) morphology, (4) hemoglobin level, (5) platelet count, (6) serum immunoglobulin levels, (7) presence or absence of mediastinal mass, and (8) presence or absence of palpable external lymphadenopathy of abdominal organomegaly [115]. The prognosis of ALL varies significantly with respect to age being favorable in children 1-10 years of age, intermediate outcome in adolescents and young adults and unfavorable in patients below 2 years and above 50 years of age [63,115]. Table 10 shows risk stratification in ALL patients based on their clinical and cytogenetic prognostic features in addition to survival rates in each risk category [115,116].

Several studies have shown that the poor prognostic feature in patients with ALL are: (1) age < 2 years or above 50 years, (2) WBC count ≥ 50 × 10^9/liter, (3) presence of extramedullary disease, particularly CNS involvement, at presentation, (4) presence of residual disease following induction chemotherapy (≥ 5% BM blasts on days 15, 19,29,35 and 43), (5) unfavorable immunophenotypic profiles: precursor T, pro-B and mature B-cell types, and (6) presence of adverse or unfavorable cytogenetic abnormalities or molecular mutations such as: hypodiploidy with < 44 chromosomes, near hypodiploidy, translocation (t) 4,11 (q21;q23), t8,14 (q24;q32), complex cytogenetics, MLL gene rearrangement, FLT 3 (13q12) mutation, BCR-ABL1-like

Risk category	Cytogenetic abnormalities	Other risk factors	4 year event free survival	Overall survival
Low-Risk	**Favorable**	Children with favorable age	> 95%	> 50%
	Del (9p)			
	High hyperdiploid			
	Low hyperdipoid	Low white blood cell count at diagnosis		
	Tetradiploid			
	ETV6-RUNX1 rearrangement	Rapid response to treatment		
Standard-Risk	**Intermediate**	1- t (10;14)	90-95%	40-50%
	- Patients with favorable age	2- abn 11q		
		3- del (12p)		
	- Low WBC count at presentation	4- del (13q) 1-13		
		5- Normal		
	- Favorable response to treatment	6- Other		
High-Risk	**High-risk** 1- Del (6q) 5- t (1,19)	- Patients older than 10 years of age	88-90%	30-40%
	2- - 7 6- other TCR	- Residual disease in bone marrow after induction		
	3- del (7p) 7- 14 q 32			
	4- other 11q 23 - other			
Very high-risk	**Very high-risk** 1- t (4,11) – KMT_2A (MLL rearrangement)	Age < 1 year and >13 years	< 80%	< 30%
	2- t (8,14)	Failure to achieve complete remission at the end of induction therapy		
	3- del (7p)			
	4- + 8			
	5- tx			
	6- Extreme / Low hypodipioidy (44 chromosomes)			
	7- t (9,22) in Adults (bcr/abl)			
	8- iAMP21; intrachromosomal amplifn of chr. 21			

Table 10: Risk stratification in ALL.

(IKZF1, CRLF2 and JAK mutations), iAMP21 (RUNX1/P2RY8-CRLF2), and IgH (14q32) rearrangement with multiple fusion partners [61,63,114,115,117]. Also, several studies have shown that the following factors are associated with good prognosis in patients with ALL: (1) age between 2 and 10 years, (2) high hyperdiploidy, (3) del (9q), and t12,21 (p13;q22) (ETV6-RUNX1; TEL-AML1) [63,114,115,117]. However, intermediate outcome is associated with the following factors: (1) adolescents and young adults, (2) t9,22 (q34;q11), and (3) t1,19 (q23;p13) [63,115,117].

Drug Resistance and Relapse in ALL Patients

The biology and treatment of ALL continue to attract attention as areas for which targeted therapies offer great promise [118]. Host genetic polymorphisms are associated with: (1) predisposition to childhood ALL, (2) response to therapy, and (3) adverse effects of medications [100]. Prospective testing and therapy modifications can be achieved in real time, allowing patients who might have suffered relapse to achieve

adequate control for HR ALL [118]. The discovery of relapse-specific genetic mutations provides an explanation for clonal evolution, drug resistance and the possibility of designing therapies to prevent relapse [100]. Risk factors for relapse in ALL include: (1) WBC count > 100 × 10^9/liter, and (2) having genes that express drug resistance such as: MLL-AF4, TEL-AML1, BCR-ABL and NT5C2 mutations that are associated with resistance to 6-mercaptopurine and 6-thioguanine [52,53]. In patients with B-cell ALL, genetic mutations that are associated with HR of treatment failure and relapse include: CDKN2A, CDKN2B, ETV6, IKAROS-IKZF1, CREBBP, SETD2, KDM6A, MSH6 and MLL2 [117]. The genetic mutations that are associated with relapse in patients with T-cell ALL include: TP53, KRAS, NRAS, NT5C2, USP7, IL7R, CNOT3 and MSH6 [119].

In patients with relapsed ALL, the poor prognostic factors for CR include: (1) serum albumin < 3 gram/liter (g/L), (2) duration of first CR< 3 months, (3) hemoglobin < 10 g/L, (4) platelet count ≤ 50 × 10^9/L, (5) BM blasts > 50%, and (6) peripheral blood blasts ≥ 1% [120]. Poor prognostic factors for survival in relapsed ALL include the above 6 factors in addition to WBC count > 20 × 10^9/L [120].

The integration of targeted therapies remains a challenge both in identifying targetable genomic alterations and on how to integrate targeted therapies into clinical practice [118]. Multiple studies have shown that, in addition to allogeneic HSCT, the following novel therapies are effective in patients with drug resistant or relapsed ALL: clofarabine, nelarabine, dasatinib alone or in combination with hyper-CVAD, sphingosomal vincristine, pegylated asparaginase, blinatumomab, ponatinib, chimeric antigen receptor (CAR) T-cells, SRC inhibitor KX2-391 in T-cell ALL and the BCL2 inhibitor venetoclax [54,81,86,100,120-122]. The latter drug has been found to be highly effective in pre-B ALL with MLL-AF4 and TCF3-HLF mutations. Venetoclax has also been shown to be effective in T-cell ALL. It can be used as a single agent or in combination with vincristine or dexamethasone [47]. Novel immunotherapeutic approaches using BiTE antibodies and CAR T-cells offer great promise in treating relapsed disease which is a leading cause of death in patients with ALL [100]. In order to reduce neurophysiological sequelae of radiotherapy, CNS irradiation can be limited to a very small subset of patients at initial diagnosis or even abandoned completely [100].

Recent Therapeutic Modalities

Novel therapies and precision medicine

ALL is a malignancy that is influenced by epigenetics. Therefore, epigenetic targeted therapies may look particularly appealing in the post-remission setting in order to target specific sub-clones that may be resistant to standard cytotoxic chemotherapy [123]. Current therapeutic regimens that are used in the treatment of ALL patients are mostly non-targeted and most of the chemotherapeutic agents included in these regimens have a narrow therapeutic index that frequently causes life-threatening toxicities and even deaths [124,125]. Even targeted therapies have their own toxicities that may be severe [125]. Hence, new treatments that are tailored to patient-specific tumor vulnerabilities are required to further improve treatment outcomes and limit toxicities [124]. Examples of the novel agents and targeted therapies that are either currently used or being under development are shown in Table 11 [60,66,126-132].

Recent genomic sequencing studies have provided multiple insights into: classification of ALL subtypes and genetic basis of ALL [124]. Examples of the main molecular markers and genetic targets in ALL are: BCR-ABL1, BCR-ABL1-like, ETV6-RUNX1, IKZF1, CDKN2A, CDKN2B and NOTCH1 [123]. A detailed list of genetic abnormalities and molecular markers that have been found in patients with ALL are included in Tables 4-7 [33,58,59,63-69]. Examples of main novel and targeted therapies that are currently used in the treatment of ALL are: rituximab, nelarabine, TKIs, blinatumomab and CAR-T cells [123]. Drug development data has shown different effects on the use of HSCT, highly effective novel agents may replace HSCT while less effective novel drugs may improve the outcome of patients when combined with HSCT [133].

1	**Monoclonal antibodies, CD marker and conjugated CD marker antibodies:** Rituximab, obintuzumab, ofatumomab, epratuzumab, inotuzumab ozogamicin, gemtuzumab ozogamicin, moxetumomab pasudodotox (reformulation of BL22), coltuximan ravtansine (SAR3419), denintuzumab mefodotin (GN-CD19A), ADC-402 (newest CD19 monoclonal antibody), combotox (anti- CD19 and anti- CD22) and blinatumomab (anti- CD3; CD 19 construct)
2	**FLT3 inhibitors:** lestaurtinib, midostaurin, sunitinib and tandutinib
3	**Tyrosine kinase inhibitors (TKIs) and spleen TKIs:** imatinib, dasatinib and nilotinib
4	**Multikinase inhibitors:** sorafinib
5	**Proteasome inhibitors:** bortezomib
6	**JAK and TAM tyrosine kinase inhibitors**
7	**DNA methyltransferase:** dectitabine and azacytidine,
8	**Histone deacetylase inhibitors:** vorinostat, LBH 589 and PDX 101
9	**mTOR inhibitors:** RAD001 and rapamycin (sirolimus)
10	**Aurora inhibitors:** MLN 8237
11	**BCL2 antagonists:** obatoclax
12	**Farnesyl transferase inhibitors**
13	**Trail receptor antagonists**
14	**Survivin inhibitors**
15	**Microtubule, destabilizing agents:** ENMD-1198
16	**Antifolates:** pemetrexed
17	**Heat shock protein inhibitors**
18	**Chimeric antigen receptor T-cells (CAR T-cells)**
19	**Nucleoside analogues:** nelarabine, clofarabine and forodesine
20	**Liposomal and pegylated compounds:** liposomal or sphingosomal vincristine, liposomal doxorubicin, liposomal cytarabine, pegylated asparaginase and liposomal annamycin

Table 11: Novel and targeted therapies that are either available or under development for the treatment of acute lymphoblastic leukemia.

Precision or personalized medicine refers to the use of specific characteristics of an individual patient, based on his/her molecular and genetic profiles to tailor therapies during all stages of care accordingly and this implies: (1) provision of the right patient with the right drug at the right dose and at the right time, and (2) optimizing therapeutic benefits while limiting toxicities of the drugs used [123,125]. The recent findings have several implications on precision medicine approaches in ALL that include: (1) different disease subtypes have distinct associations with relapse risk, hence the intensity of treatment will be tailored accordingly, (2) several ALL subtypes have genetic alterations making them candidates for genomic studies and individualized targeted therapies, (3) identification of several genes and genetic pathways that are selectively mutated at relapse including epigenetic regulators and these require local and targeted therapeutic interventions, and (4) genomic sequencing is being utilized to develop highly sensitive approaches to detect and track responses to treatments [124].

Knowledge concerning ALL has advanced greatly to the extent that precision medicine may become a reality in the near future [132]. However, the latest successes in the treatment of childhood ALL can be

attributed to the careful personalized adaptation of treatment by means of risk stratification [134]. The use of genetic information obtained by advanced technology such as molecular genetics, DNA sequencing and genetic and epigenetic assays plays a major role in the design of personalized medicine [123]. Genomic profiling of ALL using next generation technologies has refined our understanding of the biology of ALL subtypes and has improved risk stratification of patients [135]. Identification of somatic alterations and leukemia-associated pathways has facilitated therapeutic drug targeting by TKIs and other molecularly-targeted agents [135]. Major advances in genetic and epigenetic profiling of ALL have increased our understanding of key biological subsets of de novo and relapsed ALL and this has led to improved risk stratification [136]. The latest therapeutic successes in the treatment of childhood ALL can be attributed to the refined risk stratification which is based on: the underlying biological and clinical characteristics, the depth of initial response to therapy and the appropriate modulation of the intensity of cytotoxic chemotherapy [136]. Nevertheless, achievement of greater precision in treatment requires: druggable targets as well as specific targeting drugs [134].

The more sophisticated diagnostic techniques such as: immunophenotyping, cytogenetics, molecular genetics and genomic assays have allowed the definition of new ALL subtypes which, in some cases, has translated into specific therapies [137]. Also, progress in the: (1) diagnosis of ALL with identification of genomic-defined sub-entities, (2) evaluation of MRD, and (3) development of new targeted therapies has led to a substantial realization of precision medicine [137]. The current options, such as: (1) administration of less intensive chemotherapy, (2) reduction in the number of HSCT procedures performed, (3) incorporation of targeted therapies, and (4) optimal combination of various therapies requires prospective and collaborative research to further refine the role of individualized approach to each patient [137].

Currently, human leukemias and lymphomas are defined by: (a) common chromosomal translocations, (b) shared mutations in oncogenes, (c) immunophenotyping properties, and (d) characteristic GEP [138]. PCR for antigen receptor rearrangements (PARR) assays are used in 2 settings: (1) providing lineage and other clinically useful information in resolving ambiguous cases of leukemia or lymphoma, and (2) as a research tool to better understand the biology of HMs [138]. Polymorphism term is used to describe multiple forms of a single gene that exists in a population [125]. Genetic polymorphisms are one of the most important factors that may contribute to ethnic sensitivity of a drug. Mutation in a gene is a change in DNA sequence away from normal allele and forms an abnormal variant. Polymorphism analysis, mutational analysis and genome sequencing are the backbone of "discipline of pharmacogenetics" [125]. Molecular-based diagnostic tests in patients with HMs can be classified into 3 main categories: (1) detection of individual mutations in oncogenes. (2) detection of chromosomal translocations, deletions and duplications, and (3) detection of clonality in leukemia or lymphoma by taking advantage of the unique antigen receptor genes of B- and T-cells [138]. Thus, the goal of "discipline of pharmacogenetics" is to make personalized medicine applicable to various patient groups [125].

Drug repurposing in ALL

Multidrug resistance is a major cause of chemotherapy failure that requires development of new drugs [139,140]. Development of a new or de novo drug is very expensive and often takes 9-17 years [141,142]. Drug repositioning or repurposing means exploration of new clinical indications for existing drugs [141,142]. It is a potential alternative to new drug discovery that takes plenty of time, effort and cost [142-144]. Drug repositioning requires collaboration between academic institutions, research centers, drug industry and nonprofit charitable organizations [142]. Computational drug repositioning by integrating data from various sources and knowledge of the transcriptional information related to gene expression signatures are new gates to drug repositioning [141,143].

Examples of drugs that have been repositioned to treat ALL include: (1) dasatinib was originally used for CML and currently it is used for Ph+ALL; (2) arteannuin which was discovered by the Chinese to treat malaria in the 1970s, recently it has been found to have activity against CD10 (neprilisin) common ALL; (3) the natural semisynthetic compound (artesunate, ART) and its novel analogue (ART-838) that are currently recommended for the treatment of severe malaria by the WHO have been found to have antileukemic activity against T-cell ALL, B-cell ALL and AML; (4) the natural product (triptolide) that is used for the treatment of inflammatory arthritis has been found to reverse cytarabine and doxorubicin resistance in ALL, so it can be used as salvage therapy for relapsed and refractory ALL; (5) the anthelmintic drug, niclosamide, has been found to have activity against multidrug resistant leukemia in addition to its uses in other solid tumors, (6) the HIV protease inhibitor, nelfinavir, has been found to be effective against all leukemia cell lines including HR ALL with MLL and HR AML with FLT3-ITD; and (7) the angiotensin II receptor blocker, telmisartan, has recently been found to induce significant cell growth inhibition and apoptosis in adult T-cell leukemia cell lines via caspase activation [139,140,142,145-147].

Conclusions and Future Directions

The recent advances in cytogenetic and molecular assays as well as genomic and epigenetic techniques have resulted in tremendous progress in: understanding the biology of ALL, the diagnosis of ALL and its classification into specific subtypes, risk stratification, prognosis, development of novel agents and targeted therapies, monitoring of response to treatment as well as the recent introduction of precision medicine. These technical advances have translated into improved outcomes and have resulted in improving the prognosis of certain disease subtypes starting with Ph+ ALL and then moving to other subtypes such as Ph-like and T-cell types of ALL. Prior to the introduction of targeted therapies such as TKIs, these disease subtypes were associated with poor outcome. Nowadays, with the availability of several novel therapies, these aggressive ALL subtypes are potentially curable even without HSCT. The incorporation of these novel therapies at various stages in the therapeutic paradigm of ALL will further improve the outcome of patients.

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