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REVIEW ARTICLE

Intracellular Signaling Pathways Involved in Childhood Acute Lymphoblastic Leukemia; Molecular Targets

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Abstract Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by an uncontrolled proliferation of immature lymphoid cells. ALL is the most common hematologic malignancy in early childhood, and it reaches peak incidence between the ages of 2 and 3 years. The prognosis of ALL is associated with aberrant gene expression, in addition to the presence of numerical or structural chromosomal alterations, age, race, and immunophenotype. The Relapse rate with regard to pharmacological treatment rises in childhood; thus, the expression of biomarkers associated with the activation of cell signaling pathways is crucial to establish the disease prognosis. Intracellular pathways involved in ALL are diverse, including Janus kinase/Signal transducers and transcription activators (JAK-STAT), Phosphoinositide-3-kinase–protein kinase B (PI3K-AKT), Ras mitogen-activated protein kinase (Ras-MAPK), Glycogen synthase kinase-3β (GSK-3β), Nuclear factor-kappa beta (NF-κB), and Hypoxia-inducible transcription factor 1α (HIF-1α), among others. In this review, we present several therapeutic targets, intracellular pathways, and molecular markers that are being studied extensively at present.

Keywords Acute lymphoblastic leukemia · Biological markers · Signaling pathways · Targets · Therapeutic

Introduction

The acute leukemia is the most common cancer in children, adolescents, and young adults. These diseases are characterized by great clinical variability, prompting an on-going search for accurate outcome predictors. Using algorithms based on clinical manifestations at presentation, response to therapy, and several molecular analyses, some patients are diagnosed with the features of high-risk disease and are at comparatively greater risk for relapse [1].

Molecular analyses of patients with high-risk acute leukemia have resulted in an improved understanding of how deregulated cellular signaling can affect resistance to conventional therapy. In this regard, molecular therapies that target genetic abnormalities in leukemic cells and their affected signaling pathways have been emerging in pediatric Acute lymphoblastic leukemia (ALL). Expression profiling, whole-genome sequencing, and other molecular analyses have provided insight into the signaling pathways that are mechanistically related to chemotherapy resistance. For pediatric acute leukemia, the molecular determinants of risk have yet to be fully defined, and target therapies remain in the earliest stages of discovery [1]. Whereas exciting discoveries continue to be made in the identification of relevant molecular biomarkers and targeted therapies, the challenges and opportunities associated with these findings continue to await being clearly defined in future clinical trials [1].

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Acute Lymphoblastic Leukemia

The clinical presentation of ALL is variable with an insidious initial manifestation of about 4 weeks. This type of leukemia is more common in early childhood, and reaches its highest incidence between the ages of 2 and 3 years (>80 per million per year, pmpy), with rates decreasing to 20 pmpy in children between 8 and 10 years. In many cases, some leukemic cells have begun to accumulate in different organs such as the liver, lymph nodes, spleen and central nervous system (CNS) when the diagnosis is confirmed [1]. The complete blood count generally reflect marrow failure conditioned by the invasion of leukemic cells such as anemia, thrombocytopenia and neutropenia, therefore, in the study of acute leukemia, morphology and cytochemical stains are essential in the initial characterization of the disease [1, 2].

The genetic basis of this disease has been revolutionized with the study of long cohorts [2]. The identification of new ALL subtypes, structural and numerical genetic alterations, transcriptomic profile, key genes in outcome and resistance to the treatment are critical keys to redefine the ALL classification in the shortcoming time [2, 3].

Classification and Staging

French- American -British (FAB) Classification

Cell morphology criteria classifies ALL as L1, L2 and L3 based on the characteristics of the leukemic cell. Approximately 70–85 % of all enrolled pediatric patients belong to the ALL L1 group. While the morphological distinction between L1 and L2 variety losses prognostic value, the L3 morphology has been associated with the mature B- range [2].

Risk Groups in Childhood ALL

Children with ALL are classified into four risk groups according to the National Cancer Institute (NCI): very high, high, standard and lower risk [4].Participants of a recent workshop sponsored by the NCI defined standard-risk ALL as B-precursor cases with age between one and 10 years and an initial leukocyte count of $<50 \times 10^9$/L. All other patients are considered high-risk [4].

The type of leukemia determines the initial response to treatment; therefore, the predictive factors have a very important role in childhood acute lymphoblastic leukemia (chALL) (Table 2) [3, 5].

Leukemic Cells Immunophenotype

The immunophenotype is a highly significant prognostics feature. With this diagnostic method, the target is to identify the affected cell line, whether B or T. Children with acute pre-B cell leukemia or early pre-B cell leukemia early respond better than those with T-cell leukemia and mature B-cell leukemia [6–10].

The prognostic significance of cell surface markers in ALL is an integral and important part of disease diagnosis, classification, and prognosis. These cells have been classified by mature, differentiation, and activation markers, according to the expression of these surface markers: for example, CD45 is a blastic cell marker, CD34, CD117, HLA-DR, and TdT are mature cell markers, CD10, CD19, CD20, CD22, and CD79A are B-lymphoid lineage markers, and CD1a, CD2, CD3, CD5, and CD7T- are lymphoid lineage markers.

The previously mentioned cell surface markers have been correlated with the prognosis in ALL; accordingly, early T-precursor ALL and T-ALL demonstrate poorer prognosis than B-cell lineage leukemia [5, 11, 12]. Similarly, the presence of CD20 has been associated with a high WBC count and a lower platelet count [12].

Cytogenetic Abnormalities

The cytogenetic abnormalities include an important number of structural and numerical alterations present in chALL. According with the World Health Organization (WHO) the classification of acute leukemia includes two lineages; B lymphoblastic leukemia and T lymphoblastic leukemia (Table 1) [13].

Leukemic cells are usually associated with an increased number of chromosomes (hyperdiploid), the most common of this phenomenon affects the 4, 10, 17, and 18 chromosomes; moreover, leukemic cells can also be associated with a decreased number of chromosomes related with a poor prognosis. In fact, a translocation between chromosomes 12 and 21 t(12;21) is more likely to be cured [13]. On the contrary, children with a translocation between chromosomes 9 and 22, or between 1 and 19, have a lower cure rate. Children with a translocation affecting chromosomes 4 and 11 or all (q23) translocations also have a lower cure rate [5, 14, 15]. Chromosomal translocations that possess prognostic significance may be detected in a substantial number of cases of pediatric ALL, and some of these rearrangements are described later.

Cryptic translocation t(12;21) favors the fusion of *TEL (ETV6)* gene on chromosome 12 to the *AML1(CBFA2)* gene on chromosome 21, resulting in the *TEL-AML1* fusion

Table 1 WHO classification of acute leukemia

B lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma, NOS
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);*BCR-ABL 1*
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1* (*ETV6-RUNX1*)
B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH*
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
T lymphoblastic leukemia/lymphoma

that can be detected in 20–25 % of cases of B precursor ALL, giving a favorable clinical outcome. Patients with *TEL-AML1* fusion, have a favorable clinical outcome, although there is controversy concerning whether the final cure rate is actually higher than that of other patients with B-precursor ALL, or whether the final cure rate is similar, but time-at-relapse occurs significantly later in patients with *TEL-AML1* fusion compared with other patients with B-precursor ALL.

Linka et al. [16] recently identified, directly and indirectly, regulated target genes with an inducible design *TEL-AML1* system to explain the mechanism associated with cellular proliferation and transport, cellular migration, and stress responses in chALL. Therefore, the Philadelphia chromosome (Ph chromosome) implicates the t(9;22) translocation that is present in approximately 4 % of pediatric ALL which confers a poor prognosis, especially when associated with either a high WBC count or a slow, early response to initial therapy. The Ph chromosome is more common among older patients with B-precursor ALL and high WBC count [13].

Another translocation involved in chALL is the t(11;19) that occurs in about 1 % of cases B or T precursors. The t(11;19) have a worse prognosis but the result appears relatively favorable for children with T-cell ALL and the t(11;19) [14, 15].

Finally, t(1;19) presents in 5–6 % of cases of chALL, and consists of *E2A* gene to the *PBX1* gene fusion located on chromosome 19 and 1 respectively. t(1;19) could present either as an unbalanced translocation or as primarily associated with pre-B ALL (cytoplasmic immunoglobulin-positive). *E2A-PBX1* fusion was initially associated with inferior outcome in the context of antimetabolite-based therapy [5]. It has been demonstrated that the poor prognosis associated with t(1;19) can be largely overcome by more intensive therapy. However, improvement in the results appears to be primarily for patients with t(1;19) who, whether unbalanced and balanced; remain at higher risk of treatment non-response [14].

Intracellular Signaling Pathways and Outcome

The outcome for children with ALL is determined by a variety of clinical and laboratory variables. Risk stratification provides a tool to adjust the treatment, avoiding intensive therapies or unnecessary dose escalation for patients with a favorable prognosis. However, there is an important percentage of patients who have been classified with low risk, but they present relapse; currently, despite the use of multiple, independent prognostic features to assess the chance for relapse, approximately one half of patients who relapse are found to have favorable clinical features and an excellent response to induction therapy. Hence, the search for additional variables is necessary to redefine risk stratification [17]. The emergence of resistant clones poses a difficult challenge regardless of the previous treatment intensity [14]. It is possible that the size of a resistant subclone will determine whether a patient fails induction or has an early relapse, and also may govern ease-of-identification of an underlying high-risk molecular feature [18]. At present, a redefinition of risk stratification has been proposed based on the study of altered genes during oncogenesis [17, 18]. Actually, a redefinition of risk stratification has been proposed based on the study of altered genes during oncogenesis [17].

JAK-STAT Pathway

The *JAK-STAT* pathway consists of Janus family protein tyrosine kinases JAK1, JAK2, JAK3 and and TYK2 and a family of seven cytosolic transcription factors, and made up of STAT (Signal transducers and transcription activators), a complex of six subtypes including 1, 2, 3, 4, 5a, 5b, and 6 with the same activation process mediated by cytokine and other stimuli. Several hematologic malignancies appear to be driven by molecular aberrations that affect the *JAK-STAT* pathway, including JAK2 and JAK1 mutations [19, 20], leading to the targeted development of small molecules that might control deregulated signaling [21].

JAK mutations have been described in high-risk chALL; in mutated cases a gene expression signature similar to *BCR-ABL* pediatric ALL had been identified. The results of Mullighan et al. suggest that JAK signaling inhibition is a target for therapeutic intervention in *JAK* mutated ALL; the authors reported activating mutations in *JAK1* [3], *JAK2* [16], and *JAK3* [1], which represent 10.7 % of high-risk chALL cases negative to *BCR-ABL* [22].

Patients with *JAK* mutations have a poor outcome; the primary structure of *JAK* 1, -2 and -3 exhibit different changes; including missense, insertions and deletion mutation [21]. *JAK1* is activated by a wide variety of cytokines; *JAK* 1, -2 and -3 are ubiquitously expressed and interact with many different cytokine receptors [23–26]. Based on this data, *JAK* inhibitors could be used in these hematological diseases, in that they avoid the proliferation of malignant cells [27, 28].

JAK1 in Cytokine-Induced Biologic Responses Associated with chALL

JAK1 affects a wide variety of interleukins (IL), including IL-2, -4, -6, -7, -9, -10, -15, the Leukocyte inhibitory factor (LIF) and all interferons that participate in the hematopoiesis process; *JAK1* mutations impair lymphoid development and defective responses to class 2 cytokines and those using gp130 receptor subunits [21, 23]. Cytokines that bind to class I or class II cytokine receptors employ the *JAK-STAT* signaling pathway [23].

JAK2 in Cytokine Responses and Erythropoietin Receptor Activity

Janus-kinase 2 (*JAK2*) is required for the function of a variety of cytokine receptors. In addition, the mutations of *JAK2* affects the erythropoiesis process and inhibits the correct evolution [29]. Initial studies that suggested the JAK2-EPO relation reported an important number of kinases that have been implicated in signaling [30]. *JAK2* gives rise to a regulator effect in EPO, Thrombopoietin (TPO), IL-3, IL-5, (GM-CSF), and Interferon gamma (IFN-γ) expression [29, 31].

JAK2 is constitutively bound to cell surface erythropoietin receptor (EpoR) and is crucial in signaling that include the growth hormone receptor [32]. *JAK2* is crucial for EpoR signaling; *JAK2* phosphorylated tyrosine residues in EpoR cause docking sites for SH2 and subsequently the activation of *STAT5*, Ras, mitogen-activated protein kinase (*MAPK*), *JNK*, P38, PI3-kinase–*AKT*, *SHP1, SHP2, SHIP*, and *BCL-xL* [33].

A *JAK2* inhibitor such as Ruxolitinib has been reported as an active therapeutic in preclinical models of Polycythemia vera (PV) and other myeloproliferative neoplasms associated with somatic gain-of-function *JAK2* mutations [34].

The fusion of *JAK2* to PCM1 is a product of the translocation t (8; 9)(p22; p24); an uncommon case classified as myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*, in this case there are an aberrant tyrosine kinase expression [35].

Yu et al. [36, 37] tested *JAK2* inhibitors with a purified enzyme assays in a high-throughput using a line of Ba/F3 cells that expressing individually the translocated ETS leukemia (*TEL*) fusions of each *JAK* family member (*TELJAK Ba/F3*). This technique should provide a more meaningful understanding of selectivity and facilitate the development of additional JAK inhibitors.

JAK3 in the Lymphoid Development

JAK3 mutations are associated with defective lymphoid development, deregulated myelopoiesis, and the affectation of different cytokines, such as IL-4, -7, -9, and -15 [38, 39]. Signaling regulation of proliferation and apoptosis by JAK3 has been found in the physiopathology of different leukemia types, including acute megakaryoblastic leukemia and T-cell lymphoma, and PEPT1 and -2 (peptide transporters) are regulated by JAK3, a powerful regulator of these peptide transporters [40].

Ross et al. [41] have tested JAK3 inhibitors considering the important role of JAK3 in T-cell malignancies, including T-cell ALL (T-ALL), and the authors used a selective and orally active small molecule with high rates of effectiveness to induce therapeutic response in T-cell malignancies with less toxic therapies.

Mutations in receptor of Interleukin 7 (IL7R) and others altered cytokine IL-7 stimulate the differentiation of multipotent hematopoietic stem cells (HSC) from lymphoid lineage. The receptor of Interleukin 7 (IL-7R) which is formed by IL-7Rα (encoded by IL-7R) is essential for normal T-cell development and homeostasis [42]. IL-7 plays an important role in the development and homeostasis of cells and is involved in the proliferation, maturation and survival of of B, T and Natural Killer (NK) cells (Table 2).

Ott et al. [43] have investigated the chemical compound JQ1 as possible therapeutic option as it reduces the viability of B-ALL cell line through the BET protein inhibition especially in patients with high-risk cytogenetic. Therefore plasma concentration of IL-2 receptor, IL-8, -12 and -15 or C–X–C motif chemokine 10 (CXCL10) in primary myelofibrosis is important due to their association with prognostic. In addition to these cytokines, IL-10 and CXCL9 have been included in the prognosis of large-cell lymphoma [44, 45].

Table 2 chALL characteristics of risk

Characteristics of risk disease	Precursor B-Cell	T-linage ALL
Age at presentation	<1 year, >10 years	>10 years
Initial WBC count	>50,000	
Extramedullary disease	CNS/Testes	CNS/Testes
Phenotypic subset		Early T-precursor and mixed phenotype acute leukemia. (ETP/MPAL)
Common karyotype abnormalities	t(9;22), (q34;q22), rearranged MLL, hypodiploidy (<0.84)	Complex anomalies
Common gene mutations	FLT3/ITD, BCR/ABL, CRLF2, JAK1, JAK2, IKZF	ABD, ME2KC, IL-7R
Expression profiling signatures revealing target-able pathways.	Overexpression of PI3K-AKT, RAS-MAPK, BCR-ABL-like	Dysregulation TGFβ1, G_0/G_1 cell, cycle arrest
Clinic presentation	Frequent fever	
	Hepatosplenomegaly and lymphadenopathy as an expression of extramedullary disease	
	Petechiae	
	Purpura	
	Bone pain	
Blasts morphology	Small, large core, scant cytoplasm homogeneous chromatin	
Prognosis	Standard risk: 85 %, High risk: 75 %, Infants <50 %	
Immunophenotype	CD10, CD19, CD22, TdT	CD2, CD3, CD5, CD7, TdT

CNS central nervous system

Signaling of *TYK2* in ALL

Tyk2 is the last *JAK* family member with an effect mediated by. Recently, Sanda et al. [46] utilized RNA interference (RNAi) to identify the TYK2-STAT1 pathway in T-ALL, promoting it as a novel oncogenic pathway that upregulates *BCL2* expression.

STATs

STAT is a protein complex with an important role in the regulation of hematopoiesis mediated by cytokine signal transduction [47, 48]. The majority of critical processes are regulated by STAT, including cellular proliferation and survival. To our knowledge, *STAT* activation is mediated by a receptor complex, and it translocates to modify nuclear gene expression [21].

STAT1

A region required of the EpoR for the STAT1 has been identified in a *JAK2* dependent process; this transcription factor is activated after EPO treatment and is mediated by the Tyr 432 residue of the human EpoR gene, suggesting its role in the development of normal erythropoiesis [47]. Thus, Interferon (INF) and innate immune responses are associated with the regulation of STAT1 expression [21].

STAT2

Alterations in *STAT2* affect the type 1 INF response [21].

STAT3

STAT3 inhibitors have been employed with high effectiveness levels in cellular lines. *STAT3* plays an important role in signaling pathways related with oncogenic processes. Despite that in normal cells STAT inhibitors do not demonstrate significant effects, they continue to be molecular targets in malignant cells [48]. In other hematological disorders that affect myeloid linage, the *BCR-ABL* activates *STAT3* in human cells mediated by the *JAK* pathway including *MEK*. Coppo et al. [49, 50] established that the activation mechanism of STAT3 has been mediated by JAK and Erk/MAP-kinase, leading to the increase of STAT3 mRNA, which should contribute to CML progression and to increased risk in patients with the Ph chromosome.

STAT4

This STAT is associated with IL-12 response, IFN γ-Rα production by Th1 cells, lymphocyte proliferation, and other Th2 cell responses [21].

STAT5 a/b

STAT5 a/b affects the proliferation signaling, including CFU-Mix, Eos, G, GM, and the absence of NK cells [21]. The active forms, A and B, are mediators of the oncogenic process that utilize a similar mechanism to that of STAT3. In essays employing Pimozide to evaluate the in vitro colony formation of healthy cells, there have been favorable results without hematopoietic toxicity. STAT5 is associated with a normal hematopoiesis process mediating cytokine responses; this latter notion supports the concept that healthy cells are not affected by STAT5 inhibitors, an interesting target in ALL [48].

STAT6

Mutations of this STAT have been identified in cases of IL-4 response failure; these Th2 cell affectations should be reduced simultaneously the lymphocyte proliferation [21]. In fact, in mice models the induction of *STAT6* deficiencies affect the biological responses in B cells associated with IL-4 levels [51].

The *PI3K-AKT/Ras-MAPK* Pathway

RAS proteins are regulated by guanine-nucleotide-exchange factors prior to stimulation of the nucleotide process. Mitogen-activated protein kinase (MAPK) and Phosphatidylinositol 3-kinase (PI3K) pathways are regulated by the formation of an intracellular docking site able to regulate cell proliferation, organization, and survival [52].

The *RAS* gene family includes *KRAS*, *HRAS*, and *NRAS* genes with similar effects in the development of cell cycle, although Ras genes have specific differences due to the presence of a posttranslational modification [52].

The *PI3K-AKT* and *Ras-MAPK* prosurvival signaling pathways are required for normal homeostasis in nonmalignant cells, but there is a continued reliance or "addiction" by leukemia cells on these pathways that has made them popular for targeted therapies. The PI3K-Akt pathway is often constitutively upregulated in many lymphoid malignancies (reviewed by Lee-Sherick et al.) [53], leading to the development of targeted therapies against the mammalian Target of Rapamycin (mTOR) and the TOR complexes [54, 55]. Inhibition of mTOR has been found to be effective in pediatric ALL [55].

The interaction between the PI3K-Akt pathway and the active forms STAT5a and STAT5b is mediated by Grb2 growth factor receptor bound protein 2 (Grb2). There are two different important roles of Gab2 in hematological disorders; Gab2 is associated with the activation PI3K/Akt and Ras/MAPK pathway mediated by STAT5 protein activation, and with ERK and Akt activation [56].

Inhibition of the PI3K/AKT/mTOR pathway has been a therapeutic tool in ALL; unfortunately, resistance to therapy is emerging. The use of selective inhibitors of this pathway, such as NVP-BKM120, RAD001, BEZ235, and NVP-BGT226, were compared in the cell cultures of ALL cells and probed in an experimental design; in this case, the combined use of dual PI3K/mTOR inhibitors provided an interesting therapeutic approach, especially in patients with the ALL linage of pre-B cells [57].

Glycogen Synthase Kinase-3β *(GSK-3β)—NF-κB* Pathway

Glycogen synthase kinase-3β (GSK-3β) has recently been found to positively regulate the activity of Nuclear factor-kappa B (NF-κB). The association between GSK-3β inhibition and NF-κB has been studied in pediatric primary leukemia cells obtained from newly diagnosed children with ALL. Hu et al. [58] isolated Bone marrow mononuclear cells (BMMC) by density gradient centrifugation from the heparinized aspirates of children with ALL. This group utilized immunofluorescence staining to detect nuclear GSK-3β in these cells, as well as western blot and electrophoretic mobility shift assays, testing the GSK-3β inhibitor in vitro treatment. By using this approach, these authors showed that inhibition of GSK-3β downregulates the NF-κB activation pathway, leading to suppression of the expression of an NF-κB-regulated gene and the promotion of apoptosis in ALL cells in vitro.

Therefore, *GSK-3β* is a multi-faceted kinase that is regulated by different growth factor signaling pathways and that affects a diverse range of physiological processes. Although crosstalk among signaling pathways is a feature of development, homeostasis, and disease, it is widely accepted that the Wnt pathway is associated with other signaling pathways: in Wnt-stimulated cells, inhibition of GSK-3β exerts a profound influence on β-catenin and Axin phosphorylation, but does not substantially affect the phosphorylation of other GSK-3β target substrates, such as tau and glycogen synthase [59].

The mTOR and *GSK-3β* cooperate to control the activity of S6K1, an important regulator of cell proliferation and growth [60]. The 40S ribosomal protein S6 kinase (*S6K*) is a major substrate of mTOR and is a crucial effector of mTOR signaling [61]. One of the *S6 K* isoforms, *S6K1*, plays important roles in cell growth, proliferation, and cell differentiation by regulating ribosome biogenesis, protein synthesis, cell cycle progression, and metabolism [62, 63].

Hypoxia-Inducible Transcription Factor-1α (*HIF-1α*)—*GSK-3β* Pathway

Hypoxia-inducible transcription factor-1α (HIF-1α) is a major regulator of carcinogenesis and various processes by which cells adapt to hypoxic conditions, being an important target for the understanding of angiogenesis and different cancer phenotypes and for unraveling new therapeutic options [61]. Flüel et al. [64] proposed an interactive mechanism between *HIF-1α* and *GSK-3* across induced phosphorylation and recruitment of the ubiquitin ligase and tumor suppressor F-box and WD protein Fbw7.

CBFA2/RUNX1

RUNX1 is a runt-related transcription factor 1 also known as *CBFA2* (corebinding factor α2) or *AML1* (acute myelogenous leukemia 1). *RUNX1* is a heterodimeric transcription factor with multiple splice variants that plays a role in normal hematopoiesis. Defects in *RUNX1* are associated with several types of leukemia.

At present, detection of human RUNX1/CBFA2 by Western blot comprises a necessary tool to determine this frequent translocation variant between intron 5 of TEL and intron 2 of AML [65].

Mutations in RUNX1 have been identified in T-ALL. Dowdy et al. [66] induced triple-point mutations in mouse model to characterize the effects in different hematopoietic lineages. In this case, induction with a RUNX1 HTY350-352AAA causes several alterations in embryonic development mediated by a mechanism related with transactivation and co-factor interaction TGF (Tumor growth factor)-b/BMP pathway. In specific domains of RUNX1, mutations have been reported that provide abnormal subnuclear targeting and interaction with co-factor processes, inducing alterations in early stages of hematopoiesis and leading to a pre-leukemic phenotype; this punctual mutation is induced by RUNX1 HTY350-352AAA, and the results indicate the crucial role of this transcription factor in the control and development of definitive hematopoiesis at different stages and its importance as a therapeutic target.

Moreover, RUNX1 is not exclusively associated with ALL, as it is also important in myeloid neoplasm suppression. In mouse model, overexpression of RUNX1 inhibited the growth of normal umbilical cord-blood cells and promoted the growth of AML1-ETO. These results are promising for AML treatment [67].

Lymphoid Transcription Factor *IKZF1* (IKAROS)

The lymphoid transcription factor IKZF1 (IKAROS) has been associated with poor outcome in patients with ALL. Currently, determination of abnormalities in IKZF1 and other genes by multiplex ligation-dependent probe amplification contributes to promoting the physiopathology and prognosis of the disease [68].

HOXA Cluster

HOX genes have importance in normal development of lymphopoiesis and leukemogenesis processes and there are two classes of *HOX* genes class I and class II. The first group includes four clusters (*HOXA, HOXB, HOXC* and *HOXD*) and the second group *TLX1* and *TLX3*. *HOXA, TLX1* and *TLX3* have been strongly associated with T-ALL development [15, 69].

Starkova et al. [69] analyzed 61 pediatric samples with ALL diagnosis and quantified RNA expression of *HOX* genes associated with ALL development: *HOXA, HOXB*, and *CDX1/2* using qRT-PCR. Considering the diagnosis, the patients were analyzed in karyotype subgroups; in BCP-ALL and T-ALL patients expressed *HOXA 3–4, HOXA 7*, and *HOXB 3–4* genes including abnormal karyotype as *MLL/AF4*, *TEL/AML1* and *BCR/ABL*. In immature cells and progenitors were identified *HOXA 7–10*, 13 and *HOXB 2–4*, moreover *HOXB 6* and *CDX 2* were detected only in leukemic cells. Therefore, they found a particular expression in each karyotype anomaly. Information related to the most common caryotype associated to chALL is shown in Table 3.

Abnormal expression of HOXA9 has been correlated with the development of T-cell and myeloid leukemia. However, the study of overexpression in hematopoietic tissues demonstrated the HOXA9 role in accelerated lymphoid, but not in myeloid, leukemia. Transformation utilizing Vav regulatory elements in mouse model has provided valuable information about the overexpression of other HOXA cluster genes; however, lower levels of HOXA9 stimulate the development of a T-cell precursor to lymphoblastic leukemia [70]. Finally, *HOX11* activation has been associated with a favorable prognosis in T-ALL [71].

LMO1 and *LMO2*

The presence of chromosomal rearrangements in ALL is common: LMO1 and LMO2 overexpression in the loci of the T-cell receptor has been found at around 45 % of total cases. Therefore, LMO1 and -2 encode in the LIM-domain: 11p15 and 11p13, respectively. LMO proteins are associated with LYL1 or TAL1 dysregulation and the latter is associated with the induction of T-cell malignancies in co-expression with TAL1 [15, 72]. LMO2 has been associated with other chromosomal aberrations, specifically with TAL1 in a same cluster, suggesting that LMO2 and TAL1

Table 3 Karyotype abnormalities associated with chALL development

Karyotype abnormalities	Affected genes	Phenotype abnormalities	Detection method	Frequency		Ref.
				Ch.	A.	
Hyperdiploid	4, 6, 10, 14, 17, 18, 21 and X	More than 50 chromosomes. Common. Patients present mutations in the receptor of tyrosine kinase *FLT3*.	Cytogenetic techniques; karyotype analysis, FISH, PCR	15 %	6 %	[15, 81, 82]
Hypodiploidy		Less than 45 chromosomes. Uncommon.				[15]
t(12;21)(p13;q22)	TEL-AML1 (ETV6-RUNX1)	Good prognosis. Marked sensitivity asparaginase.		22 %	2 %	[6, 15, 16, 65]
t(9;22)(q34;q11.2); Ph chromosome.	BCR-ABL1	Poor prognosis. Hyperleukocytosis.		3 %	30 %	[6, 15]
t(1;19)(q23;p13.3)	E2A- PBX1 (TCF3-PBX1)	Pre-B phenotype hyperleukocytosis. Need intensive treatment		5 %	3 %	[6, 15]
t(4; 11)	MLL-AF4	Associated with infant ALL. Hyperleukocytosis. Poor prognosis		8 %	10 %	6,
t(11; 19)		Poor prognosis. Relatively favorable for children with T- cell ALL.		8 %	10 %	[15]
t(5;14)(q31; q32)	IL3- IGH	Poor outcome in T-chALL		20 %	13 %	[15]
	TLX3 (HOX11L2)			T-ALL	T-ALL	
1p32, t(1;14)(p32;q11) and t(1;7)(p32;q34)	TAL1	Is not expressed in normal T- cell development. Nonrandom genetic defect in T-chALL	Cytogenetic techniques; karyotype analysis, FISH, multiplex FISH, SKY, CGH, Microarray analysis	40 % T-ALL		[15, 76]
1p34, t(1;7)(p34;q34)	Lymphocyte-specific protein tyrosine kinase; LCK	LCK is highly expressed in T-cells; it has interactions with TCR signaling				[15]
8q24	MYC	Juxtaposing of *C-MYC*				[15]
9q34, t(7;9)(q34;q32)	TAL2 TAN1/NOTCH1	TAL2 Is not expressed in normal T- cell development. Both proteins promote T-ALL		<2 % T-ALL		[15, 76]
10q24	HOX11	Overexpression of *HOX11* in 10q24 absence. Alterations in normal leukemogenesis.				[15]
t(11;14)(p15;q11)	*LMO1 (RBTN1* or *TTG1*, 11p15)	*LMO1* or *LMO2* is strictly required to induce T cells malignances in co-expression with TAL1		45 % T-ALL		[15]
t(11;14)(p13;q11)	*LMO2 ((RBTN2* or *TTG2*, 11p13)					[15]
14q32	TCL1	Control of immunoglobulin (Ig) gene enhancers of the heavy chain (*IGH*- 14q32)				[15, 76, 83–88]
19p13, t(7;19)(q35;p13)	LYL1	Is not expressed in normal T- cell development. LYL1 is expressed in some types of leukemia is possible that it participates in T-cell leukemogenesis				[15]

Table 3 continued

Karyotype abnormalities	Affected genes	Phenotype abnormalities	Detection method	Frequency		Ref.
				Ch.	A.	
t(8;14)(q24;q32)	MYC/IG	Burkitt lymphoma mature B-ALL (BL)		5 %	10 %	[15]
t(10;14)(q24;q11), t(7;10)(q35;q24)	TLX1 (HOX11)	Nonrandom alteration identified in T-ALL	5 % T-ALL	30 % T-ALL	[15, 89, 90]	
inv(7)(p15q34), t(7;7)(p15;q34), t(7;14)(p15;q11)	HOXA@ cluster	Elevated *HOXA10* and *HOXA11* expression in developing thymocytes. Participate in positive development of CD4 and CD8	5 % T-ALL		[15, 91]	
Duplication and t(6;7)(q23;q34)	MYB	T-cell differentiation.	Microarray analysis	8–15 % T-ALL		[15]

Ref. reference, *A.* adult's prevalence, *Ch.* Children's prevalence, *FISH* fluorescence in situ hybridization, *SKY* spectral karyotypes analysis, *CGH* comparative genomic hybridization

rearrangements affect similar pathways. In a cohort of 117 samples of patients with an ALL diagnosis, Homminga et al., by Fluorescent in situ hybridization (FISH) and real-time quantitative PCR (RQ-PCR), the authors found, in their results, that LYL1 translocation and LMO2 rearrangement conform a TAL-LMO subgroup present in immature cells [73]. Moreover, LMO2 gene expression in mouse model has been associated with the development of T-ALL [74].

TAL1 and *TAL2*

TAL1 (also known as SCL) is not expressed in normal T-cell development, but is rather a non-random genetic defect in T-chALL; moreover, TAL1 gene expression in mouse model has been associated with T-ALL development [74]. TAL1/SCL activation in patients with ALL has been identified in 50–60 % [71]. In leukemogenesis, the TAL1/SCL product could interfere in E2A and HEB transcriptional activity. Thus, Kusy et al. [75] characterized the oncogenic pathways associated with TAL1 activation, corroborating that in T-ALL cells, NKX3.1 is activated by TAL1 in fusion with THE LMO-Ldb1 complex, causing a suppression of heterochromatin protein 1 (HP1) and subsequently the NKX3.1 gene promoter, the latter required for T-ALL proliferation. The authors conclude that TAL1 or NKX3.1 knockdown inhibit the capacity of T-ALL cells to induce leukemia in mouse model. TAL2 is not expressed in normal T-cell development and promotes T-ALL [15, 76]. TAL2 is a helix-loop helix protein, such as TAL1 and LYL1, and is a mediator of T-cell leukemogenesis that is identified in T-ALL [77].

Lymphocyte-Specific Protein Tyrosine Kinase (LCK)

Lymphocyte-specific protein tyrosine kinase (LCK) is predominantly expressed in T cells. LCK gene location is susceptible to chromosomal aberrations and, as a Src family kinase member, it activity is regulated by tyrosine phosphorylation at Tyr 394 and Tyr 505 [78]. LCK has been associated with a deregulation of the tyrosine kinases family and the regulation of the LIM domain. The constitutive activation of LCK has been related with ALL and other lymphoid malignancies. LCK regulates LMO2 expression and mediates a similar mechanism in the nuclear Janus kinase 2 (JAK2). Overexpression of LCK is implicated in hematological malignances [79].

TAN1/NOTCH1 and LYL1

NOTCH1 is another oncogene, like TAL1 and LMO2. In mouse model, the relation between NOTCH1 and LIC was

established through treatment with a vehicle of the γ-secretase inhibitor [74]. The role of NOTCH1 in the development of T-ALL is mediated by its influence in signaling pathways, encoding a transmembrane receptor on HSC that induces proteolysis cleavage, allowing the intracellular-to-nucleus translocation of NOTCH1 followed by the activation of HES1 (Hairy/enhancer of split) and Deltex (DTX1), both present in about 50 % of T-ALL cases [52]. Furthermore, LYL1 is a basic Helix-loop-helix protein (bHLH) that is non-expressed in normal T-cell lineages. LYL1 ectopic expression is caused by juxtaposition on chromosome 19 and chromosome 7 T-cell receptor t(7; 19). The LYL1 structure contains a bHLH domain and a conserved region with binding sites [80]. LYL1 is a transcription factor that exhibits protein homology with TAL1, which is also overexpressed in hematopoietic malignancies [73].

In conclusion, there are under development at present an extensive number of inhibitors of molecular targets associated with intracellular signaling pathways in chALL (Table 4). Throughout this review, we compiled information on the main signaling pathways involved in ALL, one of the most diverse and complex diseases that greatly affect the child population in Mexico and worldwide.

The presence of fusion translocation products and other cytogenetic abnormalities and their role as protein markers, such as LMO1, LMO2, LCK, TAL1, TAL2, TAN1/NOTCH1, LYL1, TCL1, and MYB, have already been extensively described and are crucial to the understanding and development of new therapies given its frequency in the appearance of chALL.

Concerning the widely described and unconventional intracellular signaling pathways, extensive evidence supporting the key role of JAK-STAT pathway point mutations in chALL pathophysiology provides the key to some family members as oncogenic mediators against which are directed the majority of therapeutic targets. However, although the treatment response rate in pediatric population is favorable at present, it is important to continue the characterization of these, especially STAT3 and STAT5 transcriptional activity as JAK proteins effectors, including the recently identified regulators of action.

Another important topic of lymphoid malignancy comprises the determination of molecular prognostic factors that could establish the likelihood of relapse more accurately, including the HOXA cluster. LYL1 and other proteins implicated in cell maturation might be useful as molecular risk factors, at least in T-cell malignancies. NKX3.1 and its relationship with the TAL1 protein as related with the proliferation of T-cells in ALL have gained prominence for its role in leukemogenesis.

Finally, we must take GSK-3β inhibitors into account as potential therapeutic targets, not only in hematologic malignancies but also in metabolic processes, including alterations in the signaling pathways of insulin and Alzheimer. Much research remains to investigate the involvement of cellular pathways in leukemia, and probably a single molecule will not be the gold standard for

Table 4 Trials of therapeutic targets in ALL

Trial	Mechanism/target	Phase of trial/disadvantages	Ref.
Recombinant human CD19L-sTRAIL fusion protein	Heterogeneous expression of surface TRAIL receptors in patients with B cell precursor ALL.	Preclinical studies of CD19L-sTRAIL in rodents/toxicity	[92]
mTOR inhibitors	Decreased leukemia proliferation and growth in pre B-ALL	Preclinical studies in mice models	[54, 55, 57]
		Preclinical setting treating individual patient-derived ALL in vivo	[93]
	Synergize with histone deacetylase inhibitors to kill B-ALL.	Preclinical studies in Balb/c and C57Bl/6 mice	[94]
GSK-3β inhibitors	Regulate the *NF-κB* transcriptional activity.	Tests in cell culture obtained from bone marrow mononuclear cells and mice models/Drug toxicity	[58, 59]
Sphingosine kinase inhibitors	Regulate the autophagy and apostosis in T-cell ALL	Cell culture and primary samples	[95]
Epigenetic drugs: histone 3 lysine 27 demethylases, JMJD3 and UTX	The JMJD3 modulation affects the initiation and maintenance of ALL, modulate H3K27 methylation	Tests in a knockout mouse models of JMJD3 and UTX	[96]
VLA-5 blocking	VLA-5 block may increase susceptibility to therapy in BCL/ABL for Ph+ ALL treatment	Tests in human bone marrow stromal cell line HS-5	[97]

Ref. reference

treatment or prognosis, but rather a mixed expression of many of these.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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