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1. Introduction

Acute lymphoblastic leukemia (ALL) is mainly a disease of childhood that arises from recurrent genetic alterations that block precursor B- and T-cell differentiation and drive aberrant cell proliferation and survival [1]. Due to the advances in the cytogenetic and molecular characterization of the acute leukemias in the past two decades, genetic alterations can now be identified in more than 80% of cases of ALL [2]. These genetic lesions influence the prognosis and therapeutic approach used for treatment of ALLs [3]. This chapter describes genetic subtypes of ALL according to the hematological malignancies classification (WHO) 2008, risk groups, frequency of cytogenetic abnormalities, and their relationship with the prognosis of ALL, copy number alterations and somatic mutations in ALL.

2. Acute Lymphoblastic Leukemia (ALL) — Genetic subtypes

2.1. Definition and genetic subtypes according to the hematological malignancies classification (WHO) 2008

Acute lymphoblastic leukemia (ALL) is mainly a disease of childhood that arises from recurrent genetic alterations that block precursor B- and T-cell differentiation and drive aberrant cell proliferation and survival [1]. ALL is characterized by the accumulation of malignant, immature lymphoid cells in the bone marrow and, in most cases, also in peripheral blood. The disease is classified broadly as B- and T-lineage ALL [1].

ALL occurs with an incidence of approximately 1 to 1.5 per 100,000 persons. It has a bimodal distribution: an early peak at approximately age 4 to 5 years with an incidence as high as 4 to 5 per 100,000 persons, followed by a second gradual increase at about age 50 years with an
incidence of up to 2 per 100,000 persons. ALL, the most common childhood malignancy, represents about 80% of all childhood leukemias; but only about 20% of adult leukemias [4]. The rate of success in the treatment of ALL has increased steadily since the 1960s. The five-year event-free survival rate is nearly 80 percent for children with ALL and approximately 40 percent for adults [5].

Diagnosis of ALL relies on an assessment of morphology, flow cytometry immunophenotyping, and identification of cytogenetic-molecular abnormalities [4]. Conventional and molecular genetics allow the identification of numerical and structural chromosomal abnormalities and the definition of prognostically relevant ALL subgroups with unique clinical features [6, 7]. However, acute lymphoblastic leukemia subtypes show different responses to therapy and prognosis, which are only partially discriminated by current diagnostic tools, may be further determined by genomic and gene expression profiling [4]. More accurate delineation of genetic alterations can also provide information important for prognosis. Minimal residual disease (MRD) detection and quantification have proven important in risk-group stratification for both pediatric and adult ALL [7].

It seems likely that one or several changes in the genome are required for a blast cell to evolve into a leukemic clone, and that all cases probably harbor some form of genetic alteration [7]. Due to the advances in the cytogenetic and molecular characterization of the acute leukemias in the past two decades, genetic alterations can now be identified in greater than 80% of cases of ALL [2]. Improvement in recognizing abnormalities in the blast cells will help in understanding the mechanisms that underlie leukemogenesis.

The cloning and characterization of recurrent chromosomal translocations has allowed the identification of genes critical for understanding of the pathogenesis and prognosis of ALL [5, 8, 9]. These genes are implicated in cell proliferation and/or survival, self-renewal, cell differentiation and, and cell cycle control [10, 11]. The main causes of gene deregulation are: (i) oncogene activation with ensuing ectopic or over-expression, which is mainly due to juxtaposition with T-cell receptor loci; (ii) gain of function mutations; (iii) tumor suppressor gene haploinsufficiency or inactivation, which is usually the result of deletion and/or loss of function mutation; and (iv) chromosomal translocations producing fusion proteins which are associated with specific subgroups of ALL [10].

Efforts to define the genetic lesions that underlie ALL have identified a number of different subtypes of ALL based on their lineage (T-versus B-cell), chromosome number, or the presence or absence of chromosomal translocations. Collectively, these genetic lesions account for approximately 75% of cases, and their presence significantly influences the therapeutic approach used for treatment [3].

B-lineage ALL (B-ALL) shows considerable genetic heterogeneity. Within the category “B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities”, the 2008 World Health Organization classification of hematopoietic neoplasms recognizes seven recurrent genetic abnormalities including t(9;22) (q34;q11.2) BCR-ABL1, t(v;11q23) MLL rearranged, t(12;21)(p13;q22) TEL-AML1 (ETV6-RUNXI), t(5;14)(q31;q32) IL3-IGH, t(1;19) (q23;p13.3) E2A-PBX1 (TCF3-PBX1), hypodiploidy and hyperdiploidy [12].
Burkitt lymphoma/mature B-ALL (BL) was included in the category of mature lymphatic neoplasms in the new WHO classification [12]. BL is characterized by translocation of MYC at 8q24.21 with an immunoglobulin gene locus, which in most cases is the immunoglobulin heavy chain locus (IGH, 14q32.33) with rare translocations with the light chain genes for kappa (IGK, 2p11.2) and lambda (IGL, 22q11.2). These translocations result in constitutive expression of the MYC gene in peripheral germinal-center B cells, driven by the immunoglobulin gene enhancer [13]. The BL clone must acquire chromosomal aberrations secondary to the IG-MYC fusion. The most frequent secondary changes in BL detected by conventional cytogenetics are gains in 1q as well as in chromosomes 7 and 12 [14, 15].

In B-ALL, malignant cells often have additional specific genetic abnormalities, which have a significant impact on the clinical course of the disease. In contrast, although the spectrum of chromosomal abnormalities in T-lineage ALL (T-ALL) has been further widened by the finding of new recurrent but cryptic alterations, no cytogenetically defined prognostic subgroups have been identified [16, 17].

T-ALL is mainly associated with the deregulated expression of normal transcription factor proteins. This is often the result of chromosomal rearrangements juxtaposing promoter and enhancer elements of T-cell receptor genes TRA@ (14q11), TRB@ (T-cell receptor b, 7q34-35), TRG@ (T-cell receptor g, 7p15) and TRD@ (T-cell receptor d, 14q11) to important transcription factor genes [18]. In most cases, these rearrangements are reciprocal translocations, and lead to a deregulation of transcription of the partner gene by juxtaposition with the regulatory region of one of the TCR loci (e.g. TCRB/HOXA, TCRA/D-HOX11, TCRA/D-LOMO1). These chromosomal aberrations affect a subset of genes with oncogenic properties, such as 1p32(TAL1), 1p34(LCK), 8q24(MYC), 9q34(TAL2), 9q34(TAN1/NOTCH1), 10q24(HOX11), 11p13 (RBTN2/LMO2), 11p15(RBTN1/LMO1), 14q32(TCL1), 19p13 (LYL1), 21q22(BHLHBI) and Xq28 (MTCP1) [10, 17].

Other type of rearrangement in T-ALL, mostly translocations, results in formation of ‘fusion genes’ that are associated with specific subgroups of T-ALL (CALM-AF10, NUP98-t, MLL-t and ABL1-fusions). In these translocations, parts of both genes located at the chromosomal breakpoints are fused ‘in frame’ and encode a new chimeric protein with oncogenic properties [10]. Chromosomal translocations producing fusion proteins also are associated with specific subgroups of T-ALL.

In addition, gain of function mutations (NOTCH1 and JAK1) and tumor suppressor gene haploinsufficiency or inactivation, which is usually the result of deletion (CDKN2A-B) and/or loss of function mutation (PTEN); are frequent in T-ALL. These genetic alterations could be concomitant with other genomic changes [10, 19].

2.2. Risk groups in ALL

During the past three decades, the prognosis of has been improved and the treatment achieved cure rates exceeding 80%. ALL in adults has followed the same trend with long-term survival of about 40%. One main factor behind this improvement is the development of risk-adapted
therapy, that permit to stratify the patients in different clinical categories according to risk factors with prognostic influence and to define the intensity and duration of treatment [20].

The prognosis of patients with ALL is influenced by clinical, hematologic and genetic factors, including age, leukocyte count at diagnosis, percentage of blast in peripheral blood, immunophenotype, central nervous system (CNS) involvement, the presence or absence of mediastinal tumor, cytogenetic and molecular alterations and the presence of minimal residual disease (MRD) in different stages of treatment which is currently a defined risk of adapted therapy strategies [20-24].

With respect to age, children less than 24 months and adults more than 50 years old have a worse prognosis, while the better results are achieved for children between 1 and 10 years, followed by adolescents and young adults. The leukocytosis (>30x10^9/L in B-ALL and >100x10^9/L in T-ALL), the phenotype Pro-B ALL, and T-ALL, are related to a poor outcome and are used to stratify patients as high risk [23].

The study of these prognostic factors allows recognition of three subgroups with outcome clearly differentiated in children: standard risk (40% of cases - 90% survival), intermediate (45-50% - 70-80% survival) and high risk (10-15%-less than 50% survival) [23, 25], and two subgroups in adult, standard-risk (20-25% of cases, 60% survival) and high risk (75-80% - 30% survival) [23, 26].

3. Cytogenetic alterations in ALL

3.1. Cytogenetic alterations in B-cell precursor ALL (BCP-ALL)

A correlation between prognosis and the karyotype at diagnosis in ALL was firstly demonstrated by Secker-Walker (1978) [24]. Subsequently, during the third International Workshop on Chromosomes in Leukemia (IWCL, 1983), the first large series of newly diagnosed ALL were analyzed to establish cytogenetic and prognostic correlations. Sixty-six percent of the patients analyzed showed clonal aberrations, which were identified both high-risk and low-risk ALL patients [27]. Since then it has been considered that the cytogenetic alterations have prognostic value of first order in the ALL.

Development of methods in cytogenetics has contributed to the understanding that ALL is not a homogeneous disease. Chromosome abnormalities have been detected by conventional G-banding in approximately 60-70% of all cases [7, 28]. Abnormal karyotypes have been reported in up to 80% of children and 70% of adults with ALL [29, 30]. There had been considerable developments in fluorescence in situ hybridization (FISH) for the detection of significant chromosomal abnormalities in leukemia in the 1990s [31]. The development of 24-color fluorescence in situ hybridization (FISH), interphase FISH with specific probes, and polymerase chain reaction (PCR) methods has improved the ability to find smaller changes and decreased the proportion of apparently normal karyotype to less than 20% in ALL [7].

In cases with B-ALL (excluding mature B-ALL), the most important subgroups for modal number are hypodiploidy, pseudodiploidy, and hyperdiploidy with a chromosome number
greater than 50 [32]. The most structural rearrangements include translocations that generate fusion transcripts with oncogenic potential. The most important of the translocations are t(1;19) (q23;p13) (TCF3-PBX1 fusion gene; alias E2A-PBX1), t(4;11)(q21;q23)(MLL-AFF1 fusion gene; alias MLL-AF4), t(9;22)(q34;q11)(BCR-ABL1), and t(12;21)(p13;q22)(ETV6-RUNX1 fusion gene; previously TEL-AML1) [32]. These cytogenetic subgroups have distinctive immunophenotypic characteristics as well as age and prognostic associations [24].

3.1.1. Ploidy alterations

The presence of hypodiploidy (less than 45 chromosomes) is found in only 2% of ALL, and is associated with a very poor outcome [33]. The high hyperdiploidy (with more than 50 chromosomes) is the most common cytogenetic subgroup in childhood BCP-ALL, and associated to a long survival. Hyperdiploidy is more frequent in children (15%) than in adults (6%) [34].

The gain of chromosomes is nonrandom, the eight chromosomes that account for 80% of all gains are: +4(78%), +6 (85%), +10 (63%), +14 (84%), +17 (76%), +18 (76%), +21 (99%), and +X (89%) [24]. Trisomy 4, 10, and 17 are associated to favorable outcome in children [33]. Unlike hypodiploid ALL patients, hyperdiploid ALL cases have an extremely good prognosis with event-free survival rates near 90% [21]. These patients seem to particularly benefit from high dose methotrexate [33].

Approximately 20% of hyperdiploid ALL have activating mutations in the receptor tyrosine kinase FLT3. These mutations are interesting because not only they trigger the activation of the tyrosine kinases as potential oncogenes in hyperdiploid ALL, but also in that it suggests that tyrosine kinase inhibitors could be of benefit to patients with this leukemia. [9].

3.1.2. E2A-PBX1 fusion t(1;19) (q23;p13)

The t(1;19) (q23;p13) represents 5% of children ALL, and 3% in adults ALL, this translocation is frequently associated with the pre-B immunophenotype, in approximately 25% of cases [5, 34, 35]. The t(1;19) (q23;p13) forms a fusion gene that encodes a chimeric transcription factor, E2A-PBX1 (TCF3-PBX1 fusion). It disrupts the expression the expression of HOX genes and the targets of the E2A transcription factor [5]. The t(1;19) has good prognosis with high-dose methotrexate treatment; however this translocation is a risk factor for CNS relapse [1, 21]

3.1.3. BCR-ABL fusion t(9;22) (q34;q11)

As a result of the t(9;22) (q34;q11)/Philadelphia chromosome (Ph+), the BCR gene at 22q11.2 is joined to the ABL protooncogene at 9q34, giving rise to the BCR-ABL fusion gene. The fusion gene encodes an oncogenic fusion protein with enhanced tyrosine kinase activity that interacts with RAS, AKT, and JAK/STAT pathways [1]. This translocation is found in approximately 3% of children and 30% of adults, and is associated with unfavorable prognosis [34]. Imatinib mesylate plus intensive chemotherapy improve early treatment outcome against Philadelphia chromosome-positive (Ph+) in ALL, one of the highest risk pediatric ALL groups, however imatinib resistance develops rapidly [36].
3.1.4. 11q23-MLL rearrangements

Chromosomal rearrangements of the human MLL gene are the most common genetic abnormality in the first year of life, but it occurs in only 8% of children and 10% adults with ALL [34]. The 11q23-MLL rearrangements are associated with high-risk pediatric, adult and therapy-associated acute leukemias [37].

Some 104 different MLL rearrangements of which 64 fused translocation partner genes (TPGs) are now characterized at molecular level [37]. The five most common MLL rearrangements, present about 80% of MLL-translocated acute leukemia (MLL-t AL), are t(4;11)(q21;q23) encoding MLL-AF4, t(9;11)(p22;q23) encoding MLL-AF9, t(11;19) (q23;p13.3)-ncoding MLL-ENL, t(10;11)(p12;q23)encoding MLL-AF10, and t(6;11)(q27;q23)encoding MLL-AF6 [38].

MLL rearrangements are associated with unfavorable prognosis. However, outcomes could be improved with high-dose cytarabine for some rearrangements [1]. MLL fusions with AF4, AF9 and ENL recruit small serine/proline-rich proteins with nuclear localization signals, which may generate unique chimeric transcriptional transactivators [1]. The t(4;11)(q21;q23) have poor prognosis and predominance in infancy, especially those < 6 months of age. This rearrangement has been associated with overexpression of FLT3 [21].

3.1.5. ETV6-RUNX1 t(12;21) (p13;q22)

The t(12;21) (p13;q22) leads to a fusion ETV6-RUNX1 (TEL-AML1). It occurs in 22% of children and 2% adults with ALL [34]. This translocation t(12;21)(p13;q22) is the most common translocation in childhood BCP-ALL [39]. Moreover, is associated to an excellent prognosis with intensive chemotherapy, including asparaginase therapy [1, 21]. TEL-AML1 is a leuke-mogenic, chimeric transcription factor encoding the amino-terminal basic helix-loop-helix (bHLH) domain of the ETS family member TEL fused to the AML1 DNA-binding Runt and transactivation domains [40]. TEL-AML1 may generate a pre-leukemia clone by repression of activated AML1 target genes or by TEL inhibition of other ETS family proteins via binding through the TEL’s pointed domain [1].

3.2. Cytogenetic alterations in Burkitt lymphoma/mature B-ALL (BL)

3.2.1. MYC/IG (t(8;14), t(2;8) and t(8;22))

The t(8;14)(q24;q32) and its variants t(2;8)(p11;q24) and t(8;22)(q24;q11) are associated with BL [13]. The t(8;14) is most common, found in 85%, whereas t(2;8) and t(8;22) are found in around 5 and 10%, respectively [24]. The crucial event in all three reciprocal translocations is the juxtaposing of C-MYC (from 8q24) under the control of immunoglobulin (Ig) gene enhancers of the heavy chain (IGH-14q32), kappa light chain (IGK-2p12), or lambda light chain (IGL-22q11), leading to deregulation and increased transcription of MYC [13]. The 2008 World Health Organization classification of hematopoietic neoplasms, established that MYC translocations are not specific for BL. Most MYC/IG breakpoints in endemic BL originate from
aberrant somatic hypermutation. On the other hand, in sporadic cases the translocation involves the IG change regions of the IGH locus at 14q32 [12].

The abnormalities of C-MYC are an important step in the development of BL. MYC is a transcription factor with both activating and repressing function and is involved in the regulation of roughly 10–15% of all human genes. MYC regulates a number of critical biologic processes such as cell cycle control, cell growth, protein synthesis, angiogenesis, and apoptosis [41]. The upregulation of C-MYC disrupts many aspects of cell function, such as cell cycle progression, differentiation, metabolism, apoptosis, telomerase activity, and cell adhesion. These effects of C-MYC are likely to be of pathogenetic relevance in human tumors [42].

3.2.2. Secondary chromosome changes in BL

Several cytogenetic reports have correlated the presence of cytogenetic abnormalities with the outcome of patients with non-Hodgkin lymphomas, showing that secondary chromosome changes may influence the clinical phenotype of lymphoid tumors [43].

Most of the secondary chromosome changes are unbalanced rearrangements, leading to DNA gains or losses. These changes have been studied in Burkitt’s lymphoma-derived cell lines and primary tumors by cytogenetic techniques including karyotype analysis [44-48], fluorescence in situ hybridization (FISH) [49], multiplex FISH (M-FISH) [50], spectral karyotype analysis (SKY), comparative genomic hybridization (CGH) [43, 51-54], and microarray analysis [55].

Additional recurrent chromosomal abnormalities have involved chromosomes 1, 6, 7, 12, 13, 17, and 22. Gains of the long arm of chromosomes 1 (+1q) or 7 (+7q) or 12 (+12q), deletion (del) 17p13 and abnormalities of band 13q34 usually occur in adult BL, without or in the setting of an HIV infection [13, 44-46, 51, 56]. Some secondary abnormalities have been associated with tumor progression, such as abnormalities on 1q, +7q and del(13q) which have been independently associated with a worse outcome [43-46, 49, 50].

3.3. Cytogenetic alterations in T-ALL

Conventional karyotyping identifies structural chromosomal aberrations in 50% of T-ALL. Numerical changes are rare, except for tetraploidy which is seen in approximately 5% of cases. The presence of chromosomal abnormalities is not associated to the prognosis [19]. Some nonrandom translocations that are specific to T-lineage malignancies have been identified. They involve genes coding for transcriptional regulators transcriptionally deregulated in malignancies [57].

Extensive characterization of specific chromosomal abnormalities for T-ALL led to the identification of several oncogenes whose expression was up-regulated under the influence of the transcriptional regulation elements of genes which are normally expressed during T-cell differentiation [58]. T-cell malignancies are often associated with unfavorable features compared with childhood precursor B-cell ALL. However, the use
of more intensive treatments and risk adapted therapy has significantly improved the outcome of patients with T-ALL. Event-free survival rates of 60% to 70% are now reported in children [57].

3.3.1. Rearrangements involving TCR genes

3.3.1.1. Deregulation of homeobox genes

The homeobox (HOX) family of transcription factors is divided into two classes. Class I HOX genes are organized in four distinct clusters (HOXA, HOXB, HOXC and HOXD) at four chromosomal loci (7p15, 17q21, 12q13, and 2q31 whereas class II HOX genes are dispersed throughout the whole genome. In the class I HOX genes, the HOXA cluster is involved in T-ALL, while that in the class II HOX genes, TLX1 (HOX11) and TLX3 (HOX11L2) have been extensively studied in the context of T-ALL [18].

3.3.1.1.1. TLX1 (HOX11) (t(10;14)(q24;q11) and its variant t(7;10)(q35;q24))

The translocation t(10;14)(q24;q11) and its variant t(7;10)(q35;q24) are a nonrandom alteration identified in T-ALL. Either of these is present in 5% of pediatric to 30% of adult T-ALL [1]. Both of them lead to the transcriptional activation of a homeobox gene, HOX11 gene (TLX1; TCL3), that is not expressed in healthy T-cells, by bringing the HOX11 coding sequence under the transcriptional control of regulatory sequences of the T-cell receptor gene (TRA or TRB genes, respectively). However, the overexpression of HOX11 in thymocytes has been also demonstrated in the absence of a 10q24 rearrangement, suggesting that other, trans-acting mechanisms could lead to this aberrant gene expression, probably by disrupting gene silencing mechanisms that operate during normal T-cell development [18, 57, 58].

There is some evidence that HOX11 may play an important role in leukemogenesis. It has been particularly shown that constitutive expression of HOX11 favors expansion and, in some instances, immortalization of murine hematopoietic progenitors in vitro [59, 60]. However, HOX11 has better prognosis than other T-ALL molecular subtypes [1].

3.3.1.1.2. TLX3 (HOX11L2) (t(5;14)(q35;q32))

The cryptic translocation, t(5;14)(q35;q32), is restricted to T-ALL, is present in approximately 20% of childhood T-ALL and 13% of adult cases. This translocation is associated with strong ectopic expression of another homeobox gene called HOX11L2 (RNX; TLX3) [17, 58, 61], because of possibly the influence of regulatory elements of CTIP2 (BCL11B), a gene highly expressed during T-lymphoid differentiation [17, 57]. Other variant chromosomal aberrations, each targeting TLX3, have been observed as well, including a t(5;7)(q35;q21), in which the CDK6 gene is involved on 7q21[18].

Although TLX1 and TLX3 themselves and the gene expression profiles of TLX1 and TLX3 expressing T-ALL samples are very similar [18], the t(5;14) and/or HOX11L2 ectopic expression has been associated with a very poor outcome in children with T-ALL [57].
However, the exact prognostic meaning of TLX3 expression alone or in combination with other markers is not clear [18].

3.3.1.3. HOX@ cluster (inv(7)(p15q34))

Other rearrangement involving TCR genes that affecting HOX@ cluster (7p15) is associated with the inv(7) (p15q34), t(7;7)(p15;q34), and t(7;14)(p15;q11). The chromosomal inversion inv(7)(p15q34) has been observed in approximately 5% of T-ALL cases. This inversion juxtaposes part of the TRB@ locus (7q34-35) to part of the HOX@ cluster (7p15), resulting in elevated HOXA10 and HOXA11 expression. In addition, 2% of the cases showed elevated HOXA10 and HOXA11 expression in the absence of inv(7), suggesting that other activating mechanisms may exist [18].

In contrast to TLX1 and TLX3, which are normally not expressed in the hematopoietic system, HOXA10 and HOXA11 are expressed in developing thymocytes. While HOXA11 is expressed at different stages of T-cell differentiation, HOXA10 expression is only detected at the earliest stages of differentiation, suggesting that its downregulation is required for full maturation of T-cells to the CD4 and CD8 single positive stages [18].

3.3.1.2. Deregulation of TAL1-related genes

TAL1-related genes (TAL1, TAL2 and LYL1), encode a distinct subgroup of basic helix-loop-helix (bHLH) proteins that share exceptional homology in their bHLH sequences [62]. The malignant potential of these proteins is likely to reside largely within their HLH domains that potentially mediate sequence-specific DNA recognition [63].

Although expression of TAL1, TAL2 or LYL1 has not been observed during normal T-cell development, the rearranged alleles of these genes are readily transcribed in T-ALL cells, and the ectopic expression of these genes in T-lineage cells may be a contributing factor in T-ALL pathogenesis [62].

3.3.1.2.1. TAL1 (SCL,TCL5) ( t(1;14)(p32;q11), t(1;14)(p34;q11) and t(1;7)(p32;q34))

Alteration of the TAL1 (SCL, TCL5), a gene located on chromosome 1p32, is considered as the most common nonrandom genetic defect in childhood T-ALL. TAL1 disruption is associated with a t(1;14)(p32;q11), t(1;14)(p34;q11) and t(1;7)(p32;q34) (TRA@/TRA@-TAL1 respectively) in 1% to 3% of childhood T-ALL [1, 57]. In other 9% to 30% of childhood T-ALL, TAL1 is overexpressed as a consequence of a nonrandom submicroscopic interstitial deletion between a locus called SIL and the 5' untranslated region (UTR) of TAL1 at 1p32, giving rise to an SIL-TAL fusion transcript [19].

As the translocation as interstitial deletion disrupt the coding potential of TAL1 in a similar manner, leading to its ectopic overexpression in T-cells [57]. Nevertheless, high expression levels of TAL1 in the absence of detectable TAL1 rearrangement are observed in about 40% of T-ALL [19].
The deletions aberrantly trigger activated TAL1 during thymocyte maturation, promoting transformation [1]. TAL1 alteration leads to silencing of genes target encoding E47 and E12 variants of E2A transcription factors. Several studies have proposed that the reactivation of silenced genes by administering histone deacetylase (HDAC) inhibitors may prove efficacious in T-ALL patients expressing TAL1 [18, 57].

3.3.1.2.2. TAL2 (t(7;9)(q34;q32))

As a consequence of t(7;9) (q34;q32), the TAL2 gene is juxtaposed to the TRB@ locus. The TAL2 gene is activated as a result of this translocation. The activation of the TAL2 or LYL1 genes is less common, affecting <2% of T-ALL patients [18, 62]. The properties of TAL2 broadly resemble those described previously for TAL1. Therefore, this support the idea that both proteins promote T-ALL by a common mechanism [64].

3.3.1.2.3. LYL1 (t(7;19)(q34;p13))

In the t(7;19)(q35;p13), the LYL1 coding sequences are juxtaposed to the TRB@ locus. This gene is constitutively expressed in T-ALL, whereas its expression is absent in normal T-cells. The ectopic LYL1 expression is found in some human T-cell leukemias, suggesting that it may participate in T-cell leukemogenesis. Similar to TLX1, TLX3, and TAL1, the ectopic expression of LYL1 is mutually exclusive, although rare exceptions to this rule have been described [18].

LYL1 encodes another class II basic helix-loop-helix (bHLH) transcription factor that forms heterodimers with class I bHLH proteins, including E2A (E47 and E12) and HEB. LYL1-transgenic mice developed CD4+CD8+ precursor T-cell ALL (pre-T-LBL), probably by dimerization with E2A, inhibition of CD4 promoter activity, and downregulation of a subset of E2A/HEB target genes, suggesting a block in cell differentiation [1, 65]

3.3.1.3. Deregulation of LIM-domain only genes LMO1 and LMO2

3.3.1.3.1. LMO1 (t(11;14)(p15;q11) and LMO2 (t(11;14)(p13;q11))

The genes encoding the LIM-domain only proteins LMO1 (RBTN1 or TTG1, 11p15) and LMO2 (RBTN2 or TTG2, 11p13) are frequently rearranged with the T-cell receptor loci in T-ALL, resulting in overexpression of LMO1/LMO2 [1, 66]. The most common alterations are t(11;14) (p15;q11) and t(11;14)(p13;q11) juxtaposing LMO1 or LMO2 to the TRA@ or TRAD@ loci [1], nevertheless other genetic alterations have also been reported like t(7;11)(q34;p15) and t(7;11) (q34;p13) translocations, involving TCRB and LMO1 or LMO2 loci [17].

Generally the ectopic expression of LMO1 and LMO2 are not mutually exclusive, because abnormal expression of LMO1/2 has been found in 45% of T-ALL, even in the absence of typical chromosomal changes, but often in association with deregulation of LYL1 (LMO2) or TAL1 (LMO1 and 2) [19]. Studies in transgenic mice have showed that TAL1 expression in itself is not sufficient to induce T-cell malignancies and that co-expression of LMO1 or LMO2 is strictly required [18].
3.3.1.4. Deregulation of family of tyrosine kinases — LCK gene (t(1;7)(p34;q34))

The lymphocyte-specific protein tyrosine kinase (LCK), a member of the SRC family of tyrosine kinases, is highly expressed in T-cells and plays a critical role in proximal TCR-based signaling pathways [67]. The LCK gene is activated due to the t(1;7)(p34;q34) that juxtaposing LCK with TRB@ loci [18]. ABL1 is located downstream of LCK in the TCR signaling pathway. Based on these results, SRC kinase inhibitors and the dual SRC/ABL kinase inhibitors could be used for treating T-ALL patients with hyperactive LCK [18].

3.3.1.5. Deregulation of MYB gene — Duplication and t(6;7)(q23;q34)

MYB is the cellular homolog of the V-MYB oncogene of the avian myeloblastosis virus. A t(6;7) (q23;q34), juxtaposing MYB to TCRβ regulatory elements, and a submicroscopic amplification of the long arm of chromosome 6 at 6q23.3 caused by ALU-mediated homologous recombination, has been detected in 8–15% of T-ALL. It leads to upregulation of MYB expression and a blockade in T-cell differentiation that could be reversed with MYB knockdown [1, 68]. The upregulation of MYB has raised expectations that MYB may be used as a molecular target for therapy in these patients [66].

Finally, other rearrangements involving TCR genes affect genes like BCL11B (inv(14)(q11q32); and t(14;14)(q11;q32)), TCL1 (inv(14)(q11q32), and t(14;14)(q11;q32)), CCND2 (t(7;12) (q34;p13.3), and t(12;14)(p13;q11)), NOTCH1 (t(7;9)(q34;q34.3)), and OLIG2 gene (t(14;21) (q11;q22)) [19, 24].

3.3.2. Fusion genes rearrangements

3.3.2.1. PICALM-MLLT10 (CALM-AF10) — t(10;11)(p13;q14)

The PICALM-MLLT10 (CALM-AF10) fusion gene is caused by a recurrent translocation, t(10;11) (p13;q14). It is detected in about 10% of childhood T-ALL and it has been associated with a poor prognosis [11]. This translocation has also been observed in other leukemias, including acute myeloid leukemia [18].

The precise mechanism for CALM-AF10 mediated transformation is not known, although CALM-AF10 T-ALL are characterized by overexpression of HOXA cluster genes, including HOXA5, HOXA9, and HOXA10. CALM-AF10+ T-ALL has also showed overexpression of several AF10 downstream genes (DNAJC1, COMMD3, BMI1 and SPAG6) located close to the AF10 gene breakpoint. From these four AF10 downstream genes, BMI1 is the only one known to be associated with an increase of self-renewal of hematopoietic stem cells and oncogenesis [69].

3.3.2.2. MLL-fusions

Translocations implicating MLL with various partners represent about 4% of T-ALL cases [18]. The t(11;19)(q23;p13) MLL-MLLT1 (ENL) gene fusion is the most common MLL translocation partner in T-ALL. Nevertheless, other MLL translocations also occur in T-ALL [11].
CALM-AF10+ T-ALL and MLL-t AL share a specific HOXA overexpression, triggering activate common oncogenic pathways [69]. MLL fusion proteins enhance transcriptional activity, resulting in increased expression of HOXA9, HOXA10, HOXC6, and overexpression of the MEIS1 HOX coregulator [18].

MLL controls skeletal patterning, regulates the establishment of functional hematopoietic stem cells, and early hematopoietic progenitor cell development [1, 70]. T-ALL cells with MLL fusions are characterized by differentiation arrest at an early stage of thymocyte differentiation, after commitment to the TCR gammadelta lineage [11].

3.3.2.3. ABL1-fusions

Translocations of ABL1 are rare, except for NUP214-ABL1 fusion (t(9;9)(q34;q34)) identified in up to 6% of T-ALL as a result of episomal formation with amplification. Recurrent translocations involving NUP98, such as the t(4;11)(q21;p15) with the NUP98/ RAP1GDS1 gene fusion), another protein of the nucleopore complex, are reported very rarely [19]. The t(9;12)(p24;p13) encoding ETV6-JAK2 fusion gene, with an important leukemogenic role, results in constitutive tyrosine kinase activity in positive T-ALL patients [71].

4. Copy number alterations in acute lymphoblastic leukemia

In spite of continually improving event-free (EFS) and overall survival (OS) for ALL, particularly in children, a number of patients on current therapies will relapse. Therefore it is important to know the group of patients with high risk of relapse [72, 73]. As the risk-stratification of ALL is partly based on genetic analysis, different genomic technologies designed to detect poor-risk additional genetic changes are being expanded substantially. Analyses of somatic DNA copy number variations in ALL aided by advances in microarray technology (array comparative genomic hybridization and high density single nucleotide polymorphism arrays) have allowed the identification of copy gains, deletions, and losses of heterozygosity at ever-increasing resolution [74].

Several microarray platforms have been used for the analysis of DNA copy number abnormalities (CNAs) in ALL, such as array-based comparative genomic hybridization (array-CGH), bacterial artificial chromosome (array-BAC) array CGH and oligonucleotide array CGH (oaCGH), single nucleotide polymorphism array (aSNP) and single molecule sequencing [75]. These microarray platforms vary in resolution, technical performance, and the ability to detect DNA deletions, DNA gains, and copy neutral loss of heterozygosity. These techniques have improved the detection of novel genomic changes in ALL blast cells [76]. The aCGH also detects the majority of karyotypic findings other than balanced translocations, and may provide prognostic information in cases with uninformative cytogenetics [77, 78]. In addition, the use of these methods documented multiple regions of common genetic cryptic alterations. These analyses provide information about multiple submicroscopic recurring genetic alterations including target key cellular pathways. However, many aberrations are still undetected in most cases, and their associations with established cytogenetic subgroups remain unclear [28, 79].
4.1. CNA in BCP-ALL

Most of ALL (79-86%) showed alterations in the number of copies (CNA) by aCGH techniques. The CNA frequently involved chromosomes 1, 6, 8, 9, 12, 15, 17 and 21; and rarely chromosomes 2, 3, 14 and 19. The losses have been more frequent than gains [6, 7, 28, 35, 77, 78, 80-85].

In precursor B-cell ALLs, most of the abnormalities have been gains of 1q (multiple loci), 9q, 17q, amplification of chromosome 21 (predominantly tetrasomy 21), and loss of 1p and 12p. Other recurrent chromosomal rearrangements have been found in both B-and T ALLs, such as loss of 6q (heterogeneous in size), 9p (9p21.3), 11q, and 16q, as well as gain of 6q and 16p. Other recurrent findings have included dim (13q), dim (16q) and enh (17q) [6, 7, 28, 35, 77, 78, 80-85] (Figure 1).

![Figure 1. Copy number alterations in two newly diagnosed BCP-ALL patients. A. Male patient with losses of 7, 9p24-q21 and 20 chromosome and gain of 21q chromosome. B. Male patient with whole gains of 4, 10, 14,18, 21, and X chromosome.](http://dx.doi.org/10.5772/55504)

Several observations suggest that the CNAs are biologically important. The identification of these recurrent chromosomal rearrangements in ALL has defined Minimal Critical Regions (MCR), which are target small regions of the genome, that are often small enough to pinpoint the few candidate genes that present in these chromosomal regions [75].

Many of these MCR contain genes with known roles in leukemogenesis of ALL. These lesions include deletions of lymphoid transcription factors and transcriptional coactivators (e.g. PAX5, EBF1, E2-2, IKZF1-Ikaros, ETV6 (TEL), ERG, TBL1XR1, and LEF1), tumor suppressor and cell cycle regulatory genes (e.g. CDKN2A/B, NF1, PTEN, RB1, and ATM), as well as genes with other established roles in B-cell development, such as RAG1 and RAG2, FYN, PBEF1 or CBP/
Moreover putative regulators of apoptosis (e.g. BTG1), lymphoid signaling molecules (e.g. BTLA/CD200, TOX), micro-RNAs (e.g. mir-15a/16-1), steroid receptors (e.g. NR3C1, NR3C2), genes at fragile sites (e.g. FHIT, DMD), and genes with unknown roles in leukemogenesis of ALL (e.g. C20orf94/MKKS, ADD3, and DMD) have been located in these regions. It is notable that about 40% of B-progenitor ALL cases present genomic alterations in genes that regulate B-lymphocyte differentiation [6, 7, 28, 35, 77, 78, 80-86] (Table 1).

<table>
<thead>
<tr>
<th>Loss/gain</th>
<th>Chromosome</th>
<th>Cytoband</th>
<th>Size (Mb)</th>
<th>Start position (Mb)*</th>
<th>End position (Mb)*</th>
<th>Candidate genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>loss 1</td>
<td>1</td>
<td>p33</td>
<td>0.039</td>
<td>47.728</td>
<td>47.767</td>
<td>TAL1</td>
<td>[75, 85]</td>
</tr>
<tr>
<td>loss 1</td>
<td>1</td>
<td>q44</td>
<td>1.74</td>
<td>245.113</td>
<td>246.853</td>
<td>LOC440742</td>
<td>[80, 110]</td>
</tr>
<tr>
<td>loss 2</td>
<td>1</td>
<td>p21</td>
<td>0.287</td>
<td>43.425</td>
<td>43.712</td>
<td>TRNA2</td>
<td>[75, 85]</td>
</tr>
<tr>
<td>loss 3</td>
<td>1</td>
<td>p22.3</td>
<td>0.396</td>
<td>35.364</td>
<td>35.670</td>
<td>No annotated gene ARPP-21</td>
<td>[85, 110]</td>
</tr>
<tr>
<td>loss 3</td>
<td>1</td>
<td>p14.2</td>
<td>0.254</td>
<td>60.089</td>
<td>60.343</td>
<td>FHT</td>
<td>[75, 83, 85, 110]</td>
</tr>
<tr>
<td>loss 3</td>
<td>1</td>
<td>q13.2</td>
<td>0.148</td>
<td>112.055</td>
<td>112.203</td>
<td>CD200, BTLA</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td>loss 3</td>
<td>1</td>
<td>q26.32</td>
<td>Various</td>
<td></td>
<td></td>
<td>TBL1XRF</td>
<td></td>
</tr>
<tr>
<td>loss 4</td>
<td>4</td>
<td>q7</td>
<td>0.469</td>
<td>109.035</td>
<td>109.084</td>
<td>LEF1</td>
<td>[75, 83, 85, 110]</td>
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<tr>
<td>loss 4</td>
<td>4</td>
<td>q11.23</td>
<td>0.145</td>
<td>149.697</td>
<td>149.842</td>
<td>None; telomeric to ARPP-21</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td>loss 5</td>
<td>5</td>
<td>q31.3</td>
<td>0.087</td>
<td>142.780</td>
<td>142.867</td>
<td>NR3C1, LOC389335</td>
<td>[75, 85]</td>
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<tr>
<td>loss 5</td>
<td>5</td>
<td>q31.3</td>
<td>0.553</td>
<td>157.946</td>
<td>158.499</td>
<td>EB1</td>
<td>[75, 83, 85, 110]</td>
</tr>
<tr>
<td>loss 6</td>
<td>6</td>
<td>p22.22</td>
<td>0.023</td>
<td>26.237</td>
<td>26.260</td>
<td>Histone cluster, MIST1/HR, MIST1/HAG, MIST2/ABI</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 6</td>
<td>6</td>
<td>q21</td>
<td>0.088</td>
<td>109.240</td>
<td>109.328</td>
<td>MIR22</td>
<td>[75, 83, 85, 110]</td>
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<tr>
<td>loss 7</td>
<td>7</td>
<td>7p</td>
<td>Whole p-arm</td>
<td></td>
<td></td>
<td>LOC645008X2, GATA6/7, KLF4/5/6/7,</td>
<td>[35, 85]</td>
</tr>
<tr>
<td>loss 7</td>
<td>7</td>
<td>q21.2</td>
<td>0.209</td>
<td>92.255</td>
<td>92.464</td>
<td>PCK1, DEGPSM5G5OS23, LOC442176</td>
<td>[80, 110]</td>
</tr>
<tr>
<td>loss 7</td>
<td>7</td>
<td>q12.2</td>
<td>0.048</td>
<td>50.419</td>
<td>50.467</td>
<td>IL21R/2 (ZNF79, JUN11)</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 8</td>
<td>8</td>
<td>q12.1</td>
<td>0.944</td>
<td>60.332</td>
<td>60.126</td>
<td>Immediately 5' of TGL2</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 9</td>
<td>9</td>
<td>p21.3</td>
<td>0.237</td>
<td>21.804</td>
<td>22.131</td>
<td>CDX2, CDX2NB1, MTA1, MLL13</td>
<td>[6, 75, 83, 85, 88]</td>
</tr>
<tr>
<td>loss 9</td>
<td>9</td>
<td>q15.2</td>
<td>0.088</td>
<td>36.912</td>
<td>37.020</td>
<td>FRAT/CMAH or sequence mutation</td>
<td>[75, 83, 85, 110]</td>
</tr>
<tr>
<td>loss 10</td>
<td>10</td>
<td>q25.31</td>
<td>0.062</td>
<td>89.616</td>
<td>89.738</td>
<td>PTEN</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 10</td>
<td>10</td>
<td>q24.1</td>
<td>0.178</td>
<td>97.889</td>
<td>98.067</td>
<td>BEEK</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 10</td>
<td>10</td>
<td>q25.1</td>
<td>0.078</td>
<td>111.182</td>
<td>111.860</td>
<td>ADD3</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 11</td>
<td>11</td>
<td>p13</td>
<td>0.155</td>
<td>33.917</td>
<td>34.072</td>
<td>No gene; Immediately 5' of MLL2</td>
<td>[75, 85]</td>
</tr>
<tr>
<td>loss 11</td>
<td>11</td>
<td>q12</td>
<td>0.068</td>
<td>36.618</td>
<td>36.626</td>
<td>KAG1/2, LOC119710</td>
<td>[75, 83, 110]</td>
</tr>
<tr>
<td>loss 11</td>
<td>11</td>
<td>q22.3</td>
<td>0.054</td>
<td>36.600</td>
<td>36.654</td>
<td>ATM</td>
<td>[80, 110]</td>
</tr>
<tr>
<td>loss 11</td>
<td>11</td>
<td>q23.3</td>
<td>0.274</td>
<td>118.369</td>
<td>118.643</td>
<td>16 genes distal to MLL breakpoint, including 3' MLL</td>
<td>[80, 85]</td>
</tr>
<tr>
<td>loss 12</td>
<td>12</td>
<td>p12.1</td>
<td>0.45</td>
<td>19.109</td>
<td>23.889</td>
<td>KDM5</td>
<td>[75, 110]</td>
</tr>
<tr>
<td>Loss/gain</td>
<td>Chromosome</td>
<td>Cytoband</td>
<td>Size (Mb)</td>
<td>Start position (Mb)*</td>
<td>End position (Mb)*</td>
<td>Candidate genes</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
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<td>----------</td>
<td>---------------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>12</td>
<td>p13.2</td>
<td>0.086</td>
<td>11.813</td>
<td>11.899</td>
<td>ETV6, B3A4-D family</td>
<td>[6, 15, 75, 80, 83, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>12</td>
<td>q13.33</td>
<td>0.218</td>
<td>92.391</td>
<td>92.589</td>
<td>BTG1</td>
<td>[75, 80, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>13</td>
<td>q14.11</td>
<td>0.031</td>
<td>41.535</td>
<td>41.586</td>
<td>FLJ3, C13orf71, LOC430128</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>13</td>
<td>q14.2</td>
<td>0.148</td>
<td>49.016</td>
<td>49.165</td>
<td>RB7</td>
<td>[6, 75, 80, 83, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>13</td>
<td>q14.2-3</td>
<td>0.889</td>
<td>50.573</td>
<td>51.462</td>
<td>DLEU2, AIP2, XWNG, MIRN18-2, MMN15A, DLEU1, FAM1064</td>
<td>[75, 85]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>15</td>
<td>q12</td>
<td>0.038</td>
<td>26.036</td>
<td>26.074</td>
<td>AIP10A</td>
<td>[80, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>15</td>
<td>q14</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>SPRED1 (5')</td>
<td>[75, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>15</td>
<td>q15.1</td>
<td>0.752</td>
<td>41.258</td>
<td>42.030</td>
<td>18 genes including IGF1 and MIRNA26</td>
<td>[85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>17</td>
<td>17p</td>
<td></td>
<td></td>
<td></td>
<td>TP53</td>
<td>[83]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>17</td>
<td>q11.2</td>
<td>0.169</td>
<td>29.066</td>
<td>29.235</td>
<td>LOC299840, SUFT2P, CRLF3</td>
<td>[75, 83, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>17</td>
<td>q21.1</td>
<td>0.045</td>
<td>37.931</td>
<td>37.976</td>
<td>IGF1, LMNW1A3, Akt2</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>19</td>
<td>p13.3</td>
<td>0.229</td>
<td>1.351</td>
<td>1.580</td>
<td>63 genes telomeric to TCF3, region may include TCF3</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>20</td>
<td>20q12.1</td>
<td>0.035</td>
<td>10.422</td>
<td>10.457</td>
<td>CXORF94</td>
<td>[75, 85]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>20</td>
<td>q11.22</td>
<td>1.426</td>
<td>32.304</td>
<td>33.730</td>
<td>Several genes, UPREBT</td>
<td>[6, 110]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>21</td>
<td>q22.12</td>
<td>0.004</td>
<td>36.428</td>
<td>36.432</td>
<td>No gene, but immediately distal to RUNX1</td>
<td>[75, 85]</td>
</tr>
<tr>
<td><em>loss</em></td>
<td>21</td>
<td>q22.2</td>
<td>0.027</td>
<td>39.784</td>
<td>39.807</td>
<td>ERG</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>1</td>
<td>q23.3-q44</td>
<td>81.326</td>
<td>164.759</td>
<td>qter</td>
<td>719 genes telomeric of PEX1, including 3' region of PEX1</td>
<td>[75, 85]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>6</td>
<td>q23.3</td>
<td>0.182</td>
<td>135.492</td>
<td>135.674</td>
<td>MEF2, MIRN548A2, AHI1</td>
<td>[75, 80, 85]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>9</td>
<td>9p</td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>[83, 85]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>9</td>
<td>q14.12-q24.3</td>
<td>7.876</td>
<td>133.857</td>
<td>qter</td>
<td>158 genes telomeric of ABL1, including 3' region of ABL1</td>
<td>[75, 85, 110]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>10</td>
<td>10p</td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>[83, 85]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>21</td>
<td>21</td>
<td>46.8</td>
<td>Whole chromosome</td>
<td>Whole chromosome</td>
<td>Several genes</td>
<td>[5, 83]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>21</td>
<td>21q</td>
<td></td>
<td>Whole q-arm</td>
<td>Whole q-arm</td>
<td>AMN1, Bcl2, ERG</td>
<td>[35, 83]</td>
</tr>
<tr>
<td><em>Ampl</em></td>
<td>21</td>
<td>1AMP21**</td>
<td>13.713</td>
<td>–</td>
<td>–</td>
<td>Several genes</td>
<td>[5]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>21</td>
<td>q22.3</td>
<td>0.589</td>
<td>42.775</td>
<td>43.364</td>
<td>7 genes</td>
<td>[30]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>21</td>
<td>q22.11-12</td>
<td>4.002</td>
<td>32.322</td>
<td>36.344</td>
<td>M4 genes</td>
<td>[30]</td>
</tr>
<tr>
<td><em>gain</em></td>
<td>21</td>
<td>q22.11-q22.12</td>
<td>2.303</td>
<td>33.974</td>
<td>36.277</td>
<td>33 genes including RUNX1</td>
<td>[75, 85]</td>
</tr>
</tbody>
</table>
Table 1. Recurring regions of copy number alteration reported in ALL and involved genes with known or putative roles on leukemogenesis and cancer.

<table>
<thead>
<tr>
<th>Loss/gain</th>
<th>Chromosome</th>
<th>Cytoband</th>
<th>Size</th>
<th>Start position</th>
<th>End position</th>
<th>Candidate genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td>22</td>
<td>q11.1-q11.23</td>
<td>21.888</td>
<td>ptel</td>
<td>23.563</td>
<td>277 genes telomeric (5’) of BCR, including 5’ region of BCR</td>
<td>[75, 85]</td>
</tr>
</tbody>
</table>

* Assembly GRCh37/hg19 from Genome Reference Consortium

The average number of CNAs per ALL case is usually low, suggesting that this disease is not characterized by inherent genomic instability. This has been shown in a large SNP array study performed on pediatric ALL cases (B-progenitor and T-lineage). It allowed to identify a relatively low number of CNAs in ALL -a mean of 6.5 lesions per case- indicating that gross genomic instability is not a feature of most ALL cases [75, 85], although it is higher that the number of genomic changes in myeloid malignancies. Furthermore, similar studies have found 4.2 lesions per case in the precursor B-cell childhood ALLs (3.1 losses and 1.1 gains), and 2.6 lesions per case in the T-ALLs (1.7 losses and 0.9 gains) [80].

In spite of the large number of novel alterations, most of them have been focal deletions (less than a megabase) that involve only one or a few genes in the minimal region of genetic alteration. Apart from high hyperdiploid ALL, gains of DNA have been specifically uncommon and a few of them were focal gains [75, 85].

The pattern and number of CNAs is similar in the genetic ALL subtypes. Notably, less than one deletion per case was observed in MLL-rearranged ALL, typically presenting early in infancy. Therefore it has been suggested that a few additional genetic lesions are required for inducing leukemia. In contrast, other ALL subtypes such as ETV6-RUNX1 and BCR-ABL1, typically presenting later than childhood, had over 6 copy number alterations per case, and some cases had over 20 lesions. These results are consistent with the concept that the initiating translocations are developed early in childhood, previous to clinically manifest leukemia (particularly for ETV6-RUNX1 leukemia). Additional lesions are subsequently required for establishment of the leukemic clone. The deletion of IKZF1 is also a lesion in BCR-ABL1 ALL, but it is exceptionally uncommon in ETV6-RUNX1 ALL [75, 76, 85-87].

High-resolution genomic profiling studies in childhood ALL also reveals recurrent genetic lesions, affecting genes with an established and critical role in leukemogenesis such as CDKN2A, ETV6 (TEL), RUNX1 (AML1) and other genes, such as MLL, that are used to stratify the patients [80]. Furthermore, many of these recurrent CNA were different between B-ALL and T-ALL subtypes. For example, deletions involving ADD3, C20orf94, ERG, ETV6, the fragile histidine triad gene FHIT, TBL1XR1, and a histone cluster at 6p22 were common in B-ALL but rare in T-ALL, whereas deletion of CDKN2A/B (9p21), are more frequent in T-ALL (72%-90%) than B-ALL (34%) [11, 76, 85].
4.2. CNA in T-ALL

Genome-wide profiling in T-ALL has been used to identify copy number alterations accompanying novel structural abnormalities, such as the *NUP214-ABL1* and *SET-NUP214* fusion genes. The amplification on extrachromosomal episomes of *ABL1* has been associated with the cryptic fusion of *NUP214* to *ABL1* gene, in around 6% of individuals with T-ALL. This fusion gene triggers the constitutive expression of the chimeric protein tyrosine kinase *NUP214-ABL1* and it is sensitive to the tyrosine kinase inhibitor imatinib. This amplification could improve outcome or decrease treatment-related morbidity of T-ALL cases, but large studies are needed to confirm these results [88]. Moreover, the cryptic and recurrent deletion, del(9)(q34.11q34.13), in pediatric T-ALL cases, results in a conserved *SET-NUP214* fusion product, that contribute to T-ALL pathogenesis by inhibition of T-cell maturation by the transcriptional activation of the HOXA genes [89].

Using SNP, BAC, or oligo-array CGH platforms, focal deletions have also identified in T-ALL, leading to deregulated expression of *TAL1* [85] and *LMO2* [90]; deletions of the *RB1* [85]; deletion and mutation of *PTEN* [85, 91]; deletion or mutation of the U3 ubiquitin ligase *FBXW7* [92]; and duplications of the protooncogene *MYB*, present in about 8% of T-ALL cases, that occur in combination with other genetic rearrangements contributing to T-cell differentiation arrest (*TAL/LMO, TLX1, TLX3, HOXA*) [68, 75, 93].

4.3. CNA in BL

High rates of CNAs have been reported in BL. CNAs have been observed in 65% [53] and 76% [43] of BL cases by conventional CGH. CNAs have been reported in 54% and 100% of BL patients by oaCGH and aSNP respectively [14, 55]. In addition, high-resolution molecular inversion probe (MIP) SNP assay have been reported 64% of CNAs in BL [94].

CGH and aCGH studies on cases of BL have shown that the increased number of gains and losses are significantly associated with shorter survival [43]. Gains are more frequent than losses in a range from 52% to 65% [14, 53, 94]. These studies have reported gains on chromosomes 1q, 7, 8q, 12, 13, 22 and Xq and losses in 6q, 13q, 14q, 17p, and Xp [14, 15, 43, 51, 53-55, 94, 95]. Some studies have also identified cases with gains on 2p [43, 55], 3q27.3 [14], 4p [43], 15q [51, 55], and 20p12-q13 [51].

It has been demonstrated that chromosomal gains or losses in the most frequently altered regions in BL, such as 1cen-q22, 1q31-q32, 7q22-qter, 8q24-qter, 13q31-q32, and 17p13-pter, influence changes in locus-specific gene expression levels of many genes that probably are associated with pathogenesis of BL. For example, the chromosomal region 1q showed increased gene expression levels in cases with gains, and correlates with the expression of germinal center-associated genes. By contrast, genetic losses in the chromosomal region 17p13 lead to a down regulation of genes located in this region, not only *TP53*, but also many other genes such as *AURKB*, that may influence the biological behavior as a consequence of deregulated expression [53].
4.4. CNA analysis of paired diagnostic and relapse ALL samples

Detailed comparative analysis of paired diagnostic and relapse ALL samples, using high resolution genomic profiling, have showed the next findings: i) frequent changes in DNA copy number abnormalities have been observed at relapse, ii) there are loss of copy number lesions present at diagnosis in ALL relapse samples, and acquisition of new additional (secondary) lesions in the relapse samples in nearly all analyzed patients, iii) deletions were more common than gains about newly acquired copy number abnormalities in the relapse samples. These data support the clonal evolution in ALL. The pattern of deletions on the antigen receptor loci was comparable between relapse and diagnosis, suggesting the emergence of a related leukemic clone, rather than the development of a distinct second leukemia. It should be noted that several cases were found in which the diagnosis and relapse samples carrying alternative lesions affecting the same gene(s), including CDKN2A and PAX5, suggesting that the inactivation of these genes were secondary but essential events required to develop a full-blown leukemia. Additionally, genomic abnormalities distinct from those presented at diagnosis has been identified lately, involved genes such as, IKZF1, IKZF2, IKZF3, RAG, ADD3, ETV6, BTG1, DMD and IL3RA/CSF2RA, suggesting that they confer a selective advantage and resistance to therapy in ALL [75, 96, 97].

These findings indicate that relapse is frequently the result of the emergence of a leukemic clone that shows significant genetic differences from the diagnostic clone. Whether these represent rare clones at the time of diagnosis or are the emergence of new clones should be further investigated [96].

5. Somatic mutations in acute lymphoblastic leukemia

Genome-wide profiling of DNA copy number alterations (CNA) coupled with focused candidate gene resequencing has identified novel genetic alterations in key signaling pathways in the pathogenesis of both B-progenitor and T-ALL. These findings are associated with leukemogenesis, treatment outcome in ALL, and are being exploited in the development of new therapeutic approaches and in the identification of markers of poor prognosis [72, 98].

5.1. Gene mutations in BCP-ALL

Somatic mutations in several genes are present in BCP-ALL. These mutations have identified in genes which are involved in RAS signaling (48%), B-cell differentiation and development (18%), JAK/STAT signaling (11%), TP53/RB1 tumor suppressor (6%) and noncanonical pathways and in other/unknown genes (17%) [72]. The incidence of the most recurrently mutated genes in ALL is described in the Table 2.

The frequency of alterations in the TP53/RB1, RAS, and JAK signaling pathways is much higher in High Risk B-Precursor Childhood Acute Lymphoblastic Leukemia (HR B-ALL)
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>Frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCP-ALL</strong></td>
<td></td>
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<tr>
<td><strong>RAS signaling</strong></td>
<td>NRAS</td>
<td>17%</td>
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<tr>
<td></td>
<td>KRAS</td>
<td>16%</td>
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<tr>
<td></td>
<td>FLT3</td>
<td>7%</td>
<td></td>
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<td></td>
<td>PTPN11</td>
<td>5%</td>
<td></td>
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<tr>
<td></td>
<td>NF1</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td><strong>B-cell differentiation and development pathway</strong></td>
<td>PAX5</td>
<td>15%</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>IKZF1 (IKAROS)</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td><strong>JAK/STAT signaling</strong></td>
<td>JAK1</td>
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<tr>
<td></td>
<td>JAK2</td>
<td>9%</td>
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</tr>
<tr>
<td><strong>TP53/RB1 pathway</strong></td>
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<tr>
<td></td>
<td>CDKN2A/CDKN2B</td>
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</tr>
<tr>
<td><strong>Others</strong></td>
<td>TBL1XR1</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ETV6</td>
<td>4%</td>
<td></td>
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<tr>
<td></td>
<td>CREBBP</td>
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<tr>
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<td>Unknown genes</td>
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<td><strong>T-ALL</strong></td>
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<td></td>
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<tr>
<td><strong>Cell cycle defects</strong></td>
<td>CDKN2A/CDKN2B</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP53, RB, p27</td>
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<td><strong>Differentiation impairment</strong></td>
<td>TAL1 plus LMO1/2</td>
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<td>LYL plus LMO2</td>
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<td>TLX1</td>
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<td>HOXA10/11</td>
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<td>PICALM-MLLT10</td>
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<tr>
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<td>MLL-fusions</td>
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<tr>
<td><strong>Proliferation and survival</strong></td>
<td>ABL1-fusions</td>
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<tr>
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<td>NRAS</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FLT3</td>
<td>5%</td>
<td></td>
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<tr>
<td></td>
<td>LCK</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>ETV6-PBL2</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ETV6-JAK2</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTEN</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td><strong>Self-renewal capacity</strong></td>
<td>NOTCH</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown genes</td>
<td>*/&gt;44%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Frequency of the different mutations observed in ALL.
cohort than reported for unselected pediatric B-precursor ALL patients. In this subgroup of patients have been recently proposed new targeted therapeutics, such as the RAS/MAPK signaling pathway [98].

5.1.1. Ras signaling

Deregulation of the RAS-RAF-mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK signaling cascade is often caused by somatic mutations in genes encoding proteins that influence the activity of this pathway, such as NRAS, KRAS2, FLT3, PTPN11, and BRAF [99]. As observed in myeloid malignancies, up-regulated RAS signaling, due to mutations in RAS genes or in genes coding for proteins controlling RAS function, represent a major pathway driving the aberrant growth of malignant B-cell precursors [100].

In BCP-ALL, a number of associations with other genetic changes are already known, such as the link between mutations of genes within the RAS signaling pathway and high hyperdiploidy [79, 99, 101]. These mutations have been found in ~60% of high hyperdiploid childhood cases ALL. They are invariably mutually exclusive, and additional cooperative genetic events in this subgroup of patients [99, 101, 102].

5.1.1.1. NRAS and KRAS

RAS genes are part of the small GTPase family and consist of three separate genes, NRAS, KRAS2, and HRAS. HRAS is rarely mutated in hematologic tumors and is expressed at a low level compared to the other two isoforms in the hematopoietic cells in leukemia [102]. The RAS proteins activate several downstream pathways to promote proliferation, differentiation, survival, and apoptosis, depending on cellular conditions [102].

Mutations in NRAS and KRAS have been recognized as a recurring molecular event in childhood ALL, with a reported incidence of between 15% and 30% [98, 100, 102]. The incidence and spectrum of mutations at diagnosis and relapse are similar, although the presence is not a significant risk factor [99, 101]. Moreover, it has not been found any association of RAS mutation with an adverse clinical outcome [103]. The presence or number of mutations in the RAS signaling pathway have not been associated with relapse-free survival [98].

5.1.1.2. FLT3

Activating mutations in the receptor tyrosine kinase FLT3 have been identified in approximately 20-25% of hyperdiploid and MLL-rearranged ALL samples [9, 104]. This observations supports the idea that the activation of tyrosine kinases as potential oncogenes in hyperdiploid ALL, as well as that leukemogenic fusion proteins such as MLL fusions cooperate with activated kinases to promote leukemogenesis [9].

Furthermore, small molecule tyrosine kinase inhibitors have activity against MLL-rearranged and hyperdiploid ALL with activating mutations in FLT3. Therefore FLT3 inhibitors are
validated as a potential therapeutic target in this leukemia [9]. The presence of FLT3 mutations in those patients with relapsed ALL harbored these alterations at diagnosis, suggested that FLT3 inhibition could represent a therapeutic opportunity in at least a subset of patients with relapsed ALL [104].

5.1.1.3. PTPN11

PTPN11 encodes SHP2, a protein tyrosine phosphatase that positively controls RAS function. Somatic missense mutations in PTPN11 cause SHP2 constitutive activation and enhance signaling through the mitogen-associated protein (MAP) kinase pathways [5].

PTPN11 mutations occur in approximately 6 to 7.3% of children with B-cell precursor ALL [5, 100]. Although PTPN11 defects have been negatively associated with most of the gene rearrangements (TEL-AML1, E2APBX1, BCR-ABL, and AF4-MLL), and other gene lesions (NRAS and KRAS2), it has been observed higher prevalence of PTPN11 mutations in children and adolescents with hyperdiploid DNA content [100].

PTPN11 mutations have been observed at disease presentation but are undetectable at remission, supporting the presence of the mutated gene in the leukemic clone and role of PTPN11 lesions in leukemogenesis. Nevertheless, the prognostic significance of these mutations remains unknown [100].

5.1.1.4. BRAF

The BRAF gene, a member of RAF family, intermediates downstream in the RAS/RAF/MAP kinase pathway. This gene has been described mutated in most of hairy cell leukemias, but is less frequently mutated in acute leukemias, indicating that the RAS-RAF kinase pathway in some leukemias may be desregulated by somatic mutations of BRAF [105].

Mutations in BRAF have been reported with a frequency of 20% in B-cell ALLs cases [105, 106]. BRAF is expressed in hematopoietic cells, and the expression of activated BRAF could relieve the cytokine dependence and could result in the transformation of hematopoietic cells [105]. The functional significance of the most of the BRAF mutations is unknown, though all mutations are located within the kinase activation domain of BRAF [106]. Therapies that target RAS-RAF-MEK-ERK-MAP kinase pathway would be very valuable in treating tumors with activating mutations of BRAF [105].

5.1.2. B-cell differentiation and development pathway

5.1.2.1. PAX5

PAX5 (paired box 5) encodes a transcription factor which is known as B-cell specific activator protein. This protein plays a key role in B-cell commitment by activating essential components of B-cell receptor signaling and repressing the transcription of genes that are necessary for T-lymphopoiesis [107]. PAX5 is the most common transcription factor which is altered in both children and adults B-ALL (32% of cases) [108]. Alterations of PAX5, including deletions, focal
amplifications, novel translocations, and sequence mutations, have not influence treatment outcome [107].

By SNP arrays, monoallelic deletion of PAX5 has been observed in about 30% of children and adults with B-ALL, resulting in loss of PAX5 protein expression or in the production of a PAX5 isoform lacking the DNA binding domain and/or transcriptional regulatory domain [107, 109]. It has been demonstrated that the PAX5 deletions are present in a dominant leukemic clone, consistent with a role in leukemogenesis during the establishing the leukemic clone [85, 110]. By sequencing, inactivating mutations of PAX5 have been observed between 7–30% of B-ALL cases [107]. These somatically acquired mutations have different patterns of alterations among some genetic subtypes of pediatric ALL [85]. The most point mutations of PAX5 are hemizygous reducing or inhibiting normal PAX5 functional activity [85].

Inactivating point mutations in PAX5 have more effect on the intracellular transcriptional network within primary leukemic cells. These mutations are clustered in exons encoding the DNA-binding or transcriptional regulatory domains, which leads to lose or to alter DNA-binding or transcriptional regulatory function [85].

Chromosomal translocations PAX5 are relatively rare, occurring in 2.5% of B-ALL cases; it has been reported at least 12 different fusion partners including transcription factors, structural proteins, and protein kinases (e.g. ETV6, ENL, FOXP1, ZNF521, PML, C20ORF112, AUTS2, JAK2, POM121, HIPK1, DACH1, LOC392027, SLCO1B3, ASXL1, and KIF3B) [107, 111]. In PAX5 rearrangements, the DNA binding domain of PAX5 and/or a variable amount of the C-terminal trans-activating domains are fused to functional domains of the partner genes, resulting in a loss of PAX5 function rather than in a gain of functional elements [107, 110]. The fusion proteins may also influence the expression of genes which are normally regulated by the partner protein, each of which has been implicated in B-cell development or hematopoietic malignancies [85].

5.1.2.2. IKZF1 (IKAROS)

IKZF1 has been established as one of the most clinically relevant genes in pathogenesis of ALL, because it plays a key role in tumor suppression in pediatric B-cell ALL and in high-risk B-cell ALL [112]. Deletions or mutations of this gene have been described in 15% of all pediatric B-ALL. However the incidence in BCR-ABL ALL is higher (80%) and is associated with a poor outcome. In addition, recent genomic profiling studies (GEP) have produced strong evidence that IKAROS plays a key role in tumor suppression in pediatric B-cell ALL and in high-risk B-cell ALL. Thus the GEP of ALL cases with losses in IKZF1 is similar to the observed in BCR-ABL1 positive ALL [112]. Further studies, in larger series of patients, are needed to assess the clinical value of the deletion/mutations in IKAROS in the other subtypes of ALL.
5.1.3. JAK/STAT signaling

5.1.3.1. JAK

Activating mutations involving the pseudokinase and kinase domains of Janus kinases (primarily JAK2, but also JAK1 and JAK3) have been reported in 10% of BCR-ABL1-negative high-risk pediatric ALL cases [86, 98, 113]. The childhood high-risk ALL cases, which harbor activating mutations JAK, have a gene-expression profile similar to BCR-ABL1 pediatric ALL ("BCR-ABL1-like"-Ph-like), and are associated to a poor outcome [98].

These mutations are transforming in-vitro, and trigger constitutive JAK-STAT activation of the mouse Ba/F3 hematopoietic cell line expressing the erythropoietin receptor transduced with mutant JAK alleles [108]. This transformation is abrogated by pharmacologic JAK1/2 inhibitors, suggesting that these agents may be a useful approach for treating patients harboring these mutations [108, 113].

The presence of JAK mutations have been associated with concomitant IKZF1 and CDKN2A/B alterations, suggesting that genetic lesions target multiple cellular pathways, including lymphoid development (IKZF1), tumor suppression (CDKN2A/B), and activation of tyrosine kinase signaling (BCR-ABL1, JAK, or other kinase mutations) that cooperate to induce aggressive lymphoid leukemia in high-risk BCR-ABL1-ALL [113].

Particularly, gain-function mutations in JAK2 are a common molecular event which is present about 18% of ALL Down’s syndrome (DS-ALL) cases [114]. These findings suggest that JAK2 inhibition might be a useful therapeutic approach in JAK2-mutated acute ALL associated with Down syndrome, because children with DS-ALL are especially sensitive to toxic effects of conventional chemotherapy [115].

5.1.3.2. Mutations in JAK regulators. CRLF2 and IL7R

CRLF2 encodes cytokine receptor–like factor 2 (also known as TSLPR-thymic stromal lymphopoietin receptor), a lymphoid signaling receptor molecule that forms a heterodimeric complex with interleukin-7 receptor alpha (IL7R) and binds TSLP [116]. CRLF2-mediated signaling promotes B lymphoid survival and proliferation [117].

Signaling from the TSLP receptor activates signal transducer and activator of transcription (STAT5) by phosphorylation of JAK1 and JAK2 through association with IL-7R and CRLF2, respectively [118]. Genetic alterations dysregulating CRLF2 expression may contribute to the pathogenesis of ALL [117], by induced activation of STAT proteins, especially STAT5 and STAT1 [119].

CRLF2 rearrangements, such as IGH@-CRLF2 or P2RY8-CRLF2 fusion, are present in up to 60% of children with Down Syndrome ALL (DS-ALL) and about 10–15% of high-risk BCR-ABL1 negative childhood and adult ALL [22]. In both DS-ALL and non-DS-ALL, approximately half of CRLF2 rearranged cases have concomitant activating JAK mutations (the most common in JAK2 but occasionally in JAK1), suggesting that the two alterations cooperate downstream in the signal transduction and transformation [108, 116].
Furthermore, in high-risk ALL, \textit{IKZF1} alterations, \textit{CRLF2} rearrangement and \textit{JAK} mutations are frequently observed together. They are associated with very poor outcome, even with current maximal intensive therapy [108, 117]. These leukemias may be sensitive to \textit{JAK} inhibitors, suggesting the potential for a targeted therapy. Thereby, detection of \textit{IKZF1}, \textit{CRLF2}, and \textit{JAK} mutations should be considered at diagnosis in childhood ALL [117].

Moreover, somatic mutations of Interleukin-7 receptor (\textit{IL7R}) (the heterodimeric partner of \textit{CRLF2}) have been reported in pediatric B and T ALL. Some \textit{IL7R} mutations have been observed in both diagnosis and relapse, but other mutations have been only present in relapse, whereas \textit{CRLF2} expression have been already described at diagnosis, suggesting that the \textit{IL7R} mutation may be a progression event [120]. Mutations of \textit{IL7R} are gain-of-function mutations that cooperate with \textit{CRLF2} to form a constitutively activated TSLP receptor. \textit{IL7R} activating mutations trigger cytokine-independent growth of progenitor lymphoid cells, and constitutive activation of STAT and mTOR pathways [120].

5.1.4. TP53/RB1 pathway

Mutations of the tumor suppressor gene \textit{TP53} have been associated with resistance to treatment and worse prognosis of patients in several tumors. Alterations of the \textit{TP53} gene are important at relapse in childhood ALL, in which they independently predict high risk of treatment failure in a significant number of patients [121].

The presence of \textit{TP53} mutations is associated with a reduced response rate to reinduction therapy. In addition, \textit{TP53} mutations correlate with a shortened duration of survival (from time of relapse and from time of diagnosis), even after successful reinduction therapy [122].

The clinical significance of exclusive deletions might be explained by \textit{TP53} haploinsufficiency. Moreover, an additional mutation appeared during or after relapse therapy in some relapse patients with exclusive deletion and nonresponse to treatment or second relapse, indicating outgrowth of fully \textit{TP53} altered clones that might contribute to the poor outcome [121].

5.2. Gene mutations in T-ALL

T-ALL has been associated with four different classes of mutations: (i) Affecting the cell cycle (\textit{CDKN2A/CDKN2B}); (ii) Impairing differentiation (\textit{HOX} genes, \textit{MLL}, \textit{LYL1}, \textit{TAL1/2} and \textit{LMO1/2}); (iii) Providing a proliferative and survival advantage (\textit{LCK} and \textit{ABL1}); (iv) Providing self-renewal capacity (\textit{NOTCH1}) [10, 11, 18, 123]. The genes most recurrently mutated in T-ALL are described in Table 2.

5.2.1. CDKN2A/CDKN2B

In up to 90% of ALL cases, the \textit{CDKN2A/2B} genes, located in tandem at chromosome 9p21, are inactivated by cryptic deletions, promoter hypermethylation, inactivating mutations or (post)-transcriptional modifications. Homozygous or heterozygous inactivation of the genomic \textit{CDKN2A} and \textit{CDKN2B} loci are the most frequent genetic abnormalities in T-ALL [124].
Inactivation of \textit{CDKN2A} and \textit{CDKN2B} by homozygous deletion has been described in 65% and 23% of T-ALL samples, respectively. Hemizygous \textit{CDKN2A} and \textit{CDKN2B} deletions are observed in approximately 10% and 15% of the samples [18].

The haploinsufficiency or inactivation of these tumor suppressor genes are involved in the development of T-ALL, because they not only promote uncontrolled cell cycle entry, but also disable the p53-controlled cell cycle checkpoint and apoptosis machinery. Thus, \textit{RB1} and \textit{TP53} pathways have been identified as possible targets for therapy of T-ALL [10, 11, 18, 19, 123].

**5.2.2. Tp53**

The acquisition of mutations in \textit{TP53} has been described in T-cell lines and T-ALL patients [123, 125]. The \textit{TP53} mutations are infrequent at diagnosis (5% of T-ALL cases) and tend to be associated with poor clinical outcome [123]. Copy number and sequence alterations of \textit{TP53} have been observed in 6.4%-24% of patients with T-cell ALL relapse, suggesting the importance of these alterations in the progression of the disease, in which they independently predict high risk of treatment failure in a significant number of patients [121, 123].

**5.2.3. NOTCH1**

Gain-of-function mutations in \textit{NOTCH1} have been identified in more than 50% of T-ALL samples resulting in constitutive NOTCH signaling [126]. They have been associated with a favorable early treatment response [11, 127]. \textit{NOTCH1} is a transmembrane receptor that plays a role in normal hematopoiesis as an early transcription factor and regulates self-renewal of stem cells and lineage commitment of lymphoid progenitor cells towards T-cell development [11, 128]. The intracellular NOTCH (ICN) released after proteolytic cleavage step of \textit{NOTCH1} mediates in the nucleus the expression of various target genes including \textit{HES1}, \textit{HEY1}, \textit{MYC}, \textit{PTCRA}, \textit{DTX1} and members of the NFkB pathway. At the protein level, activation of \textit{NOTCH1} mutations could also cause phosphorylation of multiple signaling proteins in the mTOR pathway [11]. \textit{NOTCH1} receptor is a promising target for drugs such as gamma-secretase inhibitors which block a proteolytic cleavage required for \textit{NOTCH1} activation signaling pathway [91].

The presence of subclonal duplications of the chromosomal region 9q34 are present in about 33% of pediatric T-ALL patients; the critical region encloses many genes including \textit{NOTCH1}. Although this duplication appears as an independent genetic event from both the episomal \textit{NUP214-ABL1} amplification and the \textit{NOTCH1} mutations, it could induce subtle changes in \textit{NOTCH1} expression levels and contribute to global \textit{NOTCH1} activation in T-ALL [11, 129].

**5.2.4. FBXW7**

F-box protein \textit{FBXW7} is an E3-ubiquitin ligase that regulates the half-life of other proteins including CyclinE, cMYC and cJUN [11]. Heterozygous \textit{FBXW7} single mutations have been identified in 8-30% of T-ALL patients, and usually are combined with \textit{NOTCH1} mutations affecting the heterodimerization (HD) domain. \textit{FBXW7} mutations render \textit{FBXW7} inactive to prime target proteins like \textit{NOTCH1} for proteosomal degradation, therefore these mutations
represent an alternative mechanism for NOTCH1 activation in T-ALL [11]. The presence of both FBXW7 and NOTCH1 mutations has been associated with good treatment response in T-ALL patients [130].

5.2.5. JAK1

Somatic activating JAK1 mutations occur about 10-20% of adults with T-cell precursors ALL, and have low prevalence in children and adolescents T-ALL [131]. JAK1 gene defects are associated with a poor response to therapy, frequent relapse, and reduced overall survival, identifying such mutations as a novel informative prognostic marker in adult T-ALL [132]. JAK1 gene encodes a cytoplasmic tyrosine kinase that it is noncovalently associates with a variety of cytokine receptors and plays a nonredundant role in lymphoid cell precursor proliferation, survival, and differentiation. T-cell origin with mutated JAK1 share a gene expression signature which is characterized by transcriptional up-regulation of genes positively controlled by JAK signaling [132].

Gain-of-function mutations in JAK1 may be concomitant with other genomic changes, such as NOTCH1 defects. The activation of JAK1 and NOTCH1 transduction pathways might cooperate in T-ALL pathogenesis and/or progression [10, 19, 132].

5.2.6. PTEN

PTEN loss of function mutation and deletions occur in approximately 25% to 35% of cases of T-cell ALL [98]. PTEN mutations and loss of PTEN protein could be also found as a secondary event during disease progression, thereby it could represent a progression marker rather than an initiating event in T-ALL [11].

The PTEN phosphatase has been identified as an important regulator of downstream (pre)TCR signaling. It directly opposes the activity of the phosphor-inosital-3 kinase (PI3K) functioning as a negative regulator of the oncogenic PI3K-AKT signaling [11, 133]. Inactivation of PTEN has been associated with activation of the PI3K-AKT pathway resulting in enhanced cell size, glucose uptake and proliferation [91]. Furthermore, the detection of abnormalities in the PTEN, PI3K, and AKT genes in a large subset of primary T-ALL samples have demonstrated a prominent role for oncogenic PI3K-AKT signaling in the pathogenesis of T-ALL [133].

Independent from activation following (pre)TCR stimulation, PTEN is negatively regulated by NOTCH1 [11]. There are some small molecule inhibitors of γ-secretase (GSIs) which block NOTCH1 activation in T-ALL cell lines with prototypical activating mutations in NOTCH1. However some of them are GSI-resistant. This resistance to GSI action is mediated by molecular abnormalities in signaling pathways that promote cell growth downstream of NOTCH1 [91]. It has been reported that mutational loss of PTEN is associated with human T-ALL resistance to pharmacological inhibition of NOTCH1 performed by GSIs [91]. Therefore PTEN deletions appeared to impart a high risk of induction failure with contemporary chemotherapy in T-ALL patients [91].
5.2.7. RAS

In T-ALL, activating RAS mutations have been identified only in 4–10% of cases without a prognostic impact [98, 123, 134, 135]. Nevertheless, it has been identified an alternative RAS activation mechanism in T-ALL cases with NF1 microdeletions on chromosome 17 without clinical evidence for neurofibromatosis with mutations on the remaining NF1 allele. NF1 is a negative regulator of the RAS signaling pathway. The presence of mutations on the remaining NF1 allele, confirmed the potential NF1 inactivation as an alternative RAS activation mechanism in these T-ALL cases. Therefore, T-ALL patients with activated RAS could potentially benefit from additional treatment with RAS inhibitors, such as farnesylthiosalicylic acid [11].

5.2.8. WT1

WT1 mutations is a recurrent genetic alteration in T-ALL. They are present in around 10% of T-ALL both in childhood and adults [136]. These mutations are highly associated with direct or indirect aberrant HOX genes expression in T-ALL cases with aberrant rearrangements of the oncogenic TLX1, TLX3, and HOXA transcription factor oncogenes [137]. Survival analysis have demonstrated that WT1 mutations do not confer adverse prognosis in either pediatric and adult T-ALL cases [136].

5.2.9. Mutated genes in Early Thymic Progenitors (ETP)-ALL

A new T-ALL subgroup, which is defined by a specific gene expression profile and a characteristic immunophenotype (CD1a-, CD8-, CD5weak with expression of stem cell or myeloid markers), has been recently described in pediatric T-ALL patients with poor outcome. This subgroup likely originates from early thymic progenitors (ETP) and has been called ETP-ALL. Recently, it has been described the high presence of FLT3 mutations in ETP-ALL [138] while in T-ALL patients with a non-ETP immunophenotype are rare (1-3%). In some patients, these mutations are only present in leukemic subclones [139, 140], indicating that FLT3 mutations may represent a T-ALL progression marker rather than an initiating event [11].

Moreover a recent study of whole-genome sequencing in ETP-ALL cases, has identified activating mutations in genes regulating cytokine receptor and RAS signaling in 67% of cases (NRAS, KRAS, FLT3, IL7R, JAK3, JAK1, SH2B3 and BRAF), inactivating lesions disrupting hematopoietic development involving 58% of patients (GATA3, ETV6, RUNX1, IKZF1 and EP300) and histone-modifying genes in 48% of patients (EZH2, EED, SUZ12, SETD2 and EP300) [141]. The global transcriptional profile of ETP ALL was similar to normal and myeloid leukemia hematopoietic stem cells. These findings could be related to the prognosis of ETP ALL patients [141].

In summary, the recent development of the genome wide analysis has provided new and critical knowledge of genetic changes in ALL. These new chromosomal imbalances and mutations could provide new insights for the management of the disease that is still associated with a dismal prognosis in the adult patients.
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