Molecular Biology of B- and T-ALL

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11.1 Introduction

Acute lymphoblastic leukemia (ALL) is traditionally classified into precursor B-, precursor T-, and B-cell (Burkitt) and then subclassified on the basis of recurrent cytogenetic changes that include aneuploidy and translocations (75% of precursor B and 15% of T-ALL patients) [1].

ALL is biologically heterogeneous. Improving outcome of treatment for such a heterogeneous group is what has driven much of the research into the biology of the disease. Elucidation of biologically and prognostically important molecular cytogenetic groups enabled stratification of patients in clinical trials that used chemotherapeutic agents developed in the 1950s through the 1980s. The steady improvement in the 5-year survival that this approach led to enabled predictions to be made that the survival rate in children with ALL would climb to over 90% [2]. High-resolution genomic methods like microarray analysis of gene expression, DNA copy number assays, and next-generation sequencing now contributing majorly toward this goal are also revealing new diseases and new targets of therapy and have thus brought ALL into the realm of targeted therapy and precision medicine [3].

Together, molecular cytogenetic and genomic methods of study have shown ALL to be a heterogeneous group of genetic diseases driven by sentinel genetic alterations (generally chromosome rearrangements and aneuploidy) and cooperating copy number and sequence mutations in genes that encode transcription factors regulating lymphoid development, tumor suppressors, protein regulators of the cell cycle and perturb cytokine receptor, kinase, and Ras signaling; and epigenetic/chromatin modifications. Several of these pathways, particularly kinase-activating lesions and epigenetic alterations, are candidates for novel precision medicine therapies [3, 4].

Based on the background, this chapter summarizes the key features of the major molecular subgroups of B-cell precursor ALL (BCP-ALL) and T-ALL. Table 11.1 represents WHO classification of acute lymphoblastic leukemia, 2016.

11.2 Precursor B-ALL (B-Cell Precursor ALL; BCP-ALL)

Approximately 75% of BCP-ALL patients can be risk-stratified in treatment protocols, based on NCI recommendations combined with chromosomal changes—BCR-ABL1, ETV6-RUNX1, E2A-PBX1, MLL rearrangements, hyperdiploid and hypodiploid karyotypes—into

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Table 11.1 WHO classification of acute lymphoblastic leukemia, 2016 [5]

B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
B-cell lymphoblastic leukemia/lymphoma, with recurrent genetic abnormalities
B-cell lymphoblastic leukemia/lymphoma with hypodiploidy
B-cell lymphoblastic leukemia/lymphoma with hyperdiploidy
B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2)[*BCR-ABL1*]
B-cell lymphoblastic leukemia/lymphoma with t(v;11q23)[*MLL* rearranged]
B-cell lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22)[*ETV6-RUNX1*]
B-cell lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3)[*TCF3-PBX1*]
B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32)[*IL3-IGH*]
B-cell lymphoblastic leukemia/lymphoma with intrachromosomal amplification of chromosome 21 (iAMP21)[a]
B-cell lymphoblastic leukemia/lymphoma with translocations involving tyrosine kinases or cytokine receptors ("BCR-ABL1–like ALL")[a]
T-cell lymphoblastic leukemia/lymphomas
Early T-cell precursor lymphoblastic leukemia[a]

[a]Provisional entity

Table 11.2 Common sentinel cytogenetic abnormalities in acute lymphoblastic leukemia

Cytogenetic abnormalities	Frequency	Clinical significance
t(9;22); *BCR-ABL1*	1–3% in children; 25–30% in adults	Poor prognosis
t(v;11q23); *MLL* rearrangements	75% of infants; 1–2% in older children; 4–9% in adults	Poor prognosis
t(12;21); *TEL-AML1*	25% in children; 0–4% in adults	Good prognosis
t(1;19); *E2A-PBX1*	1–6% in children; 1–3% in adults	Intermediate to favorable prognosis
Hyperdiploidy (>50 chromosomes)	25–30% in children; 7–8% in adults	Favorable prognosis
Hypodiploidy (<44 chromosomes)	6% in children; 7–8% in adults	Poor prognosis

"low" (high hyperdiploidy with favorable chromosome trisomies or (ETV6-RUNX1)), "standard/intermediate," "high," or "very high" (hypodiploidy, BCR-ABL1, and induction failure) risk groups (Table 11.2) [6–10]. More recently, IKZF1 mutation/deletion, predictive of poor outcome, has been recommended for risk stratification in treatment protocols [11].

About 25% of genetically unclassified children and a greater proportion of adults having no known cytogenetic abnormality constitute the *B-other* group [6, 12–14], referred to in the WHO 2016 book as *B-cell lymphoblastic leukemia/lymphoma, not otherwise specified*. With recent work showing a large proportion of this to be comprised of BCR-ABL-like, CRLF2-rearranged (non-BCR-ABL-like), ERG-dysregulated, iAMP21, and others, the size of the true B-other group has got reduced [15].

The following entities are considered in some detail below:

1. Ph-positive ALL
2. ERG-deleted ALL
3. B-ALL with intrachromosomal amplification of chromosome 21 (iAMP21)
4. Ph-like ALL

Brief accounts of the other entities can be checked from one of the reviews cited in the text [5].

11.2.1 Ph-Positive ALL

The t(9,22)(q34;q11) translocation or "Philadelphia" chromosome is the most common and a highly unfavorable sentinel abnormality in ALL and is present in 20–30% adults and 2–3% children with BCP-ALL. The translocation results in a fusion gene BCR-ABL that encodes an oncogenic protein that has constitutively active tyrosine kinase activity, which by altering signaling pathways regulates cell survival and proliferation and self-renewal of stem cells [16]. The p190 BCR-ABL transcript is more commonly seen (50–80% patients) in BCP-ALL, though the p210 transcript, that is characteristic of chronic myeloid leukemia (CML), may also be present; both p190 and p210 L transcripts may coexist (Fig. 11.1).

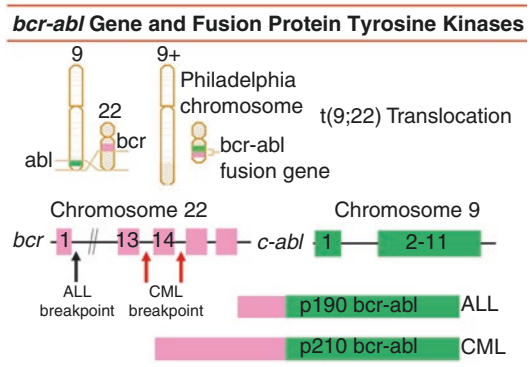

Fig. 11.1 P210 and 0190 transcripts in Ph+ ALL

Though Ph+ ALL has been frequently treated with intensive chemotherapy followed by hematopoietic stem cell transplantation, the prognosis of Ph+ BCP-ALL has been unsatisfactory with a 5-year event-free survival of approximately 50% in both children and adults. Tyrosine kinase inhibitors (TKIs), such as imatinib and dasatinib that target BCR-ABL1 fusion protein, have significantly improved treatment outcome; this is not true of all patients, which indicates the need to identify more biological features of the disease that could explain the heterogeneity.

To determine oncogenic lesions that work with BCR-ABL1 to induce ALL, Mullighan et al., 2008 [17] performed a genome-wide analysis of a large cohort of ALL patients, including BCR-ABL1 BCP-ALL. IKZF1 gene was found to be deleted in over 80% of BCR-ABL1 ALL patients. IKZF1 gene encodes IKAROS, a zinc-finger transcription factor associated with chromatin remodeling, which is expressed in fetal and adult hematopoietic system and functions as a regulator of lymphocyte proliferation and differentiation.

IKZF1 gene deletion could be of the whole gene or involve only the starting codon located in exon 2, resulting in haploinsufficiency. Additionally, there could be loss of the DNA-binding domain in exons 4–7 (what is called isoform 6) exerting a dominant negative effect over the unaffected allele, causing a loss of the tumor suppressor function of the wild-type IKZF1.

Studies have shown that in both Ph-negative BCP-ALL, in which it is fourfold less frequent, and Ph+ ALL patients, presence of IKZF1 deletion of any kind affects the outcome adversely, partially offsetting the positive effect even of imatinib [18–20].

IKZF1 deletion thus marks patients who will do particularly poor and have a high chance of relapse even in the poor prognosis Ph+ ALL [20]. IKZF1-deleted patients thus are candidates for more intensive and/or alternative therapy.

Additional interest in *IKZF1* alteration stems from the demonstration in animal models that this molecular lesion has a stem cell-like phenotype, aberrant expression and signaling of adhesion molecules, adhesion of the leukemic cell to stem cell niche in the bone marrow, and poor treatment response. Churchman et al., 2015 [21] showed that FAK1, a cytoplasmic, non-receptor protein tyrosine kinase involved in integrin-mediated signaling arising from binding of integrin to extracellular matrix proteins, upregulated in Ph+ B-ALL, is further overexpressed in IKZF1-altered cells; it is also a potential therapeutic target. They demonstrated that a FAK inhibitor VS-4718 potently inhibits aberrant FAK signaling and leukemic cell adhesion, thereby potentiating responsiveness to tyrosine kinase inhibitors, inducing cure in vivo. Thus, targeting FAK with VS-4718 has emerged as a promising way of overcoming the deleterious effects of FAK overexpression in Ph+ B-ALL, especially in abrogating the Ikaros alteration-induced adhesive phenotype; this warrants evaluation in clinical trials of combined ABL and FAK inhibition in Ph+ B-ALL, regardless of *IKZF1* status.

11.2.2 ERG-Deleted ALL

In a comparative genomic hybridization analysis of a group of NCI high-risk ALL patients who had a distinct gene signature but lacked a sentinel chromosomal cytogenetic change [22], Mullighan et al. [23] showed ERG deletion as defining genetic lesion in them. The ERG gene is located on chromosome 21 and is a member of the erythroblast transformation-specific (ETS) (v-ets erythroblastosis virus E26 oncogene homolog) family of transcriptions factors involved in the

regulation of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis. ERG has been shown to regulate hematopoiesis, and the differentiation and maturation of megakaryocytic cells are involved in chromosomal translocations TMPSSR2-ERG and NDRG1-ERG in prostate cancer, EWS-ERG in Ewing's sarcoma, and FUS-ERG in acute myeloid leukemia.

The frequency of ERG-deleted ALL has been reported to be approximately 3–7% of all BCP-ALL [24]. It is frequently associated with IKZF deletion, but whereas IKZF deletion is an adverse factor in Ph+ ALL and Ph-like ALL, it does not impact the favorable outcome of ERG-deleted ALL. ERG-deleted BCP-ALL has CD2 positivity and shows dysmorphic monocytes [24].

11.3 Newly Recognized B-ALL Subtypes (WHO)

In 2016 WHO classification of tumors of hematopoietic and lymphoid tissues, two new provisional B-ALL subtypes have been recognized: B-lymphoblastic leukemia/lymphoma with iAMP21 and B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like. These two new entities identify B-ALL patients with inferior clinical outcome and who may benefit from more aggressive or targeted therapy [5].

11.3.1 B-ALL with Intrachromosomal Amplification of Chromosome 21 (iAMP21)

Intrachromosomal amplification of chromosome 21 (iAMP21) accounts for 2% of pediatric B-ALL. It is more common in older children and adolescents, but rare in adults. This leukemia is recognized by FISH with a RUNX1 probe that reveals extra signals (≥5 copies per interphase nucleus, or ≥3 copies on a single abnormal chromosome 21 in metaphase FISH) [25]. This makes chromosome 21 unstable and has been shown to be a primary genetic event [26]. Patients with this abnormality present with leukopenia and display common B-lymphoblast immunophenotype with a subset of blasts displaying aberrant myeloid-associated antigen expression. These patients have poor event-free and overall survival when treated with standard risk therapy that improves with more aggressive chemotherapy [27].

11.3.2 BCR-ABL1-Like ALL

BCR-ABL1-like also called Ph-like ALL is a newly described subtype of BCP-ALL defined by a GEP similar to that of Ph+ ALL, produced in the absence of BCR-ABL1 translocation, by kinase-activating alterations, amenable to treatment with currently available TKIs. It is characterized by alterations of B-lymphoid transcription factors, a high frequency of IKZF1 alterations and a high risk of relapse when treated with conventional chemotherapy [13, 28–30].

The disease constitutes 10–20% of BCP-ALL in children [13] and over 25% of young adults [31]. Ph-like subgroup is thus more common than Ph+ ALL and has emerged the most common childhood ALL with poor prognosis [13]. Ph-like ALL was discovered independently by two groups. Mullighan et al. (2009) discovered that BCP patients whose poor prognosis could be predicted by copy number alterations of IKZF1, but not other genes encoding transcriptional regulators of B-lymphocyte development and differentiation, had GEP similar to that of BCR-ABL+ ALL [17, 19, 23].

Some B-other patients share a GEP with Ph + B-ALL which was shown independently by den Boer et al. (2009) [13], who studied the subjects from an alternative perspective of using a classifier based on GEP and then characterizing the genetic abnormalities thus revealed by comparative genomic hybridization and molecular cytogenetics. GEP analysis of high-risk ALL employing ROSE outlier-identifying program has also shown Ph-like ALL as unique group. Using a candidate gene approach, identified in 10.7% BCR-ABL1-negative ALL, mutations in JAK1, JAK2, and JAK3, especially JAK2, as oncogenic drivers producing BCR-ABL gene signature in the absence of BCR-ABL translocation. JAK mutation nearly always coexisted with CRLF2

gene lesions. Conversely, only about half the CRLF2-mutated patients had JAK mutation. CRLF2 abnormality has been reported also in half the BCR-ABL-ALL patients. Using transcriptome and whole genome approaches [32], the same group of investigators showed, employing functional assays, that in patients without JAK mutations, the disease was driven in all but 9% of 154 BCR-ABL patients, by activated kinases, resulting from 30 chromosomal rearrangements or sequence mutations that dysregulated 13 cytokine receptor and kinase signaling [29]. Gene expression data from transcriptome sequencing showed that patients with ABL-class, EPOR, or JAK2 rearrangements clustered separately from those with other JAK-STAT or Ras pathway alterations. Based on this, BCR-ABL-like ALL patients could be put into several key subgroups with therapeutic implications: (1) ABL class rearrangements (~13%) targeting ABL1, ABL2, CSF1R, and PDGFRB which are sensitive to ABL1 inhibitors; (2) EPOR (involving IGH, IGL) and JAK2 (several 5′ fusion partner genes) rearrangements (~11%), sensitive to JAK inhibitors; (3) CRLF2 rearrangements (~50%), frequently with concomitant activating JAK point mutations, that may also be sensitive to JAK inhibition; (4) other JAK-STAT activating mutations (11%) and deletions, including those involving IL7R, FLT3, etc. (e.g., JAK inhibitors for patients with IL7-R mutation); and (5) infrequent targets of rearrangement (less common kinase alterations) including NTRK3 (ETV6-NTRK3; sensitive to crizotinib). In addition, approximately 4% patients had RAS pathway mutations, but no other kinase lesions and several were associated with hypodiploidy. Genomic studies also revealed non-kinase lesions, including rearrangement of transcriptional regulators and chromatin modifiers, consistent with the notion that deregulation of multiple pathways, including kinase signaling, lymphoid maturation, and epigenetic modification contribute to leukemogenesis (Fig. 11.2) [31].

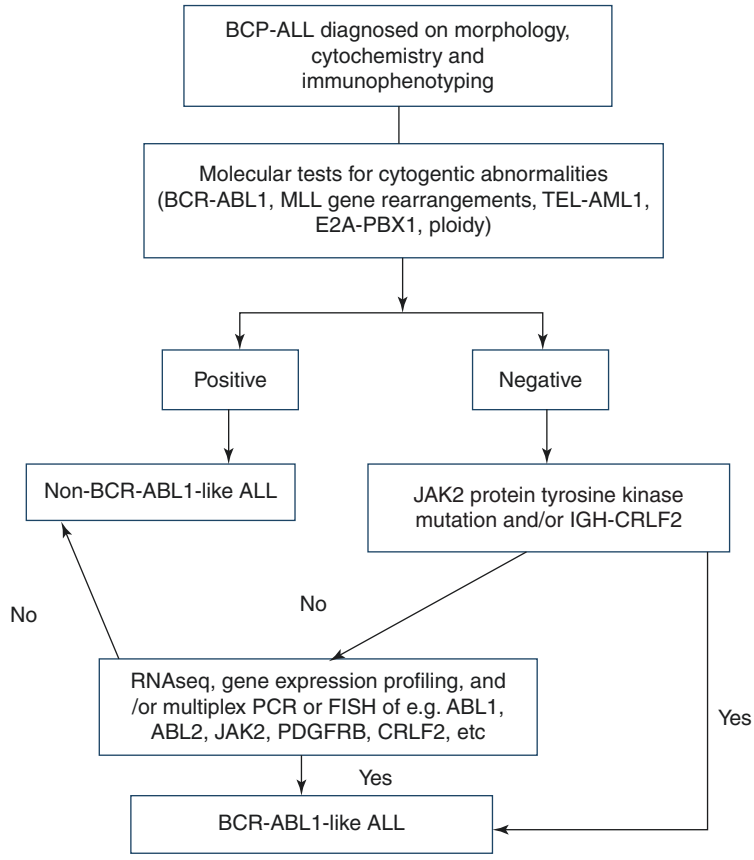

Fig. 11.2 Flowchart for the diagnosis of BCR-ABl1-like ALL

11.4 T-Acute Lymphoblastic Leukemia

11.4.1 Introduction

T-cell transformation, that has been shown to arise from transformation of T-cell progenitors blocked early or late in differentiation, is a multistep process in which different genetic alterations in key cellular pathways cooperate to produce the T-ALL phenotype. It has been estimated that every T-ALL patient probably has over 10 genetic lesions that cooperate to alter normal mechanisms that control cell growth, proliferation, survival, differentiation, metabolism, epigenetic control, and homing properties in normal developing T-cells [33, 34].

Current understanding of the molecular basis of T-ALL has come largely from analysis of recurrent chromosomal translocations and intrachromosomal rearrangements that are present in about half the T-ALL cases. In addition, translocations have been uncovered in genomic studies. These abnormalities typically juxtapose strong promoter and enhancer elements located in the *TCRB* (7q34) or *TCRA-TCRD* (14q11) loci, responsible for high levels of expression of T-cell receptor genes, next to a small number of developmentally important transcription factor genes, including basic helix-loop-helix (bHLH) family members such as *TAL1*, *TAL2*, *LYL1*, and *BHLHB1*; LIM-only domain (LMO) genes such as *LMO1* and *LMO2*; the *TLX1/HOX11*, *TLX3/HOX11L2*, *NKX2.1*, *NKX2.2*, *NKX2.5*, and *HOXA* homeobox (HOX) genes; *MYC*; *MYB*; and *TAN1*, a truncated and constitutively activated form of the NOTCH1 receptor [33, 35]. It has been shown that aberrant activation of these key transcription factor genes often in the absence of chromosomal rearrangements is the principal transforming event in the disease. Apart from promoter swap due to translocations in the TCR loci as mentioned above, oncogene activation occurs also due to gene fusion encoding chimeric proteins (Graux et al. 2006) [36].

Gene expression profiling studies have revealed a limited number of well-defined molecular groups of T-ALL [35–39], which share unique gene expression signatures that indicate distinct stages of arrest during T-cell development. Early immature T-ALLs, an amalgam of pro- and pre-T-ALL immunological groups proposed by EGIL [40], show an early block at the double-negative stage of thymocyte development [35, 38, 41]. In contrast, early cortical T-ALLs are characteristically CD1a, CD4, and CD8 positive and are typically associated with the activation of the *TLX1*, *TLX3*, *NKX2.1*, and *NKX2.2* homeobox genes [35, 41]. Finally, late-cortical thymocyte T-ALLs express CD4, CD8, and CD3 and show activation of the *TAL1* transcription factor oncogene [33, 35]. Immature T-ALL often is CD34+ and CD7+ while lacking CD3, CD4, and CD8; the reverse is not always true as cortical and mature T-ALL patients may be CD34+ [42].

Type A and Type B Genetic Alterations: The driving lesions in the leukemogenic process, the so-called type A genetic alterations, are mutually exclusive genomic rearrangements, mainly translocations. They define specific T-ALL subgroups: (1) TAL/LMO; (2) TLX1, TLX3, and HOXA (MLL, CALM-AF4, and SET-NUP214), NKX2.1/NKX2.2; (3) MEF2C; and (4) MYB.

The much more numerous type B abnormalities such as chromosome translocations, mutations, and genomic imbalances cooperate with the driving type A lesions and are present in diverse genetic subgroups. These include CDKN2A/2B, Notch1, FBXW7, PHF6, PTEN, FBXW7, RUNX1, EZH2, SUZ12, NRAS, JAK1, IL7R, ETV6, BCL11b, LEF1, and WT1.

Table 11.3 lists cytogenetic and molecular changes in T-ALL.

The above can be considered from another perspective, that of the systems and processes affected by the molecular cytogenetic changes.

1. Transcription factor oncogenes
2. Notch1 pathway
3. Cell cycle
4. Transcription factor tumor suppressors
5. Signal transduction
6. Chromatin remodeling
7. Ribosomal proteins and translation

Table 11.3 Cytogenetic and molecular changes in T-ALL (based on [29, 33, 41])

	Involved gene(s) oncogene	Protein(s)	Function of fusion gene or expressed oncogene	Consequence of rearrangement/ mutation
t(**7**;10)(q34;q24) and t(10;**14**)(q24;q11) *(T-cell receptor α, β, and δ are involved)*	*TLX1 (HOX11)*	Class II homeodomain-containing	Transcription factor	Ectopic TLX1 expression driven by TCR enhancer
t(5;**14**)(q35;q11) *(T-cell receptor δ at 14q11)* t(5;**14**)(q35;q32) (cryptic)[a] *(BCL11B at 14q32)*	*TLX3 (HOX11L2)*	Class II homeodomain-containing	Transcription factor	Ectopic TLX3 expression driven by TCR enhancer Ectopic TLX3 expression driven by BCL11B
inv(**7**)(p15q34), t(**7**;**7**)	*HOXA cluster*	Class II homeodomain-containing	Transcription factor	
t(1;**14**)(p32;q11) and t(1;**7**)(p32;q34)	TAL1	bHLH type II (basic helix-loop-helix)	Transcription factor	Ectopic TAL1 expression driven by TCR enhancer
t(**7**;19)(q34;p13)	*LYL1*	bHLH type II	Transcription factor	
t(11;**14**)(p13;q11) and t(**7**;11)(q35;p13)	LMO2	LIM-only domain	Protein–protein interaction	
t(1;**7**)(p34;q34)	LCK	SRC family of tyrosine kinase		
(**7**;9)(q34;q34.3)	*Notch1*	Notch receptor family	Cell fate determination, differentiation	
t(**7**;12)(q34;p13) and t(12;**14**)(p13;q11)	*CCND2*	D-type cyclin	Cell cycle activator	
1p32 deletion (cryptic)	*SIL/TAL1*	bHLH type II		Ectopic TAL1 expression driven by STIL promoter
t(10;11)(p13;q14) (often cryptic) CALM/AF10[b] (HOXA group)	*CALM/*	ENTH motif containing		
	AF10	Zinc fingers/leucine zipper containing		
t(11;19)(q23;p13)MLL/ ENL[b] (HOXA group)	*MLL/*	Mammalian homolog of *Drosophila trithorax*		
	ENL	Nuclear targeting sequence containing		
t(6;11)(q27;q23)	*MLL/AF6*	GLGF motif containing		
t(10;11)(p13;q23)	*MLL/AF10*	See earlier		
t(X;11)(q13;q23)	*MLL/AFX1*	Forkhead family		
t(4;11)(q21;q23)	*MLL/AF4*	Nuclear targeting sequence containing		
t(9;9)(q34;q34) (episomal)[c] Amplification	(*NUP214-ABL1*) *NUP214/*	Nuclear pore complex component		
	ABL1	Intracellular tyrosine kinase	ABL1—signal transduction	
t(9;14)(q34;q32) (cryptic)	*EML1/ABL1*	Echinoderm microtubule-associated/…		

(continued)

Table 11.3 (continued)

	Involved gene(s) oncogene	Protein(s)	Function of fusion gene or expressed oncogene	Consequence of rearrangement/ mutation
t(9;12)(q34;p13)	*ETV6(TEL)/ ABL1*	ETS DNA binding containing		
(9;12)(p24;p13)	*ETV6(TEL)/ JAK2*	…/Intracellular tyrosine kinase	JAK2—Signal transduction	
inv14(q13;q32.33) t(7;14)(q34;q13)	*NKX2.1*			
t14;20 (q11;p11)	*NKX2.2*			
t(4;11)(q21;p15)	*NUP98/*	Nuclear pore complex component		
	RAP1GDS1	Cytoplasmic		
del9p21 (homozygous/ hemizygous)	*CDKN2A/p15* and *CDKN2B/ p16* loci *P16*	INK4/ARF		
del(9)(q34.11q34.13)[d]	*SET-NUP214*	SET	Chromatin remodeling and transcriptional activation	
NOTCH1	*NOTCH1*	Notch receptor family	Cell fate determination, differentiation	
FLT3 ITD	*FLT3*	Receptor tyrosine kinase	Development of hematopoietic stem cells	
N-RAS	*N-RAS*	Signaling protein	Signal transduction	

Notes: (1) Normal thymic expression: TLX1 and TLX3 are not expressed; TAL1, LYL1, and LMO2 are expressed in early stages. (2) Homeobox family members: TLX1, TLX3, HOXA, HOXA (CALM-AF10), HOXA (MLL-ENL), HOXA (SET-NUP214, NKX2.1, and NKX2.2. (3) Duplications (*MYB*) and amplifications also occur

[a]TLX3 is juxtaposed to the distal region of BCL11B, a gene universally expressed during T-cell differentiation. Other variants have been described

[b]HOXA group because of elevated expression of HOXA genes

[c]Fusion of NUP214 to ABL1 on amplified episomes, hence described as episomal *NUP214-ABL1* amplification, is an example of a genetic change that is acquired during leukemic growth

[d]SET-NUP214 is very similar to the DEK-NUP214 fusion as previously identified in t(6,9)(p23;q34)$^{+}$ patients with AML [43]

11.4.2 Biological Subgroups of T-ALL

There are three clinically relevant biological groups of T-ALL defined by distinct gene expression and having immunophenotypes that reflect thymocyte arrest at different stages of development. These include early immature T-ALL including ETP-ALL and early cortical/thymic and mature T-ALL [44].

Early immature T-ALL, including early thymic precursor T-ALL (ETP-ALL) is discussed below.

11.4.3 Early Immature T-ALL, Including Early Thymic Precursor T-ALL (ETP-ALL)

Immunological underpinnings: Assessment of T-ALL by immunophenotypic analysis has allowed prognostically relevant classification of the disease into developmental groups, based on maturity. The EGIL classification system (1995) distinguishes pro-T (CD7^{+}, CD2^{-}, CD5^{-}), pre-T (CD7^{+}, CD2^{+} and/or CD5^{+} and/or CD8^{+}, CD1a being negative), cortical-T (CD1a^{+}), and mature

T-cell stages (mCD3$^+$). More recent studies have not made a distinction between pro-T (CD1a$^-$, CD5$^-$) and pre-T (CD1a$^-$, CD5$^+$) ALL (EGIL), and instead, combine pro- and pre-T-ALLs, to make an early or immature T-cell ALL group (CD1a$^-$, CD5$^{-/+}$), while retaining the remaining two groups, thymic (cortical) and mature T-ALL. In contrast to the CD1a$^+$ thymic (cortical) T-ALL that has been shown in most studies to carry a relatively better prognosis, immature T-ALL cases (pro-/pre-T-cell immunophenotype; CD5$^{+/-}$, CD1a$^-$, CD8) have a lower remission induction, early relapse, and shortened overall survival [40, 41].

Immature T-ALL as a transcriptionally defined entity: Ferrando et al., 2002 [35], in the first gene expression profiling study in T-ALL showed the existence of a transcriptionally defined immature group of T-ALLs whose gene expression indicated arrest at an early stage of T-cell differentiation. This group had high expression of LYL1 and coexpression of LMO2 genes and was positive for early hematopoietic marker CD34 and myeloid antigens CD13 and CD33 and generally CD4 and CD8 negative. This immature T-ALL group was shown to be lacking deletion of short arm of chromosome 9 that deletes CDKN2A/B gene in over 70% T-ALL. These findings have been confirmed in other studies [37, 39].

Transcriptional likeness to early thymic precursors and best immunologically delineated poor prognosis group: Using gene expression profile of normal early thymic precursors to identify their leukemic counterparts and define their immunophenotype, Dario Campana's group in their study in children identified a novel poor prognosis group of T-ALL that was termed ETP-ALL, defined by a characteristic gene expression profile, increased genomic instability, and a distinct cell immunophenotype that made easy recognition possible: CD8$^-$ CD1a$^-$ defined by positivity in <5% blasts; negative or weak CD5 defined by positivity in <75% blasts; and positivity (>25% blasts) for one or more stem cell and myeloid markers CD34, CD117, HLA-DR, CD13, CD33, CD11b, CD65. ETP-ALL immunophenotype is the most validated prognostic marker for identification of high-risk early immature T-ALL [45].

Biallelic deletion of TCRγ locus as a marker of immaturity: Using array comparative genomic hybridization and GEP, Guiterrez et al. [46] showed that absence of biallelic deletion (ABD) of TCRγ chain, a marker of developmental arrest at the earliest stages of thymocyte development, detected by QPCR, was a very good predictor of induction failure. ABD possibly represents early maturation arrest before the onset of T-cell receptor rearrangements [45, 46]. Given that *TCR*γ rearrangements occur early in normal T-cell development, it is not surprising that *TCR* loci deletions were found to be significantly less frequent in ETP T-ALL [38], and the majority of ABD patients possessed the ETP T-ALL gene expression signature. In marked contrast to this, however, only a minority of the ABD T-ALL patients [46] met the ETP T-ALL immunophenotype criteria, with most expressing CD5 on >75% of blasts. Because CD5 is expressed at low levels but is generally not absent on ETP-ALL blasts [38, 46] accurately distinguishing between the low (but not absent) CD5 expression characteristic of ETP-ALL lymphoblasts and the higher CD5 expression present in most T-ALL patient samples may be a problem. These discrepant results may thus very well reflect, at least in part, the challenge to accurately assess the ETP T-ALL immunophenotype across different laboratories. In fact many investigators studying immature T-ALL define it less narrowly than ETP-ALL and include CD5+ patients as well, thus defining an early immature group that is different from ETP-ALL, in that it includes CD5+ patients as well [39, 41].

Overlapping myeloid and T-ALL characteristics in early immature T-ALL—studies in adult-ALL: Investigating the possibility that early immature adult T-ALL (CD5$^{+/-}$) may be transcriptionally and genetically related to acute myeloid leukemias, based on the observation of marked enrichment in hematopoietic stem cell and granulocyte monocyte/macrophage precursor gene sets, Van Vlierberghe et al., 2011 [39], performed mutation analysis *of* AML oncogenes

and tumor suppressor genes and found mutations in IDH1, IDH2, DNMT3A, FLT3, and NRAS *in 14/29 (48%)* of immature adult T-ALL cases. Prevalence of prototypical T-ALL genetic alterations such as activating mutations in the *IL7R* gene and in *NOTCH1* and *FBXW7* that activate the NOTCH-signaling pathway was low. These results suggested that early immature adult T-ALLs are a heterogeneous group with features of both myeloid and T-lymphoid genetic alterations. This analysis also revealed the presence of *ETV6* mutations, truncated forms of ETV6 with dominant negative activity, in approximately 25% early immature T-ALLs, but not in other groups, highlighting the potential role of ETV6 mutations in these tumors. A later reanalysis of the gene expression signatures associated with immunophenotypically defined pediatric ETP-ALLs also showed that the gene expression programs of these leukemias are most closely related to those of human hematopoietic stem cells and myeloid progenitors (Zhang et al. 2012).

MEF2C as a driving lesion in early immature T-ALL: The first indication of a specific genetic lesion possibly associated with the pathogenesis of early immature leukemias was the identification via 4C analysis of rare but recurrent rearrangements resulting in aberrantly high levels of expression of the *MEF2C* gene in this group. *MEF2C* encodes an important transcriptional regulator of lymphoid development which is expressed at high levels in very early pre-DN1 and DN1 thymocytes and whose expression dramatically drops beyond the DN2 stage of thymocyte development. Notably, and supporting a potential role as a master regulator of the early immature T-ALL transcriptional signature, MEF2C can directly induce the expression of *LYL1*, *LMO2*, *HHEX*, three oncogenic transcription factors expressed at high levels in early immature leukemias.

Early immature T-ALLs include ETP T-ALLs and related what are described as "close to ETP" leukemias: Cross examination of gene sets associated with the ETP T-ALL [38], the *LYL1* leukemias [35], and the immature T-ALL clusters [38, 39] has shown these to be closely related groups. Also, early immature T-ALLs identified by gene expression signature encompass a broader patient group than the ETP T-ALLs defined on the basis of immunophenotype. The original report of Dario Campana's group [38], for example, showed that a few T-ALL cases that clustered together with ETP T-ALLs in an unsupervised gene expression analysis were $CD5^+$ showing ETP-ALL to be a group larger than what would appear from its immunophenotypic definition. Conversely, in the study of Vlierberghe et al. (2011), the transcriptionally and immunophenotypically (positive for stem cell and myeloid-associated antigens) immature cluster was a mixture of $CD5^-$ as well as $CD5^+$ cases, showing that only a fraction of these leukemias seem to strictly meet the immunophenotype criteria of ETP-ALL.

Genetics of ETP and early immature T-ALLs: Zhang et al. addressed the question of the absence of unifying genetic alteration by analyzing 12 ETP T-ALL cases using whole-genome sequencing and showed the presence of a high frequency of activating mutations in molecules mediating (1) cytokine receptor and RAS signaling—NRAS, KRAS, FLT3, IL7R, JAK3, JAK1, SH2B3, and BRAF; (2) inactivating mutations in genes encoding key transcription factors involved in hematopoietic development including GATA3, ETV6, RUNX1, IKZF1, and EP300; and (3) genes encoding histone modifiers such as EZH2, EED, SUZ12, SETD2, and EP300. Several ETP T-ALL cases showed multiple genomic rearrangements suggestive of genomic instability and deletions in the short arm of chromosome 9 encompassing the CDKN2A/B/B tumor suppressor genes were less prevalent in ETP T-ALLs compared with non-ETP leukemias.

11.5 Conclusion

Early immature T-ALLs encompass ETP-ALL and the related "close to" or "near" ETP-ALL and are defined by a gene expression signature that is most related to that of hematopoietic stem cells and myeloid progenitors. ETP T-ALL immunophenotype and the absence of biallelic deletion of the *TCRG* locus are associated with

very poor prognosis in children. Though they harbor a distinct transcriptional signature, early immature T-ALLs seem to lack a distinct unifying genetic alteration and have a lower prevalence of NOTCH1 activating mutations, mutations in myeloid oncogenes and tumor suppressor genes, and genetic alterations disrupting key transcription factors involved in hematopoietic and lymphoid development.

Early immature T-ALLs encompassing ETP T-ALLs and related "close to" ETP tumors constitute a genetically heterogeneous group of leukemias characterized by a gene expression signature most related to that of hematopoietic stem cells and myeloid progenitors. Even though they harbor distinct transcriptional signature, early immature T-ALLs seem to lack a distinct unifying genetic alteration; they have a lower prevalence of NOTCH1 activating mutations, but have mutations in myeloid oncogenes and tumor suppressor genes, and genetic lesions that disrupt key transcription factors involved in hematopoietic and lymphoid development. Two recent studies have reported a possible worse prognosis for immature adult T-ALL leukemias harboring mutations in *RUNX1* and DNMT3A [14, 15]. Further evaluation of this heterogeneous leukemia group is needed to address the prognostic implications of specific immunophenotypes, transcriptional signatures, and genetic alterations. Therapeutically, drugs active in myeloid tumors, validated in relevant animal models may help in ETP T-ALLs. In addition, targeted therapies may help in those leukemias that have activating mutations in druggable factors and signaling pathways like FLT3, JAK1, JAK3, IL7R, and NOTCH1.

Early cortical/thymic T-ALL: This good prognosis CD1a+, CD4+, CD8+ favorable prognosis group corresponds to early stage of thymocyte maturation. These tumors are characterized by activation of TLX1, TLX3, NKX2.1, NKX2.2 homeobox genes and have the highest frequency of Notch 1 mutations, and CDNK2A is deleted in almost all cases.

Mature T-ALL: This third group has the mature T-ALL immuophenotype (sCD3+, CD4+, CD8+) and typically has activation of TAL1 gene.

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