Chapter 1
Molecular Pathways and Targets in B-Cell Progenitor Acute Lymphoblastic Leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) is a neoplasm of B- or T-lineage lymphoid progenitors, with B-cell precursor ALL (BCP-ALL) representing the more common lineage of disease in both children and adults. BCP-ALL comprises over 20 subtypes characterized by constellations of genetic alterations, including aneuploidy, chromosomal rearrangements, DNA copy number alterations, and sequence mutations and, typically, distinct gene expression profiles [1–3]. As described in this review, the subtypes of B-ALL show variability in the nature of the initiating lesion (e.g., single or multiple chromosomal rearrangements, sequence mutations, or aneuploidy), secondary genetic alterations, and outcome. The prevalence and prognosis of each subtype is age dependent. Moreover, there is growing appreciation of the role of germline coding and non-coding variants in predisposing to ALL, both in familial and sporadic cases, and, in some instances, predisposing to specific subtypes of ALL, a striking example being germline *TP53* alterations and low hypodiploid ALL [4]. In the majority of subtypes of B-ALL, secondary genomic alterations are important events required for leukemogenesis, and also influence the risk of relapse [5, 6] (Fig. 1.1). Indeed, it is now recognized that in the majority of cases of B-ALL, the disease is usually polyclonal at the time of diagnosis, and when relapse occurs, there is substantial genomic evolution with clonal rise and fall and mutational extinction, convergence, and emergence [7–9]. Herein, we review the genomic landscape of BCP-ALL, including discussion of the role of germline predisposition and the genetics of clonal evolution and relapse. This review will emphasize illustrative examples of recently defined subtypes of ALL and highlight

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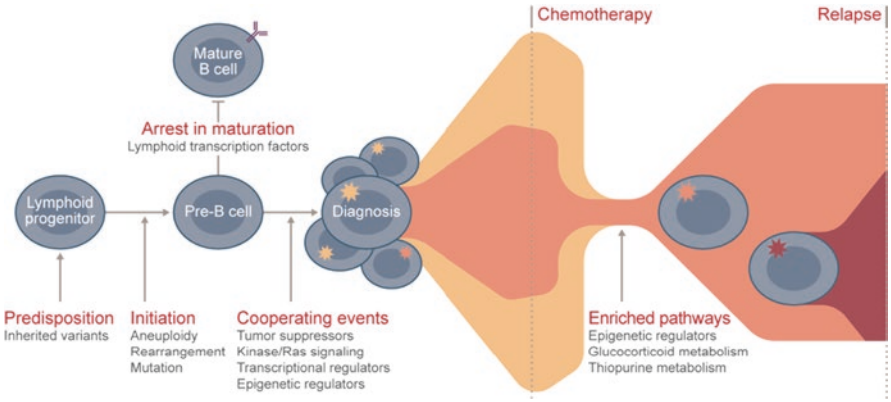

Fig. 1.1 Schema of the temporal pathogenesis of BCP-ALL

potential avenues for diagnostic implementation and therapeutic targeting of relapsed ALL with an emphasis on newly described entities and targets during the past decade.

Historic Aspects of Genetic and Genomic Classification of B-ALL

For many years, genetic classification of B-ALL was performed by cytogenetic karyotyping and complementary targeted fluorescence in situ hybridization (FISH) and molecular assays for specific chromosomal rearrangements and fusions [10]. These identified aneuploid B-ALL with high hyperdiploidy and hypodiploidy; chimeric fusions including *ETV6-RUNX1*, *BCR-ABL1*, and *TCF3-PBX1*; and rearrangement of *KMT2A* (*MLL*) in approximately two thirds of childhood ALL. Due to the low prevalence of high hyperdiploidy and *ETV6-RUNX1* in older individuals, over 50% of adult cases were unclassified [11]. This, coupled with the observation that many of these alterations were insufficient for leukemogenesis in experimental models, and the ability to detect several alterations at birth in cord blood or blood spots [12] years prior to the onset of leukemia, indicated that many cases of ALL had unidentified drivers and that collaborating genetic alterations are required for leukemogenesis in many cases.

The advent of microarray profiling of gene expression and DNA content (array-based comparative genomic hybridization, array-CGH, and single-nucleotide polymorphism (SNP) arrays) demonstrated that known subtypes of B-ALL exhibited relatively distinct gene expression profiles and could identify cases and subgroups that lacked a known driver [13–15]. SNP arrays identified multiple recurring DNA copy number alterations (CNA), particularly alterations in transcriptional regulators

of lymphoid development (*PAX5, IKZF1*, *EBF1*), providing valuable insights into the nature of co-alterations in B-ALL [16, 17]. These approaches were largely incapable of robustly identifying subtype-defining new alterations, in part due to the limited ability to identify chromosomal rearrangements and chimeric fusion oncoproteins.

Transcriptome sequencing (RNA-seq) has been the most powerful single experimental approach in enabling a near-complete understanding of the molecular classification of B-ALL and the genomic drivers responsible. Although not able to fully identify all sequence and structural alterations, RNA-seq provides a wealth of data regarding gene expression, gene rearrangement, chromosomal aneuploidy, and mutations. The combination of all four data types has proven necessary in classifying B-ALL. The first advance in subtyping of B-ALL using RNA-seq was the identification of Ph-like (BCR-ABL1-like) ALL, a subtype first recognized using microarray gene expression profiling [18, 19], but requiring RNA-seq to resolve the remarkable diversity in genetic alterations, particularly chromosomal rearrangements resulting in enhancer hijacking and chimeric fusion oncoprotein formation, characteristic of this subtype of ALL [20, 21].

In the last 5 years, multiple groups from the USA, Europe, Japan, and China have generated or used B-ALL RNA-seq data to identify new targets of recurring rearrangement (e.g., *DUX4*, *MEF2D*, and *ZNF384*) associated with distinct gene expression profiles [22–29] and the presence of cases with alterations that phenocopy additional canonical B-ALL drivers, e.g., *ETV6-RUNX1*-like ALL [27]. Several of these subtypes have diverse rearrangements involving a single gene, some of which are cryptic and eluding classification by conventional cytogenetic analysis. Several large-scale B-ALL RNA-seq generation/aggregation studies encompassing up to almost 2000 samples enabled additional observations: additional, less prevalent subtypes driven by chromosomal rearrangements (e.g., rearrangement of *NUTM1* and *BCL2MYC/BCL6*), identification of subtypes driven by initiating sequence mutations rather than chromosomal rearrangements (e.g., PAX5 P80R and IKZF1 N159Y), and subtypes with relatively distinct gene expression but diverse alterations targeting a single gene (PAX5alt, with fusions, sequence mutations, and intragenic amplification of this DNA-binding transcription factor) [5, 6, 26, 29] (Fig. 1.2).

By extending these studies across the age spectrum, these data have been particularly valuable in defining the genetic basis of B-ALL in older individuals, which is more parsimonious in the repertoire of subtypes, and commonly driven by alterations that are now recognized as inherently high risk: *BCR-ABL1*, Ph-like, low hypodiploid, and *KMT2A*- rearranged ALL, providing a partial explanation for the historically poor outcomes of B-ALL in adults [30] (Fig. 1.3).

Collectively, these studies have enabled classification of over 90% of childhood and adult ALL cases (Table 1.1). A minority of cases remain unclassified, but their driver alterations will likely be identified by the application of WGS that can identify non-coding mutations and rearrangements that deregulate genes without generating a chimeric RNA molecule and thus are not detected by RNA-seq alone.

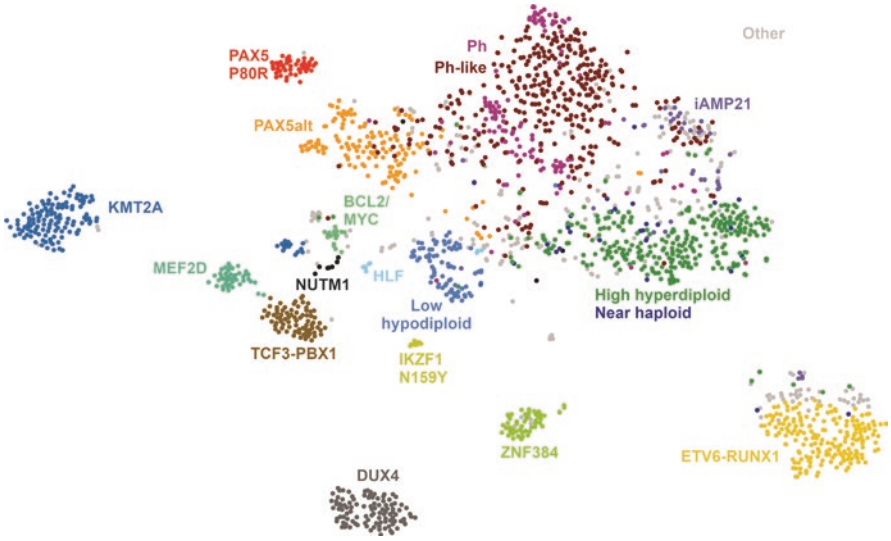

Fig. 1.2 t-SNE plot of gene expression data showing major B-cell precursor acute lymphoblastic leukemia (BCP-ALL) subtypes based on gene expression profiling of 1988 cases [5]. Each dot represents an individual case

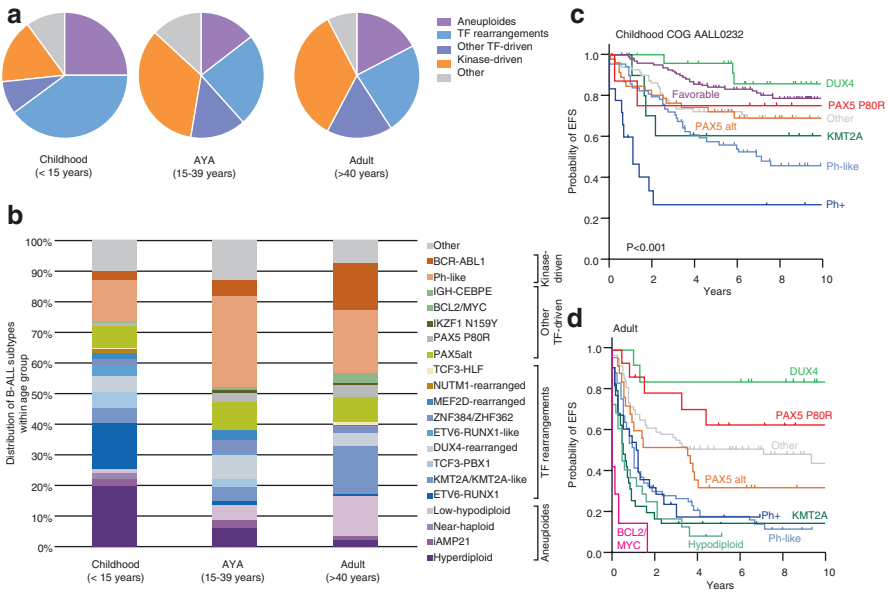

Fig. 1.3 Prevalence of each major subtype in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) across age or risk. AYA, adolescent and young adult; SR, standard risk; HR, high risk. (**a**) Distribution of key groups of ALL according to age. (**b**) Cumulative prevalence of ALL subtypes by age. Outcome of selected subtypes for (**c**) high-risk childhood B-ALL and (**d**) adult ALL. (Data taken from Gu et al. [5])

Table 1.1 Genetic and clinical characteristics of B-ALL subtypes

Molecular subtype	Median age (years)	Peak prevalence	Genomic alterations	Characteristics	Therapeutic approach	Ref
High hyperdiploid (51–67 chromosomes)	4	Children (25%)	Ras pathway, FLT3, epigenetic modifiers	Excellent prognosis; association of *CREBBP* mutations with relapse; FLT3, Ras alterations	FLT3 inhibition?	[57, 59]
Low hypodiploid (32–39 chromosomes)	47	Adults (10–15%)	*IKZF2* deletion, biallelic *TP53* alterations	Poor prognosis; *TP53* alterations are commonly inherited in children, but not in adult	BCL2/BCL-XL inhibitors	[4, 67]
Near haploid (24–31 chromosomes)	5.4	< 3% in all ages	Ras pathway (*NF1*), *IKZF3* deletions	Intermediate prognosis; No loss of chromosome 21 is observed	BCL2/BCL-XL inhibitors	[4, 67]
iAMP21	10	< 3% in children and AYA	Gain of three or more extra copies of a region of chromosome 21 including *RUNX1*	Good prognosis with intensive therapy; low WBC at diagnosis; the germline Robertsonian translocation rob(15;21) or a germline ring chromosome 21 is associated with increased risk of iAMP21		[69, 70, 74]
ETV6-RUNX1	4	Children (25%)	Deletion of the non-rearranged *ETV6* allele, *PAX5* deletions, *WHSC1* mutations	Excellent prognosis; expression of CD27 and low/negative expression of CD44; acquired in utero		[16, 77]
ETV6-RUNX1-like ALL	3	Children (3%)	Alterations (fusions/deletions) in *ETV6*, *IKZF1*, *TCF3*	Intermediate to favorable prognosis; similar gene expression profile to *ETV6-RUNX1* ALL; expression of CD27 and low/negative expression of CD44		[5, 27, 76]
TCF3-PBX1	8	Children (8%)		Good prognosis; pre-B immunophenotype	ROR1, BCL2 inhibitors	[80, 183]
TCF3-HLF	15	< 1% in all ages	*PAX5* deletions, Ras pathway	Very poor prognosis, sensitivity to BCL2 inhibitors	BCL2 inhibitors	[88, 89]
KMT2A rearranged	40	Infants (80%) and adults (15%)	Ras pathway (subclonal), PI3K pathway	Poor prognosis; relation to therapy-related leukemia (topoisomerase II inhibitors); acquired in utero (infant ALL)	DOT1L, MENIN inhibitors	[92, 98, 99]
BCR-ABL1 (Ph+)	40–45	Adults (40–50%)	*IKZF1* alterations, *CDKN2A/B* deletions	Prognosis improved with TKI	Second-/third-generation TKI, rexinoids, FAK inhibitors	[43, 102, 108, 184, 185]

(continued)

Table 1.1

Molecular subtype	Median age (years)	Peak prevalence	Genomic alterations	Characteristics	Therapeutic approach	Ref
Ph-like	21	AYA (25–30%)	Multiple kinase alterations, *IKZF1* alterations, *CDKN2A/B* deletions	Poor prognosis, amenable to TKI therapy; similar gene expression profile to *BCR-ABL1* ALL	JAK, ABL1, TRK inhibitors; combination with PI3K, BCL2, BCL-XL inhibitors; CRLF2 CAR-T	[20, 116, 131, 133, 135, 139]
DUX4 rearranged	14.3	AYA (8%)	*ERG* deletions (polyclonal), *IKZF1* deletions, Ras pathway	Excellent prognosis; distinct immunophenotype (CD2 and CD371 positive)	Potentially deintensify therapy	[22–24, 140, 145]
MEF2D rearranged	14	AYA (7%)	Ras pathway	Intermediate to unfavorable prognosis; low or absent expression of CD10 and high expression of CD38	Bortezomib, panobinostat	[24, 145]
ZNF384 rearranged	15	AYA (5%)	Ras pathway, epigenetic modifiers	Peak age of onset and prognosis varies by fusion partners; low CD10 expression and aberrant CD13 and/or CD33 expression; frequent in childhood B/M MPAL	FLT3 inhibition	[22, 147, 148]
PAX5alt	10	Children (10%)	*PAX5* rearrangements	Intermediate prognosis; loss of heterozygosity or acquisition of compound heterozygosity of *PAX5*		
PAX5 P80R	22	Adults (4%)	Ras pathway, JAK-STAT pathway	Intermediate to favorable prognosis; association with dic(9:20)		[5, 159, 160, 186]
NUTM1 rearranged	3	Children (1%)		Excellent prognosis		[7]
IKZF1 N159Y		< 1% in all ages	Gain of whole chromosome 21	Retain non-mutated wild-type allele of *IKZF1*		[7]
BCL2/MYC rearranged	48	AYA and adults (3%)		Poor prognosis		[5]

Heritable Susceptibility to Leukemia

Several lines of evidence support genetic predisposition for many subtypes of BCP-ALL, including (a) Down syndrome and other rare constitutional syndromes with increased risks for leukemia; (b) kindreds with familial BCP-ALL; (c) genome-wide association studies (GWAS) that have identified non-coding DNA polymorphisms which influence risk of BCP-ALL; and (d) a growing number of genes harboring germline non-silent variants presumed to confer risk of sporadic HM.

Children with constitutional syndromes such as Down syndrome, Noonan syndrome, neurofibromatosis type 1, ataxia-telangiectasia, Fanconi anemia, and other bone marrow failure syndromes (severe congenital neutropenia, dyskeratosis congenita, Shwachman-Diamond syndrome, and Diamond-Blackfan anemia) have an increased risk of leukemia. The spectrum of risk is syndrome specific. For example, Down syndrome is associated with a markedly increased risk of AML and B-ALL; Noonan syndrome and neurofibromatosis type 1 have increased risk of JMML (discussed later in this chapter), ataxia-telangiectasia increases T-ALL risk, and bone marrow failure syndromes primarily increase risk of AML [31–34].

Familial cancer syndromes such as Li-Fraumeni syndrome, constitutional mismatch repair deficiency syndrome, or DNA repair syndromes (Bloom, Werner, Nijmegen breakage) have increased incidence of malignancy, including ALL in a proportion of cases [35, 36]. Familial BCP-ALL is uncommon, but genomic analyses of such kindreds has been tremendously informative by identifying non-silent germline variants in transcription factor and tumor suppressor genes segregating with ALL that in many cases are also present as germline events in sporadic BCP-ALL. Key examples are *TP53* germline mutations and low hypodiploid B-ALL, *ETV6* variants and hyperdiploid and *ETV6-RUNX1*-like ALL [37], and *PAX5* mutations and B-ALL with dicentric/isochromosome 9 [4, 38–40]. These susceptibility genes are targets of somatic mutation in ALL: *ETV6* and *PAX5* are rearranged, amplified, deleted, and mutated in B-ALL [5, 16]. Germline variants of *IKZF1* predispose to a syndrome with immunodeficiency, autoimmunity, and sporadic/familial BCP-ALL [41, 42]; somatic *IKZF1* alterations are enriched in *BCR-ABL1*, Ph-like, and *DUX4*-rearranged B-ALL [19, 23, 43].

Genome-wide association studies (GWAS) have identified at least 13 loci with primarily non-coding variants associated with BCP-ALL. The relative risk associated with these variants is modest compared with constitutional syndromes or familial leukemia. Risk variants are frequently at or near hematopoietic transcription factor or tumor suppressor gene loci, including *ARID5B, BAK1, CDKN2A/CDKN2B, BMI1-PIP4K2A, CEBPE, ELK3, ERG, GATA3, IGF2BP1, IKZF1 IKZF3*, *USP7*, and *LHPP* [36, 44, 45]. Several variants display ancestry and ALL subtype-specific associations, such as *GATA3* with Hispanics and Ph-like B-ALL, *ERG* with African Americans and *TCF3-PBX1* B-ALL, and *USP7* with African Americans and T-ALL with *TAL1* deregulation [46–48].

Genomic analyses have identified additional susceptibility variants in sporadic hyperdiploid B-ALL (*NBN*, *ETV6*, *FLT3*, *SH2B3*, and *CREBBP*), Down syndrome-associated B-ALL (*IKZF1*, *NBN*, *RTEL1*), and T-ALL (Fanconi-BRCA pathway mutations) [49–51].

Prenatal Origin of Leukemia

Several observations indicate that a proportion of childhood leukemia cases are initiated before birth [52–54]. Chromosomal translocations such as *ETV6-RUNX1* may be detected at birth in blood spots and cord blood, years before the clinical onset of leukemia, providing support for a multistep process of leukemogenesis. This is supported by genomic analyses of monozygotic, monochorionic twins concordant for leukemia, showing genetic identity of initiating lesions and discordance for secondary genetic alterations, indicating inter-twin, intrauterine transmission of leukemia [53, 55]. Evidence for in utero origin is strongest for *KMT2A*-rearranged and *ETV6-RUNX1* ALL. Anecdotal evidence supports in utero origin for other subtypes of B-ALL, including hyperdiploid and *ZNF384*-rearranged leukemia [56].

Aneuploid BCP-ALL: Hyperdiploidy, Hypodiploidy, and Intrachromosomal Amplification of Chromosome 21

High hyperdiploidy (51–67 chromosomes) comprises approximately 30% of pediatric BCP-ALL and is associated with a favorable prognosis (Table 1.1) [57]. High hyperdiploidy is characterized by a nonrandom gain of chromosomes, typically +X, +4, +6, +10, +14, +17, +18, and +21 [57]. In particular, combined gain of chromosome 4, 10, and 17 is associated with favorable prognosis [58]. Alterations involving the Ras pathway (*KRAS*, *NRAS*, *FLT3*, *PTPN11*) and epigenetic modifiers (*CREBBP*, *WHSC1*) are frequent genetic events, with deletions leading to enhancer hijacking and deregulation of FLT3 particularly common in high hyperdiploid ALL [57, 59]. These secondary genomic alterations and the gene expression profiles of high hyperdiploid and the near-haploid subset of hypodiploid ALL are similar, suggesting a common origin [60]. Low hyperdiploid cases (47–50 chromosomes) harbor a diverse range of chromosomal changes and alterations rather than representing a genetically distinct subtype of ALL.

Hypodiploid ALL comprises three subtypes, two of which have an unfavorable prognosis: near-haploid ALL (24–31 chromosomes) and low hypodiploid ALL (32–39 chromosomes) [61–63]. Notably, chromosome 21 is never lost in hypodiploid ALL nor in other forms of ALL, suggesting an essential role in tumor cell fitness [4]. High hypodiploid ALL (40–44 chromosomes) is genetically heterogeneous, is not a distinct subtype of B-ALL, and does not share the unfavorable outcome of the other two groups. Accurate identification of low/near-haploid ALL is important in view of the poor prognosis and inherited genetic basis of low hypodiploid ALL in children [4]. Duplication of the aneuploid genome, or masked hypodiploidy, is common and may be mistaken for high hyperdiploidy [64]. These entities can be distinguished by the patterns of chromosomal gain and loss of heterozygosity observed on cytogenetic or SNP array analysis: masked hypodiploidy typically has

diploid and tetraploid chromosomes, whereas hyperdiploidy has a mixture of triploid and some tetraploid (e.g., 21, X); masked hypodiploid cases typically have LOH of the duplicated chromosomes. Flow cytometric analysis of DNA index frequently demonstrates peaks for both non-duplicated and masked clones in hypodiploid ALL, even if cytogenetic analysis demonstrates an apparently predominant masked clone.

Near-haploid ALL presents at a younger age and commonly exhibits alterations activating the Ras pathway (particularly *NF1*) and inactivating mutations/deletions of *IKZF3* (AIOLOS) [4]. Low hypodiploid ALL is rare but increases with age. Frequent secondary alterations include *IKZF2* (HELIOS), *RB1*, and *CDKN2A*/*CDKN2B*. The mechanistic differences between the IKAROS gene family members in leukemogenesis (*IKZF1* in kinase-driven and *DUX4*-rearranged leukemia and IKZF2/3 in hypodiploid ALL) remain to be determined. Importantly, almost all cases of low hypodiploid ALL in children and adults have biallelic alterations of *TP53* due to mutation (or less commonly focal deletion) and aneuploidy of the second chromosome [4]. In approximately half of pediatric cases (but not adult), the *TP53* mutations are germline, indicating that low hypodiploid ALL is a manifestation of Li-Fraumeni syndrome [4, 65]. Although still associated with an unfavorable prognosis, minimal residual disease (MRD) risk-stratified therapy has improved the outcome of hypodiploid ALL [66]. Hypodiploid ALL cells are sensitive to BCL2 inhibition, and BCL2 inhibitors are being evaluated in prospective clinical trials of newly diagnosed and relapsed/refractory ALL [67].

Intrachromosomal amplification of chromosome 21 (iAMP21) is more common in older children and is characterized by gain of three or more extra copies of a region of chromosome 21 including *RUNX1* generated by breakage-fusion-bridge cycles and chromothripsis [68–71]. The germline Robertsonian translocation rob(15;21) or a germline ring chromosome 21 is associated with a markedly elevated risk of iAMP21 [72]. Patients with iAMP21 usually lack other key cytogenetic alterations, although it is observed as a secondary event in *ETV6-RUNX1* and *BCR-ABL1* ALL in a minority of cases. Historically associated with an unfavorable outcome, intensive therapy improves prognosis [73, 74]. The key driver gene(s) located on chromosome 21 resulting in requirement for this chromosome in ALL, and mediating leukemogenesis in iAMP21 ALL, remains to be determined.

ETV6-RUNX1 and ETV6-RUNX1-Like ALL

The t(12:21)(p13:q22) translocation encodes *ETV6-RUNX1*, the most common fusion in BCP-ALL (20–25% in children) that is associated with a favorable prognosis [5, 75]. This translocation is frequently cryptic on cytogenetic analysis, and leukemic cells have a distinct immunophenotype (CD27 positive and CD44 low/negative) [76]. The *ETV6-RUNX1* fusion may be identified in umbilical cord blood and, thus, is considered to arise in utero as a leukemia-initiating alteration [75]. However, *ETV6-RUNX1* itself is insufficient to induce overt leukemia and

requires the prolonged latency with additional genetic events including deletion of the non-rearranged *ETV6* allele, focal deletion of *PAX5*, and mutation of *WHSC1* [2, 16, 17, 75, 77]. This is supported by heterogeneity in the subclonal composition of *ETV6-RUNX1* ALL [75, 78, 79].

ETV6-RUNX1-like ALL exhibits a similar GEP and immunophenotype to *ETV6-RUNX1* ALL despite the lack of *ETV6-RUNX1* fusion [5, 6, 27, 76]. *ETV6-RUNX1*-like ALL is also most common in children and has relatively favorable outcome [27, 76]. This subtype includes several alternate rearrangements in *ETV6* (e.g., *ETV6-ELMO1*), *IKZF1* (e.g., *IKZF1-ETV6*), *TCF3* (e.g., *TCF3-FLI1*), and *FUS-ERG* as well as copy number alterations in *ETV6*, *IKZF1*, and *ARPP21*, suggesting that alteration of multiple ETS and other transcription factors are converging on the same mechanism of transformation (although not *ERG*, which is distinct in the *DUX4*-rearranged ALL) [5, 27, 76].

TCF3-PBX1 and TCF3-HLF BCP-ALL

The t(1;19)(q23;p13) translocation encoding *TCF3-PBX1* fusion is present in 5–6% of pediatric BCP-ALL and is associated with a pre-B in transition (cytoplasmic immunoglobulin heavy chain positive) immunophenotype [80]. Previously considered high risk due to higher central nervous system involvement and relapse [15, 81, 82], *TCF3-PBX1* ALL is classified as favorable or intermediate risk with current treatment regimens [83]. Conditional activation of *TCF3-PBX1* in B-cell progenitors results in enhanced self-renewal and eventual development of leukemia with *PAX5* deletion and activation of JAK-STAT or Ras signaling pathways [84]. Importantly, *TCF3-PBX1* ALL exhibits sensitivity to dasatinib and ponatinib, but not imatinib, which occurs as a result of inhibition of pre-BCR signaling by SRC kinases. Due to compensatory upregulation of *ROR1* expression, combination with ROR1 inhibition may enhance the sensitivity of dasatinib [85].

A variant of the t(1;19) translocation, t(17;19)(q22;p13), encodes the *TCF3-HLF* fusion, a rare subtype of ALL associated with an extremely poor prognosis [86, 87]. *TCF3-PBX1* and *TCF3-HLF* ALL have distinct gene expression profiles and mutational landscapes [88]. *TCF3-HLF* ALL exhibited stem cell and myeloid features with enrichment of *PAX5* deletions and alterations of Ras pathway genes [88]. The TCF-HLF fusion may act as a pioneer transcription factor, recruiting EP300 to activate MYC, with vulnerability to EP300 inhibition [89]. *TCF3-HLF* leukemic cells are sensitive to the BCL2 inhibitor venetoclax (ABT-199), representing a potential targeted therapeutic approach [88].

KMT2A-Rearranged ALL

KMT2A (*MLL*) on chromosome 11q23 is rearranged to more than 80 different partner genes, and these rearrangements describe a distinct subtype of leukemia with variable immunophenotype spanning ALL, AML, and mixed phenotype leukemia

with both lymphoid and myeloid features and poor outcome [90]. *KMT2A*-rearranged BCP-ALL is typically of the pro-B phenotype, lacking CD10 expression, with co-expression of myeloid markers. Approximately 80% of *KMT2A*-rearranged ALL is observed in infants, in whom *KMT2A* rearrangement is acquired in utero. There is also a second peak in prevalence in adults, and more than 75% of cases are fused to *AFF1*. *KMT2A*-rearranged leukemia may also follow exposure to topoisomerase II inhibitors, with similar breakpoints to infant leukemia suggesting a common mechanism of leukemogenesis [91]. In infant ALL, the most commonly perturbed pathways include PI3K and Ras pathways [92–94]. *KMT2A* rearrangement results in assembly of a large multi-protein complex that results in aberrant transcriptional and epigenetic dysregulation via H3K79 methylation and recruitment of the H3K79 methyltransferase DOT1L, which interacts with multiple *KMT2A* rearrangement partners [95–97]. Multiple therapeutic approaches are being pursued, including inhibition of DOT1L, bromodomain, Menin, and the polycomb repressive complex [90, 97–99].

Kinase-Driven BCP-ALL: *BCR-ABL1* ALL and Ph-like ALL

The derivative chromosome 22, Philadelphia chromosome (Ph), arises from the reciprocal t(9;22)(q34;q11) translocation and encodes *BCR-ABL1* [7, 41, 100]. Although *BCR-ABL1* ALL is associated with poor prognosis, the addition of tyrosine kinase inhibitors (TKIs) to the conventional chemotherapy has improved outcome in children and adults [101–104]. In contrast to *BCR-ABL1*-positive chronic myeloid leukemia at chronic phase, *BCR-ABL1* ALL is characterized by a high frequency of secondary genetic alterations, particularly of the lymphoid transcription factor gene *IKZF1* and *CDKN2A/B* encoding the INK4/ARF cell cycle regulators [43, 105], and *IKZF1* alterations are associated with unfavorable outcome irrespective of TKI exposure [102, 105]. Moreover, mutations in the kinase domain of *ABL1* (most frequently T315I) induce TKI resistance and are more commonly observed in patients treated with TKI monotherapy or in adults treated with less intensive chemotherapy and less common in children treated with intensive chemotherapy [106]. Current treatment approaches to mitigate the poor outcome of *BCR-ABL1* ALL include frontline treatment with the third-generation TKI ponatinib with chemotherapy [101]. The adverse effect of *IKZF1* mutations in the pathogenesis of BCR-ABL1 ALL is in part due to loss of IKZF1 repression of stemness and cell-cell adhesion [107, 108]. This may be reversed by rexinoids (via agonism of rexinoid X receptor alpha, which induces expression of wild-type IKZF1) and focal adhesion kinase inhibitors (which inhibit downstream integrin signaling pathways) [108, 109].

Before consensus guidelines for MRD assessment in *BCR-ABL1* ALL were provided [110], several approaches have been tested for MRD monitoring (genome or transcriptome *BCR-ABL1* and Ig/TCR rearrangements) [111]. Importantly, some patients showed discrepancy of MRD results as assessed by measurement of Ig/TCR and *BCR-ABL1* transcript levels, due to the presence of the *BCR-ABL1* fusion in progenitors in addition to the blast population [111]. This *BCR-ABL1*-positive

clonal hematopoiesis is suggestive of a CML-like disease exhibiting lymphoid blast crisis.

Ph-like or *BCR-ABL1*-like ALL exhibits a gene expression profile similar to *BCR-ABL1* ALL despite the lack of the *BCR-ABL1* fusion [18, 19]. The prevalence and outcome of Ph-like ALL are similar to those of *BCR-ABL1* ALL, increasing in incidence with age and associated with elevated MRD levels and/or higher rates of treatment failure [20, 112–118], although the prevalence of Ph-like ALL is higher than *BCR-ABL1* ALL in the adolescent and young adult (AYA) population [20, 117, 119, 120]. Similar to *BCR-ABL1* ALL, *IKZF1* alterations are common, which result in acquisition of stem cell-like features and poor responsiveness to TKI. The heterogeneous genetic alterations driving Ph-like ALL may be classified into four main groups (Table 1.2., Fig. 1.4): (1) alterations driving JAK-STAT signaling, including rearrangements and mutations/deletions of *CRLF2*, *JAK2*, *EPOR*, *TYK2*, *IL7R*, *SH2B3*, *JAK1*, *JAK3*, *TYK2*, and *IL2RB*; (2) fusions involving ABL-class genes (*ABL1*, *ABL2*, *CSF1R*, *LYN*, *PDGFRA*, *PDGFRB*); (3) mutations activating Ras signaling (*NRAS*, *KRAS*, *PTPN11*); and (4) less common fusions (*FLT3*, *FGFR1*, *NTRK3*) [2, 121, 122]. Of these, *CRLF2* alterations are found in almost half of Ph-like ALL in adolescents, young adults, and older adults, as well as in half of ALL associated with Down syndrome ALL [123–125]. These alterations are rearrangements of *CRLF2* to IGH or P2RY8 resulting in enhancer hijacking or promoter swapping, respectively, and aberrant expression of CRLF2 as part of a heterodimer with IL-7 receptor alpha. *CRLF2*-rearranged ALL commonly has concomitant alterations that facilitate JAK-STAT signaling pathway activation, including sequence mutations of Janus kinases (most commonly at R683 of the pseudokinase domain of JAK2), IL-7RA, and deletions of negative regulators of JAK-STAT signaling (*SH2B3* and *USP9X*) [126, 127]. *CRLF2* rearrangement is associated with Hispanic ancestry and a germline *GATA3* non-coding variant [46, 128].

Importantly, most kinase-activating alterations in Ph-like ALL can, theoretically, be targeted by FDA-approved TKIs: JAK-STAT signaling (JAK inhibition), ABL-class fusions (ABL inhibitor), and *FLT3* and *NTRK3* fusions (FLT3 and NTRK3 inhibitor) with emerging evidence of efficacy in human leukemia, although evidence for efficacy of TKIs, at least as monotherapy, is strongest for ABL1-class and ETV6-NTRK3 Ph-like ALL [20, 129–133]. In contrast JAK inhibitor monotherapy in preclinical and clinical studies of CRLF2-rearranged Ph-like ALL is less effective [134]. Combination of kinase inhibitors against multiple signaling shows synergistic effects in PDX models of *CRLF2*/*JAK* mutant (JAK and PI3K/mTOR inhibitors) and *ABL*/*PDGFR* mutant (dasatinib and PI3K/mTOR inhibitor) [135]. Several of these (ruxolitinib, imatinib, dasatinib, ponatinib) are being tested in frontline studies [120, 133, 136]. As kinase-activating lesions also drive signaling through additional signaling pathways (e.g., PI3K, MEK, etc.), it is likely that additional therapeutic approaches will be required for optimal therapeutic response. Additional therapeutic approaches include BCL2 inhibitors, which exhibit synergy with TKIs in preclinical models [137, 138], and chimeric antigen receptor T cells directed against CRLF2 [139].

Table 1.2 Kinase-activating alterations in Ph-like ALL

Category	Kinase gene	Representative alterations	Targeted therapy
JAK-STAT	*CRLF2*	Mutations (F232C), fusions (*CSF2RA*, *IGH*, *P2RY8*)	JAK inhibitor
	EPOR	Truncating rearrangement to enhancers (*IGH, IGK, LAIR1, THADA*)	JAK inhibitor
	TYK2	Fusions (*MYB, SMARCA4, ZNF430*)	TYK2 inhibitor
	TSLP	Fusions (*IQGAP2*)	JAK inhibitor
	SH2B3	Deletion/mutations	JAK inhibitor
	IL7RA	Mutations, indels	JAK inhibitor
	JAK1	Mutations (e.g., V658F)	JAK inhibitor
	JAK2	Mutations (of R683; most commonly R683G, also kinase domain mutations), fusions (*ATF7IP, BCR, EBF1, ETV6, HMBOX1, PAX5, PCM1, PPFIBP1, RFX3, SMU1, SNX29, SSBP2, STRN3, TERF2, TPR, USP25, WDR37*, *ZNF274, GOLGA5, SMU1, HMBOX1, SNX29, ZNF430*)	JAK inhibitor
	JAK3	Mutations (usually kinase domain)	JAK inhibitor
	IL2RB	Fusions (*MYH9*)	JAK inhibitor
	USP9X	USP9X-DDX3X interstitial deletion and fusion	JAK inhibitor
ABL	*ABL1*	Fusions (*CENPC, ETV6, FOXP1, LSM14A, MYO18B, NUP214, NUP153, RCSD1, RANBP2, SFPQ, SNX2, SPTAN1, ZMIZ1*)	Imatinib/ dasatinib
	ABL2	Fusions (*ATF7IP, EBF1, ETV6, PAG1, RCSD1, SSBP2, TNIP1, ZEB2, ZC3HAV1, ZMYND8*)	Imatinib/ dasatinib
	CSF1R	Fusions (*MEF2D, SSBP2, TBL1XR1*)	Imatinib/ dasatinib
	LYN	Fusions (*GATAD2A, NCOR1*)	Imatinib/ dasatinib
	PDGFRA	Fusions (*FIP1L1*)	Imatinib/ dasatinib
	PDGFRB	Fusions (*ATF7IP, EBF1, ETV6, SNX29, SSBP2, TNIP1*, *ZEB2, ZMYND8*)	Imatinib/ dasatinib
Ras	*NRAS*	Mutations	MEK inhibitor
	KRAS	Mutations	MEK inhibitor
	PTPN11	Mutations	MEK inhibitor
	NF1	Mutations/deletions	MEK inhibitor
	BRAF	Mutations	MEK inhibitor
	CBL	Fusions (*KANK1*)	MEK inhibitor
Other	*FLT3*	*FLT3*-ITD, fusions (*AMYM2*)	FLT3 inhibitor
	NTRK3	Fusions (*ETV6*)	NTRK3 inhibitor
	FGFR1	Fusions (*BCR, MYO18A*)	Ponatinib
	PTK2B	Fusions (*KDM6A, STAG2, TMEM2*)	FAK inhibitor
	DGKH	Fusions (*ZFAND3*)	
	BLNK	Fusions (*DNTT*)	

Clinical trials of TKI in Ph-like ALL include dasatinib (newly diagnosed, NCT03117751 and NCT03020030; relapsed, NCT02420717) and ruxolitinib (newly diagnosed, NCT02723994, NCT03571321, NCT03117751; relapsed, NCT02420717). Data updated from Gu et al. [5]

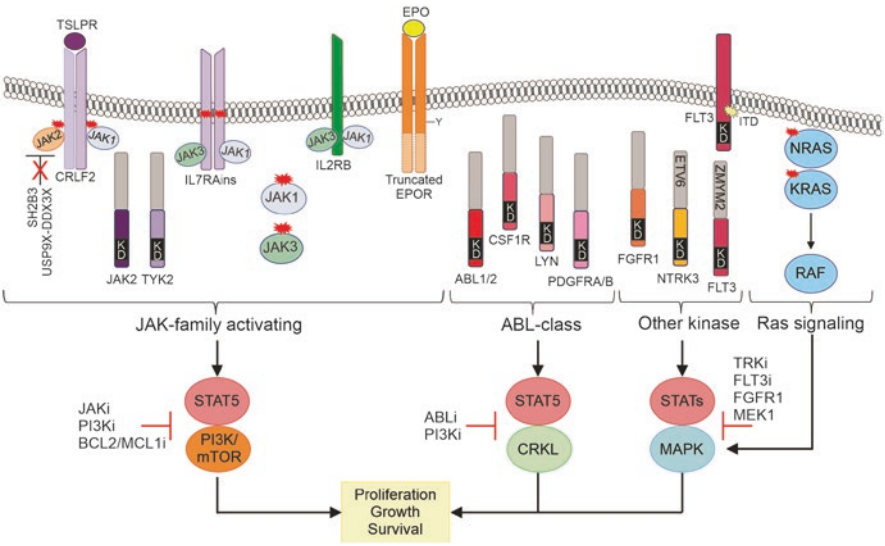

Fig. 1.4 Cartoon depicting targets of genetic alteration and type of mutation in Ph-like ALL

DUX4-Rearranged ALL

Rearrangement and overexpression of the homeobox transcription factor gene *DUX4* defines a distinct subgroup of BCP-ALL [5, 22, 23, 27]. This subtype also exhibits deregulation of the ETS family transcription factor *ERG* and comprises up to 5–10% of BCP-ALL with a slight peak in the AYA population. It has a distinct immunophenotype (CD2 and CD371 positive) and favorable outcome [140]. The pathogenesis of this form of leukemia is remarkable for the interrelated, sequential genetic events that deregulate two DNA-binding transcription factors characteristic of this disease (Fig. 1.5). Deregulation of *DUX4* is induced by rearrangement to strong enhancer elements, most commonly the immunoglobulin heavy chain (IGH) enhancer, which results in expression of a C-terminal truncated DUX4 protein that is not normally expressed in B cells [22, 23]. This truncated isoform of DUX4 then binds to an intragenic region of *ERG* resulting in transcriptional deregulation and expression of multiple aberrant coding and non-coding *ERG* isoforms and deletion of *ERG* in up to 70% of *DUX4*-rearranged cases [23]. One isoform is ERGalt, a C-terminal fragment which retains the DNA-binding and transactivating domain of ERG, that exerts a dominant negative effect and is transforming [23]. The deletions of *ERG* are commonly polyclonal [141], supporting a model in which an initiating rearrangement of *DUX4* results in gross transcriptional deregulation of ERG and primes the locus for RAG-mediated deletion. Loss of ERG activity, either through deletion and/or expression of ERGalt, cooperates with DUX4 deregulation in leukemogenesis [23, 141]. *DUX4* rearrangement is associated with a favorable outcome in children and adults, even with *IKZF1* deletion [142]. As clonal *ERG* deletions are

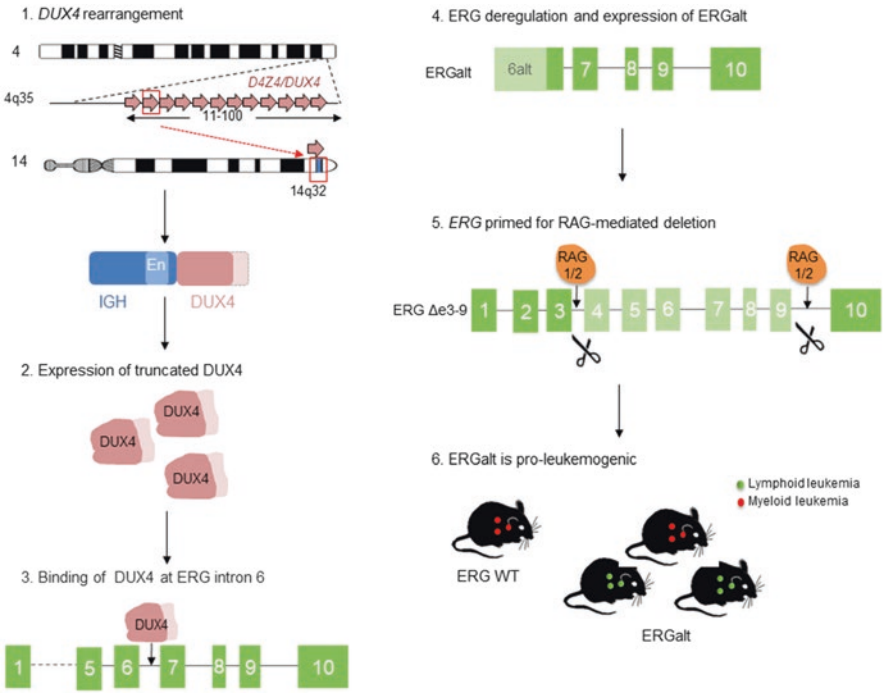

Fig. 1.5 Schema of the sequence of transcription factor alterations driving leukemogenesis in *DUX4*-rearranged ALL: rearrangement of DUX4 to strong enhancers results in deregulation of DUX4 expression with truncation of the C-terminus. This shortened form of DUX4 binds to intron 6 of *ERG,* resulting in gross transcriptional deregulation and expression of multiple coding, non-coding, and enhancer RNA species, including a C-terminal isoform initiated by a novel first exon, ERGalt. This aberrancy also permits deletion of *ERG* as a secondary event

not present in all *DUX4*-rearranged cases, the use of *ERG* deletion as a surrogate for this subtype, as is used in the definition of IKZF^{plus} [143], is suboptimal and should be avoided. Accurate identification of this favorable subtype of ALL requires identification of *DUX4* rearrangement (either directly or through identification of elevated *DUX4* expression) [23]. In this regard, detection of strong CD371 cell surface expression by flow cytometry might serve as a promising surrogate marker for this subtype [140].

MEF2D-Rearranged ALL

Rearrangement of *MEF2D* is associated with older age of onset and relatively inferior outcome due to early relapse [24, 26, 144, 145]. *MEF2D*-rearranged ALL is characterized by an aberrant immunophenotype (low or absent expression of CD10, high expression of CD38, and cytoplasmic μ-chain), mature B-ALL-like

morphology, and distinct expression profiles. The N-terminal of *MEF2D* is fused to several partner genes, retaining its DNA-binding domain [24, 144, 145]. High expression of MEF2D fusion protein is induced by evasion from miRNA-mediated degradation [146] and results in transcriptional activation of MEF2D targets [24]. Dysregulated MEF2D targets include overexpression of HDAC9, which confers therapeutic sensitivity to HDAC inhibitors such as panobinostat [24].

ZNF384-Rearranged ALL

ZNF384 rearrangement defines a distinct subtype of leukemia that can be diagnosed as BCP-ALL or B/myeloid mixed phenotype acute leukemia (MPAL) [147]. ZNF384 is rearranged as the C-terminal partner to multiple genes, including the histone acetyltransferases and transcriptional regulators EP300 and CREBBP, SWI/SNF proteins SMARCA2 and ARID1B, and others (TAF15, EWSR1, TCF3, NIPBL, and CLTC) [5, 6, 22, 24–26, 29, 147–154]. The most common are EP300-ZNF384 (particularly in BCP-ALL) and TCF3-ZNF384 (in both BCP-ALL and B/myeloid MPAL). In BCP-ALL, peak age of onset and prognosis vary by fusion partners: *EP300-ZNF384* (median age 11, excellent outcome) and *TCF3-ZNF384* (median age 5, frequent late relapse) [5, 148, 155]. In contrast, *ZNF384*-rearranged ALL shows uniformly distinct immunophenotype (weak CD10 and aberrant CD13 and/or CD33 expression) and gene expression profiles [147, 148]. The secondary genomic alterations and gene expression profiles of *ZNF384*-rearranged BCP-ALL and MPAL cases are similar, and both have lineage plasticity at diagnosis and relapse (lymphoid disease to myeloid disease and vice versa) [147]. Transplantation of purified lymphoid or myeloid subpopulations of cells from *ZNF384*-rearranged leukemia showed that each subpopulation could reconstitute the immunophenotypic diversity of the primary leukemia, indicating that this plasticity is inherent to all leukemic cells [69]. These data support the notion that *ZNF384*-rearranged cases should be treated uniformly rather than tailoring therapy according to predominant lineage. In this regard, *FLT3* overexpression without mutation is characteristic of *ZNF384*-rearranged leukemia and in anecdotal reports can be targeted with the multi-kinase inhibitor sunitinib [156]. Due to the propensity of *ZNF384*-rearranged ALL to change lineage, CD19-directed CAR-T cell therapy may fail due to CD19-negative escape [147, 157, 158].

PAX5-Driven BCP-ALL: PAX5alt and PAX5 P80R

The paired box DNA-binding transcription factor PAX5 is required for B-cell lineage commitment and differentiation. *PAX5* alterations are important in the pathogenesis of BCP-ALL as initiating or cooperating lesions. These include (1) disease initiating alterations (PAX5 rearrangements in chimeric fusion oncoproteins and the

P80R mutation [5, 16, 159–161], rearrangements/focal intragenic amplifications in *PAX5*-altered ALL [PAX5alt]) [5, 162], (2) secondary lesions (e.g., *PAX5* focal deletions in 30% of *ETV6-RUNX1* ALL [16, 77] and *PAX5* mutations in multiple subtypes), and (3) germline alterations that predispose to ALL [39]. In mouse models, *Pax5* heterozygosity cooperates with constitutive activation of the JAK-STAT pathway in the development of BCP-ALL, supporting its role as a haploinsufficient tumor suppressor [163].

PAX5alt is a subtype of BCP-ALL with similar leukemic cell gene expression profiles but diverse nature of underlying *PAX5* alterations. These include (1) cases with diverse (>20) *PAX5* rearrangements that typically preserve the N-terminal DNA-binding domain of PAX5, but with loss of the C-terminal transactivation domain, (2) cases with focal intragenic amplification of the *PAX5* DNA-binding paired domain (PAX5amp), and (3) cases with sequence mutations. Within this group, specific lesions are associated with variation in gene expression profile, for example, cases with *PAX5-ETV6* rearrangement, or compound heterozygosity for p.Arg38 and p.Arg140 mutations in the DNA-binding paired domain, have distinct gene expression profiles. PAX5alt is most common in children and the AYA population and is associated with intermediate outcome [5].

The PAX5 P80R subtype is characterized by the presence of the PAX5 P80R mutation with inactivation of the wild-type *PAX5* allele by deletion, loss-of-function mutation, or copy-neutral loss of heterozygosity [5, 159, 160]. Notably, heterozygous $Pax5^{P80R/+}$ knock-in mice develop transplantable BCP-ALL, with genetic inactivation of the wild-type *Pax5* allele [5]. Thus, biallelic *PAX5* alterations are a hallmark of this subtype, and sequence mutations of lymphoid transcription factors such as PAX5 P80R and IKZF1 N159Y (see below) may be initiating events in leukemogenesis. The prevalence of PAX5 P80R increases with age and is associated with intermediate to favorable prognosis [5, 159, 160]. Additional important cooperating lesions include structural rearrangements of chromosomal arms 9p and 20q, which associate with the presence of dic(9:20). Moreover, mutations in the Ras and JAK-STAT pathway members are particularly enriched, highlighting the potential for targeted therapies.

Other Subtypes of BCP-ALL

BCP-ALL with *NUTM1* rearrangements is a rare subtype observed exclusively in children [5, 6]. NUTM1 is a chromatin modifier, recruiting EP300 to increase local histone acetylation [164]. While the common partner, *BRD9-NUTM1*, is reported in BCP-ALL, *BRD4-NUTM1* is a hallmark of NUT midline carcinoma (NMC) and acts to repress differentiation in NMC by widespread repression of histone acetylation, indicating therapeutic approach with bromodomain and HDAC inhibitors. *NUTM1* is rearranged to multiple genes in BCP-ALL (and less commonly, T-ALL) [165] in addition to BRD9 [92, 166], including ACIN1 [24, 26, 92, 167, 168], AFF1 [6, 151], BPTF [165], CUX1 [24, 167], IKZF1 [6, 24, 27, 167],

SCL12A6 [6, 24, 167], and ZNF618 [6, 24, 29, 151], with emerging evidence that these fusions are enriched in non-*KMT2A*-rearranged BCR-ALL in infants [92, 168]. The potential for bromodomain inhibition as a therapeutic strategy has not yet been tested in *NUTM1*-rearranged BCP-ALL.

IKZF1 alterations, like *PAX5*, are also common across the spectrum of B-ALL (particularly in *BCR-ABL1*-positive, Ph-like, and *DUX4*-rearranged cases), but a specific mutation, IKZF1 p.Asn159Tyr, defines a subtype with gene expression profile [5, 6]. In this subtype, the non-mutated wild-type allele of *IKZF1* is retained, and most cases have concurrent gain of chromosome 21. Notably, this mutation is located at a residue that is critical for DNA binding of IKZF1 [169] and is also mutated in germline syndromes with immunodeficiency and autoimmunity [42, 170], although most commonly to serine but not tyrosine, suggesting genotype-phenotype variation of different IKZF1 mutations. The IKZF1 p.Asn159Tyr mutation induces misregulation of IKZF1 transcriptional activation, in part through distinctive nuclear mislocalization and enhanced intercellular adhesion [108].

Relapsed ALL

Genomic analyses of paired primary and relapsed ALL samples have revealed that these secondary mutations are acquired during disease progression with Darwinian patterns of selection, and highly branched clonal architectures, especially in early relapse (9–36 months) [8, 9, 78, 171–175]. Furthermore, chemotherapy of ALL has been postulated to induce bona fide drug resistance mutations including *NT5C2*, *PRPS1*, *NR3C1*, and *TP53* [9]. However, recent studies integrating genome sequencing of matched diagnosis and relapse samples, and xenografts propagated from these samples, coupled with drug sensitivity testing of the relapse fated clones have shown that relapse-fated subclones present at diagnosis commonly exhibit drug resistance prior to the administration of any therapy [174] (Fig. 1.6).

One of the representative relapse-specific somatic alterations is *CREBBP* alterations which occur in up to 20% of relapsed B-ALL and impair glucocorticoid sensitivity [60]. Early relapse is commonly associated with 6-MP resistance, as a result of *NT5C2* gain-of-function mutations [175–178], *PRPS1* mutations [179], and loss of *MSH6* [180]. *NT5C2* mutations confer resistance to purine analogs at the cost of impaired tumor cell growth and reduced leukemia-initiating cell activity [175]. While the development of NT5C2 inhibitors may be promising, several problems are anticipated such as the development of mutant specific inhibitors [176]. Importantly, *NT5C2* and *PRPS1* mutations are not detectable in primary samples even in a minor clone [7, 9, 175]. Other recurrent somatic alterations in relapsed ALL include mutations in [78] *SETD2*, *KDM6*, and *KMT2D* (*MLL2*) [9, 173, 181]. Tracking of these mutations as MRD may offer the opportunity to identify the relapse-fated clone early in disease evolution and modulate therapy accordingly to

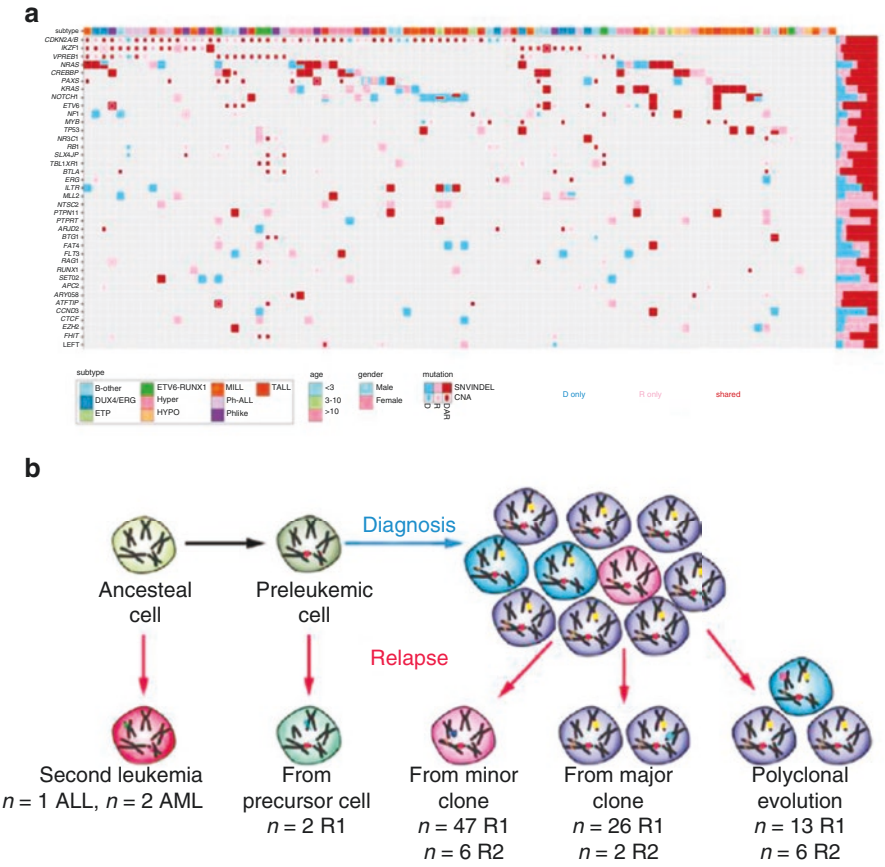

Fig. 1.6 (**a**) Oncoprint of the most common targets of mutation at relapse in childhood B- and T-ALL. (**b**) Patterns of clonal evolution in relapsed ALL. (Data taken from Waanders et al. [7])

circumvent relapse. Detailed, genome-wide analyses of large ALL cohorts have enabled several additional important observations: hypermutation becomes increasingly frequent during disease progression, is enriched in leukemic cells with mutations in mismatch repair genes and hypodiploidy, and results in a predicted increase in expressed neoantigen formation. Thus, strategies to promote autologous T cell reactivity may be efficacious in this setting. Secondly, careful analysis of the nature and structure of coding and non-coding sequence and structural variants has shown that most cases presumed to be second leukemias are indeed clonally related to the primary tumor, including cases with lineage shift/switch, indicating relapse from an ancestral, pre-diagnosis clone [7] (Fig. 1.6b). These observations confirm hypotheses from SNP array analyses of relapsed ALL [78] and are of therapeutic importance for disease monitoring and selection of therapy.

Summary

Genomic analyses have transformed our understanding of the molecular basis of BCP-ALL, in terms of identification of new subtypes and dysregulated pathways associated with therapeutic targets. Many clinically important alterations are not evident using conventional cytogenetic and molecular approaches, and optimal ALL diagnosis requires next-generation sequencing, with RNA-seq capturing the most relevant information required for risk stratification, disease monitoring, and the development of precision medicine approaches [136]. While clinical implementation of genome and transcriptome sequencing is not trivial, it is now clearly apparent that targeted molecular approaches such as fusion-specific PCR and exome/gene panel capture sequencing are not optimal as they do not capture the diversity of genomic alterations in ALL. Moreover, integrated genome, exome and transcriptome sequencing has been shown to have excellent sensitivity and specificity in detection of the various driver alterations in pediatric cancer [182]. Even if sequencing is not available, several key alterations can be detected by alternative approaches, such as flow cytometry for *CRLF2* (which correlates well with *CRLF2* overexpression) and FISH assays for gene rearrangements in Ph-like ALL.

These genomic discoveries are partly responsible for a wave of new therapeutic approaches entering the clinic in BCP-ALL including small molecules (TKI, BCL2 inhibitors, MEK inhibitors), antibody-based therapy (blinatumomab, inotuzumab), and cellular immunotherapy. Future challenges and opportunities include (1) determining the tumor intrinsic and extrinsic determinants of response in the era of targeted therapies and immunotherapy, (2) developing efficacious approaches to directly target transcription factor alterations that underlie over 50% of BCP-ALL, and (3) integrating genomic and functional genomic approaches to identify therapeutic vulnerabilities both in the research and clinical setting.

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