**Review Article**

**Advances in biology of acute lymphoblastic leukemia (ALL) and therapeutic implications**

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**Abstract:** Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer and also occurs in adults. Although the outcomes of multi-agent chemotherapy regimens have greatly improved, high toxicity and relapses in many patients necessitate the development of novel therapeutic approaches. Advances in molecular profiling and cytogenetics have identified a broad range of genetic abnormalities, including gene mutations, chromosome translocations and aneuploidy, which has provided a more comprehensive understanding of the biology and pathogenesis of ALL. This understanding has also led to new targeted therapeutic approaches, including the use of selective small molecule inhibitors, nucleic acid-based therapies and immune-based therapies mediated by specific monoclonal antibodies and cellular immunotherapy, which are poised to revolutionize the treatment of various ALL subtypes. The main focus of this review is to highlight the latest advances in ALL biology, including the identification of prognostic factors and putative therapeutic targets. We also review the current status of, and ongoing progress in, the development of targeted therapies for ALL.

**Keywords:** Acute lymphoblastic leukemia, leukemia, cytogenetics, molecular subtypes, targeted therapy, immunotherapy

**Introduction**

Acute lymphoblastic leukemia (ALL) is a malignancy of hematopoietic stem cells that originates from B- and T-lineage lymphoid precursors and is driven by a spectrum of genetic aberrations including mutations, chromosome translocations and aneuploidy in genes involved in the development of lymphoid cells and regulation of cell cycle progression [1]. ALL is the most common childhood cancer (Figure 1) with 5-year survival rates of about 90% in children [2-4], and 75-85% in adolescents and young adults. The outcomes in older adults are inferior, with overall survival rates of 35-55% in middle age adults and under 30% in those over age 60 [5-8].

The standard front-line therapeutic approach for treatment of pediatric and adult ALL patients consists of multiagent chemotherapy regimens, followed by hematopoietic stem cell transplantation (HSCT) in high-risk groups [5, 9-12] Although there have been major advances in the treatment of ALL that has resulted in improved overall survival (OS), significant drawbacks of conventional therapies persist, including severe toxicities and the development of chemoresistance leading to relapse. Accordingly the development of novel therapeutic strategies is of utmost importance to improve treatment outcomes [2, 13-15]. This requires a thorough understanding of the biology of this heterogeneous group of diseases.

Both B-ALL and T-ALL subtypes harbor distinct groups of chromosomal rearrangements and sequence mutations affecting lymphoid development, tumor suppression, cytokine receptors, and kinase and other signaling pathways [16] (Table 1). Differential gene expression patterns in various ALL subtypes have been identified by a number of techniques, including polymerase chain reaction (PCR), genome-wide sequencing, single nucleotide polymorphism (SNP) array analysis and microarrays. These have provided valuable insights into the biology and pathogenesis of ALL, permitting differential-
In this review, advances in the understanding of ALL biology will be discussed. We will then summarize recent studies evaluating molecular and immune-based targeted therapies, and discuss potential novel therapeutic strategies based on our understanding of disease biology.

Genetic subsets of B-cell lymphoblastic leukemia/lymphoma (B-ALL)

**BCR-ABL1**+ ALL

The t(9;22)(q34;q11) translocation, associated with the Philadelphia (Ph) chromosome, is the most common cytogenetic abnormality in adult ALL. The frequency increases with age, occurring in 2-5% of pediatric ALL cases, and 20% of young adults and 30-40% of older adult patients [18, 19]. The Ph chromosome encodes the BCR-ABL1 fusion oncogenic protein with constitutively active tyrosine kinase activity. The major breakpoint, which creates a 210-kDa protein, is detected in 24-50% of adult Ph+ ALL [20, 21], but is rare in childhood Ph+ ALL [22]. The minor breakpoint, which encodes a 190-kDa protein, is more prevalent and can be identified in 50-77% of adult Ph+ ALL [18, 21] and more than 90% of pediatric cases [23].

Upregulation of BCR-ABL1 fusion gene leads to activation of multiple signaling pathways such as MAPK, Ras, NF-kB, c-Myc, PI-3 kinase, and JAK-STAT [24]. It also promotes proliferation of lymphoblasts by the alteration of pro- and anti-apoptotic proteins [13]. One of the main genetic alterations in BCR-ABL1 positive patients is the mutations and deletions in *IKZF1* gene, encoding for the transcription factor Ikaros which is associated with the unfavorable outcomes and poor prognosis in both Ph+ and Ph- ALL [17, 25, 26]. One study on 83 Ph+ patients demonstrated that 10% lacked *IKZF1* due to chromosome 7 monosomy. Moreover, 63% of patients had a 7p12 deletion of *IKZF1* with different patterns. The most frequent deletions were the loss of exons 4 to 7, detected in 37% of patients, and the loss of exons 2 to 7, detected in 20%. This type of abnormality led to shorter disease-free survival (DFS) compared to patients with *IKZF1* wild type (10 vs. 32 months, P=0.02) [27]. In addition, the time of cumulative incidence of relapse (CIR) was significantly shorter in patients with *IKZF1* deletions versus patients
### Table 1. Genetic aberrations and targeting agents associated with different ALL subtypes

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[26, 88, 89]
without this aberration (10.1 vs. 56.1 months, respectively; P=0.001) [27].

**BCR-ABL** positive ALL has been associated with an adverse prognosis and is virtually incurable with chemotherapy alone. The advent of **BCR-ABL1**-directed tyrosine kinase inhibitors (TKIs) has significantly improved the response rates and overall survival rates, particularly when used in combination with chemotherapy, although relapse remains a problem [28, 29].

**BCR-ABL1-like (Ph-like) B-ALL**

This high-risk subtype of ALL was first detected by Mullighan and colleagues from the Children’s Oncology Group (COG) and St. Jude Children’s Research Hospital, (SJCRH) and den Boer and colleagues from the Netherlands in 2009. This subtype is characterized by a gene expression pattern similar to that of the **BCR-ABL1** positive ALL cases [17, 30, 31], but without **BCR-ABL1** expression. This so-called Ph-like ALL is more prevalent in adolescents and young adults with B-ALL, comprising about 15% of pediatric B-ALL patients age 12-18 and 20-25% of young adult B-ALL cases [15, 32-35]. It has been associated with an adverse response to induction chemotherapy, a higher frequency of persistent minimal residual disease (MRD) and poor survival [25, 32, 36]. It is the most frequently occurring pediatric and young adult ALL subtype associated with an unfavorable prognosis, with a 5-year disease free survival of about 60% [17, 32].

Different types of genomic alterations have been identified in Ph-like ALL, which are involved in the activation of kinase and cytokine receptor signaling. In addition, more than 80% of Ph-like ALL cases have deletions and/or mutations in genes involved in B-cell development including **IKZF1** (the most frequent aberration), paired box 5 (**PAX5**), **EBF1**, transcription factor 3 (**TCF3**) and **VPREB1** which encodes the immunoglobulin iota chain [9, 37].

Translocations of **CRLF2** such as **P2Ry8-CRLF2** fusion (detectable by RT-PCR) or **IGH-CRLF2** rearrangements (detectable by FISH), or translocations resulting in truncation and activation of the erythropoietin receptor (**EPOR**) involving four partner genes (**IGH**, **IGK**, **LAIR1** and **THADA**), are the common genomic characteristics of Ph-like ALL [38-42]. It has been observed that **EPOR** rearrangements, overexpression of **CRLF2** (detectable by flow cytometry), translocations and point mutations involved in activating JAK proteins, rare deletions of **SH2B3** (encodes the JAK2-negative regulator **LNK**) and activation mutations of **IL7R** result in the constitutive activation of JAK-STAT signaling, which explain the resemblance of kinase activity profiles to those of Ph- ALL [25]. B-ALL children with Down syndrome (30-50% of cases) are more likely to have CRLF2 translocations along with point mutations in **JAK** genes (**JAK1** (V617F), **JAK2** (R683G), and **JAK3**) [15, 38-40, 42-46]. Upregulation of the thymocyte stromal lymphopoietin receptor (TSLPR) encoded by **CRLF2** gene can be detected by flow cytometry in leukemic cells. This receptor, which is induced by the cytokine TSLP, is involved in the activation of numerous signaling pathways, including PI3K/AKT/mTOR and JAK/STAT, that are associated with aberrant proliferation and survival of ALL blasts. Therapeutic approaches targeting PI3K/mTOR, JAK-STAT, and BCL2 signaling pathways have been effective in preclinical models. With the development of JAK inhibitors, JAK mutations can be considered as potential targets for treatment of this subgroup of ALL patients [31, 47-50].

Another Ph-like-associated genetic aberration involves ABL-class fusion genes, including translocations of **ABL1** (with partners other than **BCR**), **ABL2**, **PDGFRB** and **CSF1R** (encoding the macrophage colony-stimulating factor receptor) which have been observed in cases with translocations in tyrosine kinases genes. These types of abnormalities have been detected in about 3-5% of childhood ALL patients and 2-3% of adult ALL patients [31, 32, 50]. Preclinical studies suggest that TKIs, including imatinib and dasatinib, may represent effective treatment options for the Ph-like ALL patients with ABL-class fusions [28, 31, 32, 50]. In addition, cases with **ETV6-JAK2** and **BCR-JAK2** rearrangements are considered as Ph-like variants. It has also been suggested that TKI therapy could be very effective for patients with **EBF1-PDGFRB** translocations [15, 25, 28, 31, 32, 50, 51].

Other rearrangements involving kinase genes such as **ETV6-NTRK3** fusion, **FGFR1**, **TYK2**, **IL2RB**, **BLNK**, **DGKH**, **LYN**, **PTK2B**, **FLT3** and **RAS** subfamily genes are identified in Ph-like ALL cases [15, 32, 33, 52-55]. Patients with
tyrosine receptor kinase (TRK) fusions are sensitive to TRK inhibitors [56] and ponatinib, which is a kinase inhibitor of FGFR1 can be considered for cases with FGFR1 fusion [31].

**MLL rearrangements**

The t(4;11)(q21;q23) translocation, resulting in the MLL-AF4 fusion gene, is the most frequently occurring aberration in infants with ALL. This abnormality is also detected in 3-7% of adult ALL cases and confers a poor prognosis [19, 57]. Other MLL gene rearrangements can be seen, including MLL-AF10 in t(10;11), MLL-AF9 in t(9;11) and MLL-ENL in t(11;19). The MLL gene, also called KMT2A, codes for a histone methyltransferase that regulates gene transcription. Gene expression profiling has identified distinct MLL-associated gene signatures in ALL [58].

**Hypodiploid B-ALL**

This group, characterized by having less than 44 chromosomes, composes 2-3% of ALL patients, and is associated with poor outcomes [59]. It has different subtypes with distinct genetic alterations: Low hypodiploid ALL, with 32 to 39 chromosomes, is associated with several abnormalities, including TP53 mutations (in 91% of patients), RB1 (41% of cases) and IKZF2 alterations (53% of cases). Another subset is the near-haploid ALL with 24 to 31 chromosomes, associated with aberrations such as IKZF3 alterations (13% of patients) and mutations involved in Ras signaling pathway activation (71% of cases). In pediatric hypodiploid ALL cases, aberrations detected by next-generation sequencing (NGS) include RAS signaling (NRAS, Kras, FLT3 and NFI), receptor tyrosine kinases (70% of near haploid cases), IKZF2 and TP53 mutations% of low hypodiploid cases) [26, 60].

As Ras and PI3K signaling pathways are activated in both near haploid and low-hypodiploid subtypes, these pathways can be considered as potential targets in the treatment of hypodiploid ALL [9, 60, 61].

**B-ALL with intrachromosomal amplification of chromosome 21 (iAMP21)**

The amplification of a portion of chromosome 21, which can be recognized by FISH using the RUNX1 gene probe, is the main characteristic of this subtype of ALL. The result of metaphase FISH reveals ≥5 or ≥3 extra copies of genes on a single anomalous chromosome 21. This subtype rarely occurs in adults, but accounts for about 2% of childhood ALL with a higher incidence rate in older children, and patients typically have low WBC counts and a poor prognosis. Patients with this abnormality have been categorized as a standard-risk group; however, studies have revealed a shorter event-free survival (EFS) and overall survival upon treatment based on the standard-risk protocols [11, 62, 63]. More intensified chemotherapy regimens appear to improve the poor prognosis of this entity [64].

**B-ALL with DUX4 and ERG deregulation**

This recently identified subgroup of B-ALL accounts for about 7% patients. It is characterized by deregulation of the double homeobox 4 gene (DUX4) and the ETS transcription factor gene (ERG). In this subtype, the expression of a truncated isoform of DUX4 has been identified in the B-cell lineage as a result of DUX4 rearrangement, while DUX4 cannot be expressed in normal B cells. This isoform has the ability to bind to the ERG intron 6, leading to the deregulation of ERG and expression of a noncanonical first exon and transcript, ERGalt. This results in the inhibition of the transcriptional activity of wild-type ERG and plays an important role in the early initiation of leukemogenesis [16, 64-67]. In general, DUX4/ERG deregulated ALL is associated with a good response; however, the presence of other genetic aberrations, including IKZF1 deletions along with DUX4/ERG deregulation, is associated with an unfavorable outcome [16, 68, 69].

**Other molecular aberrations in B-ALL**

Hyperdiploidy occurs in about 25% of childhood ALL and has a favorable prognosis. These cases have been associated with an approximately ten-fold over-expression of the SH3BP5 gene, which encodes SH3-binding protein 5 located on chromosome 3p24. This protein may be involved in transferring signals from Bruton’s tyrosine kinase (BTK) receptor, suggesting this receptor or its downstream signaling pathways may constitute potential therapeutic targets [17, 70]. Disruptions in CREBBP gene have also been identified in relapsed hyperdiploid cases [71].

The t(12;21)(p13;q22) translocation, resulting in the TEL-AML1 (ETV6-RUNX1) fusion gene,
accounts for 20-25% of childhood B-ALL. The erythropoietin receptor, which is found in myeloid lineage progenitor cells, can be expressed ~7-fold higher in ETV6-RUNX1-positive cases. The upregulation of this gene implies that either ETV6-RUNX1-positive cases can express myeloid associated markers, or this gene might have non-erythropoietin related functions [17, 72-74].

Translocation (1;19)(q23;p13.3), occurring in adult B-ALL subtype, generates a chimeric E2A-PBX1 gene, is seen in ~5% of cases, and has been associated with poor outcome in some studies [19, 75].

About 80% of ALL cases have epigenetic alterations, such as promoter hypermethylation of tumor-suppressor genes, leading to inactivation of tumor-suppressor genes or hypomethylation of oncogenes. Evaluation of epigenetic changes by the use of methylation-specific PCR in ALL is of the utmost importance because different steps involved in this process can be potential targets for many current chemotherapeutic agents including DNA methyltransferase inhibitors [5, 76-81].

Genetic alterations in T-cell acute lymphoblastic leukemia/lymphoma (T-ALL)

T-ALL, comprising 10-15% of ALL cases, has been associated with a number of genetic lesions [61, 82]. The most common T-cell receptor (TCR) breakpoint is at 14q11.2, which are the alpha and delta T-cell receptor loci [TRA and TRD]. In adult T-ALL, HOX11 gene overexpression resulting from t(10;14)(q24;q11.2) is the most frequent TCR rearrangement [19, 83]. However, in childhood T-ALL, t(1;14)(p32;q11.2) leading to SCL (also called TAL1 or TCL5) overexpression (identified in about 3% of cases) and the SIL-SCL deletion (chromosome 1 deletion juxtaposing SCL and SIL genes, which is recognized in 6-26% of cases) are the most common aberrations [83]. Moreover, upregulation of HOX11L2, resulting from a cryptic t(5;14) (q35;q32) translocation, is detected in 20-30% of pediatric T-ALL [84, 85]. The TCR gene can also be rearranged to other fusion partners such as TAL2, LYL1, OLG2, LMO1, LMO2, NXXZ-1, NXXZ-2, NXXZ-5, HOXA genes, MYC, and MYB [16, 86]. In addition, in-frame fusion genes encoding oncogenic proteins such as PICALM-MLLT10, MLL gene rearrangements, SET-NUP214 fusion, EML1-ABL1, ETV6-ABL1 and NUP214-ABL1 fusion formed on episomes, can be caused by chromosomal rearrangements [26].

Common sequence mutations in T-ALL detected by re-sequencing and NGS consist of those in translocation-associated Notch homolog 1 (NOTCH1) (detectable in >60% of cases), FBW7 (detectable in >20% of cases), PTPN2, and MYB, genes involved in the RAS/PI3K/AKT (NRAS, KRAS, and PTEN) and JAK-STAT (JAK1, JAK3, IL7R, and STAT5B) pathways, in transcription regulators (BCL11B, LEF1, WT1, and ZEB2), in epigenetic regulators (SUZ12, PHF6, EZH2, TET2, H3F3A, and KDM6A) and in genes associated with the maturation of mRNA and activity of ribosomes (CNOT3, RPL5, and RPL10) [87, 88]. Cases with mutations in NOTCH1, a gene expressing a transmembrane receptor involved in T-cell development, and FBW7 genes are considered as low-risk patients, while patients who lack these mutations or have mutations involving RAS/PTEN are defined as high-risk cases [89-91]. Moreover, JAK1 mutations are associated with poor prognosis in T-ALL [92, 93].

Early T-precursor (ETP) ALL has recently been identified as a distinct subtype. This heterogeneous subtype shows limited early T-cell differentiation phenotype and has some myeloid and hematopoietic stem cell associated genetic and immunophenotypic features. This subset overexpresses myeloid transcription factors such as CEBPA, CEBPB, CEBPD and a group of micro-RNAs, including miR-221, miR-222 and miR-223 [26, 94-98].

Studies of ETP ALL blasts have revealed the presence of mutations in several cellular pathways including Ras, kinase and cytokine receptor signaling genes (NRAS, KRAS, IL7R, JAK1, JAK3, NF1, PTPN11, and SH2B3), myeloid-associated genes (FLT3, DNMT3A, IDH1, IDH2 and ETV6), genes involved in lymphoid and hematopoietic development (RUNX1, IKZF1, GATA3, and EP300) and epigenetic regulators with loss-of-function mutations (EZH2, SUZ12, EED, and SETD2). In this subtype, common gene mutations in typical T-ALL such as mutations in NOTCH1 (detectable in >60% of T-ALL cases) or CDKN1/2 are rarely observed [97]. In general, this subgroup has an unfavorable
response to standard therapy; however, current risk adapted therapy may improve the therapeutic outcome [99, 100]. As JAK-STAT and PRC2 pathways are active in ETP-ALL, JAK inhibitors and chromatin-modifying agents may be potentially beneficial as therapeutic options [101].

**Targeted therapeutic approaches**

**Small molecule inhibitors**

**BCR-ABL1-directed TKIs:** This class of agents has revolutionized the treatment of BCR-ABL+ B-ALL. When used as single agents combined with corticosteroids, imatinib or dasatinib can produce complete responses in virtually 100% of cases, but usually leads to the rapid emergence of resistant clones, most commonly due to point mutations within the *BCR-ABL* kinase domain, resulting in relapse [28, 29]. Other mechanisms of resistance include increased drug efflux and recruitment of alternative active cell signaling pathways and kinases including Src-family kinases leading to cell proliferation and inhibition of apoptosis [102, 103].

These agents have also resulted in significant improvement in treatment outcomes when used in combination with conventional chemotherapy [5, 11, 13]. Imatinib mesylate combined with chemotherapy dramatically increased the 3-year EFS rate from 35% to 80% in pediatric Ph+ ALL compared with chemotherapy alone [104]. The COG AALL0031 study, using the combination of imatinib and intensive chemotherapy, reported equivalent or better 5-year DFS (70%) compared to those who received allogeneic HSCT from related or unrelated donors (65% and 59%, respectively) [105]. Children with refractory Ph+ B-ALL demonstrated remissions following treatment with imatinib and dasatinib in combination with chemotherapy [106, 107]. Dasatinib and nilotinib, which are more potent and have activity in some imatinib-resistant clones, have produced remissions in some cases that have relapsed on imatinib therapy [5, 108, 109].

A number of studies have evaluated the use of imatinib, dasatinib and nilotinib, in combination with chemotherapy in adults with Ph+ B-ALL [110-112], which was recently reviewed [113]. Although results are superior to previous studies with chemotherapy alone, relapse due to the emergence of resistant clones remains a problem. The use of dasatinib has been associated with a high frequency of relapse with resistant T315I mutations [112]. More recently, the addition of ponatinib, which is active against resistant T315I clones, to chemotherapy has produced encouraging 3-year EFS in adults with Ph+ ALL [114, 115], and a number of further studies with this agent are either in progress or are being planned.

In Ph-like ALL patients with ABL-class fusions, adding imatinib or dasatinib to combination chemotherapy regimens has resulted in the induction of remissions and clearance of minimal residual disease (MRD) in isolated cases [116-119]. Furthermore, dasatinib was effective in a pediatric Ph-like ALL case with persistent post-transplant MRD [120]. A Phase II clinical trial by MDACC (NCT02420717) is evaluating the use of either ruxolitinib or dasatinib, based on molecular profiling, as initial mono-therapy, followed by the addition of Hyper-CVAD chemotherapy [31, 121]. A COG AALL1131 trial (NCT02883049) is currently evaluating the efficacy of dasatinib in Ph-like ALL patients with ABL-class mutations.

**PI3K/mTOR inhibitors:** Several small molecule inhibitors have been developed to efficiently target different aberrantly activated signaling pathways [9, 13, 14]. One of the constitutive activated signaling pathways in B- and T-ALL is the PI3K/Akt/mTOR pathway which can promote drug resistance, cell proliferation and metabolism. Mammalian target of rapamycin (mTOR) which is a downstream target of Akt, functions as a serine/threonine kinase and comprises a core component of two protein complexes: mTORC1 and mTORC2 [2, 9]. mTOR kinase activity can be inhibited by small molecules such as everolimus, temsirolimus, and sirolimus [2, 9]. Studies reported that everolimus induced apoptosis in B-ALL cell lines and reduced enzyme phosphorylation in Akt and mTOR signaling [122-124]. In addition, a Phase I/II trial in relapsed childhood ALL treated with everolimus in combination with hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) reported a CR rate of 25% [125]. Temsirolimus is being evaluated in combination with intensive re-induction therapy through a phase I COG study ADVL1114 in relapsed ALL cases (NCT014-
While mutations of FMS-like FLT3 (MDACC) trial (NCT02420717) in adult and adolescent Ph-like patients with JAK pathway lesions. In this trial, patients receive 3 weeks of ruxolitinib monotherapy followed by multi-agent chemotherapy for patients with an incomplete response. A combination of ruxolitinib, dasatinib and dexamethasone is being assessed in a Phase I trial (NCT02494882) in older patients with newly diagnosed Ph+ ALL. A recent preclinical study has suggested that targeting STAT5 directly may have therapeutic potential in both BCR-ABL+ and BCR-ABL-like B-ALL [242].

MEK inhibitors: MAPK/ERK pathway deregulation has been observed in hematologic malignancies as well. 40% of children with relapsed ALL and 6% of Ph-like ALL patients contain mutations in KRAS and NRAS genes and MAPK signaling pathway [2, 13, 31, 32, 139]. Selumetinib, which is an inhibitor of mitogen-activated protein kinase (MEK), has been effective in RAS mutated ALL cell line models [2, 139]. Moreover, the cytotoxic effect of concomitant inhibition of MEK and PI3K/AKT pathways was revealed in T-ALL cells [140]. Pimasertib and trametinib are other inhibitors of MEK1/2; however, their effects have not been clinically evaluated in ALL [13]. A preclinical study investigated the efficacy of trametinib alone and in combination with BCL-2 inhibitors, ABT-199 and ABT-263, in different B-ALL cell lines and primary B-ALL patient cells. The results demonstrated that trametinib alone could modestly affect the cell viability; however, a combination of MEK and BCL-2 inhibitors showed a synergistic effect and significantly suppressed proliferation and induced apoptosis in B-ALL cells through a MEK/ERK signaling-dependent mechanism mediated by the pro-apoptotic factor BIM [6].

JAK/STAT inhibitors: In Ph-like ALL with JAK/STAT pathway alterations, including CRLF2 rearrangements, JAK mutations, JAK2 fusions and EPOK rearrangements, using ruxolitinib as a selective JAK inhibitor, USP9X inhibitors, and givinostat as a histone deacetylase inhibitor may constitute potential therapeutic strategies [31, 48, 135-138]. Currently, two clinical trials, COG AALL1521 Phase II trial (NCT02723994) and a subset of SJCRH Total XVII, are evaluating the efficacy of adding ruxolitinib to multi-agent chemotherapy in Ph-like patients with JAK pathway lesions. Moreover, ruxolitinib in combination with chemotherapy is also being investigated by the MD Anderson Cancer Center (MDACC) trial (NCT02420717) in adult and adolescent Ph-like patients with JAK pathway lesions. In this trial, patients receive 3 weeks of ruxolitinib monotherapy followed by multi-agent chemotherapy for patients with an incomplete response. A combination of ruxolitinib, dasatinib and dexamethasone is being assessed in a Phase I trial (NCT02494882) in older patients with newly diagnosed Ph+ ALL. A recent preclinical study has suggested that targeting STAT5 directly may have therapeutic potential in both BCR-ABL+ and BCR-ABL-like B-ALL [242].

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**PARP inhibitors:** Veliparib is an inhibitor of poly(ADP-ribose) polymerase (PARP), a protein involved in DNA repair, genomic stability, and programmed cell death. It was observed that veliparib could inhibit cell proliferation by apoptosis induction in human T-ALL cell line models [13, 143]. Multiple clinical trials are now evaluating veliparib in combination with other inhibitors and chemotherapeutic agents in various ALL subgroups including a Phase I, multi-center trial (NCT01139970) investigating veliparib and temozolomide, another Phase I study (NCT00588991) assessing veliparib and topotecan with or without carboplatin and a Phase I/II trial (NCT01326702) evaluating veliparib, bendamustine, and rituximab combination.

**Apoptosis inhibitors:** YM155 is a small molecule inhibitor of survivin [144], which is a member of the Inhibitor of Apoptosis Protein (IAP) family involved in the cancer development. It was originally suggested to act as a transcriptional suppressor of survivin, but recent evidence is suggesting a multitude of activities in its mechanism of action [145]. Since survivin overexpression was observed in relapsed childhood ALL, [146] the efficacy of YM155 in combination with dasatinib was evaluated in various subtypes of primary ALL samples and ALL cell lines including Ph+ ALL. The results revealed significant sensitivity of ALL cells to YM155 treatment. In addition to its action on downregulation of survivin expression, its activation of the DNA damage pathway is leading to apoptosis induction and chemosensitivity in ALL cells [147].

The B cell lymphoma-2 (BCL-2) proteins are involved in the cell death regulation and can prevent apoptosis through binding to anti-apoptotic proteins. BCL-2 inhibition by selective inhibitors can induce apoptosis in malignant cells. Navitoclax (ABT-263) is believed to inhibit the binding of BCL-2 protein to apoptotic effectors Bax and Bak proteins [148]. It has been evaluated through preclinical xenograft models of B-ALL, T-ALL and MLL-mutated ALL and the results were promising [13, 149]. However, navitoclax could not be evaluated in childhood ALL due to dose-limiting thrombocytopenia. Venetoclax (ABT-199) is also a second generation of BCL-2 inhibitor which showed promising activity in xenograft models of ALL [149]. One study reported that high level of BCL-2 expression induced by MLL/AF4 fusion protein was significantly decreased by venetoclax in MLL-rearranged ALL cells and also showed a synergistic effect of venetoclax and standard-induction-type chemotherapeutic agents on both MLL-rearranged cell lines and xenograft models [150]. A Phase I study has opened evaluating venetoclax and navitoclax in combination with chemotherapy in children and adults with relapsed B-ALL (NCT03181126).

Moreover, BCL-2 can be upregulated through the tyrosine kinase 2 (TYK2) signaling and its downstream effector phospho-STAT1. It has been observed that inhibition of heat shock protein 90 (HSP90) by its specific small molecule inhibitor NVP-AUY922 (AUY922) resulted in the blockage of TYK2 signaling and downregulation of phospho-STAT1 and BCL-2 in T-ALL cells. In addition, pro-apoptotic proteins BIM and BAD were upregulated by AUY922 which in combination with BCL-2 downregulation led to induction of apoptosis in T-ALL cells [151].

**Proteasome inhibitors:** Another aberration in ALL is the deregulation of nuclear factor kappa-B (NF-κB), a transcription factor involved in the expression of oncogenes leading to protecting cells from apoptosis. It was observed that NF-κB is constitutively activated in different malignancies; inhibition of the activity of NF-κB by proteasome inhibitors including bortezomib, carfilzomib, and ixazomib can induce apoptosis in tumor cells or increase the sensitivity of cells to anti-tumor agents [2, 13, 152].

Bortezomib, which inhibits the 26S proteasome reversibly, has been effective in combination with other chemotherapeutics such as dexamethasone, asparaginase, vincristine, doxorubicin, and cytarabine in pre-clinical studies of ALL [153-156]. Different clinical trials have investigated bortezomib in combination with re-induction chemotherapy in ALL patients. A Phase I/II trial of the TACL recruited children with relapsed ALL, with a response rate of 73% [155]; a Phase II AALL07P1 COG study in relapsed B-ALL (61 cases) and T-ALL (17 cases) patients showed CR rates of 69% and 65%, respectively [157]. A Phase II study (NCT01769209) in adult ALL patients, another Phase II trial (NCT02535806) in childhood ALL patients, and a randomized Phase III COG trial (AALL1231) recruiting newly diagnosed young T-ALL patients or stage II-IV T-ALL cases receiv-
ing chemotherapy with or without bortezomib (NCT02112916), are investigating the effectiveness of bortezomib. The efficacy of bortezomib in combination with other inhibitors including HDAC inhibitors which target epigenetic-related abnormalities has been evaluated; however, the results are yet to be published.

Carfilzomib, a more potent proteasome inhibitor with higher specificity [13], was investigated by a Phase I trial (NCT01137747) for AML and ALL cases in 2014 but the outcomes were not published. Moreover, a Phase I study (NCT-02293109) is evaluating the optimal tolerable dose of carfilzomib in combination with hyper-CVAD, and another study (NCT02228772) is exploring the tolerability and safety of carfilzomib combined with re-induction chemotherapy in patients with refractory ALL. Ixazomib, another proteasome inhibitor, is under investigation in combination with chemotherapy by a Phase I trial (NCT02228772) for ALL patients.

**Inhibitors of epigenetic modifications:** Epigenetic abnormalities have been also considered a significant source of transformations in ALL. Deacetylation of lysine residue on histones mediated by histone deacetylase inhibitors (HDACs) is an epigenetic abnormality leading to silencing of the transcription of tumor suppressor genes [152]. Therefore, inhibition of HDACs can stop cell proliferation and induce programmed cell death. Belinostat, vorinostat, and panobinostat are HDAC inhibitors, [13] which have been investigated through several clinical trials in ALL patients. Relapsed ALL patients were treated in a Phase II study (NCT01483690) with a combination of vorinostat, decitabine and chemotherapy; however, high toxicity resulted in the termination of the
study. A Phase I trial (NCT00348985) investigated the effect of the combination of belinostat and bortezomib on adult patients with refractory T-ALL, but the outcomes were not published. The effectiveness of panobinostat in combination with bortezomib, liposomal vincristine and salvage therapy is being evaluated in a Phase II study (NCT02518750) in children and young adults with refractory T-ALL.

DNA methylation of cytosine-phosphate dinucleotides (CpG) islands by DNA methyltransferases (DNMTs) is another epigenetic silencing mechanism resulting in suppression of tumor suppressor genes. Cytosine analogs, including azacitidine and decitabine, are hypomethylating agents which inhibit DNMTs through incorporation into DNA and RNA and induction of apoptosis in abnormal hematopoietic cells. Azacitidine and decitabine with and without HDAC inhibitors demonstrated promising results in relapsed ALL cell line models [158]. Furthermore, patients with refractory AML and ALL were treated in a Phase I TACL trial with azacitidine in combination with chemotherapy and was well-tolerated. However, the high toxicity associated with the combination of decitabine, vorinostat and chemotherapy in children with relapsed ALL caused termination of the pilot TACL study (NCT01483690).

Antibody-based immunotherapy

Recently, several mAbs have been developed to target specific markers mostly expressed on B-cell lymphoblasts including CD20, CD19, CD22 and CD52 (Figure 2). In addition, cytotoxic T-cell responses can be activated by new immunotherapeutic approaches [2, 9, 13].

Anti-CD20 mAbs: CD20, which is expressed on the surface of 30-50% of B cell lymphoblasts, can be targeted by rituximab, ofatumumab, and obinutuzumab [13, 159] (Figure 2). Rituximab is a chimeric anti-CD20 mAb which was approved in 1997 for non-Hodgkin lymphoma [160]. Binding of rituximab to CD20 removes B cells from circulation through complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), and apoptosis induction (Figure 2). It has been observed that rituximab improved the efficacy of induction and consolidation chemotherapy and increased the CR and OS in CD20+ ALL patients [13, 159]. In the GRAALL-2005 study, rituximab was combined with chemotherapy and improved 2-year EFS and 2-year OS (65% vs. 52%; P=0.038 and 74% vs. 63%; P=0.018, respectively) in patients with Ph-, CD20+ B-ALL [161, 162].

Ofatumumab, which was approved in 2009 for CLL [163], is a humanized type I mAb, and obinutuzumab (GA101) is a glycoengineered humanized type II anti-CD20 mAb [164, 165] (Figure 2). Ofatumumab targets the small-loop epitope on CD20 and it has higher complement-dependent cytotoxicity compared to rituximab, while obinutuzumab has lower complement- and antibody-dependent cytotoxicity and little direct cytotoxicity [164, 165]. Ofatumumab is being investigated in a Phase II clinical trial (NCT02419469) in combination with the augmented BFM in B-ALL patients. In another Phase II trial, ofatumumab in combination with hyper-CVAD increased the 3-year continuous CR rate to 78% and the 3-year OS to 68% in newly diagnosed ALL patients [166]. In addition, preclinical studies revealed that the cell death was induced in rituximab-sensitive or rituximab-resistant precursor B-ALL xenografts by obinutuzumab [167].

Anti-CD19 mAbs: CD 19, which is highly expressed on the surface of >90% of B-ALL blasts, is the target for blinatumomab and denintuzumab mafodotin [13] (Figure 2). Blinatumomab is a bispecific T cell engager (BiTE) antibody, which binds to CD3 on T cells and CD19 on B lymphoblasts, resulting in the release of inflammatory cytokines, proliferation of T cells and CD19+ cell lysis (Figure 2). Initial studies in relapsed/refractory pediatric B-ALL patients demonstrated promising results, with 40-60% of patients achieving CR [168]. In 2016, blinatumomab was approved by the US FDA for the treatment of adult patients with relapsed Ph+ ALL based on Phase II data [169]. It has also been shown to be capable of eradicating MRD in ALL [170], and this may improve outcomes post-HSCT [171]. The Phase III TOWER study assessed the efficacy of blinatumomab compared to the standard chemotherapy in adults with relapsed/refractory B-ALL [172]. This trial demonstrated a superior CR rate and improved OS with blinatumomab compared to standard therapy (7.7 vs. 4 months and 39 vs. 19%, respectively). Treatment was more effective in patients with lower tumor burdens. Other trials (NCT02143414, NCT02003-
222 and NCT02807883) are evaluating blinatumomab in different settings, including frontline and maintenance therapy. Side effects associated with blinatumomab, including cytokine release syndrome and neurologic toxicities, necessitate close observation during the early stages of infusion [13].

Denintuzumab mafodotin (SGN-CD19A) which is an antibody drug conjugate (ADC) links a humanized anti-CD19 antibody to the monomethyl auristatin F (MMAF), a drug that induces apoptosis by inhibiting the microtubule assembly and triggering G2/M arrest upon binding to CD19 and internalization [173] (Figure 2). Denintuzumab mafodotin was applied for the treatment of relapsed B-ALL patients in a Phase I trial, and 22% and 35% of patients demonstrated CR or PR following the weekly treatment and once in 3 weeks of treatment, respectively [174].

ADCT-402 is also an ADC which links a humanized anti-CD19 antibody to a cytotoxic dimer, pyrrolobenzodiazepine, leading to inhibition of both DNA replication and proliferation of CD19+ blasts (Figure 2). ADCT-402 demonstrated significant cytotoxicity in CD19+ cell lines and improved the survival of xenograft models [175]. The efficacy of ADCT-402 is currently under investigation for relapsed B-ALL cases in a Phase I clinical study (NCT02669264).

DT2219 is a bispecific recombinant mAb targeting both CD19 and CD22 and contains the catalytic and translocation domains of diphtheria toxin (DT390) and two sFv subunits recognizing CD19 and CD22. In vitro studies demonstrated favorable results in B cell leukemia models [176, 177] (Figure 2). In a Phase I dose escalation study, only one patient out of 25 adolescents and adults with relapsed CD22+ CD19+ B-ALL achieved a partial response [178]. Efficacy of DT2219 is now being evaluated in a Phase II trial in adults and children older than 12 years (NCT02370160).

Anti-CD22 mAbs: CD22 is another specific B cell lineage antigen expressed by 90% of B-ALL lymphoblasts and is a potential target for inotuzumab ozogamicin, moxetumomab pasudotox, coltuximab ravtansine, and epratuzumab [13, 159, 179] (Figure 2). Inotuzumab ozogamicin, which is an ADC, includes a humanized anti-CD22 IgG4 mAb and calicheamicin, a cytotoxic agent resulting in DNA breakage following the linkage and internalization of CD22 and anti-CD22 [180, 181] (Figure 2). In the Phase III INO-VATE trial (NCT01564784), relapsed/refractory CD22+ ALL patients treated with inotuzumab ozogamicin experienced higher CR rates and superior OS compared to a control group treated with standard chemotherapy (80.7 vs. 29.4%; P < 0.001, 7.7 vs. 6.7 months; P=0.04) [182]. This agent has also been approved by the FDA and other regulatory bodies. Other ongoing clinical trials are also evaluating inotuzumab ozogamicin in combination with chemotherapy in relapsed ALL patients (NCT01925131), in combination with frontline chemotherapy regimens (NCT03150693, NCT-01371630), and in combination with bendamustine, fludarabine +/- rituximab as conditioning therapy with an allogeneic HSCT (NCT01-664910).

Moxetumomab pasudotox is a recombinant antibody comprising the variable fragment of an anti-CD22 antibody and part of a Pseudomonas exotoxin which can induce apoptosis upon internalization [183]. This agent is being studied in a Phase I/II study in refractory ALL patients (NCT01891981). Coltuximab ravtansine (SAR3419) is also an ADC which includes an anti-CD22 antibody and maytansinoid (DM4), a cytotoxic agent that induces cell cycle arrest through the inhibition of microtubule assembly and tubulin polymerization following internalization. However, a phase II trial in relapsed ALL did not yield favorable results [183].

Epratuzumab is a humanized anti-CD22 mAb that is internalized upon binding to the third extracellular domain of CD22. It can induce CD22 phosphorylation, inhibition of proliferation, B-cell activation and cytotoxicity (Figure 2). The results of a Phase II COG trial showed that combining epratuzumab with standard re-induction chemotherapy could not improve the rates of achieving a second CR in relapsed B-ALL [13, 185]. Epratuzumab is being evaluated in combination with cytarabine and clofarabine in another Phase II trial (NCT00945815) in refractory ALL. Adult with relapsed ALL have shown an overall response rate of 40-52% following treatment with epratuzumab and chemotherapy [186].

Anti-CD52 mAb: Alemtuzumab is a recombinant mAb against CD52, expressed in 36-66% of leukemic blasts, which causes ADCC-mediated lysis of CD52+ cells [13, 159, 187]
(Figure 2). However, there are limitations and side effects associated with its use in ALL patients, including lymphopenia resulting in severe and prolonged immunosuppression, and it did not show promising results in several trials [159]. Alemtuzumab is currently being applied in a clinical trial for refractory chronic or acute adult T-cell leukemia in combination with recombinant human IL-15 (NCT02689453).

Anti-PD-1 mAb: Nivolumab, which is a humanized mAb against anti-programmed cell death protein-1 (PD-1), can induce immunosurveillance of malignant cells [13, 188]. The efficacy of this mAb in combination with dasatinib is being assessed in a Phase I study for relapsed Ph+ ALL patients (NCT02819804). Furthermore, the poor-risk relapsed CD19+ B-ALL patients treated with the combination of blinatumomab and nivolumab with or without ipilimumab, are being evaluated by a Phase I study (NCT02879695).

Cellular immunotherapy

A novel targeted therapeutic approach for B-ALL utilizes chimeric antigen receptor-modified T-cells (CAR T-cells) which are specific for B-cell antigens. In this approach, an antibody is expressed by the patients’ own genetically engineered cytotoxic T cells recognizing B-cell antigens. In the CAR structure, an extracellular antigen-recognition domain from a mAb fragment (scFv) is linked to the intracellular signaling domains of the T-cell receptor complex by CARs. This results in the activation of T-cells in a major histocompatibility complex-independent manner yielding a potent cytotoxic response [2, 189]. In the second and third generations of CAR T-cells, one (second) or two (third) costimulatory domains including CD28 or CD27, CD137 (4-1BB), ICOS, and CD134 (OX40) are incorporated to increase the persistence of engineered T cells and achieve higher cytokine production and replicative capacity [190-193]. Preclinical data indicated that CD19-targeted CAR T-cells display enhanced cytotoxicity in vitro and in murine xenograft models [194].

CAR T-cell therapy has yielded remarkable activity in patients with relapsed and refractory B-ALL, with CR rates in the 70-90% range [195-197]. In a Phase I study in 2014, 21 ALL patients (children and young adults) were treated with CD19 CAR T-cells (maximum tolerated dose: 1 × 10^6 CD19-CAR T-cells/kg), 70% achieving CR [197]. In another report in 2014, 90% (27 out of 30) of childhood and adult patients with relapsed ALL achieved CR with CTL019 cells, and these cells were detectable at least for 6 months in 68% of cases [198]. In Sept. 2017, the FDA approved the first CAR T-cell therapy (Tisagenlecleucel) for relapsed/refractory B-ALL in patient under age 25, and many other CAR T trials in B-ALL are in progress. CAR T-cell therapy is associated with several acute adverse effects including hypotension related to the inflammatory cytokine level in serum, fevers and cytokine release syndrome (CRS), which can develop into macrophage activating syndrome [198, 199]. These effects can be mitigated by tocilizumab, an anti-IL6 monoclonal antibody [200, 201].

The outcome of CAR T therapy is affected by the durability of CAR T-cells in patients. This limitation can be addressed by reinfusion of ‘fresh’ CAR T-cells [202, 203]. Emergence of CD19 negative B cells is another reason for CAR-T therapy failure. A study by Lacey et al. identified a relapsed CD19 negative leukemia originated from a CTL019 treated clone [204]. These CD19 negative B-cells escaped from CAR T therapy by downregulation of surface CD19 target antigen in a cell autonomous manner. Developing CAR T-cells to target additional antigens on the surface of B-cells is a promising approach to prevent the relapse. CAR T-cells targeting CD22 have been explored to treat CD19 negative B-cells in some studies, and the results revealed that CD22 is a promising target in relapsed B-ALL patients pre-treated with CD19 CAR T-cells [205-207]. Innovative strategies to improve the efficacy of CAR T-cell therapy include using combined CD19 and CD22 CAR T-cells, vaccination to increase the CAR T-cells persistence, use of allogeneic T-cells or even cord blood T-cells, induction of apoptosis in CAR T-cells, and sleeping beauty transposon system [208, 209].

RNAi-mediated and related therapy

The development of drug resistance leading to relapse and toxicity due to off-target effects are serious limitations associated with conventional therapeutic strategies [2, 9, 11, 210, 211]. To address these limitations and develop a...
more specific approach, RNA interference (RNAi) based therapy is being explored with promising results in pre-clinical settings [212, 213]. The RNAi targets the complementary mRNA for degradation or inhibition of translation and, therefore, can selectively silence the expression of the aberrant proteins involved in uncontrolled cell proliferation. As RNAi functions at a molecular level to downregulate its target mRNA with a high degree of specificity, its activity is minimally affected by point mutations which results in drug resistance [211, 214].

RNAi-mediated therapy can be carried out by delivery of short hairpin RNA (shRNA) encoded by an expression vector (viruses or plasmid DNA for non-viral means), antisense oligonucleotides (ASO; typically 16-20 b.p. single-stranded DNA polynucleotides) or double stranded small interfering RNA (siRNA; typically 19-27 polynucleotides) [211, 215]. However, the latter approaches require an efficient delivery system since polynucleotides are highly unstable in serum due to presence of nucleases, and their anionic nature prevents them from traversing cellular membranes. Among different carrier systems, cationic hydrophobic biomolecules are the most frequently used carriers for intracellular delivery of anionic polynucleotides due to safety reasons; unlike viral vectors, they do not integrate into host genome, offering safe delivery of polynucleotides. They can be chemically modified for different purposes [211, 214, 216].

A number of potential targets in B-ALL have been identified for downregulation by RNAi (Table 2). These targets can be categorized based on their role in the development of leukemia and include those mediating proliferation, apoptosis, chemo-sensitivity, mediating B-cell differentiation and regulating the mobility of cancer cells [211, 217]. One of the potential targets in ALL is CD22 without exon 12 (CD22ΔE12) which normal B cells lack. Therefore, siRNA mediated silencing of this specific target will not affect the normal B cell function. One study showed that CD22ΔE12 siRNAs delivered by liposome and cationic peptide carriers reduced clonogenicity of B-ALL cells and added to the cytotoxic effects of chemotherapy agents in vitro. Furthermore, infusion of this construct inhibited the growth of B-ALL in a mouse xenograft model [216, 217] (Table 2).

High expression of certain isoforms of histone deacetylases (HDAC) is also associated with poor outcomes in ALL. One study reported that the chemo-sensitivity of T-cells increased in an ALL model treated by HDAC siRNA [220]. Another study investigated silencing of MAX dimerization protein 3 (MXD3) which is a basic-helix-loop-helix-leucine zipper transcription factor involved in cellular proliferation [116, 221, 222]. It has been demonstrated that MXD3 functions as an anti-apoptotic protein, and therefore, downregulation of MXD3 may be beneficial for B-ALL [223, 224].

Ab-mediated delivery of polynucleotides, which can specifically target the leukemic cells, is also promising, as it reduces their non-specific delivery into non-leukemic cells. To knockdown MXD3, a nanoparticle (NP) formulation of super paramagnetic iron oxide (SPIO) with MXD3 ASO conjugated to anti-CD22 Ab (αCD22 Ab) was developed to target B-ALL cells. The results revealed significant in vitro and downregulation of MXD3 mRNA, with induction of apoptosis and sensitization to chemotherapeutics; it also demonstrated anti-leukemic activity in B-ALL xenograft mouse models [225] (Table 2).

Hsp32, which functions as a survival factor in cancer cells, is another target for RNAi-mediated therapy. The role of Hsp32 was explored in ALL patient and cell line models by Cerny-Reiterer et al. [226]. ATL1102 is a second-generation antisense oligonucleotide against human α4 integrin (CD49d/ITGA4) RNA. CD49d is involved in signal transduction, adhesion and proliferation of cells [227, 228] and its silencing by ATL1102 in chemo-resistant human Kasumi-2 B-ALL cells decreased the expression of CD49d protein; however, these results could not be confirmed in mouse xenograft models [229] (Table 2).

A recent preclinical study evaluated the therapeutic effect of lipid NP encapsulated TCF3-PBX1 siRNA in vitro on the TCF3-PBX1-expressing 697 cells and in vivo on a patient-derived xenograft (PDX) model from a TCF3-PBX1-positive B-ALL patient [230, 231]. TCF3-PBX1 was significantly downregulated by the specific siRNA at both mRNA and protein levels. Moreover, an efficient uptake of the NP/siRNA
### Table 2. A list molecular targets, delivery systems and cell models applied to investigate nucleic acid-based therapy in ALL

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formulation was observed in difficult-to-transfect patient CML and ALL cells, which confirmed the potency of the siRNA delivery system. In vivo studies demonstrated that siRNA-mediated silencing of the TCF3-PBX1 fusion oncogene improved survival in TCF3-PBX1 dependent B-ALL PDX mice compared to the control group (230) (median OS 45 days vs. 32 days, \( P=0.0026 \)) (Table 2).

MicroRNAs (miRNA) are endogenous non-coding RNA molecules (typically 19 to 25 nucleotides with partial base pairing) that are involved in regulating the expression of genes as well as other mRNAs [213, 215]. Long non-coding RNAs (lncRNAs) are a new category of non-coding RNAs that are capable of regulating different cellular processes leading to tumorigenesis. Recent studies have reported that some specific lncRNAs are deregulated in pediatric B-ALL and they can be utilized as potential therapeutic targets [215, 232-235]. RP11-137H2.4 is a lncRNA which plays an important regulatory role in apoptosis, proliferation, and migration of leukemic blasts. It has been observed that silencing the expression of RP11-137H2.4, through its specific siRNA, induced apoptosis and inhibited migration in NALM6 B-ALL cells. In addition, transducing Reh prednisolone-resistant B-ALL cells with a shRNA targeting RP11-137H2.4, sensitized cells to glucocorticoids by modulating the expression of MAPK cascade genes [232] (Table 2).

RNAi prodrugs were recently derived from modified short interfering Ribonucleic Neutrals (siRNNs), which can enter the cells without a delivery system. Upon internalization, cytoplasmic enzymes cleave the siRNNs into regular siRNAs which are capable of inhibiting their target mRNAs [236]. The therapeutic efficacy of RNAi prodrugs against polo-like kinase 1 (Plk1), which has a key role in mitosis regulation and its upregulation is associated with unfavorable outcomes [237-240], was evaluated by a preclinical study in pediatric T-ALL cell line models and patient samples. Plk1 siRNNs significantly inhibited Plk1 at both mRNA and protein levels and induced apoptosis and G2/M arrest in T-ALL patient cells, with less toxic effect on normal cells [239].

Concluding remarks and future perspectives

Strategies to analyze the molecular genetic and epigenetic aberrations in leukemic cells have resulted in a more comprehensive understanding of the molecular mechanisms leading to chemotherapy drug resistance and adverse outcomes in ALL. The genomic approaches, combined with transcriptional profiling and proteomics, are yielding ever greater insights into ALL pathogenesis and biology, and will result in an expanding repertoire of targeted therapies for clinical evaluation. Ultimately, these emerging technologies should lead to a new era of individualized molecular medicine, resulting in more effective and less toxic regimens [5, 83]. While improved diagnosis and individualized therapy are expected to improve outcomes, it will also further fragment patient populations into sub-populations based on possible “therapeutic” targets. Libraries of different TKIs, Abs targeting different epitopes, and CAR T-cells with different specificities will all help the physician in the choice of individualized therapies. Molecular therapies relying on nucleic acids (RNAi and similar approaches) will be more convenient to adopt since changing target specificity of nucleic acids can be accomplished relatively easily. This approach will provide an effective way to combat relapsing disease, provided the molecular signature of the emerging population is understood.

Advances in targeted therapeutics have already yielded a series of effective targeted immunotherapies. However, with the exception of BCR-ABL1-directed TKIs in Ph+ ALL, the use of therapeutic approaches to downregulate aberrant signaling and apoptotic pathways in ALL remains investigational. Considerable clinical research will be required to evaluate the potential utility of selective potent small molecule inhibitors targeting other aberrant pathways in ALL. Given the likely emergence of drug resistance against small molecular entities, and the multiplicity and redundancy of such signaling pathways, these agents will likely need to be used in combination with other chemotherapeutic or immunotherapeutic approaches. It will be important to systematically investigate upstream (e.g., JAK) vs. downstream signaling pathways (e.g., STATs) for their relative efficacy. Combinational inhibition of signaling pathways will be useful, but whether interfering with multiple sequential steps in a signaling pathway, or interfering parallel pathways will need to be evaluated for their relative efficacy. RNAi offers another promising avenue of targeted therapy,
potentially avoiding the emergence of drug resistance; however, effective delivery systems will need to be optimized before this approach can be widely applied. Detection of minimal residual disease using flow cytometry, and molecular analysis for identifying mutations and genetic signatures [14, 85], will be necessary to optimally evaluate the response to such therapies [241].

Disclosure of conflict of interest

None.

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