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Novel targeted drug therapies for the treatment of childhood acute leukemia

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Abstract

The cure rates for childhood acute leukemia have dramatically improved to approximately 70% overal, with treatments that include intensive cytotoxic chemotherapy and, in some cases, hematopoietic stem cell transplantation. However, many children still die of their disease or of treatment-related toxicities. Even in patients that are cured, there can be significant and, not uncommonly debilitating, acute and late complications of treatment. Improved understanding of the molecular and cellular biology of leukemia and the increasing availability of high-throughput genomic techniques have facilitated the development of molecularly targeted therapies that have the potential to be more effective and less toxic than the standard approaches. In this article, we review the progress to date with agents that are showing promise in the treatment of childhood acute leukemia, including monoclonal antibodies, inhibitors of kinases and other signaling molecules (e.g., BCR–ABL, FLT3, farnesyltransferase, mTOR and γ-secretase), agents that target epigenetic regulation of gene expression (DNA methyltransferase inhibitors and histone deacetylase inhibitors) and proteasome inhibitors. For the specific agents in each of these classes, we summarize the published preclinical data and the clinical trials that have been completed, are in progress or are being planned for children with acute leukemia. Finally, we discuss potential challenges to the success of

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molecularly targeted therapy, including proper target identification, adequate targeting of leukemia stem cells, developing synergistic and tolerable combinations of agents and designing adequately powered clinical trials to test efficacy in molecularly defined subsets of patients.

Keywords

acute lymphoblastic leukemia; acute myeloid leukemia; BCR-ABL; epigenetics; FLT3; immunotherapy; mTOR; notch; proteasome; RAS

The past few decades have yielded remarkable improvements in long-term outcomes for children with acute leukemia, primarily resulting from sequential, controlled, multicenter clinical trials evaluating intensification of chemotherapy in a risk-adjusted paradigm based upon clinical and biologic features [1]. These improvements have been particularly striking for acute lymphoblastic leukemia (ALL), the most common malignancy in children. In ALL, prolonged event-free survival (EFS) rates now approach 80%. This progress unequivocally represents one of the greatest success stories in modern medicine [2,3].

However, despite the extraordinary success in ALL, many patients still relapse. Unfortunately, the outlook for children with relapsed ALL is grim, especially if the relapse occurs within 3 years of diagnosis [4]. Equally disconcerting is the price associated with curative therapeutic approaches in terms of the significant, and not uncommonly debilitating, acute and late complications of treatment [5,6].

Similar therapeutic success has unfortunately not yet been duplicated in acute myeloid leukemia (AML), although prolonged EFS rates for AML are now approximately 50% [7]. In both AML and the higher-risk subsets of ALL, a limit in the effectiveness of chemotherapy intensification to improve outcomes has been reached due to offsetting increases in unacceptable toxicities. Furthermore, intensification of therapy with allogeneic hematopoietic stem cell transplantation (HSCT) only modestly improves the outcome of these patients, while introducing even higher risks of acute and late complications [8,9].

Further improvements in outcome for childhood acute leukemia will probably depend on the development of new therapeutic strategies. To accomplish this, several interdependent and complimentary areas of research are required. These areas include:

- Refinement in the definitions of specific subclasses of these diseases that warrant varying intensities of therapy due to the relative risk of treatment failure [2,10];
- An improved understanding of the biology of the leukemic stem cell (LSC), since eradication of the LSC (as opposed to mere reduction in the numbers of bulk leukemia cells) is emerging as a relevant goal of leukemia therapy [11];
- The discovery and characterization of genetic and epigenetic alterations that are important in the pathogenesis of these diseases, either overall or for specific molecularly defined subsets, and can be potentially targeted by novel therapeutic approaches.

This review will focus on these novel molecularly targeted drug therapies for childhood ALL and AML. In this context, ‘targeted’ refers to agents that selectively disrupt a tumor-specific biological pathway or a subset of cells within the bulk population that is essential for leukemia maintenance.

Molecular pathogenesis of leukemia: past, present & future

Recurrent translocations provide important clues to the molecular pathogenesis of leukemia (and cancer in general) and, in some notable cases, have facilitated the development of successful molecularly targeted therapies. Perhaps the best example is found in chronic myelogenous leukemia (CML), where the discovery of the reciprocal translocation t(9;22)(q34;q11) (the Philadelphia chromosome [Ph]) sequentially led to identification of the BCR–ABL fusion protein, an understanding that resultant activation of the ABL kinase was central to the pathogenesis of CML and, finally, the development of novel agents (e.g., imatinib and others) that could eradicate CML cells by inhibiting ABL kinase activity. This example is also helpful in understanding the importance of the LSC concept in the development of novel, molecularly targeted therapies. While a high proportion of patients with CML can achieve remission with imatinib therapy alone, it is unknown whether imatinib therapy successfully eradicates the CML stem cell, since it is not yet clear that long-term remissions after cessation of imatinib therapy will be widely achievable [12,13]. Longer follow-up of patients treated in this manner will answer this important question.

Another example is acute promyelocytic leukemia (APL), which is characterized by the reciprocal translocation t(15;17)(q22;q21). The differentiation block induced by the resultant PML–RARα fusion protein has been targeted successfully with all-*trans* retinoic acid (ATRA), which, in combination with chemotherapy, has turned a poor-prognosis disease into one in which most cases are cured. In this case, the APL stem cell appears to be effectively eradicated by this approach. Interestingly, the APL stem cell appears to have distinct characteristics compared with the stem cell for other subtypes of AML, in that it has the more differentiated phenotype of a committed myeloid precursor (as opposed to the primitive phenotype reminiscent of the normal hematopoietic stem cell [HSC], which is characteristic of the other subtypes of AML) [14]. This may, in part, explain the relative ease with which APL can be treated successfully in children and adults with the combined use of chemotherapy and differentiating agents.

Other recurrent cytogenetic abnormalities are characteristic of unique biological subsets of childhood acute leukemias. For example, in B-precursor ALL, the t(12;21) translocation that results in the ETV6–RUNX1 fusion gene is correlated with a good outcome, as are cases with ‘high’ hyperdiploidy (>50 chromosomes). By contrast, cases showing rearrangements of the *MLL* gene (11q23), hypodiploidy (<44 chromosomes) and the t(9;22) (Ph+ ALL) fare poorly. LSCs are less well characterized for childhood ALL compared with AML, but evidence suggests that their phenotype varies depending on the biological subtype [15–17]. While these abnormalities have led to a better understanding of leukemogenesis and improved prognostication, they have not as yet (except in the case of Ph+ ALL) led to the development of targeted therapies analogous to imatinib or ATRA.

The ability to move beyond standard cytogenetic approaches for the discovery and characterization of molecular alterations in ALL and AML has begun to pave the way for the development of additional novel, targeted therapies that will hopefully improve the outcome for children with these diseases (Table 1). The recent development of high-throughput technologies to assess global gene expression has led to the discovery of gene expression ‘signatures’ that correlate with morphologic, immunophenotypic and biological subtypes [18–22]. Moreover, emerging data suggest that these signatures may enhance current risk stratification schemes that allow therapy to be tailored to risk of relapse soon after diagnosis [23]. In addition, genome-wide analysis of single nucleotide polymorphisms (SNPs) can be used to detect gene copy number abnormalities (CNAs; e.g., deletion and amplification), and has revealed that ALL cells contain a small number (approximately six to seven per case), with deletions outnumbering amplifications by a ratio of 2:1 [24]. Again, CNA changes correlate

with the biological subtype and prognosis. For example deletion of *IKZF1* (*Ikaros*), which is seen in approximately 8–9% of ALL overall, is associated with a poor prognosis [25,26]. Furthermore, deletion of *IKZF1* may be seen at relapse when not present at initial diagnosis, emphasizing a role for *IKZF1* deletion in chemoresistance [27]. Finally, high-throughput approaches for assessing noncoding or miRNA expression and gene methylation have been applied to many hematopoietic malignancies [28,29]. Data examining childhood acute leukemia are just emerging.

It is important to consider the idea of 'oncogene addiction' as one thinks about new targeted therapies [30]. The activity of the BCR–ABL kinase in CML is the prototype for this concept. BCR–ABL plays a critical role in the pathogenesis of CML and continued BCR–ABL kinase activity is critical for maintenance of the malignant phenotype. Therefore, pharmacologic inhibition of BCR–ABL kinase activity has potent therapeutic benefit as it directly attacks a key step in leukemogenesis. The most effective targeted therapies will probably also be directed at molecules to which cancer cells are 'addicted'. However, there can still be significant clinical benefit to therapies that do not directly target oncogenic processes, as shown by the effectiveness of anti-CD20 monoclonal antibodies in adults with non-Hodgkin's lymphoma [31].

Monoclonal antibodies

Targeted immunotherapy involves the use of monoclonal antibodies directed against cell surface antigens expressed on the surface of malignant cells. Additional moieties (e.g., cytotoxins or radioactive isotopes) may be conjugated to the antibodies in an effort to enhance their anticancer activity. The ideal antigens to target are those that are expressed on the bulk of the leukemic clone, but are not expressed on normal HSCs or other normal tissues.

Anti-CD33 (AML): gemtuzumab ozogamicin

CD33 is a cell surface sialoglycoprotein that is increasingly expressed on normal myeloid cells as they differentiate and is expressed on the surface of leukemia cells in 85–90% of AML cases in adults and children [32,33]. Gemtuzumab ozogamicin (GO) is a humanized monoclonal anti-CD33 antibody conjugated to calicheamicin, a potent cytotoxin. When used as a single agent for adults and children with relapsed or primary refractory AML, GO has demonstrated response rates of approximately 30–35% [34–37]. In a pediatric Phase I/II trial, the response in children with primary refractory disease was the same as that for patients with relapsed disease who had achieved a prior remission, suggesting that some conventional resistance mechanisms can be circumvented with this agent [36]. The major toxicities of GO in this study were myelosuppression and veno-occlusive disease (VOD). The incidence of VOD was particularly high (46%; six out of 13) in patients undergoing allogeneic stem cell transplant (SCT) less than 3.5 months after GO administration.

The Children's Oncology Group (COG) conducted a pilot study that determined the maximum-tolerated dose of GO in combination with two high-dose cytarabine (HiDAC)-based reinduction regimens in children with relapsed, refractory or secondary AML, finding a maximum-tolerated dose of 2 or 3 mg/m^2 depending on the regimen used [38]. Another COG pilot study established the safety of GO combined with intensive Medical Research Council (MRC)-based chemotherapy in children with newly diagnosed AML, with GO added to the first induction course (cytarabine, daunomycin and etoposide) and the second intensification course (mitoxantrone and cytarabine) [39]. The current COG randomized Phase III study for *de novo* AML is testing the hypothesis that GO added to the MRC-based chemotherapy backbone will improve overall survival by reducing primary refractory disease and by reducing later relapses without increasing treatment-related mortality.

Clinical response to GO has been inversely correlated with the level of expression of MDR1 P-glycoprotein and/or drug efflux rates [36,40], suggesting that reduced efficacy of the antibody/toxin conjugate could be due to the increased cellular efflux of calicheamicin via these drug transporters. This observation raises the possibility of increasing the efficacy of GO by combining it with inhibitors of drug transporters, although a previous randomized comparison of the addition of the P-glycoprotein inhibitor cyclosporine to standard consolidation chemotherapy did not demonstrate clinical benefit for children with newly diagnosed AML [41].

One potential limitation in therapies based on CD33 targeting is the seemingly inconsistent expression of CD33 by LSCs, despite high-level expression of CD33 by bulk leukemia cells [42–44]. Since it appears that eradication of the LSC is a prerequisite for inducing prolonged disease-free survival, there is some concern that, despite its proven cytoreductive activity, the addition of GO to standard therapy may not result in significant improvements in long-term outcome. Ongoing randomized Phase III studies for childhood AML conducted by the COG will help to address this concern. The potential limitations of CD33-targeted therapies in eradicating LSC is likely to be less of a problem in APL, where the LSC appears to express a more-differentiated phenotype, including consistent expression of CD33 [14]. This, coupled with characteristically very bright surface expression of CD33 on bulk APL cells, may explain the impressive clinical responses that have been reported with Mylotarg in patients with both newly diagnosed and relapsed/refractory APL [45,46].

Anti-CD22 (ALL): epratuzumab

CD22 is a B-cell-restricted 135-kD glycoprotein that is initially present in the cytoplasm of developing B cells, but later expressed on the cell surface at more mature stages of B-cell differentiation. CD22 is widely expressed in B-cell lymphomas and B-precursor ALL [47].

Epratuzumab is a humanized monoclonal antibody (IgG1) directed against CD22 [48]. Proposed mechanisms of anti-tumor activity include antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and direct induction of apoptosis. These mechanisms of action are distinct from those of cytotoxic agents, making combination chemoimmunotherapy an attractive approach. The *in vitro* properties of epratuzumab have been characterized in B-cell lines as well as in primary B cells from healthy individuals and those with B-lineage non-Hodgkin's lymphoma [49]. Epratuzumab binds to the CD22 extracellular domain with an affinity similar to that seen with rituximab, resulting in rapid internalization of the CD22–antibody complex.

Epratuzumab has been studied as a single agent [50,51], in combination with rituximab [52, 53] and in combination with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) in adult patients with B-cell lymphomas [54]. Toxicities in the single-agent and epratuzumab–rituximab studies were mild; most were transient and manageable infusion-related toxicities. The epratuzumab–chemotherapy study suggested that epratuzumab does not add significantly to the expected toxicities of the chemotherapy alone. Dosing of epratuzumab in these studies was 360 mg/m^2 administered weekly (in the single-agent and epratuzumab–rituximab studies) or once every 3 weeks (in the epratuzumab–chemotherapy study).

Epratuzumab is being studied in relapsed childhood ALL in the COG. In a pilot study, 12 evaluable patients were treated with a single-agent phase (360 mg/m^2 twice weekly for four doses) followed by a 4-week combination phase in which standard reinduction chemotherapy (vincristine, prednisone, L-asparaginase and doxorubicin [VPLD]) was combined with four weekly doses of epratuzumab 360 mg/m^2 [55]. In the single-agent phase, toxicities were minimal (primarily infusion related) and responses were modest, with two patients showing

evidence of peripheral cytolytic responses. In the combination phase, two dose-limiting toxicities occurred: one grade 4 seizure of unclear etiology and one asymptomatic grade 3 ALT elevation. In all but one patient, surface CD22 was not detected by flow cytometry on peripheral blood leukemic blasts within 24 h of drug administration, indicating effective targeting of leukemic cells by epratuzumab. Nine patients (75%) achieved a complete remission after the 6 weeks of chemoimmunotherapy, seven of whom were minimal residual disease (MRD) negative. Based on these promising results, an ongoing COG Phase II study is testing the VPLD–epratuzumab combination in an expanded cohort of children with relapsed ALL.

Anti-CD22 immunotoxins are also in development. BL22 is a pseudomonas exotoxin A (PE) conjugate that has been studied clinically in adults with various B-cell malignancies [56,57]. HA22 (CAT-8015) is a second-generation anti-CD22 PE immunotoxin with improved potency, decreased immunogenicity and decreased animal toxicity that is currently being tested in adults with CLL and hairy cell leukemia [58–60]. Another immunotoxin product (Combotox) combines anti-CD22 and anti-CD19 monoclonal antibodies conjugated with deglycosylated ricin A. Combotox has shown preclinical activity against childhood ALL samples *in vitro* [61] and in nonobese diabetic (NOD)/severe combined immunodeficiency (SCID) xenografts [62], has been tested in adults with B-non-Hodgkin's lymphoma [63] and is currently being tested in adults with relapsed B-lineage ALL.

Tyrosine kinase inhibitors

BCR–ABL (ALL): imatinib & dasatinib

Although EFS for childhood ALL now approaches 80%, children with Ph+ ALL have a dismal prognosis, with EFS of only approximately 20% when treated with chemotherapy alone [64]. As mentioned earlier, efforts to develop small molecular inhibitors of the BCR–ABL protein led to the development of imatinib, which binds to and inhibits its tyrosine kinase activity. Imatinib has potent clinical activity in CML and has revolutionized treatment of this disorder [65]. However, both *de novo* and acquired imatinib resistance are observed in CML and represent the major cause of treatment failure [66]. In Ph+ ALL, 60–70% of previously treated patients respond to single-agent imatinib therapy, but responses are short-lived, with median time-to-progression of 2–3 months [67,68]. Clinical trials in adults have shown that the addition of imatinib to hyper-cyclophosphamide, vincristine, adriamycin and dexamethasone (CVAD) is safe and is associated with at least a short-term improvement in survival in Ph+ ALL [69]. The combination of imatinib plus induction chemotherapy has been shown to be superior to induction chemotherapy alone in quickly lowering disease burden, assessed by day 14 marrow status, and showed a trend toward improved induction rate [70].

The recently completed COG AALL0031 trial established the safety and tested the efficacy of an intensive chemotherapy backbone plus imatinib in treating children with Ph+ ALL [71]. The addition of imatinib was safe and associated with relatively minor additional toxicity (mild asymptomatic transaminitis requiring intermittent rather than continuous dosing during maintenance therapy). While the long-term impact of imatinib on EFS of pediatric Ph+ ALL patients remains to be determined, recent results in adult and pediatric patients indicate a favorable response in the context of multiagent chemotherapy, as assessed by early end points, such as complete response rate (for patients receiving imatinib in induction), levels of MRD and a greater proportion of patients proceeding to transplant [72]. In addition, the early outcome data from AALL0031 are promising [71]. The 3-year EFS of patients receiving continuous imatinib (n = 44) is 80.5 ± 11.2%, including those (n = 13) assigned to a sibling HSCT and those (n = 6) receiving off-protocol alternative donor HSCT, and excluding those (n = 6) failing to achieve remission after 4 weeks of standard induction chemotherapy. This is significantly higher than historical controls, both from previous Pediatric Oncology Group studies (n = 120; 3-year EFS 35.0 ± 4.4%) and from other published data (n = 267; 2-year EFS 40.9 ± 5.4%)

[64]. While these results are encouraging, longer follow-up is required to determine if these impressive early results will hold up over time. It is hypothesized that further gains in outcome for patients with Ph+ ALL are most likely to derive from integration of more potent targeted agents or incorporating agents that act synergistically with imatinib into intensive combination chemotherapy regimens.

A variety of agents could potentially improve upon the activity of imatinib and increase the efficacy of the current treatment program for Ph+ ALL. The new class of ABL/Src kinase inhibitors, specifically dasatinib, are the most promising of these agents. Signaling through the Src family kinases HCK, LYN and FGR is required for leukemic transformation in Ph+ ALL (but not Ph+ CML) [73]. In June 2006, dasatinib was granted accelerated approval by the US FDA for treatment of adults with chronic-phase, accelerated-phase, or myeloid or lymphoid blast-phase CML, and regular approval for adults with Ph+ ALL with resistance or intolerance to prior therapy. *In vitro*, dasatinib is 325-times more potent than imatinib at blocking ABL kinase activity [74]. Furthermore, dasatinib is active *in vitro* against most known imatinib-resistant BCR–ABL mutants [75,76]. Dasatinib has displayed a high level of efficacy for patients with imatinib-resistant CML with minimal side effects, including mild myelosuppression and nausea [77]. Dasatinib is a particularly attractive agent for treatment of Ph+ ALL owing to its dual targeting of ABL and the Src kinases, more powerful suppression of BCR–ABL signaling and efficacy in imatinib-resistant leukemia. Consequently, an ongoing COG study is testing intensification of tyrosine kinase inhibitor therapy by combining dasatinib with the AALL0031 backbone of intensive chemotherapy beginning in the latter 2 weeks of induction and continuing with all postinduction courses. As dasatinib is highly effective as a single agent at targeting imatinib-resistant Ph+ leukemic clones, it is possible that substitution of dasatinib for imatinib will prove effective in preventing resistance and increasing EFS in children with Ph+ ALL.

FLT3 (AML & ALL): lestaurtinib

Another aberrantly activated kinase against which targeted therapies are being developed is FLT3 [78,79]. FLT3 is a receptor tyrosine kinase that is expressed in over 90% of cases of AML [80]. Mutations in FLT3 that lead to constitutive (ligand-independent) phosphorylation occur in approximately 30–35% of adults and 20–25% of children with AML [81–83]. Approximately two-thirds of these FLT3 mutations are internal tandem duplications (ITDs) in the juxtamembrane region of the receptor and a third are point mutations (PMs) in the kinase domain. Several studies have shown that the presence of FLT3/ITDs confers an increased risk of relapse and decreased survival in childhood AML [82,84–86]. In a retrospective study of children with *de novo* AML enrolled on the Children's Cancer Group (CCG) CCG-2891 Phase III clinical trial, patients with FLT3/ITD had an 8-year overall survival and EFS of 13 and 7%, respectively, versus 50 and 44% for patients without FLT3/ITD [82]. Further studies in both adult and pediatric AML have demonstrated that, in FLT3/ITD-positive samples, the ratio of mutant to wild-type alleles has additional prognostic significance, such that patients with high ITD allelic ratios have a worse prognosis [86–88].

The demonstrated importance of FLT3 signaling in AML has led to the development of small molecules with selective FLT3 inhibitory activity. Lestaurtinib (CEP-701) is an orally bioavailable indolocarbazole derivative with an IC_{50} of 3 nM for inhibition of phosphorylation of ITD, PM and wild-type FLT3 [89]. Preclinical studies have shown that lestaurtinib selectively kills primary adult and pediatric AML blasts with FLT3 mutations [89,90]. In the study of pediatric AML samples, for example, those with FLT3/ITDs were particularly likely to demonstrate *in vitro* sensitivity to lestaurtinib, with 14 out of 15 samples (93%) noted to be responders. While samples with FLT3/PM (four out of 15 [27%]) and those with FLT3/wild-type (four out of 14 [29%]) were significantly less likely to respond, over a quarter of these

samples did demonstrate pronounced sensitivity and the sensitive samples were shown to express high levels of activated FLT3.

There is growing clinical experience with lestaurtinib. In a Phase II single-agent study in adults with relapsed/refractory FLT3-mutant AML, lestaurtinib was well-tolerated at doses up to 80 mg orally twice daily, with common toxicities of mild nausea and fatigue [91]. Successful inhibition of FLT3 phosphorylation to less than 10% of baseline levels was demonstrated at this dose. Clinical responses (reduction in peripheral blood or bone marrow blast percentage) were seen in five out of 14 patients, all of whom had been shown to be refractory to chemotherapy. Another Phase II study tested lestaurtinib as front-line mono-therapy (80 mg orally twice daily for 8 weeks) for older adults with AML that were not considered fit for chemotherapy [92]. Clinical responses were seen in three out of five (60%) patients with FLT3 mutations and in five out of 22 (23%) patients with wild-type FLT3. An ongoing study has randomized adults with relapsed/refractory FLT3-mutant AML to receive chemotherapy alone (mitoxantrone, etoposide and cytarabine; or high-dose cytarabine) or chemotherapy in sequential combination with lestaurtinib (80 mg orally twice daily) [93].

Lestaurtinib has been well-tolerated in this trial, with mild-to-moderate gastrointestinal symptoms and fatigue attributed to the drug. Ten out of 17 (59%) patients on the lestaurtinib arm achieved a complete or partial response, compared with four out of 17 (24%) patients randomized to chemotherapy alone. Cytotoxicity analysis showed that 80% of pretreatment samples were sensitive to lestaurtinib *in vitro*. In total, 13 out of 17 (76%) patients on the lestaurtinib arm achieved high enough levels of drug in plasma to fully inhibit FLT3 phosphorylation. Remarkably, the ten patients who met both criteria predictive of a good response (i.e., had pretreatment leukemia cells that were sensitive *in vitro* to lestaurtinib and achieved sufficient plasma levels of lestaurtinib) were the clinical responders. Conversely, the seven patients with insensitive cells or insufficient drug plasma levels did not respond. Lestaurtinib is now being tested in the COG for children with relapsed/refractory FLT3-mutant AML. Lestaurtinib is given in sequential combination with reinduction chemotherapy (HiDAC and idarubicin). The sequence of exposure in this trial (chemotherapy followed by FLT3 inhibitor) is based upon preclinical studies demonstrating that maximal synergy between FLT3 inhibition and chemotherapy is achieved with this approach, compared with simultaneous exposure (which is additive rather than synergistic) and FLT3 inhibitor followed by chemotherapy (which is antagonistic) [94,95]. The antagonism seen with the sequence of FLT3 inhibition followed by chemotherapy is due to the cell cycle arresting properties of FLT3 inhibitors, which protect the arrested cells from the cytotoxic effects of chemotherapy agents (which are most toxic to actively cycling cells). Plans to incorporate FLT3 inhibitors into COG Phase III clinical trials for *de novo* pediatric FLT3/ITD-positive patients are in progress.

Similar concerns to those described for CD33-targeted therapy have been raised regarding the relevance of FLT3-targeted therapy to the goal of eradicating the LSC. The discovery that approximately 10–20% of patients whose leukemia is FLT3/ITD positive at diagnosis will lack the mutation at relapse [96] suggests that, in a subset of patients, FLT3 mutations may be a secondary event that arises in a subclone of the leukemic population. However, the presence of FLT3/ITD mutations in the cells responsible for long-term engraftment of AML patient samples in NOD/SCID mice (which is the strongest functional evidence of LSC activity) provides direct evidence that, in the majority of cases, FLT3/ITD mutations are present in the LSC [97]. Further evidence comes from a study showing a correlation between the presence of the FLT3/ITD mutation in $CD34^{+}/CD33^{-}$ myeloid progenitors with a significantly poorer outcome (compared with cases where FLT3/ITD was present only in more differentiated $CD34^{+}/CD33^{+}$ progenitors) [98].

FLT3 has also been implicated in the pathogenesis of infant and childhood ALL. Gene-expression studies have shown that the highest levels of FLT3 mRNA expression occur in cases of infant and childhood ALL with rearrangements of the *MLL* gene (which account for 80% of infant and 5% of childhood ALL cases) and in cases of ALL with hyperdiploidy with more than 50 chromosomes (which account for 25% of childhood ALL cases) [99,100]. The correlation of high FLT3 expression and these cytogenetic abnormalities is very strong. Moreover, several laboratories have demonstrated that leukemic blasts from cases of *MLL*-rearranged and hyperdiploidy ALL also express high levels of FLT3 at the protein level, and that FLT3 is constitutively phosphorylated in these cases, even in the absence of FLT3-activating mutations, suggesting autocrine activation via coexpression of FLT3 ligand (FL) in these cases [101–104]. In addition, activating mutations of FLT3 (specifically, point mutations in the activation loop of the kinase domain) occur in approximately 15% of infants and children with ALL with *MLL* gene rearrangements or hyperdiploidy [100,103,105]. Small insertion/deletion mutations in the juxtamembrane domain have also been reported in an additional 12% of high-hyperdiploid ALL cases, for a total FLT3 mutation rate of approximately 30% in hyperdiploid ALL cases.

Given the importance of FLT3 in the pathogenesis of ALL, FLT3 inhibitors are being evaluated. *In vitro*, lestaurtinib selectively kills primary infant and childhood ALL cells with high-level expression of constitutively activated FLT3. For those with *MLL* rearrangements, marked lestaurtinib sensitivity was observed in 82% (nine out of 11 patients) of *MLL*-rearranged samples versus 8% (one of 13 patients) of samples that lacked *MLL* rearrangement and expressed low levels of FLT3 [102]. Since monotherapy with any single molecularly targeted agent is unlikely to be curative in acute leukemia, targeted agents are more likely to be effective as components of combination chemotherapy regimens. Lestaurtinib has been shown to result in synergistic killing of *MLL*-rearranged ALL cells when combined with multiple chemotherapy agents [95]. The degree of synergy is markedly dependent upon the sequence of exposure to the agents. Exposure to chemotherapy followed by lestaurtinib results in consistent and strong synergistic cell killing, while simultaneous exposure is, in most cases, additive. Exposure to lestaurtinib followed by chemotherapy is, in many cases, antagonistic. This sequence dependence is due to the effects of FLT3 inhibition on cell cycle progression.

Based on these data, lestaurtinib is being tested in the COG for infants with newly diagnosed *MLL*-rearranged ALL. Lestaurtinib is being added in a randomized fashion to the multicourse chemotherapy regimen used in the previous clinical trial for infant ALL (COG P9407). The design of this study takes into account the preclinical data regarding combinations of lestaurtinib and chemotherapy, as lestaurtinib will be administered immediately following exposure to standard cytotoxic chemotherapy in an effort to maximize potential synergy, and will not be administered for at least 24 h prior to chemotherapy to avoid potential antagonism.

Unlike rearrangements of the *MLL* gene, hyperdiploidy is a favorable prognostic feature in childhood ALL [1]. Nonetheless, 10–20% of these patients will suffer relapse of their disease. Data indicate that FLT3 delivers crucial survival signals in high-hyperdiploid cells, as indicated by their exquisite sensitivity to FLT3 inhibition in cytotoxicity and apoptosis assays [102]. FLT3 inhibitors, therefore, may have a role in the treatment of high-hyperdiploid ALL, either in the setting of relapsed disease or as part of up front therapy to enable the decreased use of chemotherapeutics (with their significant short- and long-term toxicities) while maintaining high cure rates.

Proteasome inhibitors (AML & ALL): bortezomib

NF-κB is a transcriptional activator that is known to have anti-apoptotic activity and is thereby considered to be a key survival factor for several types of cancer. While unstimulated

$CD34^+$ HSCs do not express activated NF-κB [106], NF-κB is constitutively activated in primary AML specimens, and specifically in the $CD34^+/CD38^-$ LSC population [107]. Proteasome inhibitors are a new class of drugs that block degradation of the NF-κB regulator IκBα and result in loss of NF-κB activity. These drugs have been shown to induce apoptosis in several malignant cell types. In primary AML specimens, the combination of a proteasome inhibitor and idarubicin demonstrates rapid and extensive apoptosis of AML blasts and the LSC population *in vitro* and *in vivo*, while leaving normal HSCs viable [108]. These data have led to clinical investigation of the proteasome inhibitor bortezomib in AML, with the hypothesis that, by selectively targeting the LSC population, this approach will result in more durable responses than is achievable with standard therapies alone.

A Phase I single-agent trial of bortezomib in adults with refractory or relapsed acute leukemias established tolerability with twice-weekly dosing of 1.25 mg/m^2 for 4 weeks every 6 weeks [109]. Another Phase I trial studied the combination of bortezomib (administered twice weekly for 2 weeks) and pegylated liposomal doxorubicin (administered once on day 4) in adults with advanced hematologic malignancies [110]. Doses of bortezomib 1.3 and 30 mg/m^2 were tolerable, and clinical responses were seen in two out of five patients with AML. The COG has reported the results of a Phase I single-agent trial of bortezomib in pediatric patients with recurrent/refractory leukemia [111]. Bortezomib was tolerated at a dose of 1.3 mg/m^2 administered twice weekly for 2 weeks, followed by a 1-week rest period. The COG is planning a pilot/Phase II study of bortezomib combined with reinduction chemotherapy for pediatric patients with relapsed/refractory AML.

There has also been interest in bortezomib for the treatment of ALL. *In vitro* and *in vivo* activity of bortezomib has been noted using ALL cell lines and primary sample xenografts [112], and *in vitro* synergy between dexamethasone and bortezomib has been demonstrated in primary ALL samples [113]. A pediatric Phase I study of bortezomib combined with standard ALL reinduction chemotherapy in relapsed ALL demonstrated tolerability and a bone marrow complete response in eight out of ten patients treated [114]. The COG is planning a pilot/Phase II study of bortezomib combined with reinduction chemotherapy for pediatric patients with relapsed/refractory ALL.

mTOR inhibitors (AML & ALL): sirolimus & temsirolimus

The serine/threonine kinase mTOR is an integrator of several signal-transduction pathways and is involved in the regulation of cell cycle, apoptosis and angiogenesis. The mTOR pathway is aberrantly activated in many tumor types and the inhibition of mTOR signaling (using sirolimus or one of its analogues, such as temsirolimus or everolimus) has shown anti-tumor activity in several model systems, as well as early-phase clinical trials. In fact, temsirolimus was recently US FDA approved for the treatment of advanced renal cell carcinoma [115]. In primary AML samples, activation of mTOR has been demonstrated in over 70% of cases, often associated with constitutive activation of the PI3K/Akt pathway [116–118]. Sirolimus markedly reduces clonogenic growth of primary patient AML samples, but has little effect on colony-forming unit–granulocytic/monocytic (CFU-GM) or burst-forming unit-erythroid (BFU-E) production by normal bone marrow [118]. Clinical responses were reported in five out of nine adults with relapsed/refractory or poor-risk AML treated with single-agent sirolimus continuously for 28 days [118]. A Phase I/II trial of single-agent everolimus in adults with relapsed/refractory hematologic malignancies demonstrated tolerability, inhibition of mTOR targets in patient samples and modest clinical activity [119]. The COG is planning a pilot/Phase II study of temsirolimus combined with reinduction chemotherapy for pediatric patients with relapsed/refractory AML.

There is significant preclinical evidence that mTOR inhibition may also be a useful therapeutic strategy in childhood ALL. Sirolimus (and the related mTOR inhibitor temsirolimus) inhibits the growth of B-precursor ALL cell lines *in vitro* and is also active against murine transgenic models of ALL and human ALL xenografts [120]. In further studies, mTOR inhibitors were shown to synergize with methotrexate in killing human ALL xenografts; combination therapy was much more potent than either agent alone [121]. Moreover, sirolimus has been shown to overcome glucocorticoid resistance in ALL cells via downregulation of the antiapoptotic protein MCL1 [122]. These studies provide a strong rationale for clinical trials of mTOR inhibitors in childhood ALL.

Epigenetic agents (AML & ALL): *HDAC* inhibitors & DNA methyltransferase inhibitors

Epigenetic modifications are known to play crucial roles in gene expression. Transcriptional silencing of genes important in preventing malignant transformation (including tumor suppressors, such as *p15INK4b*, and mismatch repair genes, such as *hMLH1*) is important in the pathogenesis of many cancers, including AML [123–126]. Other genes found to be epigenetically dysregulated in leukemias include the secreted frizzled-related proteins (SFRPs), E-cadherin, retinoic acid receptor β2 and the suppressor of cytokine signaling (SOCS)-1. Two interactive processes that culminate in transcriptional silencing include methylation of CpG islands in gene promoter regions and changes in chromatin conformation mediated by histone acetylation. Both processes can be targeted therapeutically (by DNA methyltransferase inhibitors and histone deacetylase [HDAC] inhibitors, respectively) in an effort to upregulate expression of the epigenetically silenced genes, potentially modifying the leukemic phenotype [127].

The recurrent chromosomal translocations that characterize many cases of childhood acute leukemia fuse the DNA-binding domain of a transcriptional activator to a transcriptional repressor, leading to decreased expression of target genes that regulate myeloid differentiation and causing the block in myeloid differentiation that characterizes acute leukemia [128,129]. The processes of histone acetylation and promoter methylation have been shown to contribute to transcriptional repression activity of some of these fusion proteins, including AML1–ETO [130–132], PML–RARA [133–135] and TEL–AML1 [136]. In addition, the constitutive upregulation of HOX genes essential to leukemogenesis associated with abnormalities in the *MLL* gene at 11q23 has been shown to involve DNA methyltransferase and histone acetylase activity [137–139].

Various combinations of small-molecule inhibitors of HDAG (e.g., vorinostat and entinostat) and DNA methyltransferases (e.g., azacitidine and decitabine) are being studied extensively in adults with myelodysplastic syndrome and acute leukemia, with promising initial results [127,140,141]. Pediatric Phase I studies of decitabine, valproic acid, MS275 and SAHA are underway. The impact of epigenetic therapeutic strategies on the goal of eradicating LSC remains to be determined.

Other agents

Farnesyltransferase inhibitors (AML & ALL): tipifarnib

RAS signaling is an important downstream component of cytokine receptor signaling. Activating mutations of *N-RAS*, *K-RAS* or other genes known to activate RAS signaling (e.g., *FLT3* or *PTPN11*) have been observed in 35–45% of pediatric AML and ALL [142,143]. In addition, signaling through the RAS pathway has been shown to play a significant role in leukemogenesis in AML as well as in juvenile myelomonocytic leukemia (JMML) [144–147]. Since RAS activation is dependent on post-translational farnesylation [148],

farnesyltransferase inhibitors (FTIs) have been investigated as a potential RAS-targeted therapy for AML [149–153]. A COG pediatric Phase I trial of tipifarnib (R115777, Zarnestra™), a potent FTI, for children with refractory leukemias has been completed, demonstrating tolerability at a dose sufficient to inhibit farnesyltransferase activity in leukemic blasts. A randomized study of tipifarnib in the postallogeneic SCT setting for pediatric patients with relapsed AML is being planned by the COG. An interesting finding in studies of tipifarnib in adult AML is that neither RAS mutations nor baseline activation of RAS-dependent signaling pathways (e.g., ERK or AKT) were correlated with clinical response, suggesting that the antileukemic activity of tipifarnib may be mediated through modulation of other farnesylated proteins or through mechanisms independent of effects on farnesyl transferase activity [149,150,153].

Notch/γ-secretase inhibitors (ALL)

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10–15% of childhood ALL and these patients require more intensive therapy to achieve cure. The NOTCH1 pathway has a crucial role in the embryonic development of the hematopoietic system and is critical to T-cell differentiation [154]. The NOTCH family is composed of four proteins that function as transmembrane heterodimeric receptors. Notch proteins are activated by ligands of the Delta–Serrate–Lag2 family located on neighboring cells. Two successive cleavage events, the first mediated by an ADAM metalloproteinase and the second by the γ-secretase complex, release the Notch intracellular domain. Active Notch associates with the DNA-binding CSL protein to function as a transcription activator.

The role of Notch in T-ALL was first suggested by the identification of the t(7;9)(q34;q34.3), which is rare in T-ALL but fuses the 3′ region of NOTCH1 to the TCRB locus, resulting in constitutive expression of intracellular active Notch protein [155]. More recently, over 50% of T-ALLs have been shown to harbor Notch mutations, either in the heterodimerization domain (HD) resulting in ligand-independent proteolytic cleavage or in the PEST domain preventing interaction with the F-box protein FBW7 [156]. Association of Notch with Fbw7 leads to ubiquitin-mediated degradation by the proteasome so that PEST mutations prolong the half-life of Notch. Approximately 26% of cases show Notch mutations occurring in the HD domain, 13% in PEST and 18% in both regions. Finally an additional 20% of patients show inactivating mutations in FBW7, creating a dominant-negative protein [157]. Thus, activation of the NOTCH pathway is a dominant feature of T-ALL.

Particularly novel agents of interest in T-cell ALL are inhibitors of γ-secretase, a multiprotein enzyme complex responsible for the cleavage and cytoplasmic release of various cellular proteins, including the NOTCH1 receptor and amyloid precursor proteins. In murine models, activated NOTCH1 results in the development of T-cell leukemia [158]. Although originally developed for the treatment of Alzheimer's disease based on their likely inhibition of amyloid peptide formation, inhibitors of γ-secretase have been studied in early-phase trials in patients with T-cell ALL [156]. While limited antileukemic activity and significant gastrointestinal toxicity have hampered clinical development to date, recent data have shown that both problems may be ameliorated by combining these agents with glucocorticoids [159]. Moreover, since Notch1 activation results in the downstream activation of the Myc and NF-κB pathways, simultaneous disruption of these pathways (e.g., with bortezomib, as described above) might also be considered [160,161].

Expert commentary

Childhood leukemia represents a diverse collection of biological subtypes. While subgroups of ALL and AML have been known for decades, the molecular basis of these subgroups and new, clinically relevant subgroups have been discovered through application of burgeoning

genetic technology. There is a clear and compelling rationale for developing therapies that specifically target the molecular abnormalities that cause leukemia. Such therapies hold the promise of being more effective and less toxic than the standard approaches using chemotherapy and stem cell transplantation. The successful treatment of chronic-phase CML with inhibitors of BCR–ABL hints at the possibilities for acute leukemia, but it is important to understand some fundamental differences between CML and acute leukemia. CML is a myeloproliferative disease caused by a single molecular abnormality. Acute leukemia, on the other hand, is a heterogeneous group of truly malignant hematopoietic tumors, each of which is caused not by one, but by multiple molecular abnormalities that differ substantially from patient to patient and even from clone to subclone within a given patient. Thus, the development of successful molecularly targeted therapies for acute leukemia will pose tremendous challenges and will require new paradigms of clinical and translational laboratory research.

Five-year view

High-throughput genomics has greatly increased the complexity of the biological information available, and it is crucial that ‘passenger’ lesions be distinguished from ‘driver’ mutations that contribute directly to maintenance of the malignant phenotype, with the latter prioritized for targeted therapies. Similarly, agents that target molecular pathways active in bulk leukemia cells, but not in the elusive and largely quiescent population of LSCs, may prove ineffective in terms of preventing relapse and improving long-term survival. In addition, it is clear that individual patients will need to be treated with a combination of therapeutic agents. Incorporating a single molecularly targeted agent into a standard chemotherapy backbone is complex. The complexity will only increase as combinations of two or more molecularly targeted therapies are contemplated. Finally, there are particular challenges associated with clinical evaluation of molecularly targeted agents. One of these is to distinguish the toxicity attributable to the addition of a molecularly targeted agent from the toxicity of the backbone of intensive chemotherapy. Another is to determine an appropriate patient population in which to study each individual agent. Since acute leukemia is a collection of molecularly heterogeneous diseases, each agent would be expected to be effective for a subset of patients. While it seems reasonable to test a novel agent only in the patients who can be predicted to have a high likelihood of responding, clinical trials for subsets of patients with a relatively rare disease, such as childhood leukemia, will be difficult to power sufficiently using standard trial designs. As new potential targets are identified, rapid preclinical validation is required to select the most promising agents for therapeutic testing. New paradigms for clinical trials are needed to rapidly determine the effectiveness of new agents. Despite the challenges, there has been real progress in the development of molecularly targeted agents for childhood acute leukemia. We are hopeful that this progress will accelerate and will lead to improved outcomes for these children.

Key issues

- Despite undeniable progress, the current treatment for childhood leukemia results in relapse and/or significant morbidity for many patients.
- Further improvements in leukemia treatment will probably result from the addition of novel drugs targeting specific molecular pathways that drive the development and maintenance of leukemia.
- Targeted drugs have the potential of being more effective and less toxic than conventional cytotoxic chemotherapy drugs.
- The discovery of recurrent chromosomal translocations in leukemia paved the way for the development of the first molecularly targeted drugs.

- High-throughput, genome-wide analytical tools are revealing the complex molecular basis of specific subtypes of leukemia, facilitating the development of novel targeted treatments.
- A limited number of targeted agents are being tested in clinical trials for children with leukemia, including monoclonal antibodies, tyrosine kinase inhibitors, proteasome inhibitors, mTOR inhibitors and epigenetic modulators.
- The successful clinical development of targeted agents faces many challenges, including proper target identification, adequate targeting of leukemia stem cells, developing synergistic and tolerable combinations of agents and designing adequately powered clinical trials to test efficacy in molecularly defined subsets of patients.
- In the era of targeted therapy, new paradigms will be needed to efficiently evaluate and incorporate molecularly targeted drugs for the treatment of childhood acute leukemia.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med 2004;350(15):1535–1548. [PubMed: 15071128]
2. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med 2006;354(2):166–178. [PubMed: 16407512]
3. Moricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 2008;111(9):4477–4489. [PubMed: 18285545]
4. Nguyen K, Devidas M, Cheng SC, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. Leukemia 2008;22(12):2142–2150. [PubMed: 18818707]
5. Pui CH, Cheng C, Leung W, et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. N Engl J Med 2003;349(7):640–649. [PubMed: 12917300]
6. Mody R, Li S, Dover DC, Sallan S, et al. Twenty-five-year follow-up among survivors of childhood acute lymphoblastic leukemia: a report from the Childhood Cancer Survivor Study. Blood 2008;111(12):5515–5523. [PubMed: 18334672]
7. Rubnitz JE. Childhood acute myeloid leukemia. Curr Treat Options Oncol 2008;9(1):95–105. [PubMed: 18506629]
8. Oliansky DM, Rizzo JD, Aplan PD, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute myeloid leukemia in children: an evidence-based review. Biol Blood Marrow Transplant 2007;13(1):1–25. [PubMed: 17222748]
9. Mulrooney DA, Dover DC, Li S, et al. Twenty years of follow-up among survivors of childhood and young adult acute myeloid leukemia: a report from the Childhood Cancer Survivor Study. Cancer 2008;112(9):2071–2079. [PubMed: 18327823]
10. Meshinchi S, Arceci RJ. Prognostic factors and risk-based therapy in pediatric acute myeloid leukemia. Oncologist 2007;12(3):341–355. [PubMed: 17405900]
11••. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3(7):730–737. Seminal paper demonstrating that phenotypically defined subsets of primary acute myeloid leukemia (AML) samples have differential capacities to engraft immunodeficient mice. This led to a renewed interest in the concept of leukemia stem cells, and cancer stem cells in general. [PubMed: 9212098]

12. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. Nature 2005;435 (7046):1267–1270. [PubMed: 15988530]

13. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. Blood 2002;99(1):319–325. [PubMed: 11756187]

14. Grimwade D, Enver T. Acute promyelocytic leukemia: where does it stem from? Leukemia 2004;18 (3):375–384. [PubMed: 14737069]

15. le Viseur C, Hotfilder M, Bomken S, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell 2008;14(1): 47–58. [PubMed: 18598943]

16. Cox CV, Evely RS, Oakhill A, Pamphilon DH, Goulden NJ, Blair A. Characterization of acute lymphoblastic leukemia progenitor cells. Blood 2004;104(9):2919–2925. [PubMed: 15242869]

17. Castor A, Nilsson L, Astrand-Grundstrom I, et al. Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia. Nat Med 2005;11(6):630–637. [PubMed: 15908956]

18. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. Blood 2004;104(12):3679–3687. [PubMed: 15226186]

19. Ross ME, Zhou X, Song G, et al. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood 2003;102(8):2951–2959. [PubMed: 12730115]

20••. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1(2):133–143. First demonstration of the potential utility of global gene-expression analysis in improving the biological understanding of childhood acute leukemia. [PubMed: 12086872]

21. Holleman A, den Boer ML, de Menezes RX, et al. The expression of 70 apoptosis genes in relation to lineage, genetic subtype, cellular drug resistance, and outcome in childhood acute lymphoblastic leukemia. Blood 2006;107(2):769–776. [PubMed: 16189266]

22. Cario G, Stanulla M, Fine BM, et al. Distinct gene expression profiles determine molecular treatment response in childhood acute lymphoblastic leukemia. Blood 2005;105(2):821–826. [PubMed: 15388585]

23. Bhojwani D, Kang H, Menezes RX, et al. Gene expression signatures predictive of early response and outcome in high-risk childhood acute lymphoblastic leukemia: a Children's Oncology Group Study [corrected]. J Clin Oncol 2008;26(27):4376–4384. [PubMed: 18802149]

24••. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446(7137):758–764. First genome-wide analysis of copy number alterations in childhood acute lymphoblastic leukemia (ALL), revealing frequent deletions in genes important in early B-cell development. [PubMed: 17344859]

25. Mullighan CG, Su X, Zhang J, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. N Engl J Med 2009;360(5):470–480. [PubMed: 19129520]

26. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 2009;10 (2):125–134. [PubMed: 19138562]

27. Yang JJ, Bhojwani D, Yang W, et al. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. Blood 2008;112(10): 4178–4183. [PubMed: 18768390]

28. Galm O, Herman JG, Baylin SB. The fundamental role of epigenetics in hematopoietic malignancies. Blood Rev 2006;20(1):1–13. [PubMed: 16426940]

29. Garzon R, Croce CM. MicroRNAs in normal and malignant hematopoiesis. Curr Opin Hematol 2008;15(4):352–358. [PubMed: 18536574]

30. Weinstein IB. Cancer. Addiction to oncogenes – the Achilles heal of cancer. Science 2002;297(5578): 63–64. [PubMed: 12098689]

31. Cheson BD, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N Engl J Med 2008;359(6):613–626. [PubMed: 18687642]

32. Freeman SD, Kelm S, Barber EK, Crocker PR. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. Blood 1995;85(8):2005–2012. [PubMed: 7718872]

33. Brashem-Stein C, Flowers DA, Smith FO, Staats SJ, Andrews RG, Bernstein ID. Ontogeny of hematopoietic stem cell development: reciprocal expression of CD33 and a novel molecule by maturing myeloid and erythroid progenitors. Blood 1993;82(3):792–799. [PubMed: 8338945]
34. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. J Clin Oncol 2001;19(13):3244–3254. [PubMed: 11432892]
35. Larson RA, Sievers EL, Stadtmauer EA, et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer 2005;104(7):1442–1452. [PubMed: 16116598]
36. Arceci RJ, Sande J, Lange B, et al. Safety and efficacy of gemtuzumab ozogamicin in pediatric patients with advanced $CD33^+$ acute myeloid leukemia. Blood 2005;106(4):1183–1188. [PubMed: 15886328]
37. Zwaan CM, Reinhardt D, Corbacioglu S, et al. Gemtuzumab ozogamicin: first clinical experiences in children with relapsed/refractory acute myeloid leukemia treated on compassionate-use basis. Blood 2003;101(10):3868–3871. [PubMed: 12543868]
38. Aplenc R, Alonzo TA, Gerbing RB, et al. Safety and efficacy of gemtuzumab ozogamicin in combination with chemotherapy for pediatric acute myeloid leukemia: a report from the Children's Oncology Group. J Clin Oncol 2008;26(14):2390–3295. [PubMed: 18467731]
39. Franklin J, Alonzo TA, Hurwitz CA, et al. COG AAML03P1: efficacy and safety in a pilot study of intensive chemotherapy including gemtuzumab in children newly diagnosed with acute myeloid leukemia (AML). Blood 2008;112(11):136a.
40. Linenberger ML, Hong T, Flowers D, et al. Multidrug-resistance phenotype and clinical responses to gemtuzumab ozogamicin. Blood 2001;98(4):988–994. [PubMed: 11493443]
41. Becton D, Dahl GV, Ravindranath Y, et al. Randomized use of cyclosporin A (CsA) to modulate P-glycoprotein in children with AML in remission: Pediatric Oncology Group Study 9421. Blood 2006;107(4):1315–1324. [PubMed: 16254147]
42. Hauswirth AW, Florian S, Printz D, et al. Expression of the target receptor CD33 in $CD34^+/CD38^-/CD123^+$ AML stem cells. Eur J Clin Invest 2007;37(1):73–82. [PubMed: 17181570]
43. Florian S, Sonneck K, Hauswirth AW, et al. Detection of molecular targets on the surface of $CD34^+/CD38^-$ stem cells in various myeloid malignancies. Leuk Lymphoma 2006;47(2):207–222. [PubMed: 16321850]
44. Taussig DC, Pearce DJ, Simpson C, et al. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood 2005;106(13):4086–4092. [PubMed: 16131573]
45. Estey EH, Giles FJ, Beran M, et al. Experience with gemtuzumab ozogamycin ("mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia. Blood 2002;99(11):4222–4224. [PubMed: 12010830]
46. Lo-Coco F, Cimino G, Breccia M, et al. Gemtuzumab ozogamicin (Mylotarg) as a single agent for molecularly relapsed acute promyelocytic leukemia. Blood 2004;104(7):1995–1999. [PubMed: 15187030]
47. Cesano A, Gayko U. CD22 as a target of passive immunotherapy. Semin Oncol 2003;30(2):253–257. [PubMed: 12720147]
48. Leung SO, Goldenberg DM, Dion AS, et al. Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. Mol Immunol 1995;32(17–18):1413–1427. [PubMed: 8643111]
49. Carnahan J, Wang P, Kendall R, et al. Epratuzumab, a humanized monoclonal antibody targeting CD22: characterization of *in vitro* properties. Clin Cancer Res 2003;9(10 Pt 2):3982S–3890S. [PubMed: 14506197]
50. Leonard JP, Coleman M, Ketas JC, et al. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. J Clin Oncol 2003;21(16):3051–3059. [PubMed: 12837807]
51. Leonard JP, Coleman M, Ketas JC, et al. Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: Phase I/II clinical trial results. Clin Cancer Res 2004;10(16):5327–5334. [PubMed: 15328168]

52. Leonard JP, Coleman M, Ketas J, et al. Combination antibody therapy with epratuzumab and rituximab in relapsed or refractory non-Hodgkin's lymphoma. J Clin Oncol 2005;23(22):5044–5051. [PubMed: 15955901]
53. Strauss SJ, Morschhauser F, Rech J, et al. Multicenter Phase II trial of immunotherapy with the humanized anti-CD22 antibody, epratuzumab, in combination with rituximab, in refractory or recurrent non-Hodgkin's lymphoma. J Clin Oncol 2006;24(24):3880–3886. [PubMed: 16864854]
54. Micallef IN, Kahl BS, Maurer MJ, et al. A pilot study of epratuzumab and rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy in patients with previously untreated, diffuse large B-cell lymphoma. Cancer 2006;107(12):2826–2832. [PubMed: 17099879]
55. Raetz EA, Cairo MS, Borowitz MJ, et al. Chemoimmunotherapy reinduction with epratuzumab in children with acute lymphoblastic leukemia in marrow relapse: a Children's Oncology Group Pilot Study. J Clin Oncol 2008;26(22):3756–3762. [PubMed: 18669463]
56. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. Phase I trial of recombinant immunotoxin RFB4 (dsFv)-PE38 (BL22) in patients with B-cell malignancies. J Clin Oncol 2005;23(27):6719–6729. [PubMed: 16061911]
57. Kreitman RJ, Wilson WH, Bergeron K, et al. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. N Engl J Med 2001;345(4):241–247. [PubMed: 11474661]
58. Bang S, Nagata S, Onda M, Kreitman RJ, Pastan I. HA22 (R490A) is a recombinant immunotoxin with increased antitumor activity without an increase in animal toxicity. Clin Cancer Res 2005;11 (4):1545–1550. [PubMed: 15746059]
59. Onda M, Beers R, Xiang L, Nagata S, Wang QC, Pastan I. An immunotoxin with greatly reduced immunogenicity by identification and removal of B cell epitopes. Proc Natl Acad Sci USA 2008;105 (32):11311–11316. [PubMed: 18678888]
60. Weldon JE, Xiang L, Chertov O, et al. A protease-resistant immunotoxin against CD22 with greatly increased activity against CLL and diminished animal toxicity. Blood. 2008 DOI:blood-2008-08-173195v1. (Epub ahead of print).
61. Herrera L, Farah RA, Pellegrini VA, et al. Immunotoxins against CD19 and CD22 are effective in killing precursor-B acute lymphoblastic leukemia cells *in vitro*. Leukemia 2000;14(5):853–858. [PubMed: 10803517]
62. Herrera L, Yarbrough S, Ghetie V, Aquino DB, Vitetta ES. Treatment of SCID/human B cell precursor ALL with anti-CD19 and anti-CD22 immunotoxins. Leukemia 2003;17(2):334–338. [PubMed: 12592332]
63. Messmann RA, Vitetta ES, Headlee D, et al. A Phase I study of combination therapy with immunotoxins IgG-HD37-deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19($^{+}$), CD22($^{+}$) B cell lymphoma. Clin Cancer Res 2000;6(4):1302–1313. [PubMed: 10778955]
64. Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med 2000;342(14):998–1006. [PubMed: 10749961]
65. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR–ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344(14):1031–1037. [PubMed: 11287972]
66. Gorre ME, Sawyers CL. Molecular mechanisms of resistance to STI571 in chronic myeloid leukemia. Curr Opin Hematol 2002;9(4):303–307. [PubMed: 12042704]
67•. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344(14):1038–1042. First demonstration of the clinical activity of a rationally identified, molecularly targeted agent in acute leukemia. [PubMed: 11287973]
68. Ottmann OG, Druker BJ, Sawyers CL, et al. A Phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 2002;100(6):1965–1971. [PubMed: 12200353]

69. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. Blood 2004;103(12):4396–4407. [PubMed: 14551133]

70. Lee KH, Lee JH, Choi SJ, et al. Clinical effect of imatinib added to intensive combination chemotherapy for newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Leukemia 2005;19(9):1509–1516. [PubMed: 16034462]

71••. Schultz KR, Bowman WP, Aledo A, et al. Improved early event free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a Children's Oncology Group study. J Clin Oncol. 2009 (In Press). A Children's Oncology Group clinical trial shows marked improvement in 3-year event-free survival for children with Philadelphia chromosome-positive ALL by adding imatinib to standard chemotherapy.

72. Lee S, Kim YJ, Min CK, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2005;105(9):3449–3457. [PubMed: 15657178]

73. Hu Y, Liu Y, Pelletier S, Buchdunger E, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR–ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet 2004;36(5): 453–461. [PubMed: 15098032]

74. O'Hare T, Walters DK, Stoffregen EP, et al. *In vitro* activity of Bcr–Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res 2005;65(11):4500–4505. [PubMed: 15930265]

75. Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. Proc Natl Acad Sci USA 2005;102(31):11011–11016. [PubMed: 16046538]

76. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305(5682):399–401. [PubMed: 15256671]

77. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 2006;354(24):2531–2541. [PubMed: 16775234]

78. Brown P, Small D. FLT3 inhibitors: a paradigm for the development of targeted therapeutics for paediatric cancer. Eur J Cancer 2004;40(5):707–721. [PubMed: 15010072]

79. Levis M, Small D. FLT3 tyrosine kinase inhibitors. Int J Hematol 2005;82(2):100–107. [PubMed: 16146839]

80. Carow CE, Levenstein M, Kaufmann SH, et al. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. Blood 1996;87(3):1089–1096. [PubMed: 8562934]

81. Kiyoi H, Towatari M, Yokota S, et al. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia 1998;12(9): 1333–1337. [PubMed: 9737679]

82. Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood 2001;97(1):89–94. [PubMed: 11133746]

83. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001;97(8):2434–2439. [PubMed: 11290608]

84. Iwai T, Yokota S, Nakao M, et al. Internal tandem duplication of the *FLT3* gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. Leukemia 1999;13(1):38–43. [PubMed: 10049058]

85. Kondo M, Horibe K, Takahashi Y, et al. Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. Med Pediatr Oncol 1999;33(6):525–529. [PubMed: 10573574]

86. Meshinchi S, Alonzo TA, Stirewalt DL, et al. Clinical implications of *FLT3* mutations in pediatric AML. Blood 2006;108(12):3654–3661. [PubMed: 16912228]

87. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 2002;99(12):4326–4335. [PubMed: 12036858]

88. Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication

of FLT3: a cancer and leukemia group B study. Cancer Res 2001;61(19):7233–7239. [PubMed: 11585760]

89•. Levis M, Allebach J, Tse KF, et al. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells *in vitro* and *in vivo*. Blood 2002;99(11):3885–3891. Important preclinical demonstration of selective activity of FLT3 inhibitors for AML patient samples harboring activating mutations in FLT3. [PubMed: 12010785]

90. Brown P, Meshinchi S, Levis M, et al. Pediatric AML primary samples with FLT3/ITD mutations are preferentially killed by FLT3 inhibition. Blood 2004;104(6):1841–1849. [PubMed: 15166029]

91. Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. Blood 2004;103 (10):3669–3676. [PubMed: 14726387]

92. Knapper S, Burnett AK, Littlewood T, et al. A Phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. Blood 2004;108(10):3262–3270. [PubMed: 16857985]

93. Levis M, Smith BD, Beran M, et al. A randomized, open-label study of lestaurtinib (CEP-701), an oral FLT3 inhibitor, administered in sequence with chemotherapy in patients with relapsed AML harboring FLT3 activating mutations: clinical response correlates with successful FLT3 inhibition. ASH Annual Meeting Abstracts 2005;106(11):403.

94. Levis M, Pham R, Smith BD, Small D. *In vitro* studies of a FLT3 inhibitor combined with chemotherapy: sequence of administration is important to achieve synergistic cytotoxic effects. Blood 2004;104(4):1145–1150. [PubMed: 15126317]

95. Brown P, Levis M, McIntyre E, Griesemer M, Small D. Combinations of the FLT3 inhibitor CEP-701 and chemotherapy synergistically kill infant and childhood MLL-rearranged ALL cells in a sequence-dependent manner. Leukemia 2006;20(8):1368–1376. [PubMed: 16761017]

96. Kottaridis PD, Gale RE, Langabeer SE, Frew ME, Bowen DT, Linch DC. Studies of FLT3 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia: implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection, and possible therapy with FLT3 inhibitors. Blood 2002;100(7):2393–2398. [PubMed: 12239147]

97. Levis M, Murphy KM, Pham R, et al. Internal tandem duplications of the FLT3 gene are present in leukemia stem cells. Blood 2005;106(2):673–680. [PubMed: 15797998]

98. Pollard JA, Alonzo TA, Gerbing RB, et al. FLT3 internal tandem duplication in $CD34^{+}/CD33^{-}$ precursors predicts poor outcome in acute myeloid leukemia. Blood 2006;108(8):2764–2769. [PubMed: 16809615]

99•. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002;30(1):41–47. Gene-expression profiling identifies *FLT3* overexpression to be highly characteristic of leukemias with rearrangements of the *MLL* gene. [PubMed: 11731795]

100. Armstrong SA, Mabon ME, Silverman LB, et al. FLT3 mutations in childhood acute lymphoblastic leukemia. Blood 2004;103(9):3544–3546. [PubMed: 14670924]

101. Zheng R, Levis M, Piloto O, et al. FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. Blood 2004;103(1):267–274. [PubMed: 12969963]

102•. Brown P, Levis M, Shurtleff S, Campana D, Downing J, Small D. FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood 2005;105 (2):812–820. Important preclinical demonstration of selective activity of FLT3 inhibitors for ALL patient samples with marked overexpression and autocrine activation of wild-type FLT3. [PubMed: 15374878]

103. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell 2003;3(2):173–183. [PubMed: 12620411]

104. Stam RW, den Boer ML, Schneider P, et al. Targeting FLT3 in primary *MLL*-gene-rearranged infant acute lymphoblastic leukemia. Blood 2005;106(7):2484–2490. [PubMed: 15956279]

105. Taketani T, Taki T, Sugita K, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. Blood 2004;103(3):1085–1088. [PubMed: 14504097]

106. Pyatt DW, Stillman WS, Yang Y, Gross S, Zheng JH, Irons RD. An essential role for NF-κB in human CD34^{+} bone marrow cell survival. Blood 1999;93(10):3302–3308. [PubMed: 10233882]
107. Guzman ML, Neering SJ, Upchurch D, et al. Nuclear factor-κB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 2001;98(8):2301–2307. [PubMed: 11588023]
108. Guzman ML, Swiderski CF, Howard DS, et al. Preferential induction of apoptosis for primary human leukemic stem cells. Proc Natl Acad Sci USA 2002;10, 99(25):16220–16225. [PubMed: 12451177]
109. Cortes J, Thomas D, Koller C, et al. Phase I study of bortezomib in refractory or relapsed acute leukemias. Clin Cancer Res 2004;10(10):3371–3376. [PubMed: 15161691]
110. Orlowski RZ, Voorhees PM, Garcia RA, et al. Phase 1 trial of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies. Blood 2004;105(8):3058–3065. [PubMed: 15626743]
111. Horton TM, Pati D, Plon SE, et al. A Phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. Clin Cancer Res 2007;13(5):1516–1522. [PubMed: 17332297]
112. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the proteasome inhibitor bortezomib by the pediatric preclinical testing program. Pediatr Blood Cancer 2008;50(1):37–45. [PubMed: 17420992]
113. Horton TM, Gannavarapu A, Blaney SM, D'Argenio DZ, Plon SE, Berg SL. Bortezomib interactions with chemotherapy agents in acute leukemia *in vitro*. Cancer Chemother Pharmacol 2006;58(1): 13–23. [PubMed: 16292537]
114. Messinger YH, Gaynon PS, Raetz EA, et al. Remarkable activity of bortezomib combined with chemotherapy in a Phase I study of relapsed childhood acute lymphoblastic leukemia. Blood 2008;112(11):1919a.
115. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon α, or both for advanced renal-cell carcinoma. N Engl J Med 2007;356(22):2271–2281. [PubMed: 17538086]
116. Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood 2003;102(3):972–980. [PubMed: 12702506]
117. Min YH, Eom JI, Cheong JW, et al. Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. Leukemia 2003;17(5):995–997. [PubMed: 12750723]
118. Recher C, Beyne-Rauzy O, Demur C, et al. Antileukemic activity of rapamycin in acute myeloid leukemia. Blood 2005;105(6):2527–2534. [PubMed: 15550488]
119. Yee KW, Zeng Z, Konopleva M, et al. Phase I/II study of the mammalian target of rapamycin inhibitor everolimus (RAD001) in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res 2006;12(17):5165–5173. [PubMed: 16951235]
120. Brown VI, Fang J, Alcorn K, et al. Rapamycin is active against B-precursor leukemia *in vitro* and *in vivo*, an effect that is modulated by IL-7-mediated signaling. Proc Natl Acad Sci USA 2003;100 (25):15113–15118. [PubMed: 14657335]
121. Teachey DT, Sheen C, Hall J, et al. mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. Blood 2008;112(5):2020–2023. [PubMed: 18544682]
122. Wei G, Twomey D, Lamb J, et al. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell 2006;10(4):331–342. [PubMed: 17010674]
123. Herman JG, Civin CI, Issa JP, Collector MI, Sharkis SJ, Baylin SB. Distinct patterns of inactivation of p15INK4B and p16INK4A characterize the major types of hematological malignancies. Cancer Res 1997;57(5):837–841. [PubMed: 9041182]
124. Seedhouse CH, Das-Gupta EP, Russell NH. Methylation of the hMLH1 promoter and its association with microsatellite instability in acute myeloid leukemia. Leukemia 2003;17(1):83–88. [PubMed: 12529664]
125. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358(11):1148–1159. [PubMed: 18337604]
126. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128(4):683–692. [PubMed: 17320506]
127. Gore SD. Combination therapy with DNA methyltransferase inhibitors in hematologic malignancies. Nat Clin Pract Oncol 2005;2(Suppl 1):S30–S35. [PubMed: 16341238]

128. Friedman AD. Leukemogenesis by CBF oncoproteins. Leukemia 1999;13(12):1932–1942. [PubMed: 10602413]
129. Tenen DG, Hromas R, Licht JD, Zhang DE. Transcription factors, normal myeloid development, and leukemia. Blood 1997;90(2):489–519. [PubMed: 9226149]
130. Liu S, Shen T, Huynh L, et al. Interplay of RUNX1/MTG8 and DNA methyltransferase 1 in acute myeloid leukemia. Cancer Res 2005;65(4):1277–1284. [PubMed: 15735013]
131. Liu S, Klisovic RB, Vukosavljevic T, et al. Targeting AML1/ETO-histone deacetylase repressor complex: a novel mechanism for valproic acid-mediated gene expression and cellular differentiation in AML1/ETO-positive acute myeloid leukemia cells. J Pharmacol Exp Ther 2007;321(3):953–960. [PubMed: 17389244]
132. Klisovic MI, Maghraby EA, Parthun MR, et al. Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells. Leukemia 2003;17(2):350–358. [PubMed: 12592335]
133. Grignani F, De Matteis S, Nervi C, et al. Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. Nature 1998;391(6669):815–818. [PubMed: 9486655]
134. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 1998;19, 391(6669):811–814. [PubMed: 9486654]
135. Di Croce L, Raker VA, Corsaro M, et al. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 2002;295(5557):1079–1082. [PubMed: 11834837]
136. Heibert SW, Lutterbach B, Durst K, et al. Mechanisms of transcriptional repression by the t(8;21)-, t(12;21)-, and inv(16)-encoded fusion proteins. Cancer Chemother Pharmacol 2001;48(Suppl 1):S31–S34. [PubMed: 11587363]
137. Whitman SP, Liu S, Vukosavljevic T, et al. The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. Blood 2005;106(1):345–352. [PubMed: 15774615]
138. Dorrance AM, Liu S, Yuan W, et al. Mll partial tandem duplication induces aberrant Hox expression *in vivo* via specific epigenetic alterations. J Clin Invest 2006;116(10):2707–2716. [PubMed: 16981007]
139. So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. Cancer Cell 2003;4(2):99–110. [PubMed: 12957285]
140. Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. Cancer Res 2006;66(12):6361–6369. [PubMed: 16778214]
141. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, et al. Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. Blood 2006;108(10):3271–3279. [PubMed: 16882711]
142. Case M, Matheson E, Minto L, et al. Mutation of genes affecting the RAS pathway is common in childhood acute lymphoblastic leukemia. Cancer Res 2008;68(16):6803–6809. [PubMed: 18701506]
143. Meshinchi S, Stirewalt DL, Alonzo TA, et al. Activating mutations of RTK/ras signal transduction pathway in pediatric acute myeloid leukemia. Blood 2003;102(4):1474–1479. [PubMed: 12702504]
144. MacKenzie KL, Dolnikov A, Millington M, Shounan Y, Symonds G. Mutant N-ras induces myeloproliferative disorders and apoptosis in bone marrow repopulated mice. Blood 1999;93(6):2043–2056. [PubMed: 10068678]
145. Loh ML, Vattikuti S, Schubbert S, et al. Mutations in *PTPN11* implicate the SHP-2 phosphatase in leukemogenesis. Blood 2004;103(6):2325–2331. [PubMed: 14644997]
146. Emanuel PD, Snyder RC, Wiley T, Gopurala B, Castleberry RP. Inhibition of juvenile myelomonocytic leukemia cell growth *in vitro* by farnesyltransferase inhibitors. Blood 2000;95(2):639–645. [PubMed: 10627474]
147. Emanuel PD. Juvenile myelomonocytic leukemia. Curr Hematol Rep 2004;3(3):203–209. [PubMed: 15087069]

148. Khosravi-Far R, Cox AD, Kato K, Der CJ. Protein prenylation: key to ras function and cancer intervention? Cell Growth Differ 1992;3(7):461–469. [PubMed: 1419908]

149. Harousseau JL, Lancet JE, Reiffers J, et al. A Phase 2 study of the oral farnesyltransferase inhibitor tipifarnib in patients with refractory or relapsed acute myeloid leukemia. Blood 2007;109(12):5151–5156. [PubMed: 17351110]

150. Lancet JE, Gojo I, Gotlib J, et al. A Phase 2 study of the farnesyltransferase inhibitor tipifarnib in poor-risk and elderly patients with previously untreated acute myelogenous leukemia. Blood 2007;109(4):1387–1394. [PubMed: 17082323]

151. Zimmerman TM, Harlin H, Odenike OM, et al. Dose-ranging pharmacodynamic study of tipifarnib (R115777) in patients with relapsed and refractory hematologic malignancies. J Clin Oncol 2004;22 (23):4816–4822. [PubMed: 15570084]

152. Cortes J, Albitar M, Thomas D, et al. Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. Blood 2003;101(5):1692–1697. [PubMed: 12411300]

153. Karp JE, Lancet JE, Kaufmann SH, et al. Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a Phase 1 clinical-laboratory correlative trial. Blood 2001;97(11):3361–3369. [PubMed: 11369625]

154. Radtke F, Wilson A, MacDonald HR. Notch signaling in T- and B-cell development. Curr Opin Immunol 2004;16(2):174–179. [PubMed: 15023410]

155. Ellisen LW, Bird J, West DC, et al. TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991;66(4):649–661. [PubMed: 1831692]

156. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306(5694):269–271. [PubMed: 15472075]

157. O'Neil J, Grim J, Strack P, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to γ-secretase inhibitors. J Exp Med 2007;204(8):1813–1824. [PubMed: 17646409]

158. Pear WS, Aster JC, Scott ML, et al. Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. J Exp Med 1996;183(5):2283–2291. [PubMed: 8642337]

159. Real PJ, Tosello V, Palomero T, et al. γ-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. Nat Med 2009;15(1):50–58. [PubMed: 19098907]

160. Vilimas T, Mascarenhas J, Palomero T, et al. Targeting the NF-κB signaling pathway in Notch1-induced T-cell leukemia. Nat Med 2007;13(1):70–77. [PubMed: 17173050]

161. Palomero T, Lim WK, Odom DT, et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci USA 2006;103(48):18261–18266. [PubMed: 17114293]

Table 1

Summary of molecularly targeted agents in clinical development for childhood acute leukemia.

Class	Molecular target	Agent(s)	Disease	Subset	Phase
Monoclonals	CD33	Gemtuzumab ozogamicin	AML		III
	CD22	Epratuzumab	ALL	$CD22^{+}$	II
TKIs	BCR–ABL	Imatinib, dasatinib	ALL	Ph+	II
	FLT3	Lestaurtinib	AML	FLT3 mutated	II
			ALL	MLL rearranged	II
Proteasome inhibitors	NF-κB	Bortezomib	AML, ALL		I
mTOR inhibitors	mTOR	Sirolimus, temsirolimus	AML, ALL		I
Epigenetic modulators	HDAC	Vorinostat, entinostat	AML, ALL		I
	DNA methyltransferase	Azacitidine, decitabine	AML, ALL		I
FTI	Farnesyltransferase	Tipifarnib	AML, ALL	RAS (or upstream) mutated	I
Notch inhibitors	γ-secretase	MK0752, LY450139	ALL	T-cell, Notch-mutant	I

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; FTI: Farnesyltransferase inhibitor; HDAC: Histone deacetylase; NF: Nuclear factor; Ph: Philadelphia chromosome; TKI: Tyrosine kinase inhibitor.