CLINICAL INVESTIGATION

Pharmacotherapy for adult acute lymphoblastic leukemia: an update from recent clinical trials and future directions

Clin. Invest. (2012) 2(7), 715–731

Brian L Burnette, Mrinal S Patnaik & Mark R Litzow*

Division of Hematology, Mayo Clinic Foundation, 200 First Street SW, Rochester, MN 55905, USA
*Author for correspondence:
Tel.: +1 507 284 2511
Fax: +1 507 266 4972
E-mail: litzow.mark@mayo.edu

In adults, acute lymphoblastic leukemia (ALL) is an aggressive malignancy that, while initially highly responsive to chemotherapy, has a high relapse rate and poor survival. The role of allogeneic transplantation in ALL, the use of pediatric strategies in adolescents and young adults, the administration of rituximab for CD20-positive B-lineage ALL, tyrosine kinase inhibitors for Philadelphia chromosome-positive ALL, and nelarabine for T-lineage ALL will be discussed in this article. Promising agents reported in early clinical trials since 2010 are herein highlighted, including antibody-drug and antibody-immunotoxins; blinatumomab (bispecific T-cell engaging antibody); second- and third-generation tyrosine kinase inhibitors; mTOR inhibitors; bortezomib (a proteasome inhibitor); liposomal vincristine (reformulated chemotherapy); and decitabine (a hypomethylating agent). Progress has been made over the last few years in the development of novel therapeutics in ALL and appears to be setting the stage for even greater progress over the next 5–10 years.

Keywords: acute lymphoblastic leukemia • allogeneic • blinatumomab • dasatinib • epratuzumab • imatinib • inotuzumab • nelarabine • Philadelphia chromosome • rituximab • sirolimus

Acute lymphoblastic leukemia (ALL) is an aggressive lymphoid malignancy with a bimodal age distribution, with peak incidences in children 2–5 years of age and in adults over the age of 50 years. It is the most common pediatric hematologic malignancy, while in adults ALL is a rare disorder. In addition, while complete hematologic remission rates are as high as 90–95% in adults and children, increased relapse rates have made the overall survival dramatically inferior in adults. There are three distinct subgroups of ALL, including T-cell ALL (T-ALL), Philadelphia chromosome-positive B-cell ALL (PhALL), and Philadelphia chromosome-negative B-cell ALL (B-ALL), each of which have different biologic features and thus different treatment considerations. Significant progress has been made over the last few decades increasing the overall survival of adult patients with ALL from approximately 25–30%, perhaps to as high as 50–55% in recent studies [1–3].

It could be argued that there have been five distinct and substantial advances in pharmacotherapy for ALL over the past 5–10 years:

- Reporting of the largest international trial in ALL (MRC UKALLXII/ECOG2993) demonstrating allogeneic hematopoietic stem cell transplantation (AlloSCT) may result in improvement in overall survival (OS) and, furthermore, demonstrating that such a large multicenter, international trial is possible in a rare disease such as adult ALL [4];
- The use of pediatric-intensive regimens in young adults appears to significantly

FUTURE SCIENCE part of fsg

improve outcomes compared to traditional adult regimens [5–7];

- The demonstration that monoclonal antibody treatment of B-ALL is safe and effective;
- The incorporation of tyrosine kinase inhibitors (TKI) into the treatment of PhALL;
- The development of nelarabine for the treatment of relapsed/refractory T-ALL.

AlloSCT & the MRC UKALLXII/ECOG2993 trial

Only recently have comprehensive data been made available from the largest study ever performed for adult ALL. The study was a joint effort between the Medical Research Council in the UK and the Eastern Cooperative Oncology Group in the USA, and accrued patients with all subtypes of adult ALL from the early 1990s through the mid-2000s. Chemotherapy included two phases of induction; with phase one including daunorubicin, vincristine, L-asparaginase and prednisone; and phase two including cyclophosphamide, cytarabine, and 6-mercaptopurine. Intrathecal methotrexate was administered during induction. Further intensification was administered with three doses of methotrexate at 3 g/m^2 in combination with asparaginase. Assignment to allogeneic transplant (if a sibling donor was identified and the patient was considered eligible) or randomization to autologous transplantation or maintenance chemotherapy was carried out after intensification, respectively [4].

In 2008, Goldstone and colleagues reported the final results of the UKALLXII/ECOG2993 study [4]. A complete remission (CR1; for purposes of further discussion CR1 will note cytologic remission rather than molecular CR1, which we define as absence of disease, as assessed by fluorescence *in situ* hybridization or PCR) with induction therapy was achieved in 1351 of the 1646 B-ALL and T-ALL enrolled patients. Of these 1351 patients, 1031 had HLA typing data available. A survival benefit for AlloSCT was demonstrated in a donor versus no-donor analysis. For all patients with B-ALL and T-ALL, the 5-year OS was 53% in those with an HLA matched donor and 45% in those without (p = 0.01) [4].

Interestingly, in B-ALL and T-ALL the overall survival was only statistically significant in those patients considered standard risk (SR: 62 vs 52%; p = 0.02); but was not statistically significant in high-risk (HR) patients (white blood cell count >100 × 10^6/dl or age >35 years). However, the trend in HR appeared to favor patients with a donor (41 vs 35%; p = 0.2). In both groups there was a reduced risk of relapse in patients with donors (HR: 37 vs 63%; p<0.00005; SR: 24 vs 49%; p<0.00005). It is possible, given the reduced relapse rate in HR that with longer follow-up, a survival benefit will become apparent. Additionally, it is important to note that patients enrolled with CD20 positivity were not treated with rituximab (discussed below). PhALL was not discussed in detail; however, survival for PhALL was reported to be 22% at 5 years (this initial report included data prior to the incorporation of imatinib into the treatment regimen) [4].

A recent meta-analysis has demonstrated similar results as the UKALLXII/ECOG2993 study. It is important to note, however, that the meta-analysis was heavily weighted by the above mentioned study [8].

In 2007 a report from this group described the dire outcomes of patients with relapsed ALL. Of the 1372 patients in this report who achieved CR1 with induction therapy, 609 relapsed at a median of 11 months. OS at 1 year in relapsed patients was 22%. Even in patients who achieved another CR and underwent AlloSCT, the OS at 5 years was 16% with matched unrelated donors and 23% with matched sibling donors. OS with chemotherapy alone was quite poor with 4% of patients surviving at 5 years [9].

This largest assessment of survival in patients with relapsed adult ALL underscores several important issues for understanding the discussion of new therapeutics and strategies in this review. The first issue is that relapsed disease is highly resistant to currently available therapies and that once relapsed, patients will almost always die from leukemia or treatment complications, highlighting the unmet medical need and potential for drug development in the relapsed setting. On the other hand, with such initially chemotherapy-sensitive disease in the first-line setting and with such chemotherapy-insensitive disease in the relapsed/refractory setting, it is possible that agents with limited activity when assessed in relapsed/refractory settings may potentially be excluded from further investigation, when in reality, they could have substantial benefit in the chemotherapy-naive setting.

With further reporting of B-ALL as well as PhALL (discussed below) from the UKALLXII/ECOG2993 trial [10], there now appears to be evidence of the benefit of AlloSCT in eligible patients with compatible donors in CR1 when treated with conventional adult chemotherapy regimens. Ultimately however, this strategy may not be appropriate for adolescents and young adults (AYA) treated with conventional pediatric chemotherapy regimens. We acknowledge the strength of this evidence and the role of AlloSCT in CR1 is widely debated by international experts such that we do not, for the purposes of this review, draw firm conclusions for or against AlloSCT in CR1 for adults or AYAs.

As previously noted, AYA with ALL have historically had superior outcomes to adults for reasons that are not entirely clear. In a retrospective analysis, the Children's Cancer Group (CCG) and the Cancer and Leukemia Group B (CALGB) compared cohorts treated on ALL trials between the ages of 16 and 20 years. OS at 7 years appeared superior in patients treated on CCG protocols (67 vs 46%; p = 0.0002). The CCG and the CALGB protocols did not include AlloSCT in CR1. Major differences in the protocols included substantially higher doses of nonmyelosuppressive agents including L-asparaginase, dexamethasone and vincristine in the CCG protocols. CNS prophylaxis was also more aggressive in the CCG protocols [5]. Retrospective assessment by groups in the UK and in France, among others, have demonstrated similar results [6,7].

In 2009, Huguet *et al.* reported a Phase II trial assessing the use of a pediatric inspired treatment strategy for adult B-ALL [11]. The chemotherapy regimen utilized L-asparaginase, corticosteroid and vincristine doses more similar to typical pediatric regimens than adult ALL regimens. HR patients (B cell >30 × 10^9/l; T cell >100 × 10^9, HR cytogenetic abnormalities, corticosteroid resistant, chemotherapy resistant, absence of CR1 after induction, or high MRD at CR1) were offered AlloSCT in CR1, if a matched donor was identified (both unrelated and related). A total of 71 out of 139 HR patients underwent a per-protocol AlloSCT. The remainder were treated with chemotherapy alone. Patients >45 years of age did not tolerate therapy with increased induction mortality and increase mortality in CR1. However, in patients <45 years of age, prespecified end points appeared improved over previous adult protocols by the same group. Induction and consolidation deaths were low at 4 and 2%, respectively. 95% of patients achieved CR1. OS and relapse free survival at 42 months (RFS) were 66 and 58%, respectively, in patients <45 years of age [11]. A Spanish cooperative group demonstrated nearly identical results in 81 similar standard-risk patients [12]. The results of both prospective studies appear to support the retrospective observations noted above.

Clinical trials, including Intergroup Trial 10403, a combined effort between CALGB, the Eastern Cooperative Oncology Group and the Southwestern Oncology Group, is currently enrolling AYAs with B-ALL and T-ALL on a Phase II trial investigating a conventional pediatric regimen without AlloSCT. Results will ultimately be compared with historical cohorts. If similar findings of a superior OS are demonstrated in this trial, one could argue that, in AYAs treated with a conventional pediatric protocol, AlloSCT may not be appropriate in CR1.

A brief summary of articles we find to be of particular importance regarding AlloSCT and chemotherapy are included in Table 1.

Pharmacologic antibody-directed treatment of B-ALL

Before discussing currently available and investigational therapeutics directed at B cell surface markers, it is important to discuss targets of such agents. In this way, we may better understand their therapeutic potential.

While small analyses have described the prevalence and expression for several cell surface markers and targets for antibody directed therapy; comprehensive assessment has rarely been performed. Recently however, an Italian group presented data on 552 consecutive ALL patients in whom they assessed, by flow cytometry, the expression of CD19, CD20 and CD22. In B-ALL, all 451 patients with evaluable samples demonstrated CD19 expression of >20%, CD20 was expressed in 30.4% of patients and CD22 in 93% of patients [13].

In the mid-1990s drug development and the first-in-human studies of monoclonal antibodies directed at lymphocyte cell surface markers were ongoing. The promise of 'targeted' treatments for hematologic malignancies seemed to be within reach. The most promising agent during this period was a CD20 directed chimeric monoclonal antibody named rituximab. CD20 was felt to be an appropriate target given its near universal expression (as opposed to the lesser expression in B-ALL) in malignancies of mature B cells. Phase I and II studies in the mid- to late-1990s confirmed single agent activity as well as additive/synergistic activity in combination with chemotherapy [14–16]. Within 10–15 years from initial Phase I studies, rituximab has become a standard of care for initial and subsequent therapy of indolent and aggressive B-cell lymphomas [17–25].

With the decreased expression of CD20 in B-ALL as noted above, evidence for the use of rituximab in this population has developed more slowly and has only recently been reported.

The use of rituximab for B-ALL has largely been in combination with Hyper-CVAD. In a retrospective analysis at a single center, cohorts of patients with B-ALL with CD20 expression of >20% were assessed for response, complete remission duration and OS. CD20 expression was assessed by flow cytometry on bone marrow aspirates. As previous work by the same group demonstrated, CD20 expression was prognostic only in patients <60 years of age; the study authors focused on treatment in these patients [26].

Investigators identified 68 patients who had received rituximab-Hyper-CVAD and 45 who received Hyper-CVAD alone. This regimen includes hyperfractionated cyclophosphamide, vincristine, daunorubicin and dexamethasone (cycles 1, 3, 5 and 7) alternating

Table 1. Chemotherapy and allogeneic transplantation.

Authors (year)	Setting	Treatment	Mechanism of action	Response/survival data	Ref.
Fielding *et al.* (2007)	Relapsed	Outcomes after relapse	N/A	609 of 1372 CR1 patients relapsed; 5-year OS: 23% AlloSCT with matched related donor; 16% AlloSCT with matched unrelated donor; 4% chemotherapy alone	[9]
Goldstone *et al.* (2008)	Upfront	AlloSCT vs autotransplant vs maintenance chemotherapy	Graft vs leukemia effect	1913 patients; 1051 with HLA typing AlloSCT eligible, achieving CR1–5-year OS: 53% donor; 45% no donor for Ph- ALL	[4]
Stock *et al.* (2008)	Upfront, AYA only	Chemotherapy only	Chemotherapy, cytotoxicity	Child (CCG) vs adult (CALGB) for AYA; 7-year OS: CCG 67% vs CALGB 46%	[5]
Gokbuget *et al.* (2011)	Relapsed/refractory, T cell only	Nelarabine	Purine nucleoside	T cell: 36 and 10% CR	[66]

AlloSCT: Allogeneic hematopoietic stem cell transplantation; AYA: Adolescents and young adults; CALGB: Cancer and Leukemia Group B; CCG: Children's Cancer Group; CR1: Cytologic remission; HLA: Human leukocyte antigen; OS: Overall survival; Ph- ALL: Philadelphia-negative acute lymphoblastic leukemia.

with high-dose methotrexate and cytarabine (cycles 2, 4, 6 and 8). CR rate was 95% in all patients. At 3 years, disease-free survival was 78% for patients treated with rituximab (CD20 >20%) versus 38% of patients who were not ($p<0.001$). OS at 3 years was superior in patients with CD20 expression treated with rituximab (75 vs 47%; $p = 0.003$). Rituximab did not appear to add significant toxicity [27].

An assessment from the GMALL group recently reported similar findings. Investigators identified 263 patients with CD20 positive (>20% CD20 expression) B-ALL, 181 of whom received rituximab with chemotherapy and 82 of whom received chemotherapy alone. CR rate (94 vs 91%) and early induction death rate (5 vs 3%) appeared similar. Molecular complete response (molCR), however, was superior in the rituximab/chemotherapy cohort (57 vs 27% at day 24; 90 vs 59% at week 16). Disease-free survival at 5 years was 80% with rituximab/chemotherapy versus 47% with chemotherapy only. OS at 5 years was 71% with the combination versus 57% with chemotherapy alone [28].

Despite the retrospective evidence noted above and the rapid incorporation of rituximab into chemotherapy regimens for CD20 positive B-ALL, rituximab has not been approved for use for ALL in the USA.

Interestingly, while rituximab has been assessed in patients with >20% CD20 expression, it is not clear that this is the best cutoff. In an analysis of 237 pediatric B-ALL patients, CD20 expression of >20% was present in 46% of bone marrow samples and 51% of peripheral blood samples at diagnosis. Patients received a steroid prephase and a typical induction regimen. In those patients with molecular residual disease (MRD) at day 8, CD20 expression increased to 75% in peripheral blood and by day 15 had increased to 71% in bone marrow. In the few patients with MRD at day 33, 22 of 27 patients (81%) had >20% CD20 expression in a bone marrow sample. *In vitro* assessment of patients' leukemic cells also suggested rituximab cytotoxicity was enhanced by upregulation of CD20 [29]. This suggests that >20% CD20 expression may not be the best criteria for incorporating rituximab into treatment. However, to our knowledge, rituximab administration in patients with <20% CD20 expression has not been investigated.

Hopefully, the ongoing adult UKALLXIV Phase III trial randomizing patients with B-ALL to rituximab/chemotherapy or chemotherapy alone during induction will provide the Phase III evidence we are currently lacking for or against routine use of rituximab in this population. It will likely be several years before final results are reported.

CD22 has also been an attractive target for antibody development. While the development has not been as rapid as rituximab's, the anti-CD22 antibody epratuzumab has been investigated for a number of B-cell malignancies over the past 10–15 years. CD22 is highly expressed in mature B-cell lymphomas, reportedly as high as 80%. Unlike CD20, when CD22 binds to its natural ligand (or an antibody), it is rapidly internalized. This leads to increased expression of the B-cell activator complex, encouraging proapoptotic signaling in neoplastic B cells.

Initial Phase I/II studies in heavily pretreated relapsed/refractory aggressive and indolent lymphomas, demonstrated activity (more so in indolent vs aggressive) and a good safety profile with similar toxicity to that historically observed with R-CHOP alone [30–32]. Epratuzumab was first investigated for pediatric relapsed/refractory B-ALL with reinduction chemotherapy. Patients had >25% expression of CD22. Treatment included a 2-week prechemotherapy phase of twice-weekly epratuzumab. Chemotherapy was then initiated with a typical block I induction regimen consisting of vincristine, prednisone, PEG-asparaginase, doxorubicin and dexrazoxane. Epratuzumab was administered once weekly for 4 weeks during chemotherapy.

After the prechemotherapy phase, 11 out of 15 patients had stable disease, one patient had a partial response (PR) and three patients progressive disease. All but one patient had a significant reduction in peripheral blast count, suggesting drug activity. The saturation of the CD22 target was nearly 100%. Nine out of 15 patients achieved a CR after induction block I. Seven out of nine CRs were molCRs after block I. One additional CR became a molCR after block II. Toxicity was limited, but notable for a first dose infusion reaction similar to that commonly encountered with a first dose of rituximab [33].

A Phase II component of this epratuzumab/chemotherapy study has recently been reported after accrual of 116 children, adolescents and young adults (aged 2–30 years) at first relapse, treated at the maximum tolerated dose (MTD). Survival data have not yet been reported. When compared to historical controls, pooled patients treated with epratuzumab and chemotherapy had CR rates similar to patients treated with chemotherapy alone (65%). In patients with a CR, however, molCR appeared improved with epratuzumab/chemotherapy (42 vs 25%; $p = 0.01$). Further follow-up and Phase II or III trials in the relapsed/refractory setting will be necessary to draw further conclusions [34].

In addition to monoclonal antibodies such as rituximab and epratuzumab, antibody–drug conjugates and antibody–immunotoxin conjugates have been developed with varying degrees of success in other hematologic malignancies. Notably, brentuximab vedotin recently became the first antibody–drug conjugate to receive US FDA approval for the treatment of Hodgkin lymphoma. While preliminary data suggested adequate binding to CD30 (near universal expression in Hodgkin's lymphoma and anaplastic large-cell lymphoma) with a naked antibody, clinical activity (6% response rate) was disappointing. The naked antibody was then conjugated to an antitubulin agent, monomethyl aurostatin E and renamed brentuximab vedotin. The first trial of this agent was reported in 2010 with 50% of heavily pretreated patients treated at the MTD experiencing a response. Radiographic benefit was experienced in 86% of patients and in patients with symptoms related to lymphoma, 81% had resolution of symptoms [35]. This has been a breakthrough in drug development and has encouraged further development of similar agents for other hematologic malignancies.

With the high levels of expression of CD22, as noted above in B-cell malignancies, as well as its rapid internalization when bound to ligand, CD22 antibodies appear to be ideal agents for conjugation to toxins or to chemotherapy. Inotuzumab is such an agent and is a chimeric monoclonal CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic. Upon internalization of the anti-CD22 and calicheamicin complex, rapid hydrolysis occurs causing intracellular release of calicheamicin.

In an initial Phase I/II study, this agent appeared to be active and well tolerated with Grade 3/4 thrombocytopenia as the dose limiting toxicity, but requiring no treatment [36]. An ongoing Phase II trial of inotuzumab in relapsed/refractory adult and pediatric B-ALL in patients with CD22 expression >50% is ongoing; however, an interim analysis has recently been presented. Toxicity appeared similar to that in the Phase I trial. Of 49 heavily pretreated patients, nine patients experienced a CR, five experienced a bone marrow CR and 14 a CR with incomplete blood count recovery (CRi); for a response rate of 57%. In total, 20 patients proceeded to AlloSCT. Survival data are not yet mature, however, investigators have appropriately concluded that this agent is highly active [37].

Antibody-immunotoxin strategies have also been explored in B-ALL. Combotox is a CD19 and a CD22 antibody coupled to a deglygosylated ricin A chain. Preclinical studies had determined that administration of both antibodies together in a 1:1 ratio was synergistic. A Phase I trial in relapsed/refractory pediatric B-ALL demonstrated in a heavily pretreated population that ten out of 17 patients achieved a hematologic improvement (>75% reduction in peripheral blasts), three patients a CR and 6 patients experienced >95% reduction in leukemic blasts. Several patients experienced early death in close proximity to treatment; however, causality appeared more likely to be disease related [38].

While Combotox was initially reported in pediatric B-ALL, it was not until recently that a Phase I study in adult B-ALL has been reported. The study enrolled 17 heavily pretreated relapsed/refractory patients with CD19 or CD22 expression of ≥50%. All patients experienced a decrease in peripheral blasts with one patient experiencing a PR. The pharmacokinetic studies demonstrated serum drug levels not only correlated with the dose, but also correlated just as closely with the number of circulating blasts, with a much shorter half-life in patients with the most blasts. This led the authors to speculate that in molecularly relapsed disease, without the 'antigen sink' of the bone marrow and peripheral circulating blasts, this agent could

demonstrate efficacy, eradicate molecular disease and possibly cure patients. Additionally, it was speculated that there may be therapeutic potential if administered after chemotherapy had 'debulked' the leukemia and decreased the 'antigen sink' [39].

Moxetumomab is an additional antibody-immunotoxin targeting CD22. The agent is an anti-CD22 antibody, linked to pseudomonas exotoxin A. A Phase I study in pediatric CD22 expressing B-ALL enrolled 21 patients with relapsed/refractory disease. Capillary leak syndrome was the dose limiting toxicity and was experienced in two patients. Subsequently, corticosteroids were routinely administered, with no further capillary leak syndrome. Otherwise, toxicity was mild and reversible. Hematologic response occurred in five out of 17 evaluable patients (29%), with four CRs and one PR. A decrease of >50% in circulating blasts was observed in seven (41%) patients. The tolerability and response was felt to be impressive enough for the investigators to pursue further Phase II studies [40].

Despite the fact that it has been over 10 years since the first reports of dramatic B-cell cytotoxicity in cell lines from a new class of novel therapeutic agents called bispecific T-cell engagers; data in humans were not presented until recently. Blinatumomab is a single-chain bispecific antibody targeting cytotoxic T cells with an anti-CD3ε. Upon attachment to T cells, the other arm of the bispecific antibody binds to CD19 on B cells. The activated cytotoxic T cells then induce perforin-mediated lysis of B cells. CD19 is one of the cell surface markers expressed earliest in B-cell maturation and its expression in B-ALL blasts has been found to be universal, suggesting that CD19 would be a very attractive target.

The first report of blinatumomab was a Phase I trial in B-cell lymphoma patients reported in 2008. Of 38 evaluable patients, 11 obtained a major response, with the agent demonstrating some degree of cytotoxicity in all patients. At the maximum achieved dose (dose escalation was ongoing at the time of publication), all seven patients demonstrated an objective response. Responses were durable and the agent was well tolerated with common toxicities including lymphopenia, leucopenia, pyrexia and chills. No anaphylactoid reactions to the initial infusion were noted, a feature common with chimeric monoclonal antibody administration [41].

The potential efficacy of this agent in ALL was described in a recent Phase II trial from Germany. The trial enrolled 16 B-ALL and five PhALL patients who had a CR1 to initial therapy, but had either MRD or molecularly relapsed disease. Blinatumomab was administered as a continuous infusion for 4 weeks followed by 2 weeks free from treatment. Patients responding could receive three additional cycles. Patients with a matched donor were permitted to pursue AlloSCT at anytime after the first cycle. One patient experienced grade 3 seizures during the first cycle and discontinued treatment (seizures resolved within 24 h). Toxicity was mostly limited to cytopenias (leucopenia and lymphopenia) with four documented grade 3/4 infections, mild pyrexia and mild chills.

Out of 20 evaluable patients, 16 achieved durable molCR. Similar response rates were demonstrated for each level of MRD (high, intermediate and low), as well as in molecularly refractory versus relapsed patients. After a median of 405 days, all 16 responding patients remained in CR. Eight patients proceeded to AlloSCT, none of whom had relapsed at the time of the report. In addition, four out of five patients with PhALL refractory to imatinib and/or dasatinib achieved a molCR. Investigators felt that patients fared better than historical controls, exhibiting molecular evidence of disease only [42].

An additional 18 patients with morphologic relapsed or refractory disease treated with blinatumomab, have recently been reported by the same group as an interim analysis of an ongoing Phase II trial. Toxicity was similar to that seen in previous trials with this agent. Remarkably, efficacy was similar in this much higher risk group with 12 of 18 patients achieving a CR within two cycles. Of these 12 patients, nine (75%) had complete recovery of blood counts within two cycles. In addition, and most impressively, all 12 patients with a CR achieved a molCR within two cycles. This response rate and depth of response with single agent therapy in the relapsed/refractory setting is unprecedented [43].

With the reporting over the last 1–2 years of Phase I and II trials of promising agents for molecularly relapsed B-ALL, as well as relapsed/refractory B-ALL, such as moxetumomab, epratuzumab, inotuzumab, combotox and blinatumomab, new clinical options for patients appear to be on the horizon. Given the activity and limited toxicity in the relapsed/refractory setting, it is anticipated such agents will be assessed prospectively in the first-line setting in the next few years. The difficulty will be with the limited prevalence of B-ALL in adults and the number of agents currently being investigated. It does appear, however, that there is great potential for a number of these agents to be of benefit in the first-line setting and result in increased cure rates.

A brief summary of articles we find to be of particular importance regarding antibody directed therapies of B-ALL is included in Table 2.

Table 2. Immunotherapy/antibody-directed therapy for Philadelphia chromosome-negative B-lineage acute lymphoblastic leukemia.

Authors (year)	Setting	Treatment	Mechanism of action	Response/survival data	Ref.
Thomas *et al.* (2010)	Upfront	Rituximab/ hyper-CVAD	Anti-CD20 antibody with chemotherapy	5-year OS: R-chemotherapy 71%; chemotherapy 57%	[27]
Raetz *et al.* (2011)	Relapsed/ refractory	Epratuzumab	Anti-CD22 antibody	MolCR: E-chemotherapy 42%; chemotherapy 25%	[34]
O'Brien *et al.*(2011)	Relapsed/ refractory	Inotuzumab	Anti-CD22 antibody conjugated to chemotherapy	Response rate: 57%	[37]
Schindler *et al.* (2011)	Relapsed/ refractory	Combotox	Anti-CD19 and anti-CD22 antibody conjugated to immunotoxin	All 17 patients decrease blasts, one PR	[39]
Wayne *et al.* (2011)	Relapsed/ refractory	Moxetumomab	Anti-CD22 antibody conjugated to immunotoxin	CR: 29%; PR: 7%	[40]
Topp *et al.* (2011)	Relapsed/ refractory	Blinatumomab	Bispecific T-cell engaging antibody (CD3/CD19)	67% rapid/durable CR and molCR	[43]

CR: Complete remission; molCR: Molecular complete remission; OS: Overall survival; PR: Partial response.

TKIs in PhALL

One of the most important advances in hematology over the past several decades was the reporting of the early studies of the TKI imatinib in chronic myelogenous leukemia (CML), in chronic phase as well as blastic phase or PhALL. In chronic phase CML, an unprecedented 98% response rate for doses >300 mg daily was reported with an exceptional tolerability [44]. In blastic phase CML or relapsed/refractory PhALL, a remarkable 70% response rate was demonstrated. While the median duration of response was 58 days, the results were no less remarkable in such an aggressive, therapy refractory disease [45].

Soon after the initial Phase I trials, a number of Phase I and II trials, which combined imatinib with chemotherapy, were completed and reported. Importantly, GMALL compared the safety and efficacy of sequential versus concurrent imatinib with a standard intensive ALL regimen. With a somewhat limited follow-up, no difference in OS or RFS was noted. Concurrent therapy, however, was tolerated similarly and was associated with superior molCR after consolidation of 52 versus 19% in the sequential group [46].

A group from Japan also reported results of a Phase II trial investigating conventional chemotherapy with concurrent imatinib in 80 adult patients with PhALL. The imatinib was initiated at day 8 of induction therapy, excluded from consolidation one, and then continued from the start of consolidation two through 2 years of maintenance (maintenance also including monthly vincristine and pulse corticosteroids). CR1 was achieved with this strategy in 96% of patients, with 50% achieving molecular CR1 on day 63. Notably, death during induction therapy occurred in only 2.5% of patients. AlloSCT was recommended for patients with an identified HLA-identical sibling donor if the patient had achieved CR1. Event-free survival (EFS) and OS at 1 year were 60 and 76%, respectively. Both EFS and OS appeared superior to historical controls. At 1 year, outcomes appeared similar in the 49 patients undergoing AlloSCT versus those that did not. With results reported of 1-year EFS and OS rates, results between patients undergoing AlloSCT and those not undergoing AlloSCT should be interpreted with caution [47]. With such remarkable results in two Phase II studies incorporating concurrent imatinib with conventional chemotherapy, concurrent therapy thus became the standard for ongoing clinical trials.

While TKIs have been rapidly accepted as the standard of care with concurrent chemotherapy, data supporting this approach have not been optimal. It was not until 2010 that high-level evidence was presented from UKALLXII/ECOG2993 clearly demonstrating the benefit of the addition of a TKI to chemotherapy in untreated PhALL. The trial was not originally designed to include imatinib, as the study was designed in the pre-imatinib era, but enrollment opened in 1993. With the introduction of imatinib and the exciting activity of this agent, in March 2003 patients enrolled with PhALL were initiated on imatinib during the second phase of the first induction cycle (week 5) or the start of the first consolidation cycle (week 9). Patients not proceeding to AlloSCT continued with daily imatinib (600 mg once daily) for 2 years with maintenance therapy. In patients undergoing AlloSCT, imatinib was initiated and continued for 2 years after AlloSC, if tolerated.

Updated results of 441 patients with PhALL were presented in late 2010. Despite a greater median age (not reported) due to an increase in the maximum allowable

age, EFS and OS at 3 years was superior in patients receiving imatinib at 36 versus 19% (p = 0.0001) and 42 versus 25% (p = 0.0001), respectively. In addition, more patients treated with imatinib proceeded to AlloSCT (44% imatinib vs 28% no imatinib). In patients undergoing AlloSCT, 3-years OS was superior in patients receiving imatinib (59 vs 40%). Patients undergoing a per-protocol AlloSCT experienced double the 3-year OS as those that did not (59 vs 28%; p = 0.0001). While the imatinib cohort appeared to fare significantly better than the pre-imatinib cohort, it has been speculated that the results were inferior to the previously reported Phase II trials, due to the later initiation of imatinib at week 5 or 9, as opposed to at day 8 or week 5. Indeed, in subgroup analysis, the early imatinib subgroup (week 5) had improved outcomes compared with the late imatinib subgroup (week 9), and appeared similar to those patients in the previous Phase II trials. Comprehensive toxicity data have not been reported in the imatinib cohort, but are likely to be similar to those patients not treated with imatinib [10].

A recently reported Phase II trial from China investigated a less intensive induction regimen consisting solely of videsine (4 mg/m^2 weekly) and dexamethasone (10 mg/m^2 on days 1–4 each cycle) combined with 400 mg of imatinib once daily in untreated PhALL. Three intensification cycles were administered, but the details of the drugs and doses of these cycles were not reported. Maintenance for patients unwilling, unsuitable or having no available matched allogeneic donor included 400 mg imatinib daily, 10 mg/m^2 per day dexamethasone on days 1–5 and 11–15, and videsine 4 mg/m^2 on day 1 and 11. The maintenance was administered for 3 years. A total of 36 patients were enrolled and median follow-up was short (8 months). Median OS appeared shorter than the two studies previously discussed, estimated at 22.1 months. Comprehensive toxicity data have not been made available, however, treatment-related death occurred in three patients (8.3%) during intensification [48].

Dasatinib and nilotinib, both second-generation TKIs, have been developed and assessed in imatinib-relapsed/refractory disease and subsequently in imatinib-naive disease. Clear benefit has been demonstrated in imatinib-relapsed/refractory disease; however, the benefit in imatinib-naive disease has been less clear [49–52]. Both agents bind to the active and inactive *ABL* kinase, but do so with greater affinity than imatinib. Dasatinib and nilotinib have greater *ABL* inhibition at 325- and 20-times that of imatinib, respectively, in preclinical models and have been found to have activity in resistant mutations (with the exception of *T315I*). In addition, dasatinib has excellent CNS penetration as well as having 'off target' inhibition of SRC family kinases, which have been demonstrated to be upregulated in PhALL [53,54].

Over the past 2 years, several groups have reported results from Phase II trials combining conventional chemotherapy with the second-generation TKIs. Building on promising early data from the same group combining the less potent TKI, imatinib with Hyper-CVAD [55]. Phase II data have recently been reported combining dasatinib with this regimen.

In total, 35 patients with previously untreated PhALL received 50 mg of dasatinib twice daily or 100 mg daily for the first 14 days of each 28-day chemotherapy cycle. Patients also received intrathecal chemotherapy with methotrexate and cytarabine. Maintenance with monthly vincristine and 200 mg prednisone daily for 5 days every month was continued for 2 years. Dasatinib was administered concurrently from completion of chemotherapy through 2 years of maintenance and indefinitely thereafter. Methotrexate and 6-mercaptopurine were omitted from maintenance. Patients with an available donor had the option of AlloSCT in CR1 at any point during intensive and maintenance treatment [56].

Two patients (6%) died due to infection during the first cycle. The remaining 33 patients achieved a CR1, while 20 out of 35 patients (57%) achieved a molCR. The estimated 2-year OS was 62% and EFS was 57% (median follow-up 14 months). Only four patients proceeded to AlloSCT (all of whom were reported to be alive and disease free). While the authors proposed this combination to be well tolerated, notably two patients died during induction treatment (6%) and grade 3/4 toxicity was very common and included: infections (69% induction, 84% post-remission), hemorrhage (10% induction, 35% post-remission) and metabolic (60% induction, 35% post-remission) [56].

A similar strategy was recently reported from Korea, demonstrating similar findings with the administration of chemotherapy with nilotinib. A simplified schedule of chemotherapy induction with daunorubicin, vincristine and prednisolone was administered. If CR1 was obtained, patients with donor availability and appropriate clinical status were offered AlloSCT. Patients not proceeding AlloSCT received five cycles of consolidation (regimen not described) and maintenance for 2 years with methotrexate, 6-mercaptopurine and nilotinib. Nilotinib was administered from day 8 until either AlloSCT or the completion of maintenance therapy. Compared with the report of Hyper-CVAD/dasatinib, many more patients proceeded to AlloSCT. In fact, of 45 patients surviving after induction therapy, 33 underwent AlloSCT. While not reported, we suspect that this was all or nearly all patients in remission who were candidates for AlloSCT and had an available donor.

This strategy (although with greatly reduced chemotherapy compared to hyperCVAD/dasatinib) yielded promising 2-year outcomes with an EFS of 49.4% (chemotherapy/dasatinib 57%) and OS of 66.2% (chemotherapy/dasatinib 62%). In those patients who underwent AlloSCT, 2-year estimated EFS and OS were 60 and 83%, respectively. Comprehensive toxicity has not been reported; however, it is important to note that five (10%) of 50 patients perished during induction, suggesting a similar induction toxicity as reported with Hyper-CVAD/dasatinib [57].

While historically, hematologists have tended to add promising agents to existing chemotherapy regimens in the hope of improving upon them, up until recently few have focused on the upfront treatment of PhALL with either a TKI only or a TKI with corticosteroids, with or without a lesser intensity of chemotherapy. Several groups have recently reported results investigating whether such abrogated chemotherapy can obtain similar or improved results with decreased treatment related deaths, induction deaths and toxicity.

A European group recently reported on such abbreviated chemotherapy with dasatinib in 71 patients over the age of 55 years. Treatment included a 5-day corticosteroid prephase, followed by 140 mg of dasatinib daily, intravenous vincristine 1 mg weekly and 40 mg of dexamethasone (2 consecutive days, once weekly); for a 4-week induction. This was followed by chemotherapy consisting of methotrexate (1000 mg/m^2 on day 1), 10,000 IU/m^2 L-asparaginase intramuscularly (day 2), and 100 mg of dasatinib daily (consolidation cycles 1, 3 and 5); alternating with cytarabine (1000 mg/m^2 every 12 h on days 1, 3 and 5) and 100 mg of dasatinib daily (consolidation cycles 2, 4 and 6). Maintenance included 6-mecaptopurine, weekly methotrexate, dexamethasone and vincristine along with dasatinib. Despite a greater median age of 69 years, toxicity appeared less than previously reported regimens including cyclophosphamide and anthracyclines. A similar number of deaths during induction were reported (5.7%). CR and molCR were 90 and 56%, respectively. In addition, median RFS and OS in this elderly population were 22.1 and 27.1 months, respectively. Serious adverse events of grade 3 or greater occurred in approximately half as many patients as those treated with intensive chemotherapy/TKI regimens [58].

The Italian Group of Adult Hematological Diseases, building upon previous experience with imatinib and prednisone in elderly PhALL patients [59], reported in 2011 one of the most important first-line Phase II studies in ALL over the past 10 years. A total of 53 patients were enrolled and initiated dasatinib 70 mg by mouth twice daily after an initial 7-day corticosteroid prephase. Prednisone was continued until day 24, then tapered and discontinued on day 32. Intrathecal methotrexate was administered twice. Dasatinib was continued for 84 days, at which point treatment was at the discretion of treating physicians. Post-induction treatment included no further treatment in two patients, TKI alone in 19 patients, TKI plus chemotherapy in ten patients, TKI plus autologous transplant in four patients and AlloSCT in 18 patients [60].

Remarkably, and for the first time, no patients with PhALL experienced death during induction and all patients achieved a CR. Treatment was well tolerated, with few dose reductions and only four patients discontinuing treatment, all of whom were in CR at day 64 or later. In addition to achieving a 100% CR rate, 52% achieved a molCR by day 85. With a median follow-up of 25 months, the estimated median OS was 30.8 months and an OS at 20 months of 69%. RFS at 20 months was 49%. Notably, in patients undergoing an AlloSCT in CR1 following induction, only two out of 18 patients have relapsed. Also of note was the median age, which was 56 years, with 12 patients (23%) over the age of 60 [60].

In terms of CR, molCR and OS, similar, if not improved results with dasatinib and a corticosteroid, have been demonstrated over chemotherapy/dasatinib, chemotherapy/nilotinib and chemotherapy/imatinib. It appears clear, however, that toxicity is dramatically lower with the TKI/corticosteroid approach. In addition to decreased treatment-related deaths, while data have not been presented, one could speculate as to a reduced number of hospitalizations and an improved quality of life in dasatinib/prednisone patients as compared with chemotherapy/TKI. At the time of the preparation of this manuscript, the Italian Group of Adult Hematological Diseases is currently enrolling patients investigating similar strategies. It is with great enthusiasm that we anticipate the results of the investigations of the Italian Group of Adult Hematological Diseases.

Additional TKIs have also been investigated for PhALL and CML. Recently, a Phase I/II trial of a new TKI, bosutinib, has been reported in patients resistant or intolerant to imatinib. This agent, while similar to second-generation TKIs, does not have activity against *c-KIT* nor *PDGFR*. Preclinical activity demonstrated MIC50s approximately 15- to 100-fold less than imatinib and activity against common TKI-resistant mutations with the exception of *T315I* and *V299L*. The study included 164 patients with blast or accelerated phase CML or patients with PhALL. CR and major cytogenetic responses in patients without

mutations were 39 and 37%, respectively; while CR and major cytogenetic responses in patients with mutations were 17 and 24%, respectively. Of note, it was not reported in this group of blast/accelerated phase CML and PhALL the number of imatinib resistant versus imatinib intolerant, nor has it been reported how many patients had previously received a second-generation TKI. Both issues would greatly impact the interpretation of data [61].

Another new TKI, ponatinib, has demonstrated promising preclinical activity in cell lines with dasatinib and nilotinib resistance, and in *T315I*-mutant cell lines. In addition, ponatinib appears to differ from earlier TKIs with significant inhibition in FGFR1–4, which have been implicated in a number of malignancies.

With >80% of expected enrollment, an interim analysis of a Phase II trial of ponatinib in dasatinib/nilotinib intolerant/refractory CML or PhALL, or patients with CML or PhALL with *T315I* mutation (rendering currently available TKIs ineffective) was recently reported. A high percentage of patients (88%) were resistant to dasatinib/nilotinib rather than intolerant. In this interim analysis, 30 patients with blast phase CML or PhALL with resistance/intolerance and 22 patients with *T315I* mutations were evaluable. A major hematologic response was experienced in 37 and 27% of patients with resistant/intolerant disease and *T315I* mutations, respectively. Treatment appeared to be well tolerated. Given the heavily pretreated population – most of whom had failure of both imatinib and a second-generation TKI – this agent appears promising for future development [62].

A brief summary of articles we find to be of particular importance regarding TKI targetting of BCR/ABL in PhALL is included in Table 3.

Nelarabine for T-ALL

Nelarabine is a purine nucleoside and a pro-drug of deoxyguanosine analogue ara-G. It is demethylated and phosphorylated intracellularly to form the active compound ara-GTP. Ara-GTP's mechanism of action is as a substitute for GTP in a number of biologic processes including, most importantly for our purposes, DNA replication. With the substitution of ara-GTP for GTP in DNA replication, DNA synthesis is inhibited ultimately leading to cell death. In preclinical studies nelarabine accumulated to much greater concentrations in T lymphocytes, due to a more rapid phosphorylation in T cells and a more rapid catabolism of deoxyguanosine triphosphate in B lymphocytes; ultimately leading to greater selectivity and toxicity for T lymphocytes [63]

An initial Phase I trial established a MTD and demonstrated evidence for T-cell accumulation of Ara-G. However, it was not until a number of years later that the entire dataset was reported [64]. The full Phase I trial enrolled 93 patients with refractory hematologic malignancies, of which 66% had T-cell malignancies. Dose limiting toxicity was neurotoxicity, but was otherwise well tolerated. In this heavily pretreated pediatric and adult population, nine (23%) CRs and 12 (31%) PRs were demonstrated in T cell lymphoblastic lymphoma and T-ALL. It was felt that this agent warranted further investigation for T-cell malignancies [65].

A German Phase II trial has recently reported the results of single agent nelarabine for relapsed/refractory T-ALL and T-cell lymphoblastic lymphoma. Of 126 heavily pretreated adult patients, 36% achieved a CR and 10% a PR. Of patients achieving a CR, 80% proceeded to AlloSCT. Median OS was 6 months, with a 24% 1-year OS and a 12% 3-year OS. In patients who proceeded to AlloSCt, 3-year OS was estimated at 31%. Treatment was well tolerated with toxicity similar to that in the Phase I [66]. A US group demonstrated similar findings in a US-based Phase II trial for 39 adults with either T-ALL (26 patients) and T lymphoblastic lymphoma (13 patients) with a 1-year OS of 28% and response rate of 41% [67].

Recently, nelarabine has also been combined with upfront chemotherapy for pediatric T-ALL. A novel design randomized patients with >1% MRD in the bone marrow or >5% blasts at day 29 of an induction chemotherapy regimen. In this HR group, patients were randomized to chemotherapy with or without 5 days of nelarabine in consolidation, delayed intensification and at the start of each maintenance cycle. In total, 57 HR patients were identified and randomized. Nelarabine had not, at least to the point of interim analysis, resulted in greater toxicity. Nelarabine appeared better tolerated in this group of children treated in the upfront setting compared to previous Phase I/II studies in heavily pretreated adult and pediatric populations. The limited follow-up at this time precludes assessment of survival end points. Initial analysis is expected within the next several years [68]. A similar assessment of the incorporation of nelarabine into the first-line setting for adult T-ALL in a Phase II fashion in UKALLXIV is ongoing. Initial reports should be forthcoming within the next few years.

Table 3. Tyrosine kinase inhibitors in Philadelphia chromosome-positive B-cell-lineage acute lymphoblastic leukemia.

Authors (year)	Setting	Treatment	Mechanism of action	Response/survival data	Ref.
Fielding *et al.* (2010)	Upfront	Imatinib and chemotherapy	*BCR/ABL* inhibition and chemocytotoxicity	3-year OS (n = 441): imatinib 42%; no imatinib 25%; AlloSCT 59%; no AlloSCT 29%	[10]
Ravandi *et al.* (2010)	Upfront	Hyper-CVAD with dasatib	*BCR/ABL* inhibition and chemocytotoxicity	2-year OS: 62%	[56]
Foa *et al.* (2011)	Upfront	Dasatib and prednisone	*BCR/ABL* inhibition	2-year OS: 67%; median OS: 31 months	[60]
Cortes *et al.* (2011)	Relapsed/refractory	Ponatinib	*BCR/ABL* inhibition	CR: 37% TKI resistant; 27% T315I	[62]

AlloSCT: Allogeneic hematopoietic stem-cell transplantation; CR: Complete remission; OS: Overall survival; TKI: Tyrosine kinase inhibitor.

Other therapeutic agents for all undergoing active investigation

mTOR inhibitors

The mTOR pathway has been implicated as a pathway dysregulated and leading to increased proliferation and decreased apoptosis in a number of malignancies. The pathway has been implicated in T-ALL, B-ALL and PhALL. Mature in-human studies have not progressed sufficiently at this point in time to assess efficacy of mTOR inhibitors, such as everolimus, sirolimus, temsirolimus or rapamycin.

As sirolimus has immunosuppressive properties and is regularly used in this manner for solid organ transplantation, its use has been increasingly investigated as an immunosuppressive agent in AlloSCT. With the potential for specific antitumor activity in ALL, there may be a dual role for sirolimus in ALL patients undergoing AlloSCT. This role was recently investigated in pediatric ALL patients in a Phase I/II study. The study included 35 patients in second CR and 12 patients in third CR or greater. Conditioning was myeloablative with total body irradiation, cyclophosphamide and thiotepa. Besides sirolimus, graft versus host disease (GVHD) prophylaxis included tacrolimus and methotrexate administered and tapered per routine clinical practice. Sirolimus was continued for 6 months, and then tapered to discontinuation over 4 weeks.

In total, 26 patients received a matched sibling graft, five received a matched unrelated donor graft and 30 received an unrelated cord blood stem cell transplant. Engraftment, rates of acute and chronic GVHD, toxicity and transplant related mortality all appeared similar to historical cohorts. Sirolimus was well tolerated. RFS and OS at 2 years were 64 and 73%, respectively [69]. For multiple reasons, this appears to be an appealing approach in HR or relapsed patients. A Phase III study in pediatric ALL is ongoing, hopefully with results to be anticipated within the next 3–5 years.

Preliminary findings of a Phase I trial have recently been reported both in the upfront and refractory setting combining Hyper-CVAD with once weekly rapamycin. Response and survival data are premature; however, there does not appear to be excess toxicity in the first seven patients treated on protocol. Enrollment was ongoing at the time of writing [70].

Recent preclinical studies in lymphoid cell lines, as well as in mice, have continued to support a potential role of mTOR inhibitors. Studies of rapamycin and dexamethasone, everolimus as a chemotherapy sensitizer, and combining mTOR inhibition (sirolimus or temsirolimus) with methotrexate, to mention a few, have increased the enthusiasm of pursuing further trials of mTOR inhibition for adult ALL [71–74].

Proteasome inhibitors

Bortezomib is a proteasome inhibitor with evidence of benefit in hematologic malignancies, most notably multiple myeloma and indolent lymphomas. It is now FDA-approved and has been established as a standard of care in these disorders. The clinical activity of bortezomib in ALL is less well described. Preclinical data have suggested additive or synergistic activity with a number of chemotherapy agents and corticosteroids. However, single-agent bortezomib did not appear to have significant activity in ALL [75].

With potential for additive or synergistic activity, a Phase I trial of bortezomib in combination with chemotherapy in relapsed/refractory ALL was pursued. Ten pediatric patients with relapsed/refractory ALL were administered bortezomib, which was dose escalated to 1.3 mg/m^2 on days 1, 4, 8 and 11 in combination with typical induction chemotherapy. The combination was well tolerated, with hematologic toxicity being the most prominent and not differing significantly from that historically experienced. Nine patients were evaluable (one death during induction due to invasive zygomyces), of which six patients experienced a CR [76].

With dose escalation complete, the same group proceeded to a Phase II trial in 22 pediatric ALL patients with relapsed/refractory disease after two or three previous regimens. The toxicity was similar to that experienced in the Phase I trial. Of 22 patients, 14 experienced a CR and two patients experienced a CRi. All responders were B-ALL. Results

met predefined criteria for early discontinuation. Grade 3 or greater peripheral neuropathy developed in 9% of patients [77].

Given this heavily pretreated population, an 80% CR plus CRi (in B-ALL) appears remarkable in this pediatric population. While not reported, it appears highly likely that patients had received nearly identical chemotherapy in the past, but had relapsed despite it, suggesting benefit from bortezomib. To our knowledge, this combination has not been evaluated in adults. It appears unlikely, however, that adults would tolerate a similar regimen. It should be noted that the administration of bortezomib intravenously on this schedule has been associated with peripheral neuropathy, most notably in the myeloma population. If administered with concurrent vincristine, as was done in this trial (also associated with significant neurotoxicity), it is our impression this would not be tolerated by adults. It is possible that the risk of neuropathy could be mitigated, as it has in multiple myeloma, by a change to once-weekly subcutaneous administration or with the use of an alternative proteasome inhibitor [78,79].

■ Liposomal vincristine

Vincristine has been an integral part of ALL induction, consolidation, intensification and maintenance in the upfront and relapsed setting for decades. Vincristine's mechanism of action involves the binding to microtubules, causing depolymerization, metaphase arrest and cell death. It remains one of the most active agents for ALL and lymphoid malignancies. Vincristine, however, is associated with significant and dose-limiting neurotoxicity likely due to rapid binding to neurologic tissues. Outside of its neurotoxicity, vincristine is one of the most well-tolerated chemotherapies currently administered. It has been postulated that if neurotoxicity were diminished while maintaining drug activity; the administration of higher doses could be achieved. Such was the thinking in reformulating vincristine into a liposomal compound.

Liposomal vincristine sulfate is a nanoparticle formulation that is encapsulated in sphingomyelin and cholesterol liposomes. In preclinical models this encapsulated formula appeared to prolong the serum half-life and decrease drug absorption to normal tissues, as well as increasing absorption to tissues with incomplete blood vessel endothelium (such as bone marrow and lymphatic tissues).

In a Phase I trial in adults with relapsed/refractory ALL, liposomal vincristine sulfate was administered with dexamethasone. Toxicity was similar to historical experience with standard vincristine. Peripheral neuropathy rates were also similar; however, actual dosage (of vincristine) appeared to be approximately 50% greater than the maximum (2 mg capped dose) of typical vincristine. A 22% response rate was noted, suggesting some clinical activity in this heavily pretreated population [80].

Pooled data from the Phase II expansion with additional data from an international multicenter Phase II trial were recently reported. The study enrolled 101 patients with relapsed/refractory adult ALL of which 81% of patients were in second salvage or greater. A CR or a CRi was experienced in 20% of patients. A total of 17% of patients proceeded to AlloSCT. Of the patients who underwent AlloSCT, 27% were considered long-term survivors (defined as survival >12 months). Toxicity was similar to that demonstrated in the previous Phase I trial and appeared similar to that seen with vincristine [81]. Given this modest activity, a Phase III industry-sponsored trial is in development for older patients with ALL.

■ Decitabine with hyper-CVAD

Hypermethylation has been known to promote proliferation and decrease apoptosis in a number of hematologic malignancies. Hypermethylation appears to increase chemotherapy resistance. Over the past 10 years, hypomethylating agents – including decitabine and azacytidine – have demonstrated clinical benefit in patients with acute myelogenous leukemia and myelodysplastic disorders, especially in older patients who are otherwise poor candidates for induction chemotherapy [82–84].

Hypermethylation also appears to be involved in both the pathogenesis and the resistance of ALL to chemotherapy [85–87]. It has not been until recently, however, that hypomethylating agents have been assessed in ALL. A novel therapeutic strategy has recently been reported combining Hyper-CVAD and decitabine in a Phase I fashion for relapsed/refractory pediatric and adult ALL. In the initial phase, patients were administered decitabine for 5 days every 2 weeks in a dose escalating manner. After concluding this initial phase, decitabine was then administered for 5 consecutive days at the beginning of the 28-day hyper-CVAD regimen. This was alternated per the usual fashion with high-dose methotrexate and cytarabine.

In total, 39 patients were enrolled, of whom the 24% treated with the combination achieved a CR1 or a CR with incomplete platelet count recovery. Another 28% had a bone marrow response to treatment. Responses to therapy appeared to be durable. Of note, all patients had been universally treated in the upfront setting with Hyper-CVAD (without decitabine) alternating with high-dose methotrexate and cytarabine. Toxicity appeared similar to that experienced with Hyper-CVAD alone. Hypomethylation was confirmed in companion *in vitro* studies. Investigators concluded that this regimen appears to have sufficient activity to warrant further clinical trials in a treatment naive, or less heavily pretreated population [88].

Future perspective

One could argue that, over the past 2 years, more encouraging studies evaluating exciting new treatments for adult ALL have been reported than had been reported in the previous 5–10 years. It is with great enthusiasm that we look toward a future with more efficacious and less toxic therapy for ALL, such that a cure may be achieved in an ever increasing number of patients. The new and evolving therapies and issues discussed in this review are in no way inclusive of each and every agent evaluated in clinical trials or in preclinical studies, but those that we feel have been evaluated in sufficient human studies to warrant discussion are included.

We are hopeful that the following issues will be increasingly clarified over the next 5–10 years:

- With the predominant evidence for allogeneic transplant coming from UKALLXII/ECOG2993, will the current routine use of rituximab in CD20-positive B-ALL, second-generation TKIs in PhALL, or the administration of pediatric regimens to AYAs, abrogate or eliminate the benefit of transplant in some eligible patients?
- Will other antibodies targeting highly expressed cell surface markers be of benefit and, if so, will they exceed the apparent benefit of routine rituximab in CD20 positive B-ALL?
- As brentuximab vedotin has been utilized in relapsed/refractory Hodgkin's lymphoma, will we ultimately see such a breakthrough with antibody-immunotoxin and/or antibody–drug conjugates currently in clinical trials?

Executive summary

Allogeneic hematopoietic stem-cell transplantation for adult acute lymphoblastic leukemia

- UKALLXII/ECOG2993: demonstrated benefit of allogeneic transplant in adult acute lymphoblastic leukemia (ALL) and the feasibility of performing large international multicenter trials for ALL.
- Pediatric protocols for adolescents and young adults: adolescents and young adults fare better when treated on pediatric protocols, suggesting a lesser role for transplant in complete cytologic remission for such patients.

Antibody-directed therapy of B-cell ALL

- Rituximab: a CD20-directed antibody that appears to confer a survival benefit in retrospective studies.
- Epratuzumab: a CD22-directed antibody with activity in relapsed/refractory ALL.
- Inotuzumab: a CD22-directed antibody–drug conjugate with activity in relapsed/refractory ALL.
- Combotox: CD19 and CD22 antibodies conjugated to an immunotoxin with activity in relapsed/refractory ALL.
- Moxetumomab: a CD22-directed antibody–immunotoxin conjugate with activity in relapsed/refractory ALL.
- Blinatumomab: a CD3/CD19 first-in-class novel agent called a bispecific T-cell engager demonstrates activity in relapsed refractory ALL.

Tyrosine kinase inhibitors for Philadelphia chromosome-positive B-cell ALL

- Imatinib/chemotherapy: first-in-class tyrosine kinase inhibitors (TKIs) demonstrates survival benefit in UKALLXII/ECOG2993.
- Dasatinib or nilotinib/chemotherapy: several studies demonstrating feasibility and possibly improved response rates and overall survival with second-generation TKIs.
- TKIs with corticosteroids: feasibility, decreased toxicity and possible increased survival in Phase II studies with TKIs/corticosteroids alone, or TKI/corticosteroids/decreased chemotherapy.
- Third-generation TKIs: ponatinib and bosutinib demonstrate significant activity in Philadelphia chromosome-positive ALL relapsed/refractory after imatinib/dasatinib/nilotinib.

Other therapeutic agents

- mTOR inhibitors: early-phase studies on rapamycin and sirolimus suggest tolerability and possible benefit.
- Proteasome inhibitors: bortezomib in combination with traditional chemotherapy appears to increase response rate to previously administered chemotherapy agents.
- Liposomal vincristine: reformulated vincristine appears to allow for an increase in delivered vincristine dose without increased toxicity.
- Hypomethylating agents: decitabine with Hyper-CVAD very early-phase studies suggesting safety and possible signal for efficacy.

Future perspective

- Over the next 5–10 years, we anticipate and expect significant progress through clinical trials in the understanding of novel therapies and their incorporation into the standard of care for the treatment of adults with ALL.

- Will novel bispecific T-cell engaging agents, such as blinatumomab, translate the excitement from early phase trials to actual clinical benefit for patients?
- Can more patients with PhALL survive induction therapy with less intensive chemotherapy (or without chemotherapy) and will combining corticosteroids with second-generation TKIs maintain or improve long-term survival with novel maintenance strategies and/or AlloSCT?
- In patients who have achieved a rapid and persistent molCR and have a good prognosis, can we reduce the intensity and/or duration of therapy? Alternatively, can we also identify patients with residual/recurrent molecular disease who are at HR for relapse, and treat them more aggressively or with novel and investigational treatment strategies?
- Can immunosuppressants with an mTOR inhibitor after AlloSCT help to reduce the incidence and severity of GVHD as well as reduce the risk of recurrence?
- Can nelarabine be substituted for cytarabine or added to current regimens in the upfront setting for T-ALL and result in an improved OS?
- With so many new agents with exciting potential, can we develop multicenter trials to increase availability, such that most or all adults with newly diagnosed or recurrent ALL would have the opportunity for enrollment? In this manner, can we develop and advance novel treatments for ALL in a timeframe dramatically less than that which we would currently expect.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
■■ of considerable interest

1 Fielding AK. The treatment of adults with acute lymphoblastic leukemia. *Am. Soc. Hematol. Educ. Program* 2008(1) 381–389 (2008).

2 Fielding AK. Current treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Am. Soc. Hematol. Educ. Program* 2011(1), 231–237 (2011).

3 Faderl S, O'Brien S, Pui CH *et al.* Adult acute lymphoblastic leukemia: concepts and strategies. *Cancer* 116(5), 1165–1176 (2010).

4 Goldstone AH, Richards SM, Lazarus HM *et al.* In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 111(4), 1827–1833 (2008).

■■ **Final reporting of the largest adult acute lymphoblastic leukemia (ALL) trial ever performed, with >1900 patients enrolled. A benefit for allogeneic stem cell transplant is demonstrated in a randomized prospective trial for the first time.**

5 Stock W, La M, Sanford B *et al.* What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 112(5), 1646–1654 (2008).

6 Ramanujachar R, Richards S, Hann I *et al.* Adolescents with acute lymphoblastic leukaemia: outcome on UK national paediatric (ALL97) and adult (UKALLXII/E2993) trials. *Pediatr. Blood Cancer* 48(3), 254–261 (2007).

7 Boissel N, Auclerc MF, Lheritier V *et al.* Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. *J. Clin. Oncol.* 21(5), 774–780 (2003).

8 Ram R, Gafter-Gvili A, Vidal L *et al.* Management of adult patients with acute lymphoblastic leukemia in first complete remission: systematic review and meta-analysis. *Cancer* 116(14), 3447–3457 (2010).

9 Fielding AK, Richards SM, Chopra R *et al.* Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG2993 study. *Blood* 109(3), 944–950 (2007).

10 Fielding AK, Buck G, Lazarus HM *et al.* Imatinib significantly enhances long-term outcomes in Philadelphia-positive acute lymphoblastic leukaemia; final results of the UKALLXII/ECOG2993 trial. *J. Am. Soc. Hematol.* 116(21), 169 (2010).

■■ **Analysis of the UKALLXII/ECOG2993 pre- and post-imatinib incorporation into the protocol demonstrates a substantial benefit in overall survival from 25–42% in Philadelphia chromosome-postive ALL. In addition, allogeneic transplant appeared to be of benefit in the imatinib era.**

11 Huguet F, Leguay T, Raffoux E *et al.* Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J. Clin. Oncol.* 27(6), 911–918 (2009).

12 Ribera JM, Oriol A, Sanz MA *et al.* Comparison of the results of the treatment of adolescents and young adults with standard-risk acute lymphoblastic leukemia with the Programa Espanol de Tratamiento en Hematologia pediatric-based protocol ALL-96. *J. Clin. Oncol.* 26(11), 1843–1849 (2008).

13 Raponi S, De Propris MS, Intoppa S *et al.* Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. *Leuk. Lymphoma* 52(6), 1098–1107 (2011).

14 Tobinai K, Kobayashi Y, Narabayashi M *et al.* Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) in relapsed B-cell lymphoma. The IDEC-C2B8 Study Group. *Ann. Oncol.* 9(5), 527–534 (1998).

15 Maloney DG, Grillo-Lopez AJ, White CA *et al.* IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 90(6), 2188–2195 (1997).

16 Maloney DG, Grillo-Lopez AJ, Bodkin DJ *et al.* IDEC-C2B8: results of a Phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. *J. Clin. Oncol.* 15(10), 3266–3274 (1997).

17 Salles G, Seymour JF, Offner F *et al.* Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a Phase III, randomised controlled trial. *Lancet* 377(9759), 42–51 (2011).

18 Hochster H, Weller E, Gascoyne RD *et al.* Maintenance rituximab after cyclophosphamide, vincristine and prednisone prolongs progression-free survival in advanced indolent lymphoma: results of the randomized Phase III ECOG1496 Study. *J. Clin. Oncol.* 27(10), 1607–1614 (2009).

19 Witzig TE, Geyer SM, Kurtin PJ *et al.* Salvage chemotherapy with rituximab DHAP for relapsed non-Hodgkin lymphoma: a Phase II trial in the North Central Cancer Treatment Group. *Leuk. Lymphoma* 49(6), 1074–1080 (2008).

20 Marcus R, Imrie K, Solal-Celigny P *et al.* Phase III study of R-CVP compared with cyclophosphamide, vincristine and prednisone alone in patients with previously untreated advanced follicular lymphoma. *J. Clin. Oncol.* 26(28), 4579–4586 (2008).

21 Yamanaka R, Homma J, Sano M *et al.* Salvage immuno-chemotherapy with a combination of rituximab, high-dose cytarabine, mitoxantrone and dexamethasone for patients with primary CNS lymphoma: a preliminary study. *Leuk. Lymphoma* 48(7), 1429–1433 (2007).

22 Shah GD, Yahalom J, Correa DD *et al.* Combined immunochemotherapy with reduced whole-brain radiotherapy for newly diagnosed primary CNS lymphoma. *J. Clin. Oncol.* 25(30), 4730–4735 (2007).

23 Witzig TE, Vukov AM, Habermann TM *et al.* Rituximab therapy for patients with newly diagnosed, advanced-stage, follicular grade I non-Hodgkin's lymphoma: a Phase II trial in the North Central Cancer Treatment Group. *J. Clin. Oncol.* 23(6), 1103–1108 (2005).

24 Hiddemann W, Kneba M, Dreyling M *et al.* Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 106(12), 3725–3732 (2005).

25 Habermann TM, Weller EA, Morrison VA *et al.* Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J. Clin. Oncol.* 24(19), 3121–3127 (2006).

26 Thomas DA, O'Brien S, Jorgensen JL *et al.* Prognostic significance of CD20 expression in adults with *de novo* precursor B-lineage acute lymphoblastic leukemia. *Blood* 113(25), 6330–6337 (2009).

27 Thomas DA, O'Brien S, Faderl S *et al.* Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in *de novo* Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. *J. Clin. Oncol.* 28(24), 3880–3889 (2010).

■■ **A retrospective review at a single center of Philadelphia chromosome-negative B-cell ALL (B-ALL) with CD20 expression of >20%. The addition of rituximab to Hyper-CVAD chemotherapy appears to confer an overall survival benefit despite similar responses to induction therapy.**

28 Hoelzer D, Huettmann A, Kaul F *et al.* Immunochemotherapy with rituximab improves molecular CR rate and outcome in $CD20^+$ B-lineage standard- and high-risk patients; results of 263 $CD20^+$ patients studied prospectively in GMALL study 07/2003. *J. Am. Soc. Hematol.* 116(21), 170 (2010).

29 Dworzak MN, Schumich A, Printz D *et al.* CD20 up-regulation in pediatric B-cell precursor acute lymphoblastic leukemia during induction treatment: setting the stage for anti-CD20 directed immunotherapy. *Blood* 112(10), 3982–3988 (2008).

30 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.* 10(16), 5327–5334 (2004).

31 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.* 21(16), 3051–3059 (2003).

32 Micallef IN, Maurer MJ, Wiseman GA *et al.* Epratuzumab with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy in patients with previously untreated diffuse large B-cell lymphoma. *Blood* 118(15), 4053–4061 (2011).

33 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.* 26(22), 3756–3762 (2008).

34 Raetz EA, Cairo MS, Borowitz MJ *et al.* Reinduction chemoimmunotherapy with epratuzumab in relapsed acute lymphoblastic leukemia (ALL) in children, adolescents and young adults: results from Children's Oncology Group (COG) study ADVL04P2. *J. Am. Soc. Hematol.* 118(21), 573 (2011).

35 Younes A, Bartlett NL, Leonard JP *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N. Engl. J. Med.* 363(19), 1812–1821 (2010).

36 Advani A, Coiffier B, Czuczman MS *et al.* Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a Phase I study. *J. Clin. Oncol.* 28(12), 2085–2093 (2010).

37 O'Brien S, Thomas DA, Ohanian M *et al.* Inotuzumab ozogamycin (I0), a CD22 monoclonal antibody conjugated to calecheamicin, is active in refractory-relapse acute lymphocytic leukemia (R-R ALL). *J. Am. Soc. Hematol.* 118(21), 875 (2011).

■■ **Inotuzumab, a CD19 antibody conjugated to calicheamicin, demonstrates substantial single agent activity in a Phase II clinical trial enrolling 49 adults and children with heavily pretreated relapsed/refractory B-ALL. A response rate of 57% is reported.**

38 Herrera L, Bostrom B, Gore L *et al.* A Phase I study of Combotox in pediatric patients with refractory B-lineage acute lymphoblastic leukemia. *J. Pediatr. Hematol. Oncol.* 31(12), 936–941 (2009).

39 Schindler J, Gajavelli S, Ravandi F *et al.* A Phase I study of a combination of anti-CD19 and anti-CD22 immunotoxins (Combotox) in adult patients with refractory B-lineage acute lymphoblastic leukaemia. *Br. J. Haematol.* 154(4), 471–476 (2011).

40 Wayne AS, Bhojwani D, Silverman LB *et al.* A novel anti-CD22 immunotoxin, moxetumomab pasudotox: Phase I study in pediatric acute lymphoblastic leukemia (ALL). *J. Am. Soc. Hematol.* 118(21), 248 (2011).

41 Bargou R, Leo E, Zugmaier G *et al.* Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science*

321(5891), 974–977 (2008).

42 Topp MS, Kufer P, Gokbuget N *et al.* Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J. Clin. Oncol.* 29(18), 2493–2498 (2011).

43 Topp MS, Goekbuget N, Zugmaier G *et al.* Anti-CD19 BiTE blinatumomab induces high complete remission rate in adult patients with relapsed B-precursor ALL: updated results of an ongoing Phase II trial. *J. Am. Soc. Hematol.* 118(21), 252 (2011).

■■ **Blinatumomab, a first-in-class bispecific T-cell engager that links a CD3 and CD19 antibody to promote T-cell-mediated perforin mediated cytotoxicity of B lymphoblasts, demonstrates a 67% complete response rate in heavily pretreated relapsed/refractory B-ALL. All complete remission (CR) ultimately achieved a molecular CR and all CRs also appeared to be durable.**

44 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.* 344(14), 1031–1037 (2001).

45 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.* 344(14), 1038–1042 (2001).

46 Wassmann B, Pfeifer H, Goekbuget N *et al.* Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood* 108(5), 1469–1477 (2006).

47 Yanada M, Takeuchi J, Sugiura I *et al.* High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR–ABL-positive acute lymphoblastic leukemia: a Phase II study by the Japan Adult Leukemia Study Group. *J. Clin. Oncol.* 24(3), 460–466 (2006).

48 Liu T, Kuang P, Dong T *et al.* Imatinib 400 mg daily combined with vindesine and dexamethasone as induction and maintenance therapy for Philadelphia chromosome-positive acute lymphocytic leukemia. *J. Am. Soc. Hematol.* 118(21), 4243 (2011).

49 Kantarjian H, Giles F, Wunderle L *et al.* Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N. Engl. J. Med.* 354(24), 2542–2551 (2006).

50 Talpaz M, Shah NP, Kantarjian H *et al.* Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N. Engl. J. Med.* 354(24), 2531–2541 (2006).

51 Ottmann O, Dombret H, Martinelli G *et al.* Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a Phase II study. *Blood* 110(7), 2309–2315 (2007).

52 Porkka K, Koskenvesa P, Lundan T *et al.* Dasatinib crosses the blood–brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. *Blood* 112(4), 1005–1012 (2008).

53 Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 305(5682), 399–401 (2004).

54 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.* 65(11), 4500–4505 (2005).

55 Thomas DA, Faderl S, Cortes J *et al.* Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood* 103(12), 4396–4407 (2004).

56 Ravandi F, O'Brien S, Thomas D *et al.* First report of Phase II study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. *Blood* 116(12), 2070–2077 (2010).

57 Kim D-Y, Joo YD, Lee J-H *et al.* Nilotinib Combined with multi-agent chemotherapy for adult patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia: interim results of Korean Adult ALL Working Party Phase II study. *J. Am. Soc. Hematol.* 118(21), 1517 (2011).

58 Rousselot P, Cayuela JM, Hayette S *et al.* Dasatinib (Sprycel®) and low intensity chemotherapy for first-line treatment in elderly patients with *de novo* Philadelphia-positive ALL (EWALL-PH-01): kinetic of response, resistance and prognostic significance. *J. Am. Soc. Hematol.* 116(21), 172 (2010).

59 Vignetti M, Fazi P, Cimino G *et al.* Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. *Blood* 109(9), 3676–3678 (2007).

60 Foa R, Vitale A, Vignetti M *et al.* Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood* 118(25), 6521–6528 (2011).

■■ **In Philadelphia chromosome-positive ALL in the upfront setting, a 67% 2-year overall survival and a 31-month median overall survival is achieved with dasatinib and prednisone alone for induction therapy. In addition, in those patients eligible and who proceeded to allogeneic transplant, 16 of 18 patients appeared to be long-term disease-free survivors.**

61 Khoury HJ, Cortes JE, Gambacorti-Passerini C *et al.* Activity of bosutinib by baseline and emergent mutation status in Philadelphia chromosome-positive leukemia patients with resistance or intolerance to other tyrosine kinase inhibitors. *J. Am. Soc. Hematol.* 118(21), 110 (2011).

62 Cortes JE, Kim D-W, Pinilla-Ibarz J *et al.* Initial findings from the PACE trial: a pivotal Phase II study of ponatinib in patients with CML and Ph+ ALL resistant or intolerant to dasatinib or nilotinib, or with the T315I mutation. *J. Am. Soc. Hematol.* 118(21), 109 (2011).

63 DeAngelo DJ. Nelarabine for the treatment of patients with relapsed or refractory T-cell acute lymphoblastic leukemia or lymphoblastic lymphoma. *Hematol. Oncol. Clin. North Am.* 23(5), 1121–1135, vii–viii (2009).

64 Kisor DF, Plunkett W, Kurtzberg J *et al.* Pharmacokinetics of nelarabine and 9-β-D-arabinofuranosyl guanine in pediatric and adult patients during a Phase I study of nelarabine for the treatment of refractory hematologic malignancies. *J. Clin. Oncol.* 18(5), 995–1003 (2000).

65 Kurtzberg J, Ernst TJ, Keating MJ *et al.* Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. *J. Clin. Oncol.* 23(15), 3396–3403 (2005).

66 Gokbuget N, Basara N, Baurmann H *et al.* High single-drug activity of nelarabine in relapsed T-lymphoblastic leukemia/lymphoma offers curative option with subsequent stem cell transplantation. *Blood* 118(13), 3504–3511 (2011).

■■ **In a large Phase II clinical trial enrolling 126 patients, the purine nucleoside nelarabine demonstrates substantial single-agent activity in T lineage ALL. In a heavily pretreated relapsed/refractory population, a 36% CR rate and 10% partial-response rate is demonstrated.**

67 DeAngelo DJ, Yu D, Johnson JL *et al.* Nelarabine induces complete remissions in adults with relapsed or refractory T-lineage acute lymphoblastic leukemia or lymphoblastic lymphoma: Cancer and Leukemia Group B study 19801. *Blood* 109(12), 5136–5142 (2007).

68 Winter SS, Devidas M, Wood B *et al.* Nelarabine may be safely incorporated into a Phase III study for newly diagnosed T-lineage acute lymphoblastic leukemia: a report from the Children's Oncology Group. *J. Am. Soc. Hematol.* 116(21), 865 (2010).

69 Pulsipher MA, Wall DA, Grimley M *et al.* A Phase I/II study of the safety and efficacy of the addition of sirolimus to tacrolimus/methotrexate graft versus host disease prophylaxis after allogeneic haematopoietic cell transplantation in paediatric acute lymphoblastic leukaemia (ALL). *Br. J. Haematol.* 147(5), 691–699 (2009).

70 Scott EC, Perl A, Luger SM, Carroll M, Kasner M. A feasibility study of rapamycin with hyper-CVAD chemotherapy in adults with acute lymphoblastic leukemia (ALL) and other aggressive lymphoid malignancies and evaluation of mTOR signaling using phosphoflow. *J. Am. Soc. Hematol.* 118(21), 4245 (2011).

71 Zhang C, Ryu YK, Chen TZ, Hall CP, Webster DR, Kang MH. Synergistic activity of rapamycin and dexamethasone *in vitro* and *in vivo* in acute lymphoblastic leukemia via cell-cycle arrest and apoptosis. *Leuk. Res.*(2011).

72 Saunders P, Cisterne A, Weiss J, Bradstock KF, Bendall LJ. The mammalian target of rapamycin inhibitor RAD001 (everolimus) synergizes with chemotherapeutic agents, ionizing radiation and proteasome inhibitors in pre-B acute lymphocytic leukemia. *Haematologica* 96(1), 69–77 (2011).

73 Redig AJ, Vakana E, Platanias LC *et al.* Regulation of mammalian target of rapamycin and mitogen activated protein kinase pathways by BCR-ABL. *Leuk. Lymphoma* 52(Suppl. 1), 45–53 (2011).

74 Teachey DT, Sheen C, Hall J *et al.* mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. *Blood* 112(5), 2020–2023 (2008).

75 Horton TM, Pati D, Plon SE *et al.* A Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. *Clin. Cancer Res.* 13(5), 1516–1522 (2007).

76 Messinger Y, Gaynon P, Raetz E *et al.* Phase I study of bortezomib combined with chemotherapy in children with relapsed childhood acute lymphoblastic leukemia (ALL): a report from the therapeutic advances in childhood leukemia (TACL) consortium. *Pediatr. Blood Cancer* 55(2), 254–259 (2010).

77 Messinger YH, Gaynon P, Sposto R *et al.* Bortezomib combined with VXLD chemotherapy is highly effective in advanced B-lineage acute lymphoblastic leukemia allowing early study termination due to efficacy. A Therapeutic Advances in Childhood Leukemia (TACL) consortium Phase II study. *J. Am. Soc. Hematol.* 118(21), 251 (2011).

78 Moreau P, Pylypenko H, Grosicki S *et al.* Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, Phase III, noninferiority study. *Lancet Oncol.* 12(5), 431–440 (2011).

79 Bringhen S, Larocca A, Rossi D *et al.* Efficacy and safety of once-weekly bortezomib in multiple myeloma patients. *Blood* 116(23), 4745–4753 (2010).

80 Thomas DA, Kantarjian HM, Stock W *et al.* Phase I multicenter study of vincristine sulfate liposomes injection and dexamethasone in adults with relapsed or refractory acute lymphoblastic leukemia. *Cancer* 115(23), 5490–5498 (2009).

81 Schiller GJ, Lister J, Heffner LT *et al.* Vincristine sulfate liposomes injection (Marqibo®) facilitates durable remissions and potentially curative hematopoietic stem cell transplantation in adults with advanced, relapsed and/or refractory acute lymphoblastic leukemia. *J. Am. Soc. Hematol.* 118(21), 4235 (2011).

82 Lubbert M, Suciu S, Baila L *et al.* Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized Phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. *J. Clin. Oncol.* 29(15), 1987–1996 (2011).

83 Fenaux P, Mufti GJ, Hellstrom-Lindberg E *et al.* Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J. Clin. Oncol.* 28(4), 562–569 (2010).

84 Fenaux P, Mufti GJ, Hellstrom-Lindberg E *et al.* Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, Phase III study. *Lancet Oncol.* 10(3), 223–232 (2009).

85 Vilas-Zornoza A, Agirre X, Martin-Palanco V *et al.* Frequent and simultaneous epigenetic inactivation of TP53 pathway genes in acute lymphoblastic leukemia. *PLoS One* 6(2), e17012 (2011).

86 Stumpel DJ, Schotte D, Lange-Turenhout EA *et al.* Hypermethylation of specific microRNA genes in MLL-rearranged infant acute lymphoblastic leukemia: major matters at a micro scale. *Leukemia* 25(3), 429–439 (2011).

87 Roman-Gomez J, Castillejo JA, Jimenez A, Barrios M, Heiniger A, Torres A. The role of DNA hypermethylation in the pathogenesis and prognosis of acute lymphoblastic leukemia. *Leuk. Lymphoma* 44(11), 1855–1864 (2003).

88 Garcia-Manero G, Thomas DA, Rytting ME *et al.* Final report of a Phase I trial of decitabine with or without hypercvad in relapsed acute lymphocytic leukemia (ALL). *J. Am. Soc. Hematol.* 116(21), 867 (2010).