Signaling the end of chronic lymphocytic leukemia: new frontline treatment strategies

Michael Hallek[1]

[1]*Department I of Internal Medicine, Center for Integrated Oncology Köln Bonn, Center of Excellence on "Cellular Stress Responses in Aging-Associated Diseases," University of Cologne, Cologne, Germany*

The management of chronic lymphocytic leukemia (CLL) is undergoing profound changes. Several new drugs have been approved for CLL treatment (fludarabine, bendamustine, and the monoclonal antibodies alemtuzumab, rituximab, and ofatumumab) and many more drugs are in advanced clinical development to be approved for this disease. In addition, the extreme heterogeneity of the clinical course and our improved ability to foresee the prognosis of this leukemia by the use of clinical, biological, and genetic parameters now allow us to characterize patients with a very mild onset and course, an intermediate prognosis, or a very aggressive course with high-risk leukemia. Therefore, it becomes increasingly challenging to select the right treatment strategy for each condition. This article summarizes the currently available diagnostic and therapeutic tools and gives an integrated recommendation of how to manage CLL in 2013. Moreover, I propose a strategy how we might integrate the novel agents for CLL therapy into sequential treatment approaches in the near future.

Overview

With an age-adjusted incidence of 4.3/100 000 inhabitants in the United States, chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Western countries. More than 15 000 newly diagnosed cases and ~ 4500 deaths are currently estimated.[1] The median age at diagnosis lies between 67 and 72 years. More male than female patients are affected by this disease.[1]

CLL is characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B cells within the blood, BM, lymph nodes, and spleen. The leukemic transformation is initiated by specific genomic alterations, in particular deletions on the long arm of chromosome 13 [*del(13q14)*] Some of these aberrations cause the deletion of specific micro-RNA genes and increase the resistance of B cells toward apoptosis.[2,3] Additional aberrations of the long arm of chromosome 11 [*del(11q)*], of the short arm of chromosome 17 [*del(17p)*], and trisomy 12 seem to occur later in the course of the disease and predict a worse outcome. In addition, whole-genome sequencing has uncovered recurrent somatic gene mutations that occur in CLL cells in parallel to the above-mentioned structural genomic aberrations. Of these, mutations affecting the genes *NOTCH1*, *MYD88*, *TP53*, *ATM*, and *SF3B1* seem to be more common and to have prognostic impact.[4-7]

It has become increasingly clear that survival of CLL cells is not a cell-autonomous, genetically determined process. Instead, survival of CLL cells strictly depends on a permissive microenvironment composed of cellular components such as macrophages, T cells, or stromal follicular dendritic cells,[8] which provide essential proteins (chemokines, cytokines, and angiogenic factors) for activation of crucial survival and proliferative signaling pathways of transformed cells. In addition, stimulation of the BCR also drives the activation of different tyrosine kinases, such as Bruton tyrosine kinase (BTK), spleen tyrosine kinase (Syk), ZAP70, Src family kinases (in particular Lyn kinase), and phosphatidyl inositol 3-kinase (PI3K), which stimulate malignant B-cell survival via activation of transcription factors such as NF-κB.[9,10] The importance of BCR signaling is underscored by the fact that different features of the BCR have been recognized as a prognostic marker in CLL, such as the degree of immunoglobulin heavy chain variable gene (IGHV) mutations or IGHV stereotype (for review, see Stevenson et al[9]). These pathways have recently gained in importance because they can be inhibited by specific small-molecule inhibitors that show clinical efficacy in lymphoid malignancies.[10] Excellent reviews have recently summarized these BCR-mediated pathways and the corresponding therapeutic inhibitors.[10,11] In some contrast, this review intends to provide an update of the current state-of-the-art of CLL therapy and to propose strategies on how to integrate these novel inhibitors into future CLL therapies.

Standard of care using the currently approved agents

Cytostatic agents

Monotherapy with alkylating agents has served as initial, frontline therapy for CLL, and chlorambucil (CLB) was the gold standard of CLL therapy for several decades.[12] Even today, this drug remains an appropriate option for frail elderly or unfit patients. The advantages of CLB are its moderate toxicity, low cost, and convenience as an oral drug; the major disadvantages are its low to nonexistent complete remission (CR) rate and some side effects that occur after extended use (eg, prolonged cytopenia, myelodysplasia, and secondary acute leukemia). Novel results indicate that CLB monotherapy may be used less frequently because the combination with anti-CD20 antibodies has proven more effective (see below).

Three purine analogs are currently used in CLL: fludarabine, pentostatin, and cladribine (2-CdA). Fludarabine remains by far the best-studied compound of the 3 in CLL. Fludarabine induced more remissions and more CR (7%-40%) than other conventional chemotherapies such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), CAP (cyclophosphamide, doxorubicin, prednisone), or CLB, but did not improve overall survival (OS) when used as single agent.[13-16] Similarly, cladribine monotherapy was shown to produce a higher CR rate than CLB plus prednisone (47% vs 12%) without resulting in a longer survival.[17]

More recently, bendamustine was compared with CLB in a randomized trial. Bendamustine produced improved responses but greater toxicity and no OS benefit.[18] The overall response rate (ORR) and median progression-free survival (PFS) were 67% and 22 months, respectively, for bendamustine versus 30% and 8 months for CLB (both $P < .0001$).

Antibodies
Rituximab. In CLL, the anti-CD20 antibody rituximab is less active as a single agent than in follicular lymphoma unless very high doses are used.[19,20] In contrast, combinations of rituximab with chemotherapy have proven to be very efficacious therapies for CLL (see below). Some newer CD20 antibodies challenge rituximab.[21-23]

Ofatumumab. Ofatumumab is a fully humanized antibody targeting a unique epitope on the CD20 molecule expressed on human B cells, resulting in increased binding affinity to CD20, prolonged dissociation rate, and increased cell kill due to greater complement-dependent cytotoxicity and similar antibody-dependent cellular cytotoxicity activity compared with rituximab, especially in cells expressing low levels of CD20. Ofatumumab has shown some efficacy in patients who are fludarabine and alemtuzumab refractory or who have bulky disease (> 5 cm).[24] The ORR was 58% in the fludarabine and alemtuzumab (FA)–refractory group and 47% in the bulky disease group. However, there has been no formal comparative trial of rituximab versus ofatumumab. Therefore, the value of ofatumumab for the treatment of B-cell lymphoma and CLL remains unclear.

Alemtuzumab. Alemtuzumab is a recombinant, fully humanized monoclonal antibody against the CD52 antigen. Monotherapy with alemtuzumab has produced response rates of 33% to 53%, with a median duration of response ranging from 8.7 to 15.4 months, in patients with advanced CLL who were previously treated with alkylating agents and had failed or relapsed after second-line fludarabine therapy.[25-27] In addition, alemtuzumab has proven effective in patients with high-risk genetic markers such as deletions of chromosome 11 or 17 [*del(11q)* and *del(17p)*] and *TP53* mutations.[28,29] Therefore, alemtuzumab is a reasonable therapeutic option for patients with these poor prognostic features. In a prospective, randomized study, alemtuzumab was tested against CLB.[30] Alemtuzumab led to a greater ORR and CR ($P < .0001$), superior PFS with a 42% reduction in risk of progression or death ($P < .0001$), and significantly longer median time to progression (TTP; $P = .0001$). Therefore, the drug remains a valuable treatment option for high-risk patients. Unfortunately, the drug is no longer licensed, but remains available in some countries on a compassionate use basis.

Combination chemotherapy
A major advance in CLL treatment was achieved by the combined use of different treatment modalities. Because purine analogs and alkylating agents have different mechanisms of action and partially nonoverlapping toxicity profiles, they were combined both in preclinical and clinical studies. The most thoroughly studied combination chemotherapy for CLL is fludarabine plus cyclophosphamide (FC) given as oral drugs or IV infusions.[31] In noncomparative trials, the ORRs did not appear to be better than with fludarabine alone, but the addition of cyclophosphamide appeared to improve the CR rate up to 50%.[31] Three randomized trials have shown that FC combination chemotherapy improves the CR and ORR and PFS compared with fludarabine monotherapy.[32-34] The rate of severe infections was not significantly increased by the FC combination despite a higher frequency of neutropenias. An updated analysis of the CLL4 trial of the German CLL Study Group (GCLLSG) suggested that the frontline treatment of CLL patients with FC combination might improve the OS of the non-high-risk CLL patients [all patients *not* exhibiting a *del(17p)* or *TP53* mutation].

The Polish Adult Leukemia Group (PALG) compared 2-CdA alone with 2-CdA combined with cyclophosphamide (CC) or cyclophosphamide and mitoxantrone (CMC) in 479 patients with untreated progressive CLL.[35] Surprisingly, the CC combination therapy did not produce any benefit in terms of PFS or response rates compared with 2-CdA alone.

Chemoimmunotherapy
Combinations using rituximab. Phase 2 studies suggested that rituximab combinations with fludarabine or fludarabine-based regimens would produce significant improvements in all major outcome parameters such as CR rates, PFS, and OS (for review, see Hallek and Pflug[36]). The largest of these trials, conducted on 300 patients with previously untreated CLL, showed that rituximab plus fludarabine/cyclophosphamide (FCR) achieved an ORR of 95%, with CR in 72%.[37] Six-year OS and failure-free survival were 77% and 51%, respectively, and median TTP was 80 months.

These results led the GCLLSG to conduct a randomized trial, the CLL8 protocol, on 817 patients, median age 61 years, with good physical fitness who received 6 courses of FC (n = 409) or FCR (n = 408).[38] FCR treatment was more frequently associated with Common Terminology Criteria (CTC) grade 3 and 4 neutropenia (FCR 34%; FC 21%), without any formal recommendation to use growth factor support. Other grade 3 or 4 side effects, including infections, were not increased. FCR induced a higher ORR than FC (92.8% vs 85.4%) and more CR (44.5% vs 22.9%; $P < .001$). Most importantly, this trial was the first to show that the choice of a frontline therapy has an impact on OS of CLL patients. In an updated analysis of at a median follow-up of 5.9 years, 38.0% of the patients in the FCR group were free of disease progression compared with 27.4% in the FC group (hazard ratio [HR] = 0.6; 95% confidence interval [95% CI], 0.5-0.7; $P < .0001$).[39] At the same time point, 69.4% of the patients were alive in the FCR group versus 62.3% in the FC group (HR = 0.7; 95% CI, 0.5-0.9), $P = .001$). Median OS was not reached for patients in the FCR group, whereas the median OS was 86.0 months (95% CI, 78.7-93.2 months) for the FC group ($P = .001$).[39] FCR did not improve the survival of patients with a *del(17p)*. Similar results were obtained in a trial comparing FCR without FC in relapsed CLL without demonstrating a benefit for OS.[40]

Because CLL often occurs in elderly patients with relevant comorbidity, a dose-modified FCR-Lite regimen was designed to maintain the efficacy but decrease the toxicity of the FCR regimen.[41] This

regimen reduced the dose of fludarabine and cyclophosphamide, increased the dose of rituximab, and used a maintenance regimen for rituximab given every 3 months until progression. The CR rate was 77% for 50 previously untreated CLL patients with an ORR of 100%. Grade 3/4 neutropenia was documented in only 13% of cycles, which is lower than that observed with the usual FCR regimen.

Following a similar concept, the CLL11 protocol of the GCLLSG was designed to test chemoimmunotherapies with anti-CD20 antibodies combined with a milder chemotherapeutic component, CLB, in previously untreated CLL patients with comorbidities.[42] The rationale of this study was based on encouraging results obtained in phase 2 trials using CLB in combination with rituximab (RCLB).[43,44] The CLL11 trial compared CLB alone versus a new anti-CD20 antibody, obinutuzumab (GA101; see below), plus CLB (GCLB) versus rituximab (R) plus CLB (RCLB). The study included treatment-naive CLL patients with a Cumulative Illness Rating Scale (CIRS) total score > 6 and/or an estimated creatinine clearance (CrCl) < 70 mL/min. Treatment consisted of CLB alone (0.5 mg/kg orally on days 1 and 15 for 28 days and 6 cycles), GCLB (100 mg IV on day 1, 900 mg on day 2, 1000 mg on days 8 and 15 of cycle 1, 1000 mg on day 1 for cycles 2-6), or RCLB (375 mg/m^2 IV on day 1 cycle 1, 500 mg/m^2 on day 1 cycles 2-6).[42] The median age of patients was 73 years and the median CIRS score was 8. So far, the results obtained allow the conclusion that chemoimmunotherapy with one of the 2 anti-CD20 antibodies increases the ORR, allows patients to achieve CRs (22% vs 8% vs 0%; GCLB vs RCLB vs CLB) and seems to prolong PFS from 11 months (CLB) to 16 or 23 months (RCLB or GCLB), respectively. The major pronounced side effects were grade 3-4 infusion-related reactions with GCLB at the first infusion. Taken together, chemoimmunotherapy with anti-CD20 antibodies is a potent treatment concept in CLL patients with comorbidities as well.

Other variations have been tested to further improve the efficacy of the FCR regimen: Alemtuzumab (A) was added to FCR (CFAR) in a phase 2 trial on 60 high-risk untreated patients < 70 years of age with serum beta$_2$-microglobulin ≥ 4 mg/L.[45] CR was achieved in 70%, PR in 18%, nodular PR in 3%, for an ORR of 92%. Of 14 patients with 17p deletion, 8 (57%) achieved a CR. Grade 3/4 neutropenia and thrombocytopenia occurred with 33% and 13% courses, respectively. The median PFS was 38 months and median OS was not reached. Therefore, CFAR might be helpful in cases of high-risk CLL in which an effective cytoreductive therapy is desired before an allogeneic stem cell transplantation. In another study on 72 untreated CLL patients ≤ 70 years of age, mitoxantrone was combined at 6 mg/m^2 on day 1 of each cycle with FCR.[46] The ORR, minimal residual disease (MRD)–negative CR, MRD-positive CR, and PR rates were 93%, 46%, 36%, and 11%, respectively. Severe neutropenia developed in 13% of patients. These results do not justify the broad use of this regimen outside of clinical trials.

An alternative idea was to replace fludarabine in the FCR regimen with pentostatin (PCR) to reduce myelotoxicity. In a phase 3 randomized trial comparing FCR with PCR in previously untreated or minimally treated CLL patients, there were no statistical differences between treatments in OS or response rates.[47] Moreover, this trial did not demonstrate a lower infection rate with PCR.

Bendamustine has been also combined with rituximab (BR) in 81 patients with relapsed CLL.[48] Patients received 70 mg/m^2 of bendamustine on days 1 and 2 and 375 mg/m^2 of rituximab on day 0

of the first cycle and 500 mg/m^2 on day 1 of subsequent cycles administered every 28 days for up to 6 cycles. On the basis of intent-to-treat analysis, the ORR was 59.0% (95% CI, 47.3%-70.0%). CR, PR, and nodular PR were achieved in 9.0%, 47.4%, and 2.6% of patients, respectively. ORR was 45.5% in fludarabine-refractory patients and 60.5% in fludarabine-sensitive patients. Among genetic subgroups, 92.3% of patients with del(11q), 100% with trisomy 12, 7.1% with del(17p), and 58.7% with unmutated IGHV status responded to treatment. After a median follow-up time of 24 months, the median event-free survival was 14.7 months. Severe infections occurred in 12.8% of patients. Grade 3 or 4 neutropenia, thrombocytopenia, and anemia were documented in 23.1%, 28.2%, and 16.6% of patients, respectively.

The BR regimen was also investigated as frontline therapy in 117 CLL patients (age 34-78 years).[49] Bendamustine was administered at a dose of 90 mg/m^2 on days 1 and 2 combined with 375 mg/m^2 rituximab on day 0 of the first course and 500 mg/m^2 on day 1 during subsequent courses for up to 6 courses. ORR was 88.0% (95% CI, 80.7%-100.0%) with a CR rate of 23.1% and a PR rate of 64.9%. Ninety percent of patients with del(11q), 94.7% with trisomy 12, 37.5% with del(17p), and 89.4% with unmutated IGHV status responded to treatment. After a median observation time of 27.0 months, median event-free survival was 33.9 months, and 90.5% of patients were alive. Grade 3 or 4 severe infections occurred in 7.7% of patients. Grade 3 or 4 adverse events for neutropenia, thrombocytopenia, and anemia were documented in 19.7%, 22.2%, and 19.7% of patients, respectively. Overall, these results suggest that, compared with FCR, BR is somewhat less active, yielding lower CR rates, but is also less myelotoxic. The GCLLSG is currently comparing BR with FCR in a randomized phase 3 trial, the CLL10 protocol.

Several other combinations have been investigated, such as cladribine with rituximab, methylprednisolone plus rituximab followed by alemtuzumab, or rituximab plus alemtuzumab. Their detailed description is beyond the scope of this article, because none of them has been proven to result in higher efficacy compared with FCR.

Combinations using alemtuzumab. The synergistic activity of fludarabine and alemtuzumab was initially suggested by the induction of responses, including 1 CR, in 5 of 6 patients who were refractory to each agent alone.[50] The combination of FA was investigated in a phase 2 trial enrolling patients with relapsed CLL using a 4-weekly dosing protocol.[51] This combination has proven feasible, safe, and effective. Among the 36 patients, the ORR was 83% (30/36 patients), which included 11 CRs (30%) and 19 PRs (53%); there was one stable disease. Sixteen of 31 evaluated patients (53%) achieved MRD negativity in the peripheral blood by 3 months of follow-up. Resolution of disease was observed in all disease sites, particularly in the blood, BM, and spleen. The FA therapy was well tolerated. Infusion reactions (fever, chills, and skin reactions) occurred primarily during the first infusions of alemtuzumab and were mild in the majority of patients. Although 80% of patients were CMV IgG-positive before treatment, there were only 2 subclinical CMV reactivations. The primary grade 3/4 hematological events were transient, including leukocytopenia (44%) and thrombocytopenia (30%). Stable CD4$^+$ T-cell counts ($> 200/\mu$l) were seen after 1 year.

Two phase 3 trials tested alemtuzumab in combination with FC (FCA) or fludarabine (FA). One trial comparing FCA with FCR in frontline therapy was closed prematurely due to the higher toxicity

and treatment-related mortality observed in the FCA arm.[52] The therapeutic efficacy of FCR was clearly superior to FCA. In this trial, alemtuzumab was given subcutaneously. A second randomized trial compared FA with fludarabine monotherapy in previously treated patients with relapsed or refractory CLL.[53] In this trial, alemtuzumab was given IV. FA (n = 168) resulted in better PFS than fludarabine monotherapy (n = 167; median 23.7 months vs 16.5 months; HR = 0.61; P = .0003) and OS (median not reached vs 52.9 months; HR = 0.65; P = .021) compared with fludarabine alone. Adverse events occurred in 161 (98%) of 164 patients in the FA group and 149 (90%) of 165 in the fludarabine alone group. Patients in the FA group had more CMV events (14% vs < 1%) and grade 1 or 2 infusion-related adverse reactions (62% vs 13%). Major grade 3 or 4 toxicities in the FA and monotherapy groups were leukopenia (74% vs 34%), lymphopenia (94% vs 33%), neutropenia (59% vs 68%), thrombocytopenia (11% vs 17%), and anemia (9% vs 17%). The incidence of serious adverse events was higher in the FA group (33% vs 25%); deaths due to adverse events were similar between the 2 groups (6% vs 12%).

Two phase 2 trials investigated alemtuzumab in combination with high-dose corticosteroids, which seem to be a good therapeutic option, in particular for patients with *del(17p)*. The combination of alemtuzumab and methylprednisolone was tested in the UK CLL206 trial on 17 untreated and 22 previously treated patients with *del(17p)*. The ORR and CR rate were 85% and 36% in the whole cohort and 88% and 65% in treatment naive patient group, respectively.[54] In the CLL2O trial of the GCLLSG, the combination of alemtuzumab and high-dose dexamethasone resulted in an ORR above 90% in treatment-naive high-risk patients.[55]

Selecting the right treatment: how to treat CLL with the currently approved agents?

Given the impressive number of novel drugs, the right choice of treatment for a given CLL patient has become a task that requires experience, a good clinical assessment of the patient, and an appropriate use of diagnostic tools. The following parameters should be considered before recommending a treatment for CLL[56]: (1) the clinical stage of disease, (2) the fitness of the patient, (3) the genetic risk of the leukemia, and (4) the treatment situation (frontline vs second-line, response vs nonresponse of the last treatment). With the use of these 4 parameters, the following recommendations can be given (Figure 1A,B):

Frontline treatment (Figure 1A). In a patient with advanced (Binet C, Rai III-IV) or active, symptomatic disease, treatment should be initiated. In this situation, patients need to be evaluated for their physical condition (or comorbidity). For patients in good physical condition ("go go"), as defined by a normal creatinine clearance and a low score at the "cumulative illness rating scale" (CIRS),[57] patients should be offered chemoimmunotherapies such as FCR or FR to achieve sustained remissions.

Patients with a somewhat impaired physical condition ("slow go") may be offered either CLB in combination with an anti-CD20 antibody[42] or a dose-reduced fludarabine-containing regimen with a CD20 antibody. The aim of therapy in this situation is symptom control.

Patients with symptomatic disease and with *del(17p)* or *TP53* mutations may receive FCR or BR or an alemtuzumab-containing regimen as frontline treatment. In general, these regimens all yield response rates above 50%, but the TTP tends to be shorter than 2

years. The recent results obtained with single-agent ibrutinib in refractory or relapsed patients with a *del(17p)* showed an ORR of 68%, with a PFS at 26 months of 57% and an OS of 70%.[58] Although these results appear very encouraging, none of these therapies promises long-lasting remissions. Therefore, allogeneic stem cell transplantation should still be offered and discussed in patients with sufficient physical fitness.

Second-line treatment (Figure 1B). As a general rule, the frontline treatment may be repeated if the duration of the first remission exceeds 24 months provided that the frontline therapy was well tolerated. The choice becomes more difficult and limited in treatment-refractory CLL, in patients relapsing within 24 months of treatment, or in patients with the chromosomal aberration *del(17p)*. In principle, the initial regimen should be changed. The following options exist as a relapse therapy for patients no longer responding to chemoimmunotherapy with rituximab: (1) alemtuzumab alone or in combinations, in particular with high-dose steroids[26,51,54]; (2) combinations of antibodies with lenalidomide (see below); (3) BTK inhibitors such as ibrutinib,[58] where available, or experimental protocols using novel agents (Figure 2, Table 1); and (4) allogeneic stem cell transplantation with curative intent.[59]

The choice of one of these options depends on the fitness of the patient, the availability of some drugs, and the prognostic risk of the leukemia as defined by molecular cytogenetics. According to recommendations of a European Group for Blood and Marrow Transplantation (EBMT) consensus group, physically fit patients with refractory CLL or with a *del(17p)* should be offered an allogeneic transplantation because their prognosis so far has remained extremely poor with conventional therapies.[59] Finally, it is important to emphasize that patients with refractory disease should be treated within clinical trials whenever possible. Some of the new drugs, in particular ibrutinib or ABT-199, show good responses in these patients and could become an additional option in the very near future.

New drugs targeting pathogenic pathways of CLL cells

There are an increasing number of interesting new compounds in clinical development (for reviews, see Wiestner[10] and Isfort et al[60]). The common denominator of these compounds is that their mechanism of action targets a relatively specific signaling abnormality or redirects the immune system against CLL cells. A detailed description of these fascinating agents is beyond the scope of this article. Table 1 lists the some of the most promising agents and their stage of clinical development and shows that these agents may yield high response rates above 50% even in relapsed and refractory CLL patients. Some of these agents (eg, obinutuzumab, CART19, ABT-199, idelalisib, and ibrutinib) currently elicit the greatest enthusiasm and hope both among CLL patients and their treating physicians.

New anti-CD20 antibodies

Obinutuzumab (GA101). The humanized and glycoengineered monoclonal antibody obinutuzumab showed impressive results in vitro with higher rates of apoptosis in malignant B cells compared with rituximab.[61] The humanization of the parental B-Ly1 mouse antibody and subsequent glycoengineering lead to higher-affinity binding to the CD20 type II epitope, increased antibody-dependent cellular cytotoxicity, low complement-dependent cytotoxicity activity, and increased direct cell death induction.[62] A phase 1 study with

A

Stage	Fitness	del(17p) p53mut	Therapy
Binet A-B, Rai 0-II, inactive	Irrelevant	Irrelevant	None
Active disease or Binet C or Rai III-IV	Go go	No	FCR
		Yes	Allo-SCT
	Slow go	No	CLB + anti-CD20-Mab
		Yes	Al, HD R or O

B

Response to First-Line Therapy	Fitness	Therapy	
		Standard	Alternatives (trials)
Refractory or progress within 2 years	Go go	Al-Dex, FA, FCR→Allo SCT	Lenalidomide, BR, BR² Combination with kinase inhibitors
	Slow go	Change therapy (if possible, include in trial)	Al for del(17p), FCRlite, BR, bendamustine, lenalidomide, ofatumumab, HD rituximab, kinase inhibitors
Progress after 2 years	All	Repeat first-line therapy	

Figure 1. Treatment algorithm for CLL patients in frontline (A) and second-line (B) indications. Al indicates alemtuzumab; R, rituximab; O, ofatumumab; F, fludarabine; C, cyclophosphamide; Mab, monoclonal antibody; Dex, dexamethasone; and Allo-SCT, allogeneic stem cell transplantation. Please note that performing an allogeneic transplantation usually requires the induction of a PR or CR before the procedure.

obinutuzumab showed promising results in 13 CLL patients.[63] Major side effects included infections, neutropenia, thrombocytopenia, and tumor lysis syndrome, all of which resolved. There were no dose-limiting toxicities. Encouraging results were reported in the run-in phase of the CLL11 trial on CLL patients with increased comorbidity.[64] Six subjects older than 70 years (median age 76 years; median cumulative illness rating score of 8) received 6 28-day cycles of obinutuzumab (1000 mg on days 1, 8, and 15 of cycle 1 and day 1 of cycles 2-6) plus CLB (0.5 mg/kg on days 1 and 15 of each cycle). Infusion-related reactions occurred in 5 patients, but were mild. Grade 3/4 neutropenia was seen in 5 patients. No febrile neutropenias or grade 3/4 infections were observed. All subjects completed therapy. CRs were documented in 2 patients and PRs in 4. After a median follow-up of 23 months from start of

treatment, none of the patients had progressed with CLL or died. Results of a planned interim analysis of the CLL11 trial have been recently reported and confirmed the very promising activity of obinutuzumab in CLL.[42]

Agents targeting BCR signaling
The following section describes selected kinase inhibitors that have entered testing in phase 3 trials for CLL patients (Table 1).

Idelalisib (CAL-101). Class I PI3Ks regulate cellular functions relevant to oncogenesis.[65] Expression of the PI3K p110 δ isoform (PI3Kδ) is restricted to cells of hematopoietic origin, where it plays a key role in B-cell proliferation and survival. In CLL, the PI3K pathway is constitutively activated and dependent on PI3Kδ.[66]
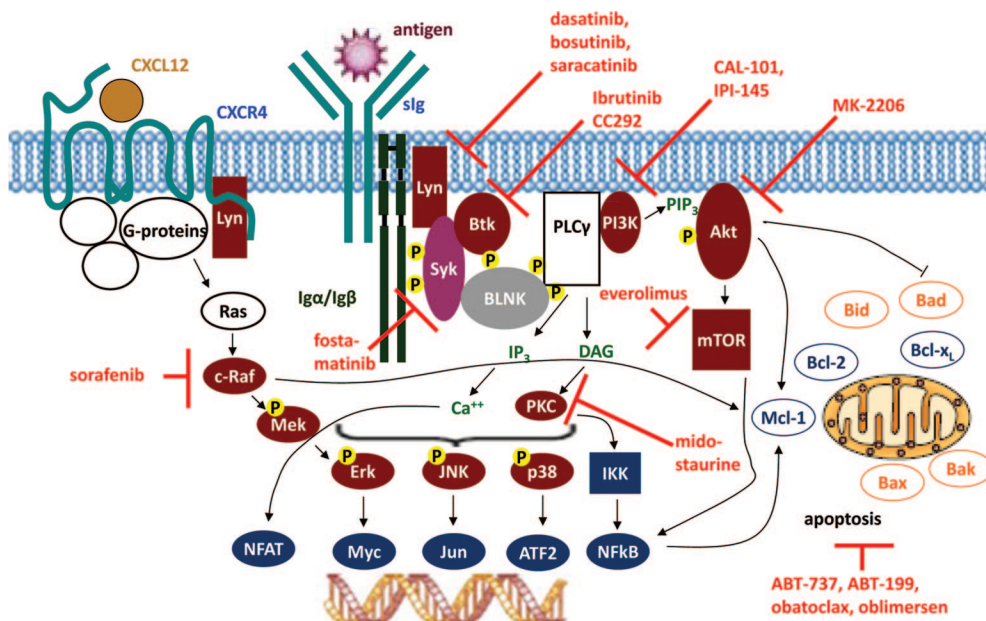
Figure 2. Targeting of BCR signaling as a therapeutic strategy in CLL. Red symbols and letters indicate new therapeutics as discussed in the text.

CAL-101 is an oral PI3Kδ isoform–selective inhibitor that promotes apoptosis in primary CLL cells in a time- and dose-dependent manner without inducing apoptosis in normal T cells or natural killer cells and without diminishing antibody-dependent cellular cytotoxicity. CAL-101 inhibits CLL cell chemotaxis toward CXCL12 and CXCL13 and migration beneath stromal cells (pseudoemperipolesis). CAL-101 also down-regulates the secretion of chemokines in stromal cocultures and after BCR triggering.[66] CAL-101 reduces survival signals derived from the BCR or from nurse-like cells and inhibits BCR- and chemokine-receptor-induced AKT and MAP kinase (ERK) activation.[66]

In a phase 1 clinical trial in 54 heavily pretreated and high-risk CLL patients, idelalisib showed acceptable toxicity, positive pharmacodynamic effects, and favorable clinical activity (ie, a high level of lymph node regression and prolonged duration of symptomatic tumor control).[67] An ORR of 56% was achieved, with 2 CRs and 28 PRs. Of the 28 patients with PRs, 6 showed persistent lymphocytosis. The majority of patients (81%) showed a lymph node response (≥ 50% reduction in the nodal sum of the product of greatest diameter). Median PFS was 17 months. Side effects were mild, with fatigue, diarrhea, pyrexia, rash, and upper respiratory tract infections being the most frequent. More importantly, there were no dose-limiting toxicities. Results on idelalisib in combination with rituximab, ofatumumab, or bendamustine/rituximab were presented in preliminary form and showed encouraging results. For example, a trial using a combination of idelalisib and rituximab as a frontline therapy in 64 patients yielded an ORR of 97% with 19% CRs.[68] Side effects were mild, with 23% diarrheas as the only relevant CTC grade 3 toxicity.

Ibrutinib (formerly called PCI-32765). BTK leads to downstream activation of cell survival pathways such as NF-κB and MAPKs via Src family kinases.[69] Ibrutinib is an orally active small molecule inhibiting BTK that plays a role in the signal transduction of the BCR. Inhibition of BTK might induce apoptosis in B-cell lymphomas and CLL cells.[69] Ibrutinib showed significant activity in patients with relapsed or refractory B-cell malignancies, including CLL.[70] Data from a phase 1b-2 multicenter study with single-agent

ibrutinib on 85 patients with relapsed or refractory CLL or small lymphocytic lymphoma, the majority of whom had high-risk disease, were published recently.[58] Patients received ibrutinib once daily (51 at a dose of 420 mg, 34 at 840 mg). Side effects were mild (predominantly grade 1 or 2) and included transient diarrhea, fatigue, and upper respiratory tract infection. The ORR was 71%, the majority being PRs (68%). Most interestingly, the response was independent of clinical and genomic risk factors, including advanced-stage disease, the number of previous therapies, and *del(17p)*. At 26 months, the estimated PFS rate was 75% and the OS rate was 83%. This study illustrates that ibrutinib may soon become an additional treatment option for CLL patients with high-risk genetic lesions.

Bcl-2 inhibitors

Proteins in the B-cell CLL/lymphoma 2 (Bcl-2) family are key regulators of the apoptotic process.[71] The Bcl-2 family comprises proapoptotic and prosurvival proteins. Shifting the balance toward the latter is an established mechanism whereby cancer cells evade apoptosis. Bcl-2, the founding member of this protein family, is encoded by the *BCL2* gene, which was initially described in follicular lymphoma as a protein in translocations involving chromosomes 14 and 18.[72]

Bcl-2 inhibitors ABT-263 (Navitoclax) and ABT-199. ABT-263 is a small-molecule Bcl-2 family protein inhibitor that binds with high affinity ($K_i \leq 1$ nM) to multiple antiapoptotic Bcl-2 family proteins, including Bcl-XL, Bcl-2, Bcl-w, and Bcl-B, and has a high oral bioavailability. Initial studies showed very promising results for this drug as a single agent.[73] However, its therapeutic use seemed somewhat limited by severe thrombocytopenias being a prominent side effect. Therefore, the compound was reengineered to create a highly potent, orally bioavailable, and Bcl-2-selective inhibitor, ABT-199.[74] This compound inhibits the growth of Bcl-2–dependent tumors in vivo and spares human platelets. A single dose of ABT-199 in 3 patients with refractory CLL resulted in tumor lysis within 24 hours.[74] In a recent update on phase 1 study data of 56 patients have been reported, of whom 16 (29%) had a *del(17p)* and 18 (32%) had F-refractory CLL.[75] Major side effects were tumor lysis syndrome and neutropenia. ABT-199 yielded an ORR of

Table 1. Status of current clinical development of novel, targeted drugs in CLL

Class	Agent	Target	Route	Current stage of clinical development	Comments, specific side effects	Response rate in relapsed, refractory CLL as a single agent			
						N	CR	PR	ORR
Antibodies	Obinutuzumab (GA101)[63]	CD20	IV	Phase 3	Cytokine release at first infusion	13	8%	54%	62%
	Dacetuzumab[95]	CD40	IV	Phase 1	Some disease stabilizations	12	0%	0%	0%
	Lucatumumab[96]	CD40	IV	Phase 2	Amylase, lipase increase	26	0%	4%	4%
Immune gene therapy	ISF35 (Adenovirus-CD154)[97]	CD40	Intra-nodal	Phase 1	Mild flu-like symptoms	15	0%	20%	20%
	CART19 (engineered T cells)[91]	CD19	IV	Phase 1/2	TLS, cytokine release syndrome	9	44%	22%	66%
Small immune-pharmaceutical	TRU-016[98]	CD37	IV	Phase 2	Neutropenia, infections, diarrhea, fatigue	17	0%	17%	17%
Bcl-2 antagonists	ABT-263 (Navitoclax)[73]	Bcl-2 and Bcl-X(L)	PO	Phase 1/2a	Thrombocytopenia	26	0%	35%	35%
	Obatoclax[99]	Pan-Bcl-2 antagonist	IV	Phase 2	Neurologic, infusion-related symptoms (somnolence, euphoria, ataxia)	26	0%	4%	4%
	ABT-199[75]	Bcl-2	PO	Phase 3	TLS, neutropenia	56	13%	72%	85%
Tyrosine kinase inhibitors	Fostamatinib[100]	Syk	PO	Phase 1/2	Diarrhea, fatigue, cytopenia, hypertension, nausea	11	0%	55%	55%
	Idelalisib (CAL-101)[67]	PI3K p110δ	PO	Phase 3	Abnormal liver function, pneumonia	54	4%	52%	56%
	Ibrutinib (PCI-32765)[58]	Bruton tyrosine kinase	PO	Phase 3	Diarrhea	85	2%	68%	71%
	Dasatinib[101]	Abl and Src family kinases	PO	Phase 2	Pleural effusion	15	0%	20% (60%)†	20% (60%)†
Cyclin-dependent kinase inhibitors	Flavopiridol[102]	CDK	IV	Phase 2	TLS	52	0%	40%	40%
	Dinaciclib[103]	CDK 1,2,5,9	IV	Phase 1	TLS, anemia, neutropenia, thrombocytopenia, hyperglycemia, hypocalcemia, diarrhea, hypokalemia, increased AST/ALT, fatigue.	23	0%	35%	35%
	SNS-032[104]	CDK 2,7,9	IV	Phase 1	TLS, myelosuppression; limited antitumor activity	19	0%	0%	0%
mTOR inhibitors	Everolimus[88,89]	mTOR	PO	Phase 1/2	Immunosuppression, severe infections	22	0%	18%	18%
						7	0%	14%	14%
Immunomodulatory drugs	Lenalidomide[80]	Multiple	PO	Phase 3	Immunosuppression, myelotoxicity, tumor flare, thromboembolic events	29*	7%	27%	34%

PO indicates per ora (orally); TLS, tumor lysis syndrome.

*Analysis included only fludarabine-refractory patients.

†60% showed nodal responses without a 50% reduction of lymphocyte counts.

85%, with 13% CRs and 72% PRs. Interestingly, 88% and 75% of patients with a *del(17p)* and F-refractory CLL, respectively, achieved at least a PR. These data indicate that selective pharmacological inhibition of Bcl-2 holds great promise for the treatment of CLL.

Immunomodulatory drugs

Lenalidomide. Lenalidomide has shown encouraging results in the treatment of high-risk patients, including carriers of a *del(17p)*.[76] In 58% of the patients, lenalidomide caused a tumor flare reaction characterized by a marked and painful increase in lymph nodes size, malaise, and fever.[77,78] This tumor flare reaction may be life-threatening and is more common in CLL than in other lymphoid malignancies. Lenalidomide may also cause relevant myelosuppression.[79] The ORR of lenalidomide monotherapy varied between 32% and 54% in different clinical trials.[78-80] Most importantly, it seems to have activity as a single agent in fludarabine-refractory CLL.[78,80]

The combination of lenalidomide and rituximab seems to increase the response rate without a higher risk of toxicity, even in patients with *del(17p)* and/or unmutated IGHV status. In a phase 2 trial, 59 patients with relapsed or refractory CLL received a combination of lenalidomide and rituximab.[81] Lenalidomide was started on day 9 of cycle 1 at 10 mg orally and was administered daily continuously. Each cycle was 28 days. Rituximab was administered for 12 cycles; lenalidomide could be continued if patients benefited clinically. The ORR was 66%, including 12% CRs and 12% nodular PRs. The median time to treatment failure was 17.4 months. The most common grade 3 or 4 toxicity was neutropenia (73% of patients). Fourteen patients (24%) experienced a grade 3 or 4 infection or febrile episode. In essence, this combination seems a helpful alternative for patients with refractory CLL and warrants further investigation.

In some contrast, the combination of lenalidomide, rituximab, and fludarabine may induce severe side effects (myelosuppression) if all drugs are started simultaneously on day 1.[82,83] Using combinations starting with lenalidomide on day 8 of the first cycle, the treatment is usually better tolerated.[83,84] The GCLLSG is currently investigating the combination of bendamustine, rituximab, and lenalidomide (BR^2) in physically fit patients (CLL2P protocol). Additional combinations currently being studied are flavopiridol plus lenalidomide, which led to a response in 7 of 15 patients [among them 4 with a *del(17p)* and 3 with a *del(11q)*][85] or lenalidomide plus ofatumumab.[86]

Everolimus (RAD001). Everolimus has shown good efficacy in some hematological malignancies,[87] but the results in CLL have so far been disappointing with low response rates and relatively severe infectious complications.[88,89]

Chimeric antigen receptors

Chimeric antigen receptors (CARs) combine the antigen recognition domain of an antibody with intracellular signaling domains into a single chimeric protein. CD19 is an ideal target for CARs because expression is restricted to normal and malignant B cells. Inclusion of the CD137 (4-1BB) signaling domain resulted in potent antitumor activity and in vivo persistence of anti-CD19 CARs in mice. June et al have recently shown that antitumor activity of CAR-modified autologous T cells targeted to CD19 (CART19 cells) yielded sustained responses even in high-risk CLL patients.[90] Recently, the outcome and longer follow-up from 10 patients treated with CART19 cells was reported.[91] Autologous T cells collected by leukapheresis were transduced with a lentivirus encoding anti-CD19 scFv linked to 4-1BB and CD3-z signaling domains. Gene-modified T cells were expanded and activated ex vivo by exposure to anti-CD3/CD28 beads. Ten patients have received CART19 cells; 9 adults of median age 65 years (range, 51-78) were treated for relapsed, refractory CLL and 1 7-year-old was treated for relapsed refractory acute lymphoblastic leukemia (ALL). Three of 9 CLL patients had a deletion of the p53 gene. All CLL patients received lymphodepleting chemotherapy 4 to 6 days before infusions (FC, PC, or bendamustine, whereas the ALL patient had an absolute lymphocyte count < 10 after prior chemotherapy and did not require further lymphodepletion). CART19 homed to the BM in the CLL patients and in the BM and CSF in the ALL patient, with detectable CART19 cells in the CSF (21 lymphocytes/μL, 78% CAR+) day 23 after infusion. Four of 9 evaluable patients achieved a CR (3 CLL, 1 ALL). Two CLL patients had a PR lasting 3 and 5 months and 3 patients did not respond. In the 4 patients who achieved CR, maximal expanded cells in the blood were detected at an average of 27-fold higher than the infused dose (range, 21- to 40-fold) with maximal in vivo expansion between day 10 and 31 after infusion. No patient with CR has relapsed. All patients who responded developed a cytokine release syndrome manifested by fever and variable degrees of nausea, anorexia, and transient hypotension and hypoxia. In responding CLL patients, cytokine levels were increased. Five patients with cytokine release required treatment. In summary, CART19 cells can induce potent and sustained responses for patients with advanced, refractory, and high-risk CLL. However, the long-term toxicity and efficacy of this approach needs to be studied further.

Outlook: signaling the end of CLL?

The above described, novel therapies all target relatively specific signaling proteins of CLL cells and their microenvironment. Therefore, their overall toxicity often is moderate and does not involve myelosuppression. Moreover, CRs have not occurred frequently so far with any of these newer agents, except CART19.[91] In addition, unlike chronic myeloid leukemia, which is initiated by a single oncogene (BCR/ABL), CLL appears to be a genetically heterogeneous leukemia. It seems that CLL may be caused by a complex array of genetic events and as a consequence is a biologically complex disease. Finally, somatic mutations in the kinase genome are very rare events in CLL,[92] unlike in other malignancies. For these reasons, it is very likely that the currently available therapeutic agents will be combined to yield optimal results. The current challenge is to identify the best combination and sequence of treatments to achieve the long-term control of CLL with optimal quality of life. Finally, it is important to keep in mind that, as in other hematological malignancies, the currently available data strongly suggest that achieving a very good disease control (using MRD negativity as a surrogate parameter) prolongs survival of CLL patients.[38,93]

How can these goals be achieved with the current treatment modalities?

In principle there are at least 3 options. The first option is combining the best agents (3, 4, or more) in a simultaneous short-term treatment (up to 6 months) aimed at MRD-negative CRs[93] that would last longer than the remissions achieved so far. For example, such an approach would be combining the best currently available chemotherapies (fludarabine or cyclophosphamide alone or in combination; bendamustine) with antibodies and with kinase inhibitors or Bcl-2 antagonists. However, these combinations will have to
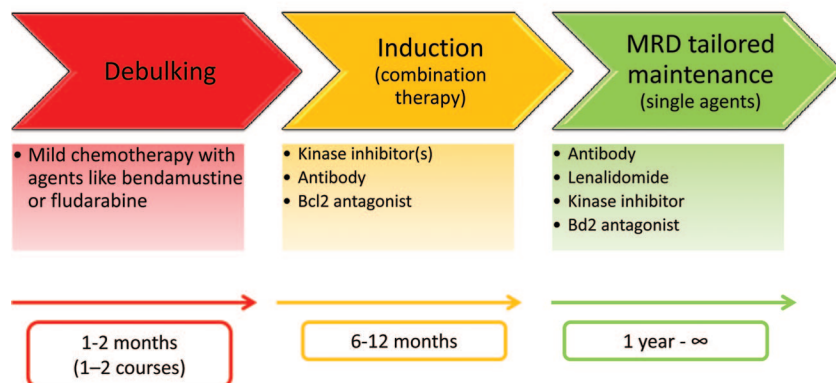
Figure 3. Future treatment concept with novel agents. For this treatment approach, I propose the term "sequential triple-T" (tailored, targeted, total eradication of MRD) to illustrate that this future approach should be a sequence of tailored measures (according to the risk of the leukemia, the tumor burden, and the fitness of the patients), should use targeted agents (ie, using the novel nonchemotherapeutic agents with a mechanism of action targeting pathogenic signaling events of CLL cells and their microenvironment), and aim at the total eradication of the leukemic clone (as assessed by MRD negativity as a clinical end point). Please note that the drugs or classes of drugs in this figure are shown as examples. Similar agents of the same class or additional classes of drugs (see Table 1) may be used as well.

be compared against the current standard (FCR) and results will not be available for a few years. Moreover, such a treatment strategy will not be without toxicity and therefore will be tolerated only by patients with good physical fitness.

A second possibility would be to use sequential monotherapies of new or old agents. Each agent would be given until maximal response was achieved. After a long-lasting response, treatment could be repeated with the same agent, whereas alternative agents would be used in case of short remissions. This strategy might be applied in elderly or nonfit patients ("slow go"), in whom the goal of treatment is symptom control rather than disease control.

A third strategy would combine the best agents in a sequence that tailors the treatment according to the initial tumor load and the response to therapy (CR vs PR; MRD-positive vs MRD-negative).[93] This strategy would have the goal of preventing the outgrowth of adverse leukemic subclones[94] and minimizing the use of chemotherapy, thereby reducing the risk for secondary mutations of the CLL clone(s) and for secondary malignancies that are frequent and prognostically unfavorable events in CLL. For this treatment approach, I propose the term "sequential TTT (triple-T)" (tailored, targeted, total eradication of MRD; Figure 3). This sequential triple-T approach will make use of all of the currently available options in a nonaggressive, nontoxic way and will aim at the complete elimination or control of the malignant clone. The advantage of such an approach would be as follows: (1) it would be available for most (fit as well as nonfit) CLL patients due to its limited toxicity, (2) it could be given in an outpatient setting, and (3) it would use the current treatment options in a tailored and response-adjusted manner and therefore use the drugs in a cost-effective, resource-saving way (Figure 3). It might also be used to monitor the evolution of new genetic subclones in CLL that may have clinical and prognostic impact.[94]

The proposed sequential triple-T strategy might consist of the following 3 steps (Figure 3):

1. Debulking. Because most of the new agents either induce a compartment shift of malignant lymphocytes (eg, ibrutinib, idelalisib), they transiently increase peripheral blood lymphocyte counts. This often causes concern in patients and physicians and prevents combination with some other drugs. Other agents (ABT-199, obinutuzumab, lenalidomide) often cause severe reactions during the first treatment phase (cytokine release syndrome, tumor lysis syndrome). Therefore, in cases with elevated lymphocyte counts (above 30 000/µl) or large lymph nodes, a short debulking therapy (eg, with 1 or 2 courses of bendamustine or fludarabine) might be an optional first treatment element. Alternatively, nonchemotherapeutic drugs could be used. This step would be performed to rapidly clear the peripheral blood of CLL cells in the majority of patients. This treatment period would last 1 to 2 months.

2. Induction. After reducing peripheral blood lymphocytes to levels below a certain threshold (eg, below 30 000 peripheral blood lymphocytes/µl), induction therapies could be initiated. These therapies would contain nonchemotherapy combinations of drugs. To avoid infusion-related reactions, antibodies such as obinutuzumab would be given first, followed a few days later by a third class of drugs, such as a tyrosine kinase inhibitor or Bcl-2 inhibitor (or both). This treatment period would typically last 4 to 6 months and the patient would be monitored at the end by MRD assessment 3 months after a CR is achieved. In general, this combination treatment during induction would last as long as the remission continues to improve or until a CR is achieved.

3. Maintenance. At the end of this induction period, the third sequential element of the triple-T strategy will ensure that a very good remission is maintained. This could be achieved by giving single agents, either oral drugs (eg, kinase inhibitors, Bcl-2 inhibitors, or lenalidomide) or repetitive infusions of antibodies (eg, obinutuzumab) in intervals of 2 to 6 months for a prolonged period. This therapeutic approach would be monitored by MRD assessment and treatment could be stopped 3 months after an MRD-negative remission has been achieved and restarted in case of MRD

American Society of Hematology

positivity. This treatment phase would last at least 1 year, but typically 2 years.

Although I am fully aware that the proposed sequential triple-T strategy needs to be validated by clinical research, it may help to design the appropriate clinical trials for the optimal use of currently available drugs. Finally, at a time when new and exciting therapeutic options are likely to change the management and outcome of CLL, hematologists and oncologists have the obligation to include their patients in clinical trials to ensure that maximal progress is achieved in the shortest possible time. By doing so, we will actively contribute to the historically unique chance to gain control over a so-far incurable disease such as CLL.

Disclosures
Conflict-of-interest disclosure: The author has received research funding from Celgene, Janssen, Gilead, Mundipharma, and Roche and has consulted for Mundipharma, Roche, Celgene, Gilead, Janssen, and GSK. Off-label drug use: ibrutinib, cal-101 (idelalisib), obinutuzumab.

Correspondence
Michael Hallek, MD, Kerpener Strasse 62, 50937 Köln, Germany; Phone: 49-221/478-4400; Fax: 49-221/478-5455; e-mail: michael.hallek@uni-koeln.de.

References
1. National Cancer Institute. SEER stat fact sheets: chronic lymphocytic leukemia. Available from: http://seer.cancer.gov/statfacts/html/clyl.html. Accessed May 15, 2013.
2. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*. 2002;99(24):15524-15529.
3. Zenz T, Mertens D, Kuppers R, Dohner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia. *Nat Rev Cancer*. 2010;10(1):37-50.
4. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011;475(7354):101-105.
5. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet*. 2012;44(1): 47-52.
6. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011;365:2497-2506.
7. Rossi D, Rasi S, Spina V, et al. Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. *Blood*. 2013;121(8):1403-1412.
8. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. *Blood*. 2009;114(16):3367-3375.
9. Stevenson FK, Krysov S, Davies AJ, Steele AJ, Packham G. B-cell receptor signaling in chronic lymphocytic leukemia. *Blood*. 2011;118(16):4313-4320.
10. Wiestner A. Emerging role of kinase-targeted strategies in chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program*. 2012;2012:88-96.
11. Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat Rev Drug Discov*. 2013;12(3):229-243.
12. CLL trialists collaborative group. Chemotherapeutic options in chronic lymphocytic leukemia. *J Natl Cancer Inst*. 1999;91: 861-868.
13. Steurer M, Pall G, Richards S, Schwarzer G, Bohlius J, Greil R. Purine antagonists for chronic lymphocytic leukaemia. *Cochrane Database Syst Rev*. 2006;3:CD004270.
14. Johnson S, Smith AG, Loffler H, et al. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. The French Cooperative Group on CLL [see comments]. *Lancet*. 1996;347(9013): 1432-1438.
15. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med*. 2000;343(24):1750-1757.
16. Leporrier M, Chevret S, Cazin B, et al. Randomized comparison of fludarabine, CAP, and ChOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. *Blood*. 2001;98(8):2319-2325.
17. Robak T, Blonski JZ, Kasznicki M, et al. Cladribine with prednisone versus chlorambucil with prednisone as first- line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. *Blood*. 2000;96(8):2723-2729.
18. Knauf WU, Lissichkov T, Aldaoud A, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27(26):4378-4384.
19. Huhn D, von Schilling C, Wilhelm M, et al. Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. *Blood*. 2001;98(5):1326-1331.
20. O'Brien S, Kantarijan H, Thomas D, et al. Rituximab dose-escalation trial in chronic lymphocytic leukaemia. *J Clin Oncol*. 2001;19:2165-2170.
21. Bauer K, Rancea M, Roloff V, et al. Rituximab, ofatumumab and other monoclonal anti-CD20 antibodies for chronic lymphocytic leukaemia. *Cochrane Database Syst Rev*. 2012;11: CD008079.
22. Cang S, Mukhi N, Wang K, Liu D. Novel CD20 monoclonal antibodies for lymphoma therapy. *J Hematol Oncol*. 2012; 5:64.
23. Maloney DG. Anti-CD20 antibody therapy for B-cell lymphomas. *N Engl J Med*. 2012;366(21):2008-2016.
24. Wierda WG, Kipps TJ, Mayer J, et al. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2010;28(10): 1749-1755.
25. Österborg A, Dyer MJ, Bunjes D, et al. Phase II multicenter

study of human CD52 antibody in previously treated chronic lymphocytic leucemia. European study group of CAM-PATH-1H treatment in chronic lymphocytic leukemia. *J Clin Oncol*. 1997;15(4):1567-1574.

26. Rai KR, Freter CE, Mercier RJ, et al. Alemtuzumab in previously treated chronic lymphocytic leukemia patients who also had received fludarabine. *J Clin Oncol*. 2002;20(18):3891-3897.

27. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood*. 2002;99(10):3554-3561.

28. Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. *N Engl J Med*. 2002;347(6):452-453.

29. Lozanski G, Heerema NA, Flinn IW, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. *Blood*. 2004;103(9):3278-3281.

30. Hillmen P, Skotnicki AB, Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. *J Clin Oncol*. 2007;25(35):5616-5623.

31. Hallek M, Eichhorst BF. Chemotherapy combination treatment regimens with fludarabine in chronic lymphocytic leukemia. *Hematol J*. 2004;5(Suppl 1):S20-S30.

32. Eichhorst BF, Busch R, Hopfinger G, et al. Fludarabine plus cyclophosphamide versus fludarabine alone in first line therapy of younger patients with chronic lymphocytic leukemia. *Blood*. 2006;107:885-891.

33. Flinn IW, Neuberg DS, Grever MR, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. *J Clin Oncol*. 2007;25(7):793-798.

34. Catovsky D, Richards S, Matutes E, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. *Lancet*. 2007;370(9583):230-239.

35. Robak T, Blonski JZ, Gora-Tybor J, et al. Cladribine alone and in combination with cyclophosphamide or cyclophosphamide plus mitoxantrone in the treatment of progressive chronic lymphocytic leukemia: report of a prospective, multicenter, randomized trial of the Polish Adult Leukemia Group (PALG CLL2). *Blood*. 2006;108(2):473-479.

36. Hallek M, Pflug N. State of the art treatment of chronic lymphocytic leukaemia. *Blood Rev*. 2011;25(1):1-9.

37. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood*. 2008;112(4):975-980.

38. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010;376(9747):1164-1174.

39. Fischer K, Bahlo J, Fink A-M, et al. Extended follow up of the CLL8 protocol, a randomized phase-III trial of the German CLL Study Group (GCLLSG) comparing fludarabine and cyclophosphamide (FC) to FC plus rituximab (FCR) for previously untreated patients with chronic lymphocytic leukemia (CLL): results on survival, progression-free survival, delayed neutropenias and secondary malignancies confirm superiority of the FCR regimen [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2012;120(21):435.

40. Robak T, Dmoszynska A, Solal-Celigny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. *J Clin Oncol*. 2010;28(10):1756-1765.

41. Foon KA, Boyiadzis M, Land SR, et al. Chemoimmunotherapy with low-dose fludarabine and cyclophosphamide and high dose rituximab in previously untreated patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27(4):498-503.

42. Goede V, Fischer K, Humphrey K, et al. Obinutuzumab (GA101) plus chlorambucil (Clb) or rituximab (R) plus Clb versus Clb alone in patients with chronic lymphocytic leukemia (CLL) and preexisting medical conditions (comorbidities): Final stage 1 results of the CLL11 (BO21004) phase III trial. *J Clin Oncol*. 2013;31:7004.

43. Hillmen P, Gribben JG, Follows GA, et al. Rituximab plus chlorambucil in patients with CD20-positive B-cell chronic lymphocytic leukemia (CLL): final response analysis of an open-label phase II study [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2010;116(21):697.

44. Foa R, Ciolli S, Di Raimondo F, et al. A phase II study of chlorambucil plus rituximab followed by maintenance versus observation in elderly patients with previously untreated chronic lymphocytic leukemia: results of the first interim analysis [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2010;116(21):2462.

45. Parikh SA, Keating MJ, O'Brien S, et al. Frontline chemoimmunotherapy with fludarabine, cyclophosphamide, alemtuzumab, and rituximab for high-risk chronic lymphocytic leukemia. *Blood*. 2011;118(8):2062-2068.

46. Bosch F, Abrisqueta P, Villamor N, et al. Rituximab, fludarabine, cyclophosphamide, and mitoxantrone: a new, highly active chemoimmunotherapy regimen for chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27(27):4578-4584.

47. Reynolds C, Di Bella N, Lyons RM, et al. A phase III trial of fludarabine, cyclophosphamide, and rituximab vs pentostatin, cyclophosphamide, and rituximab in B-cell chronic lymphocytic leukemia. *Invest New Drugs*. 2012;30(3):1232-1240.

48. Fischer K, Cramer P, Busch R, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol*. 2011;29(26):3559-3566.

49. Fischer K, Cramer P, Busch R, et al. Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol*. 2012;30(26):3209-3216.

50. Kennedy B, Rawstron A, Carter C, et al. Campath-1H and fludarabine in combination are highly active in refractory chronic lymphocytic leukemia. *Blood*. 2002;99(6):2245-2247.

51. Elter T, Borchmann P, Schulz H, et al. Fludarabine in combination with alemtuzumab is effective and feasible in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: results of a phase II trial. *J Clin Oncol*. 2005;23(28):7024-7031.

52. Lepretre S, Aurran T, Mahe B, et al. Excess mortality after treatment with fludarabine and cyclophosphamide in combination with alemtuzumab in previously untreated patients with chronic lymphocytic leukemia in a randomized phase 3 trial. *Blood*. 2012;119(22):5104-5110.

53. Elter T, Gercheva-Kyuchukova L, Pylylpenko H, et al.

Fludarabine plus alemtuzumab versus fludarabine alone in patients with previously treated chronic lymphocytic leukaemia: a randomised phase 3 trial. *Lancet Oncol*. 2011;12(13):1204-1213.

54. Pettitt AR, Jackson R, Carruthers S, et al. Alemtuzumab in combination with methylprednisolone is a highly effective induction regimen for patients with chronic lymphocytic leukemia and deletion of TP53: final results of the national cancer research institute CLL206 trial. *J Clin Oncol*. 2012;30(14):1647-1655.

55. Stilgenbauer S, Cymbalista F, Leblond V, et al. Alemtuzumab plus oral dexamethasone, followed by alemtuzumab maintenance or allogeneic transplantation in ultra high-risk CLL: updated results from a phase II study of the Gcllsg and fcgcll/MW [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2012;120(21):716.

56. Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? *Nat Rev Clin Oncol*. 2011;8(1):38-47.

57. Extermann M, Overcash J, Lyman GH, Parr J, Balducci L. Comorbidity and functional status are independent in older patients. *J Clin Oncol*. 1998;16(4):1582-1587.

58. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369(1):32-42.

59. Dreger P, Corradini P, Kimby E, et al. Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. *Leukemia*. 2007;21(1):12-17.

60. Isfort S, Cramer P, Hallek M. Novel and emerging drugs for chronic lymphocytic leukemia. *Curr Cancer Drug Targets*. 2012;12(5):471-483.

61. Patz M, Isaeva P, Forcob N, et al. Comparison of the in vitro effects of the anti-CD20 antibodies rituximab and GA101 on chronic lymphocytic leukaemia cells. *Br J Haematol*. 2011;152(3):295-306.

62. Mossner E, Brunker P, Moser S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood*. 2010;115(22):4393-4402.

63. Morschhauser F, Cartron G, Lamy T, et al. Phase I study of RO5072759 (GA101) in relapsed/refractory chronic lymphocytic leukemia [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2009;114(22):884.

64. Goede V, Fischer K, Busch R, et al. Chemoimmunotherapy with GA101 plus chlorambucil in patients with chronic lymphocytic leukemia and comorbidity: results of the CLL11 (BO21004) safety run-in. *Leukemia*. 2013;27(5):1172-1174.

65. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol*. 2003;3(4):317-330.

66. Hoellenriegel J, Meadows SA, Sivina M, et al. The phosphoinositide 3′-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood*. 2011;118(13):3603-3612.

67. Brown JR, Furman RR, Flinn IW, et al. Final results of a phase 1 study of idelalisib (GS-1101) a selective inhibitor of phosphatidylinositol 3-kinase p110 delta (PI3Kdelta) in patients with relapsed or refractory CLL. *J Clin Oncol*. 2013;31(Suppl):7003.

68. O'Brien SM, Lamanna N, Kipps TJ, et al. A phase 2 study of the selective phosphatidylinositol 3-kinase delta (PI3Kdelta)

inhibitor idelalisib (GS-1101) in combination with rituximab in treatment-naïve patients ≥65 years with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). *J Clin Oncol*. 2013;31(Suppl):Abstract 7005.

69. Herman SE, Gordon AL, Hertlein E, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood*. 2011;117(23):6287-6296.

70. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol*. 2013;31(1):88-94.

71. Chao DT, Korsmeyer SJ. BCL-2 family: regulators of cell death. *Annu Rev Immunol*. 1998;16:395-419.

72. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science*. 1984;226(4678):1097-1099.

73. Roberts AW, Seymour JF, Brown JR, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J Clin Oncol*. 2012;30(5):488-496.

74. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202-208.

75. Seymour J, Davids M, Pagel J, et al. Updated results of a phase I first-in-human study of the BCL-2 inhibitor ABT-199 (GDC-0199) in patients with relapsed/refractory (R/R) chronic lymphocytic leukaemia (CLL). *Hematol Oncol*. 2013;31(Suppl 1):115.

76. Sher T, Miller KC, Lawrence D, et al. Efficacy of lenalidomide in patients with chronic lymphocytic leukemia with high-risk cytogenetics. *Leuk Lymphoma*. 2010;51(1):85-88.

77. Moutouh-de Parseval LA, Weiss L, DeLap RJ, Knight RD, Zeldis JB. Tumor lysis syndrome/tumor flare reaction in lenalidomide-treated chronic lymphocytic leukemia. *J Clin Oncol*. 2007;25(31):5047.

78. Chanan-Khan A, Miller KC, Musial L, et al. Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J Clin Oncol*. 2006;24(34):5343-5349.

79. Ferrajoli A, Lee BN, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood*. 2008;111(11):5291-5297.

80. Chanan-Khan AA, Czuczman MS, Padmanabhan S, et al. Clinical efficacy of lenalidomide in fludarabine-refractory chronic lymphocytic leukemia patients [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2007;110(11):3108.

81. Badoux XC, Keating MJ, Wen S, et al. Phase II study of lenalidomide and rituximab as salvage therapy for patients with relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2013;31(5):584-591.

82. Brown JR, Abramson J, Hochberg E, et al. A phase I study of lenalidomide in combination with fludarabine and rituximab in previously untreated CLL/SLL. *Leukemia*. 2010;24(11):1972-1975.

83. Flinn IW, Berdeja JG, Waselenko JK, et al. Preliminary results from a phase I/II study of fludarabine, rituximab, and lenalidomide in untreated patients with chronic lymphocytic leukemia

(CLL) [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2010;116(21):2461.

84. Egle A, Steurer M, Melchardt T, Stoll M, Greil R. The REVLIRIT CLL5 AGMT Study: a phase I/II trial combining fludarabine/rituximab with escalating doses of lenalidomide followed by rituximab/lenalidomide in untreated chronic lymphocytic leukemia (CLL): results of a planned interim analysis [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2009;114(22):3453.

85. Blum KA, Jones JA, Andritsos L, et al. Phase 1 trial of flavopiridol and lenalidomide in patients with previously treated chronic lymphocytic leukemia (CLL) [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2010;116(21):2472.

86. Badoux X, O'Brien S, Wierda WG, et al. Combination of ofatumumab and lenalidomide in patients with relapsed chronic lymphocytic leukemia: initial results of a phase II trial [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2010; 116(21):2464.

87. 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.

88. Decker T, Sandherr M, Goetze K, Oelsner M, Ringshausen I, Peschel C. A pilot trial of the mTOR (mammalian target of rapamycin) inhibitor RAD001 in patients with advanced B-CLL. *Ann Hematol.* 2009;88(3):221-227.

89. Zent CS, LaPlant BR, Johnston PB, et al. The treatment of recurrent/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) with everolimus results in clinical responses and mobilization of CLL cells into the circulation. *Cancer.* 2010;116(9):2201-2207.

90. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011;365(8):725-733.

91. Porter DL, Grupp SA, Kalos M, et al. Chimeric antigen receptor T cells directed against CD19 induce durable responses and transient cytokine release syndrome in relapsed, refractory CLL and ALL [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2012;120(21):717.

92. Brown JR, Levine RL, Thompson C, Basile G, Gilliland DG, Freedman AS. Systematic genomic screen for tyrosine kinase mutations in CLL. *Leukemia.* 2008;22(10):1966-1969.

93. Bottcher S, Hallek M, Ritgen M, Kneba M. The role of minimal residual disease measurements in the therapy for CLL: is it ready for prime time? *Hematol Oncol Clin North Am.* 2013;27(2):267-288.

94. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell.* 2013;152(4):714-726.

95. Furman RR, Forero-Torres A, Shustov A, Drachman JG. A phase I study of dacetuzumab (SGN-40, a humanized anti-CD40 monoclonal antibody) in patients with chronic lymphocytic leukemia. *Leuk Lymphoma.* 2010;51(2):228-235.

96. Byrd JC, Kipps TJ, Flinn IW, et al. Phase I study of the anti-CD40 humanized monoclonal antibody lucatumumab (HCD122) in relapsed chronic lymphocytic leukemia. *Leuk Lymphoma.* 2012;53(11):2136-2142.

97. Castro JE, Melo-Cardenas J, Urquiza M, Barajas-Gamboa JS, Pakbaz RS, Kipps TJ. Gene immunotherapy of chronic lymphocytic leukemia: a phase I study of intranodally injected adenovirus expressing a chimeric CD154 molecule. *Cancer Res.* 2012;72(12):2937-2948.

98. Awan FT, Pagel JM, Andritsos LA, et al. Phase 1 Study of Tru-016, An Anti-CD37 SMIP Protein in Naive and Relapsed and/or Refractory CLL Patients [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2011;118(21):1792.

99. O'Brien SM, Claxton DF, Crump M, et al. Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. *Blood.* 2009;113(2):299-305.

100. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Blood.* 2010;115(13):2578-2585.

101. Amrein PC, Attar EC, Takvorian T, et al. Phase II study of dasatinib in relapsed or refractory chronic lymphocytic leukemia. *Clin Cancer Res.* 2011;17(9):2977-2986.

102. Phelps MA, Lin TS, Johnson AJ, et al. Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. *Blood.* 2009;113(12):2637-2645.

103. Flynn JM, Jones JA, Andritsos L, et al. Update on the phase I study of the cyclin dependent kinase inhibitor dinaciclib (SCH 727965) in patients with relapsed or refractory chronic lymphocytic leukemia (CLL): confirmation of clinical activity and feasibility of long-term administration [abstract]. *Blood (ASH Annual Meeting Abstracts).* 2010; 116(21):1396.

104. Tong WG, Chen R, Plunkett W, et al. Phase I and pharmacologic study of SNS-032, a potent and selective Cdk2, 7, and 9 inhibitor, in patients with advanced chronic lymphocytic leukemia and multiple myeloma. *J Clin Oncol.* 2010;28(18): 3015-3022.

American Society of Hematology