Targets for the treatment of drug resistant chronic lymphocytic leukemia
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Chapter 8

Standards for the treatment of relapsed CLL: a case-based study

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Abstract

In recent years, considerable advances have been made in first-line treatment strategies for chronic lymphocytic leukemia (CLL). Combination of conventional chemotherapy with immunotherapeutic agents is currently considered the most active strategy, with improved progression-free survival and overall survival. However, patients are not cured and invariably experience relapsing disease requiring treatment. In contrast to the advances made in first-line treatment strategies, much less progress has been made for patients with relapsed and especially refractory CLL. The activity of most chemotherapeutic drugs used in CLL relics on intact p53 function, and repeated cycles of therapy might eventually result in drug resistance because of acquired cytogenetic alterations, mainly affecting genes involved in the p53 response. As a consequence, most commonly used treatment regimens are ineffective in patients with refractory disease. A number of promising alternative treatment approaches are currently under investigation. In this review, the approach to patients with relapsed and refractory CLL and current promising experimental treatment options for these distinct clinical patient categories are discussed.
Introduction

Chronic lymphocytic leukemia (CLL) is a CD5^+ B cell malignancy that is considered to be incurable. Until recently, the first-line treatment consisted of alkylating agents, which resulted in responses in up to 70% of patients but did not improve survival 1. Treatment regimens containing nucleoside (purine) analogues, such as fludarabine or pentostatin, were found to yield higher response rates 2, especially in combination with cyclophosphamide 3-5. However, despite increased complete response (CR) rates, treatment with purine analogues alone does not appear to improve overall survival (OS) 2. In newer first-line treatment regimens, different classes of chemotherapy are combined with monoclonal antibodies (MoAb) 6. Although treatment with such combinations might provide for a first-time-observed survival benefit 7, such therapy still is not considered curative. Most patients treated with these (immuno-) chemotherapeutic regimens will have an initial CR or partial response (PR). However, with the exception of some patients who subsequently undergo hematopoietic stem cell transplantation (SCT), disease relapse invariably occurs after treatment has been discontinued. In this review, the approach to and current promising experimental treatment options for relapsed and refractory CLL will be discussed.

Example case studies in chronic lymphocytic leukemia

Case 1: Relapsed chronic lymphocytic leukemia

The patient is a 79-year-old man with a history of hypertension, hypercholesterolemia, and mild congestive heart failure following an acute coronary syndrome 8 years ago that is controlled with diuretics. Five years ago, he was diagnosed with Rai stage II CLL with mutated immunoglobulin heavy chain variable region (IgV_H) genes. Two years after diagnosis, he developed progressive lymphadenopathy and anemia and thrombocytopenia because of marrow infiltration, which prompted treatment with chlorambucil (20 mg, 5 days, every 28 days).

After 6 cycles he had a PR with normalization of his blood counts. Three years later, he again developed progressive disease. Cytogenetic analysis was performed by fluorescent in situ hybridization (FISH) and revealed a 13q deletion in 80% of the leukemic cells without additional abnormalities. What should be the treatment of choice for this patient?

Case 2: Refractory chronic lymphocytic leukemia

The patient is a 64-year-old woman with otherwise no significant medical history who was diagnosed with rapidly progressive CLL, Rai stage III with unmutated IgV_H. Because of her rapidly progressive disease, immunochemotherapy treatment was initiated consisting
of fludarabine, cyclophosphamide, and rituximab (FCR). Six cycles of FCR resulted in a PR. Yet, 2 months after the last cycle, lymphocytes started to rise, and another 3 months later, she developed a relapse with progressive bulky lymphadenopathy. FISH analysis was performed and revealed a 17p deletion in 67% of the leukemic cells. What should be the treatment of choice for this patient?

**Definition of relapsed or refractory chronic lymphocytic leukemia**

When patients develop progressive disease following first-line treatment, it needs to be established whether the patient has relapsed or refractory disease. In 2008, the International Workshop on Chronic Lymphocytic Leukemia developed formal criteria for relapsed and refractory CLL: relapse is defined as evidence of disease activity after a period of at least 6 months in a patient who has previously achieved a CR or a PR. Patients who do not achieve either a PR or CR following treatment or those who develop disease progression within 6 months of therapy have refractory disease.

Distinction between relapsed and refractory CLL is clinically relevant because the majority of patients with progressive disease occurring more than 6-12 months after discontinuation of first-line treatment (relapsed CLL) can be successfully re-treated with either the very same treatment regimen, or with other available treatment options. In patients with refractory disease, however, it is extremely unlikely that responses will occur with previously applied therapies, and overall, these patients have a much poorer prognosis.

It is important to reconfirm the diagnosis of CLL before a patient is considered to have relapsed or refractory CLL. Especially, disorders such as transformation of CLL into large B cell lymphoma or other types of hematologic malignancy should be ruled out by means of appropriate biopsy and histologic studies.

After establishing the diagnosis of either relapsed or refractory CLL, cytogenetic analysis using FISH should be performed. Patients with refractory disease more frequently have leukemia cells that harbour deletions in the short arm of chromosome 17 (del(17p)), which is associated with loss of functional p53, or in the long arm of chromosome 11 (del(11q)) in and/or around the gene encoding the ataxia telangiectasia mutated (ATM) protein, which is a kinase required for p53 function. In consequence, many chemotherapy-refractory patients have leukemia cells that have lost functional p53. Because the cytoreductive activity of most chemotherapy agents requires functional p53, the emergence of a p53-dysfunctional clone is thought to result from selection of chemotherapy-resistant CLL subclones during treatment. Appearance of del(11q), and especially del(17p), is highly predictive for unresponsiveness to subsequent immunochemotherapy.
Treatment of Relapsed Disease

Currently, the optimal treatment for patients with relapsed disease is not known. In the absence of del(11q) or del(17p), patients can be successfully treated with either the same regimen as used upfront, or by switching to other more potent treatment combinations. Patients who relapse following treatment with alkylating agents and who are re-treated with the same category of agents have overall response rates (ORRs) ranging from 22% to 62%; however, most responses are of short duration, and few patients achieve a CR. Response rates to purine analogues in these circumstances are higher and of longer duration, with an ORR ranging from 13% to 59% and CR rates of 3% to 37%. A landmark crossover study in treatment-naive patients compared treatment with chlorambucil with treatment with fludarabine. Patients who failed chlorambucil had an ORR to subsequent treatment with fludarabine of 46%, whereas only 7% of the patients who failed fludarabine responded to chlorambucil. This observation indicates that there is little role for treatment with alkylating agents in patients in whom previous purine analogues have failed.

Combination therapy of purine analogues with alkylators is superior to treatment with fludarabine alone with respect to ORR, CR, and progression-free survival (PFS) in patients with previously untreated CLL, as has been shown in 3 large, prospective, randomized, multicenter studies. Furthermore, in relapsed CLL, various nonrandomized studies using this regimen showed an ORR rate of 40%-80% with 3%-15% CRs. The most promising results in previously treated CLL have been attained by a group from the M. D. Anderson Cancer Center (MDACC) with the chemoimmunotherapeutic combination regimen FCR. Fludarabine 25 mg/m² and cyclophosphamide 250 mg/m² on days 2-4 of cycle 1 and days 1-3 of cycles 2-6, and rituximab 375 mg/m² on day 1 of cycle 1 and 500 mg/m² on day 1 of cycles 2-6, were given every 28 days for 6 cycles to 177 patients with relapsed CLL. Toxicity was acceptable, and infectious complications were manageable. The ORR was 73%, with 25% CR and 16% nodular PR, with a molecular CR in 32% of the patients in CR.

These very promising results with FCR prompted the study of the benefit of adding rituximab to the combination of a nucleoside analogue and an alkylating drug in relapsed patients in a large, international, prospective, randomized study, the REACH trial. This study randomized 552 relapsed patients to fludarabine and cyclophosphamide with or without rituximab. Patients had been treated previously with single-agent alkylator therapy (66%), purine analogues (16%), or other combinations (CHOP (cyclophosphamide/doxorubicin/vincristine/prednisone), COP (cyclophosphamide/vincristine/prednisone), 18%). Patients who were treated before with the combination of fludarabine and cyclophosphamide were excluded. ORR, CR, and median PFS favored FCR (70%, 24%, and 31 months, respectively) over FC (57%, 13%, and 21 months, respectively). Patients from different risk groups (eg, IgVH mutation status, ZAP-70 status) did equally well except for those with del(17p), who did much worse. The study showed an acceptable safety profile for...
both FCR and FC without new or unexpected findings for the addition of rituximab to chemotherapy.

All of the above studies dealt with patients who relapsed after either first-line single-agent therapy or less potent chemotherapy combinations. Yet, based upon recent results from the German CLL Study Group (GCLLSG) CLL08 study that showed superior results of up-front treatment with FCR over FC in terms of ORR, PFS, and OS, FCR is likely to become the first-line treatment in previously untreated fit patients. However, despite a PFS of more than 50 months and an improved OS, patients will eventually relapse, and responses to second-line treatment following FCR have not yet been prospectively studied.

In an attempt to gain more insight in treatment outcomes in patients who relapse after treatment with FCR, the MDACC group retrospectively analyzed 300 patients treated with up-front FCR. After a median follow-up of 6 years, 116 patients (39%) had failed FCR therapy, of whom 13 patients had primary refractory disease and 103 relapsed disease. Patients received treatment chosen at the discretion of individual treating physicians. The CR rates of second-line therapy were: FCR, 17%; rituximab, 4%; alemtuzumab with or without rituximab, 31%; FCR and alemtuzumab (CFAR), 56%; lymphoma-type chemotherapy, 0%; other treatment, 0%. None of the regimens showed a significant survival benefit. The median OS from time of relapse was 33 months with a 40% 5-year survival rate. Allogeneic stem cell transplantation (alloSCT) was usually performed as second or subsequent salvage therapy with a 5-year survival fraction of approximately 35%. From these studies, it can be concluded that more than half of the patients who relapsed after FCR can achieve at least a PR with salvage therapy. The optimum treatment for this group of patients, however, remains ill defined and should be studied in prospective trials unabatedly.

**Treatment of Refractory Disease**

Almost all patients with CLL will ultimately develop refractory disease. As most patients are treated with fludarabine-based regimens, either up front or at first relapse, the majority of information on refractory disease is derived from patients refractory to fludarabine. The prognosis of such patients is poor. In a retrospective review of 147 patients with fludarabine-refractory CLL, Keating et al reported a 22% response rate (CR plus PR) to the first salvage regimen and a median survival of 10 months. In recent years, various new drugs and treatment combinations have been tested in this patient category. The most promising strategies are discussed here.

**Monoclonal Antibody Treatment**

**Alemtuzumab.** Alemtuzumab (Campath-1HR), an anti-CD52 humanized MoAb, has been extensively investigated in CLL. It has significant anti-leukemic activity, predominantly in
the peripheral blood compartment, bone marrow, and spleen, whereas activity is lower in lymph nodes. The US Food and Drug Administration (FDA) approved alemtuzumab for patients with CLL who have been treated previously with alkylating agents and in whom fludarabine therapy has failed. At least 4 single-arm prospective studies have evaluated the efficacy of single-agent alemtuzumab in patients with relapsed or refractory CLL after treatment with fludarabine (Table 1). The two largest and most compelling trials will be further discussed. In a pivotal multicenter international clinical trial, 93 fludarabine-refractory patients received alemtuzumab 30 mg intravenously (I.V.) in a 3-times-weekly schedule for a maximum of 12 weeks. Most patients were heavily pretreated with a median number of 7 previous therapies. The ORR in an intention-to-treat analysis was 33% (CR, 2%; PR, 31%). Alemtuzumab induced responses in the blood in 83% and in the bone marrow in 26% of the patients. The median time to disease progression for all patients was 4.7 months, and the median OS duration was 16 months. Among responders, however, the median time to progression was 9.5 months, and the median survival duration was 32 months. This study demonstrated that alemtuzumab increases the median survival of patients with fludarabine-refractory CLL compared with historical controls. Similar results were found in the GCLLSG CLL2H trial, in which alemtuzumab was administered subcutaneously at 30 mg 3 times weekly to 103 fludarabine-refractory patients. The response rate was 34% (CR, 4%), and the median PFS and OS durations were 7.7 months and 19.1 months, respectively. In this study, a thorough analysis of responses in different risk groups was performed. Responses (CR or PR) were observed in 24% of the patients with del(11q), 39% of the patients with del(17p), and 33% of the patients with mutations in the TP53 gene (irrespective of del(17p)) . Importantly, PFS and OS did not differ significantly among these genetic subgroups. This study confirmed that alemtuzumab had activity in patients with high-risk CLL, including patients with poor-risk cytogenetic abnormalities.

Although both of these studies clearly demonstrated clinically useful effects of alemtuzumab in patients with refractory disease, cures are never obtained, and PFS remains relatively short if treatment is not followed by consolidation with allogeneic hematopoietic SCT. Furthermore, some special considerations need to be taken into account when alemtuzumab is prescribed: first of all, alemtuzumab proved least effective in patients with bulky lymphadenopathy. As an example, one study reported ORRs of 87%, 40%, and 9% in patients with no lymphadenopathy, those whose largest node was \( \leq 5 \) cm, and those whose largest node was \( > 5 \) cm, respectively. Secondly, because CD52 is ubiquitously expressed on normal B and T lymphocytes as well as monocytes, the clinical use of alemtuzumab is hampered by serious infection-related toxicities. In most studies alemtuzumab increased the already existing vulnerability of patients with advanced-stage CLL to opportunistic infections, including potentially lethal bacterial, viral (especially cytomegalovirus (CMV)), fungal, and protozoal infection, indicating that appropriate antibacterial and antiviral prophylaxis must be instituted when this agent is used. A thorough discussion on choices of prophylaxis regimens is out of
the scope of this review, but these regimens should at least include prophylaxis against *Pneumocystis carinii* infections as well as antiviral therapy and close monitoring on CMV (re)activation.

Based on the above studies, alemtuzumab monotherapy should be the treatment of choice for patients with del(17p) without bulky lymphadenopathy outside of clinical trials. As described in the next paragraph, combinations of alemtuzumab with other agents are potential treatment options for patients with del(17p).

**Ofatumumab.** Ofatumumab (HuMax-CD20R) is a fully humanized high-affinity MoAb that targets a different epitope of CD20 than that targeted by rituximab and that activates complement derived cytotoxicity more effectively than rituximab. Results of a phase I/II clinical trial of ofatumumab in recurrent/refractory CLL were encouraging. Thirteen of 26 patients responded (1 nPR, 12 PR) with a median time to progression of 106 days in responders and median time to next therapy of 366 days\(^{37}\). Grade 1/2 infectious toxicity was seen in 48% of the patients, but 1 patient developed grade 4 interstitial pneumonitis. A pivotal phase II study of ofatumumab in relapsed patients showed impressive activity both in patients refractory to both fludarabine and alemtuzumab (double refractory (DR), n = 59) and in patients with bulky lymphadenopathy refractory to fludarabine (bulky fludarabine refractory (BFR), n = 79)\(^{38}\). ORR, PFS, and OS were similar for the DR (58%, 5.7 months, and 13.7 months, respectively) and BFR groups (47%, 5.9 months, and

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Number of patients</th>
<th>Patient category</th>
<th>TRM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osterborg et al (^{35})</td>
<td>Alem 3/weekly x 12</td>
<td>29</td>
<td>Relapsed and refractory CLL</td>
<td>0</td>
</tr>
<tr>
<td>Keating et al (^{27})</td>
<td>Alem 3/weekly x 12</td>
<td>93</td>
<td>Relapsed and refractory CLL</td>
<td>6.5</td>
</tr>
<tr>
<td>Rai et al (^{36})</td>
<td>Alem 3/weekly x 16</td>
<td>24</td>
<td>Relapsed and refractory CLL</td>
<td>17</td>
</tr>
<tr>
<td>Stilgenbauer et al (^{28})</td>
<td>Alem 3/weekly x 4-12</td>
<td>103</td>
<td>Refractory CLL</td>
<td>*</td>
</tr>
</tbody>
</table>

* After a median follow-up time of 37.9 months, there were 75 deaths, 31% due to infection, and 13% not related to CLL. Abbreviations: CR = complete response; ORR = overall response rate; TRM = treatment-related mortality

Table 1. Alemtuzumab treatment in relapsed and refractory chronic lymphocytic leukemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Number of patients</th>
<th>Patient category</th>
<th>TRM, %</th>
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<td>Alem 3/weekly x 4-12</td>
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<td>*</td>
</tr>
</tbody>
</table>

* After a median follow-up time of 37.9 months, there were 75 deaths, 31% due to infection, and 13% not related to CLL. Abbreviations: CR = complete response; ORR = overall response rate; TRM = treatment-related mortality

Table 2. Pivotal trial efficacy data of ofatumumab in refractory chronic lymphocytic leukemia

<table>
<thead>
<tr>
<th>Efficacy measure</th>
<th>Double refractory (n=59)</th>
<th>Bulky fludarabine refractory (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, %</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>58</td>
<td>46</td>
</tr>
<tr>
<td>SD, %</td>
<td>31</td>
<td>41</td>
</tr>
<tr>
<td>Median time to response, months</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Median duration of response, months</td>
<td>7.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>5.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Median OS, months</td>
<td>13.7</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Abbreviations: CR = complete response; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; PR = partial response; SD = stable disease
Because it has been shown that the use of intensified rituximab therapy—especially when combined with high-dose methylprednisolone—also is effective in refractory patients, it remains to be established whether the results of ofatumumab could be attributed to drug-specific mechanisms or to the dose used. Based on these results, ofatumumab has received FDA approval in patients with CLL refractory to both fludarabine and alemtuzumab.

### Chemoimmunotherapy

Different regimens have been studied (or are currently under investigation) in which chemotherapeutic agents are combined with MoAb in chemorefractory CLL (Table 3).

A small study in patients with CLL refractory to fludarabine alone and alemtuzumab alone showed a considerable response rate upon combining fludarabine with alemtuzumab, suggesting synergy between these two agents. This result formed the basis of a multicenter, open-label, randomized phase III study in order to compare the efficacy and safety of fludarabine and alemtuzumab versus fludarabine alone as second-line therapy in 335 patients with relapsed or refractory CLL. Patients received fludarabine (30 mg/m²) with or without alemtuzumab (30 mg) on days 1-3 of a 28-day cycle. Combination therapy resulted in significantly higher ORR and CR rates (combination: ORR, 84.8%; CR, 30.4%; fludarabine single agent: ORR, 67.9%, CR, 16.4%). Interim analysis after a median follow-up time of 17 months revealed a significantly improved PFS for the combination treatment arm (29.6 months vs. 20.7 months), whereas toxicity was highly comparable between the two arms. Although the results of this study appear encouraging, the exact percentage of patients with true refractory disease was not yet disclosed.

Following the observed synergy between alkylating agents and fludarabine, the GCLLSG initiated a multicenter phase II trial combining fludarabine, cyclophosphamide, and alemtuzumab in 61 fludarabine-refractory patients. In this single-arm study, patients received fludarabine 25 mg/m², cyclophosphamide 200 mg/m², and alemtuzumab 30 mg on days 1-3, every month for up to 6 cycles. Grade 3/4 thrombocytopenia and neutropenia were the most common serious side effects. Twelve of 56 patients died during or within 6 months after last chemoimmunotherapy, of which 5 were related to therapy. The ORR for the remaining patients was 68% (CR, 22%), independent of FISH
status. Also in this study, the exact percentage of patients with true refractory disease was not disclosed.

Based upon the observed synergy between purine analogues, alkylating agents, and either alemtuzumab or rituximab, FCR combined with alemtuzumab (CFAR) has been tested in patients with previously treated CLL. Eighty patients with relapsed CLL, of whom 31 with fludarabine-refractory disease received fludarabine (25 mg/m² on days 2-4), cyclophosphamide (250 mg/m² on days 2-4), rituximab (375 mg/m² (cycle 1) or 500 mg/m² (cycles 2-6) on day 2), and alemtuzumab (30 mg I.V. on days 1, 3, and 5) every 28 days for up to 6 cycles. Grade 3/4 neutropenia was the most common side effect. ORR of the whole group of patients was 67% (CR, 27%), and for the fludarabine-refractory patients, it was 52% (CR, 6%). Median OS and time to treatment failure (TTF) were 16.6 months and 10.6 months, respectively, for all patients.

For patients achieving CR, median OS (50+ months) and TTF (28+ months) have not been reached. So, although the addition of alemtuzumab to FCR improved efficacy, CFAR seems significantly less active in patients with fludarabine-refractory disease.

Another regimen that is currently being studied in refractory CLL is the combination of a platinum-based compound (oxaliplatin), with fludarabine, cytarabine, and rituximab (OFAR).

Combining platinum-based compounds with cytarabine has been proven successful in relapsed and refractory non-Hodgkin lymphomas. A phase I/II trial using this regimen consisting of increasing doses of oxaliplatin (17.5, 20, or 25 mg/m² on day 1), fludarabine (30 mg/m² on days 2-3), cytarabine (1 g/m² on days 2-3) and rituximab (375 mg/m² on day 3) yielded moderate though encouraging results in patients with CLL with chemorefractory disease, with an ORR of 33% for the patients with fludarabine-refractory CLL and 37% for the patients with documented del(17p). In an ongoing phase II clinical trial of the OFAR regimen in patients with fludarabine-refractory CLL or Richter syndrome, the interim response rate is approximately 63%.

Based upon effectiveness of the R-DHAP regimen (rituximab, dexamethasone, cytarabine, and cisplatin) in relapsed B-cell non-Hodgkin lymphoma and the apparent

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Number of patients</th>
<th>ORR (CR), % PFS, months</th>
<th>Results in refractory patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engbert et al 41</td>
<td>F-alem</td>
<td>335</td>
<td>85 (30) 30</td>
<td>Unknown</td>
</tr>
<tr>
<td>Elter et al 42</td>
<td>FC-alem</td>
<td>56</td>
<td>68 (11) Unknown</td>
<td>Equally effective</td>
</tr>
<tr>
<td>Badoux et al 43</td>
<td>CFAR</td>
<td>80</td>
<td>67 (27) 11</td>
<td>Less effective</td>
</tr>
<tr>
<td>Tsimeridou et al 46</td>
<td>OFAR</td>
<td>91</td>
<td>44 (7) Unknown</td>
<td>Equally effective</td>
</tr>
<tr>
<td>Tonino et al 47</td>
<td>R-DHAP</td>
<td>10</td>
<td>80 (10) Unknown</td>
<td>Equally effective</td>
</tr>
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</table>

Abbreviations: CFAR = fludarabine/ cyclophosphamide/ alemtuzumab/ rituximab; CR = complete response; F-alem = fludarabine/ alemtuzumab; FC-alem = fludarabine/ cyclophosphamide/ alemtuzumab; OFAR = oxaliplatin/ fludarabine/ cytarabine/ rituximab; ORR = overall response rate; PFS = progression free survival; R-DHAP = rituximab/ dexamethasone/ cytarabine/ cisplatin
clinical activity of platinum in refractory CLL, a retrospective analysis has been performed on the activity of the R-DHAP regimen (dexamethasone 40 mg on days 1-4, cisplatin 100 mg/m² on day 1, cytarabine 2 × 2 g/m² on day 2, and rituximab 375 mg/m² on day 5, every 3 weeks) in 10 patients with fludarabine-refractory CLL. Eight of the 10 patients responded with 1 CR. Interestingly, a significant decrease in lymphadenopathy was attained in 6 of 8 patients with bulky disease. Six patients subsequently underwent reduced SCT. Based upon these promising results, R-DHAP is currently being tested in a prospective Dutch/Belgian HOVON trial studying the efficacy and tolerability of this regimen before alloSCT in CLL patients with fludarabine-resistant disease. Although combinations of immunochemotherapy seem to have an effect in fludarabine-refractory CLL, long-term results of prospective studies must be awaited, and patients should not be treated with these combinations outside of clinical trials.

**Novel agents for relapsed and fludarabine-refractory chronic lymphocytic leukemia**

Several exciting novel therapeutic agents are currently under either preclinical or early clinical investigation and include small molecule inhibitors of BcL-2, cyclin-dependent kinase (CDK) inhibitors, bendamustine, and inhibitors of phosphatidylinositol 3-kinases. Because of the nature of this review, only promising agents beyond phase Ia trials will be discussed.

**Flavopiridol**

Flavopiridol is a synthetic flavone with many divergent effects in vitro. It broadly inhibits CDK, downregulates expression of key anti-apoptotic proteins, and induces apoptosis independent of p53. A small study that indicated marked effectivity in CLL was hampered by serious side effects, especially hyperacute tumour lysis syndrome. Therefore, a modified protocol was used in a phase II study including 64 patients with relapsed CLL in which flavopiridol was combined with dexamethasone to suppress cytokine release syndrome. Thirty-four patients (53%) achieved responses, of whom 1 achieved a CR. A majority of high-risk patients responded, including patients with del(11q) and del(17p) irrespective of lymph node size. PFS among responders was 10-12 months across all cytogenetic risk groups. The use of prophylactic dexamethasone resulted in improved tolerability and treatment delivery. Based upon these encouraging results, a registration study in patients with relapsed CLL is ongoing.

**Lenalidomide**

Lenalidomide is a so-called immunomodulating drug that has proven efficacy in multiple myeloma and myelodysplastic syndrome. The exact mechanism of action is unknown, but this class of drugs is believed to be effective via pleiotropic immunomodulatory mechanisms. In the first phase II study of lenalidomide in CLL, the same dose was used as in the treatment of multiple myeloma, namely 25 mg/day on days 1 through 21 of a
28-day cycle. Although an impressive ORR of 30% was observed among 23 patients with fludarabine-refractory CLL, clinical applicability seemed hampered by major toxicity, which consisted of a so-called tumour flare reaction (swelling of involved lymph node sides) and tumour lysis syndrome. To diminish significant toxicity, in the next study, the dose of lenalidomide was decreased to a starting dose of 10 mg followed by slow titration upward to a maximum daily dose of 25 mg. Twelve patients with fludarabine-refractory CLL were included in this study. Twenty-five percent of these patients responded to lenalidomide, compared with a response rate of 38% in the patients with fludarabine-sensitive disease. Treatment with lenalidomide was associated with an ORR of 31% in the patients with del(11q) or del(17p). Furthermore, this dose-regimen was much better tolerated.

To study potential synergy, a phase II study was initiated by the MDACC to evaluate the activity of the combination of lenalidomide and rituximab in patients with relapsed CLL, including 27% with fludarabine-refractory disease. Sixty CLL patients who relapsed after purine analogue–based therapy, received rituximab 375 mg/m² I.V. on days 1, 8, 15, and 22 of cycle 1, and then once every 4 weeks during cycles 3-12. Lenalidomide (10 mg/day) was given orally starting on day 9 of cycle 1 and continued daily for 12 cycles. An interim analysis after 6 cycles of treatment showed that 25 patients achieved a response (6 nodular PRs (16%) and 19 PRs (51%)), resulting in an ORR of 68%. Six patients (16%) attained stable disease or clinical improvement and are continuing treatment, and 6 patients (16%) failed to respond, including 1 death that occurred on day 34 as a result of infectious complications. The most common side effect was neutropenia; the occurrence of lenalidomide-associated tumour flare reaction was limited to grade 1 and 2. Results of this study suggest that the combination of lenalidomide and rituximab is superior to single-agent lenalidomide, although subgroup analysis on patients with refractory disease has not yet been disclosed.

Bendamustine

Bendamustine has structural similarities to both alkylating agents and purine analogues. Phase II studies from the former East Germany using empirical dosing in relatively small numbers of patients with CLL provided evidence that bendamustine was effective with response rates of 65%-93%, with a favorable safety profile. Based on these results, the GCLLSG initiated a phase II study to investigate the combination of bendamustine (70 mg/m² on 2 consecutive days) and rituximab (day 1, at a dose of 375 mg/m² for the first course and 500 mg/m2 during subsequent cycles) in 81 patients with relapsed CLL. Included patients had a median age of 66 years and had received a median of 2 previous therapies. In the first 31 patients evaluable for response, the ORR was 65%, including 13% CRs. However, there were no molecular remissions as assessed by flow cytometry. The main adverse effect was reversible myelosuppression (48 patients experienced grade 3/4 adverse events). In conclusion, bendamustine seems active in relapsed CLL, but until now, efficacy in refractory CLL remains to be explored.
Allogeneic Hematopoietic Stem Cell Transplantation.

The only means by which long-term clinical remissions can be achieved is allogeneic hematopoietic SCT (alloHSCT). Following alloHSCT the relapse incidence decreases over time, not only following myeloablative conditioning, but also following nonmyelotoxic reduced-intensity conditioning (RIC). Evidence that this clinical effect is because of an ongoing graft-versus-leukemia (GVL) effect in CLL comes from the following observations: (1) the relapse risk is reduced in the presence of chronic graft-versus-host disease (GVHD); (2) the relapse risk is increased when T-cell–depleted allografts are used; and (3) donor lymphocyte infusions (DLI) following alloHSCT can effect reductions in tumour burden. The existence of a GVL effect is further supported by a study from Ritgen and colleagues, who measured the kinetics of minimal residual disease following RIC alloHSCT. Although the conditioning regimen did not eliminate overt disease, durable remissions were observed between 100 and 200 days following alloHSCT in patients who had GVHD or who received DLI following transplantation.

Long-term PFS rates have been reported in 30%-60% of patients who have undergone transplantation by RIC alloSCT. Outcome is independent of fludarabine refractoriness or presence of del(17p) as has been shown in different trials: in 2003 a relatively small trial of 30 patients found a probability of 2-year OS and PFS of 72% and 67%, respectively. Univariate analysis of clinical risk factors for 2-year PFS did not show a significant effect of fludarabine-refractory disease. Also in the CLL3X trial of the GCLLSG, which included 113 patients, fludarabine refractoriness did not emerge as an adverse factor for PFS in multivariate analysis. In a 5-year follow-up study of the Seattle Consortium, which included 82 patients (of whom 87% had fludarabine-refractory disease), a 5-year PFS of 39% and an OS of 50% were reported. Patients with fludarabine-refractory disease and/or patients with del(17p) did equally well as patients without poor-risk disease. The strongest prognostic factor for relapse was the presence of bulky lymphadenopathy at time of transplantation.

Based upon these data the European Group for Blood and Marrow Transplantation (EBMT) published a consensus paper on the indications for HSCT in CLL. In summary, according to EBMT, alloHSCT should be considered for eligible patients with previously treated, poor-risk CLL. Poor risk is defined as lack of response or disease recurrence within 12 months after purine analogue-containing therapy, recurrence within 24 months after purine analogue combination therapy and patients with recurrent/refractory disease with evidence of p53 deletion or mutation. Preferential induction treatment for alloHSCT has not yet been established. Regimens that are active in patients with bulky disease, such as R-DHAP or ofatumumab (approved for patients with double-refractory CLL; see above) are most promising, but the outcome of prospective trials need to be awaited.
Conclusion

The distinction between relapsed disease and refractory disease has a major effect on the choice of salvage regimen. The first-line treatment regimen or another chemotherapy-based (more effective) treatment strategy may be repeated if the duration of the first remission exceeded 12 months. Treatment choices are far more limited for refractory disease (defined as relapse within 6 months after the last treatment) or in case of del(17p). In this event, patients should be included in clinical trials whenever possible. If no trial is available, treatment choices highly depend on the physical fitness of the patient (Figure 1). In fit patients, we recommend treatment with alemtuzumab monotherapy for 12 weeks, followed by a RIC. If the patient is also alemtuzumab refractory, ofatumumab therapy should be considered. In unfit patients, prognosis is very poor, and we would prefer a nontoxic palliative regimen along with supportive care.

Example Case Studies Follow-up

The patient described in case 1, who had relapsed CLL and considerable comorbidity, was again treated with chlorambucil. This resulted in a PR lasting for more than 1 year.

The patient described in case 2 (refractory disease) was treated with R-DHAP in a clinical trial. She had a good PR without bulky disease and is now prepared for alloHSCT.

Figure 1. Algorithm for the treatment of relapsed and refractory chronic lymphocytic leukemia outside clinical trials. Abbreviations: RIC alloHSCT = reduced intensity conditioning allogeneic hematopoietic stem cell transplantation.


7. Hallek M, Fingerle-Rowson G, Fink AM et al. First-Line treatment with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) improves overall survival (OS) in previously untreated patients (pts) with advanced chronic lymphocytic leukemia (CLL): results of a randomized phase III trial on behalf of an international group of investigators and the German CLL Study Group. ASH Annual Meeting Abstracts 2009;114:535.


24. Robak T, Moiseev SI, Dmoszynska A et al. Rituximab, fludarabine, and cyclophosphamide (R-FC) prolongs progression free survival in relapsed or refractory chronic lymphocytic leukemia (CLL) compared with FC alone: final results from the international randomized phase III REACH trial. ASH Annual Meeting Abstracts 2008;112:1ba-1.


38. Osterborg A, Kipps TJ, Mayer J et al. Ofatumumab (HuMax-CD20), a novel CD20 monoclonal antibody, is an active treatment for patients with CLL refractory to both fludarabine and alemtuzumab or bulky fludarabine-refractory disease: results from the planned interim analysis of an international pivotal trial. ASH Annual Meeting Abstracts 2008;112:328.


41. Engert A, Gercheva L, Robak T et al. Improved progression-free survival (PFS) of alemtuzumab (Campath(R), MabCampath(R)) plus fludarabine (Fludara(R)) versus fludarabine alone as second-line treatment of patients with B-Cell chronic lymphocytic leukemia: Preliminary results from a phase III randomized trial. ASH Annual Meeting Abstracts 2009;114:537.


