The management of chronic lymphocytic leukemia (CLL) is currently undergoing a profound change. First, several new drugs have been approved (fludarabine, bendamustine and two monoclonal antibodies, alemtuzumab and rituximab). In addition, novel monoclonal antibodies targeting CD20, CD23, CD37 or CD40, as well as drugs designed to interfere with central pathways regulating the cell cycle, the apoptotic machinery, or the leukemic microenvironment (flavopiridol, oblimersen, ABT-263 or lenalidomide) are being tested in clinical trials. Furthermore, improved protocols using reduced-intensity allogeneic progenitor cell transplantation makes it possible to offer this procedure to more patients with CLL. Finally, new prognostic markers that may influence therapeutic decisions have been identified. This review attempts to summarize the current knowledge in this rapidly moving field.

Key words: Chemoimmunotherapy, CLL, therapy, MRD

**introduction**

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder that accounts for ~30% of adult leukemia and 25% of non-Hodgkin lymphoma (NHL). It is the most common form of leukemia in the western world (incidence 5/100 000). Elderly people are mainly affected with <10% of the patients being <40 years of age [1]. The median age at diagnosis is ~70 years [2]. Treatment of CLL is especially challenging as it is a heterogeneous disease with an extremely variable course. Recent insights into the pathogenesis of the disease are providing new starting points for more potent treatment options. Particularly the advent of chemoimmunotherapy has revolutionized CLL therapy and leads to treatment-free intervals of >5 years. Due to pronounced myelotoxicity those treatment regimens are reserved for physically fit patients. However, CLL is the classical leukemia of the elderly which requires tailoring the treatment according to the patient’s fitness and ability to tolerate more toxic combination therapies. As a consequence, the management of CLL becomes increasingly personalized, requiring detailed knowledge of diagnostic and treatment options. This review attempts to summarize our current understanding of CLL treatment and gives an outlook on new exciting strategies in this field.

**monotherapy**

**cytostatic agents**

Treatment with the alkylating agent chlorambucil has long been the ‘gold standard’ in front-line CLL therapy [3]. Due to its low toxicity profile, low cost and convenient oral application, chlorambucil is still an appropriate option especially for non-fit elderly patients. Major disadvantages, however, are its low to non-existent complete remission (CR) rate and some side-effects that occur after extended use (prolonged cytopenia, myelodysplasia and secondary acute leukemia).

Another widely utilized class of drugs for CLL therapy is the purine analogues. Fludarabine, pentostatine and cladribine are currently used in CLL. Of these fludarabine is the most extensively studied compound. Compared with chlorambucil or corticosteroids fludarabine monotherapy elicits superior overall response rates (ORRs) and induces a significantly higher number of CRs [7%–40%] [4–7]. Even in comparison with conventional combinatory regimens like cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) or cyclophosphamide, doxorubicin, prednisone (CAP), fludarabine monotherapy demonstrates superior efficacy with regard to ORR and CR [8, 9]. However, it has repeatedly been shown that fludarabine fails to improve overall survival (OS) when used as single agent [6, 7, 10, 11]. Similarly, cladribine monotherapy produced a higher CR rate than chlorambucil plus prednisone (47% versus 12%) without resulting in longer survival [12].

Bendamustine hydrochloride is an alkylating agent containing a benzimidazole ring. It was approved by the US Food and Drug administration (FDA) for the treatment of CLL in 2008, after having been developed in East Germany over 40 years ago. In March 2010 the European Medicines Agency (EMEA) followed in granting marketing authorization for bendamustine for patients with CLL. Both approvals were based upon the results of a randomized phase III trial comparing bendamustine with chlorambucil [13]. The overall response (OR) and median progression-free survival (PFS) rates were 67% and 22 months, respectively, for bendamustine versus 30% and 8 months for chlorambucil (both P < 0.0001).

Even though fludarabine and bendamustine when used as single agents tend to show higher efficacy (response rates, CR
medians OS was 19.1 months. Due to similar efficacy at later weeks. An ORR of 34% (complete response: 4%, partial response: 30%) was observed, indicating that both treatments were effective. This study supports the use of alemtuzumab in patients with high-risk markers who do not benefit from standard therapies.

However, there remain several limitations, especially as alemtuzumab is highly toxic and leads to severe immunosuppression. The drug was also recently approved as front-line therapy for CLL by the FDA based on the results of the CAM307 trial. In this prospective randomized study alemtuzumab was compared with chlorambucil (Table 1) [20]. Alemtuzumab led to better OR and CR rates (P < 0.0001), superior PFS with a 42% reduction in risk of progression or death [hazard ratio (HR) = 0.58, P < 0.0001] and significantly longer median time to alternative treatment (TTP) (HR = 0.54, P = 0.0001). However, there remain several limitations, especially as alemtuzumab is highly toxic and leads to severe immunosuppression. It should therefore be reserved for fit patients with high-risk markers. The question of whether those patients benefit from alemtuzumab in terms of OS has not yet been answered. This end point was not addressed in the Cam307 trial.

In a phase II study by the German CLL Study Group (GCLLSG) 103 patients with fludarabine-refractory CLL were treated with alemtuzumab s.c. three times a week for up to 12 weeks. An ORR of 34% [complete response: 4%, partial response (PR) 30%]. The median PFS was 7.7 months, and the median OS was 19.1 months. Due to similar efficacy independent of the administration route, a registration trial (CAM203) is currently being conducted to support approval of subcutaneously administered alemtuzumab [21].

In CLL, the anti-CD20 monoclonal antibody rituximab is less active as a single agent than in follicular lymphoma, unless very high doses are used [22, 23]. In contrast, combinations of rituximab with chemotherapy are efficacious therapies for CLL (see below).

### Combination Chemotherapy

Since the late 1990s combinatory chemotherapies have emerged as the standard of care. Purine analogues and alkylating agents have different mechanisms of action and show partially non-overlapping toxicity profiles. This suggested a synergistic effect when combining these two compounds. This was first demonstrated in vitro: exposure of CLL cells to fludarabine and cyclophosphamide resulted in synergistic cytotoxicity [24]. Fludarabine was in parallel established as the backbone of CLL therapy in 2000 by Rai et al. [6], who showed an advantage of fludarabine over chlorambucil. Fludarabine has since been evaluated in a variety of combination regimens. The combination of fludarabine with cytarabine appeared to be less effective than fludarabine alone, while the combination of fludarabine with chlorambucil or prednisone increased hematological toxicity without improving the response rate compared with fludarabine alone (response rates 27%–79%) [6, 25]. Three randomized trials shifted the standard of care for CLL towards the combination of fludarabine and cyclophosphamide (FC) for young patients (Table 2) [7, 26, 27] (reviewed in [25]). The FC combination significantly improves CR and ORR as well as PFS when compared with fludarabine alone (Table 2) [7, 26, 27]. Despite inducing a higher rate of grade 3 and 4 neutropenias, FC did not increase the rate of severe infections. A recent analysis of the CLLA trial of the GCLLSG suggests that the first-line treatment of CLL patients with the FC combination may improve the OS of non-high-risk CLL patients [all patients not exhibiting a del(17p) or p53 mutation] significantly compared with fludarabine alone [29].

A recent prospective randomized phase III trial of the Polish Adult Leukemia Group (PALG-CLL3) demonstrated that cladribine in combination with cyclophosphamide (CC) is as effective as the standard of care. Purine analogues and alkylating agents have different mechanisms of action and show partially non-overlapping toxicity profiles. This suggested a synergistic effect when combining these two compounds. This was first demonstrated in vitro: exposure of CLL cells to fludarabine and cyclophosphamide resulted in synergistic cytotoxicity [24]. Fludarabine was in parallel established as the backbone of CLL therapy in 2000 by Rai et al. [6], who showed an advantage of fludarabine over chlorambucil. Fludarabine has since been evaluated in a variety of combination regimens. The combination of fludarabine with cytarabine appeared to be less effective than fludarabine alone, while the combination of fludarabine with chlorambucil or prednisone increased hematological toxicity without improving the response rate compared with fludarabine alone (response rates 27%–79%) [6, 25]. Three randomized trials shifted the standard of care for CLL towards the combination of fludarabine and cyclophosphamide (FC) for young patients (Table 2) [7, 26, 27] (reviewed in [25]). The FC combination significantly improves CR and ORR as well as PFS when compared with fludarabine alone (Table 2) [7, 26, 27]. Despite inducing a higher rate of grade 3 and 4 neutropenias, FC did not increase the rate of severe infections. A recent analysis of the CLLA trial of the GCLLSG suggests that the first-line treatment of CLL patients with the FC combination may improve the OS of non-high-risk CLL patients [all patients not exhibiting a del(17p) or p53 mutation] significantly compared with fludarabine alone [29].

### Table 1. Monotherapy: randomized trials comparing novel agents with chlorambucil

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Reference</th>
<th>N</th>
<th>Age (median)</th>
<th>Advanced stage (%)</th>
<th>ANC, toxicity grade 3–4 (%)</th>
<th>CR (%)</th>
<th>OR (%)</th>
<th>PFS (months)</th>
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<tbody>
<tr>
<td>F</td>
<td>Rai [6]</td>
<td>179</td>
<td>64</td>
<td>39</td>
<td>27</td>
<td>20</td>
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<tr>
<td>Clb</td>
<td>Eichhorst [19]</td>
<td>193</td>
<td>62</td>
<td>41</td>
<td>19</td>
<td>4</td>
<td>37</td>
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<td>55</td>
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<tr>
<td>B</td>
<td>Knauf [13]</td>
<td>162</td>
<td>63</td>
<td>28</td>
<td>23</td>
<td>31</td>
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<td>29</td>
<td>11</td>
<td>2</td>
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<td>8</td>
</tr>
</tbody>
</table>

A, alemtuzumab; ANC, absolute neutrophil count; toxicity grade 3 or 4; B, bendamustine; Clb, chlorambucil; F, fludarabine.

*bRai III–IV.

*Study included only patients >65 years.

*Binet C.
effective as FC [30]. The CR and ORRs were 47% and 88% in the CC arm and 46% and 82% in the FC arm (P = 0.25 and P = 0.11, respectively). PFS, OS and grade 3/4 infections were comparable in both arms. Both combinations showed unsatisfactory activity in patients with a deletion of 17p.

However, an earlier randomized trial of the same group comparing cladribine (2-CdA) alone with CC and CC combined with mitoxantrone (CCM) in 508 patients with untreated progressive CLL showed no difference in OR, PFS and OS between treatment groups [31]. Grade 3/4 neutropenia occurred more frequently with CC (32%) and CCM (38%) than with cladribine (20%) (P = 0.01 and P = 0.004, respectively). Infections were more frequent with CCM compared with cladribine (40% versus 27%, P = 0.02). Compared with 2-CdA, CMC induced a higher CR rate (36% versus 21%, P = 0.004), and a trend towards higher CR rates was observed with CC (29% versus 21%, P = 0.08).

Based on these results, it remains doubtful whether cladribine combination therapies offer a major advantage when used as first-line treatment for CLL (Table 2).

Two phase II trials examined the addition of mitoxantrone to FC (FMC). In 37 patients with relapsed/refractory CLL, FMC produced a high CR rate (50%), including 10 cases of minimal residual disease (MRD) negativity, with a median duration of response of 19 months [32]. All MRD-negative patients were alive at analysis; the median duration of response had not been reached in the CR patients compared with 25 months in non-CR patients. In 69 treatment-naive patients with active CLL FMC produced an ORR of 90% including 26% of MRD-negative CRs and 38% of MRD-positive CRs [33]. Patients with del(17p) failed to attain CR. Treatment toxicity was acceptable.

**chemoimmunotherapy**

**combining chemotherapy with rituximab**

The combination of a chemotherapeutic backbone with the monoclonal antibody rituximab has opened up new horizons for CLL therapy, especially for younger and physically fit patients. Particularly, fludarabine-based regimens were investigated in several phase II trials. The trial of Schulz et al. [34] enrolled 31 previously treated or untreated CLL patients, receiving the combination of fludarabine with rituximab (FR). The ORR was 87% including 10 (32%) CRs. Another randomized phase II study in previously untreated CLL patients (CALGB 9712) addressed the question of concurrent or sequential administration of rituximab [35]. One hundred and four patients were randomized to receive six cycles of fludarabine with or without rituximab followed 2 months later by four once-weekly doses of rituximab as consolidation therapy. Patients receiving the concurrent regimen experienced more grade 3 or 4 neutropenia (74% versus 41%) and grade 3 or 4 infusion-related toxicity (20% versus 0%) as compared with the sequential arm; however, overall and complete response rates were clearly higher in the concurrent arm as well (90% and 47% versus 77% and 28%). Moreover, all patients of the CALGB 9712 protocol treated with fludarabine and rituximab were compared retrospectively with 178 patients from the previous CALGB 9011 trial, who received only fludarabine [36]. The patients receiving fludarabine and rituximab had a significantly better PFS and OS than patients receiving fludarabine alone. Two-year PFS probabilities were 67% versus 45% and 2-year OS probabilities were 93% versus 81%.

Based upon those promising results two phase II studies adding rituximab to the in terms of efficacy superior combination FC (FCR) were conducted at the MD Anderson Cancer Center. The first trial investigated FCR in 177 previously treated patients with CLL [37]. A complete response (CR) was achieved in 25% of the patients; the ORR was 73%. Even more encouraging results were attained in previously untreated CLL patients treated with FCR. In 2008 Tam et al. [38] reported the long-term results of 300 patients with a median follow-up of 6 years. The ORR was 95% with CRs in 72%, nodular partial remission in 10%, partial remission due to cytopenia in 7%, and partial remission due to residual disease in 6%. Six-year overall and failure-free survival rates were 77% and 51%, respectively. Median time to progression was 80 months.

These promising results led to the initiation of two prospective randomized phase III trials comparing FCR with FC in CLL. The trial conducted by Robak et al. [30] enrolled 552 previously treated CLL patients. The ORR (FC: 58%, FCR: 70%) and CR rate (FC: 13%, FCR: 24.3%) were significantly higher with FCR compared with FC. After a median follow-up of 23 months the addition of rituximab significantly improved PFS for patients with previously treated CLL (HR = 0.65;
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P < 0.001, median PFS: 30.6 months for FCR versus 20.6 months for FC).

The GCLLSG conducted a prospective randomized trial, including 817 treatment-naïve CLL patients with good physical fitness (CLL8 trial) [39]. Patients received six courses of either FCR or FC. A significantly higher ORR (FCR: 95.1% versus FC: 88.4%; P = 0.001) and CR rate (FCR: 44.1% versus FC: 21.8%) were achieved with FCR treatment compared with FC treatment. Median PFS was 32.8 months for FC-treated patients versus 51.8 months for FCR-treated patients (P < 0.001). At 3 years post-randomization 87.2% of patients in the FCR arm compared with 82.5% of patients in the FC arm were still alive (P = 0.01). FCR treatment was more frequently associated with grade 3 and 4 neutropenia (FCR: 33.7% versus FC: 21.0%; P < 0.001); however, notably other side-effects including severe infections were not increased. In each arm eight treatment-related deaths occurred (2%). These results demonstrate a clear benefit for the addition of rituximab to first-line CLL therapy in physically fit patients.

As CLL is predominantly a disease of the elderly and often occurs in patients with relevant comorbidities two trials investigated regime modifications to maintain efficacy while reducing toxicity. Foon et al. [40] included 50 untreated CLL patients in a single-arm study of FCR-Lite. Patients received six cycles of FCR-Lite, consisting of an elevated dose of rituximab (375 mg on day 1 of cycle 1/500 mg on day 1 of cycles 2–6 plus 500 mg on day 14 of each cycle) compared with the standard regime, while reducing the dose of administered chemotherapy (fludarabine to 20 mg/m² and cyclophosphamide to 150 mg/m² on days 2, 3 and 4 of cycle 1 and on days 1, 2 and 3 of cycles 2–6). The ORR and CR rate were 100% and 77%, respectively, using the IWCLL 2008 guidelines [41]. At a median follow-up of 2.4 years all complete responders remained in CR except for one patient who died of a myocardial infarction while still in remission. Five patients with PRs died within 2 years of completing FCR-Lite. Grade 3/4 neutropenia was noted in 13% of cycles, which is lower than observed with the usual FCR regime. Subject to criticism is, however, that the median age of patients included was 58 years, although investigating a regimen designed to be especially beneficial for fragile patients. This regime requires further testing in larger trials. The second phase II trial conducted by the Memorial Sloan-Kettering Cancer Center group introduced the concept of a sequential chemoimmunotherapy approach [42]. They report 236 treatment-naïve patients with active CLL who initially received treatment with fludarabine 25 mg/m² on days 1–5 every 4 weeks for six cycles, followed by consolidation with cyclophosphamide 3000 mg/m² every 3 weeks for three cycles, and then followed by rituximab consolidation for 4 weeks (F→C→R). The ORR achieved was 89%, including 22 CRs (61%). Consolidation with cyclophosphamide improved responses in 13 patients (36%), nine patients (25%) further improved their response with rituximab. Twenty patients (56%) achieved flow cytometric CRs, and 12 patients (33%) achieved a molecular CR (PCR negative). The 5-year survival rate was 71%. In summary, the trials by Foon et al. [40] and Lamanna et al. [42] confirm the efficacy of FCR; however, further investigation in larger trials is needed to evaluate their relevance for CLL therapy.

To further improve its efficacy additional variations of the FCR regime have been tested. Based on their experience with the chemotherapy-only regimen FCM [33]. Bosch et al. [43] conducted a phase II trial including 29 previously untreated CLL patients receiving rituximab plus FCM, followed by rituximab maintenance every 3 months for up to 2 years. The OR, MRD-negative CR, MRD-positive CR and PR rates were 93%, 46%, 36% and 11%, respectively. The regime is highly effective; however, whether it adds any further benefit to FCR alone needs to be investigated in larger randomized trials.

The MD Anderson Cancer Center conducted two trials combining FCR with alemtuzumab (CEFAR). The first trial included 80 previously treated CLL patients, who received CEFAR consisting of 250 mg/m² cyclophosphamide days 3–5, alemtuzumab 30 mg i.v. days 1, 3, 5; rituximab 375–500 mg/m² i.v. day 2, every 28 days for an intended total of six courses. One patient was not evaluable for response. Of 79 patients 21 achieved a CR (27%), the ORR was 67%. The most common cause of early cessation of therapy was infection (n = 22). Grade 3/4 hematological toxicities included neutropenia (62% of courses), thrombocytopenia (34%) and anemia (16%). Patients on prophylactic valganciclovir had a lower rate of cytomegalovirus (CMV) reactivation compared with those on valacyclovir (24% versus 3%; P = 0.01). The second trial enrolled 60 high-risk treatment-naïve CLL patients including patients with del(17p) [44]. This CEFAR regime produced more MRD eradication than FCR, but at the expense of greater myelosuppression. The ORR was 92% including 70% CRs. Grade 3/4 neutropenia and thrombocytopenia occurred in 31% and 13% of courses. The median OS for all patients has not been reached (49+ months) and the median TTP is 38 months. Although CR rates in patients with high-risk features such as 17p deletion and unmutated IgVH were >50%, TTP was significantly shorter for these patients than for patients without these features.

An alternative approach to reduce myelotoxicity was the substitution of fludarabine in the FCR regime by pentostatin (PCR). In a phase III randomized trial of FCR versus PCR in previously untreated and minimally treated CLL patients there were no statistical differences between treatments in OS or response [45]. Infection rate (fever >101°F requiring antibiotics) was the primary end point of this study, which also showed no significant difference between the two arms (31% in FCR and 34% in PCR). To evaluate whether the tolerability of this regime could be enhanced without sacrificing efficacy Kay et al. [46] conducted a phase II study in 33 untreated patients of pentostatin and rituximab without cyclophosphamide, using a higher pentostatin dose (4 mg/m²). The ORR was 76% with nine CRs (27%). Comparison of this trial with the previous PCR trial demonstrated that patients treated with PCR have a higher ORR (91% versus 76%) and CR rate (41 versus 27%). Also the median treatment-free survival was notably longer in patients treated with PCR compared with PR (30 months versus 16 months). The results of this trial indicate that increasing the dose of pentostatin does not compensate for the omission of cyclophosphamide.

The GCLLSG also tested the combination bendamustine plus rituximab (BR) in two phase II trials. Fischer et al. [47] enrolled 81 patients with relapsed CLL. Patients received 70 mg/m² of
since none of them resulted in higher efficacy compared with cladribine with rituximab, methylprednisolone plus rituximab treatment of CLL. The GCLLSG has therefore initiated to BR. In conclusion, BR is effective and safe in the first-line treatment cycles administered every 28 days for up to six cycles. Grade 3/4 neutropenia and thrombocytopenia occurred in 12% and 9% of all courses, respectively. There were 16 episodes (5%) of grade 2–3 infections with treatment-related deaths in 4% of patients. ORR was 77% with 15% CRs. These results compare favorably with the FCR regimen in that BR achieves similar response rates, but induces less neutropenias than FCR.

The second trial assessed the BR combination in previously untreated CLL patients [48]. One hundred and seventeen patients with untreated CLL requiring therapy were enrolled into the protocol. Bendamustine was given at a dose of 90 mg/m² on days 1 and 2, combined with 375 mg/m² rituximab for the first cycle and 500 mg/m² for subsequent cycles. BR treatment was administered every 28 days for up to six courses. As of June 2009, the median observation time was 15.4 months. 114 patients were evaluable for toxicity, 110 for response and 113 for PFS. The most frequent adverse events based on 583 treatment cycles were myelosuppression and infections: grade 3/4 anemia occurred in 4.9%, grade 3/4 leukopenia in 14.6%, grade 3/4 neutropenia and thrombocytopenia in 6.5% and 6.1% of all given courses, respectively. Treatment-related mortality occurred in 2.6% of the patients. The ORR was 90.9% including 32.7% (36 patients) clinical CR. Ten patients (9.1%) had stable disease whereas none of the patients had progressive disease (PD). After 18 months 75.8% of the patients were still in remission, median PFS has not been reached. An MRD level below 10E-4 was observed after completion of therapy in 29 of 50 evaluable patients in peripheral blood, while 7 of 25 patients achieved MRD negativity in bone marrow. Differences in response were observed between the genetic subgroups: 19 of 21 patients with 11q− achieved remission including nine CRs (ORR: 90.5%). Accordingly, 17 of 19 patients with +12 responded (three CRs, ORR: 89.5%). In the high-risk group with 17p−, 3 of 7 patients showed a PR (ORR: 42.9%); 56 of 63 patients (ORR: 88.9%) with unmutated IgVH status responded to BR. In conclusion, BR is effective and safe in the first-line treatment of CLL. The GCLLSG has therefore initiated a randomized phase III trial comparing BR with FCR (CLL10).

Several other combinations have been investigated, like cladribine with rituximab, methylprednisolone plus rituximab followed by alemtuzumab, or rituximab plus alemtuzumab. Their detailed description is beyond the scope of this paper since none of them resulted in higher efficacy compared with FCR.

**Combining chemotherapy with alemtuzumab**

The potential benefit of alemtuzumab in combination with chemotherapy was first suggested by a small study conducted by Kennedy et al. [49], who treated six pretreated patients with the combination of fludarabine and alemtuzumab (FA). Although the patients had beforehand received a median of eight courses of fludarabine and 16 weeks of alemtuzumab, five patients responded, including one who had a complete response. Moreover, the responses observed were better in each patient than responses after each agent used singly. These findings were confirmed by a phase II trial conducted by Elter et al. [50] enrolling 36 patients with relapsed or refractory CLL. Alemtuzumab 30 mg and fludarabine 30 mg/m² were administered intravenously for three consecutive days. The ORR observed was 83%, including 11 complete responses. Sixteen of 31 evaluated patients (53%) achieved MRD negativity in the peripheral blood by 3 months’ follow-up. Resolution of disease was observed in all disease sites, particularly in the blood, bone marrow 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. While 80% of patients were CMV immunoglobulin G (CMV IgG) positive before treatment; there were only two subclinical CMV reactivations. The primary grade 3/4 hematological events were transient, including leukocytopenia (44%) and thrombocytopenia (30%). Stable CD4+ T-cell counts (>200/μl) were seen after 1 year. A prospective randomized phase III trial investigated the combination of fludarabine and alemtuzumab (FCCam) compared with fludarabine alone as second-line treatment. Three hundred and thirty-five patients were randomized to receive either fludarabine 30 mg/m² i.v. followed immediately by alemtuzumab 30 mg i.v. on days 1–3 of a 28-day cycle or fludarabine alone 25 mg/m² i.v. on days 1–5. The median PFS for FCCam was significantly prolonged compared with fludarabine alone (29.6 months versus 20.7 months; P = 0.005; HR 1.63). FluCam resulted in significantly higher OR and CR rates (ORR: FluCam 84.8% versus 67.9%; P < 0.001; and CR: FluCam 30.4% versus Flu 16.4%; P = 0.002). No difference in survival has been observed (FluCam 37 deaths and Flu 41 deaths) after a median follow-up of 17 months. FluCam has so far proved to be feasible, safe and effective.

Based upon those convincing results the GCLLSG decided to conduct a phase II study evaluating the combination of fludarabine, cyclophosphamide and alemtuzumab (FCCam) in patients with relapsed or genetic high-risk CLL. Up to six 28-day cycles of fludarabine 25 mg/m² i.v., cyclophosphamide 200 mg/m² i.v. and alemtuzumab 30 mg i.v. were administered on days 1–3. Fifty-six patients were evaluated for response and adverse events. Five of 61 recruited patients had to be excluded due to protocol violations (withdrawn consent, secondary malignancies, death before therapy). CTC grade 3/4 thrombocytopenia and neutropenia were the most common serious side-effects. Twelve patients died during or within 6 months after last chemotherapy. Five of those deaths were related to therapy. Three were related to concomitant disease and four patients died due to progressive disease. Four of 56 treated patients were excluded from response analysis: one patient stopped therapy because of a secondary malignancy, three patients died within therapy. The ORR for the remaining 52 patients was 68%, including 11 CRs (22%). Nine (17%) patients had PD. Response was independent of FISH status. Notably a correlation of response with prior treatment was observed, with 81% ORR for fludarabine-alone pretreatment and 63% for those patients pretreated with FC. In summary the results of this phase II trial indicate that FC plus alemtuzumab (FCCam) is effective in second-line CLL treatment; however, due to its marked toxicity FCCam cannot be recommended for further application in CLL therapy outside clinical trials.

Two phase III trials evaluating the activity of alemtuzumab were conducted by the French study group/GOELAMS.
The toxicity profile was comparable to toxicities observed with combination with a 28-day cycle of FCR for up to six cycles. CD23 study evaluating lumiliximab plus fludarabine, initiated a phase I/II open-label, dose-escalation, multicenter elevated antitumor effect when lumiliximab was combined with refractory CLL patients, suggested acceptable toxicity, but of a phase I study, including 46 previously treated and Lumiliximab is a primatized anti-CD23 antibody. Clinical data combinations using lumiliximab evaluation [52].

Interestingly, the percentage of observed grade 4 neutropenia was stable during FCR treatment (17.6% for cycle 1 and 17.9% for cycle 6) but increased during FCA treatment. Seven deaths all in the FCA arm were reported. Three of those occurred due to diffuse large B-cell lymphoma (one EBV positive), one patient died of mucormycosis, another patient died of septic shock due to Pseudomonas aeruginosa and two patients died of heart failure during neutropenia. The ORR in the first 100 patients was 96% in the FCR arm compared with 85% in the FCA arm (P = 0.086). The CR rate was 78% (FCR arm) versus 58% (FCA arm) (P = 0.072). These results confirm the observation that FCA treatment is associated with severe toxicity and can so far not be recommended for CLL therapy outside clinical trials. The first-line trial of the HOVON group compares FC treatment with FCR treatment for high-risk CLL patients; however, efficacy results are still pending.

The question of whether monoclonal antibodies may be used more effectively in combination was addressed by Faderl et al. [51]. The combination of alemtuzumab with rituximab for treatment of patients with lymphoid malignancies, including those with refractory/refractory CLL, produced an ORR of 52% [8% CR; 4% nodular PR (nPR), 40% PR]. To optimize dose, schedule and route of alemtuzumab, a study was designed exploring continuous intravenous infusion (c.i.v.) followed by s.c. alemtuzumab together with weekly i.v. rituximab in 40 patients with previously treated CLL. Approximately 64% of the patients included were fludarabine refractory. The ORR was 53% including seven patients (18%) who achieved a complete response (CR). The combination did not generate unexpected toxicities. CMV reactivations occurred in six patients (15%) and responded well to anti-CMV therapy. The combination of alemtuzumab plus rituximab has activity in some patients with recurrent/refractory CLL and needs to be investigated further in other settings of CLL, accompanied by pharmacokinetic evaluation [52].

**combinations using lumiliximab**

Lumiliximab is a primatized anti-CD23 antibody. Clinical data of a phase I study, including 46 previously treated and refractory CLL patients, suggested acceptable toxicity, but limited clinical activity [53]. Preclinical data demonstrated an elevated antitumor effect when lumiliximab was combined with fludarabine and rituximab. For this reason Byrd et al. [54] initiated a phase I/II open-label, dose-escalation, multicenter study evaluating lumiliximab plus fludarabine, cyclophosphamide and rituximab (L + FCR) for relapsed CD23+ B-cell CLL in 31 patients. Patients received either 375 mg/m² (n = 3) or 500 mg/m² (n = 28) lumiliximab in combination with a 28-day cycle of FCR for up to six cycles. The toxicity profile was comparable to toxicities observed with FCR alone. The ORR was 65% including 52% CR [53]. Based upon these data, a multicenter, global, randomized study of L + FCR versus FCR alone was conducted. An interim analysis of available data in the third quarter of 2009 led the sponsor to abandon the further clinical development of this drug in CLL, because the expected response rates were not achieved.

**new agents in CLL treatment**

**lenalidomide**

Lenalidomide, an immunomodulatory agent, has shown activity in CLL in the relapsed/refractory as well as in the untreated setting. In the original phase II study noting activity of lenalidomide Chanan-Khan [56] utilized a 25-mg daily dose of lenalidomide every 21 days of a 28-day cycle in 45 pretreated CLL patients. The dosing schedule was modified after 29 patients were enrolled to the study due to two patients having tumor lysis syndrome. The new schedule stipulated a dose escalation beginning at 5 mg/day and a target dose of 25 mg/day. The ORR was 47% including a CR in 9% of patients. The median PFS was 19.4 months. A subsequent study conducted by Ferrajoli et al. [57] enrolled 45 patients with relapsed CLL. The dosing started at 10 mg daily, followed by a 5-mg dose escalation every 28 days. The median dose administered due to toxicity at higher doses was 10 mg/day. ORR was 32% with 7% of patients achieving CR. Notably 13% of patients with del(17p) and 39% of patients with del(11q) responded to therapy. A phase I/II dose-defining trial is currently ongoing. Two trials of lenalidomide in previously untreated patients were presented at ASH 2008 [58, 59]. Both trials observed high toxicities, including tumor flare and tumor lysis syndrome, especially with higher doses. The ORRs ranged between 54% and 65%, so far no CR has been achieved.

A currently ongoing trial combines lenalidomide and rituximab. Sixty patients with relapsed CLL were enrolled by Ferrajoli et al. [60]. To date, 37 patients are evaluable for response. The ORR was 68%, no CR was achieved. The results obtained suggest that the combination of rituximab and lenalidomide is superior to single-agent lenalidomide. Especially interesting is the observation that there was no increase in toxicity and lenalidomide-associated tumor-flare reaction was even less frequent and less severe compared with lenalidomide alone.

The combination of lenalidomide with fludarabine and rituximab was investigated by Brown et al. [61] and Egle et al. [62]. In the phase I trial by Brown et al. [61] lenalidomide was administered concurrently with fludarabine and rituximab. The trial had to be closed due to significant myelotoxicity and idiosyncratic tumor flare. The protocol by Egle et al. [62] combines six cycles of fludarabine (40 mg/m²) p.o. on days 1–3 and rituximab and lenalidomide (375 mg/m² i.v. on day 4 in cycle 1 and 500 mg/m² i.v. on day 1 in cycles 2–6). Lenalidomide is administered at a starting dose of 2.5 mg daily (day 7–21 in cycle 1) with the dose being escalated to 25 mg/day from day 1 to day 21 of subsequent cycles. A maintenance therapy with lenalidomide and rituximab is planned for 6 months following the end of induction therapy. To date 10 previously untreated patients have been enrolled in the trial. Preliminary efficacy data show that all patients achieved at least a PR after two cycles of therapy, except for one patient with Richter transformation.
Fifty percent of patients had to be dose-limited due to not clearly dose-dependent skin and vascular toxicities. Ongoing trials are currently further assessing tolerability of dosing schedules, toxicity and different combinatory regimes of lenalidomide. To date, lenalidomide should be reserved for treatment in clinical trials.

**flavopiridol**
Flavopiridol, a synthetic flavon, inhibiting cyclin-dependent kinases, shows high activity in CLL patients with relapsed high-risk CLL [63, 64]. A phase II trial on relapsed CLL patients with genetically high-risk features achieved an ORR of 53%, including one CR [65]. Based upon those promising results a registration study for flavopiridol in relapsed CLL is currently being conducted in the United States and Europe.

**Bcl2 antagonists**
Oblimersen, an anti-Bcl2 antagonist, was added to the FC regimen and compared with FC in a randomized study on 241 pretreated patients [66]. Fludarabine 25 mg/m²/day plus cyclophosphamide 250 mg/m²/day were administered i.v. for 3 days with or without oblimersen 3 mg/kg/day as a 7-day continuous i.v. infusion (beginning 4 days before chemotherapy) for up to six cycles. CR/nPR was achieved in 20 (17%) of 120 patients in the oblimersen group and eight (7%) of 121 patients in the chemotherapy-only group (P = 0.025) (Table 3). Achievement of CR/nPR was correlated with both an extended TTP and survival (P < 0.0001). The OS and the PFS were improved in those patients that achieved at least a PR.

ABT-263, an orally bioavailable BH3 mimicetic that binds and inhibits several members of the Bcl2 family, is currently being evaluated in clinical trials. Marked thrombocytopenia seems to be the most significant toxicity observed.

**new monoclonal CD20 antibodies**
Ofatumumab is a fully humanized monoclonal CD20 antibody, which is currently being investigated in clinical trials. Ofatumumab is tolerated well. ORR with 2000 mg dosage was 50% [68]. In patients with fludarabine- and alemtuzumab-refractory CLL and patients with fludarabine-refractory CLL with bulky disease ORRs of 5% and 47%, respectively, were observed [69]. A phase II study evaluating the combination of ofatumumab (500 mg and 1000 mg) with FC in previously untreated CLL patients is currently ongoing. In the 500 mg dosing arm an ORR of 77% including a CR rate of 32% was achieved. Following administration of 1000 mg ofatumumab/cycle in combination with fludarabine and cyclophosphamide an ORR of 73% and CRs in 50% of patients were observed [70].

GA101 is the first humanized and glycoengineered type II monoclonal CD20 antibody investigated in clinical trials. Compared with rituximab GA101 demonstrates in vitro elevated antibody-dependent cytotoxicity as well as a markedly higher induction of direct cell death. In a phase I study by Morschhauser et al. [71] GA101 showed a comparable safety profile to rituximab. Of 13 treated high-risk CLL patients, eight responded to therapy, including one CR and seven PRs. GA101 is currently being investigated further as monotherapy in a phase II trial as well as in combination with chlorambucil in the GCLLSG CLL11 trial.

**future perspectives: great expectations?**
Future CLL research will need to address a multitude of important questions. CLL is predominantly a disease of the elderly often associated with a high comorbidity rate. New treatment options for those patients with good efficacy and low toxicity profile are needed. The CLL11 of the GCLLSG comparing chlorambucil with chlorambucil and monoclonal CD20 antibodies is addressing this question. Furthermore the place of novel and highly potent agents such as lenalidomide and flavopiridol within the treatment concept has to be determined. Several phase II and III trials evaluating these new treatment options and stratifying risk groups are currently ongoing.

The role of MRD as a treatment criterion for clinical trials and clinicians needs to be established. The results of several trials indicate that detectable MRD after therapy predicts...
relapse or shorter survival or PFS [32, 72–73]. To date the role of maintenance therapy has not been established. Results of a phase III trial showed improved PFS with alemtuzumab consolidation therapy compared with the observation arm (no progression versus 24.7 months; \( P = 0.036 \)) when calculated from the start of fludarabine-based treatment [72]. In a similar approach, an OR of 53% was achieved by an alemtuzumab consolidation therapy [39% at a 10-mg dose and 63% at a 30-mg dose (\( P = 0.066 \)) [75]. MRD was efficiently cleared from the bone marrow in most patients, with 38% of the patients achieving a molecular remission. Median time to disease progression had not yet been reached for patients who achieved MRD negativity, compared with 15 months for patients who still had residual disease after alemtuzumab consolidation treatment [75]. However, this approach may cause considerable myelotoxicity, lymphocytopenia and sometimes life-threatening infections, in particular if conventional doses of alemtuzumab are administered within 3–6 months after the last chemotherapy in patients with a low tumor load [76, 77]. MRD might be a tool to identify those patients profiting from a maintenance treatment and might possibly help to answer the question of how long maintenance treatment should be administered.

MRD might also be helpful as an objective end point in clinical trials. The quantitative assessment of MRD in 471 patients of the CLL8 trial receiving FC or FCR has provided additional insight into the clinical significance of MRD as assessed by four-color flow cytometry [78]. MRD levels <10\(^{-4}\) were correlated with longer PFS. The FCR regimen produced lower median MRD levels compared with FC, resulting in longer PFS.

Therefore, MRD assessment is recommended in clinical trials using standardized protocols of either four-color flow cytometry or allele-specific oligonucleotide PCR (with a sensitivity of one CLL cell per 10,000 leukocytes) [79]. These trials should assess the benefits and risks (toxicity) of therapies aimed at decreasing the number of CLL patients with detectable MRD levels, before this strategy can be recommended for general practice [41].

**Conclusion**

**First-line treatment**

With the increasing potential of newer chemoimmunotherapy combinations, selecting the right treatment for a patient with CLL requires a high degree of professional experience. Table 4 proposes an algorithm for the selection of therapies based on three potentially relevant points to consider:

- the physical condition (fitness and comorbidity) of the patient, which is independent of calendar age;
- the individual prognostic risk as determined by genetic and other prognostic factors;
- the Rai or Binet stage of the disease.

Patients at early stage (Binet A and B, Rai 0–II) without symptoms usually do not require therapy. The role of early treatment for patients with high-risk features is currently assessed in clinical trials. In patients with advanced (Binet C, Rai III–IV) or active, symptomatic disease, treatment should be initiated. In this situation, the physical condition of the patients has to be taken into account. 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) [80], should be offered an FCR combination therapy. Patients with relevant comorbidity (‘slow go’) may be offered either chlorambucil, bendamustine or a dose-reduced fludarabine-containing regimen for symptom control.

Patients with symptomatic disease and with del(17p) or p53 mutations respond poorly to fludarabine or FC.

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**Table 4. Proposal of an algorithm for first and second-line therapy of CLL.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Fitness</th>
<th>Molecular cytogenetic</th>
<th>First line treatment</th>
<th>Alternatives (partly tested in phase III trials)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic Binet A or B (Rai 0, I, II)</td>
<td>Irrelevant</td>
<td>Irrelevant</td>
<td>Standard</td>
<td>High-risk patients should only be treated in clinical trials</td>
</tr>
<tr>
<td>Binet C (Rai III–IV), or symptomatic disease (all stages)</td>
<td>Go Go</td>
<td>No del(17p) Del(17p)</td>
<td>FCR</td>
<td>BR, FR, FA</td>
</tr>
<tr>
<td></td>
<td>Slow Go</td>
<td>No del(17p)</td>
<td>CLB</td>
<td>CLB+R, CLB+GA101, B, dose reduced F, FC or FCR</td>
</tr>
<tr>
<td>Relapse</td>
<td>Fitness</td>
<td>Molecular cytogenetic</td>
<td>Relapse therapy</td>
<td>Alternatives</td>
</tr>
<tr>
<td>Early (&lt;1 year) = refractory disease</td>
<td>Go go</td>
<td>No del(17p) Del(17p)</td>
<td>A or FA followed by Allo SCT</td>
<td>BR, flavopiridol, lenalidomide</td>
</tr>
<tr>
<td>Slow go</td>
<td>No del(17p) Del(17p)</td>
<td>A</td>
<td>A or FA followed by Allo SCT</td>
<td>Flavopiridol, lenalidomide</td>
</tr>
<tr>
<td>Late (&gt;1 year)</td>
<td>Go go and Slow go</td>
<td>Repeat first line</td>
<td>A</td>
<td>BR, lenalidomide</td>
</tr>
</tbody>
</table>

A, alemtuzumab; Allo SCT, allogeneic stem cell transplantation; B, bendamustine; C, cyclophosphamide; Clb, chlorambucil; F, fludarabine; GA101, novel anti-CD20 antibody; R, rituximab.
alemtuzumab monotherapy or FCR a response can be achieved in up to 50% of those patients. However, those responses are usually of short duration and only last a few months up to 1.5 years. For this reason it is advisable to treat these patients within experimental protocols and offer allogeneic stem cell transplant whenever possible.

**second-line treatment**

While an extensive review of all treatment options of relapsed or refractory CLL is beyond the scope of this paper, Table 4 summarizes some principles of the management of patients at relapse according to the duration of remission and their physical fitness. A general rule is that first-line treatment may be repeated, if the duration of the first remission exceeds 12 months (or 24 months after FCR or similar potent chemoimmunotherapy). Treatment decisions become more difficult and limited in treatment-refractory CLL (as defined by an early relapse within 6 months after the last treatment) or in cases with the chromosomal aberration del(17p). Here the principle rule is that the initial regimen should be changed. The following treatment options exist:

- altemuzumab alone or in combination [15, 50];
- flavopiridol (if available or in clinical trials) [64];
- lenalidomide (if available or in clinical trials) [56];
- allogeneic stem cell transplantation with curative intent [81].

The decision for one of these options is strongly dependent on the physical condition of the patient. According to recent recommendations of an EBMT consensus group, physically fit patients with refractory CLL or with del(17p) should be offered allogeneic transplantation, since their prognosis has remained extremely poor with conventional therapies [81]. Finally, it is important to emphasize that patients with refractory disease should be treated within clinical trials whenever possible.

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