Treatrment of Fludarabine-refractory Chronic Lymphocytic Leukemia

Apostolia-Maria Tsimberidou, MD, PhD1 and Michael J. Keating, MB, BS2

The development of resistance to purine analogs defines a poor-risk subset of patients with chronic lymphocytic leukemia (CLL). Although in recent years chemoimmunotherapeutic combinations such as fludarabine, cyclophosphamide, and rituximab have induced response rates of 95% in previously untreated patients and increased the rates of failure-free survival, CLL remains incurable for many patients because of a lack of disease response or the development of refractoriness to fludarabine. Fludarabine-refractory disease is defined as CLL that does not respond to fludarabine or that recurs within 6 months of treatment with a fludarabine-containing regimen. The natural course of the disease is associated with poor survival. Salvage therapeutic strategies include alemtuzumab-containing regimens, targeted agents, and allogeneic stem cell transplantation. Single-agent alemtuzumab induces response in up to 40% of patients with fludarabine-refractory CLL, but responses are not durable, and the median survival is approximately 1 to 2 years. Alemtuzumab is also combined with fludarabine, cyclophosphamide, and/or rituximab, and other agents such as lenalidomide and flavopiridol, as well as targeted agents, and used in fludarabine-refractory CLL. Cumulative evidence suggests that allogeneic stem cell transplantation is an efficacious therapeutic strategy for patients who do not respond to fludarabine or who develop disease recurrence within 12 months after purine analog treatment. In conclusion, chemoimmunotherapy regimens that include alemtuzumab and/or rituximab and allogeneic stem cell transplantation improve the prognosis of this disease, but there is a continued need for novel, more effective therapies. Cancer 2009;115:2824–36. © 2009 American Cancer Society.

KEY WORDS: chronic lymphocytic leukemia, fludarabine refractory, alemtuzumab, rituximab, lenalidomide.

Resistance to purine analogs is a challenging issue in the therapeutic management of patients with B-cell chronic lymphocytic leukemia (CLL). Treatment of CLL is usually deferred until indications for therapeutic intervention are present.1 When patients do require treatment, they are typically treated with purine analogs, most commonly fludarabine. In untreated patients, single-agent fludarabine induced response in 63% to 80% (complete remission [CR], 20% to 38%) of patients.2,3 The median time to disease progression was reported to be 31 months in 1 study,2 and the progression-free survival was 20 months in the other study.3 With fludarabine and cyclophosphamide combination therapy, the response rates ranged from 74% to 94% (CR, 23% to 35%),4 and the median progression-free survival was 32 to 48 months.5,6 Fludarabine, cyclophosphamide, and rituximab (FCR) combination therapy induced an overall response (OR) rate of 95% (CR, 70%), and the 5-year disease-free survival rate was 70%.7,8
In contrast to the encouraging clinical outcomes of patients with fludarabine-sensitive CLL, the prognosis of patients with fludarabine-refractory CLL is poor. Several factors predict a poor response to purine analogs. Deletion of the \( p53 \) gene is the strongest independent adverse prognostic factor for survival, and it is associated with the shortest median treatment-free interval and the shortest survival compared with other cytogenetic groups in patients with CLL. Other factors predicting poor clinical outcomes include unmutated immunoglobulin heavy chain variable region gene (\( IGHV \)) and expression of ZAP-70. Patients with unmutated \( IGHV \) genes have been reported to express higher proportions of CD38 B-CLL cells, and patients with both unmutated \( IGHV \) genes and \( >30\% \) CD38 have lower rates of response to chemotherapy (including fludarabine) and of survival compared with patients with mutated \( IGHV \) genes and \(<30\% \) CD38. The adverse prognostic significance of CD38 reported by other investigators has not been confirmed in our series. Other investigators have reported that patients with CLL who were treated with fludarabine and rituximab with unmutated \( IGHV \) or high-risk interphase cytogenetics (del[17p] or del[11q]) appeared to have a shorter duration of progression-free survival and overall survival. In patients treated with fludarabine or fludarabine plus cyclophosphamide, 17p or 11q deletion was associated with reduced progression-free survival, but did not appear to affect the response rates. \( IGHV \) mutational status, CD38 expression, and ZAP-70 status did not predict outcome of fludarabine-based chemotherapy. In contrast, in our series 11q deletion was associated with favorable outcomes in patients treated with fludarabine and rituximab combination therapies.

Fludarabine-refractory CLL is defined as CLL with a response less than a partial remission (PR) to a fludarabine-based regimen or a remission lasting <6 months on discontinuation of treatment with a fludarabine-based regimen. Most of these patients have already been exposed to and have disease that is refractory to alkylating agents. This review focuses on treatments for patients with fludarabine-refractory CLL.

**Clinical Outcomes With Salvage Chemotherapy**

In a retrospective review of 147 patients with fludarabine-refractory CLL, Keating et al. reported a 22% response (CR plus PR) rate to the first salvage regimen and a median survival of 10 months. The response rate was highest in the purine analog and alkylating agent combination therapy group (37%; 16 of 43 patients). The response rates for alkylating agent-refractory, alkylating agent-native, and alkylating agent-sensitive CLL were 18%, 22%, and 40%, respectively; the respective survival rates were 8 months, 10 months, and 14 months. As expected, patients who responded lived longer than nonresponders. Among patients treated with a second salvage regimen, the best response rates were noted in patients who underwent stem cell transplantation (6 of 9 patients responded) and in those who received alemtuzumab (4 of 6 patients responded).

In patients with fludarabine-refractory CLL, the response rate to fludarabine and cyclophosphamide combination therapy was 39% (CR, 3%). With fludarabine and mitoxantrone combination therapy, the response rate was 25% in patients with fludarabine-refractory disease. Fludarabine, cyclophosphamide, and mitoxantrone induced a response in 78% (CR, 50%) of patients with resistant or recurrent CLL.

**Alemtuzumab**

Alemtuzumab (Campath-1H), an anti-CD52 humanized monoclonal antibody, has been extensively investigated in CLL (Table 1). It has significant antileukemic activity, which is higher in the blood, bone marrow, and spleen and lower in lymph nodes. The US Food and Drug Administration has approved alemtuzumab for patients with B-cell CLL who have been treated with alkylating agents and for whom fludarabine therapy has failed.

In a pivotal multicenter clinical trial, patients from 21 centers in the US and Europe were treated with alemtuzumab. The study objective was to assess the OR rate, using the 1996 National Cancer Institute working group (NCI-WG) criteria, in patients with B-cell CLL who had received an alkylating agent and in whom fludarabine treatment had failed. Fludarabine failure was defined as...
<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen and Schedule</th>
<th>No. of Patients</th>
<th>Fludarabine-refractory, %*</th>
<th>OR, %</th>
<th>CR, %</th>
<th>Median PFS, mo</th>
<th>Median Survival, mo</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keating 2002</td>
<td>Alemtuzumab, 30 mg 3 times wkly × 12 wk</td>
<td>93</td>
<td>48</td>
<td>33</td>
<td>2</td>
<td>5</td>
<td>16</td>
<td></td>
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<tr>
<td>Rai 2002</td>
<td>Alemtuzumab, 30 mg 3 times wkly × 16 wk</td>
<td>24</td>
<td>71</td>
<td>33</td>
<td>0</td>
<td>7</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Lozanski 2004</td>
<td>Alemtuzumab, 30 mg 3 times wkly × 12 wk</td>
<td>36</td>
<td>81</td>
<td>31</td>
<td>6</td>
<td>Remission duration, 10</td>
<td>NR</td>
<td>Response, 40% (6/15 patients) if p53 mutations/deletions vs 19% (4/21 patients) if other</td>
</tr>
<tr>
<td>Moreton 2005</td>
<td>Alemtuzumab, 30 mg 3 times wkly until maximum response</td>
<td>91</td>
<td>50</td>
<td>53</td>
<td>35</td>
<td>NR</td>
<td>20% of patients achieved MRD negativity</td>
<td></td>
</tr>
<tr>
<td>O’Brien 2001</td>
<td>Rituximab, escalating dose 375-2250 mg/m² wkly × 4 wk</td>
<td>40</td>
<td>53</td>
<td>36</td>
<td>0</td>
<td>8</td>
<td>80% at 1 y</td>
<td>Survival data include 10 patients with other lymphoid malignancies</td>
</tr>
<tr>
<td>Byrd 2001</td>
<td>Rituximab, 100-375 mg/m² 3 times wkly × 4 wk</td>
<td>33</td>
<td>52</td>
<td>52 (45%, intent-to-treat)</td>
<td>3</td>
<td>In responders, 11</td>
<td>NR</td>
<td>6 patients were previously untreated</td>
</tr>
<tr>
<td>Huhn 2001</td>
<td>Rituximab, 375 mg/m² wkly × 4 wk</td>
<td>30</td>
<td>77</td>
<td>25 (23%, intent-to-treat)</td>
<td>0</td>
<td>16</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Itala 2002</td>
<td>Rituximab, 375 mg/m² wkly × 4 wk</td>
<td>24</td>
<td>75</td>
<td>35</td>
<td>0</td>
<td>3</td>
<td>NR</td>
<td>&gt;50% decrease in the size of LN in 17 (85%) of 20 patients vs 2 (11%) of 18 with bone marrow involvement</td>
</tr>
<tr>
<td>Faderl 2003</td>
<td>Alemtuzumab (30 mg 3 times wkly × 4-8 wk) plus concurrent rituximab (375 mg/m² wkly × 4 wk)</td>
<td>32</td>
<td>54</td>
<td>63</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>Survival data include 15 patients with other lymphoid malignancies</td>
</tr>
<tr>
<td>Chanan-Khan 2006</td>
<td>Lenalidomide, 25 mg/d orally × 21 d every 28 d</td>
<td>45</td>
<td>51</td>
<td>47</td>
<td>9</td>
<td>81% at 1 y</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Byrd 2007</td>
<td>Flavopiridol, 60-80 mg/m² wkly × 46 wk</td>
<td>42</td>
<td>83</td>
<td>45</td>
<td>0</td>
<td>13</td>
<td>NR</td>
<td>Phase 2 results are reported</td>
</tr>
<tr>
<td>O’Brien 2005</td>
<td>Oblimersen sodium, 3 mg/kg/d × 5 d (Cycle 1) or × 7 d (subsequently) every 3 wk</td>
<td>26</td>
<td>69</td>
<td>8</td>
<td>0</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>O’Brien 2007</td>
<td>Fludarabine (25 mg/m²/d, Day 1-3) plus cyclophosphamide (250 mg/m²/d, Day 1-2) with vs without oblimersen sodium (3 mg/kg/d × 7 d, from d -3 to 3)</td>
<td>120 vs 121</td>
<td>57 vs 59</td>
<td>17 vs 7</td>
<td>9 vs 3</td>
<td>Time to disease progression, 6 vs 9 (NS)</td>
<td>Survival benefit with 3-drug arm in fludarabine-sensitive patients (P = .05)</td>
<td></td>
</tr>
<tr>
<td>Itala 2002</td>
<td>Rituximab, 375 mg/m² wkly × 4 wk</td>
<td>24</td>
<td>35</td>
<td>0</td>
<td>3</td>
<td>NR</td>
<td>&gt;50% decrease in LN in 17 (85%) of 20 patients vs 2 (11%) of 18 with bone marrow infiltration</td>
<td></td>
</tr>
</tbody>
</table>

CLL indicates chronic lymphocytic leukemia; OR, overall response; CR, complete remission; PFS, progression-free survival; NR, not reported; MRD, minimal residual disease; (−), negative; (+), positive; PR, partial remission; LN, lymph nodes; NS, not significant (difference).

* Fludarabine-refractory CLL is defined as CLL that does not respond to fludarabine or progresses within 6 months of treatment.
failure to achieve PR or CR to at least 1 fludarabine-containing regimen, disease progression while receiving fludarabine treatment, or disease progression in responders within 6 months of the last dose of fludarabine.

Alemtuzumab was administered intravenously at doses that increased gradually; the target dose was 30 mg, 3 times weekly, for a maximum of 12 weeks. Infection prophylaxis was mandatory, from Day 8 until a minimum of 2 months after treatment was completed. Responses were assessed at Weeks 4, 8, and 12, and patients were observed for 34 months. Ninety-three patients were treated. The median age of the patients was 66 years (range, 31-86 years), and the median number of prior therapies was 7. Twenty-four percent of patients had Rai stage 0-II, 17% had Rai stage III, and 59% had Rai stage IV disease. The OR rate in an intent-to-treat analysis was 33% (CR, 2%; PR, 31%) (Table 1). Alemtuzumab induced response in the blood in 83% and in the bone marrow in 26% of patients. The median time to response was 1.5 months (range, 0.4-3.7 months), and the median time to disease progression for all patients was 4.7 months. Sixty-six patients died, and the median overall survival duration was 16 months (95% confidence interval [95% CI], 11.8-21.9). Among responders, the median time to progression was 9.5 months, and the median survival duration was 32 months. Clinical benefit was observed both in responders and in patients with stable disease. The most common adverse events were transient injection-site reactions, generally grade 1 to 2, which were usually observed during the 1st week. Grade 3 to 4 infections were reported in 25 patients (27%), but only 3 (10%) of the 31 patients who responded to alemtuzumab treatment developed grade 3 to 4 infections. This study demonstrated that alemtuzumab increases the median survival of patients with fludarabine-refractory CLL compared with historical controls.22

Similar results were reported by Stilgenbauer et al., who administered alemtuzumab subcutaneously at 30 mg three times weekly.23 Overall, 109 patients, who had received a median of 3 prior therapies, were treated. The response rate was 33% (CR, 4%), and the median progression-free survival and overall survival durations were 7.7 months and 19.1 months, respectively. Among patients with del11q and del17p cytogenetic abnormalities, the response rates were 39% and 24%, respectively. Responses (CR or PR) were observed in 22% of unmutated immunoglobulin heavy chain variable region (IgVH), 24% of 11q del, 39% of 17p del, and 33% of mutated p53 cases. Progression-free survival and overall survival did not differ significantly among the genetic subgroups, particularly mutated p53, 11q del, and 17p del. Alemtuzumab was administered on an outpatient basis, and a temporary interruption was required in 68 patients because of neutropenia (43%), anemia (6%), thrombocytopenia (3%), or infections (40%; cytomegalovirus [CMV] reactivations 30%). Treatment was discontinued early in 63 patients because of insufficient response (44%), hematotoxicity (16%), infection (17%), or CMV reactivation (13%). The most common toxicities included hepatotoxicity, hematologic toxicities (grade 3-4 anemia, 42%; thrombocytopenia, 52%; and neutropenia, 54%), and infections (grade 3-4). With a median follow-up time of 21.4 months, the investigators reported 56 deaths because of disease progression (52%), infections (39%), or non–CLL-related factors (9%). This study confirmed that alemtuzumab had activity in patients with high-risk CLL, including those with unmutated IgVH, 11q del, or 17p del.23

Other phase 2 clinical trials have demonstrated that alemtuzumab therapy results in favorable clinical outcomes (Table 1).24,25 In a phase 2 clinical trial, patients previously treated with fludarabine and other chemotherapy regimens received alemtuzumab at 6 centers in the US.24 Alemtuzumab was administered at a target dose of 30 mg over 2 hours, 3 times weekly, for up to 16 weeks. Twenty-four patients were treated, and responses were evaluated by an independent panel of experts using 1996 NCI-WG criteria. In contrast to the aforementioned study, antimicrobial prophylaxis was not mandatory. The OR rate was 33% (all PRs), and the median time to response was 4 months (range, 2-5 months). The median duration of response was 15 months (range, 5 to ≥38 months), and the median time to disease progression was 20 months (range, 8 to ≥42 months). The median survival duration was 36 months (range, 9 to ≥47 months). Acute infusion-related events, mainly grade 1 to 2, which were more common and severe during the first week, were also noted. Eight nonresponders and 2 responders developed major infections. Pneumocystis carinii (or Pneumocystis jiroveci) pneumonia (PCP) was reported in 2 patients who had not received PCP prophylaxis. The median CD4⁺ and CD8⁺ counts decreased and started recovering.
at the end of the study, with further recovery noted after 1 month of follow-up. This study confirmed the antileukemic activity of alemtuzumab in patients with CLL previously treated with fludarabine and demonstrated that alemtuzumab protocols should include mandatory prophylaxis because of a lymphopenia-associated high incidence of opportunistic infections.24

Intravenous alemtuzumab also induces response in patients with fludarabine-refractory CLL bearing del17p or mutated p53.25-27 CLL with these cytogenetic characteristics is typically resistant to chemotherapy.9 In 1995, Dohner et al. reported that none of 12 patients with a p53 deletion detected by fluorescence in situ hybridization responded to fludarabine or pentostatin, compared with 20 (56%) of 36 patients without the deletion (P < .001). Deletion p53 was also an independent factor predicting shorter survival in multivariate analysis.9

Some investigators suggest that CLL with p53 mutations or deletions may have higher response rates to alemtuzumab than CLL without these abnormalities.25 Among 36 patients with fludarabine-refractory CLL treated with alemtuzumab, response rates were higher in patients with p53 mutations and/or deletions (40%), compared with others (19%), and the median response duration for the subset of patients with p53 abnormalities was 8 months (range, 3-17 months).25

**Alemtuzumab and Rituximab**

Alemtuzumab has been evaluated in combination with rituximab in recurrent/refractory CLL.36,37 At our institution, concurrent alemtuzumab and rituximab therapy induced responses in 63% of patients (Table 1). The most common toxicities were infections and infusion-related reactions. CMV antigenemia was noted in 27% of the patients, but only 15% were symptomatic and required therapy.36

**Rituximab**

Rituximab (IDEC-C2B8) is a chimeric antibody that binds to the B-cell surface antigen CD20. Although highly effective in follicular lymphoma, with an OR rate of 60%, single-agent rituximab has inferior clinical activity in B-cell CLL, where it is less effective for clearing disease from the bone marrow and has a short response duration.28-32 Single-agent rituximab induces responses in 23% to 45% (CR, 0%-3%) of previously treated CLL patients (Table 1).28-30,32 A phase 2 study demonstrated a dose-response relationship, as response rates increased with increasing rituximab dose.28 Response rates (all PRs) increased from 22% at a dose of 500 to 825 mg/m² to 43% at a dose of 1000 to 1500 mg/m² to 75% at the highest dose of 2250 mg/m² (P = .007).28 In another study, rituximab was administered at a dose of 375 mg/m² weekly for 4 consecutive weeks as a first-line therapy in CLL and was repeated at 6-month intervals.31 Overall, 44 patients were treated and the response rate after the first course of rituximab was 51% (CR, 4%); after the completion of ≥1 additional courses (28 patients), the OR rate was 58% (CR, 9%). However, the progression-free survival duration of 18.6 months was inferior to that reported with first-line plus maintenance rituximab in patients with follicular lymphoma.31 Rituximab has been used in combination with granulocyte-macrophage–colony–stimulating factor (GM-CSF) on the basis of in vitro33 and clinical data in indolent lymphoma demonstrating increased antitumor activity when combined with GM-CSF.34 This regimen resulted in a 79% response rate (CR, 36%) in patients with indolent lymphoma,34 but in those with CLL who were aged ≥70 years, response rates were 61% (CR, 7%).35

**Lumiliximab**

Lumiliximab (IDEC-152) is an immunoglobulin (Ig) G1 macaque-human anti-CD23 monoclonal antibody. In a phase 1 study in patients with CLL, single-agent lumiliximab was administered at doses ranging from 125 mg/m² weekly to 500 mg/m² 3 times weekly, for 4 weeks.38 Overall, 25 patients with fludarabine-refractory CLL were treated. Lumiliximab-induced adverse events were headache, constipation, nausea, and cough. A decrease in lymphocyte counts was noted in 91% (42 of 46) of all patients and in 59% of patients with baseline lymphadenopathy, but no confirmed responses were noted.38 On the basis of these observations, a phase 1-2 study of lumiliximab and FCR combination therapy was initiated in recurrent CD23⁺ B-cell CLL. Lumiliximab was administered at a dose of 375 mg/m² or 500 mg/m² in combination with FCR every 4 weeks.39 Subsequently, results of this study led to an ongoing multicenter, global, randomized study of lumiliximab and FCR versus FCR alone.40

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### Table 2. Selected Targeted Therapies in CLL

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Route of Administration</th>
<th>Overall Response in Previously Treated CLL, %</th>
<th>Key Toxicities</th>
<th>Ongoing/Planned Use</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>iv, sc</td>
<td>31-53 (CR, 0-35)</td>
<td>Immunosuppression, CMV reactivation</td>
<td>Combination with cytotoxics (CFAR, etc), MRD eradication, 17p deletion</td>
<td>CMV monitoring and prophylaxis; activity in CLL with 17p del</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>iv</td>
<td>52-77 (CR, 0-3)</td>
<td>Infusion-related events</td>
<td>Combination with cytotoxics or other targeted therapies</td>
<td></td>
</tr>
<tr>
<td>Ofatumumab (HuMax-CD20)</td>
<td>CD20 novel epitope</td>
<td>iv</td>
<td>50 (CR, 0)</td>
<td>Infusion-related events</td>
<td>Phase 3 pivotal study in recurrent CLL after fludarabine failure; phase 2 studies in combination with fludarabine, cyclophosphamide in untreated CLL</td>
<td>HuMax-20 kills rituximab-resistant cells and fresh CLL cells expressing low levels of CD20</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>Immunomodulatory down-regulation of cytokines (TNF, VEGF, IL-6, etc); activation of immune effector cells</td>
<td>Oral</td>
<td>32-47 (CR, 5-9)</td>
<td>Fatigue, thrombocytopenia, neutropenia</td>
<td>Ongoing phase 2 study in recurrent/refractory CLL; phase 2-3 study of 2 lenalidomide dose regimens in recurrent or refractory CLL</td>
<td></td>
</tr>
<tr>
<td>Lumiliximab</td>
<td>CD23</td>
<td>iv</td>
<td>0 (decrease in ALC and lymphadenopathy)</td>
<td>Headache, GI toxicity</td>
<td>Ongoing phase 2/3 randomized international study of FCR with or without lumiliximab</td>
<td>May enhance activity of FCR</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>Cyclin-dependent kinase; p53 independent</td>
<td>iv</td>
<td>45%</td>
<td>Hyperacute tumor lysis syndrome</td>
<td>Ongoing phase 2 in previously treated CLL; ongoing phase 1 as consolidation after cytoreductive therapy</td>
<td>Other phase 1 or 2 studies are ongoing to optimize dose and schedule</td>
</tr>
<tr>
<td>Oblimersen</td>
<td>BCL-2</td>
<td>iv</td>
<td>8% (CR, 0)</td>
<td>Cytokine release syndrome, fatigue, gastrointestinal toxicity</td>
<td>Ongoing phase 1-2 in combination with rituximab in previously treated CLL</td>
<td>Phase 3 randomized trial of FG ± oblimersen demonstrated superior rates of CR and nodular CR85</td>
</tr>
<tr>
<td>Gossypol</td>
<td>Bcl-2 homology 3 of BCL-2 antagonists</td>
<td>Oral</td>
<td>0 (decrease in ALC and lymphadenopathy in untreated CLL, phase 1)</td>
<td>Gastrointestinal toxicity, fatigue, neutropenia</td>
<td>Ongoing phase 1 as single agent in lymphoproliferative disorders; ongoing phase 1-2 in combination with rituximab in recurrent/refractory CLL</td>
<td>Gossypol + rituximab; PR, 42%</td>
</tr>
<tr>
<td>CNF1010 (17-AAG)</td>
<td>Heat shock protein 90</td>
<td>iv</td>
<td>Too early</td>
<td></td>
<td>Phase 1 in ZAP-70–positive CLL; phase 1, 17-AAG ± rituximab in recurrent CLL</td>
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</tr>
<tr>
<td>CNF 2024</td>
<td>Heat shock protein 90</td>
<td>Oral</td>
<td>Too early</td>
<td></td>
<td>Ongoing phase 1 in recurrent CLL</td>
<td></td>
</tr>
</tbody>
</table>

CLL indicates chronic lymphocytic leukemia; iv, intravenously; sc, subcutaneously; CR, complete remission; CMV, cytomegalovirus; CFAR, cyclophosphamide, fludarabine, alemtuzumab, and rituximab; MRD, minimal residual disease; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; IL-6, interleukin-6; ALC, absolute lymphocyte count; GI, gastrointestinal; FCR, fludarabine, cyclophosphamide, and rituximab; PR, partial remission.
**Ofatumumab**

Ofatumumab (HuMax CD20), a monoclonal antibody that targets a different epitope of CD20 than that targeted by rituximab, is believed to kill rituximab-resistant cells (Table 2). Results of a phase 1/2 clinical trial of ofatumumab in recurrent/refractory CLL were encouraging. The maximum tolerated dose was not reached, and the most common toxicities were infusion reactions. These results led to an ongoing international study of ofatumumab for patients with CLL that failed to respond to treatment with fludarabine and alemtuzumab or patients with disease that failed to respond to fludarabine and who were unable to tolerate or ineligible to receive alemtuzumab.

**High-dose Methylprednisolone**

High-dose methylprednisolone-containing therapy also has activity in fludarabine-refractory CLL, including CLL with a p53 deletion (5 of 10 patients responded). In combination with rituximab, high-dose methylprednisolone induces high rates of OR and CR (in a study of 14 patients, 93% and 36%, respectively) in patients with fludarabine-refractory CLL.

**Lenalidomide**

Immunomodulating drugs such as lenalidomide are believed to exert their antileukemic activity via pleiotropic immunomodulatory mechanisms. These agents decrease the production of cytokines, including tumor necrosis factor-α, vascular endothelial growth factor, and interleukin-6. Lenalidomide also modulates an immune effector cell response through activation of T cells and natural killer cells and directly induces apoptosis in tumor cells. Phase 2 clinical trials demonstrated that lenalidomide is clinically active in CLL.

Treatment with lenalidomide at a dose of 25 mg orally on Days 1 through 21 of a 28-day cycle demonstrated that among 23 patients with fludarabine-refractory CLL, 7 (30%) patients responded to lenalidomide (CR in 1 patient and PR in 6 patients). In our institution, lenalidomide was administered starting at a dose of 10 mg daily, followed by titration upward in 5-mg increments every 28 days to a maximum daily dose of 25 mg. Three (7%) patients achieved a CR, 1 a nodular partial remission, and 10 a PR, for an OR rate of 32%. Twelve patients with fludarabine-refractory CLL were treated, and 3 (25%) patients responded to lenalidomide, compared with a response rate of 38% in 32 patients with fludarabine-sensitive disease. Treatment with lenalidomide was associated with an OR rate of 31% in patients with 11q or 17p deletions, 24% in patients with unmutated IgVH, and 25% in patients with fludarabine-refractory disease. Adverse events included fatigue, thrombocytopenia, neutropenia, and gastrointestinal toxicities. Lenalidomide in combination with rituximab is currently being investigated in recurrent or refractory CLL.

**Flavopiridol**

Flavopiridol (alvocidib) is a broad cyclin-dependent kinase inhibitor that induces apoptosis in leukemic cell lines and in human CLL cells in vitro. Its activity is p53 independent, and it also decreases the expression of the proteins Mcl-1 and XIAP, which mediate resistance to apoptosis in CLL cells. When administered as a continuous infusion, flavopiridol had no activity, but the response rate was 11% when it was given as a 1-hour bolus in patients with recurrent CLL. When administered in 3 cohorts (cohort 1, 30 mg/m² loading dose followed by 30 mg/m² 4-hour infusion; cohort 2, 40 mg/m² loading dose followed by 40 mg/m² 4-hour infusion; and cohort 3, cohort 1 dose for treatments 1 to 4, and then a 30 mg/m² loading dose followed by a 50 mg/m² 4-hour infusion), flavopiridol resulted in a 45% PR rate, with a median survival duration that exceeded 12 months. These responses included 5 (42%) of 12 patients with del 17p and 13 (72%) of 18 patients with del 11q. The dose-limiting toxicity was hyperacute tumor lysis syndrome. Although this study suggests that flavopiridol has activity in fludarabine-refractory CLL, the investigators suggested that it be used mainly for the elimination of minimal residual disease after cytoreductive therapy.

**Gossypol**

Gossypol (AT-101) is a small molecule that mimics the inhibitory Bcl-2 homology 3 domain of endogenous antagonists of the Bcl-2 family antiapoptotic proteins (Bcl-2, Bcl-XL, Bcl-W, and Mcl-1). Gossypol induces apoptosis and enhances the cytotoxicity of rituximab in CLL cells. In a phase 2 study, gossypol and rituximab...
combination therapy was investigated in recurrent/refractory CLL. Gossypol was administered at 30 mg/d for 21 or 28 days for 3 28-day cycles. Rituximab was administered at 375 mg/m² on Days 1, 3, 5, 8, 15, 22, 29, 31, 33, 40, 57, 59, and 61. Preliminary results demonstrated that gossypol and rituximab induced a PR in 42% (5 of 12) of patients, with improved lymphocyte counts, splenomegaly, and/or lymph node size. The most frequent toxicities were gastrointestinal effects, fatigue, and neutropenia. The study was ongoing at the time of last follow-up, with different gossypol schedules being tested in an attempt to improve activity and reduce toxicity.

**Obatoclax**

Obatoclax (GX15-070) is a synthetic small molecule that inhibits the binding of the antiapoptotic properties of Bcl-2, Bcl-\(_{XL}\), Bcl-\(_{w}\), and Mcl-1 to the proapoptotic proteins Bax and Bak, and is believed to reinstate programmed cell death in transformed cells. In a phase 1 trial in CLL, the main adverse events were drowsiness and euphoria. Although the clinical activity of this agent was limited (1 unconfirmed PR and 7 cases of stable disease of 15 patients), improvement in thrombocytopenia was noted in some patients with baseline cytopenias.

**Heat Shock Protein 90 Inhibitors**

Heat shock protein 90 (Hsp90) is a ubiquitous molecular chaperone that is necessary for the expression and activity of the tyrosine kinase ZAP-70, which is expressed aberrantly in 45% of patients with B-cell CLL, particularly those with unmutated B-cell receptor genes, and has been associated with adverse prognosis. In vitro studies demonstrated that the Hsp90 inhibitor 17-allyl-amino-demethoxy-geldanamycin induces ZAP-70 degradation and apoptosis in CLL lymphocytes. Another Hsp90 inhibitor, CNF2024 (CF1983 mesylate), is currently being investigated in patients with recurrent CLL or intolerance to purine analog−based therapy (Table 2).

**Talabostat**

Talabostat is an oral small molecule inhibitor of dipeptidyl peptidases, such as CD26 and fibroblast activation protein, which is expressed in bone marrow, lymph nodes, and the stroma of solid tumors and induces cytokine and chemokine expression in lymph nodes and the spleen. On the basis of its enhancement of the activity of rituximab in patients with B-cell malignancies, talabostat and rituximab combination therapy was explored in a phase 2 study in patients with CLL who had failed to respond to fludarabine and/or rituximab therapy. Rituximab was administered at a dose of 375 mg/m² on Days 1, 8, 15, and 22, and talabostat was given at a dose of 300 mg orally twice daily for 6 days after each rituximab infusion. Toxicities were similar to those associated with rituximab alone, with the exception of edema, which occurred in 25% of patients. The OR rate was 22% (8 of 36 patients), and the median response duration was 5 months. However, these response rates appeared to be lower or equal to those achieved with single-agent rituximab.

**SNS-032**

SNS-032, a selective inhibitor of cyclin-dependent kinases 2, 7, and 9, which are involved in cell cycle regulation and regulate RNA polymerase II−dependent transcription, is being investigated in an ongoing phase 1 study in patients with CLL and multiple myeloma. Seventeen patients with CLL have been treated with total doses of 15 to 100 mg/m². Although no drug-related dose-limiting toxicities (DLTs) were observed through the 50-mg/m² dose cohort, tumor lysis syndrome was noted in all CLL patients treated at a dose of 75 mg/m², and 1 patient experienced a DLT of vascular leak syndrome. One patient treated at a dose of 100 mg/m² experienced tumor lysis syndrome and a DLT of transaminitis, but no objective responses have been observed.

**Chemoimmunotherapy**

On the basis of the observation that rituximab enhances the cytotoxicity of both fludarabine and cyclophosphamide, Keating et al. studied FCR in the treatment of CLL. The OR rate in patients with recurrent or refractory CLL was 73% (CR, 32%). The superiority of FCR compared with fludarabine and cyclophosphamide combination therapy in prolonging progression-free survival was shown in the REACH (Rituximab in the study of relapsed Chronic lymphocytic leukemia) trial, a multicenter, randomized, open-
label, international phase 3 study that enrolled 552 patients with recurrent or refractory CD20⁺ CLL.\textsuperscript{58} Progression-free survival was defined as the length of time from the date of treatment randomization to the time of disease progression, recurrence, or death from any cause; it was 30.6 months for the FCR arm and 20.6 months in the fludarabine and cyclophosphamide combination therapy arm (\(P = .0002\)).\textsuperscript{58}

Another regimen with activity against fludarabine-refractory CLL is FCR combined with the monoclonal antibody alemtuzumab (CFAR) in patients with previously treated CLL.\textsuperscript{59} CFAR consists of cyclophosphamide at a dose of 250 mg/m\(^2\) on Days 3 to 5; fludarabine at a dose of 25 mg/m\(^2\) on Days 3 to 5; intravenous alemtuzumab at a dose of 30 mg/m\(^2\) on Days 1, 3, and 5; and rituximab at a dose of 375 to 500 mg/m\(^2\) on Day 2, every 4 weeks. Among 33 patients with fludarabine-refractory CLL treated with CFAR, the OR rate was 58\% (CR, 6\%; nodular PR, 9\%; and PR, 42\%). However, in logistic regression multivariate analysis, fludarabine-refractory disease was an independent factor predicting treatment failure in this study.\textsuperscript{59}

Another regimen with significant antileukemic activity in recurrent or refractory CLL is oxaliplatin, fludarabine, cytarabine, and rituximab (OFAR) combination therapy.\textsuperscript{60,61} The rationale for combining oxaliplatin with fludarabine and cytarabine was based on preclinical data demonstrating synergistic cytotoxicity between cisplatin in combination with the nucleoside analogs cytarabine\textsuperscript{62} and fludarabine.\textsuperscript{63-65} Oxaliplatin had shown activity in recurrent/refractory non-Hodgkin lymphoma,\textsuperscript{56-69} and it had been substituted for cisplatin in the dexamethasone, high-dose cytarabine, and cisplatin regimen\textsuperscript{70,71} and combined with cyclophosphamide, doxorubicin, vincristine, and prednisone\textsuperscript{72} for the treatment of large-cell non-Hodgkin lymphoma.\textsuperscript{66,67,69,73} The OFAR regimen consists of increasing doses of oxaliplatin (17.5, 20, or 25 mg/m\(^2\)/d) on Days 1 to 4 (phase 1); fludarabine at a dose of 30 mg/m\(^2\) on Days 2 to 3; cytarabine at a dose of 1 g/m\(^2\) on Days 2 to 3; rituximab at a dose of 375 mg/m\(^2\) on Day 3; and pegfilgrastim at a dose of 6 mg on Day 6, every 4 weeks. The OR rate in patients with heavily pretreated fludarabine-refractory CLL was 36\%. Responses included 7 of 19 (37\%) patients with 17p deletions, 2 of 6 (33\%) patients with 11q deletions, 4 patients with trisomy 12, and 2 of 5 (40\%) patients with 13q deletions.\textsuperscript{60,61} In our ongoing phase 1-2 clinical trial of the OFAR regimen in patients with fludarabine-refractory CLL or Richter syndrome, oxaliplat is given at a dose of 30 mg/m\(^2\) daily on Days 1 to 4; fludarabine at a dose of 30 mg/m\(^2\) on Days 2 to 4; cytarabine at a dose of 500 mg/m\(^2\) intravenously on Days 2 to 4; rituximab at a dose of 375 mg/m\(^2\) on Day 3; and pegfilgrastim at a dose of 6 mg on Day 6, every 4 weeks. The current response rate in the phase 2 portion of the study is 64\% (unpublished data).

**Allogeneic Stem Cell Transplantation**

Cumulative evidence suggests that allogeneic stem cell transplantation (allo-SCT) is an efficacious therapeutic strategy for patients with CLL that does not respond to purine analogs or that recurs within 12 months after treatment. The Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation recommended that younger patients with CLL whose disease does not respond or recurs within 12 months after purine analog treatment should be considered for allo-SCT.\textsuperscript{74}

Other indications include 1) younger patients with disease recurrence within 24 months after having achieved a response to purine analog–based combination therapy or autologous transplantation and 2) patients with p53 abnormalities requiring treatment. The graft-versus-leukemia effect is evidenced by long-term clinical remissions in poor-risk patients with CLL who undergo SCT; long-term molecular responses with allogeneic, but not autologous, SCT; lower recurrence rates in the presence of graft-versus-host disease; an increased risk of recurrence in patients who receive T-cell-depleted allografts; and anecdotal cases of donor lymphocyte infusion efficacy.

Six published series of unmanipulated allo-SCT with reduced-intensity conditioning in CLL included 20 to 73 patients each, with 33\% to 90\% of patients having fludarabine-refractory CLL. The studies demonstrated encouraging results, with recurrence rates ranging from 5\% to 28\% at 2 years and up to 69\% at 4 years.\textsuperscript{74-79}

**Other Investigational Agents**

Ongoing clinical trials are exploring targeted therapies as single agents or in combination with chemotherapy and a monoclonal antibody in fludarabine-refractory CLL.\textsuperscript{80}
For example, the tyrosine kinase inhibitor dasatinib (BMS-354825)\(^{80}\), a Bcl-2 family inhibitor, ABT-263\(^ {80}\), and a third-generation, glycoengineered type II IgG1 anti-CD20 monoclonal antibody, GA101,\(^ {81}\) are being investigated in CLL. Other targeted agents, such as phenylethyl isothiocyanate, and nutlins,\(^ {82}\) warrant investigation in CLL, given their promising in vitro antileukemic activity.

**Conclusions**

In summary, fludarabine-refractory CLL is associated with a poor prognosis, and its treatment continues to be challenging. The introduction of salvage therapeutic strategies such as alemtuzumab-containing regimens, chemoimmunotherapeutic regimens, targeted agents, and allo-SCT has improved the prognosis of this disease. Novel, more effective therapies are needed.

**Conflict of Interest Disclosures**

The authors made no disclosures.

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