Phase II Study of Cladribine and Cyclophosphamide in Patients with Chronic Lymphocytic Leukemia and Prolymphocytic Leukemia

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Received June 6, 2002; revision received July 19, 2002; accepted July 29, 2002.

BACKGROUND. One of the mechanisms of action of cladribine is the inhibition of DNA repair of damage caused by radiation, alkylating agents, or other drugs. To determine its antitumor activity in combination with cyclophosphamide, we initiated a Phase II trial of the two agents in patients with advanced chronic lymphocytic leukemia (CLL) or prolymphocytic leukemia (PLL).

METHODS. Twenty-nine patients with refractory or recurrent CLL or PLL received cladribine 4 mg/m²/day and cyclophosphamide 350 mg/m²/day (both administered intravenously) for 3 days every 4 weeks.

RESULTS. Eleven patients (38%), nine with CLL and two with PLL, had a response. The median duration of response was 12 months. Severe extrahematologic toxicity (National Cancer Institute Grade 3–4) occurred in two patients, consisting of skin rash in one patient and progressive multifocal leukoencephalopathy in the other. The most common form of hematologic toxicity was severe neutropenia, which developed after 25% of the 84 courses was administered. Severe thrombocytopenia and anemia developed after 12% and 7% of the courses, respectively, and five episodes of anemia were immunomediated. In addition, three major infections resulted in the death of one patient.

CONCLUSIONS. Although inferior to the combination fludarabine plus cyclophosphamide, this regimen showed interesting activity in patients with advanced CLL or PLL. Myelosuppression was the major dose-limiting toxic effect. Cancer 2003; 97:114–20. © 2003 American Cancer Society.

DOI 10.1002/cncr.11000

KEYWORDS: cladribine, cyclophosphamide, CLL, PLL.

Since their introduction in the early 1980s, purine analogs have demonstrated striking activity against B-chronic lymphocytic leukemia (B-CLL). Fludarabine, the most extensively studied drug in this group, has proven to be effective in the treatment of lymphoproliferative disorders, including CLL, low-grade lymphoma, macroglobulinemic lymphoma, and prolymphocytic leukemia (PLL).1–4 Cladribine is a purine analog that closely resembles fludarabine. The major differences between the two drugs are the presence in cladribine of both a chlorine atom on the 2-carbon of the purine ring (fludarabine has a fluorine atom) and deoxyribose (fludarabine has an arabinose). These differences lead to a wide variation in the dosing schedules (20–30 mg/m² for fludarabine vs. 4–5 mg/m² for cladribine). In addition, cladribine is highly effective against hairy cell leukemia (HCL) and produces clinical results similar to those of fludarabine in the treatment of patients with advanced CLL.5,6 The usual dose for treat-
ing HCL patients is 0.1 mg/kg/day repeatedly for 7 days. This translates to an approximate dose of 4 mg/m²/day.

The current extensive knowledge of nucleoside analog metabolism and action in preclinical studies has facilitated the design and experimental validation of some treatment strategies. The most important biochemical modulation for clinical evaluation concerns fludarabine and cytosine arabinoside (ara-C). The combination of these two drugs suggested that biochemical modulation by fludarabine may provide a tool for increasing the active metabolite of ara-C, ara-CTP, when this drug is infused at a dose that otherwise saturates the rate of ara-CTP accumulation.

Alkylating agents are also active against CLL. Before the discovery of fludarabine, chlorambucil was the traditional standard agent for several decades and it is still administered frequently as the initial therapy in many centers. Cyclophosphamide, another alkylating agent effective against CLL, was chosen for combination therapy with fludarabine. Preclinical studies had shown that the two agents had additive or synergistic activity. In addition, DNA interstrand crosslinks induced in CLL lymphocytes after in vitro exposure to activated cyclophosphamide, 4-hydroperoxycyclophosphamide (4-HC), were repaired rapidly and were not detected after 6–8 hours of incubation. However, when CLL cells were exposed to small doses of fludarabine and incubated with 4-HC, 80% of DNA crosslinks were still detectable 24 hours later. These results are consistent with the hypothesis that fludarabine treatment interferes with the rate of crosslink repair, giving strong support to the rationale behind combining cyclophosphamide and fludarabine. Interesting clinical results were published concerning the use of this drug combination to treat CLL and other indolent lymphoproliferative disorders.

Inhibition of DNA repair is a well understood mechanism of action common among purine analogs. In addition, the metabolism and activation of cladribine within cells to the triphosphate form are similar to the mechanism of fludarabine. Based on these observations, we evaluated the clinical efficacy of the combination of cyclophosphamide and cladribine in the treatment of patients with advanced CLL and PLL.

**MATERIALS AND METHODS**

Twenty-nine pretreated patients with CLL or PLL requiring therapy entered this study from August 1996 to February 1998 in the Department of Leukemia at the University of Texas M. D. Anderson Cancer Center. Informed consent was obtained from the patients according to institutional guidelines. The patients underwent pretreatment examination, including a medical history and physical examination, complete blood counts, differential and platelet counts, liver and renal function studies, bone marrow aspiration, and biopsy and marrow samples for immunophenotyping.

Patients who were 16 or older and had Rai Stage III–IV disease were eligible for the study. Patients with Rai Stage 0–II disease had to fulfill one or more of the criteria for active disease as defined by the National Cancer Institute (NCI)-sponsored Working Group. In addition, patients were required to have adequate renal and hepatic function (creatinine level < 2 mg/dL, bilirubin level < 2 mg/dL) and be off any previous chemotherapy, radiotherapy, and immunotherapy regimens for at least 2 weeks. Localized radiotherapy delivered to an area not compromising bone marrow function did not apply. Patients who presented with a performance status (PS) greater than 3 according to World Health Organization criteria were excluded.

**Response Criteria**

The response criteria used were those previously defined by the NCI Working Group. Specifically, complete remission (CR) required normalization of physical examination findings and blood counts (polymorphonuclear neutrophil leukocytes [PMN] > 1.5 × 10⁹/L, platelets > 100 × 10⁹/L, hemoglobin [Hb] > 11 g/dL), a bone marrow aspirate lymphocyte percentage less than 30%, and no evidence of disease on bone marrow biopsy analysis. A nodular partial remission (PRN) required the same criteria as those for CR with the exception that lymphoid nodules could be seen on bone marrow biopsy analysis. A partial remission (PR) required a reduction in measurable disease of 50% or more as well as one or more of the remaining features: PMN greater 1.5 × 10⁹/L or 50% improvement over baseline, platelets greater than 100 × 10⁹/L or 50% improvement over baseline, and Hb level greater than 11 g/dL or 50% improvement over baseline without transfusions. Bone marrow evaluation was not required to determine PR.

After completing therapy, patients were reexamined at 3-month intervals, consisting of a medical history and physical examination and blood counts. In addition, bone marrow biopsy analysis was performed every 6 months.

**Treatment**

Patients received 4 mg/m² cladribine intravenously over 60 minutes daily for 3 consecutive days and 350 mg/m² cyclophosphamide over 30 minutes daily for 3 days 4 hours after receiving cladribine to ensure that an adequate intracellular level of 2-CdATPs were present in neoplastic cells. This treatment course was
repeated every 4 weeks. A maximum of six courses was given.

Patients also received prophylactic trimethoprim-
sulfamethoxazole (320–1600 mg) twice a week to pre-
vent *Pneumocystis carinii* infection if they had re-
ceived corticosteroids within the preceding 3 months.

Toxicity was evaluated according to the NCI common
toxicity criteria. Dose-limiting toxicity was de-
finite as higher than Grade 2 extrahematologic toxicity (nau-
sea, vomiting, alopecia excluded), Grade 4 thrombo-
cytopenia, Grade 4 neutropenia lasting more than 7
days, or febrile neutropenia.

**Patient Characteristics**

The clinical characteristics of the 29 patients in this
study are shown in Table 1. Their median age was 58
years (range, 39–77 years) and the PS was 0–1 in 27
patients and 2 in the remaining 2 patients. The leuke-
emia diagnosis was B-CLL in 20 cases and B-PLL in 9
cases. Eleven patients presented with Rai Stage I or II
disease and 18 presented with Rai Stage III or IV
disease. The distribution according to the Binet
stage\(^1\) was as follows: 3 cases at Stage A, 12 at Stage B,
and 14 at Stage C.

All of the patients had undergone previous treat-
ment, with the median number of previous regimens
being two (range, 1–5). At the time of treatment using
cyclophosphamide and cladribine, 17 patients had
disease progression after a previous response to che-
motherapy and 12 patients had disease refractoriness
to previous regimens. Before starting treatment, 22
patients presented with anemia, 16 patients were
thrombocytopenic, and four patients had neutro-
penia.

**RESULTS**

**Responses**

A median of three chemotherapy courses was admin-
istered to the 29 patients (range, 1–6). Of the 29 pa-
tients, 1 with PLL has been lost to follow-up after
undergoing one course of chemotherapy and is con-
sidered a treatment failure. Therapy was discontinued
in five patients because of severe myelosuppression.

An overall response rate (ORR) of 38% has been
achieved so far: four patients had a CR, two had a PRN,
and five had a PR. Ten patients had stable disease and
the remaining six patients had disease progression.
One patient died of infection after the first course of
chemotherapy. The results obtained for the 29 pa-
tients are reported in Table 2. All patients showed
disease progression after a median of 12 months
(Fig. 1).

Among the nine patients with PLL, there was one
CR and one PR. None of the five PLL patients whose
disease was refractory to previous treatment using
purine analogs showed a response. The response rate
in the 20 patients with CLL was 45%. Five responses
were observed in the eight patients who had recurrent
disease after treatment with purine analogs and four
responses were observed in the 11 patients whose
disease was refractory to purine analogs.

**Toxicity**

Seven patients (24%) experienced Grade 1–2 nausea
and vomiting. Four patients (14%) experienced skin
rash, three of whom had a Grade 1 rash that resolved
rapidly. The fourth patient had a severe rash (Grade 3).
Multifocal leukoencephalopathy developed after the
third cycle in a patient with progressive disease.

The rate of Grade 3–4 neutropenia, which oc-
curred in 12 patients (41.3%), was 25% after all 84
courses of chemotherapy were administered. In three
patients, long-lasting Grade 4 neutropenia required
the discontinuation of treatment (after two courses of
therapy in one patients and one course in the remain-
ing two). Growth factors were administered to reduce
the impact of neutropenia in two patients: granulo-
cyte–colony-stimulating factor (G-CSF) in one patient
and granulocyte-macrophage–colony-stimulating fac-
tor in the other.

In addition, seven patients developed Grade 3–4

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**TABLE 1**

Clinical Characteristics of the 29 B-CLL and B-PLL Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range)</td>
<td>58 yrs (39–77 yrs)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (55)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (45)</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>27 (93)</td>
</tr>
<tr>
<td>2</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>B-CLL</td>
<td>20 (69)</td>
</tr>
<tr>
<td>B-PLL</td>
<td>9 (31)</td>
</tr>
<tr>
<td>Rai stage</td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>11 (38)</td>
</tr>
<tr>
<td>III–IV</td>
<td>18 (62)</td>
</tr>
<tr>
<td>Binet stage</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3 (10)</td>
</tr>
<tr>
<td>B</td>
<td>12 (42)</td>
</tr>
<tr>
<td>C</td>
<td>14 (48)</td>
</tr>
<tr>
<td>No. of previous regimens</td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>18 (62)</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>11 (38)</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>17 (59)</td>
</tr>
<tr>
<td>Refractory</td>
<td>12 (41)</td>
</tr>
</tbody>
</table>

B-CLL: B-chronic lymphocytic leukemia; B-PLL: B-chronic prolymphocytic leukemia.
thrombocytopenia. Three had severe thrombocytopenia before starting treatment with cyclophosphamide and cladribine and the remaining four patients had mild thrombocytopenia before starting chemotherapy.

Grade 3–4 anemia was observed in seven patients. Grade 3 anemia was already present in two patients at the start of chemotherapy. They remained transfusion dependent during treatment without any signs of hemolysis. The remaining five patients developed immunemediated anemia, consisting of autoimmune hemolytic anemia (AIHA) in four patients and pure red cell aplasia in the fifth. Three of the patients with AIHA achieved a response, two CRs and one PR. The anemia in two patients improved after treatment using cyclosporine (CyA), whereas the third patient remained anemic after treatment using both prednisone and CyA.

Finally, eight episodes of fever of unknown origin were observed, three of which occurred in patients having Grade 4 neutropenia. In addition, three and four patients experienced major and minor infections, respectively. The three major infections were pneumonia due to *Enterococcus* (which was isolated from a bronchoalveolar lavage sample) in one patient, cytomegalovirus (CMV) pneumonia in another, and radiologically documented pneumonia without specific organism in the third. The four minor infections were reactivation of herpes simplex or herpes zoster infection in one and two patients, respectively, and a urinary tract infection in the fourth patient. Toxicity and infections observed in the 29 patients are summarized in Table 3.

**DISCUSSION**

Alkylating agents such as chlorambucil, administered with or without prednisone, have long been considered standard therapy for patients with CLL. Over the past 10 years, however, purine analogs have emerged as an important new group of drugs in the management of this disease. Grever et al. were the first to report their findings of using fludarabine to treat patients with recurrent or alkylator refractory CLL. Although only 4 (13%) of 32 patients achieved a response, 15 (47%) showed substantial signs of clinical improvement. Subsequent clinical trials confirmed therapeutic activity in patients who had previously received alkylating agents. However, convincing data encourage testing the efficacy of alkylating agents in combination with purine analogs. For example, preclinical studies suggested that the formation of DNA crosslinks after exposure to activated cyclophosphamide in vitro was prolonged with concomitant administration of fludarabine, presumably because fludarabine induced inhibition of DNA repair. The results obtained with the combination of fludarabine and cyclophosphamide as salvage therapy are better than those reported for fludarabine alone. In Phase II stud-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>All patients (%)</th>
<th>CLL (%)</th>
<th>PLL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any response</td>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>Any pretreatment</td>
<td>11/29 (38)</td>
<td>4 (14)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Purine analog sensitive</td>
<td>6/10 (60)</td>
<td>3 (30)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Purine analog refractory</td>
<td>4/16 (25)</td>
<td>1 (6)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>No previous purine analogs</td>
<td>1/3 (33)</td>
<td>—</td>
<td>1 (33)</td>
</tr>
</tbody>
</table>

ies of fludarabine in patients previously treated with alkylating agents, the response rates were 45–65%.16,24 O’Brien et al.25 reported an ORR of 69% and an 85% response rate in the alkylator refractory group using the combination of fludarabine plus cyclophosphamide in 94 pretreated patients with CLL. Encouraging data were also reported by the German CLL Study Group.26

Cladribine has been evaluated less extensively in CLL patients than fludarabine. It has a high level of cytoreductive activity, although its effect in CLL patients is less dramatic than that observed in HCL patients.27 Cladribine’s mechanism of inhibition of DNA repair is similar to that of other purine analogs, although its method of metabolism and activation within the cells to the triphosphate form is similar to that of fludarabine. Therefore, clinical cross-resistance between cladribine and fludarabine would be expected. In vitro cross-resistance between these two nucleoside analogs has been demonstrated.28 However, a lack of cross-resistance between cladribine and fludarabine in vivo has also been reported but not confirmed.29,30 For this reason, the combination of cladribine and cyclophosphamide is worthy of testing in pretreated CLL patients, particularly those who have disease resistance to fludarabine. In such patients, most treatment schedules produce a response rate of less than or equal to 15%. In our series, however, the response rate in patients with previous disease refractoriness to purine analogs was 25%. These results are superior to those reported by Saven et al.31 and Delannoy et al.32 in patients who received cladribine alone after not having a response to fludarabine. In this subset of fludarabine-refractory patients, O’Brien et al.25 obtained a response rate of 39% with the combination of fludarabine and cyclophosphamide.25

In our study, the ORR was 38% and it was 45% in the 20 patients with CLL. Van Den Neste et al.23 conducted a Phase I/II study of cladribine, at a fixed dose of 5.6 mg/m²/day for 3 days, combined with cyclophosphamide, at escalated doses from 200 to 400 mg/m²/day for 3 days, increasing by increments of 100 mg/m². They treated 13 patients with CLL and obtained responses in 8 (1 CR, 1 PRN, 6 PR). Twelve of the 13 patients received cyclophosphamide 300 mg/m². Myelosuppression was the dose-limiting toxicity. In addition, although cladribine has little extramedullary toxicity, nausea and vomiting were noted more frequently in our study with the use of combination therapy than with cladribine as a single agent.

The major dose-limiting toxic effect was myelosuppression, particularly neutropenia. Almost one-half of the patients had Grade 3–4 neutropenia. The observed myelosuppression prevented further drug escalation from the initial dose of cyclophosphamide (350 mg/m² × 3 days) and was initially planned. Specifically, myelosuppression led to a relatively low dose intensity of the regimen administered because of frequent delays in course administration. Previous studies of fludarabine and alkylating agents in CLL patients were complicated by hematopoietic and infectious toxicity. In the Intergroup randomized Phase III trial comparing fludarabine and chlorambucil, the arm containing the combination of fludarabine and chlorambucil was more toxic than either agent alone.

### Table 3

Toxicity and Infections Graded According to the NCI Common Toxicity Criteria Grading System

<table>
<thead>
<tr>
<th>Toxicity and infection</th>
<th>No. of patients (%)</th>
<th>Grade 1–2</th>
<th>Grade 3–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrahematologic toxic effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>7 (24)</td>
<td>15 (18)</td>
<td>—</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>1 (3)</td>
<td>—</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>4 (14)</td>
<td>5 (6)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Hematologic toxic effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>14 (48)</td>
<td>3 (4)</td>
<td>21 (25)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7 (24)</td>
<td>2 (2)</td>
<td>10 (12)</td>
</tr>
<tr>
<td>Anemia</td>
<td>7 (24)</td>
<td>2 (2)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3 (10)</td>
<td>—</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>1 (3)</td>
<td>1 (1)</td>
<td>—</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>2 (7)</td>
<td>2 (2)</td>
<td>—</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (3)</td>
<td>1 (1)</td>
<td>—</td>
</tr>
</tbody>
</table>

NCI: National Cancer Institute.
chlorambucil was closed earlier than the others arms because of excessive toxicity.36
Laurencet et al.37 reported a lower incidence of cytopenic episodes and infectious complications with the combination of cladribine and cyclophosphamide. This can be explained by the lower dose of cyclophosphamide (500 mg/m² on Day 1) and because most patients had not received previous treatment. Poor medullary tolerance has been highlighted as a major cause of treatment failure after purine analog administration, especially if the retreatment regimen included purine analogs.38

The infections in our study were not negligible, with one patient dying of infection while neutropenic and another presenting with CMV pneumonia. We did not observe any cases of P. carinii infection and we introduced growth factors for patients with long-lasting neutropenia. The use of growth factors is recommended strongly in heavily pretreated patients. In a previous study, significant attention was paid to supportive care for patients with CLL, which included introducing P. carinii prophylaxis39 and hematopoietic growth factors. In addition, in previously treated patients with Rai Stage II–IV CLL, the addition of G-CSF following fludarabine treatment resulted in a significant decrease in the incidence of sepsis as reported by O’Brien et al.40

Neurotoxic effects, predominantly cerebral dysfunction, have been associated with the use of purine analogs. For example, in a trial at the Scripps Clinic, cladribine was used as part of a conditioning regimen that included cyclophosphamide and total body irradiation.41 Of 31 patients having chemotherapy-resistant leukemia or lymphoma, 12 (39%) experienced delayed neurologic symptoms. In addition, although the prevalence of toxic leukoencephalopathy is unknown, it is increasingly being recognized in patients in whom neurobehavioral disturbances develop after exposure to toxins. In our study, one patient presented with symptoms of leukoencephalopathy, which was confirmed upon magnetic resonance imaging showing evidence of damage at the white matter. Neurologic symptoms appeared in this patient after three courses of treatment. If the occurrence of autoimmune processes is a consequence of an imbalance between the stimulatory and suppressive effects in T-lymphocyte subpopulations, then purine analogs may trigger autoimmune phenomena in CLL patients. Conversely, as demonstrated previously, alkylating agents can trigger these autoimmune phenomena in previously treated patients with purine analogs.42

In the current study, we observed five autoimmune processes during treatment. Although their appearance did not influence treatment, the outcome was favorable in three patients whose disease responded to therapy.

In summary, the addition of cyclophosphamide confers to cladribine the ability to overcome resistance to purine analogs, with an ORR of 25% in purine analog-refractory patients. However, the results obtained with this combination of cladribine and cyclophosphamide are not superior to those obtained with the combination of fludarabine and cyclophosphamide, showing in the same setting an ORR of 39%.25 Finally, the major dose-limiting toxic effect was myelosuppression, which did not allow for proper timing of the administration of treatment and led frequently to discontinuation of therapy.

REFERENCES