Clinical and Pharmacokinetic Study of Clofarabine in Chronic Lymphocytic Leukemia: Strategy for Treatment

Varsha Gandhi,[1,2] William Plunkett,[1,2] Peter L. Bonate,[3] Min Du,[1] Billie Nowak,[1] Susan Lerner,[2] and Michael J. Keating[2]

Abstract

Purpose: Based on its mechanistic similarity to fludarabine and cladribine and the success of these analogues for treatment of chronic lymphocytic leukemia (CLL), we hypothesized that clofarabine would be effective for indolent leukemias. The present study was conducted to determine the efficacy and cellular pharmacology during clinical trials of single-agent clofarabine in CLL.

Experimental Design: Previously treated patients with relapsed/refractory CLL were eligible for this study. Clofarabine was infused over 1 hour daily for 5 days. Most patients received 3 or 4 mg/m^2/d $\times$ 5 days, whereas the other two were treated with 15 mg/m^2/d $\times$ 5 days. Clinical outcome and associated pharmacologic end points were assessed.

Results: Myelosuppression limited the maximum tolerated dose of clofarabine to 3 mg/m^2/d on this schedule. Cellular pharmacokinetic studies showed a median clofarabine triphosphate concentration in CLL lymphocytes of 1.5 μmol/L (range, 0.2-2.3 μmol/L; n = 9). In the majority of cases, >50% of the analogue triphosphate was present 24 hours after infusion, indicating prolonged retention of the triphosphate in CLL cells. Although cytoreduction was observed, no patients achieved a response. *In vitro* clofarabine incubation of leukemic lymphocytes from 29 CLL patients showed that clofarabine monophosphate accumulated to a higher concentration compared with the triphosphate. Nonetheless, the triphosphate increased in a dose-dependent fashion and upon successive clofarabine infusions, suggesting benefit from greater doses given at less frequent intervals.

Conclusion: Levels of clofarabine triphosphate at higher doses and prolonged maintenance of clofarabine triphosphate in leukemic lymphocytes provide a rationale to treat CLL in a weekly clofarabine schedule.

Clofarabine was synthesized as a rational extension of the experience with other deoxyadenosine analogues, such as fludarabine and cladribine. Both have excellent activity against indolent lymphoproliferative malignancies, but neither has proved curative. Nonetheless, these agents are among the primary treatments of indolent leukemias, either as single agents or in combinations (1–5). The search for similar but new compounds with possibly better activity led to the synthesis of a series of 2-halo-2′-halo-2′-deoxyarabinofuranosyl adenine analogues (6).

Authors' Affiliations: Departments of [1]Experimental Therapeutics and [2]Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, Texas and [3]Genzyme Oncology, San Antonio, Texas

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Requests for reprints: Varsha Gandhi, Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, Box 71, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-2989; Fax: 713-794-4316; E-mail: vgandhi@mdanderson.org.

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Both fludarabine and cladribine are derivatized with a halogen at the 2-position of the adenine to prevent deamination and hence deactivation of the compound by adenosine deaminase. Clofarabine (2-chloro-2-deoxy-fluoro-β-D-arabinofuranosyladenine) was synthesized with the specific intention of eliminating some of the undesirable characteristics of both fludarabine and cladribine while retaining their therapeutic attributes (Fig. 1). Clofarabine retains the 2-chloroadenine aglycone of cladribine and hence is resistant to deamination by adenosine deaminase. Reminiscent of fludarabine, the fluorine at critical 2′-position of the carbohydrate is in the arabinosyl configuration (6). Structure-activity studies comparing the 2′,2′-difluoro- and 2′-deoxy-2′-ribo- derivatives with clofarabine showed that the placement of the fluorine in the arabino configuration was essential for the DNA-directed activity of the compound (7). Furthermore, substitution of a fluorine at the C-2′ position decreased the susceptibility of clofarabine to phosphorolytic cleavage by purine nucleoside phosphorylase to roughly one third that of fludarabine and of cladribine (7). The 2′-*arabino*-fluoro substitution also resulted in an increased acid stability of clofarabine relative to dAdo and cladribine (8).

After cellular uptake, clofarabine is converted to its monophosphate by deoxycytidine kinase (7–9). The phosphorylation of clofarabine by deoxycytidine kinase is substantially more efficient than that of fludarabine and is similar to

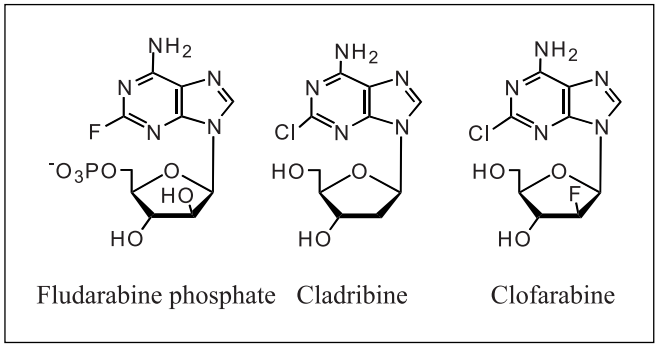

Fig. 1. Structures of deoxyadenosine analogues.

cladribine. Although eventually the triphosphate accumulates to significant concentrations, the monophosphate is also present in large quantities (10). The triphosphate of clofarabine is a substrate for DNA polymerases, and incorporation of the analogue leads to further inhibition of DNA primer extension and hence results in inhibition of DNA synthesis (11–13). The fact that clofarabine triphosphate, similar to cladribine triphosphate, is a potent inhibitor of ribonucleotide reductase further facilitates inhibition of DNA synthesis by lowering the deoxynucleotide pools required for efficient DNA synthesis (11–13).

Because of structural, metabolic, and mechanistic similarities with fludarabine and cladribine and effective cytoreduction in two patients with chronic lymphocytic leukemia (CLL) treated during the broad phase I study, additional CLL patients were entered. All patients received clofarabine on a daily times 5 schedule. The current report describes the clinical and pharmacokinetic results during these trials and also provides a rationale for a modified schedule based on *in vivo* results and *in vitro* investigations in primary CLL cells.

Patients and Methods

Patients for clinical trial. A total of 13 patients with CLL were treated with clofarabine on a daily × 5-day schedule. Whereas the majority of these patients ($n = 11$) were treated with 3 or 4 mg/m^2/d × 5-day schedule, two patients were treated on the original broad phase I trial with 15 mg/m^2/d × 5-day schedule (14). Informed consents, indicating that patients were aware of the investigational nature of this study, were signed according to institutional guidelines. A separate consent form was obtained from 11 patients to participate in correlative pharmacokinetic investigations.

Patients for* in vitro *investigations. Leukemic lymphocytes obtained from peripheral blood of 29 additional CLL patients were used for *in vitro* investigations. These patients signed an informed consent to obtain peripheral blood for *in vitro* investigations with new agents, such as clofarabine. The majority ($n = 22$) of these patients were previously untreated. Seven patients had previously received fludarabine phosphate, cytoxan, or rituximab or their combinations.

Drug and other chemicals. Clofarabine for clinical use was initially prepared by Ash Stevens, Inc. (Detroit, MI) and formulated for injection by the University of Iowa Pharmaceutical Services (Ames, IA). Subsequently, production of bulk drug was conducted by Delmar Chemicals (Lasalle, Quebec, Canada). As a high-pressure liquid chromatography standard to determine the triphosphate level, clofarabine 5′-triphosphate was synthesized by Sierra BioResearch (Tucson, AZ). All other chemicals were reagent grade.

Response criteria. The response criteria for CLL are published in 1988 by the National Cancer Institute Working Group and were revised in 1996. The main change in the criteria was that patients with residual lymphoid nodules on bone marrow biopsy would now be included with patients achieving partial response and subclassified as nodular partial remission (15).

Clinical pharmacology. Blood samples were obtained from patients who consented to blood drawing for pharmacologic determinations. Samples were obtained before therapy for baseline values, at the end of the initial infusion, and, in some cases, at 2, 4, and 24 hours after start of therapy. For some patients, samples were also obtained before and after infusion on days 2, 3, 4, and 5.

Blood samples (10 mL) were obtained and transferred to green stopper vacutainer tubes containing heparin and 1 μmol/L deoxycoformycin (obtained from the National Cancer Institute, Bethesda, MD) as a precaution to inhibit deamination of clofarabine by adenosine deaminase. The tubes were immediately placed in an ice-water bath and transported to the laboratory. Because the whole blood is placed in ice-water bath, metabolic processes and any effect of deoxycoformycin should be negligible. Control studies have shown that normal and leukemia cells are stable under these conditions with respect to size and membrane integrity (16). The cellular nucleotide content is stable for at least 15 hours under these conditions. All patients gave written informed consent for plasma and cellular pharmacology investigations.

Plasma pharmacology. The plasma was removed after centrifugation and stored at −70°C until analyses were done. Clofarabine plasma levels were analyzed at MicroConstants LLC (San Diego, CA) by a Good Laboratory Practices–validated procedure employing reversed-phase high-pressure liquid chromatography using a tandem quadrupole mass spectrometer as previously described (14, 17). The linear range of the method for the samples was 0.0033 to 16.5 μmol/L. The coefficient of variation of the assay was ~10%.

Cellular pharmacology. Cell pellets from blood samples were diluted with PBS, and mononuclear cells were isolated using Ficoll-Hypaque density-gradient step-gradient centrifugation procedures described previously (16). A Coulter electronics channelizer (Coulter Corp., Hialeah, FL) was used to determine the mean cell volume. After being washed with PBS, cells were processed for nucleotide extraction. Normal nucleotides and clofarabine triphosphate were extracted from cells using standard procedures with $HClO_4$. Triphosphates were separated on an anion-exchange Partisil-10 SAX column (Waters Corp., Milford, MA) using high-pressure liquid chromatography as described in detail previously (18). The intracellular concentration was calculated and expressed as the quantity of nucleotides contained in the extract from a given number of cells of a determined mean volume. This calculation assumes that nucleotides are uniformly distributed in total cell water. In general, the lower limit of quantitation of this assay was about 1 pmol in an extract of 2×10^7 cells, corresponding to a cellular concentration of about 0.2 μmol/L. For many samples, extract from as many as 8×10^7 cell equivalents were analyzed to detect the peak. The coefficient of variation of the assay for inter day variation was <1% (18).

In vitro *investigations in CLL cells.* For these investigations, CLL cells obtained from peripheral blood samples were diluted with PBS and suspended in RPMI 1640 with 10% fetal bovine serum. The cells from six patients were incubated for 2 hours with 3, 10, 30, and 100 μmol/L [^{3}H]clofarabine. The cells were then collected, and nucleotides were extracted using the perchloric acid extraction procedure as described above. Clofarabine triphosphate was separated and quantitated as described before (18). To determine the ratio of clofarabine monophosphate to clofarabine triphosphate, CLL cells from 29 patients were collected and incubated with 10 μmol/L clofarabine for 2 hours. Clofarabine monophosphates, diphosphates, and triphosphates were separated and quantitated as described before (10).

Results

Study group. A total of 13 patients with relapsed or refractory CLL were treated with single-agent clofarabine. Nine

of the 13 patients were male with a median age of 61 years (range, 52-78 years). All patients were heavily pretreated with a median number of therapies being 5 (range, 3-11); almost all patients (n = 11) were fludarabine refractory, and most (n = 9) were also alkylator refractory. Nine of the 13 patients were Rai stage III or IV. Additional characteristics especially the peripheral blood counts are tabulated (Table 1).

Response and outcome. Although clinical remissions were not achieved, all patients except one showed a modest reduction in the lymphocyte counts. To establish the degree of reduction of the lymphocytes, the log [lymphocytes] was calculated at the start of the first course and 8 to 10 days after induction of the 5-day course. The median log reduction in the 3 to 4 mg/m^2/d × 5-day schedule was 0.18 (range, −0.53 to +0.05). The log reductions in the two patients started at 15 mg/m^2/d × 5 days were 1.08 and 1.51. Eight patients received one course of treatment; three patients received two courses; and two patients received three courses. At evaluation, 12 of the 13 patients were Rai stages III to IV. One patient died on study. The cause of death was multiorgan failure secondary to sepsis.

Dose reduction and maximum tolerated dose. For all studies, the drug was infused i.v. over 1 hour, and the 5-day course was repeated every 3 to 4 weeks. For the initial phase I study of clofarabine (14), patients with solid tumors, acute leukemias, and indolent leukemias were entered. Among these 51 patients, two patients had CLL, and both of these were treated at a daily dose of 15 mg/m^2/d × 5 days (14). Because of grade 4 neutropenia in these individuals, which was similar in patients with solid tumors treated with this initial dose, dose was reduced to 7.5 mg/m^2/d, and patients with solid tumors were treated; dose was further reduced, and five CLL patients were treated with the reduced dose of 4 mg/m^2/d × 5 days. Again, neutropenia was evident; thus, the dose was reduced, and six additional patients received 3 mg/m^2/d, the maximum tolerated dose.

Hematologic and nonhematologic toxicity. There was almost a complete incidence of grade 3 and 4 hematologic toxicity for neutropenia (all patients) and thrombocytopenia (Table 2). The hematologic toxicity became dose limiting for this group of patients on this daily for 5-day schedule. These severe toxicities may be the reason for infections. Of the 13 patients, 5 (38%) had a major infection; 4 (31%) developed a fever of unknown origin; and 4 (31%) had minor infections. The overall infection rate per patient was 62%. Although many patients had nonhematologic toxicities, such as nausea (31%), fatigue (23%), edema (16%), rash (23%), and constipation (8%), the dose-limiting toxicity was myelosuppression.

Plasma pharmacology during therapy. The plasma concentrations of clofarabine were 0.55 and 0.28 μmol/L at the end of infusion of 15 mg/m^2 dose of clofarabine. For the nine patients receiving the lower 3 to 4 mg/m^2 dose, the median concentration of clofarabine at the end of infusion was 0.20 μmol/L (range, 0.08-0.76 μmol/L). At 24 hours before the second infusion of clofarabine, the drug concentration had decreased to 0.01 μmol/L (range, 0.008-0.04 μmol/L), a lower but detectable level of clofarabine in the plasma.

Cellular pharmacology during therapy. Clofarabine triphosphate concentrations were quantitated in the circulating CLL lymphocytes from 11 patients at the end of clofarabine infusion. Two patients were studied at 15 mg/m^2/d × 5 days; the CLL B-cells from these individuals accumulated 2.8 and 8.3 μmol/L clofarabine triphosphate. The levels of clofarabine triphosphate were less at the 3 to 4 mg/m^2/d × 5-day dose levels; the median value was 1.5 μmol/L (range, 0.2-2.3 μmol/L; n = 9). Although the variation among individuals in the intracellular concentrations of clofarabine triphosphate was similar to that observed with fludarabine and cladribine, the absolute triphosphate concentrations were less than those achieved with fludarabine, whereas similar to those of cladribine when given on the same schedule. Both clofarabine and cladribine are better substrates

Table 1. Patient characteristics

Characteristics	Median (range)
Age (y)	61 (52-78)
No. prior treatments	5 (3-11)
WBC (×10^9/L)	33.3 (1.9-194)
Absolute lymphocytes (×10^9/L)	25.1 (0.44-178.5)
Hemoglobin	11.5 (7.7-15.2)
Platelets (×10^9/L)	65 (32-236)

Table 2. Hematologic and nonhematologic toxicities

	Patient, n (%)
Hematologic (grade 3/4) toxicity	
Neutropenia	13 (100)
Thrombocytopenia	12 (93)
Anemia	5 (40)
Nonhematologic toxicity	
Nausea	4 (31)
Fatigue	3 (23)
Edema	2 (16)
Rash	3 (23)
Constipation	1 (8)

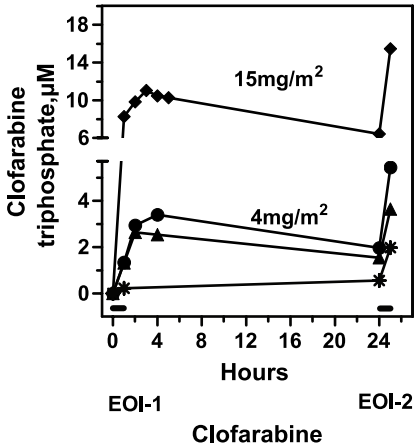

Fig. 2. Pharmacokinetics of clofarabine triphosphate during therapy. Accumulation of clofarabine triphosphate in peripheral blood leukemic lymphocytes of four patients after clofarabine infusions of 15 mg/m^2 (♦) and 4 mg/m^2 (●, ▲, and *). CLL cells were isolated and nucleotides were extracted by perchloric acid extraction procedure. The analogue triphosphate was quantitated as described in Patients and Methods. EOI, end of infusion of clofarabine.

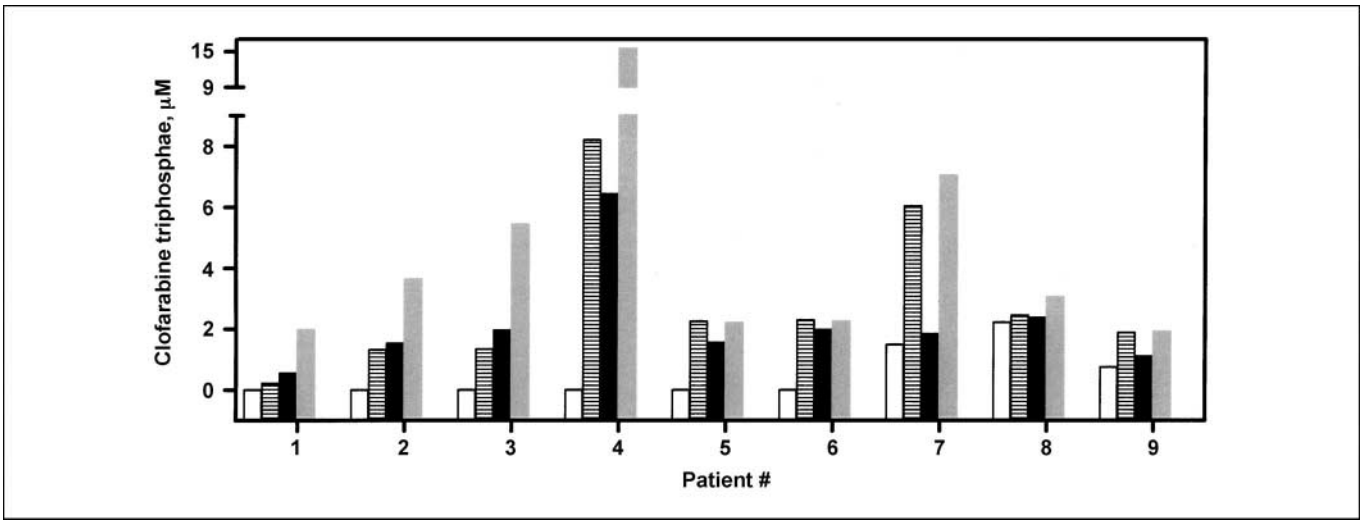

Fig. 3. Accumulation of clofarabine triphosphate during the first 2 days of therapy in nine patients with CLL. Cells were collected from peripheral blood before clofarabine infusion (*white columns*), after end of infusion (*lined columns*), at 24 hours (*black columns*), and after second infusion (*gray columns*) of clofarabine. For patients 7 and 8, the first sample was before the second clofarabine infusion (i.e., 48 hours), and for patient 9, it was before the third (72 hours) clofarabine infusion. CLL cells were isolated, and nucleotides were extracted by perchloric acid extraction procedure. The analogue triphosphate was quantitated as described in Patients and Methods.

than fludarabine for deoxycytidine kinase. It is possible that the monophosphates of clofarabine and cladribine accumulate in cells due to relatively unfavorable kinetics for the conversion to the respective diphosphates and triphosphates as has been shown to be the case for chlorinated analogues in cell line studies (7, 10, 11).

To determine the pharmacokinetic profile of clofarabine triphosphate in primary leukemia B cells during the first 24 hours, additional data points were analyzed and plotted (Fig. 2). As is apparent from these kinetics, the accumulation of clofarabine triphosphate continued after the end of infusion of clofarabine, and peak levels were generally achieved by 4 hours. At this time point, the levels were below 12 and 4 μmol/L with 15 and 4 mg/m^2/d dosing, respectively. Before the second clofarabine infusion, the remaining triphosphate concentration was still about 50% of the peak value in the circulating leukemic lymphocytes. In all cases, there was an increase in the peak level of clofarabine triphosphate after the second infusion, and the intracellular clofarabine triphosphate concentration was greater at the end of second infusion compared with that achieved after the first infusion.

To better define if such an increase occurred in lymphocytes of most patients, these investigations of clofarabine triphosphate accumulation were extended to nine additional patients after the first infusion, at 24 hours, and after the second infusion (Fig. 3). For patients 7 and 8, samples were taken after the second and third infusions (48, 49, 72, and 73 hours), and for patient 9, they were obtained after the third and fourth infusions (72, 73, 96, and 98 hours). After the first infusion (*lined columns*), clofarabine triphosphate was detected in all cases. Compared with this value, more than half of clofarabine triphosphate was retained 24 hours later (*black columns*). Three of the nine patients had a similar concentration of clofarabine triphosphate after the first and the subsequent infusions. In the remaining six patients, however, there was an increase in clofarabine triphosphate. These data showed that in the majority of CLL patients, more than half of the accumulated clofarabine triphosphate is retained for at least 24 hours after the infusion, and that additional infusions result in an increased level of analogue triphosphate. Nonetheless, the cellular concentration of clofarabine triphosphate was <6 μmol/L in eight of nine patient samples after the second infusion. Because the clinical response to this therapy was not impressive, we hypothesize that achieving a higher level of clofarabine triphosphate may be necessary for effective reduction of the circulating CLL lymphocytes. A strategy that could achieve this goal is to infuse a higher dose of clofarabine per infusion while increasing the interval between infusions.

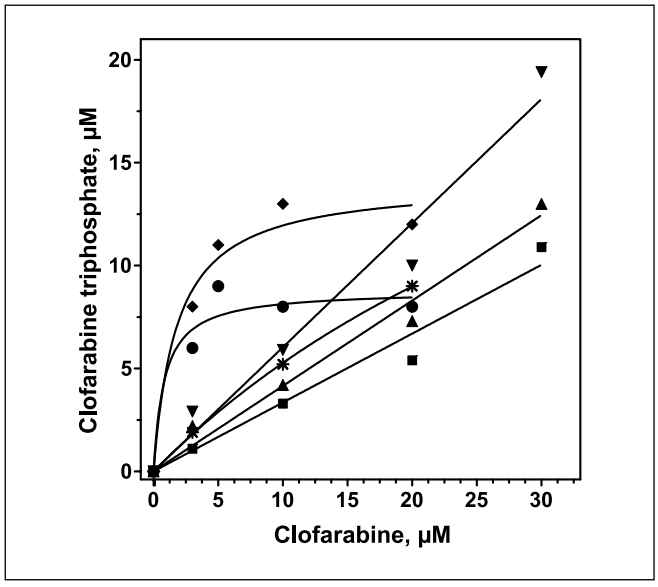

Fig. 4. *In vitro* accumulation of clofarabine triphosphate in primary CLL cells. Leukemic lymphocytes were isolated from peripheral blood of six CLL patients and placed in RPMI. Cells were incubated with indicated concentrations of exogenous clofarabine for 2 hours. Normal and analogue nucleotides were extracted from CLL cells by acid extraction and quantitated using high-pressure liquid chromatography as described under Patients and Methods.

Table 3. Accumulation of phosphorylated metabolites of clofarabine after a 2-hour *in vitro* incubation with 10 μmol/L clofarabine

Patient no.	Clofarabine monophosphate, μmol/L	Clofarabine diphosphate, μmol/L	Clofarabine triphosphate, μmol/L	Monophosphate/ triphosphate
1	11	3.4	18	0.6
2	28	1.9	21	1.3
3	15	1.2	11	1.4
4	22	0.8	15	1.5
5	27	0.7	17	1.6
6	27	1	15	1.8
7	16	1.1	13	2
8	24	1.4	11	2.2
9	18	1.1	8	2.3
10	33	1	14	2.4
11	42	1.5	17	2.5
12	13	0.5	5	2.6
13	42	2	14	3
14	19	0.4	6	3.2
15	72	3	21	3.4
16	48	2	14	3.4
17	28	0.4	8	3.5
18	9	0.2	3	3.6
19	34	1	9	3.8
20	51	0.8	13	3.9
21	57	0	13	4.4
22	35	8.9	7	4.8
23	43	0.5	9	4.8
24	20	0.3	4	5
25	36	5	7	5.1
26	102	2.3	19	5.4
27	37	0	6	6.2
28	35	1	4	8.8
29	27	0.4	3	9
Median	28		11	3.4
Range	9-102		3-21	<1-9

In vitro ***accumulation of clofarabine triphosphate.*** At the 3 to 4 mg/m^2 doses, the plasma concentration of clofarabine was <1 μmol/L. To determine if higher clofarabine concentrations would result in greater accumulation of clofarabine triphosphate, cells from six patients with CLL were isolated and incubated *in vitro* with increasing concentrations of clofarabine (Fig. 4). In four of six patient samples, there was a linear increase in clofarabine triphosphate with increasing concentration of clofarabine. The highest levels were 20 and 30 μmol/L of clofarabine. In the remaining two patients, there was a linear increase in triphosphate level for up to 5 μmol/L of exogenous clofarabine, whereas increased drug concentrations failed to generate significant increases in clofarabine triphosphate.

In vitro ***accumulation of clofarabine metabolites.*** Similar to cladribine, the major intracellular metabolites of clofarabine are monophosphates and triphosphates. Hence, it is possible that at higher level of clofarabine, such as 10 μmol/L, more monophosphate accumulates, which saturates the rate of conversion to the triphosphate. This will result in a high ratio of monophosphate to triphosphate. To evaluate this possibility, CLL cells from 29 patients were isolated and incubated with 10 μmol/L clofarabine for 2 hours, and phosphorylated metabolites were measured. Table 3 suggests that the monophosphate was the major metabolite in these cells, with a median concentration to 28 μmol/L (range, 9-102 μmol/L). The ratio of monophosphate to triphosphate concentrations ranged between <1 and 9 (median = 3.4) with a ratio of <4 in two thirds of the samples (20 of the 29 patient samples studied).

Discussion

Purine nucleoside analogues, such as deoxycoformycin, cladribine, fludarabine phosphate, and potentially nelarabine, are the backbones for the treatment of adult indolent leukemias. The active moieties of these purine nucleoside analogues are dATP (from deoxyadenosine generated by inhibition of adenosine deaminase), cladribine triphosphate, fludarabine triphosphate, and ara-G triphosphate, respectively. However, differences in optimal dosing likely reflect variations in the cellular metabolism of each drug, the cellular pharmacokinetics of the respective active metabolite, and its dominant mechanism of action. Based on the clinical success of these

analogues, we postulated that another congener (clofarabine) would also be active in CLL. This is based on several facts. First, clofarabine resembles both cladribine and fludarabine in its structure (6). Second, similar to cladribine, clofarabine has been shown to be a favored substrate for deoxycytidine kinase (7). Third, the activity of deoxycytidine kinase is high in CLL leukemic lymphocytes. Fourth, clofarabine combines the positive features of cladribine and fludarabine for its actions on ribonucleotide reductase and DNA polymerases affecting DNA synthesis (11, 12). Fifth, clofarabine triphosphate is as effective as cladribine triphosphate and is more active than dATP or fludarabine and nelarabine triphosphates as a cofactor in the conversion of procaspase-9 to active caspase-9 in an *in vitro* apoptosome assay (19). Finally, compared with dATP and the triphosphates of cladribine and fludarabine, clofarabine triphosphate was more potent in changing the mitochondrial membrane potential, a factor in the initiation of mitochondria-induced cell death (20).

Based on this information and the fact that fludarabine phosphate, cladribine, and nelarabine all have been used effectively in a daily times 5-day schedule, we tested clofarabine in CLL using the same schedule. Clofarabine was not effective in these heavily pretreated patients with CLL at these doses and administration schedule. None of the 13 patients achieved an objective response, although cytoreduction was observed in these patients, who were heavily pretreated and mostly refractory to fludarabine phosphate or alkylators. Still, these results should be viewed in light of the small study size. In addition, it was noted that the log reduction in two patients at 15 mg/m^2/d $\times$ 5 days were 1.1 and 1.5, which is much greater than that in patients who received 3 or 4 mg/m^2/d $\times$ 5 days. The DLT with clofarabine in these patients was hematologic. This may be due to the fact that clofarabine triphosphate may also be long-lived in myeloid lineage cells.

Previous investigations with clofarabine in adult and pediatric acute leukemias showed efficacy and clinical responses to clofarabine therapy on both phase I and phase II trials (21, 22, 23). Furthermore, it was shown that there was a relationship between the peak concentrations of clofarabine triphosphate after daily infusions of clofarabine and response to clofarabine therapy (21). The concentrations of clofarabine triphosphates in the circulating blasts of patients with acute leukemias at the phase II doses were much higher (median = 15 μmol/L; n = 29; ref. 21) than that achieved in leukemic lymphocytes from patients with CLL (Figs. 2 and 3). This difference may be due to the fact that the phase II dose of clofarabine was 10- to 13-fold greater in acute leukemias (40 mg/m^2/d $\times$ 5 days) compared with that in CLL (3-4 mg/m^2/d $\times$ 5 days). At 40 mg/m^2/d infusion, the peak plasma level of clofarabine varies between 1 and 2 μmol/L. In contrast, at 3 to 4 mg/m^2 infusions, the concentration of clofarabine in plasma is relatively low (median = 0.20 μmol/L). In both diseases, the analogue triphosphate was long-lived in the target leukemia cells ($t_{1/2}$ > 24 hours).

Based on these pharmacokinetic characteristics and prevalence of deoxycytidine kinase in CLL lymphocytes and the dose-response relationship observed in phase I study between clofarabine levels in plasma and infusion dose, it may be expected that at a higher dose of clofarabine will generate proportionally greater plasma clofarabine concentrations (14), facilitating accumulation of greater levels of clofarabine triphosphate in CLL cells. As the active triphosphate would be retained effectively ($t_{1/2}$ > 24 hours), the initial greater clofarabine triphosphate concentration may eliminate the need for frequent administration of the drug. When such a strategy was modeled *in vitro* (Fig. 4), it was apparent that increased accumulation of the triphosphate was achieved in CLL lymphocytes at higher clofarabine concentrations. This rationale supports a schedule with low frequency of administration, such as a weekly infusion schedule.

This approach is currently being used for patients with solid tumors. For these patients, the current infusion dose is >100 mg/m^2/wk every 3 weeks (24). Hence, the maximum tolerated dose of clofarabine is very different in patients with solid tumors, depending on the schedule of administration. Although the maximum tolerated dose is 2 mg/m^2/d for a five-consecutive-day infusion schedule in this patient population (21), the maximum tolerated dose has not been achieved in the ongoing clofarabine trial and is >100 mg/m^2 when given on a weekly schedule. Such stringent behavior in the dosing and schedule of administration was also observed with gemcitabine infusions. For example, the maximum tolerated dose on a daily $\times$ 5-day schedule is 9 mg/m^2/d, whereas single weekly infusions for 3 weeks ranges between 790 and 2,200 mg/m^2 as the maximum dose (25–27).

A burgeoning question with infusion of greater clofarabine doses in patients with CLL would be whether these cells would make higher level of clofarabine triphosphate or not. Because plasma accumulation of clofarabine is dose dependent (21), we would expect that increased doses of clofarabine would result in greater levels of plasma clofarabine. The *in vitro* data in CLL lymphocytes (Fig. 4) clearly shows that CLL cells have a clofarabine dose-dependent increase in clofarabine triphosphate. Furthermore, data for clinical activity suggested that greater level of cytoreduction was achieved at higher (15 mg/m^2/d) doses than at lower (3-4 mg/m^2/d) doses. Based on these data and rationales, we conclude that daily $\times$ 5-day schedule of clofarabine as a single agent is not effective for patients with CLL, and for this reason and the *in vitro* experiments presented in the current report, we have initiated a new trial of weekly infusions of clofarabine for 3 weeks followed by a week of rest for patients with CLL.

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