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Combination of fludarabine and arabinosylcytosine for treatment of chronic lymphocytic leukemia: clinical efficacy and modulation of arabinosylcytosine pharmacology

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Abstract. Previous studies have demonstrated that treatment with fludarabine 4 h prior to arabinosylcytosine (ara-C) potentiates the accumulation of the active triphosphate of ara-C (ara-CTP) in leukemic lymphocytes. The clinical efficacy of this combination was evaluated in 15 patients with chronic lymphocytic leukemia (CLL) that was advanced in their disease (median Rai stage, IV) and refractory to treatment with fludarabine. Patients received 0.5 g/m^2 ara-C infused i.v. over 2 h followed at 20 h by a 30-min infusion of 30 mg/m^2 fludarabine. At 24 h, an identical dose of ara-C was infused. To intensify the therapy and to determine the duration of fludarabine potentiation of ara-CTP accumulation, six additional patients with Rai stage III or IV CLL were treated with an amended 2-week protocol. On week 1, 30 mg/m^2 fludarabine was infused over 30 min, followed 4 h later by a 2-h infusion of 0.5 g/m^2 ara-C; on week 2, the fludarabine dose was followed 4 h later by a 4-h infusion of ara-C (1.0 g/m^2). In all, 1 partial remission and 7 minor responses in 1 or more disease sites were observed in the 21 patients. The major treatment-related toxic effects were myelosuppression and infection. Comparison of the ara-CTP accumulation area under the concentration-time curve (AUC) in circulating CLL cells of patients on the amended protocol demonstrated a significant ($P = 0.001$) 1.6-fold (range, 1.4- to 2.0-fold) increase after fludarabine administration. Although the initial rates of ara-CTP accumulation were similar for the 2-h and 4-h infusions, ara-CTP accumulation continued for up to 4 h in four of five patients who received the longer infusion. The activity of the fludarabine and ara-C combination is being evaluated in in vitro model systems and in phase II clinical trials in combination with other drugs.

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Introduction

Purine nucleoside analogues have recently been recognized as effective agents for the treatment of chronic lymphocytic leukemia (CLL) [1]. Activity has been reported for 2-chlorodeoxyadenosine and pentostatin [2, 3], although investigations of these drugs are not as extensive as those of fludarabine (arabinosyl-2-fluoroadenine monophosphate, or F-ara-A monophosphate). When used as a single agent, fludarabine has achieved response rates of >75% in patients with newly diagnosed CLL [4] and 45%–65% in patients with previously treated or refractory disease [5, 6]. Although it is too soon to determine whether fludarabine alters the natural history of the disease in previously untreated CLL, it now appears that the disease will recur in previously treated patients who have responded to fludarabine salvage therapy [7]. Thus, fludarabine does not appear to be curative when used as a single agent, and consideration should be given to its use in combination with other drugs to improve its efficacy.

Arabinosylcytosine (ara-C), a pyrimidine nucleoside, is an effective agent for the treatment of acute myelogenous leukemia. Response rates ranging between 25% and 50% have been observed in single-agent high-dose ara-C therapy of relapsed and refractory acute leukemia. An attenuated version of high-dose ara-C therapy resulted in a 33% response rate in patients with previously treated, advanced CLL [8]. Furthermore, high-dose ara-C in combination with cisplatin and etoposide had major cytoreductive activity in patients with CLL, including one complete and one partial remission [9]. This activity suggests that ara-C be used in combination with other agents to treat CLL.

Our previous investigations [10] demonstrated that when leukemic lymphocytes from patients with CLL were incubated first with F-ara-A, the parent nucleoside of fludarabine, and then with ara-C, a 2.2-fold increase in the accumulation of the active 5′-triphosphate of ara-C (ara-CTP) was observed as compared with the ara-CTP accumulation in CLL cells incubated with ara-C alone. These studies were extended to CLL lymphocytes obtained from patients before and after fludarabine therapy that were incubated in vitro

with ara-C. Accumulation of ara-CTP was increased by a factor of 1.7 in lymphocytes obtained after fludarabine therapy [10].

On the basis of these studies, we hypothesized that infusion of fludarabine prior to ara-C would potentiate the area under the concentration-time curve (AUC) of ara-CTP in leukemia cells. Comparison of ara-CTP pharmacokinetics in the circulating lymphocytes of eight patients with CLL who received fludarabine and ara-C therapy demonstrated that the ara-CTP AUC increased by a median of 1.5 times (range, 1.1–1.7 times) after fludarabine infusion, in agreement with laboratory studies. The rate of ara-CTP accumulation, which was the only parameter influenced by fludarabine, remained linear throughout the 2-h ara-C infusion [11].

To determine the duration during which fludarabine would potentiate the rate of ara-CTP accumulation, the treatment protocol was amended to increase the time of the ara-C infusion. We report the results of these investigations along with the clinical efficacy and toxicity of the fludarabine and ara-C combination for patients with CLL on both the original and amended protocols.

Patients and methods

Patients. Between April 1990 and September 1992, 21 patients with CLL refractory to previous fludarabine therapy were treated with these protocols (15 under the original protocol and 6 under the amended protocol). Study entry required morphologic confirmation of the diagnosis of CLL and documentation of a B-cell immunophenotype. Only patients with advanced disease (Rai stages III and IV) were entered except when there was evidence of active disease in patients with Rai stages I and II disease as defined by the National Cancer Institute Working Group (NCIWG) [12]. All patients had complete histories, physical examinations, complete blood counts, and biochemical surveys (SMA12) done. A bone marrow aspirate and a biopsy specimen were obtained from each patient. Normal renal and hepatic functions were mandatory. Patients were informed about the investigational nature of this program in accord with institutional policies, and each gave informed consent.

For the pharmacologic investigations, eight patients on the original protocol and five patients on the amended protocol were studied. These patients were selected on the basis of adequate numbers of circulating lymphocytes (>20,000/μl), laboratory preparedness, and informed consent to participate in the pharmacology studies.

Treatment protocols. In the original protocol, the first course of treatment (Fig. 1A) included one dose of 0.5 g/m^2 ara-C infused over 2 h. At 20 h, patients received a 30-min infusion of 30 mg/m^2 fludarabine; 4 h later (24 h after the start of the first ara-C dose), a second identical dose of ara-C was infused. In subsequent courses, given at 28-day intervals, one dose of fludarabine was infused 4 h before one dose of ara-C. The study design of the first course permitted evaluation of the effect of fludarabine on the pharmacokinetics of ara-CTP in circulating leukemic lymphocytes.

In vitro modeling of the fludarabine and ara-C therapy suggested that F-ara-A potentiates the accumulation of ara-CTP for up to 4 h. Hence, the protocol was amended (Fig. 1B) to evaluate a different dose schedule of greater intensity and to study the duration of fludarabine potentiation of ara-CTP metabolism. Patients treated under the amended 2-week protocol received a dose of fludarabine (30 mg/m^2) 4 h before a 2-h infusion of 0.5 g/m^2 ara-C during the 1st week and were given the same dose of fludarabine followed by a 4-h infusion of 1.0 g/m^2 ara-C during the 2nd week. Thus, intrapatient comparisons of the rate of ara-CTP accumulation and the duration of

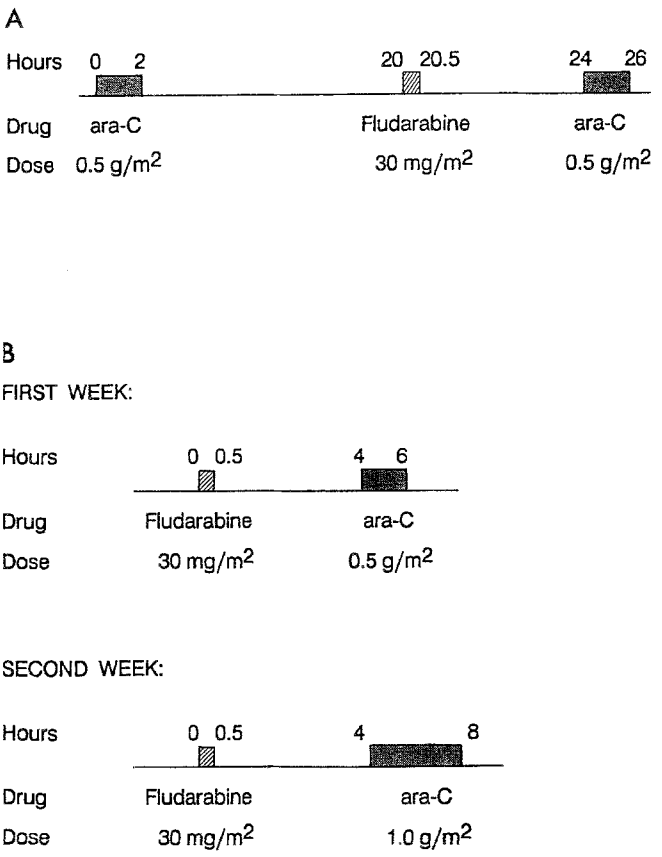

Fig. 1 A, B. First cycles of the original (**A**) and amended protocols (**B**), indicating the schedules and doses of ara-C and fludarabine administration

potentiation after fludarabine therapy were possible. This therapy was scheduled to be repeated at 4-week intervals or after recovery from myelosuppression.

Drugs. Berlex Laboratories, Inc. (Alameda, Calif.) provided fludarabine as a sterile, lyophilized powder free of antibacterial preservatives. Ara-C was obtained commercially as Cytosar-U from Upjohn Co. (Kalamazoo, Mich.). For in vitro studies, ara-C and ara-CTP were obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of reagent grade.

Response criteria. Patients who completed a single course of therapy were considered evaluable for response. Sites evaluated for response included lymph nodes, spleen, liver, peripheral blood, and bone marrow. Guidelines outlined by the NCIWG [12] were used to evaluate responses. A complete remission required the disappearance of all palpable disease, normalization of the blood counts (neutrophils, $>1.5\times10^3$/μl; platelets $>100\times10^3$/μl; and hemoglobin, >11 g/dl), and a lymphocyte content of <30% on marrow-aspiration differential analysis. A partial remission required >50% decrease in palpable disease and a >50% improvement in all abnormal blood counts.

Clinical pharmacology. Pharmacologic procedures for patients treated on the original protocol have been presented elsewhere [11]. For patients treated on the amended protocol (Fig. 1B), 10-ml samples were taken during weeks 1 and 2 at 0, 0.5, 2, and 4 h for determination of F-ara-ATP levels and at 5, 6, 7, 8, 9, and 10 h for investigation of ara-CTP pharmacokinetics. Ara-C and ara-U concentrations were determined by reverse-phase high-pressure liquid chromatography (HPLC) as described elsewhere [13]. F-ara-A levels in plasma were determined after derivatization with chloroacetyldehyde to the fluorescent arabinosyl-etheno-isoguanine. The product was separated and quantitated by HPLC with fluorescence detection as previously re-

Table 1. Patients' characteristics[a] and clinical response

Patient	Prior regimen	Rai stage	Courses	Response[b]		Toxicity
				Overall	Site(s)	
1	6	IV	1	Fail		
2	2	I	1	Fail	WBC	C. difficile colitis
3	3	IV	1	Fail	WBC	
4	3	II	3	Fail	WBC, nodes	
5	2	IV	1	Fail	Nodes	Sinusitis
6	2	IV	1	Fail		
7	3	IV	1	ED		Pneumonia, gastrointestinal hemorrhage
8	3	IV	1	ED		Congestive heart failure
9	3	IV	1	Fail		Pseudomonas septicemia
10	5	I	2	Fail		
11	4	IV	4	Fail	WBC, nodes	
12	4	II	2	Fail	WBC	Pneumonia
13	4	IV	2	Fail		
14	4	II	1	Fail		
15	3	II	2	Fail		Herpetic keratitis
16	5	IV	2	Fail		Fever of unknown origin
17	5	IV	1	Fail		Pneumonia, *Herpes zoster*
18	5	IV	1	Fail		
19	2	IV	1	PR	WBC, nodes, spleen	Pneumonia
20	4	IV	1	Fail	WBC, nodes	
21[c]	3	III	1	ED		Pneumonia, hemolysis

ED, Early death; PR, partial remission; WBC, white blood cells

[a] Patients 1–15 were on the original protocol; patients 16–21 were on the amended protocol

[b] Using NCIWG criteria [12]

[c] Follicular small-cleaved-cell lymphoma, leukemic phase

ported [14]. After the removal of plasma, mononuclear cells were isolated by Ficoll-Hypaque density-gradient centrifugation procedures. Following enumeration and cell-volume determination (Coulter Electronics, Hialeah, Fla.), normal and arabinosyl nucleotides were extracted from CLL lymphocytes by $HClO_4$ [15]. Ara-CTP and F-ara-ATP were separated from ribonucleoside triphosphates by anion-exchange chromatography on a Partisil-10 SAX column; ara-CTP and F-ara-ATP were quantified at 262 nm by electronic integration with reference to external standards [15].

In vitro ara-CTP accumulation. Leukemic lymphocytes were isolated from the peripheral blood of these patients and incubated in vitro with or without 5 μ*M* F-ara-A for 4 h. The lymphocytes were washed into fresh medium and incubated with 10 μ*M* ara-C for 4 h, and the intracellular level of accumulated ara-CTP was measured hourly for 4 h.

Calculations and statistical analysis. The levels of ara-CTP and F-ara-ATP obtained by HPLC analysis were normalized on the basis of the concentrations of endogenous nucleotides found in the corresponding cell extracts in each individual. The rate of ara-CTP accumulation in leukemic cells was calculated by a linear regression analysis that included a zero-hour value. The AUC for the accumulation of ara-CTP in leukemic cells was estimated by gravimetric procedures. The paired *t*-test was used to compare pharmacologic data obtained during the first and second doses of ara-C.

Results

Clinical investigation

Patients' characteristics. The primary objective was to evaluate the clinical efficacy of the pharmacologically guided regimen in which ara-C was the major drug used to treat CLL patients whose disease was fludarabine-refractory. The median age was 56 years (range, 36–74 years), and 62% of the patients were men. Of the 21 patients, 17 had received 3 or more prior therapies and 14 had Rai stage IV disease (Table 1). All patients had undergone prior therapy with fludarabine as a single agent or with prednisone. None of the patients responded and all were considered refractory to fludarabine. Additionally, most (71%) were refractory to alkylating agents. In all, 20 patients had morphologically confirmed B-cell CLL and 1 patient had leukemic-phase follicular small-cleaved-cell lymphoma.

Clinical responses, toxicity, and survival. All patients were evaluable for clinical response. According to the NCIWG response criteria, one patient achieved a partial remission for 4 months (Table 1). Several other patients had minor responses in one or more disease sites: four in lymph nodes and six in peripheral blood.

Severe myelosuppression was common and similar with these two regimens. Severe neutropenia ($<0.5\times10^3/\mu l$) was present in 6 patients prior to fludarabine and ara-C therapy; of the remaining 15 patients, 11 developed severe neutropenia following this treatment. All 21 patients had a platelet nadir of $<50\times10^3/\mu l$ after therapy, and severe thrombocytopenia ($<20\times10^3/\mu l$) occurred in 9 patients. The hemoglobin concentration reached a nadir of <8.5 g/dl in 14 patients. Myelosuppression did not appear to be cumulative in the seven patients who received more than one course of therapy.

The most common nonhematologic toxicity associated with these regimens was infection (Table 1). Five patients developed pneumonia, one patient developed a pseudomonas bacteremia, and fever of unknown origin was seen in one patient. Other complications included herpetic keratitis and pseudomembranous colitis. Dermatomal herpes zoster

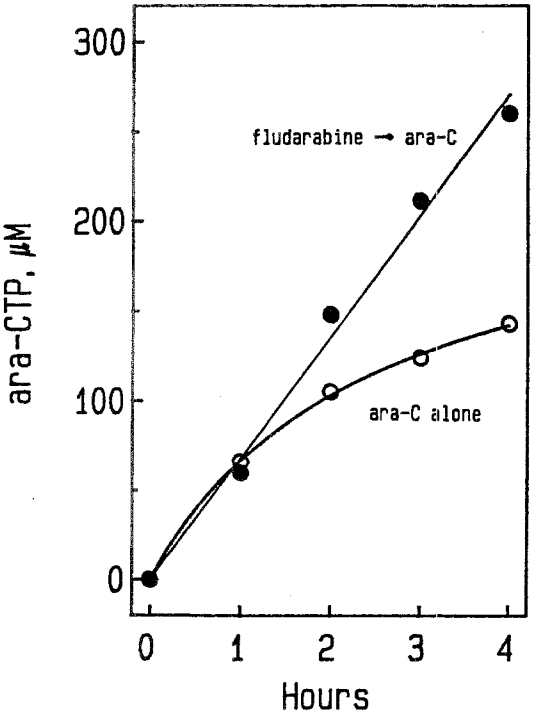

Fig. 2. In vitro modeling for 4-h ara-C infusion in a representative patient. Leukemic lymphocytes from patients were obtained and incubated with ara-C with (●) or without (○) prior incubation with F-ara-A. Accumulation of ara-CTP was quantitated by HPLC assay as described in Patients and methods

occurred in one patient. Three patients with Rai stage IV disease died early, one of congestive heart failure and two of pneumonia. No other toxic side effect was prominent. Survival analysis revealed that four patients are alive, and the median survival is approximately 9 months.

Pharmacologic investigation

Plasma pharmacokinetics. Ara-C was infused at 0.25 g/m^2 per hour because this rate achieves plasma ara-C concentrations (10 μ*M* or above [11]) that maximize the rate of ara-CTP accumulation in CLL lymphocytes during therapy [16, 17]. The pharmacokinetics were studied in two patients on the amended protocol; ara-C levels at the end of the 4-h infusion were 9 and 11 μ*M*. These findings indicate that plasma ara-C levels were adequate to maximize the accumulation of ara-CTP in circulating leukemic lymphocytes. The plasma F-ara-A pharmacokinetics for the patients on the original protocol have previously been reported [18]. For the five patients studied on the amended protocol, the peak level of 2.2 ± 0.5 μ*M* was achieved at the end of the first fludarabine infusion.

Cellular pharmacokinetics. Eight patients on the original protocol were studied for the pharmacokinetics of ara-CTP [11]. The salient features of these studies were as follows. First, the accumulation of ara-CTP in circulating lymphocytes was linear until the end of ara-C infusion. Second, the elimination of ara-CTP was monophasic and was not significantly affected by fludarabine infusion. Third, there was

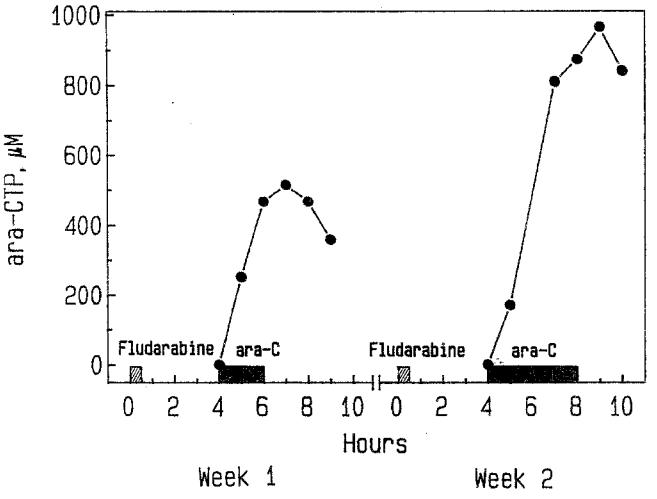

Fig. 3. Effect of the fludarabine infusion on ara-CTP accumulation in a representative patient when ara-C was infused for 2 or 4 h. Accumulation of ara-CTP was quantitated during the first (2 h) and second doses (4 h) of ara-C as described in Patients and methods

a median 1.3-fold (range, 1.2- to 1.8-fold; $P = 0.001$) increase in the rate of ara-CTP accumulation after fludarabine infusion that resulted in a significant ($P = 0.018$) augmentation of the ara-CTP AUC (median, 1.5 times; range, 1.1–1.7 times) during the second dose of ara-C.

Among the several parameters that could influence ara-CTP accumulation, only the rate of ara-C phosphorylation was affected by fludarabine infusion. This rate was dependent on the plasma ara-C level, which was above 10 μ*M* only during the ara-C infusion [11]. In vitro modeling of this combination regimen was done to test the influence of fludarabine on ara-C phosphorylation when ara-C was present for 4 h. In a representative patient, the rate of ara-CTP accumulation in cells treated with ara-CTP alone declined after 3 h as steady state was approached (Fig. 2). In contrast, after F-ara-A incubation, the rate of ara-CTP accumulation was linear until 4 h. These results were consistent in cells of six of eight patients (data not shown) and suggest that if ara-C were infused in the clinic for more than 2 h, fludarabine would continue to augment ara-CTP accumulation in leukemic lymphocytes for at least an additional 2 h.

To test this hypothesis, the protocol was amended such that each patient received ara-C first as a 2-h infusion and then as a 4-h infusion given at the same dose rate (Fig. 1 B). As shown in the cells of a representative individual (patient 19), ara-CTP accumulated at a linear rate during the 2-h ara-C infusion (Fig. 3). The rate of ara-CTP accumulation decreased following the end of this initial ara-C infusion, and ara-CTP levels declined thereafter. In the following week, ara-C was infused for 4 h after a dose of fludarabine in the same patient. Ara-CTP accumulated at a rate similar to that observed the previous week, but this rate of ara-CTP accumulation was sustained past the duration of the 4-h infusion.

Similar results were obtained in three of the four additional patients studied on this protocol (Table 2). A comparison of the ara-CTP accumulation AUC in these patients during the first 4 h after ara-C infusion demonstrated a median 1.6-fold increase (range, 1.4- to 2.0-fold) after the

second (4-h) dose of ara-C. Similarly, there was a significant increase in the peak level of ara-CTP (median, 1.9 times; range, 1.6–2.9 times) during the second dose of ara-C. The time of the peak was between 2 and 4 h during the 2-h infusion and between 3 and 5 h during the 4-h infusion of ara-C. In patient 17, however, the rate of ara-CTP accumulation in the circulating lymphocytes was potentiated only to 3 h after the start of a 4-h ara-C infusion (Table 2). The F-ara-ATP level in the circulating lymphocytes from this patient was <5 μ*M*, a concentration that was low as compared with that of other patients (range, 65–200 μ*M*). Thus, it is likely that a minimal F-ara-ATP concentration of >5 μ*M* must be maintained in leukemic lymphocytes to augment ara-CTP synthesis.

Relationship between ara-CTP and F-ara-ATP accumulation. A comparison of the peak of ara-CTP and F-ara-ATP accumulation during the 1st week of infusion suggests a direct relationship between these parameters (Table 2). Because both analogues are phosphorylated by the same enzyme, deoxycytidine kinase (dCyd kinase), this relationship could be predicted. In these patients, however, ara-C was infused after fludarabine. Thus, the ara-CTP levels represent the rate of ara-C phosphorylation and the effect of fludarabine infusion on this rate. For this reason, the rates of cellular metabolism of these two triphosphates were evaluated in the CLL cells of patients treated on the original protocol (Fig. 1 A) when ara-C was infused alone. Because the peak time of ara-CTP accumulation varied among patients, the ara-CTP concentration at the end of ara-C infusion was compared with the peak of F-ara-ATP accumulation in each individual (Fig. 4). Except for one patient (peak F-ara-ATP level, 20 μ*M*; ara-CTP level, 400 μ*M*), these data suggest a direct relationship between the rate of ara-CTP accumulation and the peak concentration of F-ara-ATP in CLL cells during therapy.

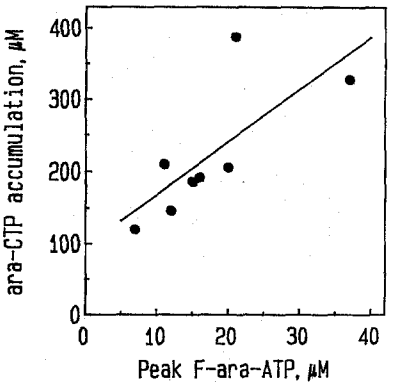

Fig. 4. Relationship between ara-CTP and F-ara-ATP accumulation during therapy in CLL lymphocytes from patients on the original protocol. F-ara-ATP and ara-CTP were quantitated as described in Patients and methods. The levels of ara-CTP accumulation at the end of ara-C infusion are plotted against the peak of F-ara-ATP

Discussion

Fludarabine was recently recognized as having major activity in both newly diagnosed CLL and advanced-stage disease [4, 5]. Although fludarabine used as a single agent induces responses in 45%–65% of patients with previously treated CLL, the apparent failure to translate this success into a survival advantage calls into question the ability of the drug to cure previously untreated patients [7]. Thus, there is the indication that additional drugs or modalities are required to achieve this goal. With this in mind, the present study investigated the clinical activity of fludarabine combined with ara-C in patients with advanced CLL who had previously failed to respond to fludarabine therapy alone.

In addition to having failed prior fludarabine therapy, the disease of 70% of the patients entered in this study had also progressed during therapy with alkylating agents. The population was characterized by advanced-stage disease (15 of 21 patients had Rai stage IV disease) that was accompanied by thrombocytopenia in two-thirds of the patients at the time of treatment. In this group of patients, a partial remission was obtained in one patient and minor responses (responses in one or more disease sites) were achieved in another one-third of the patients. Overall, the treatment was tolerable in this extremely poor prognosis group. The high incidence of thrombocytopenia and neutropenia suggests that increases in the intensity of either regimen beyond the initial level is not warranted.

The choice of ara-C for combination with fludarabine was based on two rationales. First, we had previously observed [8] that an attenuated version of high-dose ara-C therapy (3 g/m^2 given over 2 h every 12 h for one to four doses) resulted in a 33% response rate in patients with advanced, previously treated CLL. Pharmacology studies

Table 2. Pharmacokinetics of ara-CTP in circulating lymphocytes of patients on the amended protocol[a]

Patient	Peak, μ*M* (time, h)		Peak ratio:	AUC ratio:	F-ara-ATP peak, μ*M*	
	Week 1	Week 2	Week 2/week 1[b]	week 2/week 1[c]	Week 1	Week 2
17	252 (3)	431 (3)	1.7	1.6	<5	<5
18	314 (2)	703 (5)	2.2	1.5	75	80
19	515 (3)	966 (5)	1.9	1.4	140	110
20	259 (2)	749 (4)	2.9	1.6	65	80
21	450 (4)	722 (4)	1.6	2.0	200	190

AUC, Area under the concentration-time curve

[a] Week 1, 0.5 g/m^2 ara-C over 2 h; week 2, 1.0 g/m^2 ara-C over 4 h (Fig. 1 B)

[b] Median, 1.9; $P = 0.002$

[c] Median, 1.6; $P = 0.001$

conducted during that investigation demonstrated that the maximal rate of ara-CTP accumulation could be achieved at substantially lower dose rates [16, 17]. Furthermore, CLL lymphocytes accumulate high concentrations of ara-CTP and eliminate it more slowly than do other leukemic morphologies. This suggests that a daily schedule of ara-C administration is sufficient to maintain potentially inhibitory concentrations of ara-CTP. Additionally, this schedule would eliminate or decrease the inhibitory effect of intracellular ara-CTP on the phosphorylation of fludarabine [18]. Thus, for the present trial we used intermittent infusions of intermediate doses of ara-C (0.25 g/m^2 per hour), the aim being that leukemic lymphocytes would accumulate active triphosphate effectively but that the decreased total ara-C dose would result in less toxic side effects. Second, previous investigations had demonstrated that the rate of ara-CTP synthesis was increased in CLL lymphocytes that had been exposed to fludarabine, either in vitro [10] or during therapy [11], before incubation with ara-C. In contrast, when consecutive ara-C doses (without any fludarabine) were infused into patients with leukemia, the ara-CTP AUCs remained similar during these doses [17, 19], confirming the role of fludarabine in augmentation of the ara-CTP AUC. Together, these rationales provided guidance for the design of the present study and compelled us to evaluate the pharmacologic aspects of these strategies.

Earlier studies have demonstrated that in CLL cells, the potentiation of ara-CTP accumulation depends primarily on the intracellular levels of F-ara-ATP and the availability of ara-C as a substrate [20, 21]. CLL lymphocytes tend to retain F-ara-ATP effectively [22], whereas ara-C is rapidly cleared from plasma by deamination at the end of an infusion [11]. Therefore, we sought to determine the duration over which a single fludarabine dose could potentiate the rate of ara-CTP accumulation. During the 4-h infusion of ara-C, the CLL cells of four of five patients accumulated ara-CTP at a linear rate for the infusion duration or longer. The individual whose cells failed to maintain linear kinetics throughout the infusion (patient 17) had an ara-CTP peak at 3 h. This lack of continued ara-CTP accumulation may be attributed to the observation that the intracellular F-ara-ATP concentration in the CLL lymphocytes of this individual was <5 μM at the time of ara-C infusion. This is consistent with a previous report in which acute lymphocytic leukemia cells from a patient accumulated 6 μM F-ara-ATP did not potentiate the accumulation of ara-CTP [23].

A comparison of the ara-CTP and F-ara-ATP accumulation in these patients suggested a direct relationship between the rate of ara-CTP accumulation and that of F-ara-ATP (Fig. 4). This can be evaluated with a knowledge that both drugs are phosphorylated by dCyd kinase [24–27], which is also the rate-limiting step for the accumulation of the respective active triphosphates. As indicated above, the self-potentiation of fludarabine metabolism through the inhibition of ribonucleotide reductase (indirect effect of F-ara-ATP on dCyd kinase) would not be evident in these cells because of their exceedingly low deoxynucleotide pools. Therefore, the levels of analogue triphosphates represent the sum of phosphorylation and the rate at which the triphosphates are catabolized. Because the elimination of ara-CTP and F-ara-ATP is slow in CLL cells, nucleoside phosphorylation must be the major contributor to accumulation of ara-CTP and F-ara-ATP [8, 16, 22, 28, 29]. The relative rates of triphosphate accumulation are influenced by how long ara-C and F-ara-A are present at maximal concentrations in plasma and by the affinity of dCyd kinase for these analogues. Ara-C phosphorylation is favored because it is present at higher concentrations (≥ 10 μM) [11] than is F-ara-A (<3 μM) [18] for a longer time (2–4 h for ara-C vs 0.5 h for F-ara-A) and because dCyd kinase has a greater affinity for ara-C than for F-ara-A (K_m, 10–20 μM for ara-C vs 300–600 μM for F-ara-A) [18, 30]. These factors explain why accumulation of ara-CTP proceeds at a substantially greater rate than that of F-ara-ATP.

Regardless of the differential accumulation kinetics, these data indicate that quiescent lymphocytes contain sufficient levels of dCyd kinase to metabolize these drugs effectively. This opens opportunities for the design of new protocols that include the fludarabine/ara-C strategy in combination with other agents. Furthermore, both fludarabine and ara-C are among the most potent inhibitors of DNA replication and, probably, of DNA repair [31]. Thus, consideration should be given to the design of regimens that target DNA repair for inhibition by fludarabine and ara-C. For instance, combinations of these drugs with radiation [32], which elicits a DNA-repair response directly, or drugs that alkylate or otherwise cause DNA damage, thereby triggering a DNA-repair response, should be evaluated. Such strategies are presently being evaluated in model systems [33] and in the clinic [34, 35].

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References

1. Cheson B (1992) The purine analogs – a therapeutic beauty contest. J Clin Oncol 10: 352
2. Juliusson G, Elmhorn-Rosenborg A, Liliemark J (1992) Response to 2-chlorodeoxyadenosine in patients with B-cell chronic lymphocytic leukemia resistant to fludarabine. N Engl J Med 327: 1056
3. Grever MR, Leiby JM, Kraut EH, et al (1985) Low dose deoxycoformycin in lymphoid malignancy. J Clin Oncol 3: 1196
4. Keating MJ, Kantarjian H, O'Brien S, et al (1991) Fludarabine: a new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia. J Clin Oncol 9: 44
5. Keating MJ, Kantarjian H, Talpaz M, et al (1989) Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood 74: 19
6. Hiddeman W, Rottman R, Wormann B, et al (1991) Treatment of advanced chronic lymphocytic leukemia by fludarabine: results of a clinical phase-II study. Ann Hematol 63: 1
7. Keating MJ, O'Brien S, Kantarjian H, et al (1993) Long-term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. Blood 81: 2878
8. Robertson LE, Hall R, Keating MJ, et al (1993) High-dose cytosine arabinoside in chronic lymphocytic leukemia: a clinical and pharmacologic analysis. Leuk Lymphoma 10: 43
9. McCroskey RD, Mosher DF, Spencer CD, et al (1990) Acute tumor lysis syndrome and treatment response in patients treated for refractory chronic lymphocytic leukemia with short-course, high-dose cytosine arabinoside, cisplatin, and etoposide. Cancer 66: 246

10. Gandhi V, Nowak B, Keating MJ, et al (1989) Modulation of arabinosylcytosine metabolism by arabinosyl-2-fluoroadenine in lymphocytes from patients with chronic lymphocytic leukemia: implications for combination therapy. Blood 74: 2070
11. Gandhi V, Kemena A, Keating MJ, et al (1992) Fludarabine infusion potentiates arabinosylcytosine metabolism in lymphocytes of patients with chronic lymphocytic leukemia. Cancer Res 52: 897
12. Cheson BD, Bennett JM, Rai KR, et al (1988) Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute-sponsored Working Group. Am J Hematol 29: 152
13. Liliemark JO, Plunkett W, Dixon DO (1985) Relationship of 1-β-D-arabinosylcytosine in plasma to 1-β-D-arabinosylcytosine 5′-triphosphate levels in leukemic cells during treatment with high-dose 1-β-D-arabinosylcytosine. Cancer Res 45: 5952
14. Kemena A, Fernandez M, Bauman J, et al (1991) A sensitive assay for quantitation of fludarabine and metabolites in biological fluids. Clin Chim Acta 200: 95
15. Gandhi V, Danhauser L, Plunkett W (1987) Separation of 1-β-D-arabinofuranosyl-2-fluoroadenine 5′-triphosphate in human leukemic cells by high performance liquid chromatography. J Chromatogr 413: 293
16. Plunkett W, Liliemark JO, Adams TM, et al (1987) Saturation of 1-β-D-arabinosylcytosine 5′-triphosphate accumulation in leukemia cells during high-dose 1-β-D-arabinosylcytosine therapy. Cancer Res 47: 3005
17. Plunkett W, Liliemark JO, Estey E, et al (1987) Saturation of ara-CTP accumulation during high-dose ara-C therapy: pharmacologic rationale for intermediate-dose ara-C. Semin Oncol 14 [Suppl 1]: 159
18. Kemena A, Gandhi V, Shewach D, et al (1992) Inhibition of fludarabine metabolism by arabinosylcytosine during therapy. Cancer Chemother Pharmacol 31: 193
19. Plunkett W, Nowak B, Keating MJ (1987) Effect of amsacrine on ara-CTP cellular pharmacology in human leukemia cells during high-dose cytarabine therapy. Cancer Treat Rept 71: 479
20. Gandhi V, Estey E, Keating MJ, et al (1993) Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 11: 116
21. Gandhi V, Estey E, Keating MJ, et al (1993) Biochemical modulation of arabinosylcytosine for therapy of leukemias. Leuk Lymphoma 10 [Suppl]: 109
22. Gandhi V, Kemena A, Keating MJ, et al (1993) Cellular pharmacology of fludarabine triphosphate in chronic lymphocytic leukemia cells during fludarabine therapy. Leuk Lymphoma 10: 49
23. Suki S, Kantarjian H, Gandhi V, et al (1993) Fludarabine and cytosine arabinoside in the treatment of refractory or relapsed acute lymphocytic leukemia. Cancer 12: 2155
24. Schrecker AW, Urshel MJ (1968) Metabolism of 1-β-D-arabinofuranosylcytosine in leukemia L1210: studies with intact cells. Cancer Res 28: 793
25. Kessel D, Hall TC, Wodinsky I (1967) Transport and phosphorylation as factors in the antitumor action of cytosine arabinoside. Science 156: 1240
26. Brockman RW, Cheng Y-C, Schabel FM, et al (1980) Metabolism and chemotherapeutic activity of 9-β-D-arabinofuranosyl-2-fluoroadenine against murine leukemia L1210 and evidence for its phosphorylation by deoxycytidine kinase. Cancer Res 40: 3610
27. Dow LW, Bell DE, Poulakos L, et al (1980) Differences in metabolism and cytotoxicity between 9-β-D-arabinofuranosyladenine and 9-β-D-arabinofuranosyl-2-fluoroadenine in human leukemic lymphoblasts. Cancer Res 40: 1405
28. Kemena A, Keating MJ, Plunkett W (1991) Plasma and cellular bioavailability of oral fludarabine (abstract). Blood 78: 52A
29. Danhauser L, Plunkett W, Keating M, et al (1986) 9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-monophosphate pharmacokinetics in plasma and tumor cells of patients with relapsed leukemia and lymphoma. Cancer Chemother Pharmacol 18: 145
30. Krenitsky TA, Tuttle JV, Koszalska GW, et al (1976) Deoxycytidine kinase from calf thymus. Substrate and inhibitor specificity. J Biol Chem 251: 4055
31. Plunkett W, Gandhi V, Huang P, et al (1993) Fludarabine: pharmacokinetics, mechanisms of action, and rationales for combination therapies. Semin Oncol 20: 2
32. Gregoire V, Hunter N, Milas L, et al (1992) Enhancement of radiation response by fludarabine in murine tumor models (abstract). Proc Am Assoc Cancer Res 33: 85
33. Yang LY, Li L, Trujillo JM, et al (1993) Fludarabine synergizes cisplatin cytotoxicity in LoVo human colon carcinoma cells by inhibiting repair of cisplatin-induced DNA damage (abstract). Proc Am Assoc Cancer Res 34: 347
34. Feldman E, Gandhi V, Plunkett W, et al (1992) Sequential administration of fludarabine, ara-C, and mitoxantrone enhances topoisomerase II-DNA complex formation and has efficacy in acute leukemia (abstract). Blood 80: 208A
35. Robertson LE, Kantarjian H, O'Brien S, et al (1993) Cisplatin, fludarabine, and ara-C: a regimen for advanced fludarabine refractory chronic lymphocytic leukemia (abstract). Proc Am Soc Clin Oncol 12: 308