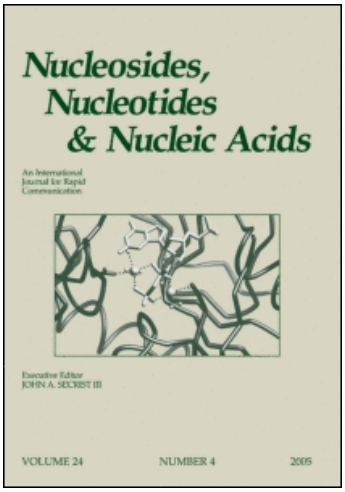

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Clofarabine in Adult Acute Leukemias: Clinical Success and Pharmacokinetics

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ABSTRACT

Clofarabine is a deoxyadenosine analog synthesized with the intention of retaining the favorable mechanistic properties of fludarabine and cladribine while eliminating their undesirable characteristics. Phase I studies among 32 patients with acute leukemia defined a maximum tolerated dose (MTD) of 40 mg/m^2/d given as a one hour infusion daily for 5 days. The dose limiting toxicity (DLT) was transient hepatotoxicity. In a phase II study, 62 patients with acute leukemias received clofarabine at the MTD over 1 hour daily for 5 days. Twenty patients (32%) achieved complete response (CR), 1 had a partial response (PR), and 9 had a CR but without platelet recovery (CRp), for an overall response rate of 48%. Pharmacokinetic studies in the phase I trial revealed marked heterogeneity in peak levels of clofarabine among patients at the end of infusion, however; there was a linear, dose dependent increase in clofarabine concentration in the plasma. Pharmacodynamically, at the MTD, DNA synthesis was inhibited by more than 80% at the end of infusion. In phase II studies, the relationship between the pharmacokinetics of clofarabine triphosphate accumulation and clinical response at the MTD was explored, revealing an accumulation advantage of the

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cytotoxic triphosphate in leukemia cells of responders. The circulating leukemia blasts of patients who respond to clofarabine therapy exhibited a favorable pharmacokinetic profile. In conclusion, clofarabine is an active agent in the treatment of acute leukemias and MDS, and cellular pharmacokinetics has prognostic significance.

Key Words: Clofarabine; AML; Leukemia; Nucleoside analog; Chloro-fluoro-ara-A; Pharmacology.

INTRODUCTION

Nucleoside analogs are widely used in the treatment of leukemias and other hematologic malignancies. Deoxyadenosine analogs, such as fludarabine, cladribine, and deoxycoformycin have shown efficacy in the treatment of lymphoproliferative disorders. Fludarabine is most active in CLL and indolent lymphoma, while cladribine is most active in hairy cell leukemia and Waldenstrom's macroglobulinemia. While these agents have proven efficacious, there are limitations including drug metabolism and extramedullary toxicities. Clofarabine (Cl-F-ara-A, 2-chloro-2′-fluoro-deoxy-9-β-D arabinofuranosyladenine) was synthesized with the specific intention of eliminating the undesirable characteristics of fludarabine and cladribine while keeping their favorable therapeutic attributes. Clofarabine retains the 2-chloroadenine aglycone of cladribine, hence it is resistant to deamination. Reminiscent of fludarabine, clofarabine is further derivatized with a fluorine molecule in the arabinosyl configuration of the carbohydrate (Fig. 1).

The 2′-halogen inhibits cleavage of the glycosidic bond, a mechanism of clearance for the parent drugs.[1] Similar to fludarabine and cladribine, clofarabine requires intracellular phosphorylation by deoxycytidine kinase to its monophosphate, which is converted to the cytotoxic triphosphate form.[2,3] Similar to cladribine triphosphate, the clofarabine triphosphate is a strong inhibitor of ribonucleotide reductase.[2–5] As with fludarabine triphosphate, clofarabine triphosphate is incorporated into DNA and inhibits further chain elongation. These actions result in inhibition of DNA synthesis and cell death. Additionally, mitochondrial actions of clofarabine triphosphate initiate cell death in DNA-independent fashion.[6,7] These previous studies combined with the favorable characteristics of clofarabine suggested use of this drug in hematological malignancies

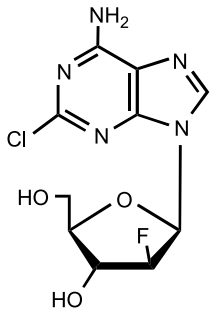

Figure 1. Structure of clofarabine.

which have led to phase I studies of clofarabine in patients with solid and liquid tumors and phase II investigations in patients with relapsed acute leukemias.

PATIENTS AND METHODS

In the phase I studies, patients 18 years or older with solid tumors, chronic leukemias, lymphoma, myeloma, or acute leukemias were eligible for the study.[8] The objective of the clinical trial was to define the MTD and the toxicity profile of single agent clofarabine. The phase II studies included adults with a diagnosis of AML, MDS, ALL, or CML-BP whose disease was refractory to frontline and/or salvage therapy.[9] The goals of this investigation included clinical efficacy of this agent for patients with acute leukemias. Plasma and cellular pharmacology of clofarabine and its triphosphate were performed to determine the relationship between clinical responses and pharmacokinetic profile.

RESULTS AND DISCUSSION

Fifty-one patients were treated on the phase I trial. Thirteen patients had metastatic solid tumors, six had CLL, and 32 had acute leukemias (Table 1). In patients with solid tumors, the dose limiting toxicity (DLT) was myelosuppression and the MTD was defined at 2 mg/m^2/d for 5 days. However, more patients need to be evaluated to precisely define the MTD. Similar to solid tumors, indolent leukemia patients also had myelosuppression, and the MTD for this group was 3–4 mg/m^2/d. In contrast, for patients with acute leukemias including AML, ALL, CML-BC, and MDS, the MTD was much higher (40 mg/m^2/d); the DLT in this group was hepatotoxicity. Among all diagnoses, objective responses were observed only in patients with acute leukemias; 5 patients had a response for an overall response rate of 16%. The two patients who achieved a CR were treated at a dose of 40 mg/m^2/d. The other three patients achieved HI and were treated with doses ranging from 11.25 to 55 mg/m^2/d for 5 days.

To determine the pharmacokinetics of clofarabine, blood samples were obtained before treatment and at the end of clofarabine infusion. Similar to the data obtained with other nucleoside analogs, the peak level of clofarabine occurred at the end of infusion and demonstrated marked heterogeneity. Despite this heterogeneity, there was a linear, dose dependent increase in clofarabine concentration in the plasma. At the

Table 1. Patient diagnosis and MTD during phase I study of clofarabine.

Tumor type	n	MTD (mg/m^2/d)
Solid tumor	13	2
CLL	6	3–4
Acute leukemias	32	40

(From Ref. [8].)

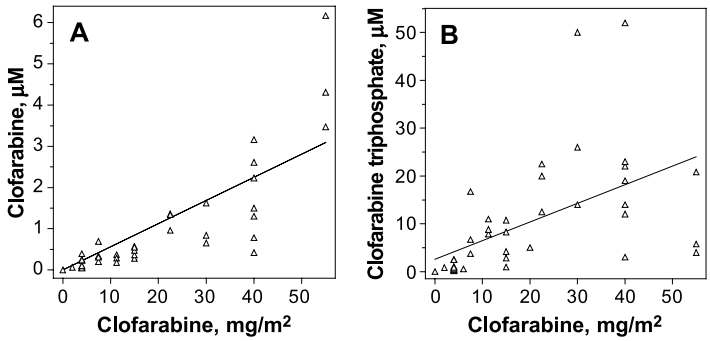

Figure 2. A and B. Relationship between the dose of clofarabine and plasma clofarabine and cellular clofarabine triphosphate, respectively. (From Ref. [8].)

MTD of 40 mg/m^2/d, the median plasma level of clofarabine was 1.5 µM (Fig. 2A). Levels of clofarabine triphosphate were analyzed in 40 patients at the end of infusion. There was a wide variation of clofarabine triphosphate accumulation, which was more apparent at higher doses ($\geq$ 30 mg/m^2). At the MTD for acute leukemias, a median 19 µM (range 3 to 52 µM) was achieved in circulating leukemia blasts (Fig. 2B). Pharmacodynamically, at the MTD, DNA synthesis was inhibited by more than 80% at the end of infusion and was maintained throughout the 24 hours.[8]

In conclusion, during the phase I studies in acute leukemias, clofarabine showed efficacy at a MTD of 40 mg/m^2 for five days. The favorable cellular pharmacokinetics of clofarabine triphosphate at the MTD, pharmacodynamic actions on DNA synthesis inhibition, and response rate in this heavily pretreated subgroup of patients supported a detailed phase II investigation in adults with acute leukemias.

To define the drug efficacy profile, phase II studies were conducted in a total of 62 patients with acute leukemias.[9] In this cohort, 39 patients had AML or high risk MDS, 11 patients had Ph-positive CML in blastic phase (CML-BP), and 12 had ALL. Overall, 20 patients (32%) achieved a CR, 1 had a PR (2%), and 9 had HI or CRp (15%), for an overall response rate of 48% (Table 2). In patients with AML, 17 patients (55%) achieved an objective response. Response rates were higher in patients who had achieved longer first remissions. Similar to AML, in MDS, half of the patients had an objective response. In both diseases the karyotype did not have an effect on overall

Table 2. Patient diagnosis and response during phase II study of clofarabine.

Diagnosis	n	CR	CRp/PR	OR
AML	31	13	4	17
MDS	8	2	2	4
CML-BC	11	4	3	7
ALL	12	1	1	2

CR, complete remission; CRp, Complete response with low platelet counts; PR, partial remission; OR, overall response rate.
(From Ref. [9].)

response rate. In CML blastic phase, 7 patients (64%) achieved objective responses. Compared to these myeloid diseases, lymphoid malignancies had a moderate response rate (17%). Common side effects of the patients treated with clofarabine include transient liver dysfunction, skin rashes, palmoplantar erythrodydesthesia and mucositis. The most common toxicity was myelosuppression complicated by febrile episodes in 45/62 patients.

Similar to phase I data, there was heterogeneity for accumulation of plasma clofarabine at the end of the first infusion. The median clofarabine concentration was 1 μM. At 24 hours after clofarabine infusion, a median of 0.038 μM plasma clofarabine was still detected. Although this concentration is relatively low, it is highly likely that this could get phosphorylated to maintain clofarabine triphosphate. There was heterogeneity in concentrations of clofarabine triphosphate measured in circulating blasts in patients on the study. This heterogeneity did not appear to be diagnosis dependent. Elimination profiles of clofarabine triphosphate indicated that presence of the cytotoxic metabolite was maintained in the leukemia cells with a half-life of 24 hours or more. Therefore, there would be residual triphosphate at 24 hours when a second infusion occurred. To support this data, the relationship between the pharmacokinetics of clofarabine triphosphate accumulation and clinical response at the MTD was pursued (Table 3).[9] A total of 30 patients – 19 responders and 11 non-responders, were studied for cellular pharmacokinetics of clofarabine triphosphate in leukemia blasts during therapy.

As shown in Table 3, at the end of the day 1 infusion, responders had accumulated a median of 18 μM of the triphosphate, while non-responders accumulated a median of 10 μM. Although there was a wide variation in the level of the triphosphate, there seems to be an accumulation advantage of the cytotoxic triphosphate in the leukemia cells of responders. At 24 hours after the first infusion of clofarabine, the values of the intracellular clofarabine triphosphate were 11 μM and 5 μM in responders and non-responders, respectively. These data indicate that in all patients, clofarabine triphosphate is retained well in the circulating leukemia blasts. To determine if there would be an incremental increase in the cytotoxic triphosphate after next infusion, cellular pharmacokinetics were compared after the end of 2nd infusion. At the end of the day 2 infusion, leukemia cells from responders had a median triphosphate accumulation of 30 μM while in the cells of non-responders the analog triphosphate level was 9 μM. This suggests that circulating leukemia blasts of patients who respond to clofarabine therapy exhibited a favorable pharmacokinetic profile.

Table 3. Relationship between pharmacokinetics of clofarabine triphosphate and clinical responses in patients with acute leukemias treated during phase II study.

	Median clofarabine triphosphate, μM (range)		
	Responders, 19	Nonresponders, 11	p
d1 end of infusion	18 μM (5–44)	10 μM (1–23)	0.032
at 24 hour	11 μM (1–44)	5 μM (1–20)	0.039
d2 end of infusion	30 μM (1–67)	9 μM (1–23)	0.015

(From Ref. [9].)

In summary, clofarabine is a promising new deoxyadenosine analogue that has shown activity in adult acute leukemias at a dose that is well tolerated. Phase II studies have established that a favorable pharmacokinetic profile of clofarabine triphosphate is associated with clinical responses observed with this agent. Additionally, clofarabine has shown efficacy as a single agent in the treatment of pediatric patients with refractory or relapsed AML or ALL.[10] The favorable toxicity profile, pharmacokinetics, and clinical activity of clofarabine have led to several multi-center trials with this nucleoside analog used as a single agent. As a potent inhibitor of ribonucleotide reductase, clofarabine is ideal to be incorporated into biochemical modulation strategies such as those tested and validated with fludarabine and ara-C.[11–13] The clinical responses of single agent clofarabine in acute leukemias and the biochemical modulation of cytarabine triphosphate[14] have led to the mechanism based combination therapy of clofarabine and cytarabine in the treatment of adult acute leukemias.[15]

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