



## The bioavailability of oral fludarabine phosphate is unaffected by food

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**Introduction:** A prospective, open and randomized, two-way crossover study was conducted to evaluate the pharmacokinetics and bioavailability of oral fludarabine phosphate when taken on a full *versus* an empty stomach. The effectiveness of therapy was also assessed after two cycles of treatment, four weeks apart

**Materials and methods:** Patients with chronic lymphocytic leukemia or low-grade non-Hodgkin's lymphoma were randomly assigned to two groups, both of which received two cycles of treatment with 90 mg of oral fludarabine phosphate administered when either fed or fasted. Patients in Group 1 ( $n=8$ ) received oral treatment on a full stomach for the first cycle then on a fasted stomach for the second, while those in Group 2 ( $n=10$ ) received their treatment in the reverse sequence. Oral fludarabine phosphate was administered on the first day of the two study cycles and intravenous fludarabine phosphate was administered on days 3–6.

**Results and conclusion:** Of 22 patients recruited, 18 (CLL  $n=10$ ; NHL  $n=8$ ) were eligible for efficacy and safety evaluation, and 16 for bioavailability and pharmacokinetic analyses. The response to oral 2-F-ara-AMP was rapid: by two treatment cycles, 12 out of 18 patients (66.7%) had achieved partial response. Of the six patients who did not respond, five patients (27.7%) had stable disease. There was no notable difference in the rate of response between patients with B-CLL and Ig-NHL. There was a marginal increase in total systemic availability of fludarabine phosphate when administered orally on a fed stomach (2-F-ara-A  $AUC_{(0-24\text{ h})} = 3.28 \pm 1.48 \mu\text{g}\cdot\text{h}/\text{ml}$ ) compared to a fasted stomach (2-F-ara-A  $AUC_{(0-24\text{ h})} = 3.05 \pm 1.56 \mu\text{g}\cdot\text{h}/\text{ml}$ ). Time to peak plasma concentration was slightly extended by the presence of food ( $2.2 \pm 1.0$  *versus*  $1.3 \pm 0.74$  h) but the terminal half-life was unaffected. The minor differences in the pharmacokinetics of oral fludarabine phosphate when taken after food were not statistically significantly different and seem unlikely to be clinically relevant. The efficacy and safety data closely paralleled previous experience with the intravenous formulation.

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### Introduction

Fludarabine phosphate (2-Fluoro-9-(5-O-phosphono- $\beta$ -D-arabinofuranosyl)-9H-purin-6-amine; 2-F-ara-AMP) is a fluorinated nucleotide analog of the antiviral drug vidarabine, and has been licensed for the treatment of patients with relapsed or refractory B-cell chronic lymphocytic leukemia (CLL). In this indication, fludarabine phosphate is the drug of choice.<sup>1</sup> Usually given at a dose of 25 mg/m<sup>2</sup>/day for five days (repeated every four weeks), this drug has proven to be a highly

effective second-line agent in CLL in a number of clinical trials with objective response rates (i.e. the sum of complete remissions [CR] and partial remissions [PR]) of 40 to 60% being reported.<sup>2,3</sup> Response and survival following treatment with fludarabine phosphate in CLL appear to be related to the stage of the disease, the extent of previous treatment, and a failure to respond to alkylating agents.<sup>4–7</sup>

Fludarabine phosphate was compared with the combination therapy cyclophosphamide, doxorubicin and prednisone (CAP), and the overall response rate was significantly higher in the fludarabine phosphate group (60% *vs* 44%;  $P=0.023$ ).<sup>8</sup> The overall response to fludarabine phosphate in previously treated patients was also significantly higher (48% *vs* 27%;  $P=0.036$ ).

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When compared with chlorambucil as first line treatment in a randomized, parallel-group study, the overall response rate of patients who received fludarabine phosphate was considerably higher (64% vs 39%), as was the CR rate (20% vs 5%). In addition, the progression-free survival interval was longer in the fludarabine phosphate group (20 months vs 14 months).<sup>9</sup> Fludarabine phosphate has also been investigated as a single-agent therapy in low-grade non-Hodgkin's lymphoma (Ig-NHL), with overall response rates of 52–75% being reported in previously treated patients.<sup>10–14</sup>

An oral formulation of fludarabine phosphate comprising an immediate-release tablet, containing 10 mg fludarabine phosphate, has been developed, and pharmacokinetic studies have been carried out to compare the oral and intravenous formulations in patients with indolent NHL and CLL.<sup>15,16</sup> In these cases oral administration resulted in dose-dependent increases in the maximum plasma concentration ( $C_{max}$ ) and the 24-h area under the concentration-time curve ( $AUC_{(0-24\text{ h})}$ )—the latter being similar to that seen following intravenous administration (after dose adjustment) with a lower  $C_{max}$ . Linear increases in  $AUC_{(0-24\text{ h})}$  corresponded well with increases in oral dosage, and bioavailability was approximately 55%. There was low intra-individual variation, and both time to  $C_{max}$  ( $t_{max}$ ) and the terminal half-life ( $t_{1/2z}$ ) have proven to be dose independent.<sup>15</sup>

The conclusions drawn from this and other pharmacokinetic studies are that a once-daily oral dose of approximately 40–50 mg/m<sup>2</sup> would provide systemic exposure equivalent to 25 mg/m<sup>2</sup>/day given intravenously.<sup>15–18</sup>

The present study aimed to establish the pharmacokinetics and systemic availability of fludarabine phosphate when administered orally to fasted and non-fasted patients. Although food was not anticipated to interfere with the absorption of fludarabine phosphate, a particularly fatty meal was selected in order to determine whether it would be necessary to place restrictions on administration of the drug – for example, recommending that it be taken only on an empty stomach. In addition to assessing the effects of food on the absorption of fludarabine phosphate, the study objectives also included an evaluation of the efficacy and safety of fludarabine phosphate after two cycles of treatment.

The primary pharmacokinetic parameters that were determined are the AUC for the periods 0–24 and 0–48 h ( $AUC_{(0-24\text{ h})}$  and  $AUC_{(0-48\text{ h})}$ , respectively), and the secondary parameters  $C_{max}$ ,  $t_{max}$  and  $t_{1/2z}$ .

## Patients and methods

The prospective, open, randomized, two-way crossover study was conducted at five hospitals in the United Kingdom. The study was conducted in accordance with the ICH Guidelines for Good Clinical Practice (July, 1996; CPMP/ICH/135/95) and in compliance with the

Declaration of Helsinki (Hong Kong Amendment, 1989). The protocol met all the requirements of the appropriate regulatory authority, and received formal approval from the Medical Ethics Committees of each collaborating center prior to any patient receiving treatment at that center.

## Patients

The inclusion criteria were: patients with previously treated CLL or previously treated Ig-NHL, of either gender, aged over 18 years, with WHO performance status grade 0–2, and willing and able to give written informed consent to participate.

Patients in Group 1 received their first oral dose of 2-F-ara-AMP on an empty stomach and their second oral dose on a full stomach. Patients in Group 2 received their first oral dose of 2-F-ara-AMP on a full stomach and their second dose on an empty stomach. Both groups received intravenous 2-F-ara-AMP on days 3–6.

## Exclusion criteria

The exclusion criteria for the study included: severe cardiac, pulmonary, metabolic, renal, hepatic, psychiatric or neurological diseases; patients with a body surface area less than 1.6 m<sup>2</sup> or greater than 2.2 m<sup>2</sup>; AIDS or AIDS-related complex; gastrointestinal disorders that could affect absorption of the drug; autoimmune hemolytic anemia or thrombocytopenia; and more than one previous cycle of fludarabine phosphate in a course of treatment immediately prior to enrollment. In addition, patients with prior malignancies, hairy cell leukemia or more aggressive B-cell cancers and those requiring regular systemic steroids were excluded. Pregnancy and lactation were also exclusion criteria. Furthermore, participants were instructed to practice an appropriate form of contraception during the study, and for at least six months after treatment with fludarabine phosphate.

Alternative cytotoxic drugs, regular systemic corticosteroids and medications that could influence drug absorption (e.g. H1-antihistamines, antacids, metoclopramide and charcoal) were prohibited.

## Clinical and laboratory assessments

Patients underwent full physical examinations at baseline, and again 48 h prior to each cycle of treatment. Height and weight were measured and body surface area calculated. In addition, full blood cell counts and 14-point blood biochemistry analyses were carried out at each examination. For the safety evaluation, the blood tests were repeated intermittently between the two cycles and again 28 days after the second cycle.

All adverse events were recorded and classified using the WHO toxicity criteria (grade 0=no toxicity, through to grade 4=severe toxicity). The investigators also assigned a relationship with the study medication

to each of the reported adverse events (from 'not related', through to 'definitely related').

### Study design

Although designed as a pharmacokinetic study, efficacy was evaluated by comparing each patient's disease status after two cycles of treatment with his or her baseline status, using the standard Binet (CLL) or Ann Arbor (NHL) staging criteria.

Following randomization, patients received two cycles of treatment containing 90 mg (as 10 mg tablets) of oral fludarabine phosphate (active ingredient: 2-Fluoro-9-(5-O-phosphono- $\beta$ -D-arabinofuranosyl)-9H-purin-6-amine) on the first day of each cycle, and intravenous fludarabine phosphate (25 mg/m<sup>2</sup>/day) on days 3–6. Patients in Group 1 received oral treatment on a fasted stomach for the first cycle then on a full stomach for the second, while those in Group 2 received their treatment in the reverse sequence.

For the cycle of treatment on a full stomach, patients ate a standardized breakfast consisting of bacon, egg, fried potatoes, toast, butter, jam and milk. The caloric content was estimated to be approximately 960 kilocalories (kCal) and comprised 57 g of fat (513 kCal), 30 g of protein (120 kCal) and 82 g of carbohydrate (327 kCal).

### Pharmacokinetic procedures

For the determination of pharmacokinetic parameters, blood samples were taken at time zero and then at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after the oral fludarabine phosphate dose on day 1. The samples were of approximately 5 ml volume and drawn from a peripheral vein through a catheter, or by direct venepuncture. Samples were immediately transferred to heparinized tubes and stored in the refrigerator (4°C approximately) prior to centrifugation. Subsequently, after centrifugation at 500 g for 20 min at 4°C, or at 2100 g for 10 min at ambient temperature, the plasma was separated and stored at –20°C until analysis.

Fludarabine phosphate (2-F-ara-AMP) is rapidly and quantitatively metabolized to its dephosphorylated nucleoside, 2-F-ara-A, when delivered intravenously, or by the oral route, in humans and in a variety of animal species. Determination of the bioavailability of fludarabine phosphate was therefore based on plasma levels of the metabolite 2-F-ara-A.

Protein removal from the plasma samples was effected using solid-phase extraction with Worldwide Monitoring™ C18 cartridges (200 mg Clean-Up® bonded silica) pre-conditioned with 2 ml of alkaline methanol and subsequently rinsed with 6 ml of water. Samples of 0.5–1.0 ml of thawed plasma were then applied to the cartridge and rinsed with 2.5 ml of water. After drying for 2 min, 2-F-ara-A was eluted with 2 ml of the alkaline methanol solution. All steps were carried out at approximately 700 mbar using a membrane diaphragm pump. After complete methanol

evaporation under a gentle stream of nitrogen, samples were again stored at –20°C.

The dry sample extracts were re-dissolved by vortex mixing in 0.1 ml of 0.6 M citrate buffer (pH 4.0) before the addition of 0.2 ml of 45% aqueous chloroacetaldehyde solution. The samples were subsequently incubated for 20 h at 50°C with gentle shaking. After derivatization, the samples were diluted to 3 ml with 0.1 M citrate buffer (pH 4.0).

Levels of 2-F-ara-A were determined by a valid and sensitive HPLC method with fluorescence detection after derivatization with chloroacetaldehyde.<sup>19</sup> Samples of 100  $\mu$ l volume were injected into a Kontron HPLC system (Kontron Instruments S.P.A., Milan, Italy) comprising a pump, autosampler and multiport connected to a Perkin Elmer LC240 fluorescence detector (Perkin-Elmer Ltd., Beaconsfield, UK). Ultra-sphere ODS columns with Spherisorb ODS2 pre-columns were used. The mobile phase consisted of a ternary solution with 2% (v/v) methanol and 5% (v/v) N,N-dimethylformamide in water. 2-F-ara-A was detected with a retention time of approximately 8 min at a flow rate of 1.0 ml/min.

Detection was performed with an excitation wavelength of 296 nm and total emission. The chromatograms were evaluated using ACCESS\*CHROM (Version 1.9.3; Perkin Elmer, 1994).

The samples were compared with external standards comprising human plasma spiked with 2-F-ara-A to produce concentrations of 1–200 ng/ml. Quality control samples (10, 50 and 200 ng/ml nominal concentrations of 2-F-ara-A in human plasma) were analysed in triplicate. The accuracy and precision of the assay were calculated using the quality control sample sets. The mean intra-assay variability was 7.1% (10 ng/ml), 7.3% (50 ng/ml) and 5.5% (200 ng/ml). The corresponding inter-assay variability was 14.1, 6.4 and 8.5%, respectively. The mean accuracy was 102.7, 102.7 and 101.0%, respectively, for the quality control sample sets.

Based on the quality control analyses, a quantitation limit of 0.167 ng was set (equivalent to 5 ng/0.5 ml sample volume). Pharmacokinetic evaluations were based on individual 2-F-ara-A plasma levels, and the parameters calculated by means of TOPFIT software (Version 2.1  $\beta$ ; Thomae, Gödecke, Schering) at Pharmacokinetics Therapeutics 1, Schering AG, Berlin, Germany, using a non-compartmental approach.

### Results

In compliance with the 1997 FDA Draft Guidance on Food-effect Bioavailability and Bioequivalence Studies (minimum acceptable number of 12 patients for such studies), 22 patients were initially enrolled in the study. Three of these failed to meet one or more of the study criteria for the following reasons: one patient with body surface area greater than the upper limit allowed by the protocol, withdrawn prior to dosing on cycle 1;

one patient with insulin-dependent diabetes, withdrawn prior to dosing on cycle 1; one patient judged too ill to enter the study; one patient showed myelosuppression and abnormal liver enzymes at the baseline examination. These four patients did not receive treatment and were therefore excluded.

The intent-to-treat (ITT) population of 18 patients comprised 14 males and four females (Table 1). Ten of the ITT patients presented with CLL and eight with NHL. Two patients were withdrawn from the pharmacokinetic study after the first dose of treatment; one for failing to eat sufficient breakfast and the other because her body surface area was below the stipulated minimum of 1.6 m<sup>2</sup>. Both patients were excluded from pharmacokinetic analyses and the calculation of bioavailability but were included in the safety and efficacy evaluations.

No pre-dose plasma samples contained detectable levels of 2-F-ara-A. Maximum plasma levels ranged from 0.16–0.78 µg/ml when the tablets were taken with food, and 0.17–1.06 µg/ml when taken on an empty stomach.

The oral administration of 2-F-ara-AMP to patients with CLL and NHL, when fed or fasted, resulted in slightly different plasma levels of 2-F-ara-A (Table 2). The rate of absorption was marginally reduced in the presence of a high fat meal and C<sub>max</sub> was reached slightly later, although t<sub>1/2z</sub> was not affected. A slightly increased total systemic availability of 2-F-ara-A was observed in patients taking the oral formulation of fludarabine phosphate after eating the high fat meal compared with the patients who fasted (Figure 1) although the mean values for AUC<sub>(0–24 h)</sub> and AUC<sub>(0–48 h)</sub> were not statistically significantly different (Table 2).

The response to oral 2-F-ara-AMP was rapid: after two cycles of treatment, 12 (66.7%) of the ITT population were deemed to be showing PR. A further five patients (27.7%) showed stable disease. There was no marked difference between the response rates amongst the patients with CLL and those with NHL.

**Table 1** Demographic characteristics of the ITT population

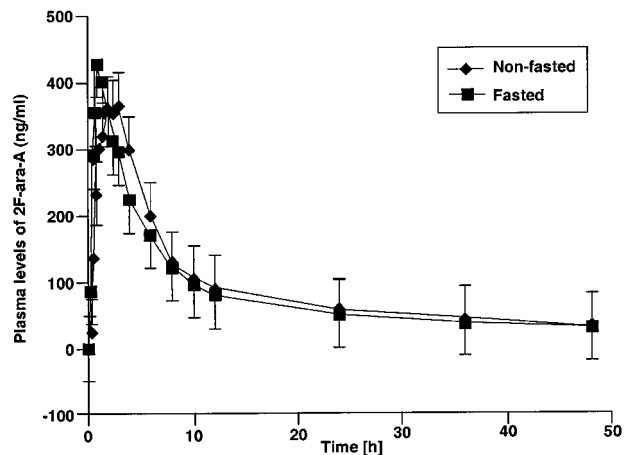
Characteristic	ITT population
Male	14
Female	4
Median (range) age in years	63 (44–79)
Median (range) height in cm	176 (153–190)
Median (range) weight in kg	79 (54–102)
Median (range) body surface area in m <sup>2</sup>	2.0 (1.54–2.19)
CLL baseline status (n=10; male 9, female 1)	
Binet stage A	3
Binet stage B	4
Binet stage C	3
NHL baseline status (n=8; male 5, female 3)	
Ann Arbor stage IIA	1
Ann Arbor stage IIIA	1
Ann Arbor stage IVA	5
Ann Arbor stage IVB	1
Group 1 (cycle 1 = non-fasted, cycle 2 = fasted)	8
Group 2 (cycle 1 = fasted, cycle 2 = non-fasted)	10*

\*Two patients withdrew after the first cycle.

Fifteen of the patients experienced at least one adverse event (AE) and eight patients (44.4%) had serious AEs during the entire treatment period (up to six cycles), of which one (severe thrombocytopenia) was considered probably related to treatment. This patient later required splenectomy. A total of 31 AEs were reported during the study, the most common being infection, pneumonia, fever, nausea, dizziness and pain. The most frequent toxicity was myelosuppression: 10 patients (55.6%) experienced grade 3 or grade 4 granulocytopenia and eight (44.4%) showed similar degrees of leukocytopenia. Other toxicities were infrequent and, in general, there were no unexpected signs of toxicity during the study.

## Discussion

In this population of patients with previously treated B-CLL (Binet stage B or C) or Ig-NHL (stages II–IV), 66.7% patients responded to treatment with oral fludarabine, a response rate comparable to previously reported figures (48.8% B-CLL,<sup>8</sup> 31% Ig-NHL<sup>20</sup>). Treatment with oral fludarabine was effective and well tolerated in this group of patients with severe underlying disease. The safety profile appeared similar



**Figure 1** Mean ± s.d. 2-F-ara-A plasma levels from fludarabine phosphate when taken orally with food and without food.

**Table 2** Summary of pharmacokinetic parameters

Parameter	Fasted Mean ± s.d. (n)	Non-fasted Mean ± s.d. (n)
AUC <sub>(0–24 h)</sub> µg·h/ml	3.05 ± 1.56 (16)	3.28 ± 1.48 (16)
AUC <sub>(0–48 h)</sub> µg·h/ml	3.91 ± 1.77 (15)	4.23 ± 1.76 (15)
C <sub>max</sub> ng/ml	488 ± 279 (16)	442 ± 181 (16)
t <sub>max</sub> h	1.3 ± 0.74 (16)	2.2 ± 1.0 (16)
t <sub>1/2z</sub> h	26.5 ± 10.3 (14)	26.9 ± 12.7 (14)

to that previously reported,<sup>15</sup> with myelosuppressive effects, infection and fever being the most common adverse events.

Gastrointestinal AEs are of particular importance in the assessment of any oral formulation, since nausea, vomiting or diarrhea not only make the drug unpalatable or unacceptable to the patient but, more significantly, affect the plasma concentration of the drug. The low incidence of such gastrointestinal effects in the present study suggests that oral fludarabine phosphate is well tolerated.

As patients with chronic hematological malignancies such as CLL are typically over 60 years of age, it is sometimes difficult to obtain adequate venous access—particularly in individuals who have undergone several prior treatments. Furthermore, the increased risk of concomitant infection in this age group makes the use of indwelling catheters for intravenous administration less attractive.

In the scenario of palliative therapy, the oral formulation of fludarabine phosphate appears to offer certain advantages, including high efficacy, a relatively benign gastrointestinal AE profile, ease of administration and the fact that doses may be taken on a fed or a full stomach. The added advantage of a more convenient dosing form that can be self-administered at home would also alleviate the potential stress of travel and in-patient stays for patients to whom quality of life is a significant factor.

It is also important to establish whether an oral formulation is sufficiently robust to be used without specific dietary restrictions or, if any restrictions do prove necessary, that they are clearly defined. The objective of the present study was to evaluate the effect of a particularly fatty meal on the bioavailability of oral fludarabine phosphate.

That there were no significant differences in the pharmacokinetics of oral fludarabine phosphate when taken with or without food, confirms that the drug may be taken without dietary restrictions. The marginal (<10%) increase in the total systemic availability and the slight delay in reaching maximum plasma concentration when taken after food intake, were considered unlikely to have any clinical relevance. The fact that terminal half-life was unaffected indicates that digestion of fatty foods does not interfere with the excretion of the oral formulation.

In a multicenter, uncontrolled, open-label study<sup>21</sup> involving 78 patients with previously treated B-CLL who received oral fludarabine phosphate at a dose of 40 mg/m<sup>2</sup>/day for five days every four weeks for six cycles, there were 16 complete and 20 partial remissions according to the IWCLL criteria. The overall response rate was 46.2% (36 out of 78 patients with a 95% confidence interval of 34.8 to 57.8%), comparable to published response rates with the intravenous formulation.<sup>8</sup> The toxicity profile in this study was also similar to that observed with intravenous fludarabine phosphate. Thus, considering the low incidence of nausea in patients taking the drug on an empty stomach in the current study, combined with lack of any significant alteration in the bioavailability of fludarabine phosphate if taken with food, it appears that the formulation is robust, and may offer a potentially valuable alternative to intravenous delivery in the treatment of CLL or NHL.

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