Cancer Chemother Pharmacol (2000) 45: 423–427

ORIGINAL ARTICLE

**John D. Roberts · Stephen Shibata · Darcy V. Spicer
Howard L. McLeod · Mary Beth Tombes · Brenda Kyle
Mary Carroll · Beth Sheedy · Mary A. Collier
Yazdi K. Pithavala · Linda J. Paradiso
Neil J. Clendeninn**

Phase I study of AG2034, a targeted GARFT inhibitor, administered once every 3 weeks

Received: 18 May 1999 / Accepted: 25 October 1999

Abstract *Purpose*: To identify a recommended phase II dose for the second generation glycinamide ribonucleotide transformylase (GARFT) inhibitor, AG2034, administered by intravenous bolus every 3 weeks without folate supplementation and to describe AG2034 pharmacokinetics. *Methods*: Adults with advanced malignancies were enrolled in cohorts of three per dose level with expansion to six upon observation of dose-limiting toxicity (DLT). The maximum tolerated dose (MTD) was defined as the dose at which two of up to six patients experienced DLT. Upon identification of an MTD and evidence of cumulative toxicity, a lower intermediate dose was explored as a candidate phase II dose. AG2034 plasma concentrations were measured using an ELISA assay. *Results and conclusions*: The recommended phase II dose is 5.0 mg/m^2. DLTs were anemia, thrombocytopenia, mucositis, diarrhea, hyperbilirubinemia, fatigue, and insomnia. Toxicities were modestly cumulative over three courses. Pharmacokinetic analysis showed a dose-AUC_{0-24} relationship and a progressive increase in AG2034 AUC_{0-24} over three courses. Both pharmacokinetic and pharmacodynamic factors may contribute to the modest cumulative toxicity observed with AG2034.

Key words AG2034 · Glycinamide ribonucleotide transformylase inhibitor · Phase I study

This work was supported by Agouron Pharmaceuticals, Inc.

J. D. Roberts (✉) · M. B. Tombes · B. Kyle
Massey Cancer Center, Richmond, VA 23298-0037, USA
e-mail: roberts@mcc1.mcc.vcu.edu
Tel.: +1-804-8280450; Fax: +1-804-8285406

J. D. Roberts · M. B. Tombes · B. Kyle
Massey Cancer Center,
Virginia Commonwealth University,
Richmond, VA 23298-0037, USA

S. Shibata · M. Carroll
City of Hope Medical Center, 1500E. Duarte Road,
Duarte, CA 91010, USA

D. V. Spicer
USC/Norris Comprehensive Cancer Center,
University of Southern California, 1441 Eastlake Avenue,
Los Angeles, CA 90033-0800, USA

H. L. McLeod
Department of Medicine and Therapeutics,
University of Aberdeen, Aberdeen, AB25 2ZD, UK

B. Sheedy · M. A. Collier · Y. K. Pithavala · L. J. Paradiso
N. J. Clendeninn
Agouron Pharmaceuticals, Inc.,
10350 North Torrey Pines Road, San Diego, CA 92037, USA

Introduction

Glycinamide ribonucleotide formyltransferase (GARFT), a folate-dependent enzyme, is an essential enzyme of the de novo purine synthesis pathway. In comparison with normal tissues, most tumor cell lines have elevated activity of the de novo pathway and decreased activity of the salvage pathway [1]. This suggests that selective inhibitors of de novo purine biosynthesis might have use as anticancer agents. Lometrexol, or 5,10-dideaza-5,6,7,8-tetrahydrofolate, the prototype selective GARFT inhibitor, was synthesized as a methotrexate analog and was only subsequently shown to have this novel mechanism of action [2]. Lometrexol demonstrates a broad range of antitumor activity in murine and human tumor xenograft models [3, 4] and has produced objective responses against a variety of human cancers in phase I trials [5–9]. Lometrexol has yet to enter phase II trials, however, largely due to serious problems with cumulative toxicity, especially myelotoxicity, in numerous phase I trials. In numerous clinical studies it has been shown that the toxic dose of lometrexol is highly dependent upon recent folate intake [5, 7–9]. Concurrent folic acid supplementation not only increases the toxic dose of lometrexol, but also largely eliminates the cumulative nature of lometrexol toxicity. Preclinical studies suggest that this is not a threshold effect reflecting folic acid "deficiency", but rather the toxic dose of lometrexol varies across a wide range of levels of folate intake [10].

AG2034 is a second generation GARFT inhibitor designed using knowledge of the X-ray crystal structures of GARFT from *Escherichia coli* and of the GARFT domain of the human trifunctional enzyme (Fig. 1) [11]. Analysis of the GARFT active site using the program GRID [12] suggests that sulfur atoms should have particular affinity for two regions of the folate cofactor binding site. The design of AG2034 represents an attempt to satisfy this condition while retaining substrate specificity for the reduced folate carrier and for folylpolyglutamate synthetase (FPGS) [13]. In preclinical studies, AG2034 has been shown to be similar to lometrexol as a GARFT inhibitor and as a substrate for FPGS, but to bind more avidly to the membrane folate binding protein. In in vitro and in vivo anticancer test systems, AG2034 has shown a broad spectrum of activity similar to that of lometrexol with somewhat more potency.

In anticipation of clinical trials, AG2034 toxicity studies were performed in mice and dogs (Agouron Pharmaceuticals, data on file). Dogs were observed to be more sensitive to the effects of AG2034. This may be due to the relatively low folate intake and tissue folate levels of laboratory dogs (similar to humans) as compared with laboratory mice. The highest no-effect dose in dogs when AG2034 was administered daily for 5 days was 4 mg/m^2 per day. Toxic effects appeared to target the hematopoietic and gastrointestinal systems. Whereas lometrexol is eliminated almost exclusively in the urine, studies in the rat using ^{14}C-AG2034 have shown significant excretion in both the urine and feces. The basis for this difference is unknown. Pharmacokinetic studies in the dog and rat have shown half-times measured in hours.

We set out to study the dose-related toxicity of AG2034 administered by intravenous bolus every 3 weeks. Although preclinical studies had suggested that AG2034 might show more antitumor activity with frequent administration schedules, the clinical experience with lometrexol of severe cumulative toxicity suggested that it might be more prudent to study an infrequent administration schedule first. Although from the lometrexol experience one might infer that optimal administration of other GARFT inhibitors would require coadministration of folate, it remained possible that biochemical, disposition, and possibly other unknown differences between lometrexol and AG2034 would render coadministration of folate unnecessary. We elected to initiate clinical study of AG2034 without scheduled folate supplementation.

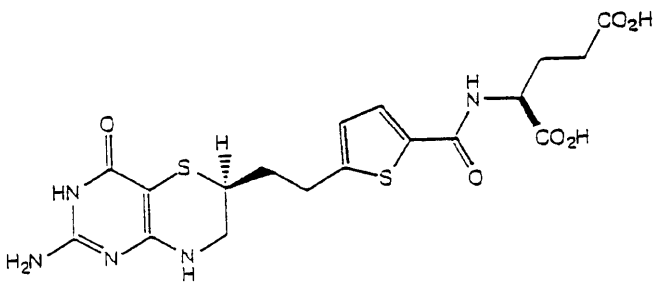

Fig. 1 Structure of AG2034

Methods

The study was conducted at the Massey Cancer Center, Virginia Commonwealth University, the City of Hope Medical Center, and the University of Southern California. Sample analysis and pharmacokinetic modeling were performed at the University of Aberdeen. AG2034 [4-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4-6][1,4]thiazin-6-yl)-(*S*)-ethyl]-2,5-thienoyl-L-glutamic acid] as the disodium salt was provided by Agouron Pharmaceuticals in 5-ml glass vials as a lyophilized powder containing 20 mg AG2034 (calculated as the free acid), 28.4 mg Na_2HPO_4, and 100 mg mannitol.

Patients were adults with histologically or cytologically confirmed malignancies for whom no satisfactory treatment existed at the time of enrollment; WHO performance status < 2; life expectancy of at least 3 months; 4 weeks from prior chemotherapy and radiation therapy; and acceptable bone marrow (absolute granulocyte count $\geq 1.5 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobin ≥ 10 g/dl), renal (creatinine < 1.5 mg/dl or calculated creatinine clearance > 60 ml/min), and liver (AST or ALT not more than twice the institutional upper limit of normal unless clearly due to the presence of tumor; total bilirubin ≤ 5 mg/dl) status. Pregnant or nursing women and patients with a continuing requirement for allopurinol treatment, prior therapy with other GARFT inhibitors, or other medical conditions that might compromise realization of study objectives were not eligible. Measurable or evaluable disease was not an eligibility requirement, but responses were assessed in patients in whom this was possible. Prestudy evaluation included history and physical examination; performance status assessment; electrocardiogram; urinalysis; complete blood cell count with differential, platelets, and reticulocytes; serum chemistries including electrolytes, calcium, total protein, albumin, BUN, creatinine, uric acid, total bilirubin, LDH, alkaline phosphatase, ALT or AST, serum iron and iron binding capacity; calculated creatinine clearance; and tumor assessment by physical examination and/or imaging. Patients gave informed consent and agreed to practice a medically acceptable form of contraception. Appropriate approvals were obtained from the institutional review boards of the participating institutions.

AG2034 was administered as a short (<5 min) infusion every 3 weeks. Extradietary folate supplementation was prohibited, except that leucovorin was permitted to attempt to reverse exceptional toxicity (but, in fact, was not used). A complete blood cell count and reticulocyte percentage was obtained twice weekly during the first three cycles and at least once weekly thereafter. An interval history, serum chemistries, and urinalysis by dipstick were obtained weekly. A physical examination was performed prior to each dose. The first scheduled tumor reassessment was performed following the third dose. All adverse events were identified, and those considered to be possibly related to AG2034 were graded according to the Agouron Adverse Events Criteria. This instrument is an adaptation from the NCI Common Toxicity Criteria Version 1.0. As it happened, these criteria were identical to the NCI CTC v1.0 for adverse events that defined dose-limiting toxicity (DLT) for the study. DLT was defined as grade ≥ 3 anemia and nonhematological toxicity (excluding nausea and vomiting) and grade 4 granulocytopenia and thrombocytopenia.

Patients were enrolled in cohorts of three per dose level with expansion to six upon observation of DLT. Within a dose level, patients receiving only a single course for reasons other than toxicity were replaced. Intrapatient dose escalation was not permitted. The starting dose was based upon toxicology studies in dogs, as this species is more sensitive to AG2034 than mice. The highest no-effect dose in dogs upon administration of AG2034 daily for 5 days was 4 mg/m^2 per day. Upon this basis, a starting dose of 1 mg/m^2

was selected. Dose escalation was permitted following observation of first courses in three patients and a second course in one patient. Doses were escalated by 50% to 100% according to prospective criteria concerning the nature and severity of toxicity observed in the previous cohort. DLT occurring within a patient's first three treatment courses was considered in the evaluation of maximum tolerated dose (MTD), which was defined as the dose at which two of up to six patients experienced DLT. Upon identification of an MTD and evidence of cumulative toxicity, a lower, intermediate dose was explored as a candidate phase II dose.

During the first course of treatment, blood samples were obtained prior to drug administration and 5, 15, 30 and 45 min, and 1, 1.5, 2, 4, 6, 8,12, 24, 48, 72 and 96 h after bolus injection for the evaluation of AG2034 pharmacokinetics. During the second and third courses of treatment, blood samples were obtained prior to therapy and 5 and 30 min, and 1 and 24 h after drug injection. AG2034 plasma concentrations were measured using an ELISA assay, which is described in detail elsewhere (McLeod et al., in preparation). The assay has a linear range of 1–500 ng/ml and interassay coefficient of variation of 6.7–8.2%. AG2034 area under the concentration-time curve (AUC) was determined for each patient using the trapezoidal rule. Noncompartmental pharmacokinetic analysis was restricted to the first 24 h after injection (AUC_{0-24}) to allow greater comparison between different courses of study drug, as sampling times in course 1 were different from the remaining courses. A European phase I study of AG2034 was conducted concurrently with this study and will be the subject of a separate report. A more comprehensive pharmacokinetic analysis, incorporating data from both studies and including evaluation of a prolonged terminal elimination phase, is described elsewhere (McLeod et al., in preparation).

Results

A total of 32 patients were treated with AG2034 (Table 1). Patient characteristics, prior therapies, and primary tumor sites were typical for subjects enrolled in phase I trials of new agents.

Of the 32 patients, 18 were treated at dose levels ranging from 1.0 to 5.0 mg/m^2 without DLT (Table 2). Three patients completed first courses at 7.5 mg/m^2 without DLT, and four patients were enrolled at 11.0 mg/m^2. Subsequently, two patients enrolled at 7.5 mg/m^2 experienced DLT following second and third courses. In view of this, two patients enrolled at 11.0 mg/m^2 and continuing treatment received subsequent courses at reduced doses. Of the four patients enrolled at 11.0 mg/m^2, two experienced DLT. Finally, of seven patients enrolled at 6.0 mg/m^2, three experienced DLT. Five of seven DLT events occurred with second or third cycles, consistent with a cumulative aspect to toxicity, although in general toxicities rapidly reversed between courses and following cessation of therapy. One patient with stable disease underwent nine courses of treatment. In this patient, anemia and thrombocytopenia caused dose delays during courses seven through nine and incompletely resolved subsequently, although previously unsuspected bone marrow involvement with tumor may have contributed to this. Details of all DLTs are shown in Table 2. Other grade ≥3 toxicities observed were anorexia, back pain, chills, constipation, and nausea.

AG2034 pharmacokinetics were evaluable in 25 patients receiving 1 to 11 mg/m^2. AG2034 AUC_{0-24} demonstrated a linear relationship with dose ($r^2 = 0.51$), with variable drug exposure at each dose level (Table 3). There was evidence of drug accumulation as AG2034

Table 1 Patient characteristics[a]

Gender	
Male	21 (66%)
Female	11 (34%)
Age (years)	
Median	57
Range	26–80
Ethnicity	
Caucasian	26 (82%)
Hispanic	3 (9%)
Asian	1 (3%)
Black	2 (6%)
Performance status	
0	7
1	20
2	5
Prior therapy	
Chemotherapy and radiation therapy	15 (47%)
Chemotherapy	14 (45%)
No chemotherapy or radiation therapy	3 (9%)
Tumor types	
Non-small-cell lung cancer	8
Colorectal	8
Renal	2
Mesothelioma	2
Melanoma	2
Head and neck squamous cell	2
Adenocarcinoma of unknown primary	1
Breast	2
Gastric	1
Ovary	1
Cervix	1
Sarcoma	1
Adenoid cystic carcinoma	1

[a] Two patients enrolled but not treated are not included

Table 2 Dose escalation and DLT events

Cohort	Dose (mg/m^2)	Patients	Courses	Patients with DLT
1	1	3	12	0
2	1.5	5	14	0
3	2.25	3	9	0
4	3.4	4	11	0
5	5	3	15	0
6	7.5	3	8	2[a]
7	11[b]	4	10	2[c]
8	6	7	17	3[d]

[a] One patient with grade 3 diarrhea and grade 3 mucositis following the second course; one patient with grade 4 thrombocytopenia and grade 3 anemia following the third course

[b] Dose reduced to 7.5 mg/m^2 or lower following first cycle in two patients

[c] One patient with grade 3 fatigue, grade 3 insomnia, grade 3 anemia, and grade 3 thrombocytopenia following the second course; one patient with grade 3 insomnia despite dose reduction to 7.5 mg/m^2 at the second course

[d] One patient with grade 3 fatigue following the first course; one patient each with grade 3 and grade 4 hyperbilirubinemia following the second course

Table 3 AG2034 AUC_{0-24} (μg/ml · min). Values are medians and range, or individual values for data sets less than three (*n* number of patients)

Dose (mg/m^2)	Course 1		Course 2		Course 3	
	n	AUC	*n*	AUC	*n*	AUC
1	3	20.4 (12.2–35.0)	2	51.7, 59.0	2	61.1, 92.7
1.5	5	32.0 (24.9–50.6)	4	70.8 (24.1–91.2)	3	95.1 (92.8–99.7)
2.25	2	64.5, 68.9	1	225.4	1	144.4
3.4	2	118.5, 190.9	2	239.3, 348.2	2	194.6, 266.8
5	2	99.4, 113.7	2	127.7, 256.0	1	329.4
6	4	191.6 (167.0–337.9)	4	465.8 (228.8–478.1)	2	732.3, 705.0
7.5	3	118.3 (66.1–179.3)	3	390.5 (330.5–448.3)	2	208.8, 480.3
11	4	300.2 (95.7–546.3)	2	576.4, 785.5	0	

AUC_{0-24} demonstrated a stepwise increase from course one to three in 9 of 13 patients with complete studies at all three courses (Fig. 2).

Although five patients were observed to have stable disease and received one to six courses additional to the first three, no patients experienced tumor responses.

Discussion

AG2034 showed much less cumulative myelosuppression than has been reported with lometrexol. This may reflect intrinsic differences in the drugs, or it may be an artifact of the manner in which each was studied. Most of the experience with lometrexol has involved doses in the range 1.0–6.0 mg/m^2 administered approximately weekly. Sessa et al. abandoned a daily for 3 days every 4 weeks schedule upon identification of cumulative myelosuppression with doses in the range of 3.0–4.0 mg/m^2 [8]. Their experience with lometrexol at 1.5–2.25 mg/m^2 daily for 3 days every 4 weeks may have been similar to ours with AG2034 at 5.0 mg/m^2 administered every 3 weeks. Thus, it is unclear whether the absence of severe, cumulative myelosuppression in our study represents an intrinsic difference between the two drugs or a decision on our part, influenced by familiarity with the lometrexol experience, not to explore higher doses.

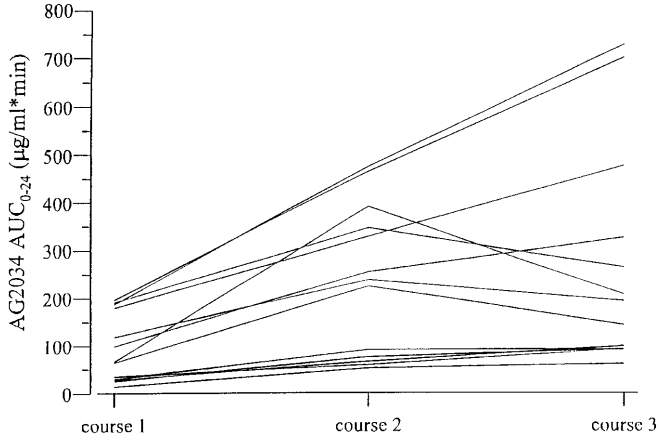

Fig. 2 AG2034 AUC_{0-24} in 13 patients with complete data for courses one through three

Like lometrexol, AG2034 is associated with mucositis and diarrhea. Unlike lometrexol, hyperbilirubinemia, fatigue, and insomnia contribute to the AG2034 toxicity profile. Whereas sporadic responses have commonly been observed in the lometrexol phase I experience, no responses were observed in this study. This, again, could reflect intrinsic differences between the drugs or failure to explore higher (albeit, potentially intractable) dose levels in our study.

There have been two preliminary reports of phase I studies with the GARFT inhibitor, LY309887, which differs chemically from AG2034 in that it does not have a sulfur substituent within the pteridine ring [14, 15]. One study involved a weekly schedule, another involved administration every 3 weeks. In both studies, based upon the lometrexol experience, all patients received concurrent folic acid. The preliminary results suggest that, despite concurrent folic acid, cumulative toxicity is a feature of LY309887. Also of interest, these studies reveal peripheral neuropathy as a DLT for this agent. In contrast, three patients experienced grade I peripheral neuropathy after receiving AG2034.

AG2034 pharmacokinetics are highly variable, similar to those described for lometrexol [16]. However, a dose-AUC_{0-24} relationship was observed, suggesting that nonlinear metabolism is not a feature of AG2034 pharmacokinetics at the doses used in this study. There was a progressive increase in AG2034 AUC_{0-24} over three courses of treatment, but the rate of accumulation demonstrated a high level of interpatient variability. The basis for accumulation has not been elucidated. This phenomenon might contribute toward cumulative toxicity. It should not be inferred, however, that cumulative toxicity with AG2034 is exclusively due to cumulative pharmacokinetics. Although lometrexol pharmacokinetics beyond the first course of treatment have not been reported, first-course lometrexol pharmacokinetics are not affected by concurrent folic acid administration, yet the cumulative nature of lometrexol toxicity is dramatically altered by this intervention. This suggests that pharmacodynamic factors play a role in cumulative lometrexol toxicity. Similar factors may affect AG2034 as well.

AG2034 can be administered safely every 3 weeks at doses up to 5.0 mg/m^2 without folate supplementation.

DLTs are anemia, thrombocytopenia, mucositis, diarrhea, hyperbilirubinemia, fatigue and insomnia. Within this dose limit, toxicities are modestly cumulative over three courses. Doses in the range 6.0 to 11 mg/m^2 generally are tolerated as single doses, but repeated dosing usually is associated with DLT. Further study of AG2034, including studies of alternate schedules and studies with concurrent folic acid administration, is warranted.

References

1. Jackson RC, Harkrader RJ (1981) The contributions of the de novo and salvage pathways of nucleotide biosynthesis in normal and malignant cells. In: Tattersall MHN, Fox RM (eds) Nucleosides and cancer treatment. Academic Press, Sydney, pp 18–31
2. Beardsley GP, Moroson BA, Taylor EC, Moran RG (1989) A new folate antimetabolite, 5,10-dideaza-5,6,7,8-tetrahydrofolate, is a potent inhibitor of de novo purine synthesis. J Biol Chem 264: 328–333
3. Shih C, Grindey GB, Houghton PJ, Houghton JA (1980) In vivo antitumor activity of 5,10-dideazatetrahydrofolic acid (DDATHF) and its diastereomeric isomers. Proc Am Assoc Cancer Res 29: 283
4. Taylor EC (1990) New pathways from pteridines. Design and synthesis of a new class of potent and selective antitumor agents. J Heterocycl Chem 27: 11
5. Young CW, Currie VE, Muindi JF, Saltz LB, Pisters KMW, Esposito AJ, Dyke RW (1992) Improved clinical tolerance of lometrexol with oral folic acid. Proc Am Assoc Cancer Res 33: 406
6. Ray MS, Muggia FM, Leichman CG, Grunberg SM, Nelson RL, Dyke RW (1993) Phase I study of (6R)-5,10-Dideazatetrahydrofolate: a folate antimetabolite inhibitory to de novo purine synthesis. J Natl Cancer Inst 85: 1154–1159
7. Laohavinij S, Wedge SR, Lind MJ, Bailey N, Humphreys A, Proctor M, Chapman F, Simmons D, Oakley A, Robson L, Gumbrell L, Taylor GA, Thomas HD, Boddy AV, Newell DR, Calvert AH (1996) Phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid. Invest New Drugs 14: 325–335
8. Sessa C, Jong J de, D'Incalci M, Hatty S, Pagani O, Cavilli F (1996) Phase I study of the antipurine antifolate lometrexol (DDATHF) with folinic acid rescue. Clin Cancer Res 2: 1123–1127
9. Roberts JD, Poplin EA, Tombes MB, Kyle B, Spicer DV, Grant S, Synold T, Moran R (2000) Weekly lometrexol with daily oral folic acid is appropriate for phase II evaluation. Cancer Chemother Pharmacol 45: 103–110
10. Alati T, Worzalla JF, Shih C, Bewley JR, Lewis S, Moran RG, Grindey GB (1996) Augmentation of the therapeutic activity of lometrexol [(6-R)5,10-dideazatetrahydrofolate] by oral folic acid. Cancer Res 56: 2331–2335
11. Almassy RJ, Janson CA, Kan CC, Hostomaska Z (1992) Structure of Apo and complexed *Escherichia coli* glycinamide ribonucleotide transformylase. Proc Natl Acad Sci USA 89: 6114–6118
12. Goodford PJ (1985) A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J Med Chem 28: 849–857
13. Boritzki TJ, Barlett CA, Zhang C, Howland EF, Margosiak SA, Palmer CL, Romines WH, Jackson RC (1996) AG2034: a novel inhibitor of glycinamide ribonucleotide formyltransferase. Invest New Drugs 14: 295–303
14. Aylesworth C, Baker SD, Stephenson J, Monroe P, Sanchez I, Walling J, Johnson R, Von Hoff D, Rowinsky E (1998) Phase I and pharmacokinetic (PK) study of the glycinamide ribonucleotide formyltransferase inhibitor LY309887 as a bolus every 3 weeks with folic acid (FA). Proc Am Soc Clin Oncol 17: 225a
15. Budman DR, Barile B, Johnson R, Vinciguerra V, Allen SL, Kolitz J, Kreis W, Zervos P, Walling J (1998) Phase I trial of LY 309887: a specific inhibitor of purine biosynthesis. Proc Am Soc Clin Oncol 17: 224a
16. Wedge SR, Laohavnjj S, Taylor GA, Boddy A, Calvert AH, Newell DR (1995) Clinical pharmacokinetics of the antipurine antifolate (6R)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) administered with an oral folic acid supplement. Clin Cancer Res 1: 1479–1486