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ORIGINAL ARTICLE

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# Phase I and pharmacokinetic study of LY309887: a specific inhibitor of purine biosynthesis

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**Abstract** *Purpose*: In this phase I trial in humans the safety and pharmacology of LY309887 on a weekly schedule combined with daily oral 5-mg doses of folic acid were evaluated. *Background*: LY309887 is an inhibitor of folate-dependent enzymes involved in de novo purine biosynthesis and has a broad preclinical antitumor activity. In murine systems, combining this agent with exogenous folic acid results in an enhanced therapeutic index. *Methods*: This study was a single-institution, open-label, clinical trial of dose escalation with toxicity and pharmacokinetic parameters determined. The dose range studied was 0.5–4 mg/m$^2$ per week ×6 and then a modified schedule weekly ×3 every 6 weeks. *Results*: Dose-limiting toxicities were of delayed onset and associated with hematologic, neurologic, and mucosal effects. Pharmacokinetic parameters revealed dose linearity for AUC and $C_{max}$. Low circulating levels of drug persisted for over 200 h. Urinary excretion accounted for approximately 50% of the parent drug but was highly variable. The urinary excretion was near maximal within 24 h of dosing. *Conclusions*: The modified dosing schedule allowed repetitive dosing in patients. Further evaluation of the 2 mg/m$^2$ per week ×3 every 6 weeks with daily oral folate supplement as a potential phase II dose may be warranted.

**Keywords** GARFT inhibitor · Phase I · Pharmacology

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## Introduction

The use of inhibitors of folic acid function and metabolism has been the backbone of many clinically useful treatments of human malignancies for the last four decades [1]. Unfortunately, despite the development of numerous folate antagonists, only methotrexate and trimetrexate which have major effects on the enzyme dihydrofolate reductase have been commercially released in the United States, with the latter being most useful in *Pneumocystis carinii* infections [2]. A parallel course of development has been to look at inhibition of other folate-dependent enzymes with glycinamide ribonucleotide formyltransferase (GARFT) as a major target, as this enzyme is the first folate-dependent enzyme in de novo purine synthesis [3].

In the 1980s, a series of dideaza-aminopterin compounds with inhibitory activity against GARFT were synthesized and demonstrated to have antitumor activity in preclinical systems [4, 5]. Inhibition of this enzyme was associated with preclinical antitumor activity in cell lines not sensitive to methotrexate. These agents show weaker inhibition of other folate-dependent purine biosynthetic enzymes such as aminoimidazolecarboximide ribonucleotide formyltransferase (AICARFT) [6]. Lometrexol (5,10-dideaza-5,6,7,8-tetrahydrofolate), the first GARFT inhibitor developed for clinical use [7, 8], is associated with delayed severe mucositis and myelosuppression when administered in humans. This unexpected finding led to an intensive evaluation of the role of the functional folate status of the treated patient, the expression of functional folate receptors [9], the effect of folate balance on polyglutamylation of the drug, and finally of organ drug disposition of the antifol [10, 11].

In the mouse, low total body folate stores are associated with increased retention of lometrexol in the liver with enhanced polyglutamylation thus leading to a prolonged drug effect [10]. The addition of oral folic acid supplementation in the mouse enhances the therapeutic

index. However, exogenous folate beyond an optimal range inhibits the antitumor effect of this drug [12]. An initial human study using oral 5 mg folic acid daily for 2 weeks with monthly bolus injections of lometrexol demonstrated enhanced plasma folate concentrations and the ability to administer three- to tenfold higher dosages of this drug than could be done without folate supplementation [13]. A pronounced intersubject variability in dose-related toxicities has been noted [13]. Another clinical study using a high dose of folate (60 mg folinic acid per day) has shown the ability to deliver a fivefold increase in drug dose [14]. In other studies using an intravenous bolus schedule of folate administration, it was not possible to reduce the occurrence of mucositis and thrombocytopenia [15]. In the latter study, lometrexol was shown to accumulate in red blood cells and to correlate with cumulative toxicity [16].

To enhance the antitumor effect and potentially lessen drug toxicity, analogues of lometrexol have been developed with properties of higher binding affinities for GARFT (as much as 70-fold compared to lometrexol), lower polyglutamylation, higher affinity for the $\alpha$ isoform of the folate receptor overexpressed in many tumors, less affinity for the membrane folate-binding protein ($\beta$ isoform) found in the liver, and activity in lometrexol-resistant cell lines [3]. The racemic mixture (LY254155) [3] of one of these second-generation compounds and the resulting isomers LY309886 and LY309887 were studied as candidate drugs in humans.

LY309887 (6*R*-2′,5′-thienyl-5,10-dideazatetrahydrofolic acid; Fig. 1) with a molecular weight of 493.4 is a potent inhibitor of GARFT ($K_i$ 6.5 n*M*), is freely soluble in aqueous solutions, and has potent antitumor activity against human colon, lung, mammary, and pancreatic xenograft models [17]. LY 309887 is attractive for clinical development because of the following properties: an affinity ratio of 10.5 for the $\alpha$ isoform of the membrane folate-binding protein compared to the $\beta$ isoform, more activity than the *S*-diastereomer, less polyglutamylation than LY309886, and activity over a broad dose range when used in an intermittent schedule in the mouse [18]. In the mouse, folate supplementation enhances the therapeutic index of this compound [18]. Chronic dosing has revealed that the dog is the most sensitive animal with dose-limiting toxicity of gastroenteritis. On a weekly schedule with folate supplementation, the low toxic dose in the dog is 0.25 mg/kg per week (body surface area equivalent of 5 mg/m$^2$ per week) [17].

**Chemical Structure**

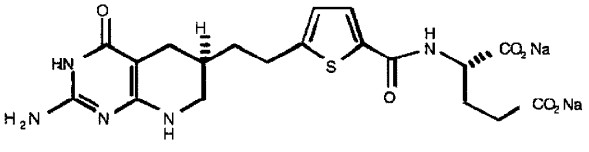


**Fig. 1** LY309887 is an antifolate with particular specificity for GARFT

This report describes a single-institution, open-label study in which the clinical findings and pharmacology of LY309887 were evaluated following administration to cancer patients using dosages from 0.5 to 4.0 mg/m$^2$ on a weekly schedule with daily folic acid supplementation. The study built upon the prior experience of folate supplementation in the human trials of lometrexol, with the addition of the major cytotoxic effect observed in preclinical systems following frequent repetitive dosing.

## Materials and methods

Patients with histologically confirmed, inoperable, malignant solid tumors who had previously failed conventional therapy or where no consensus on therapy existed were recruited for the study. All patients had to sign informed written consent as per institutional, Federal Drug Administration, and Occupational Safety and Health Administration guidelines. Entry criteria included ECOG performance status 0–2, age greater than 18 years, recovery from previous therapy with at least 3 weeks from cytotoxic treatment (6 weeks for mitomycin or nitrosourea), negative pregnancy test for females and use of an effective contraceptive method if premenopausal, white blood cell (WBC) count $\geq 3.0\times10^3$/mm$^3$, hemoglobin $\geq$9 g/dl, hematocrit $\geq$27%, platelets $> 100\times10^3$/mm$^3$, bilirubin $\leq$ 2.0 mg/dl, transaminase not more than three times the normal laboratory values, protime and activated partial thromboplastin time within 1.5 times normal, serum creatinine $\leq$ 1.5 mg/dl, electrolytes within 10% of normal values, and calcium $\leq$ 11.0 mg/dl. Patients with clinically significant third-space fluid, a serious co-morbid condition, a history of bowel disease, a history of brain metastasis, or extensive radiotherapy to the pelvis were excluded. Patients requiring allopurinol, probenecid, phenytoin, trimethoprim, cotrimoxazole, pyrimethamine, or nephrotoxic drugs were excluded.

LY309887 can be stored at room temperature as the lyophilized powder and is stable in solution for 24 h. The drug was supplied in 1- and 10-mg vials, reconstituted in normal saline to a final volume of 50 ml and then administered as a 10-min intravenous infusion. The initial starting dose was based on chronic dosing in the dog with a conservative initial treatment dosage of 0.5 mg/m$^2$ per week (one-tenth of the dose-limiting toxicity in the dog) because of the prior cumulative toxicity seen with lometrexol.

Three patients were entered at the baseline level (0.5 mg/m$^2$) with the dose escalation initially determined by the modified continual reassessment method [19] which is a more conservative approach than the continual reassessment method as originally proposed [20]. The maximum tolerated dose (MTD) for this study was defined as the dose which resulted in "unacceptable" toxicity defined as grade 3 or higher nonhematologic toxicity or grade 4 hematologic toxicity using the NCI common toxicity rating [21]. Because drugs of this class may manifest toxicity weeks after administration, the first patient was observed for 21 days for chronic toxicity prior to entering additional patients. If the initial dose level during the first 21 days of treatment was found to be tolerable, two additional patients were then to be entered on the starting dose level. If no unacceptable toxicity was noted in this initial cohort of patients on day 21 after initiation of treatment, then additional patients could be entered at higher dose levels. No patient was dose-escalated after being assigned to a dose level. As per the modified continual reassessment method, at least one patient was to be enrolled at each nontoxic or subtoxic dose level (grade 0–1 nonhematologic or grade 0–2 hematologic toxicity using the NCI common toxicity rating [21]) before dose escalation.

On a weekly dosing schedule ×6, the projected dosage levels were 0.5, 1.0, 2.0, 4.0, 6.0, and 9.0 mg/m$^2$. The MTD was further defined as the dose level at which 30% or more of patients would experience grade 3 or more nonhematologic toxicity or grade 4 hematologic toxicity. The intention of the study was to add additional patients at the dose level just below the MTD to define a

clinically useful phase II dose. In the event of delayed toxicity not apparent on the first cycle (after 21 days of study), additional patients were to be entered on the next lower dose level to define a chronic dosing schedule. As indicated in the Results, the appearance of cumulative toxicity in some patients was delayed until 42 days after initiation of treatment. This late cumulative toxicity led to an interactive discussion among investigators and abandonment of the initial dose escalation approach. As the initial strategy of the modified continual reassessment method resulted in patients having intolerable cumulative toxicity on repetitive dosing, a weekly ×3 schedule (treatment days 1, 8, 15) every 42 days was adopted for exploration as most toxicity was evident after more than three dosages of drug had been administered and the 6-weekly dosage schedule was not believed to be feasible for repetitive cycle treatment.

Folic acid tablets were obtained from Danbury Pharmaceutical Company (lot no. C5 E1138) for the first 15 patients on this study and from Westwood Pharmaceutical Company (lot no. 548958) for the remaining patients. Oral folate at a dose of 5 mg/day was administered continuously throughout the study period beginning 2 days prior to commencement of LY309887 treatment.

Patients were evaluated for toxicity weekly with physical examinations, complete blood counts and platelet counts, reticulocyte counts, chemistries, and amylase. Urinalysis was repeated every 42 days. Antitumor effects in patients with measurable disease were evaluated weekly if assessable on physical examination and every 42 days if only detected by imaging (chest film, or CT scan). Response was defined from the first objective assessment of antitumor effect until the time of first progression or death.

### Sample collection and handling

In the case of administration of LY309887 through an arm vein, blood was obtained from the contralateral arm. In the case of administration via a central venous access device, either arm was used for sampling. For pharmacokinetic measurements, peripheral blood (7 ml) was collected into Becton-Dickinson Vacutainer tubes (Franklin Lakes, N.J.), centrifuged at 1000 *g* in a refrigerated (5°C) Sorvall centrifuge (Dupont Co., Wilmington, Del.) for 15 min and then the supernatant was removed, transferred to polypropylene tubes and frozen at –20°C or more until batch analyzed. Samples were obtained before treatment, at 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, 72, 96, 192 (time prior to second injection) and 360 h (time prior to third injection), and on day 43. Urine samples were obtained prior to treatment, and during hours 0–6, 6–12, 12–24, 24–36, and 36–48, and processed in a similar manner.

### Radioimmunoassay of LY309887

Serum concentrations of LY309887 were determined by a validated competitive radioimmunoassay [22]. Briefly, serum samples (0.05 ml) were combined with 0.5 ml [$^{125}$I]LY-389753 (50 pg/tube), a *p*-tyramine adduct of LY309887, and 0.1 ml of a specific rabbit anti-LY309887 antiserum in a 12×75 mm polypropylene tube. After an overnight incubation at ambient temperature, the bound and free forms of radioiodinated LY-389753 were separated by adding 0.25 ml of a suspension of goat anti-rabbit IgG coated on superparamagnetic particles, followed by 1 ml of assay buffer. The liquid phase was decanted and the radioactivity adhering to the particles was counted in a $\gamma$-counter. A VAX computer was used to analyze the radioimmunoassay data by a weighted four-parameter logistic model algorithm. Standard curves were prepared in assay buffer and analyzed in duplicate. The specificity of the assay was determined by competition assay using the $ED_{50}$ (the concentration of a compound needed to displace 50% of bound radiolabeled LY309753) and comparing that value with the LY309887 value which was normalized to 100%. The LY309887 concentrations in test samples were estimated from a standard curve of LY309887 in human serum that ranged in concentration from 0.1 to 100 ng/ml. The validated range of the assay was 0.5–5 ng/ml in plasma and 25–250 ng/ml in urine. The interassay precisions (percent coefficient of variation) were 15.4% at 0.5 ng/ml, 9.2% at 1 ng/ml, and 8.1% at 5 ng/ml for serum [22]. Epitope mapping experiments demonstrated that the 5,10-dideazapteridine moiety is critical for recognition by the antiserum. All endogenous folates tested cross-reacted less than 0.01% compared to LY309887.

### Pharmacokinetic methods

Pharmacokinetic parameters for LY309887 were calculated using noncompartmental methods [23]. Maximum plasma concentration ($C_{max}$) and the corresponding sampling time ($T_{max}$) were identified from the observed data. Concentration-time data were plotted on a semilogarithmic scale and the terminal log-linear phase was identified by visual inspection. Blood samples were obtained up to at least 192 h after drug administration. The terminal slope ($\lambda_z$) was determined by linear regression for the terminal log-linear portion of the concentration-time curve up to 24 h. The curve after 24 h became virtually flat and varied widely between patients and thus was not believed to represent excretion. A predicted concentration ($\tilde{C}$) at the last sampling time at which the assay value was above the limit of quantification was calculated from the regression equation. Area under the plasma concentration versus time curve ($AUC_{0\text{-}t}$) and area under the first moment curve ($AUMC_{0\text{-}t}$) were calculated by the trapezoidal method and extrapolated to infinite time using the predicted concentration ($\tilde{C}$) at the last measurable sampling time (T), and the apparent terminal elimination rate constant $\lambda_z$ values as:

$$AUC_{0-\infty} = AUC_{0-t} + \tilde{C}/\lambda_Z \quad (1)$$

$$AUMC_{0-\infty} = AUMC_{0-t} + \tilde{C}/\lambda_Z \cdot \left(T + 1/\lambda_Z\right) \quad (2)$$

Mean residence time (MRT), plasma clearance ($CL_p$), renal clearance ($CL_r$), and volume of distribution at steady-state ($V_{ss}$) were calculated as:

$$MRT = (AUMC_{0-\infty}/AUC_{0-\infty}) - (\tau/2) \quad (3)$$

$$CL_p = Dose/AUC_{0-\infty} \quad (4)$$

$$CL_r = Ae_{0-\infty\,24}/AUC_{0-\infty\,24} \quad (5)$$

and

$$V_{ss} = CL_p \cdot MRT \quad (6)$$

where $\tau$ is the duration of infusion (10 min), and $Ae_{0\text{-}24}$ is the total amount of drug excreted in the urine over 24 h. The fraction of unchanged drug excreted in the urine (Fe) was determined using the total amount excreted in the urine over 48 h (the last time point of collection) divided by the administered dose. Terminal half-life was calculated as $\ln(2)/\lambda_z$. Dose linearity was determined by linear regression.

## Results

As demonstrated in Table 1, 17 patients were entered onto this study with a twofold predominance of males to females. The average age of the study patients was 63 years. The patients had a wide range and a diverse variety of solid tumor types with a slight predominance of colorectal tumors. The mean performance status of the patients was 1 with the majority of patients having been previously exposed to fluoropyrimidines. Significant co-morbid conditions (hypertension) were present in six patients.

**Table 1** Patient demographics

| | |
|---|---|
| Number of patients | |
| Total | 17 |
| Male | 12 |
| Female | 5 |
| Age (years) | |
| Mean | 63 |
| Range | 26–83 |
| Oncologic diagnosis | |
| Adenocarcinoma (unknown primary) | 1 |
| Colon | 4 |
| Esophageal | 1 |
| Gallbladder | 1 |
| Lung | 3 |
| Ovarian[a] | 1 |
| Prostate | 2 |
| Rectal | 3 |
| Uterine | 1 |
| Number of known metastatic sites | |
| Mean | 1.8 |
| Range | 1–4 |
| Body surface area ($m^2$) | |
| Mean | 1.9 |
| Range | 1.6–2.2 |
| ECOG performance status | |
| Mean | 1 |
| Range | 0–2 |
| Prior cytotoxic treatments | |
| Mean | 3 |
| Range | 1–8 |
| Nephrotoxic drugs (number of patients) | 6 |
| Mitomycin or anthracyclines (number of patients) | 7 |
| Prior radiation | 9 |

[a] This patient had concurrent colon and ovarian carcinoma

Table 2 indicates the dose levels studied, the median number of total infusions delivered to each patient per dose level, and the resulting toxicities at grade 2 or more. Minor levels of hematologic toxicities and paresthesia were seen at dosages less than 2 mg/m$^2$ per week. Hence, patients on 0.5 or 1.0 mg/m$^2$ per week received their treatments on time. The dose escalation schema initially specified that only one patient should be entered on to the 2.0 mg/m$^2$ dose level. As the initial schema of the dose escalation schedule allowed dose escalation to a higher level of a new cohort of patients if no limiting toxicity was noted at the current level after 21 days of observation, the toxicities at 2.0 mg/m$^2$ in this single patient did not become evident before patients were entered at 4.0 mg/m$^2$. As the dose on the weekly schedule was escalated, more cumulative toxicities became apparent (Table 2). Four additional patients were therefore studied at the dose level of 2.0 mg/m$^2$ when it became apparent that dosing at 4.0 mg/m$^2$ was intolerable.

Evaluated as a group, patients at 2.0 mg/m$^2$ per week had cumulative toxicities of grade 3 or more (occurring between days 21 and 42 from the start of treatment) consisting of mucositis, loss of taste, neuropathy (asthenia, paresthesia), orthostatic hypotension, anemia, leukopenia, and thrombocytopenia which resulted in dose-limiting toxicities in four of five patients. The hematologic toxicities at a weekly dose of 2 mg/m$^2$ reversed within 2–3 weeks but the neurologic toxicities persisted for months. When the toxicities resolved, additional weekly dosages were administered as the next cycle of treatment with cumulative toxicity once again noted. Five patients were treated at 4.0 mg/m$^2$ as this was initially believed to be the MTD level. Patients treated at the 4.0 mg/m$^2$ weekly dose all showed grade 3 toxicities (asthenia, blurred vision, diarrhea, hyperbilirubinemia, vomiting) which were delayed in onset and prevented completion of the 6-week course of weekly treatment. Hence, the 4.0 mg/m$^2$ dose level was deemed to be above the MTD. The 2 mg/m$^2$ dose and schedule was declared the MTD.

As the significant toxicities were delayed in onset and thus mimicked previous findings with lometrexol, the dosing schedule of LY 309887 was modified to once weekly ×3 every 6 weeks in an attempt to develop a schedule that would allow repetitive dosing. Three patients were entered on this schedule with the finding of delayed grade 2 thrombocytopenia and grade 3 anemia (dose-limiting toxicity) in one patient on day 64, delayed anemia on days 36 and 64 in two additional patients, and grade 2 amylasemia (dose-limiting toxicity) without symptoms in one patient. The grade 3 anemia was believed to be secondary to progressive disease. No patient achieved a major response. Because of delayed hematologic and neurologic toxicities seen in all the ongoing phase I studies of this agent, the sponsor elected to end entry to further patients pending further review of all studies.

## Pharmacokinetic results

Of the 17 patients, 16 had adequate samples for pharmacokinetic measurements. The mean plasma disposition curves are shown in Fig. 2. Plasma LY309887 concentrations declined rapidly after termination of infusion up to 36 h after administration. After this initial decline, plasma concentrations remained fairly constant over a long period of time with samples obtained up to 200 h after treatment. The relatively flat plasma concentration-time profile observed after 24 h suggests a long apparent terminal phase for LY309887.

A comparison of mean pharmacokinetic parameters at doses of 0.5, 1, 2, and 4 mg/m$^2$ are presented in Table 3. Both $AUC_{0-\infty}$ and $C_{max}$ increased linearly with dose (Fig. 3A,B). The clearance of this agent and the volume of distribution at steady-state ($V_{ss}$) were constant across the dosage range, suggesting no saturation of disposition. The measurement of clearance in these patients was closer to values seen in C3H mice (35.3) than the values in the beagle dog (3.1). The half-life ($t_{1/2}$) was also consistent across dosing levels. Trace amounts of this agent could be detected in the plasma 200 h after administration. Predose plasma samples prior to administration in subsequent courses of therapy had low but detectable concentrations. As a result of the nearly flat concentration-time profile during the prolonged

**Table 2** Number of patients exhibiting toxicities of grade 2 or more in relation to dose level

| | Dose level, weekly ×6 (old) schedule (mg/m$^2$/week) | | | | Dose level, weekly × 3 (new) schedule (mg/m$^2$/week) | Total number of patients |
|---|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 | 2 | |
| Number of patients entered | 3 | 1 | 5 | 5 | 3 | 17 |
| Number of doses per patient | | | | | | |
| Mean | 8 | 8 | 7 | 3 | 6 | |
| Range | 6–12 | | 6–11 | 1–6 | 6 | |
| Cardiovascular toxicity | | | | | | |
| Orthostatic hypotension | | | 2[a] | | | 2 |
| Gastrointestinal toxicities | | | | | | |
| Abdominal pain | 1 | | | | | 1 |
| Eructation | 1 | | | | | 1 |
| Diarrhea | | | | 1[a] | | 1 |
| Mouth pain | | | | 1 | | 1 |
| Pharyngitis | | | 3 | 1 | | 4 |
| Stomatitis | | 1 | 4 | 1 | | 6 |
| Vomiting | | | | 1[a] | | 1 |
| Miscellaneous toxicities | | | | | | |
| Dehydration | | | | 1 | | 1 |
| Weight loss | | | | 1 | | 1 |
| Neurologic toxicities | | | | | | |
| Amblyopia | | | | 2 | | 2 |
| Asthenia | 1 | | 1[a] | 2[a] | 2 | 6 |
| Fall | | | 2 | | | 2 |
| Taste alteration | | | 2 | | | 2 |
| Taste loss | | | 2 | | | 2 |
| Syncope | | | 1 | | | 1 |
| Unsteady gait | | 1 | | | | 1 |
| Vertigo | | | 1[a] | | | 1 |
| Laboratory toxicities | | | | | | |
| Anemia | 1 | | 4 | 3 | 2 | 10 |
| BUN increase | | | | 1 | | 1 |
| Creatinine increase | | | | 1 | | 1 |
| Hyperamylasemia | | | 1 | | 1 | 2 |
| Hyperbilirubinemia | | | | 1[a] | | 1 |
| Hypocalcemia | | | | 1 | | 1 |
| Hypouricemia | | | | 1 | | 1 |
| Leukopenia | | | 1 | 2 | | 3 |
| Thrombocytopenia | | | 3 | 1 | 1 | 5 |

[a]Dose-limiting toxicity

terminal phase, it was not possible to determine a terminal half-life. This effect therefore may lead to an underestimation of both AUC and AUMC. Therefore, the elimination half-life was determined from plasma concentrations obtained over the sampling time range of 8 to 24 h. The observed elimination half-life of about 3 to 5 h is consistent with the urinary excretion of LY309887 which is nearly maximal within 24 h of drug administration. Therefore, the calculation of $AUC_{0-\infty}$ and $CL_p$ were based upon the terminal slope ($\lambda_z$) of the disposition curve up to 24 h after treatment. Urinary excretion of LY309887 (Fe) revealed considerable variability which could not be statistically correlated with renal function, serum albumin level, or concurrent medications. Although highly variable, on average, approximately 50% of the administered LY309887 dose had been excreted unchanged in the urine by 48 h after the start of infusion with the majority of drug excreted by 24 h after treatment (Fig. 4). No gender-specific effects were identified.

As the toxicity of this agent is known to be increased in animals which are not folate-replete, and a significant proportion of the human population may not have adequate folate stores unless exposed to supplements [24], we supplemented by the oral route all the patients with 5 mg folate per day. To minimize variability between manufacturers and lots, we restricted our source of folate to two lots.

## Discussion

LY309887 was attractive for development as it has high inhibitory activity for a critical enzyme in de novo purine synthesis (GARFT) and is transported by both the reduced folate carrier and the folate-binding protein. As the drug is polyglutamated, although less so than lometrexol, this charged form of the drug is retained within cells and may be in part responsible for the prolonged and delayed cumulative toxicity seen in this phase I study. In murine

models, the addition of exogenous folate has been shown to have a protective effect in reducing toxicities and enhancing the therapeutic index. Despite oral folate supplementation, which has been shown to enhance plasma folate stores in prior lometrexol studies [13], prolonged toxicity of LY309887 was unmanageable at dosages above 1 mg/m$^2$ when administered on a weekly ×6 rapid intravenous infusion schedule. Four out of five patients at the 2 mg/m$^2$ per week dose level had to have their treatments interrupted because of toxicities. Thus, the use of this schedule resulted in the appearance of delayed and prolonged dose-limiting hematologic, mucosal, and neurologic toxicity. A revised weekly ×3 schedule every 6 weeks was adopted to lessen the potential for unexpected delayed toxicity seen on the weekly schedule. Use of this agent on the revised schedule (LY309887 at 2 mg/m$^2$ per week with daily 5 mg folic acid) was manageable and, if confirmed in larger numbers of patients, could potentially serve as a phase II schedule for future studies of this agent.

The pharmacokinetics of LY309887 could be assessed in 16 of the 17 patients in this study. After termination of the infusion, LY309887 concentrations declined rapidly for 24 to 36 h followed by a prolonged terminal phase. During the prolonged terminal phase, very low plasma concentrations remained virtually constant up to the next dose. LY309887 is transported intracellularly by a folate transport mechanism. Once inside the cell, LY309887 undergoes polyglutamation leading to prolonged retention within the cell. However, the polyglutamate can be hydrolyzed back to the parent compound thus leading to redistribution into the general circulation. Therefore, the long terminal phase observed in this study may represent drug in dynamic equilibrium between plasma and intracellular glutamates. The observed variability in renal excretion between patients remains unexplained and did not correlate with clinical

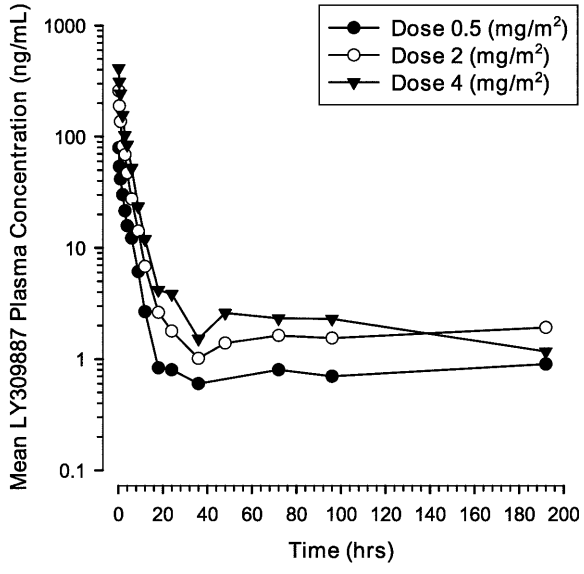


**Fig. 2** Mean plasma LY309887 concentration-time profiles by dose (mg/m$^2$)

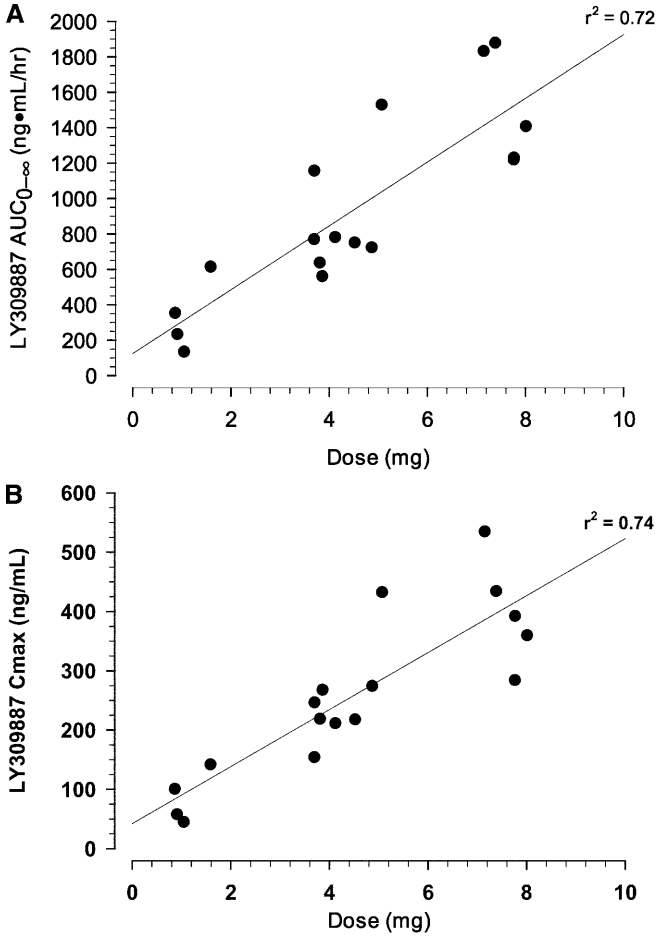


**Fig. 3** **A** Relationship between plasma AUC and absolute dose administered. **B** Relationship between maximal plasma concentration ($C_{max}$) and absolute dose administered

**Table 3** LY309887 pharmacokinetic parameters by dose. Values are arithmetic means (CV)

| Parameter | Dose (mg/m$^2$) | | | |
|---|---|---|---|---|
| | 0.5 ($n=3$) | 1.0 ($n=1$) | 2 ($n=7$) | 4 ($n=5$) |
| $C_{max}$ (ng/ml) | 68.0 (43%) | 142 | 251 (32%) | 401 (23%) |
| $AUC_{0-\infty}$ (ng·h/ml) | 241 (46%) | 614 | 907 (35%) | 1514 (21%) |
| $CL_p$ (ml/min) | 95.7 (50%) | 43 | 84 (27%) | 85.5 (22%) |
| $CL_r$ (ml/min) | 43.2 (77%) | 45.8 | 44.4 (63%) | 49.5 (39%) |
| $V_{ss}$ (l) | 28.7 (57%) | 17.6 | 29.6 (19%) | 31.3 (35%) |
| $t_{1/2}$ (h) | 3.6 (33%) | 4.7 | 4.8 (34%) | 4.2 (25%) |
| Fe | 0.36 (39%) | 0.58 | 0.39 (83%) | 0.56 (36%) |

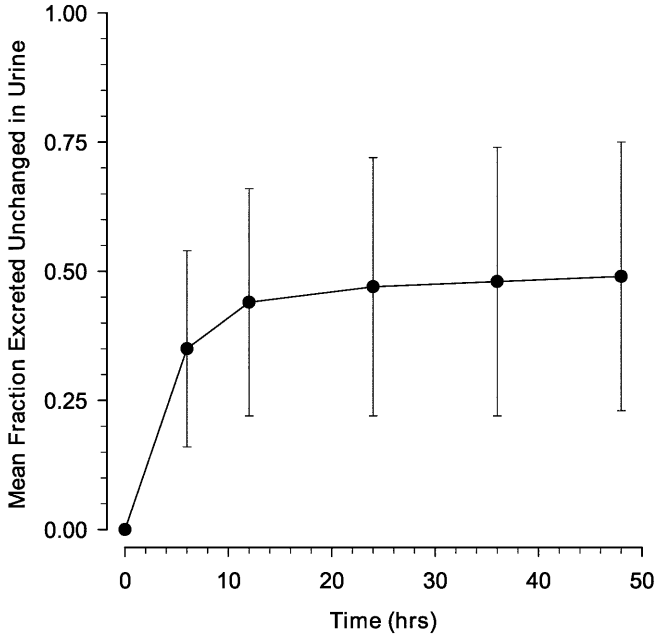


**Fig. 4** Mean cumulative fraction of LY309887 excreted unchanged in urine (*error bars* ± SD)

parameters such as creatinine clearance or plasma albumin levels.

In summary, LY309887 exhibits many of the biologic characteristics in humans seen with the first-generation broad-activity antifolates. The drug shares with this class of agents a broad antitumor spectrum of activity in preclinical model systems, and an enhanced therapeutic index in the presence of exogenously administered folates. However, this clinical phase I study also indicates the limitations of this agent with the appearance of delayed cumulative toxicity which may be lessened by an alteration of the dosing scheme.

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