Clinical Pharmacokinetic and Pharmacodynamic Studies with the Nonclassical Antifolate Thymidylate Synthase Inhibitor 3,4-Dihydro-2-amino-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone Dihydrochloride (AG337) Given by 24-Hour Continuous Intravenous Infusion


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ABSTRACT

3,4-Dihydro-2-amino-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone dihydrochloride (AG337) is a nonclassical inhibitor of thymidylate synthase (TS) designed to avoid potential resistance mechanisms that can limit the activity of classical antifolate antimetabolites. A clinical pharmacokinetic and pharmacodynamic study of AG337 given as a 24-h i.v. infusion was performed. Thirteen patients received 27 courses over the dose range 75–1350 mg/m². Plasma AG337 concentrations were achieved which, in preclinical models, were associated with antitumor effects. AG337 clearance was saturable, and the pharmacokinetics of the drug at doses above 300 mg/m² was best described by a one-compartment model with saturable elimination (median Kₘ = 6.5 µg/ml; range, 4.1–13 µg/ml; median Vₘₐₓ = 2.0 µg/ml/h/m²; range, 0.96–5.6 µg/ml/h/m²). Following the end of the infusion, AG337 was cleared rapidly (t½ = 53–193 min), and levels were less than 0.2 µg/ml in all patients by 48 h. Plasma protein binding was 96–98%, and the urinary excretion of AG337 as unchanged drug did not exceed 30% of the dose administered. Measurements of plasma deoxyuridine (dUrd) concentrations showed that doses of 600 mg/m² and above of AG337 produced a consistent elevation in plasma dUrd levels (60–290%), suggesting that TS inhibition was being achieved in patients. However, in all cases dUrd concentrations had returned to pretreatment levels 24 h after the end of the infusion, suggesting that TS inhibition was not maintained. Local toxicity, probably due to the infusate pH, was the only significant adverse effect observed. These studies have shown that cytotoxic AG337 plasma concentrations can be readily achieved without acute toxicity and that these concentrations are associated with elevations in plasma dUrd levels. The lack of prolonged dUrd elevations indicates that extended administration should be explored using central line or p.o. administration to avoid local toxicity.

INTRODUCTION

TS³ (EC 2.1.1.45) is the terminal enzyme in the de novo pathway for the biosynthesis of thymidine nucleotides. Thymidine nucleotides are used exclusively for DNA synthesis, and hence TS is an important target for antiproliferative cancer chemotherapy. TS catalyzes the methylation of dUMP to form TMP, using 5,10-methylene tetrahydrofolate as the methyl-donating folate cosubstrate. TS inhibitors based on both the pyrimidine (e.g., 5-fluouracil, 5-fluorodeoxyuridine) and folate (e.g., N¹⁰-propargyl-N⁸,N¹⁰-dideazafolic acid, tomudex, N⁴-[4-[(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)-ethyl][benzoyl]-1-glutamic acid disodium salt) cosubstrates have been developed and evaluated in clinical trials (1–7). The clinical activity seen with some of these agents confirms the importance of TS as a target for cancer chemotherapy; however, either inherent or acquired resistance to currently available TS inhibitors is a significant problem.

In the case of pyrimidine-based TS inhibitors, resistance can occur as a result of inadequate activation toFdUMP, accelerated catabolism, or structural alterations to the TS protein arising from mutations (1, 8). In addition, fluorinated pyrimidines have multiple loci of action, notably incorporation into DNA and RNA as well as TS inhibition, which may compromise their antitumor selectivity (1, 8). In the case of antifolate TS inhibitors, all of the agents that have been introduced into clinical trials to date are classical folate antagonists in that they have a terminal glutamate residue (2–7). The presence of the

¹ The abbreviations used are: TS, thymidylate synthase; AG236, 3,4-dihydro-2-methyl-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone; AG337, 3,4-dihydro-2-amino-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone dihydrochloride; ALT, alanine transaminase; AP, alkaline phosphatase; AUC, area under the plasma concentration versus time curve; Cₘₐₓ, time-averaged clearance; Cₘₐₓ, maximum clearance; UK, United Kingdom; QA, quality assurance; dUrd, deoxyuridine.
glutamate residue imparts two negative charges on the molecule at physiological pH, such that a carrier system is required for uptake into cells (9). In addition, the glutamate residue may result in the molecule being a substrate for intracellular polyglutamation, which can markedly enhance the TS inhibitory potency of antifolate inhibitors such that TS inhibition in whole cells is largely due to these metabolites (10-13). By analogy with methotrexate, resistance mechanisms involving both impaired transport and decreased polyglutamation could operate in the case of classical antifolate TS inhibitors (14), although the clinical relevance of these mechanisms remains to be established.

AG337 (Fig. 1) is a nonclassical antifolate that was designed to circumvent antifolate resistance due to mechanisms involving transport or polyglutamation, while retaining the advantages of folate as opposed to pyrimidine-based TS inhibitors (15). AG337 is the first antitumor compound to enter clinical trials whose design was aided by the crystal structure of its protein target, an approach which resulted in rapid structural optimization. AG337 is a competitive inhibitor of TS with a Ki of 16 nM (15). Against a range of rodent and human cell lines grown in vitro, AG337 is growth inhibitory at concentrations in the region of 1 μg/ml (15-18). Clonogenic assays have confirmed that AG337 is cytotoxic, and thymidine reversal, as well as cell cycle and resistance studies, have shown that TS is the major locus of cytotoxic action of the compound (16-18).

Because of the presence of high circulating levels of thymidine in mice (19), TS inhibitors do not display significant in vivo activity against standard murine tumors or human tumor xenografts. However, against thymidine kinase-deficient murine and human tumors, which therefore cannot salvage extracellular thymidine, AG337 was growth inhibitory and in some experiments curative (16, 17). Importantly, AG337 was active when given p.o. and i.p. to mice bearing tumors implanted i.m., indicating that the compound can have activity against systemic cancer.

On the basis of these in vitro and in vivo data, AG337 was selected by the Phase I/II Clinical Trials Committee of the Cancer Research Campaign for clinical evaluation. As a prelude to clinical trials, toxicological studies were performed according to established protocols (20); however, in the case of AG337, these were modified to substitute the dog for the rat as the second species. This modification was performed in light of information on the circulating levels of thymidine in rodents in comparison to dogs and patients (19).4 i.e., there was concern that the high thymidine levels in rodents might result in reduced toxicity and hence the derivation of a Phase I trial starting dose that would be unsafe. In mice, the LD10 of AG337 given as a single i.v. dose was 816 mg/m² and as a single i.p. dose was 759 mg/m², with lethal events being observed 2–5 days after AG337 administration. Despite biochemical, hematological, and histopathological studies, the cause of the lethal event was not identified. In dogs, a 24-h constant rate i.v. infusion of AG337 was well tolerated; the only toxic events being vomiting, hypokinesia, and anorexia at doses of 3500–3900 mg/m². This latter dose resulted in plasma levels of 20–50 μg/ml AG337, and after the end of the 24-h infusion, AG337 was rapidly cleared.

In initiating clinical trials with AG337, it was noted that antiproliferative side effects had not been observed in preclinical toxicity models. Furthermore, in contrast to expectations based on plasma thymidine concentration data, the mouse was the most sensitive of the two species studied, although the dose-limiting toxicity in this species could not be identified. For these reasons, and as a prelude to the formal Phase I evaluation of AG337, a pharmacokinetic/pharmacodynamic dose escalation study was performed with the following objectives: (a) to investigate whether or not plasma concentrations of AG337 that were associated with antitumor activity in preclinical models could be achieved in patients; (b) to seek evidence that TS was being inhibited in patients at clinically achievable plasma concentrations and to study the dose dependency of TS inhibition; and (c) to record any antitumor activity or toxicities encountered.

AG337 pharmacokinetics was defined using plasma and urine AG337 concentration data measured by HPLC methodology. AG337 pharmacodynamics was studied by evaluating plasma dUrd concentrations. dUrd in plasma is derived from intracellular dUMP levels which rise after TS inhibition (21-23). Hence, plasma dUrd levels may reflect TS inhibition in vivo. The starting dose for the clinical study was 75 mg/m², 0.1 of the LD10 in mice, the most sensitive species. Dose escalation was performed on the basis of AG337 plasma concentration data and the changes in plasma dUrd levels observed. The schedule chosen was a 24-h i.v. infusion on the basis of data from studies in dogs that had shown that this was a safe schedule for AG337 administration.

MATERIALS AND METHODS

Patient Characteristics and Drug Administration

Eligibility

A total of 13 patients were investigated, with full pharmacokinetic studies in 12 patients on 24 courses. Eligibility criteria were as follows: a histologically proven diagnosis of a malignant disease for which no satisfactory treatment existed or against which established treatments had failed; WHO performance status of 0, 1, or 2; no chemotherapy within 4 weeks, full recovery from all previous myelosuppressive chemotherapy,

4 G. A. Taylor, unpublished results.
and no radiotherapy, nitrosourea, or mitomycin C treatment within 6 weeks prior to entering the study; pretreatment ura, electrolytes, and bilirubin within normal ranges, and other liver function tests less than twice the upper limit of normal unless clearly due to the presence of tumor; hemoglobin $\geq 10$ g/dl, WBCs $\geq 4.0 \times 10^9$/liter, and platelets $\geq 100 \times 10^9$ /liter. Patients had to be 18–75 years and have a life expectancy of at least 3 months. This study was approved by the Regional Ethics Committee, and written informed consent was obtained from all patients. This study was conducted under the auspices of the Cancer Research Campaign Phase I/II Clinical Trials Committee. Patient characteristics are shown in Table 1.

### Exclusions

Any patient who had a severe intercurrent medical condition, evidence of bone marrow tumor infiltration, or bone marrow significantly compromised by previous extensive therapy was excluded. Patients who needed concurrent radiotherapy or administration of allopurinol, probenecid, nephrotoxic agents, trimethoprim, antiepileptics, co-trimoxazole, or pyrimethamine were also excluded. Patients with hematological malignancies were also excluded, but patients with brain metastases or primary brain tumors were eligible unless symptoms prevented them from giving informed consent.

### AG337 Formulation

AG337 was supplied by Agouron Pharmaceuticals, Inc. (San Diego, CA) in 100-mg ampules containing 5 ml of a pale yellow solution at 20 mg/ml. The solution contained sodium acetate (0.54% w/w), glacial acetic acid (0.36% w/w), and propylene glycol (48.45% w/w) as inactive ingredients. The solution also contained methyl p-aminobenzoate (0.18% w/w) and propyl p-aminobenzoate (0.02%) as preservatives, and the final pH of the solution was 3–5. The vials of AG337 were protected from light and stored at a controlled room temperature of 15–30°C. The relevant dose was diluted to 1 or 2 liters in 5% dextrose and administered either through a peripheral cannula placed in an arm vein or a central line, over 24 h, using an infusion pump. AG337 was administered every 3 weeks.

### Dose Escalation

A 0–24 h pharmacokinetic profile was determined for all patients, with a cohort of three or four patients being entered at each dose level. Doses were doubled from the starting dose (75 mg/m²) to 600 mg/m², at which point evidence of TS inhibition and pharmacokinetic nonlinearity was observed. Thereafter, doses were escalated in 50% increments, and the maximum dose administered was 1350 mg/m².

### Patient Monitoring

Before treatment and prior to each course, all patients were examined, and the full blood count, urea and electrolyte concentrations, liver function tests, and $^{51}$Cr-EDTA clearance were recorded. Patients were monitored twice weekly with full blood counts, urea and electrolyte levels, and liver function tests being determined. Common toxicity criteria for toxicity and response were used (24).

### Pharmacokinetic Studies

#### Sampling Schedule

Heparinized blood samples (3 ml, 10 IU/ml) were collected from an i.v. cannula placed in the contralateral arm. Samples were collected before AG337 administration, 0.5, 1, 2, 3, 4, 6, 12, and 18 h into the infusion, at the end of the infusion (24 h), and 5 min, 10 min, 15 min, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 5 h, 8 h, and 24 h thereafter. All samples were immediately placed on ice, and plasma was prepared within 30 min by centrifugation at $2000 \times g$ at 4°C for 15 min. Plasma samples were then stored frozen at $-20^\circ C$ until analyzed (<28 weeks). In addition, pretreatment, total 0–24 h, and 24–48 h urine samples were collected, the volumes recorded, and 20 ml aliquots stored frozen at $-20^\circ C$ prior to analysis (<20 weeks).

#### Materials

Analytical samples of AG337 and AG236, the 2-desamino 2-methyl derivative of AG337, were supplied by Agouron Pharmaceuticals, Inc. Analytical grade acetonitrile and methanol were obtained from Fisons Scientific Equipment (Loughborough, UK), and deionized water was produced using an Aquatron A4D system (Elga Ltd., High Whickham, Bucks, UK). Control plasma was kindly provided by the Red Cross Transfusion Service (Newcastle upon Tyne, UK) as outdated plasma. Control urine was collected from healthy volunteers.

#### Chromatographic Equipment

Separations were performed on a HPLC consisting of 510 HPLC pumps (Waters Associates, Northwich, Cheshire, UK), a model ISS-101 autosampler (Perkin Elmer/Cetus Ltd., Beaconsfield, Bucks, UK), and a Philips PU4021 multichannel UV/visible detector (ATI Unicam, Cambridge, UK). Analytical columns were purchased from Jones Chromatography (Hengoed, Mid-Glamorgan, UK). Solvent filtration filters and precolumn packing material were supplied by Whatman Ltd. (Maidstone, Kent, UK). HPLC control and data collection were achieved using the Minichrom Data Capture System (VG Data System; Altrincham, Cheshire, UK).
HPLC Analyses

Sample processing. Patient and QA samples were thawed at room temperature and mixed thoroughly. One hundred-μl aliquots of patient, QA, and standard curve samples were then removed and placed in 15-ml conical glass centrifuge tubes to which 20 μl of 25 μg/ml AG236 in water were added. Each sample was then individually placed on a rotary mixer for 15 s, during which time 1.2 ml acetonitrile were added. Any precipitate was removed by centrifugation at 2000 × g at 4°C for 15 min, and 1 ml of the supernatant was removed and concentrated to dryness in a stream of nitrogen at 40°C. The concentrated samples were reconstituted in 250 μl HPLC mobile phase (see below) by rotary mixing for 15 s followed by centrifugation at 2000 × g for 2 min if necessary. Plasma and urine samples were treated in an identical manner.

Preparation of Standard Curve and QA Samples. Each assay was calibrated using a 6- or 7-point standard curve prepared on the day of patient sample analysis. Each standard curve concentration was analyzed in duplicate. For the analysis of AG337 in plasma, standard curve concentrations were 0.2, 0.5, 1, 2, 5, and 10 μg/ml whereas for the analysis of AG337 in urine, concentrations were 1, 2, 5, 10, 20, 50, and 100 μg/ml. These concentrations were prepared by the serial dilution of a 1 mg/ml solution of AG337 in deionized water with control plasma or urine. The 1-μg/ml AG337 solution in water was prepared periodically (range, 16–24 weeks) and stored as frozen 650-μl aliquots at −20°C. QA samples were prepared in control plasma at 1 and 10 μg/ml, and in control urine at 1, 10, and 100 μg/ml and stored at −20°C prior to analysis.

Chromatographic Conditions. Two hundred-μl aliquots of each reconstituted plasma sample were transferred to 250-μl glass autosampler vials (Chromacol Ltd., Welwyn Garden City, Herts, UK), which were then placed in the precooled HPLC autosampler (4°C). Samples were analyzed on a 15 × 0.46-cm Spherisorb C6 3-μm column, fitted with a 2 × 0.2-cm pellicular ODS precolumn, and eluted isocratically with 75:25 (w/w) 0.01 M Na2HPO4 (pH 7.0):CH3CN at a flow rate of 1 ml/min. The column effluent was monitored at 233 and 273 nm, and peaks were identified by retention volume and 273:233-nm peak area ratio. Apart from the use of 78:22 (w/w) 0.01 M Na2HPO4 (pH 7.0):CH3CN as the HPLC mobile phase, reconstituted urine samples were analyzed in an identical manner.

Calibration and Quantitation. In addition to the standard curve samples, five pairs of QA samples were analyzed throughout each assay (five each at high and low concentrations). The AG337 and AG236 peak area at 273 nm was recorded for each sample, and the AG337:AG236 peak area ratio was calculated. A standard curve of peak area ratio versus AG337 concentration was constructed using unweighted linear regression analysis with the intercept forced through zero. The slope of the standard curve was used to calculate AG337 concentrations in both patient and QA samples. For an analysis to be valid, the intraassay coefficients of variation had to be less than 15%, and the observed concentration for all QA and standard curve samples within 15% of the nominal values, with the exception of the lowest point on the standard curve where deviation by up to 20% was acceptable. In addition, the standard curve had to be linear ($r^2 > 0.98$).

Pharmacokinetic Analyses

AG337 pharmacokinetics was analyzed using both compartmental and noncompartmental pharmacokinetics. Noncompartmental analyses were performed using the trapezoidal rule to calculate the AG337 AUC from which the clearance was calculated. This was designated $Cl_{\text{ur}}$ because it is time averaged and ignores any nonlinearity in elimination. Compartmental analyses were performed using the program ADAPT II, release 3 (1992) kindly provided by Drs. D’Argenio and Schumitzky (University of Southern California, Los Angeles, CA). In all patients studied at doses of 300 mg/m² or less, a one-compartment model with linear elimination was sufficient to describe the data. However, in seven courses (six patients) at doses of 600 mg/m² or higher, neither a one- nor a two-compartment model with linear pharmacokinetics provided an adequate fit to the data, since the plasma concentration continued to rise during the infusion period without attaining a well-defined steady-state concentration. A one-compartment model incorporating saturable elimination was therefore fitted to the data, using a Michaelis-Menten equation to describe the relationship between the rate of elimination and plasma concentration, i.e., plasma clearance at time $t$ was assumed to be equal to:

$$C(t) = \frac{V_{\text{max}} \times V}{K_m + C(t)}$$

where $V_{\text{max}}$ is the maximum elimination rate, $V$ is the volume of distribution, $K_m$ is the concentration associated with 50% maximum elimination, and $C(t)$ is the plasma concentration at time $t$. $Cl_{\text{max}} (C(t) = 0)$, was determined for each course as $(V_{\text{max}}/K_m) \times V$ and $Cl_{\text{max}}, V_{\text{max}}$, and $V$ were corrected for patient surface area. The data were weighted as the reciprocal of the output variance, assuming a constant coefficient of variation. The appropriate model was determined on the basis of the Akaike information criterion (25) as well as the ability of the model to estimate model parameters with reasonable precision. Other models, including two-compartment models with saturable distribution or peripheral elimination, were also investigated, but did not provide a good fit to the data. Half-lives were obtained from the first-order rate constant of a monoexponential equation fitted to the last four to five data points by unweighted log linear regression.

Protein Binding

Protein binding experiments were performed using radio-labeled [2-14C]AG337 (specific activity, 18.2 mCi/mmol; Agouron Pharmaceuticals, Inc.). Bound and free AG337 were separated by ultrafiltration using Amicon Centrifree micropartition units (Amicon, Stonehouse, Glouce., UK) which have a molecular weight cutoff of 30,000. Absorption of AG337 onto the ultrafiltration membranes was less than 10% at the concentrations used. Patient plasma samples containing AG337 at high and low AG337 concentrations were selected, and 2 μg (approximately 10,000 dpm) [14C]AG337 were added to give final concentrations ranging from 2 to 18 μg/ml. Plasma samples were then ultrafiltered by centrifugation (1500 × g, 10 min, 4°C). Ultrafiltrate (100 μl) was collected, and the radioactive content was compared to that of unfiltered plasma using scintillation counting.
Pharmacodynamic Studies

The pharmacodynamics of AG337 was studied by measuring the effects of the drug on the plasma concentration of dUrd. A semiautomated method was developed for the estimation of dUrd concentrations in which [3H]dUrd was added to each plasma sample to act as an internal standard. The assay was performed in two stages in which an initial separation yielded fractions containing dUrd, along with a limited number of unwanted components, which were detected by UV absorption following concentration and a second HPLC separation.

Sample Preparation

Fifty μl [3H]dUrd (5 μCi/ml, 16.7 Ci/mmol) were added to weighed samples of thawed plasma (0.6–4.5 ml), which were then extracted by the addition of 6 ml acetonitrile while vortexing vigorously. The resulting suspensions were centrifuged at 223 × g for 10 min at 4°C and the supernatants transferred to fresh tubes and dried at 40°C under a stream of nitrogen.

Initial Separation

Samples were resuspended in 200 μl HPLC mobile phase (see below), transferred to microfuge tubes, and centrifuged at 13,000 × g for 2 min at an ambient temperature. Supernatants (150 μl) were transferred to precooled (4°C) 200-μl autosampler vials and 100 μl injected onto a 10 × 0.46-cm Nucleosil 3-μm 100 Å cartridge column (Jones Chromatography) fitted with a 2 × 0.2-cm pellicular ODS guard column (Whatman). Isocratic elution at an ambient temperature with 1 ml/min 0.05 M ammonium acetate (pH 5.0) containing 4% methanol (w/w) was used, and the effluent was monitored for radioactivity. Fractions were collected automatically using a 2211 Ultrarac fraction collector (Pharmacia Biotech Ltd., St. Albans, Herts, UK) under the control of a modified Packard A500 radio detector (Canberra Packard, Pangbourne, Berks, UK). Under these conditions dUrd had a retention of approximately 4 min, and fractions (600–800 μl) were collected into 10 ml-glass tubes prior to concentration by evaporation at 40°C under a stream of nitrogen. Between each set of five plasma samples, the column was washed for 5 min with 100% acetonitrile.

Second Separation

For the quantitation of dUrd, a 15 × 0.46-cm Spherisorb 3-μm C6 cartridge column (Jones Chromatography) eluted with 0.2% formic acid (w/w; adjusted to pH 3.25 with 5 M NaOH) containing 2% acetonitrile (w/w), at a flow rate of 1 ml/min, was used. The dried fractions from the initial separation were reconstituted in 150 μl of the above mobile phase and transferred to precooled 200-μl vials. One hundred-μl aliquots were injected onto the column, and the effluent was monitored for radioactivity and UV absorption (262 and 280 nm). dUrd was identified by
Plasma AG337 Concentrations. Data from 12 patients treated with 21 courses of AG337 were studied, with dose levels ranging from 75 to 1350 mg/m². Fig. 2 illustrates typical pharmacokinetic profiles for patients at the highest four dose levels. AG337 plasma concentrations rose during the first 6 h and were maintained for the remaining 18 h of the 24-h infusion. Following the end of infusion, there was a rapid monoexponential elimination of the drug, and in all patients the concentration of the drug was below the limit of detection of the assay (0.2 µg/ml) by 48 h. Although there was a significant linear relationship between the AG337 dose administered and the AG337 AUC (Fig. 3A, \( r^2 = 0.89 \)), the relationship between dose and clearance (Fig. 3B) indicated a reduction in clearance at higher dose levels, suggesting saturable drug elimination. Compartmental analyses confirmed saturable elimination at doses above 300 mg/m². A one-compartment model with linear elimination provided an adequate fit in 17 courses (10 patients), including patient 12 who was studied at the highest dose level (Table 2). The median for the parameters of the linear model were 16 (range, 10–31) liters/m² for volume of distribution and 0.41 (range, 0.22–0.79) h⁻¹ for the elimination rate constant. For the five patients (seven courses) in whom a one-compartment model with saturable elimination was optimal, the median and range for the parameters were \( V_{\text{max}} \) 2.0 (0.96–5.6) µg/ml/h/m²; \( K_{\text{m}} \) 6.5 (4.1–13) µg/ml; and \( V \) 16 (10–20) liters/m² (Table 3). Full details of the parameter values for individual patients and for each course are given in Tables 2 and 3.

Urinary Elimination. The urinary excretion of AG337 was studied following the administration of 21 courses to 13 patients. As shown in Fig. 4, following course 1, up to 28% of the administered dose was excreted unchanged in the urine within 48 h of the start of AG337 administration. Renal excretion was not clearly dose dependent, although there was a trend toward decreased elimination in patients with reduced renal function as measured by \( ^{51} \)Cr-EDTA clearance (Fig. 4).

Protein Binding. Over the concentration range 2–18 µg/ml, unbound AG337 constituted between 2.2 and 3.3% of the total AG337 in plasma. There was no significant difference in the extent of protein binding in individual patients at the high and low AG337 concentrations studied (paired Student’s \( t \) test of the unbound concentrations, \( P = 0.5 \)).

Pharmacodynamics

Plasma dUrd concentrations were measured in all 13 patients after 23 courses of AG337 therapy. dUrd concentrations were measured before AG337 administration, at the end of the infusion, and 24 h after the end of infusion. Pretreatment plasma dUrd concentrations showed considerable variation among patients, and at doses \( \leq 300 \) mg/m², there was no consistent change in plasma dUrd levels after drug administration, although one patient at 300 mg/m² did have an increased dUrd concentration at the end of the infusion (Fig. 5C). In contrast, at doses of 600 mg/m² and above, plasma concentrations of dUrd were consistently elevated at the end of the 24-h infusion (Fig. 5, D–F). dUrd elevations ranged from 60 to 290% relative to pretreatment values, but these increases were not dose dependent. In all patients who demonstrated an increase in plasma dUrd concentrations, levels 24 h after the end of infusion had returned to, or near, pretreatment levels. In patients studied after more than one course of AG337 at \( > 300 \) mg/m² (patients 8–11), the increase in dUrd concentrations was consistently observed (Fig. 5).

With one exception (patient 5/1), all patients who displayed a dUrd elevation had an AG337 AUC of \( > 4 \) mg/ml/min and a

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**Table 2** Pharmacokinetic parameters for AG337 in patients with linear pharmacokinetics

<table>
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<tr>
<th>Patient/course</th>
<th>Dose (mg/m²)</th>
<th>( V ) (liters/m²)</th>
<th>( K_{\text{e}} ) (h⁻¹)</th>
<th>( Cl_{\text{e}} ) (ml/min/m²)</th>
<th>( t_{1/2} ) (min)</th>
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</table>

Fig. 4 Urinary excretion of AG337 in relation to dose and renal function. Each point, a separate patient with first course data only being given: ■, 75 mg/m²; ▲, 150 mg/m²; ○, 300 mg/m²; ●, 600 mg/m²; □, 900 mg/m². Satisfactory urine collections were not available for 3 of the 13 patients.
peak/end of infusion plasma AG337 concentration of >4 μg/ml. Studies were performed to investigate possible relationships between the changes in plasma dUrd concentrations (absolute and percentage) and AG337 dose (mg/m²), peak/end of infusion concentration, and AUC. However, there were no linear, sigmoidal, or hyperbolic relationships.

Clinical Results

In total, 13 patients received 27 courses with a median of two courses for each patient. Patient characteristics are shown in Table 1, and the dose escalation scheme used is given in Table 4. The starting dose was 75 mg/m², and doses were doubled until 600 mg/m², at which point plasma dUrd elevations were consistently observed (Fig. 5D). In addition, evidence of non-linearity in the relationship between AG337 dose and AUC became convincing at this dose level (Fig. 3). For these two reasons, dose increments were reduced to 50%, and dose levels of 900 and 1350 mg/m² were investigated; however, no further increases in plasma dUrd elevation were seen (Fig. 5E and F).

Three factors led to the cessation of AG337 dose escalation at 1350 mg/m². First, in all patients studied plasma dUrd concentrations returned to pretreatment values 24 h after the end of the infusion, suggesting that prolonged TS inhibition was not being achieved. Second, further dose escalation would have been complicated by the saturable clearance of AG337. Finally, there did not appear to be any increase in the magnitude of dUrd elevation at 900 and 1350 mg/m² AG337, over that seen at 600 mg/m².

The most prevalent side effect of AG337 given by peripheral administration was local toxicity, and the incidence of patients suffering from grade 2 local toxicity is given in Table 4. The local toxicity typically consisted of erythema around the
infusion site, usually associated with local tenderness and swelling. All such cases were treated with i.v. hydrocortisone (100 mg bolus), local hydrocortisone s.c. injections, and local hydrocortisone cream. In the majority of cases resolution occurred within 24–48 h. The severity of local toxicity was related to dose, the most severe reaction being in patient 13 at 1350 mg/m² (grade 2), who required i.v. antibiotics to treat local cellulitis. Apart from grade 2 toxicity, although no organisms were cultured from swabs. The apparent fall in the incidence of local toxicity, although no organisms were cultured from swabs.

The AG337 formulation in 5% dextrose solution used in this study is acidic (pH 3–5), with glacial acetic acid (0.36% w/w) a constituent. Higher doses were associated with the administration of increased amounts of glacial acetic acid, and the low pH of the infusion may explain the local toxicity of AG337. Alternatively, buffering of the acidic drug solution within the vein might have resulted in drug precipitation and subsequent vein occlusion, although venous thrombosis was not observed. In an attempt to reduce local toxicity, AG337 was diluted in 2 liters as opposed to 1 liter of 5% dextrose; however, there was no marked reduction in the incidence or severity of local toxicity.

The AG337 formulation was well tolerated. Patient 11 on courses 2, 3, and 4 at 1350 mg/m² AG337 required i.v. metoclopramide during the infusion for nausea and vomiting, which may have been drug related. However, no other patient suffered from drug-associated vomiting. Two patients developed elevated liver function tests (ALT and AP), which may have been related to drug administration. Patient 7 had aggressive metastatic bladder carcinoma, with a normal liver ultrasound prior to drug administration. The patient deteriorated rapidly because of disease progression and received only one course of AG337 before coming off study due to poor performance status. On day 5 after AG337 administration, the plasma ALT concentration had risen to 123 IU/liter from a pretreatment level of 30 IU/liter, with levels returning to 58 IU/liter on day 18. AP levels did not exceed 143 IU/liter. Despite rapid disease progression, AG337 cannot be excluded as the reason for the ALT increases in this patient. The second patient to develop elevated liver function tests (patient 10), a patient with pancreatic carcinoma, had a sustained rise in ALT and AP following AG337 therapy. Subsequent ultrasound following course 2 revealed liver metastases and nodes at the porta hepatis. Drug administration was not therefore thought to be the cause of the rise in liver enzymes in this patient.

No significant bone marrow suppression was observed, although patient 11 on course 3 at 1350 mg/m² did develop grade 1 thrombocytopenia, with a fall in the platelet count from 271 × 10⁹/liter before treatment to 145 × 10⁹/liter on day 7. By day 10 the platelet count had returned to pretreatment levels. Patient 2 suffered from grade 3 anemia 2 weeks after her first and only course at 75 mg/m², which was associated with a melaena bleed unequivocally related to disease. One patient developed grade 4 septicemia 21 days after drug administration as a result of a central line infection. Overall, hematology and clinical chemistry profiles did not demonstrate consistent or significant changes from baseline values after drug treatment. There was one patient who died while on study of causes not related to AG337 therapy.

**DISCUSSION**

The primary objectives of the study described here were to determine whether or not plasma concentrations of AG337 could be achieved in patients which in preclinical studies were associated with antitumor activity, and to investigate whether or not these levels resulted in TS inhibition. AG337 has been investigated using human colorectal tumor cell lines, continuous exposure to AG337 at concentrations of 0.04–1 μg/ml results in 50% growth inhibition (15–18). Similarly, the cytotoxicity of AG337 has been investigated using human colorectal tumor cell lines, and, following a 5-day exposure, clonogenicity was reduced to

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**Table 3** Pharmacokinetic parameters for AG337 in patients with saturable pharmacokinetics

<table>
<thead>
<tr>
<th>Patient/course</th>
<th>Dose (mg/m²)</th>
<th>Vₘₐₓ (μg/ml/h/m²)</th>
<th>Kₛ (μg/ml)</th>
<th>V (litters/m²)</th>
<th>Clₘₐₓ (ml/min/m²)</th>
<th>Clₜₜ (ml/min/m²)</th>
<th>t½/2 (min)</th>
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<tr>
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<td>4.1</td>
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<td>150</td>
<td>80</td>
<td>93</td>
</tr>
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<td>8/1</td>
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<td>88</td>
<td>53</td>
<td>94</td>
</tr>
<tr>
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<td>118</td>
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<td>93</td>
</tr>
<tr>
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<td>91</td>
</tr>
<tr>
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<tr>
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<td>6.0</td>
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<td>67</td>
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**Table 4** AG337 dose escalation and local toxicity

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>Courses</th>
<th>Central lines</th>
<th>Toxicity</th>
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<td>3</td>
</tr>
<tr>
<td>900</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1350</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

a Number of patients with central lines at each dose level.
b Number of courses associated with grade 2 local toxicity, i.e., pain, inflammation, swelling. Local toxicity was only seen in patients who received AG337 by peripheral administration.
<10% of control following treatment with AG337 at a concentration of 1 μg/ml (17). Comparison of these in vitro results with the plasma concentration data for patients indicates that cytotoxic levels of AG337 can be achieved clinically and are well tolerated for 24 h.

In comparing in vitro and in vivo data, it is important to take note of protein binding since the serum supplement used in in vitro studies is normally only 10–20% and as a result free drug concentrations in vitro may be much higher than those observed in patients. As shown here, only 2–4% of AG337 was unbound in patients, in comparison to 60% in 10% serum-supplemented tissue culture medium. Assuming that only free AG337 is cytotoxic, the total plasma AG337 concentrations of approximately 10 μg/ml observed here, i.e., free drug concentrations of 0.2–0.4 μg/ml, should still be growth inhibitory and cytotoxic. Pharmacokinetic investigations performed in association with preclinical efficacy studies in mice revealed that active doses of AG337 gave rise to plasma total AG337 concentrations in the range 5–20 μg/ml. Again, these levels are similar to those observed in patients in the current study.

In an attempt to confirm the prediction that growth inhibitory and cytotoxic concentrations of AG337 were being achieved in patients, plasma dUrd levels were measured as a surrogate indicator of TS inhibition. Data from mice treated with N10-proparagyl-N6,N8-dideazafoaic acid suggests that plasma dUrd elevations can be used to measure the pharmacodynamics of TS inhibitors (19); however, the technique has not been applied widely in clinical studies. The data obtained following AG337 administration showed a clear and consistent pattern with dUrd concentrations being elevated at the end of the 24-h infusion in all patients treated at 600 mg/m² and above. In contrast, only one patient treated below this dose level displayed a convincing dUrd elevation. In guiding the future development of AG337, two additional aspects of the effects of the drug on dUrd concentrations are noteworthy. First, in all patients, plasma dUrd concentrations had returned to pretreatment levels 24 h after the end of the AG337 infusion, suggesting that TS inhibition was dependent on the presence of extracellular drug. By virtue of its structure, AG337 cannot be polyglutamated and will probably pass readily through the cell membrane, thus, presumably, intracellular drug concentrations decline in parallel with extracellular levels, leading to a rapid recovery of TS activity following the end of the infusion. Second, the magnitude of the increase in dUrd concentrations did not appear to increase above a dose of 600 mg/m² AG337, i.e., at either 900 or 1350 mg/m². The most likely explanation for the lack of further dUrd elevations is that maximum cellular export of the nucleoside was already achieved at 600 mg/m² AG337 and that any increase in TS inhibition that was produced by higher doses was not large enough to be reflected in further dUrd elevations. It should be noted, however, that there are only limited preclinical data which address the issue of the relationship between TS inhibition and the magnitude of the dUrd elevation. As such, plasma dUrd elevation data should be used with extreme caution in guiding dose escalation with TS inhibitors.

The early clinical development of antimetabolite cytotoxic drugs presents particular problems and AG337 illustrates some of these. Preclinical toxicity models do not accurately predict the quantitative or qualitative toxicity of antimetabolites (26–28) due to interspecies differences in activating or inactivating enzymes and/or levels of endogenous metabolites capable of circumventing toxicity. In the development of AG337, neither of the species used in preclinical toxicity studies (mice and dogs) proved informative, and as a result the pharmacokinetic and pharmacodynamic study described here was performed. In the light of clinical data on AG337 given as a 24-h infusion reported here, a 5-day continuous i.v. infusion Phase I study of AG337 has been initiated, and the results from the present study have been pivotal to the design of this trial. First, a knowledge of the rapid plasma clearance of AG337 led to the selection of a continuous infusion as the mode of AG337 administration. Second, data on the relationship between plasma AG337 concentrations and dUrd elevations provided target AG337 levels that are likely to be associated with TS inhibition, and hence antiproliferative toxicity when TS inhibition is maintained for prolonged periods. Third, the starting dose chosen for the 5-day continuous study was 120 mg/m²/day X 5 (total dose, 600 mg/m²), a total dose equal to that giving dUrd elevations in the current study. Fourth, the pharmacokinetic data for AG337 in patients, in particular the presence of saturable clearance, emphasized the importance of careful pharmacokinetic monitoring and the need to adjust dose escalation in light of the pharmacokinetic data. Finally, the absence of acute systemic toxicity, even at 1350 mg/m², indicated that nonmechanism-related toxicities were not likely to be encountered; however, the occurrence of significant local toxicity indicated that central line administration would be required for additional studies of parenteral AG337.

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1284 AG337 24-h Study


Clinical pharmacokinetic and pharmacodynamic studies with the nonclassical antifolate thymidylate synthase inhibitor 3, 4-dihydro-2-amino-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone dihydrochloride (AG337) given by 24-hour continuous intravenous infusion.
