Phase I Studies with the Nonclassical Antifolate Nolatrexed Dihydrochloride (AG337, THYMITAQ) Administered Orally for 5 Days

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ABSTRACT

Phase I studies of p.o. administered nolatrexed dihydrochloride (AG337, THYMITAQ), a nonclassical thymidylate synthase inhibitor, were performed to establish the maximum tolerated dose and a recommended dose for Phase II studies. The bioavailability and pharmacokinetic and pharmacodynamic properties of oral nolatrexed were also studied. Forty-five patients were treated with oral nolatrexed every 6 h for 5 days at doses of 288-1000 mg/m²/day. The bioavailability of the oral preparation was determined, and the effect of a standard meal on nolatrexed absorption was investigated at a dose of 800 mg/m²/day. Nolatrexed plasma concentrations were analyzed by high-performance liquid chromatography. Nolatrexed was rapidly absorbed with a median bioavailability of 89% (range 33–116%), with 88% of patients above 70%. The dose-limiting toxicities were gastrointestinal, and the recommended Phase II oral dose was 800 mg/m²/day. After a standard meal, the peak plasma nolatrexed concentration achieved was lower (median, 8.3 µg/ml versus 15.0 µg/ml; P = 0.001), and the time taken to reach the peak was longer (median, 180 min versus 45 min; P = 0.00003), but the trough concentration was higher (median, 3.6 µg/ml versus 2.1 µg/ml; P = 0.004) when compared with the fasted state. The area under the nolatrexed plasma concentration versus time curve was not affected by food. Average trough nolatrexed concentration, but not dose, was significantly related to the % decrease in both thrombocytes (r² = 0.58; C₅₀ = 6.0 µg/ml, where C₅₀ is the plasma concentration associated with a 50% decrease in thrombocytes) and neutrophils (r² = 0.63; C₅₀ = 0.6 µg/ml).

Nolatrexed can be safely administered as an oral preparation at a dose of 800 mg/m²/day for 5 days. Bioavailability was close to 100% and, because inhibition of thymidylate synthase by nolatrexed is rapidly reversible, the slower absorption after a standard meal may result in a shorter duration of noninhibitory concentrations between doses.

INTRODUCTION

TS is the rate-limiting enzyme in the de novo biosynthetic pathway for thymidine nucleotides catalyzing the reductive methylation of dUMP to dTMP, a reaction that requires the folate cofactor 5,10 methylene tetrahydrofolate. Because thymidine nucleotides are used exclusively for the synthesis of DNA, TS remains an important target for anticancer drug therapy (1–3), and there is currently a high degree of interest in TS inhibitors that act at the folate binding site.

Several folate-based TS inhibitors have been studied in clinical trials. CB3717 demonstrated promising clinical activity, but because of sporadic and unpredictable nephrotoxicity and myelotoxicity, its development was abandoned (4, 5). Raltitrexed, a successor to CB3717, has shown encouraging clinical activity and can be safely and conveniently administered to patients (6, 7), and other novel agents are in early clinical development (1, 3).

Nolatrexed was designed by Agouron Pharmaceuticals (San Diego, CA) using X-ray crystallographic data from the TS folate binding site and molecular modeling (8). It is a nonclassical TS inhibitor in that it lacks a terminal glutamate side chain and is uncharged at physiological pH. Nolatrexed does not require a specific transport mechanism to gain entry into cells and, once within the cell, is not a substrate for the enzyme folyl polyglutamate synthetase. It is expected that classical antifolate resistance mechanisms involving defective cellular membrane transport and polyglutamation will not influence nolatrexed activity. The structure of the dihydrochloride salt of nolatrexed is shown in Fig. 1. All of the doses and concentrations in this article refer to the nolatrexed free base, which is the active form of the drug.

Nolatrexed has been extensively studied in Phase I and II clinical trials and has shown evidence of antitumor activity. In

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A pilot clinical study, 13 patients received 27 courses of nolatrex given as a continuous 24-h infusion (9). The starting dose was 60 mg/m² and was escalated to 1080 mg/m². No antiproliferative toxicity was observed. The rapid elimination of the drug and reversal of TS inhibition indicated that a more prolonged administration should be investigated. A Phase I study of a continuous 5-day infusion was, therefore, performed (10). To bypass the local toxicity seen in the initial study, patients received nolatrexed via a central venous catheter. The starting dose was 96 mg/m²/day, the recommended Phase II dose was found to be 800 mg/m²/day, and the dose-limiting toxicity was hematological.

Various Phase II studies using a continuous 5-day i.v. nolatrexed infusion have been undertaken in different tumor types, and preliminary results have been presented (11–17). Nolatrexed has shown antitumor activity in several types of malignancy, the most promising being head and neck squamous cell carcinoma and hepatoma.

To improve the convenience of the administration of nolatrexed and to avoid any complications from an indwelling central venous catheter, an oral preparation of the drug was developed. Preclinical data indicated a bioavailability in mice of 96% and 66% after oral or i.p. administration, respectively.

The main objectives of this Phase I study were:

(a) to determine the MTD of p.o. administered nolatrexed when given as repeated oral dosing for 5 days;
(b) to establish the dose-limiting and other toxic effects;
(c) to study the pharmacokinetics of the drug after the administration of single and repeated oral dosing and to determine the feasibility of achieving plasma levels capable of inhibiting the target enzyme TS;
(d) to document any antitumor effects; and
(e) to establish an oral dose suitable for the Phase II evaluation of activity.

PATIENTS, MATERIALS, AND METHODS

Patient Population. A total of 48 patients were enrolled in the studies, of whom 42 were evaluable for toxicity. Between one and six (median, two) courses of treatment were administered to each patient, with a total of 115 courses. Individual patient characteristics are shown in Table 1. Of the 45 patients that commenced treatment with oral nolatrexed, 53% had colorectal cancer, 56% were male, and 71% had received prior chemotherapy regimes. Patient ages ranged from 29–71 (median, 56) years. The studies were approved by the Local Research Ethics Committee and conducted under the auspices of the Cancer Research Campaign Phase I/II Clinical Trials Committee. All of the patients gave written informed consent before registration in the studies. Patient inclusion and exclusion criteria were as described previously (10).

Patient Monitoring. The primary purpose of the studies was not to assess tumor response; however, clinical measurements and scans were performed on patients who had assessable disease to document disease status. Patients received as many courses of nolatrexed as clinically indicated and were withdrawn from the study if their disease progressed, if they requested to do so or if it was felt by the investigator to be in the best interest of the patient. Common toxicity criteria (CTC) grades for toxicity and response were used.

Materials. Nolatrexed was supplied by Agouron Pharmaceuticals (San Diego, CA) as the dihydrochloride salt. The oral drug was supplied in size 4 and size 2 capsules containing 20 mg and 80 mg nolatrexed free base, respectively. The capsules contained microcrystalline cellulose, the content of which ranged, per capsule, from 4 parts to 1 part of the weight of nolatrexed together with crospovidone and magnesium stearate, which were maintained at 5% (w/w) and 0.5% (w/w), respectively, of the weight of the filled capsule. A second formulation, of higher strength, was supplied in size 3 and size 1 capsules containing 50 mg and 200 mg of drug, respectively. The capsules were stored in a cool dry place at room temperature.

Nolatrexed for i.v. infusion was used as described previously (9). The dose (200 mg/m²) for i.v. infusion was diluted in 1 liter of 5% (w/w) dextrose and administered through a peripheral vein over a period of 6 h using an infusion pump.

Analytical samples of nolatrexed and AG236, the 2-desamino 2-methyl derivative of nolatrexed, were obtained from Agouron Pharmaceuticals. Analytical grade acetonitrile and methanol were obtained from Fisons Scientific Equipment (Loughborough, United Kingdom). The Red Cross Transfusion Service (Newcastle upon Tyne, United Kingdom) kindly provided outdated plasma, which was used as control plasma. Chromatographic equipment and conditions, sample processing and preparation, and high-performance liquid chromatography analyses were as described previously (9).

Phase I Dose Escalation and Bioavailability Studies. Twenty-five patients were entered in this part of the study, which sought to establish the MTD of nolatrexed when administered p.o. for 5 days, to determine the dose-limiting side effects, and to calculate the bioavailability of the oral preparation.

Days 1 and 2 of treatment were randomized for each patient, such that patients received either 200 mg/m² nolatrexed i.v. as a 6-h infusion on day 1 and then 200 mg/m² p.o. (after an overnight fast) on day 2 or vice versa. On days 3–7 (i.e., for 5 days) patients received repeated oral dosing every 6 h. The starting dose was 1440 mg/m² for 5 days equivalent to 288 mg/m²/day. This dose was one-half that which, at the time of the commencement of this study, had been shown to be nontoxic to patients in the 5-day continuous infusion study (10). On the
second course of treatment 3 weeks later, the order of nolatrexed administration on days 1 and 2 was reversed.

Dose escalation was guided by the pharmacokinetic results obtained, which were compared to those for nolatrexed given by 5-day i.v. infusion. Doses were doubled until the predicted plasma level for the next dose exceeded the target plasma level from the 5-day i.v. infusion study, the target concentration (4 \( \mu \)g/ml) being that associated with consistent elevations of plasma deoxyuridine. Intrapatient dose escalations were permitted. Also, doses were reduced one level in the case of unacceptable toxicity.

In this part of the study, 3 ml of heparinized blood samples were taken for nolatrexed analysis before drug administration and at the following times:

![Table 1: Patient characteristics, nolatrexed dose levels, and outcome](clincancerres.aacrjournals.org)
0.5, 1, 1.5, 2, 3, 4, and 6 h after the start of the i.v. infusion and then at the end of infusion;
5, 10, and 15 min, and 0.5, 1, 1.5, 2, 2.5, 5, 8, and 18 h after infusion.

On the day of single oral dosing, samples were collected before drug administration and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, and 24 h after drug administration.

On day 3, samples were collected predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 8, 12, and 24 h following the first oral dose of the day. Sampling on day 7 was the same as day 3.

Plasma samples were treated as described previously (9).

**Food Effect Study.** Sixteen patients were studied to determine the effect of a standard meal on the absorption of p.o. administered nolatrexed. The “standard meal” consisted of 29.5 g of protein (118 Kcals), 26.4 g of fat (238 Kcals), and 78 g of carbohydrate (292 Kcals). On day 1, patients were randomized to receive 200 mg/m² of drug either after a standard meal or an overnight fast. Day 2 was the opposite of day 1. Both doses were followed by blood sampling for pharmacokinetic studies. The patients then received 200 mg/m² every 6 h on days 3–7 (i.e., for a total of 5 days). On the second course of treatment, 3 weeks later, the order of days 1 and 2 was reversed. The first 12 of these patients received a dose of 800 mg/m²/day. Patients 45–48 received a dose of 900 mg/m²/day because the drug had been well tolerated at the previous dose level.

**Higher Strength Formulation Study.** Because of the large number of 20 mg and 80 mg nolatrexed capsules that the patients were required to take, capsules of an increased strength were developed to improve patient tolerance and acceptability. The final eight patients from the food-effect study took part in a study of the absorption of nolatrexed from capsules of 50 mg and 200 mg nolatrexed. This study was performed to a similar design as that above:

Day 1, 200 mg/m² (new formulation), randomized to fasting/nonfasting;
Day 2, 200 mg/m² (new formulation), nonfasting/fasting (opposite to day 1); and
Days 3–7, 200 mg/m² q.d.s. (new formulation, but first dose on day 3 only is given with original formulation).

**PET Scanning Study.** Eight patients receiving oral nolatrexed were part of a study that was undertaken in conjunction with Hammersmith Hospital (London). This study involved the use of [13C]thymidine PET before and after nolatrexed administration, the results of which will be reported elsewhere.

**Pharmacokinetic Data Analysis.** Pharmacokinetic data were available for 45 patients, with 2 courses of data for 37 of these patients. Sampling was performed during days 1 and 5 of the 5 days of continuous administration (days 3 and 7 of the study) for 23 patients. Nolatrexed plasma concentrations were determined using a high-performance liquid chromatography assay described previously (9).

Data from the bioavailability study were analyzed by a compartmental model for estimation of parameters describing the absorption of nolatrexed. The final model included zero-order absorption with a two-compartment model and either linear or saturable elimination. This analysis was necessary because of the nonlinear pharmacokinetics of nolatrexed in some patients as described previously (9) and because of the very rapid absorption observed. Data from the subsequent 5 days of administration were used to estimate the AUC for each dose, using the trapezoidal rule with extrapolation to infinity. Correction was made for the contribution from previous doses. Peak and trough concentrations (the plasma concentration at 6 h, i.e., when the subsequent dose would be due) were also noted for different days of administration.

For the food effect study and the comparison of formulations, AUC values, peak and trough concentrations, and time to peak were compared between test doses.

**Pharmacodynamic Analysis.** For the patients with pharmacokinetic sampling throughout treatment, hematological tox-

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Toxicity grade</th>
<th>Hematological</th>
<th>Nonhematological</th>
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<tbody>
<tr>
<td></td>
<td>WBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Plts</td>
<td>Hb</td>
</tr>
<tr>
<td>1, 3 patients (7 courses)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2, 7 patients (16 courses)</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>3, 31 patients (75 courses)</td>
<td>1</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>3a, 4 patients (10 courses)</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>4, 5 patients (7 courses)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>WBC, leucopenia; Plts, thrombocytopenia; Hb, anemia; Grans, granulocytopenia; Lymphs, lymphopenia.
icity (blood cell count nadir, percentage decrease) was related to AUC and average peak and average trough concentrations, as well as to dose. Linear and sigmoid $E_{\text{max}}$ pharmacodynamic models were investigated as described previously (9, 10).

RESULTS

Clinical Study—Toxicity. A summary of the toxicities experienced is shown in Table 2. At dose level 1 (288 mg/m$^2$/day), toxicity was minimal and no grade 3 or 4 events were observed.

Dose escalation to level 2 (576 mg/m$^2$/day) resulted in grade 3 and 4 hematological toxicity with one grade 3 stomatitis. Of interest, two patients (patients 6 and 17) accounted for 12 of the 17 grade 3 and 4 hematological events. Both patients had received prior chemotherapy.

Seventy-five patient courses assessable for toxicity were administered at dose level 3 (800 mg/m$^2$/day). Along with hematological toxicity, the other major factors were nausea, vomiting, diarrhea, stomatitis, and rash. Thirty-one courses (41%) were complicated by at least one grade 3 or 4 toxicity of some description; however, 35% of the patients who received some or all of their treatment at this dose level did not experience any toxicities greater than grade 2.

From a hematological viewpoint, anemia and lymphopenia were never a clinical problem. Thirteen of the 75 courses were complicated by leucopenia of at least grade 3 severity and 19 by neutropenia of at least grade 3. Grade 3 or 4 thrombocytopenia was observed in six patients (eight courses). Gastrointestinal toxicity was effectively confined to grade 2 or below at this dose level. There was one incidence of grade 3 rash.

Five patients received seven courses of therapy at the highest dose level (1000 mg/m$^2$/day). Grade 3 or 4 toxicities were experienced in four patients (one course each), and two led to hospital admissions. Seven of the 12 major toxic events were hematological. The final four patients were treated at a dose of 900 mg/m$^2$/day (designated dose level 3a). Of the 10 courses of treatment received, 3 were complicated by at least two grade 3 or 4 events (in two of the four patients).

Other toxicities, principally hepatotoxicity, skin rash, and hair loss, were mild, transient, and not of clinical significance. Several patients did show elevations of liver enzymes but only at the higher dose levels. These generally resolved before retreatment and were associated with progression of liver metastases in at least two patients.

Clinical Study—Efficacy. Of the 48 patients who consented to the study, 37 were evaluable for response. On cessation of treatment, 31 patients had progressive disease, and 6 patients had stable disease. All of the latter patients had colorectal cancer, and two were chemotherapy naive. Patient 33, a 59-year-old man with colorectal carcinoma, experienced a partial response (60% reduction) in a liver metastasis, although, overall, his disease was stable (47% reduction in total tumor

![Fig. 2 Plasma nolatrexed concentrations and predictions of the pharmacokinetic model for patient 22. Data are for the test doses (upper panel) and for the full 5 days of subsequent administration (lower panel).](https://clincancerres.aacrjournals.org)
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Table 4: Plasma concentrations and nolatrexed AUC values during 5 days of oral administration every 6 h

<table>
<thead>
<tr>
<th>Dose level (mg/m²/day)</th>
<th>n</th>
<th>Day 3 AUC</th>
<th>Day 7 AUC</th>
<th>Day 3 C_max</th>
<th>Day 7 C_max</th>
<th>Day 3 trough</th>
<th>Day 7 trough</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/ml · min</td>
<td>µg/ml · min</td>
<td>µg/ml median (range)</td>
<td>µg/ml median (range)</td>
<td>µg/ml median (range)</td>
<td>µg/ml median (range)</td>
</tr>
<tr>
<td>288</td>
<td>4</td>
<td>1.1 (0.4–1.5)</td>
<td>1.1 (0.5–2.3)</td>
<td>6.6 (1.7–7.6)</td>
<td>7.0 (2.8–10.6)</td>
<td>1.0 (0.3–1.5)</td>
<td>1.2 (0.4–3.0)</td>
</tr>
<tr>
<td>576</td>
<td>5</td>
<td>1.6 (1.3–2.3)</td>
<td>1.7 (1.5–3.5)</td>
<td>12.5 (8.5–15.5)</td>
<td>13.4 (8.9–22.0)</td>
<td>1.5 (0.6–2.2)</td>
<td>2.3 (1.2–8.1)</td>
</tr>
<tr>
<td>800</td>
<td>9</td>
<td>2.6 (0.9–6.3)</td>
<td>2.3 (1.3–4.1)</td>
<td>13.6 (4.1–19.1)</td>
<td>14.3 (4.9–21.5)</td>
<td>2.3 (0.0–7.7)</td>
<td>4.1 (1.9–11.4)</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>3.1 (1.3–4.3)</td>
<td>3.1 (1.4–6.5)</td>
<td>13.3 (12.6–25.9)</td>
<td>19.2 (11.6–26.3)</td>
<td>3.0 (0.2–8.2)</td>
<td>3.1 (0.2–10.9)</td>
</tr>
</tbody>
</table>

a AUC is area under the plasma concentration-time curve.
b C_max is peak concentration observed.

Trough is concentration immediately before subsequent dose.

Bioavailability Study. A model incorporating saturable elimination and zero-order absorption was simultaneously fitted to the data for the two test doses. The results for days 1 and 2 of the first course in 23 patients are given in Table 3. In the majority of patients, the data could be described by a linear, two-compartment model with zero-order absorption, whereas in six patients, a model with saturable elimination provided a better fit to the data. Representative plots of observed data with the predictions of the fitted model for patient 22 are given in Fig. 2.

Overall, the bioavailability of nolatrexed was high (median, 89%; range, 33–116%), with only two patients having bioavailabilities less than 65%. When bioavailability was determined on course 2, the values for these two patients were 73 and 97%. Otherwise, the bioavailability data for the test doses on the two courses were largely comparable, with no effect from the order of administration of i.v. and oral doses. Pharmacokinetic parameters defining the elimination and distribution of nolatrexed after oral administration were similar to those seen following continuous i.v. infusion for 5 days (10).

Using the pharmacokinetic parameters obtained after the day 1 and 2 test doses, the plasma concentrations during subsequent continuous oral administration were predicted well by the model (e.g., see Fig. 2). Summary data for 23 patients at the four dose levels that formed the Phase I part of the study are given in Table 4. The AUC values increased linearly with dose, and there was no significant difference between the day 3 and day 7 AUC values for each patient. With the exceptions of patient 20 at the highest dose level and patient 15 at the 800–mg/m²/day level, there was no evidence of drug accumulation as reflected in peak and trough nolatrexed concentrations at the beginning and end of the course of treatment.

Food Effect Study. After a standard meal, the peak concentration of nolatrexed after oral administration was lower, and the time taken to reach the peak was longer (see Fig. 3). The median peak concentration (C_max) value was 8.3 µg/ml after a standard meal compared with 15.0 µg/ml in the fasted state (P = 0.0001, cross-over analysis). For T_max, the median value was 180 min after the meal, compared with 45 min after overnight fasting (P = 0.00003). The trough concentration of nolatrexed was determined at 6 h, and this was higher (median value, 3.6 µg/ml, compared with 2.1 µg/ml) when the drug was administered after a standard meal (P = 0.004). A summary of these data are given in Table 5. Overall, the nolatrexed AUC value was no different when the drug was administered after a meal or on an empty stomach (median, 2.50 µg/ml/min in the fast state versus 2.58 µg/ml/min). In this cross-over study, we also observed a small period effect on AUC and trough concentrations, in that the values on day 2 were slightly lower than those on day 1 (P = 0.03 and 0.02, respectively).

Formulation Study. The results from this part of the study are also summarized in Table 5. The median values for peak nolatrexed plasma concentrations and time to peak are almost identical—11.9 µg/ml and 55 min for the initial formulation compared with 12.7 µg/ml and 45 min for the higher strength capsules. Similarly, the nolatrexed AUC values were 2.12 µg/ml/min and 2.05 µg/ml/min, respectively.

Pharmacokinetic/Pharmacodynamic Relationships for Oral Nolatrexed. There were no significant relationships (r² < 0.1) between hematological toxicity and dose; a wide range of hematological toxicities were observed at each dose level.

Although weak relationships were observed between toxicity and nolatrexed AUC or peak concentrations (r² = 0.4–0.5), relationships with trough nolatrexed concentration were the most significant. For percentage decrease in neutrophils or platelets, an E_max model provided a good fit to the data (r² = 0.6), with average trough nolatrexed concentrations of 0.6 and 6.0 µg/ml being associated with a 50% decline of neutrophils and platelets, respectively (Fig. 4). Similarly, trough nolatrexed...
concentrations were lower in patients with ≤ grade 2 versus ≥ grade 3 neutropenia (P = 0.05, data not shown).

DISCUSSION

In early clinical studies with i.v. nolatrexed, it was shown that TS inhibition due to nolatrexed was short-lived (9) and that continuous exposure to the drug for a prolonged period of time was required to produce an antitumor effect. Consistent with these early clinical data, in studies using continuous 5-day infusions, nolatrexed has shown antitumor activity in Phase II clinical trials. In the studies described here, an oral preparation of nolatrexed has been examined because of the obvious benefits for the patient over a continuous 5-day infusion regimen through an indwelling central venous catheter. The pharmacokinetics of nolatrexed after single and repeated oral dosing were investigated, particularly with respect to the effects of a standard meal and capsule strength on the absorption of the drug.

The starting dose for the study was 288 mg/m²/day and, after three dose escalations, the MTD of 1000 mg/m²/day was reached. The recommended dose for Phase II studies of oral nolatrexed is 800 mg/m²/day. Dose-limiting toxicities were found to be nausea and vomiting; however, antiproliferative effects of neutropenia and stomatitis were observed at higher dose levels.

The drug was very rapidly absorbed in the oral form and had excellent bioavailability. The peak plasma concentration achieved when nolatrexed was taken after food was lower and later than when taken on an empty stomach, which could potentially result in less acute toxicity. However, the nolatrexed AUC was not affected by food, demonstrating that it was the time course, but not the extent, of absorption that was influenced. Consistent with a change in time course of nolatrexed absorption, trough levels measured at 6 h after the oral dose were significantly higher when the drug was administered after a meal. Interestingly, trough nolatrexed levels after a dose taken in the fasted state were below those predicted (on the basis of i.v. data; Ref. 9) to be required for maintained TS inhibition. In

Figure 4 Relationship between hematological toxicity and trough nolatrexed concentrations in plasma. Lines are fit of sigmoid E_max model to the data. Upper panel, % decrease in absolute neutrophil count (ANC; \( r^2 = 0.63, C_{\text{so}} = 0.6 \mu \text{g/ml} \)). Lower panel, % decrease in platelets (\( r^2 = 0.58, C_{\text{so}} = 6.0 \mu \text{g/ml} \)).

Table 5  Pharmacokinetic parameters for nolatrexed after an overnight fast (F) or a standard breakfast (NF), using both original and higher strength formulations (see “Discussion” concerning patient 48)

<table>
<thead>
<tr>
<th>Patient no. Courses</th>
<th>Original formulation</th>
<th>Higher strength formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (^a) (µg/ml ⋅ min)</td>
<td>C_{max} (^b) (µg/ml)</td>
</tr>
<tr>
<td>30 and 32–39 14</td>
<td>F  2.67 2.58 16.6 8.5 45 120 2.3 3.4</td>
<td>NF  1.66 1.62 5.1 7.2 30 45 1.1 1.2</td>
</tr>
<tr>
<td></td>
<td>F  5.24 5.81 25.2 13.0 180 185 5.5 7.7</td>
<td>NF  1.60 9.7 30 N/A 0.80 0.56 5.8 2.6</td>
</tr>
<tr>
<td>40–47 12</td>
<td>F  2.53 13.1 60 3.9 2.46 2.52 13.8 8.3 48 180 2.6 4.2</td>
<td>NF  1.36 6.6 30 1.1 0.89 2.11 8.3 6.1 30 60 0.5 2.2</td>
</tr>
<tr>
<td>48</td>
<td>F  7.52 19.2 94 8.0 4.72 6.48 17.0 10.8 90 245 5.0 8.3</td>
<td>NF  1.04 7.3 20 0.6 0.32 0.96 2.2 5.3 45 60 0.2 0.6</td>
</tr>
<tr>
<td></td>
<td>F  1.60 9.7 30 N/A 0.80 0.56 5.8 2.6</td>
<td>NF  1.60 9.7 30 N/A 0.80 0.56 5.8 2.6</td>
</tr>
</tbody>
</table>

\( ^a \) AUC is the area under the plasma concentration-time curve.  
\( ^b \) C_{max} is the peak concentration observed.  
\( ^c \) T_{max} is the time taken to reach the peak plasma concentration.  
\( ^d \) Trough is the concentration immediately before the subsequent dose (6 h).
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contrast, the higher trough levels observed when nolatrexed was administered with food would be predicted to be TS-inhibitory.

The results for patient 48 (Table 5) are remarkable for having very low peak plasma concentrations and AUCs when compared with the rest of the patients studied. This patient underwent a partial gastrectomy for peptic ulcer disease 40 years before receiving nolatrexed, which may account for the reduced absorption of the drug in as much as nolatrexed seems to be absorbed very rapidly from the stomach in the absence of food. The operation may have meant that gastric absorption was impaired. Alternatively, this patient may have had a higher gastric pH than the other patients studied. Nolatrexed solubility decreases markedly above pH 3–4, and this factor may also have contributed to poor oral absorption. Patient 48 had minimal toxicity as would be expected from the low plasma drug concentrations.

Pharmacokinetic analysis indicated that the most significant pharmacokinetic parameter most closely related to toxicity was the trough nolatrexed concentration between doses. The antiproliferative effects of TS inhibitors are dependent on continuous blockade of the de novo pathway of DNA synthesis. Strategies to smooth the variation in nolatrexed plasma concentrations between doses, such as sustained-release formulations, may yield more clinically effective and reproducible antitumor effects.

In summary, nolatrexed can be safely administered p.o. every 6 h for 5 days. Toxicities encountered were similar to those observed after i.v. dosing and usually of a brief duration. The recommended Phase II oral dose for nolatrexed was 800 mg/m²/day, and there may be a beneficial delay in absorption after the administration of nolatrexed capsules with food.

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Phase I Studies with the Nonclassical Antifolate Nolatrexed Dihydrochloride (AG337, THYMITAQ) Administered Orally for 5 Days

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