A Phase I Study of Raltitrexed, an Antifolate Thymidylate Synthase Inhibitor, in Adult Patients with Advanced Solid Tumors

Jean L. Grem,1 J. Mel Sorensen, Ellen Cullen, Chris H. Takimoto, Seth M. Steinberg, Alice P. Chen, J. Michael Hamilton, Susan G. Arbuck, Nanette McAtee, David Lawrence, Barry Goldspiel, Patrick G. Johnston, and Carmen J. Allegra

National Cancer Institute-Medicine Branch, National Naval Medical Center, Bethesda, Maryland 20889-5105 [J. L. G., E. C., C. H. T., A. P. C., J. M. H., N. M., P. G. J., C. J. A.]; Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland 20892-7426 [J. M. S., S. G. A.]; Biostatistics and Data Management Section, National Cancer Institute, Bethesda, Maryland 20892-8225 [S. M. S.]; Department of Radiology, National Naval Medical Center, Bethesda, Maryland 20889-5000 [D. L.]; Department of Radiology and Nuclear Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20889 [D. L.]; and Pharmacy Department, W. G. Magnusen Clinical Center, Bethesda, Maryland 20892 [B. G.]

ABSTRACT

The purpose of this study was to perform a Phase I trial of raltitrexed, a selective inhibitor of thymidylate synthase, and to determine the pharmacokinetic and toxicity profiles as a function of raltitrexed dose. Fifty patients with advanced solid tumors and good performance status were treated with raltitrexed as a 15-min i.v. infusion every 3 weeks, at doses escalating from 0.6 to 4.5 mg/m². Asthenia, neutropenia, and hepatic toxicity were the most common dose-limiting toxicities in this largely pretreated patient population, but they occurred during the initial cycle in only one of nine patients treated with 4.0 mg/m² and in two of nine patients treated with 4.5 mg/m². Only 2 of 13 patients treated with 3.5 mg/m² ultimately experienced unacceptable toxicity after three and seven cycles, respectively. The maximum raltitrexed plasma concentration and the area under the plasma concentration-time curve increased in proportion to dose. Raltitrexed clearance was independent of dose and was associated with the estimated creatinine clearance. Asthenia, neutropenia, and hepatic transaminis were dose-related and tended to occur more frequently when patients received three or more cycles of therapy. A 3-week treatment interval was feasible in the majority of patients at all doses. Although 4.0 mg/m² appeared to be a safe starting dose in this pretreated patient population, about half who received two or more courses ultimately experienced dose-limiting toxicity. A dose of 3.5 mg/m² was well tolerated in most patients.

INTRODUCTION

TS² is essential for the de novo synthesis of thymidine nucleotides from the physiological substrate dUMP. TTP is an essential precursor that is required for both DNA synthesis and repair. The pyrimidine analogues 5-fluorouracil and 5-fluoro-2′-deoxuryridine both require intracellular metabolism to FdUMP (1). In the presence of sufficient concentrations of the reduced folate cofactor, 5,10-methylene tetrahydrofolate, FdUMP forms a slowly reversible, ternary covalent complex with TS, thus inhibiting its catalytic activity. In addition, incorporation of fluoro-UTP into RNA and fluoro-dUTP into DNA may contribute to host toxicity as well as anticancer cytotoxicity. Inhibition of TS is accompanied by accumulation of dUMP, with subsequent metabolism to dUTP. Incorporation of dUTP into DNA may also interfere with nascent DNA synthesis and integrity. In some models, expansion of dUMP pools can partially reverse FdUMP-mediated TS inhibition. These considerations stimulated interest in developing metabolic inhibitors targeted against the folate-binding site of TS (2–4). Initial structure-activity investigations focused on quinazoline folate analogues of 5,10-methylene tetrahydrofolate and led to the discovery of N¹⁰-propargyl-5,8-dideazafolic acid (CB3717), a potent inhibitor of TS (Kᵢ, 3 nM; Refs. 5–7). Although clinical responses were observed with CB3717, clinical development was terminated due to severe, dose-limiting renal toxicity, which was attributed to its poor aqueous solubility and its precipitation in renal tubules (8–13).

Systematic efforts focused on optimizing the structure of a molecule that would have good aqueous solubility, be transported by the reduced folate carrier, and be a good substrate for folylpolyglutamate synthetase, thus allowing for prolonged intracellular retention. ZD1694 emerged as a leading candidate (3, 14–18). Although raltitrexed monoglutamate is considerably less potent than CB3717 as an inhibitor of purified TS in cell-free systems (Kᵢ, 60 nM), it is a much better substrate for both the reduced folate carrier (20-fold) and for folylpolyglutamate synthetase (31-fold).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at National Cancer Institute-Medicine Branch, National Naval Medical Center, 8901 Wisconsin Avenue, Building 8, Room 5101, Bethesda, MD 20889-5105. Phone: (301) 435-5382; Fax: (301) 480-1683; E-mail: jgrem@helix.nih.gov.

Received 8/13/96; revised 5/5/99; accepted 6/14/99.
Furthermore, not only are the polyglutamate forms of raltitrexed (triglutamates and above) very potent inhibitors of TS in cell-free systems (Kᵢ = 1 nM), but they also have prolonged intracellular retention. These features explain raltitrexed’s greatly improved cytotoxicity in cell culture models, compared with CB3717. A potential mechanism of resistance to inhibitors of TS is the salvaging of thymidine from the extracellular environment and subsequent conversion to TMP by thymidine kinase. In various cell culture models, concentrations of thymidine ranging from 1 to 20 μM protect cancer cells from the cytotoxicity of TS inhibitors (13, 17). Concurrent exposure to pharmacological concentrations of leucovorin decreases raltitrexed intracellular accumulation, polyglutamation, and cytotoxicity, whereas delayed administration of leucovorin is less effective in rescuing cells from prior raltitrexed exposure (13, 17).

The circulating plasma concentrations of thymidine in humans average between 0.1 and 0.2 μM and are thought to be insufficient to afford protection (19). In contrast, rodents have about 10-fold higher plasma thymidine levels, which complicates the preclinical evaluation of in vivo activity of TS inhibitors, including raltitrexed (20, 21). Administration of raltitrexed daily for 5 days was needed to observe raltitrexed activity in conventional murine tumor models (14, 21–23). However, a single dose of raltitrexed cures murine lymphoma that is thymidine kinase-deficient.

Toxicity studies in the rat and dog demonstrated that the bone marrow and gastrointestinal tract were the most sensitive tissues, and no deleterious effects were observed on the liver or kidney (21, 22). The β half-life of raltitrexed after i.p. or i.v. bolus administration in mice and rats is 30 min, but parent drug is detectable 24 h after dosing, suggesting a much longer terminal half-life (24). The levels of radiolabeled raltitrexed in L1210 ascitic leukemia cells 24 h after dosing were 50–100-fold higher than in plasma, with most of the drug present in polyglutamate forms (14). Because a long terminal plasma half-life and prolonged intracellular retention of raltitrexed polyglutamates were predicted for humans as well, a single dose of raltitrexed given every 3 weeks was chosen for clinical trials.

The starting dose for the initial human study of raltitrexed given every 3 weeks was chosen for clinical trials. The circulating plasma concentrations of thymidine in humans average between 0.1 and 0.2 μM and are thought to be insufficient to afford protection (19). In contrast, rodents have about 10-fold higher plasma thymidine levels, which complicates the preclinical evaluation of in vivo activity of TS inhibitors, including raltitrexed (20, 21). Administration of raltitrexed daily for 5 days was needed to observe raltitrexed activity in conventional murine tumor models (14, 21–23). However, a single dose of raltitrexed cures murine lymphoma that is thymidine kinase-deficient.

Toxicity studies in the rat and dog demonstrated that the bone marrow and gastrointestinal tract were the most sensitive tissues, and no deleterious effects were observed on the liver or kidney (21, 22). The β half-life of raltitrexed after i.p. or i.v. bolus administration in mice and rats is 30 min, but parent drug is detectable 24 h after dosing, suggesting a much longer terminal half-life (24). The levels of radiolabeled raltitrexed in L1210 ascitic leukemia cells 24 h after dosing were 50–100-fold higher than in plasma, with most of the drug present in polyglutamate forms (14). Because a long terminal plasma half-life and prolonged intracellular retention of raltitrexed polyglutamates were predicted for humans as well, a single dose of raltitrexed given every 3 weeks was chosen for clinical trials.

The starting dose for the initial human study of raltitrexed performed in Europe was 0.1 mg/m², one-fifth of the lowest toxic dose in dogs (0.5 mg/m²; Ref. 25). Our trial started subsequently, and a higher starting dose of 0.6 mg/m² was selected based on safety information from the other trial.

**PATIENTS AND METHODS**

**Eligibility.** Adult patients who were ≥18 years old and had a diagnosis of metastatic or recurrent cancer for which there was no effective therapy were eligible for this study, provided they had an ECOG PS of 0–2, an AGC of >2000/μl, a platelet count of >100,000/μl, serum bilirubin levels of <1.6 mg/dl, an AST level of <4 times the upper limits of normal, and a serum creatinine level of <1.6 mg/dl. Patients with active infections, pregnant or lactating females, patients with serious concurrent medical illnesses, and patients with clinically significant third space fluid collections were excluded. Measurable or evaluable disease was not required. While participating in this protocol, patients were not permitted to undergo treatment with other cancer therapies, steroids, sulfonamide antibiotics, folic acid, or vitamin supplements. Other therapies directed toward patient comfort, such as analgesics or antiemetics, were allowed. This protocol was approved by the Institutional Review Boards of the NCI and the National Naval Medical Center, and all patients gave written informed consent.

**Treatment.** Raltitrexed was provided by the Cancer Therapy Evaluation Program of the NCI; 10-mg vials (1 mg/ml solution) were supplied by Zeneca Pharmaceuticals (Wilmington, DE). Immediately prior to use, the drug was diluted in 150 ml of 0.9% sodium chloride injection USP, and raltitrexed was administered i.v. over 15 min. Treatment cycles were repeated every 21 days, provided that the toxicity from the previous cycle had resolved. Hospitalization was required for the first cycle, but outpatient administration was an option for subsequent cycles. The starting dose, 0.6 mg/m², was chosen because this was found to be safe in the ongoing European trial. A traditional modified Fibonacci scheme of dose levels was planned. As will be described later, toxicity information from the European trial led to a more cautious dose escalation plan in this trial.

Toxicity was graded using the first version of the NCI Common Toxicity Criteria (26). Dose-limiting toxicity was defined as grade 3 nonhematological or grade 4 hematological toxicity (AGC nadir of <500/μl or a platelet nadir of <25,000/μl). Because transient elevation of liver function tests had been observed with raltitrexed in the European Phase I trial without evidence of cumulative hepatic toxicity, dose reductions were not made for transitory elevations of AST, ALT, or bilirubin, provided that the enzyme levels returned to the patient’s baseline values when the next cycle was due to start. Intratreatment dose escalation was not permitted, but dose reductions were allowed for reversible dose-limiting toxicities. The MTD was considered exceeded if dose-limiting toxicity was observed in at least one-third of the patients. Patients were taken off study if they encountered debilitating toxicity, their disease progressed, or they asked to discontinue the protocol therapy for any reason.

Patients entered in this trial experienced constitutional symptoms characterized by fatigue, malaise, reduced performance status, fever, and, occasionally, myalgia. The grading of this asthenic syndrome was based on the patients’ subjective tolerance of the syndrome and whether a decline in PS occurred, as follows: grade 1, mild symptoms that did not interfere with the patient’s activities; grade 2, moderate symptoms that limited some of the patient’s activities or were associated with a decrease in performance status level to no worse than PS 2; and grade 3, severe symptoms that markedly limited the patient’s activities, associated with a decline in performance status to PS 3 or 4.

**Patient Evaluation and Follow-Up.** Pertinent radiological examinations were performed within 4 weeks of study entry to serve as a baseline for serial evaluation of the patient’s disease status. While on study, patients were monitored weekly for blood counts, WBC differential, and chemistry values to monitor liver and renal function. History and physical examinations were repeated with each return visit. Tumor response evaluations were performed every two to three courses. Standard response criteria were used (27).

**Pharmacological Methodology.** Pharmacokinetic sampling was planned in at least three patients per dose level. Blood samples were drawn into gray-top tubes that contained oxalate.
pretreatment, at timed intervals during the raltitrexed infusion, and 0, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, and 240 min after completion of the infusion; an additional sample was obtained the following morning. The blood samples were placed immediately on ice and centrifuged within 30 min, and the plasma was frozen at –70°C. Raltitrexed plasma levels were determined at Zeneca Pharmaceuticals using a sensitive RIA with a lower limit of quantification of 0.77 ng/ml (25). Assays were performed in duplicate on two occasions, and the average was calculated. During analysis of samples, assay accuracy and precision ranged from 103 to 115% and from 12.6 to 17.6%, respectively, for quality control samples prepared at 0.77, 2.4, and 14.9 ng/ml in plasma. The maximum plasma concentration ($C_{\text{max}}$) was the highest plasma value obtained during the raltitrexed infusion. The AUC from 0 to 24 h was calculated by noncompartmental analysis with WinNonLin software (Scientific Consulting, Inc., Apex, NC). TS expression in tumor tissue specimens was determined by immunohistochemistry using TS-106 monoclonal antibody, as described previously (28).

**Statistical and Graphical Analysis.** Graphs were prepared using Sigmaplot for Windows Version 4.01 (SPSS, Inc., Chicago, IL). Associations between clinically relevant toxicity (presence or absence) and dose of drug were examined using Lehmann’s nonparametric version of a Kruskal-Wallis test for ordered columns (29). The Hochberg procedure was used to indicate which of the $P$-values obtained were significant at the 0.05 level in view of the multiple comparisons performed (30). All $P$-values between groups are two-sided. The strength of the linear association between pairs of variables was determined by the Pearson correlation coefficient: $r \geq 0.70$, strong correlation; $0.5 < r < 0.70$, moderate correlation; and $r = 0.3–0.5$, weak to moderate correlation. The Wilcoxon rank sum test was used to test whether values of continuous variables differed between groups. The percentage change in leukocytes and granulocytes was determined by the following equation: $100 \times (\text{baseline value} – \text{nadir value}) \div (\text{baseline value})$. The relationship between dose and percentage change in blood counts was analyzed using a sigmoidal maximum effect model. Coefficients of determination ($r^2$) values of $>0.50$ indicate a strong fit between the model and the data, whereas values between 0.25 and 0.50 signify a moderately strong fit.

**RESULTS**

**Patient Characteristics.** Fifty adult patients were entered in this trial (Table 1). The vast majority (96%) had either no or only minor cancer-related symptoms (ECOG performance status of 0–1). Metastatic colorectal cancer was the most frequent diagnosis, reflecting our patient referral pattern. Most patients had received one or more prior chemotherapy regimens, with a median number of cycles received per patient being 2.5 (range, 1–11). The number of patients entered at the highest three doses was somewhat unusual for a Phase I trial (Table 2). The decision to expand the number of patients entered at these doses was made for several reasons. Four early deaths were observed in the European trial with unresolved drug toxicity, including 3 of 26 patients treated at 3.0 mg/m$^2$ and 1 of 6 patients treated at 3.5 mg/m$^2$ (26). Furthermore, 26% of 23 patients treated with 3.0 mg/m$^2$ in the European trial had grade 3–4 diarrhea, and 4 of 6 patients treated with 3.5 mg/m$^2$ experienced prohibitive malaise with a decline in performance status. In contrast, none of the first six patients entered at 3.5 mg/m$^2$ in our trial experienced dose-limiting toxicity. Because our patient population consisted predominately of patients with advanced cancer, we were particularly concerned about the level of toxicity that appeared above the tariff level in view of the multiple comparisons performed (30). All $P$-values obtained were significant at the 0.05 level.
Clinical Toxicity. The first patient entered in this study at 0.6 mg/m² developed a progressive peripheral sensory neuropathy during his second cycle of raltitrexed, which interfered with the ability to perform routine daily activities. No metabolic or anatomical abnormalities were evident. Of note, the patient had received two cycles of the investigational platinum analogue ormaplatin 4 months before entering this trial, which was subsequently shown to be associated with a delayed-onset peripheral neuropathy (31, 32). No further raltitrexed was given to this patient. Five additional patients were enrolled at 0.6 mg/m². Because only one of these experienced only mild nausea and mouth soreness, dose escalation continued as intended.

Clinical toxicity occurring with the first cycle of raltitrexed is summarized in Table 3. Mild fatigue and malaise during the initial cycle was reported by two patients treated with 2.1 mg/m², 12 of 13 patients treated with 3.5 mg/m², and in 8 of 9 patients treated with 4.0 mg/m².

Two additional patients were entered at 4.0 mg/m², and then dose escalation proceeded to 4.5 mg/m².

Two of the first three patients entered at the 4.0 mg/m² level who were dosed on the basis of their ideal body weight received actual doses of 3.2 and 3.3 mg/m². These two patients are included in the toxicity assessment for 3.5 mg/m², the dose level that is closest to their actual dose.
Table 4  Worst toxicity across all cycles per patient per dose level

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade</th>
<th>0.6</th>
<th>1.0</th>
<th>1.6</th>
<th>2.1</th>
<th>2.8</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no. of patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of patients</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AST</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>ALT</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two patients who were assigned to 4.0 mg/m² but dosed on their ideal body weights are included in 3.5 mg/m² cohort. Seven patients who experienced dose-limiting toxicity at their original dose level (4.5 mg/m², three patients; 4.0 mg/m², two patients; and 2.8 and 1.6 mg/m², one patient each) continued on study at a lower dose. Two of these patients subsequently required a second dose reduction (from 3.5 to 2.8 mg/m². Other infrequent toxicities continued to receive raltitrexed at reduced doses. The worst toxicities experienced by each patient across all cycles at each dose level are summarized in Table 4. Granulocytopenia of grade 3 or 4 severity occurred more often than leukopenia and was clearly dose-related. No patient treated with 1.6 mg/m² or lower raltitrexed had a granulocyte nadir of <1000/µL, compared with 18 and 32% of patients treated with 2.1–2.8 and ≥3.5 mg/m², respectively. Only one patient treated at 4.0 mg/m² had a platelet nadir that was of grade 2 severity. Because many patients were anemic when they enrolled on study, a hemoglobin nadir of <10 g/dl was not uncommon. However, only one patient (at 3.5 mg/m²) developed a hemoglobin nadir below 8.0 g/dl.

Symptoms collectively referred to as the asthenic syndrome were the most frequent clinical toxicity reported by patients and also appeared to be dose-related. Only 14% of patients treated with ≥1.6 mg/m² reported this syndrome, whereas about half of the patients treated with doses of 2.1 mg/m² or higher ultimately became symptomatic. Six patients considered these symptoms to be intolerable (grade 3); one patient each at 4.0 and 4.5 mg/m² requested to discontinue protocol therapy, two patients were removed for disease progression, and two continued on protocol with a dose reduction from 4.5 to 4.0 mg/m². Transient increases in serum transaminases above the patient’s baseline occurred frequently. At the time of study entry, 34 and 12% of the patients had elevated AST and ALT values, respectively, that met the definition for at least grade 1 toxicity. Dose reductions were avoided unless the elevated transaminase values persisted when the next cycle was due. Thus, only one patient received a dose reduction (from 1.6 to 1.0 mg/m²) for an elevated ALT value. Five additional patients who experienced transient rises in either AST or ALT values that qualified as grade 3 in severity continued on therapy at the same dose of raltitrexed. These patients received a median of two additional cycles of raltitrexed at the same dose (range, 1–8 cycles), and none experienced transaminase values worse than grade 2 in severity. Four patients had transient elevations in bilirubin values that reached grade 3 in severity before returning to baseline; two of these patients later developed a sustained increase in bilirubin after one and three additional cycles, respectively, at which time hepatic disease progression was documented. Prophylactic antiemetics were not used in this trial, and about half the patients experienced nausea and vomiting. A minority experienced diarrhea of mild to moderate severity, but this toxicity did not appear to be more common at the highest three doses compared with 2.1–2.8 mg/m². Other infrequent toxicities included mucositis, rash, and anorexia.

When all cycles were considered, asthenia occurred in 83 of 174 cycles (48%). Fatigue was the predominant complaint (70%), followed by fever (34%), malaise (13%), and myalgias (4%). The incidence of toxicities complicating each cycle are presented in Fig. 1, with the data organized into three dosage groups (low, intermediate, and high). Asthenia, granulocytopenia, and transaminitis complicated significantly more cycles at or above 3.5 mg/m² compared with lower doses. In contrast, diarrhea, mucositis, and nausea did not seem to be clearly dose-related.

The number of patients who ultimately developed dose-limiting toxicity and the cycle when this occurred are presented...
in Table 5. Only 2 of 13 patients treated with 3.5 mg/m$^2$ experienced unacceptable toxicity after three and seven cycles. Five of 12 patients treated with 4.0 mg/m$^2$ experienced dose-limiting toxicity after a median of three cycles. Five of nine patients who received 4.5 mg/m$^2$ had dose-limiting toxicity after a median of two cycles. Three of these latter patients subsequently received a reduced dose of 4.0 mg/m$^2$ for an additional one, three, and five cycles with acceptable toxicity. Overall, four of seven patients who received at least two cycles with 4.0 mg/m$^2$ raltitrexed (including new patients and those initially treated with 4.5 mg/m$^2$) eventually experienced dose-limiting toxicity.

<table>
<thead>
<tr>
<th>Dose (mg/m$^2$)</th>
<th>No. of new patients</th>
<th>No. of new patients with DLT (%)</th>
<th>Total no. of cycles at this dose</th>
<th>Total no. of patients with DLT (%)</th>
<th>Toxicities (no. of patients)</th>
<th>Cycle no. with DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 2.1$</td>
<td>15</td>
<td>1 (6.7)</td>
<td>18</td>
<td>2 (11.1)</td>
<td>Grade 3 SGPT (1)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4 AGC (1)</td>
<td>9</td>
</tr>
<tr>
<td>2.8</td>
<td>6</td>
<td>1 (16.7)</td>
<td>7</td>
<td>1 (14.3)</td>
<td>Grade 4 AGC (1)</td>
<td>6</td>
</tr>
<tr>
<td>3.5</td>
<td>11$^b$</td>
<td>1 (9.1)</td>
<td>13</td>
<td>2 (18.2)</td>
<td>Grade 3 asthenia (1)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4 AGC (1)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 3 asthenia (2)</td>
<td>2, 3</td>
</tr>
<tr>
<td>4.0</td>
<td>9</td>
<td>4 (44.4)</td>
<td>12</td>
<td>5 (41.7)</td>
<td>Grade 3 bilirubin (1)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4 AGC (2)</td>
<td>1, 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4 bilirubin (1)</td>
<td>2</td>
</tr>
<tr>
<td>4.5</td>
<td>9</td>
<td>5 (55.6)</td>
<td>9</td>
<td>5 (55.6)</td>
<td>Grade 3 asthenia (3)</td>
<td>1, 3, 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 4 AGC (1)</td>
<td>1</td>
</tr>
</tbody>
</table>

$^a$ DLT, dose-limiting toxicity.

$^b$ Two patients who were assigned to 4.0 mg/m$^2$ but dosed on their ideal body weights are included in 3.5 mg/m$^2$ cohort. New patients are those patients assigned to the specified dose for their initial cycle of therapy. The total number of patients includes both patients who received the specified dose at cycle 1 or who were treated at this dose after a dose reduction.

Dose escalation within individual patients was not allowed in this trial. To determine whether there was any evidence of cumulative toxicity, the incidence of toxicities occurring during the initial two cycles was compared with that observed in cycles three and beyond (Fig. 2). The proportion of cycles complicated by grade 3–4 granulocytopenia and grade 2–3 transaminitis and asthenia appeared to be higher for cycle 3 and beyond.

Treatment cycles were repeated at median intervals of 21 days; 84% of all cycles were given at a 3-week intervals, 10% were given at 4-week intervals, and treatment intervals were longer than 4 weeks in only 6% of cycles. No differences were noted in the cycle intervals according to either dose ($\geq 2.1$ versus $\geq 2.8$ mg/m$^2$) or cycle number (two or fewer versus three or more cycles; data not shown).

**Pharmacokinetic and Pharmacodynamic Studies.**

Raltitrexed plasma concentrations were measured in 28 patients following either the initial dose (27 patients) or the second dose (1 patient). In this latter patient, raltitrexed was detected in the pretreatment blood sample (1.6 ng/ml) obtained 20 days following the previous dose of 2.8 mg/m$^2$. Because this pretreatment value was several orders of magnitude below this individual's $C_{\text{max}}$ value (521 times lower), the pharmacokinetic data for this
AUC 0–24 h of raltitrexed was determined using all available data points and extrapolated to 24 h with WinNonLin software. The Pearson correlation coefficient was used to assess the strength of association between dose and either AUC 0–24 h or clearance.

The maximum plasma concentrations \( C_{\text{max}} \) were most likely affected by differences in duration of infusion because longer infusion durations would be expected to produce lower \( C_{\text{max}} \) values. The plasma elimination of raltitrexed was triexponential. Fifteen min postinfusion, raltitrexed plasma levels had decreased to 49.9 ± 8.3% of that measured at the end of infusion. One, 2, and 4 h postinfusion, the plasma levels had decreased to 25.5 ± 5.4%, 14.2 ± 3.8%, and 6.6 ± 2.3%, respectively. The maximum plasma concentration \( C_{\text{max}} \) correlated closely with the calculated AUC 0–24 h \((r = 0.803, P = 2.7 \times 10^{-7})\). Strong, positive correlations were noted between either AUC 0–24 h or the \( C_{\text{max}} \) and the dose of raltitrexed (AUC 0–24 h versus dose is shown in Fig. 3A). In contrast, clearance was not dose-related (Fig. 3B) and averaged 49.2 ± 13.3 ml/min/m² (mean ± SD). A moderately strong correlation was evident between the estimated creatinine clearance values for each patient and the patient’s raltitrexed clearance (Fig. 4). In contrast, no relationship was evident between raltitrexed clearance and serum albumin levels (data not shown).

Correlation analyses between raltitrexed dose and pharmacokinetic values and measures of hepatotoxicity (fold increase in AST values) and actual leukocyte and granulocyte nadirs as well as percentage decrease from baseline and asthenia were performed. No clear relationship was evident between either \( C_{\text{max}} \) or AUC 0–24 h and nonhematological toxicity (data not shown). The nadir granulocyte count tended to be lower with increasing raltitrexed \( C_{\text{max}} \) \((r = -0.437; P = 0.026)\), and increasing \( C_{\text{max}} \) and AUC 0–24 h values tended to be associated with greater percentage decreases in the granulocyte count \((r = 0.485 \text{ and } 0.471, \ P = 0.022 \text{ and } 0.027, \text{ respectively})\). When the percentage change in granulocyte count was analyzed using a sigmoidal maximum effect model, the coefficients of determination \((r^2)\) were 0.382 (\( C_{\text{max}} \)) and 0.279 (AUC 0–24 h), suggesting a moderately good fit of the data.

Similar analyses with clinical toxicity cycle one were performed using raltitrexed dose. The change in granulocyte count provided the best pharmacodynamic relationship between dose and hematological toxicity (Fig. 5). No relationship was evident between dose and platelet toxicity (data not shown). Patients who experienced an absolute decrease in their hemoglobin values by ≥10 g/dl during their initial cycle of therapy tended to receive higher doses (median 3.75 versus 2.8 mg/m²; \( P = 0.037)\). Whereas the relative increase in AST values during the first cycle showed no relationship to raltitrexed dose (data not shown), patients who experienced asthenic symptoms received significantly higher doses of raltitrexed: median 4.0 versus 2.8 mg/m² \((P = 0.001)\).

**Clinical Outcome.** The median time to treatment failure was 1.8 months (range, 0.6–8.1 months). Progression of disease was the reason for discontinuing treatment in 92% of patients. Four patients requested to stop therapy after 1, 3, 4, and 11 cycles. No partial or complete responses were observed. Thirty patients (26%) had stable disease that lasted for 3 months or longer. Initial CEA values were available in 33 of the 38 patients with colorectal cancer, and the median was 136.6 ng/ml (range, 1.3–6928 ng/ml). Three of the 31 colorectal cancer patients (9.7%) with an initially elevated CEA level of >5 ng/ml experienced a decrease in the CEA by >50% during raltitrexed therapy (39, 45, and 47% of their
Phase I Trial of Raltitrexed

When tolerance to the initial cycle of raltitrexed was considered, no dose level exceeded the definition of the MTD. Because 3.0 mg/m² had been selected for subsequent Phase II trials based on the results of the European Phase I trial, we expanded the number of patients treated on our trial with the three highest dose levels. When the tolerability of repeated cycles of therapy was considered, about half of the patients treated with 4.0 or 4.5 mg/m² experienced unacceptable toxicity, generally within the first three cycles of therapy. The asthenic syndrome occurred frequently. The etiology of this constellation of symptoms is not clearly understood but has been consistently reported in trials involving raltitrexed (Refs. 33–45; Table 6). Similar symptoms have been described in patients receiving other antifolate drugs, including AG331 and LY231514, as well as in structurally dissimilar antimetabolites like gemcitabine (46–48). A granulocyte nadir below 500/µl of any duration was considered to be dose-limiting in this trial, and colony-stimulating factors were not used. In general, however, the duration of granulocytopenia was brief. Transient elevations in serum transaminases were also commonly seen. About two-thirds of the patients in this trial had hepatic metastases, and 34% had elevated AST values when they enrolled that would qualify for at least grade 1 toxicity. The possible contribution of preexisting hepatic disease to transaminitis while a patient is receiving drug is not clear, but definite increases in both AST and ALT levels were frequently observed at midcycle. The policy of avoiding dose reductions if the transaminase levels returned to the patient’s baseline value when the next cycle of therapy was due appeared to be safe because several patients who experienced transient grade 3 AST or ALT toxicity received subsequent cycles at the same dose with no evidence of progressive hepatic dysfunction. Four patients experienced transient rises in bilirubin that reached grade 3 in severity, which were judged to be possibly treatment-related.

In our trial, mucositis and diarrhea did not appear to be dose-related, and no patient experienced worse than grade 2 toxicity. There are differences between the criteria for diarrhea specified by the WHO (49) and the NCI grading scales; the WHO criteria focus on the duration of diarrhea for the early grades, whereas the NCI criteria are based on number of bowel movements per day. The median reported frequency of grade 3 diarrhea was 10% (range, 0–24%) in clinical trials using the WHO criteria (Table 6), which seems to be somewhat higher than the frequency (median, 0%; range, 0–4%) reported in five trials that used the NCI criteria. Our observation that mucositis occurred infrequently is consistent with the results of other trials involving raltitrexed. Interestingly, the frequency of leukopenia of grade 3–4 severity in our patients treated with 3.5–4.5 mg/m² did not appear to be greater than that reported in other trials using 3.0 mg/m².

Over the dose range used in this study, the $C_{\text{max}}$ and AUC₀−2₄ h values increased in proportion to dose, whereas clearance was independent of dose. An unanticipated observation was the variation in the duration of drug infusion during the pharmacokinetic sampling periods. The precise duration of drug infusion was otherwise not routinely recorded, and it is possible that the durations of drug infusion during other treatment cycles may not have been uniform. Infusions durations longer than the planned 15 min would be expected to result in a lower $C_{\text{max}}$. Whether such variations in the infusion duration might impact on clinical toxicity is unclear. Although we had only limited data beyond four h post infusion, our results are similar to that reported by Clarke et al. (25). For example, the $C_{\text{max}}$ and AUC₀−2₄ h in eight patients treated with 3.2–3.5 mg/m² on our trial averaged 737 ± 150 ng/ml and 1163 ± 342 ng·h/ml, compared with 808 ± 128 ng/ml and 1122 ± 291 ng·h/ml in patients treated with 3.5 mg/m² in the prior trial. Although we found no relationship between pharmacokinetic parameters and nonhematological toxicity, increasing $C_{\text{max}}$ and AUC₀−2₄ h values tended to be accompanied by greater declines in the granulocyte count. Our observation that raltitrexed clearance seemed to correlate with the estimated creatinine clearance suggests that renal excretion of drug may be an important mechanism of elimination. Beale et al. (50) conducted an extended pharmaco-
had been recovered in the urine by day 10. The average plasma nantly renally excreted as unchanged drug; only 29% of the drug mg/m² [14C]raltitrexed and found that raltitrexed was predomi-

kinetic study in nine patients treated with a single dose of 3.0 

folylpolyglutamate synthetase activity significantly 

dietary folate intake modulate both folate receptor expression and 

whereas excess folate reversed the antitumor activity. Changes in 

toxicities of antifolates that require polyglutamation for activation and 

These preclinical data suggest that the tissue distribution and tox-

icities of antifolates that require polyglutamation for activation and 

clinical toxicity with antifolate compounds.

Because no objective responses were seen in 23 patients with colorectal cancer treated with raltitrexed doses of ≥2.8 mg/m², our results suggest that with disease refractory to prior leucovorin-modulated 5-FU may be cross-resistant to ralti-

trexed. Six patients were found to have high TS expression in their tumor tissue. Although the number of patients studied is too small to draw meaningful conclusions, the observation suggests that future studies incorporating molecular profiling of tumor tissue for markers that should influence raltitrexed sensitivity might provide important prognostic information.

Interestingly, one patient in our trial who received six cycles of raltitrexed without serious toxicity was subsequently found to have profound dihydropteroate synthase deficiency (58). Köhne et al. (59) reported that raltitrexed could be safely given to patients who had experienced 5-FU-associated cardiac toxicity. These clinical observations suggest that raltitrexed is a therapeutic option in patients who are 5-FU-intolerant.

In summary, we found that doses of raltitrexed up to 4.5 mg/m² for the initial cycle could be given safely, and dose-

limiting toxicity at the highest dose was observed in fewer than one-quarter of patients. With repeated dosing, however, slightly over half the patients receiving raltitrexed at doses of 4.0 and 4.5 mg/m² required a dose-reduction after a median of three and two cycles, respectively. Whereas dose-dependent clinical toxicity with raltitrexed seems apparent, there is considerable interpa-

Table 6 Incidence of grade 3–4 toxicities in clinical trials of raltitrexed (worst grade per patient across all cycles of therapy)

<table>
<thead>
<tr>
<th>No. of patients (Ref.)</th>
<th>Dose (mg/m²)</th>
<th>Type of study/cancer</th>
<th>Prior chemotherapy (%)</th>
<th>WBC</th>
<th>Mucositis</th>
<th>Diarrhea</th>
<th>Anemia</th>
<th>AST/ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>247 (33)</td>
<td>3</td>
<td>Phase III/colorectal</td>
<td>Adjunct (10)</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>223 (34)</td>
<td>3</td>
<td>Phase III/colorectal</td>
<td>Adjunct (5)</td>
<td>14</td>
<td>2</td>
<td>14</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>199 (35, 36)</td>
<td>3</td>
<td>Phase III/colorectal</td>
<td>Adjunct (13)</td>
<td>18</td>
<td>3</td>
<td>10</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>176 (37)</td>
<td>3</td>
<td>Phase II/colorectal</td>
<td>Adjunct (5)</td>
<td>15</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>73 (38)</td>
<td>3</td>
<td>Phase II/colorectal</td>
<td>Yes (71)</td>
<td>4</td>
<td>0</td>
<td>4.1⁴</td>
<td>NR⁶</td>
<td>22</td>
</tr>
<tr>
<td>33 (39)</td>
<td>3</td>
<td>Phase II/gastric</td>
<td>Yes (75)</td>
<td>12</td>
<td>3</td>
<td>0⁶</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>31 (40)</td>
<td>3</td>
<td>Phase II/ovarian</td>
<td>Yes (all)</td>
<td>13</td>
<td>3</td>
<td>13</td>
<td>NR</td>
<td>10</td>
</tr>
<tr>
<td>31 (41)</td>
<td>3</td>
<td>Phase II/ovarian</td>
<td>Yes (all)</td>
<td>4</td>
<td>1</td>
<td>4⁶</td>
<td>NR</td>
<td>3</td>
</tr>
<tr>
<td>21 (42)</td>
<td>3</td>
<td>Phase II/SCLC</td>
<td>Yes (81)</td>
<td>4</td>
<td>0</td>
<td>0⁶</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>46 (43)</td>
<td>3</td>
<td>Phase II/breast</td>
<td>Adjunct (39)</td>
<td>20</td>
<td>0</td>
<td>11</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>33 (44)</td>
<td>3</td>
<td>Phase II/liver</td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>29 (25)</td>
<td>3–3.5</td>
<td>Phase I/solid tumors</td>
<td>Yes (90)</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>29 (this study)</td>
<td>3.5–4.5</td>
<td>Phase I/solid tumors</td>
<td>Yes (90)</td>
<td>14</td>
<td>0</td>
<td>0⁶</td>
<td>21</td>
<td>31</td>
</tr>
</tbody>
</table>

⁴ NCI common toxicity criteria were used; other trials used the WHO toxicity criteria.

⁶ NR, not reported.

clinical experience with lometrexol indicated more toxicity than was predicted on the basis of preclinical in vivo studies (53). The fact that standard laboratory animal diets contain high levels of folate was suggested that a relatively low dietary folate intake would contribute to the more severe toxicity. The toxicity and therapeutic activity of lometrexol were markedly af-
fected by the folate status of mice with restricted dietary folate intake (54). Insufficient folate intake led to a marked increase in toxicity, whereas excess folate reversed the antitumor activity. Changes in dietary folate intake modulate both folate receptor expression and folylpolyglutamate synthetase activity significantly in vivo (55, 56). These preclinical data suggest that the tissue distribution and tox-
icities of antifolates that require polyglutamation for activation and cellular retention may, indeed, be influenced by the folate status of the host. Clinical studies have also suggested that folate or folic acid supplementation may ameliorate the clinical toxicity associated with lometrexol therapy, but the impact on antitumor efficacy is not clear (57). In our trial, slightly more than half the patients had received leucovorin-modulated 5-FU in the preceding 12 months. However, no clear-cut differences in toxicity were apparent when recent leucovorin exposure was considered. RBC folate levels were determined in only a minority of patients, and all were in the normal range. Whereas a markedly low RBC folate level signifies a state of relative folate depletion, the test has limited ability to distinguish more subtle differences in total body folate levels. It will be of interest to learn whether other indicators of folate status, such as homocysteine levels, may provide more useful information concerning a patient’s dietary folate status and risk for
ralitrexed have used the 3.0 mg/m² dose given as a 15-min infusion every 3 weeks. Two of three Phase III trials in colorectal cancer using 3.0 mg/m² failed to demonstrate superiority of ralitrexed compared with 5-FU/LV given on a monthly schedule; these studies were not sufficiently powered to demonstrate equivalence. The North American Phase III trial of ralitrexed initially included both a 3.0 and 4.0 mg/m² arm, but the higher dose was closed prematurely because of three deaths which occurred among the initial 32 patients (31, 32). Among the trials involving 1213 patients presented in Table 6, 26 possible treatment-related deaths were reported (2.1%). The basis for the variability in individual tolerance is not known, although data from our own and other trials suggest that impaired renal function may be a contributing factor (60). Partially on the basis of our experience, Zeneca Pharmaceuticals is now sponsoring additional trials in North America using a higher ralitrexed dose (61, 62).

ACKNOWLEDGMENTS

We thank Francis Sutcliffe and Mike Walker of Zeneca Pharmaceuticals for running the Tomudex plasma samples.

REFERENCES

A Phase I Study of Raltitrexed, an Antifolate Thymidylate Synthase Inhibitor, in Adult Patients with Advanced Solid Tumors

Jean L. Grem, J. Mel Sorensen, Ellen Cullen, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/5/9/2381

Cited articles
This article cites 57 articles, 20 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/5/9/2381.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/5/9/2381.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.