A Phase I and Pharmacokinetic Study of 1843U89, a Noncompetitive Inhibitor of Thymidylate Synthase, in Patients with Advanced Solid Malignancies

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ABSTRACT

This study was performed to assess the feasibility of administering 1843U89, a potent, noncompetitive inhibitor of thymidylate synthase that does not require polyglutamation for activity, as a 2-min i.v. infusion daily for 5 days every 3 weeks, to determine whether folic acid supplementation ameliorates the toxic effects of 1843U89 and permits further dose escalation, and to recommend doses of 1843U89 administered without and with folic acid for further clinical evaluations. The study also sought to characterize the pharmacokinetic behavior of 1843U89 and to seek preliminary evidence of anticancer activity. Patients with advanced solid malignancies were treated with escalating doses of 1843U89 as a 2-min i.v. infusion daily for 5 days every 3 weeks. Initially, patients were treated in the absence of high-dose folic acid until dose-limiting toxicity was consistently noted. Next, patients were treated with escalating doses of 1843U89 preceded by 1000 mg of folic acid administered p.o. 30 min before each of the 5 daily doses of 1843U89. Patients (32) received 101 total courses of 1843U89 at doses ranging from 1 to 6 mg/m²/day with and without folic acid. At the 2 mg/m²/day dose level without folic acid, 2 of 7 new patients experienced dose-limiting toxicity, principally neutropenia, mucositis, and malaise in 3 of 11 courses. 1843U89 doses were further increased with folic acid to 6 mg/m²/day, but repetitive treatment was not feasible at this dose level because of an unacceptable high incidence of severe neutropenia and mucositis. Other toxicities included thrombocytopenia, rash, and fever. In contrast, repetitive treatment at the 5 mg/m²/day dose level was feasible. The pharmacokinetics of 1843U89 were neither dose dependent nor affected by folic acid. On day 1, clearance, terminal half-life, and steady-state volume of distribution values averaged 47.1 ± 21.7 ml/min/m², 7.72 ± 4.09 h, and 16.7 ± 8.8 liter/m²/h, respectively. The results of the study indicate that the administration of 1843U89 as a 2-min infusion daily for 5 days every 3 weeks without and with folic acid is feasible at 1843U89 doses as high as 2 and 5 mg/m²/day, respectively. Because folic acid pretreatment results in no diminution of the antitumor activity of 1843U89 in preclinical studies and ameliorates the toxic effects of 1843U89 in both preclinical models and cancer patients, the therapeutic index of 1843U89 may be enhanced by folic acid pretreatment and, therefore, the development of 1843U89 with folic acid is warranted. However, the question of whether to administer 1843U89 at a dose of 2 mg/m²/day with folic acid, which is associated with negligible toxicity, or at its highest feasible dose with folic acid, 5 mg/m²/day, should be addressed in appropriately designed trials.

INTRODUCTION

TS¹ has been validated in the clinic as a strategic target for anticancer drug development (1). TS catalyzes the conversion of dUMP to thymidylate, using the reduced folate MTHF as a cofactor (1). Effective inhibition of TS depletes cells of thymidylate, which is an essential component of DNA, and ultimately results in cell death (1, 2). Malignant cells generally have higher TS levels than normal cells and, therefore, may be more susceptible to TS inhibition (1, 2). Malignant cells generally have higher TS levels than normal cells, which implies that cancer cells may depend on de novo pyrimidine biosynthesis to a greater degree than normal cells and, therefore, may be more susceptible to TS inhibition (3, 4). The antitumor activities of FU further support the validity of TS as a therapeutic target. After metabolism to fluorodeoxyuridine, FU forms a ternary covalent, albeit revers-

¹ The abbreviations used are: TS, thymidylate synthase; ANC, absolute neutrophil count; AUC, area under the concentration-time curve; AUC₀–₂₄, area under the concentration-time curve extrapolated to infinity; AUC₀–²₄, area under the concentration-time curve until 24 h post-treatment; Cĥ, maximum plasma concentration; CL, clearance; DLT, dose-limiting toxicity; Emax, maximal effect model; FPGS, folypolyglutamate synthetase; FU, 5-fluorouracil; MTA, multitargeted antifol; MTD, maximum tolerated dose; MTHF, 5,10-methylenetetrahydrofolate; t½₂₄, half-life of elimination; TDL, toxic dose low.
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Fig. 1 Structure of 1843U89.

iable, complex with both MTHF and TS, which, in turn, inhibits synthesis of thymidylate (5, 6). Measures that inhibit dissociation of the ternary complex in vitro, such as increasing intracellular concentrations of reduced folate cofactors and coadministration of reduced folate or folinic acid, generally enhance the antitumor effects of FU (7). Furthermore, factors that affect TS activity, such as TS kinetics, rate of TS renewal, TS gene amplification, stabilization of the ternary complex, intracellular folate concentrations, and folate polyglutamation, are also determinants of cellular sensitivity to FU (1, 8–10).

The requirement of TS activity on folate cofactors has served as the rationale for therapeutic evaluations of synthetic analogues of folate cofactors. The 3-methyl-substituted benzoquinazoline folate analogue 1843U89 (GlaxoWellcome, Inc, Research Triangle Park, NC; Fig. 1) is a very potent, noncompetitive inhibitor of human TS (Ki ≈ 90 pM; Refs. 11–14). 1843U89 was developed through chemical optimization of an initial series of benzo[1](2H)-quinoxalin-1(2H)-one compounds, which inhibit human TS with Ki values of ≈ 20 nM (11, 12). Although structurally similar to the natural folate cofactor of the TS reaction, coadministration with MTHF does not alter the binding of 1843U89 to TS, which indicates that 1843U89 is a noncompetitive inhibitor of TS (11–14). Because 1843U89 does not require polyglutamation to effectively inhibit TS, tumors with acquired resistance to polyglutamable folate analogue attributable to either low FPGS or high folylpolyglutamyl hydrolase may hypothetically retain sensitivity to the agent (14–17). On the other hand, 1843U89 is an efficient FPGS substrate for synthesis of the diglutamate (14–17). Unlike most folate-based TS inhibitors, 1843U89 does not require additional polyglutamation beyond its diglutamate species to be retained intracellularly, and the diglutamate species has been demonstrated to accumulate intracellularly, even in cells with reduced FPGS (17, 18). Furthermore, there is evidence indicating that 1843U89 and other compounds with high affinities for the reduced folate carrier may produce a tumor-selective advantage because some tumors have been demonstrated to predominately use this mechanism for folate transport (14).

1843U89 has demonstrated impressive cytotoxicity against a broad range of human tumor xenografts implanted in nude mice, when the high circulating thymidine levels of mice are reduced (14, 19). The agent has been shown to inhibit the growth or induce regression of s.c. or subrenal capsule implants of MOLT-4 leukemia, SW480 and WiDr colon, HCT-8 ileocecal, and MCF-7 breast carcinomas with IC50 values ranging from 0.2 to 0.7 nM (14, 17, 19). Regressions of advanced mammary and spinal nerve sheath malignancies have also been noted after treatment of dogs with 1843U89 and folic acid (14). Thymidine reverses the cytotoxicity of 1843U89 against human tumor xenografts, whereas folic acid has either no or a negligible effect on drug activity (10). In one study, the cytotoxicity of 1843U89 was not reduced by high folic acid concentrations (100 μM) in most cancer cell lines evaluated (14). In addition, the antitumor activity of 1843U89 was not altered in vitro after treatment with more clinically relevant folic acid concentrations (~5 μM; Ref. 14). In contrast, treatment with folic acid increased the IC50 values for 1843U89, but only by 10- to 50-fold, compared with 104- to 105-fold for other folate-based agents (13).

In preclinical toxicology studies, 1843U89 administered daily for 5 days principally affected the gastrointestinal mucosa in dogs and monkeys (14). At high doses, vomiting, diarrhea, anorexia, and decreased activity were the most common toxicities, whereas myelosuppression was uncommon. Treatment of dogs with oral folic acid (50 mg/kg) 40 min before lethal (6 mg/kg) and twice-lethal doses of 1843U89 resulted in minimal clinical toxicity and histopathological effects; however, severe gastrointestinal and hematological toxicities were observed after treatment with three times the lethal dose of 1843U89, despite pretreatment with an identical dose of folic acid (14). The minimal folic acid dose given once daily that was necessary to provide complete protection from a lethal dose of 1843U89 was between 10 and 50 mg/kg/day.

The impressive preclinical antitumor activity of 1843U89 and its unique noncompetitive mechanism of TS inhibition served as the impetus for its clinical development. The principal objectives of this study were to: (a) determine the principal toxicities of 1843U89 administered as a 2-min i.v. infusion daily for 5 days every 3 weeks without and with folic acid pretreatment; (b) determine the MTD of 1843U89 without and with folic acid and recommend doses for subsequent disease-directed evaluations; (c) describe the pharmacokinetic behavior of 1843U89 without and with folic acid; and (d) seek preliminary evidence of antitumor activity.

PATIENTS AND METHODS

Eligibility. Patients with histologically confirmed advanced solid malignancies that were unresponsive or recurred after standard therapy or for whom adequate therapy was not available were eligible for this study. Eligibility criteria also included: age ≥ 18 years; a Karnofsky performance status ≥ 70% (ambulatory and capable of self care); life expectancy ≥ 12 weeks; no chemotherapy, wide-field radiation therapy, treatment with folic acid, or immunotherapy within 4 weeks of treatment nor treatment with carboplatin, nitrosoureas, or mitomycin C within 6 weeks; adequate hematopoietic (ANC ≥ 1,500/μL, hemoglobin level ≥ 9 g/dL, platelet count ≥ 100,000/ μL), hepatic (total bilirubin ≤ 2 mg/dL, aspartate amino transaminase and alanine amino transaminase ≤ three times institutional normal upper limit), and renal [creatinine ≤ 1.5 mg/dL or calculated creatinine CL (Cockcroft-Gault method) ≥ 60 mL/ min] functions (20); a serum folic acid concentration ≥ 4 ng/mL (9 nM); a serum vitamin B12 concentration ≥ 200 pg/mL (150


Dosage and Drug Administration. The starting dose of 1843U89 was 6 mg/m²/day for 5 days every 3 weeks, which was equivalent to one-sixth of the TDL in dogs and cynomolgus monkeys. The dose was calculated on the basis of body surface area calculated on the first day of each 3-week course, rounded off to the nearest mg, and administered i.v. over 2 min. Dose escalation was to proceed according to a modified Fibonacci scheme in decreasing increments. In the initial dose escalation stage, doses were to be increased by 100, 67, 50, 33, 20, and, thereafter, 20–33% for successive cohorts as long as no DLT was observed and a minimum of 3 new patients were to be treated at each successive dose level. If DLT occurred in a new patient in the first course, as many as 6 new patients were to be treated at that dose level. If only 1 of the 6 patients experienced DLT, dose escalation resumed. In the event of DLT, dose escalation in new patients proceeded in maximum increments of 20–33%. The MTD was defined as the highest dose at which <2 of the first 6 new patients experienced DLT in course 1. Toxicity was graded according to the Southwest Oncology Group. DLT was defined as: (a) ANC < 500/µl lasting >5 days or associated with fever; (b) platelets < 25,000/µl; (c) ≥ grade 3 nonhematological toxicity, except for nausea and/or vomiting in the absence of an adequate antiemetic regimen, and possibly other brief, tolerable, Southwest Oncology Group grade 3 toxicities (e.g., transient, generalized rash); and (d) treatment delays >7 days because of unresolved drug-related toxicity.

After DLT was consistently noted at the 2 mg/m²/day dose level, which was equivalent to one-eighth of the TDL in dogs and cynomolgus monkeys, patients were treated with 1000 mg of folic acid p.o. 30 min before each of the 5 daily doses of 1843U89. This folic acid dose approximated the minimal dose equivalent to one-sixth of the TDL in dogs and cynomolgus monkeys. Patients were ineligible if they had a prior history of malabsorption, gastrointestinal disorders that could affect oral absorption, resection of the stomach or small intestine, alcohol abuse, or if they had coexisting medical problems of sufficient severity to limit compliance with the study. Patients were not able to be treated concurrently with sulfonamides, trimethoprim, pyrimethamine, or anticonvulsant medications and were instructed to avoid a list of medications that could interfere with folic acid metabolism. All medications were recorded in the case report form. All patients gave written informed consent according to federal and institutional guidelines.

Pharmacokinetic Studies. To study the pharmacokinetic behavior of 1843U89, blood was sampled from a site contralateral to the drug infusion during the first course of treatment. On treatment days 1 and 5, 4-ml blood samples were collected in EDTA-containing vacutainer glass tubes pretreatment and at 5, 10, 20, 30, 45, 60, and 90 min and 2, 4, 6, 8, and 12 h after drug administration. Blood samples were also obtained 24 and 48 h posttreatment on day 5 and pretreatment and 5 min posttreatment on days 2, 3, and 4. The samples were immediately placed in an ice bath and centrifuged within 20 min of collection. The plasma was transferred to polypropylene tubes and frozen at −20°C. To assess the renal excretion of 1843U89, a 50-ml urine sample was collected before treatment, and urine was collected continuously in pooled 8-h incremental collections after treatment on days 1 and 5. The urine collections were thoroughly mixed, the total volume was recorded, and 10-ml aliquots were removed and frozen at −20°C.

A scintillation proximity RIA, which involves fewer steps than conventional immunoassays and generally has greater accuracy and precision, was developed to measure 1843U89 concentrations in both plasma and urine. Briefly, standard solutions containing known amounts of 1843U89 or plasma samples, along with limiting quantities of rabbit polyclonal 1843U89 antiserum, radiolabeled ligand, and scintillation proximity reagent, were added to the wells of a microtiter plate. The assay was based on competition between 1843U89 and [³H]1843U89 for the limited amount of 1843U89 antibody. Next, the primary antibody complex binds to the antirabbit-IgG antibody attached to fluoromicrospheres in the scintillation proximity reagent. The radioactivity measured on the fluoromicrospheres was inversely related to the amount of unlabeled 1843U89 present in the sample. A standard curve was constructed, and the concentrations of 1843U89 in unknown samples were determined by interpolation. Intra- and interassay coefficients of variation of the method were 5.9 and 2.8%, respectively. The lower limit of quantitation was 1 ng/ml.

Pharmacokinetic and Pharmacodynamic Analyses. Individual 1843U89 plasma concentration data from days 1 and 5 were analyzed, and pharmacokinetic parameters were estimated by noncompartmental methods using the nonlinear regression program WinNonlin (Scientific Consulting, Inc., Apex, NC). The terminal rate constant (λz) was determined from the slope of the terminal phase of the drug concentration-time curve.
The $AUC_{0\rightarrow\infty}$ was calculated as AUC to the last sampling time + the last plasma concentration sampled/$\lambda_{z}$, where the last plasma concentration sampled was the last measured concentration. The $t_{1/2}$ was calculated as $ln(2)/\lambda_{z}$. The CL was calculated as dose divided by the $AUC_{0\rightarrow\infty}$. The volume of distribution at steady state was calculated as the dose/(\lambda_{z} \times AUC_{0\rightarrow\infty}). The $C_{max}$ and the time of maximum plasma concentration were determined by inspection of the plasma concentration-time curves. The accumulation ratio was calculated as the ratio of the $AUC_{0\rightarrow24}$ on day 5 to that on day 1. Parameter values were expressed as the mean value ± SD.

The Wilcoxon matched-pairs signed rank test was used to compare pharmacokinetic parameters on days 1 and 5 and explore the relationships between 1843U89 systemic exposure and toxicity in the first course. The indices of 1843U89 exposure ($C_{max}$ and $AUC_{0\rightarrow24}$) on days 1 and 5 related to the percentage decrements in the ANC and platelet counts and to categorical grades of the pertinent toxicities. The percentage decrement in the blood cell count was calculated as follows:

\[
\% \text{ decrement in blood cell count} = \frac{100 \times (\text{pretreatment count} - \text{nadir count})}{\text{pretreatment count}}
\]

The relationships between 1843U89 $C_{max}$ and AUC and hematological toxicity were described using a sigmoidal $E_{max}$ model of drug action (i.e., percentage of change hematological parameter = $E_{max} \times AUC/\left(AUC_{50} + AUC^2\right)$, where $E_{max}$ was fixed at 100% and $AUC_{50}$ is the AUC at which the effect is 50% of the maximal effect. The exponent $\gamma$ describes the sigmoidicity of the curve. The sigmoidal $E_{max}$ model was fit to the data by nonlinear least squares regression. The coefficient of determination (R$^2$) and the standard errors for the estimated parameters were used to measure the accuracy of the fit for the pharmacodynamic model.

The $AUC_{0\rightarrow24}$ on days 1 and 5 were calculated and expressed as the percentage of the dose. The total percentage of the 1843U89 dose recovered in the urine was calculated as the sum of the percentages of excreted 1843U89 dose. The renal CL of 1843U89 was calculated as follows: $CL_r = AUC_{0\rightarrow24}/AUC_{0\rightarrow24}$. In the event that the plasma concentration was not available at 24 h, $AUC_{0\rightarrow24}$ was calculated using the extrapolated or interpolated concentration.

**RESULTS**

**General.** Patients (32), whose pertinent characteristics are displayed in Table 1, were treated with 101 total courses of 1843U89 at doses ranging from 1 to 6 mg/m$^2$/day without and with folic acid. Table 2 depicts the total numbers of patients treated at each dose level, the number of evaluable courses, and the rates of DLT experienced during the first and all courses administered at each dose level. The median number of courses administered per patient was two (range, 1–11). Courses (99%) were fully evaluable for toxicity. In 2 patients during course 1 at the 5 and 6 mg/m$^2$/day dose levels, only two of the five scheduled doses were administered because of rapidly progressive disease. Two patients received dose-level modifications on one occasion, and 1 subject had dose-level modifications on two occasions.

The starting dose administered to the first patient enrolled in the study was 6 mg/m$^2$/day without folic acid. This patient subsequently developed DLT, characterized by severe (grade 3) stomatitis associated with neutropenia (grade 2), thrombocytopenia (grade 1), skin rash (grade 2), and fever (grade 2). The CL of 1843U89 in this individual was substantially <CL rates established in preclinical studies in monkeys and dogs. Because the toxicity was considered unacceptable and the CL was much lower than predicted, the dose of 1843U89 was reduced to 1 mg/m$^2$/day in the next group of new patients. Because no drug-related effects were noted after retreatment of the first subject and 2 additional new patients at the 1 mg/m$^2$/day dose level, the dose was increased to 2 mg/m$^2$/day. At 2 mg/m$^2$/day, 1 of the first 3 new patients experienced severe (grade 3) malaise in course 1, which was felt to possibly be drug related, and the dose level was expanded to 7 total patients. Two other patients experienced DLTs, including a subject who developed grade 4 neutropenia in course 1 requiring hospitalization for parenteral antibiotics and another individual who experienced grade 3 mucositis during course 2. Before elucidation of the true rate of DLT at the 2 mg/m$^2$/day dose level, 1 new patient had been enrolled at the next higher dose level (3.3 mg/m$^2$/day), which resulted in grade 3 stomatitis during course 1.

On the basis of the toxicity profile of 1843U89 without folic acid, significant dose escalation and/or repetitive treatment with 1843U89 at higher doses was not felt to be feasible, and the administration of 1843U89 with folic acid was next evaluated. At the 2 mg/m$^2$/day dose level of 1843U89 with folic acid, no DLT was noted in 3 new patients; therefore, the dose was increased by 67% to 3.3 mg/m$^2$/day with folic acid, which was also well tolerated. However, dose escalation by 50% to 5 mg/m$^2$/day with folic acid resulted in grade 4 neutropenia associated with fever and grade 4 thrombocytopenia in the lead patient during course 1. After a thorough review of this patient’s
past medical history revealed more extensive abdominal surgery than initially documented, including a gastric resection, malabsorption of folic acid was presumed to have accounted for these drug-related effects. Three additional new patients were treated at this dose level, none experiencing grade 2 or higher toxicity during both course 1 and 2, and dose escalation continued.

1843U89 dose escalation proceeded with a more conservative 20% increment to 6 mg/m²/day with folic acid supplementation. Although none of 6 new evaluable patients developed DLT during course 1, 2 patients developed DLT during subsequent courses. One subject experienced grade 3 mucositis in course 3, which was proceeded by grade 3 neutropenia, grade 2 mucositis, and grade 2 rash during both courses 1 and 2. The second patient developed DLT in course 2, characterized by grade 4 neutropenia associated with fever and grade 4 thrombocytopenia. Another individual experienced a transient grade 3 rash. Although the MTDs for 1843U89 with folic acid could not be defined precisely according to the criteria established a priori on the basis of the rate of DLT in course 1, the recommended Phase II dose was determined to be 5 mg/m²/day, because of an unacceptably high rate of severe toxicities in patients receiving repetitive treatment at 6 mg/m²/day. At the 1843U89 dose level of 5 mg/m²/day with folic acid, 1 of 6 new evaluable patients developed DLT during 16 evaluable courses.

No objective antitumor activity was observed.

**Toxicity.** Myelosuppression and mucositis were the principal toxicities of 1843U89 administered without and with folic acid. The distributions of the most common hematological and nonhematological toxicities as a function of dose level are displayed in Tables 3 and 4, respectively. Both hematological and nonhematological toxicities were more severe and frequent during courses in which 1843U89 was administered without folic acid. In fact, no patients experienced severe (grade 3–4) toxicities after treatment with 1843U89 at doses <5 mg/m²/day with folic acid, whereas both severe hematologic and nonhematologic effects occurred at all 1843U89 dose levels <5 mg/m²/day in the absence of folic acid. Table 5, which displays the principal toxicities of 1843U89 in 3 patients who received identical doses of 1843U89 with and without folic acid pretreatment, demonstrates that nonhematological toxicities were generally more severe and frequent when 1843U89 was administered alone; differences in hematological effects were more

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**Table 2** Dose escalation scheme

<table>
<thead>
<tr>
<th>No.</th>
<th>1843U89 (mg/m²/day)</th>
<th>Folic acid</th>
<th>No. of evaluate (patients)</th>
<th>No. of courses (evaluable)</th>
<th>No. of courses with DLT</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>New</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.0</td>
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<td>1 (1)</td>
<td>1 (1)</td>
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<td>1.0</td>
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<td>2 (2)</td>
<td>3 (3)</td>
<td>12 (12)</td>
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<td>7 (7)</td>
<td>7 (7)</td>
<td>11 (11)</td>
</tr>
<tr>
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<td>2.0</td>
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<td>3 (3)</td>
<td>5 (5)</td>
<td>12 (12)</td>
</tr>
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<tr>
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<td>6 (6)</td>
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<td>17 (16)</td>
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<td>7 (6)</td>
<td>7 (6)</td>
<td>27 (26)</td>
</tr>
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</table>

*a* The lead patient was initially treated at 6.0 mg/m²/day (one course) and retreated at 1.0 mg/m²/day without folic acid supplementation (five courses).

*b* One patient initially treated at 2.0 mg/m²/day dose level without folic acid (two courses) was retreated at the same dose level with folic acid (one course).

*c* One patient initially treated at 2.0 mg/m²/day dose level without folic acid (two courses) and then retreated at the 3.3 mg/m²/day dose level with folic acid (seven courses).

*d* One patient initially treated at 3.3 mg/m²/day dose level without folic acid (one course) was retreated at the same dose level with folic acid (one course).

*e* DLT presumed to be attributable to malabsorption of folic acid because of extensive gastric surgery (see text).

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**Table 3** Hematologic toxicity of 1843U89

<table>
<thead>
<tr>
<th>1843U89 dose (mg/m²/day)</th>
<th>Folic acid</th>
<th>No. of patients (new)</th>
<th>No. of courses (evaluable)</th>
<th>Median course 1 ANC nadir, µl (range)</th>
<th>No. of toxicities during all courses (course 1)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neutropenia</td>
</tr>
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<td>Grade 3</td>
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<td>1</td>
<td>1150</td>
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<tr>
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<td>3 (2)</td>
<td>12</td>
<td>4350 (3320–4740)</td>
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</tr>
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<td>7 (7)</td>
<td>11</td>
<td>4440 (400–11500)</td>
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<td>5 (3)</td>
<td>12</td>
<td>4100 (3730–4640)</td>
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<td>1</td>
<td>2090</td>
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<td>20</td>
<td>3900 (1870–11100)</td>
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<td>17 (16)</td>
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<td>7 (7)</td>
<td>27 (26)</td>
<td>2220 (950–5900)</td>
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</table>
subtle in these patients. Aside from folic acid supplementation, the propensity for severe myelosuppression, albeit relatively uncommon, appeared to be related to the extent of prior myelosuppressive chemotherapy and/or radiation therapy.

The onset of hematological toxicity, characterized by both neutropenia and thrombocytopenia, was generally on day 8; blood count nadirs were typically experienced between days 15 and 17, and both neutropenia and thrombocytopenia usually resolved by day 22. Although neutropenia was the most common drug-related hematological toxicity, the development of severe neutropenia was generally associated with other severe hematological and nonhematological effects. Severe (grade 4) prolonged (>5 days) neutropenia associated with fever was experienced by 1 heavily pretreated subject at the 5 mg/m²/day dose level who also developed thrombocytopenia (grade 4), anemia (grade 3), skin toxicity (grade 2), and mucositis (grade 2). Three other patients developed fever and/or infection associated with neutropenia, but two of these events were experienced by 2 patients who were taken off study after receiving only two of five scheduled doses because of rapidly progressive disease and diminution of performance status. Three patients developed drug-related neutropenia in three courses that was classified as grade 3 because of its generalized nature, but the skin effects were generally brief (<5 days) and not considered dose limiting. Patients typically complained of pruritis, burning, and/or discomfort, which were often treated with antihistamines and topical corticosteroids with mixed results. Both the rash and symptoms usually resolved by day 14.

Other mild to moderate (grades 1–2) complaints and nonhematological effects that were possibly related to 1843U89 included diarrhea, fever, and conjunctivitis. Eight patients experienced diarrhea (grade 1, 6 patients; grade 2, 2 patients), which was brief and never consequential. Three of these patients had also experienced concomitant grade 2 or 3 mucositis, which was the dominant nonhematological drug effect. Fever felt to be drug related was documented on day 5 or 6 in 7 patients.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>1843U89 dose (mg/m²/day)</th>
<th>Course no.</th>
<th>Stomatitis</th>
<th>Rash</th>
<th>Neutrophil count nadir (×10³/μl)</th>
<th>Platelet count nadir (×10³/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>1</td>
<td>Grade 3</td>
<td>Grade 1</td>
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<td>330</td>
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<tr>
<td></td>
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<td>None</td>
<td>3700</td>
<td>160</td>
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There were no hemorrhagic complications; platelet transfusions were administered prophylactically to 1 individual. Six (19%) of 32 patients developed grade 3 anemia requiring RBC transfusions at some time during treatment.

Mucositis and skin toxicity were the most common drug-related nonhematological effects. Mucositis of the lips and oral cavity began on days 3–8. Resolution was rapid, with complete recovery generally by days 8–10. Skin toxicity generally started on days 5–7, with manifestations persisting for ≤7 days. Dermatological effects did not worsen with cumulative treatment. Patients typically experienced a maculopapular rash involving the neck, chest, and back, but the rash was occasionally more diffuse, involving the upper and lower extremities. Three patients developed drug-related skin toxicity in three courses that was classified as grade 3 because of its generalized nature, but the skin effects were generally brief (<5 days) and not considered dose limiting. Patients typically complained of pruritis, burning, and/or discomfort, which were often treated with antihistamines and topical corticosteroids with mixed results. Both the rash and symptoms usually resolved by day 14.
fever was uncomplicated, self limited, and not associated with neutropenia. Although fever was noted across the entire 1843U89 dosing range, 6 of the 7 affected individuals did not receive folic acid. In addition, 3 patients complained of mild to moderate conjunctivitis in the peritreatment period. Alopecia, malaise, anorexia, and peripheral edema occurred infrequently.

**Pharmacokinetics.** Twenty-eight and 27 of the 32 total patients had complete plasma sampling performed on days 1 and 5, respectively, whereas 25 patients had complete plasma sampling performed on both days 1 and 5. Day 1 and 5 plasma concentration-time profiles of representative patients treated with 1843U89 at the 2 mg/m²/day dose level without folic acid (A) and with folic acid (B). A patient treated with 1843U89, 5 mg/m², with folic acid (C).

**Table 6** Noncompartmental 1843U89 pharmacokinetic parametersa

<table>
<thead>
<tr>
<th>1843U89 dose level (mg/m²/day)</th>
<th>No. of patients</th>
<th>Cmax (ng/ml)</th>
<th>CL (ml/min/m²)</th>
<th>t1/2 (h)</th>
<th>Vss (liter/m²/h)</th>
<th>AUC0–24 (ng/h/ml)</th>
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<tr>
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<td>237 (7.37)</td>
<td>47.2 (33)</td>
<td>10.2</td>
<td>26.9 (7.33)</td>
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<td>5</td>
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<td>11.7 (4.41)</td>
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<td>Mean (SD)</td>
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<td>47.1 (21.7)</td>
<td>50.5 (31.7)</td>
<td>7.72</td>
<td>16.7 (8.8)</td>
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a Values represent means (SD).

b Vss, volume of distribution at steady state.

The mean percentage of 1843U89 excreted unchanged in the urine from time 0 to 24 h after treatment on day 5 (Ae0–24)
was 10.0% ± 5.31 (range, 3.15–21.6%), and the CLr averaged 7.88 ± 4.11 ml/min. No metabolites were identified in plasma and urine.

**Pharmacodynamics.** The relationships between 1843U89 AUC and Cmax values obtained in course 1 and the mean percentage decrements in ANC, as well as the fit of these data to a sigmoidal Emax model, are depicted in Fig. 4, A and B. The relationship between AUC on day 1 and the percentage decrement in the ANC was the only pharmacodynamic relationship that was fit, albeit roughly, by the sigmoidal Emax model (R2 = 0.3578, whereas R2 < 0.3 for the other relationships). The Cmax/AUC values on day 1 in patients who did and did not experience severe mucositis were 1499 ± 1003 ng/ml / 2518 ± 1494 ng/h/ml and 1527 ± 1105 ng/ml / 1584 ± 1493 ng/h/ml, respectively [Ps (paired t testing), 0.9613 and 0.2574, respectively]. Respectively values on day 5 were 1436 ± 676 ng/ml and 2032 ± 1006 ng/h/ml for patients who experienced severe mucositis and 1234 ± 847 ng/ml and 1300 ± 700 ng/h/ml for patients who did not (Ps, 0.6565 and 0.0809, respectively). There were too few patients who had other specific types of DLT to perform adequate statistical analyses.

**DISCUSSION**

TS is a strategic target for anticancer therapeutic development because it catalyzes the reaction that provides the only de novo source of cellular thymidylate nucleotides, which are necessary for DNA synthesis and DNA repair (1). Although the fluoropyrimidines indirectly inhibit TS, fluoropyrimidine-induced TS inhibition is self limiting because pyrimidine analogues are also incorporated into DNA and RNA, and TS inhibition increases intracellular dUMP, which competes with pyrimidine analogues for binding to TS (21). Folate-based TS inhibitors have several distinct advantages over fluoropyrimidines. Appropriately designed folate analogues inhibit TS without significantly affecting other folate-dependent enzymes (1, 22). Additionally, dUMP does not reverse TS inhibition induced by folate analogues but, instead, enhances binding of folate analogues to TS (22). These considerations served, in part, as the rationale for developing 1843U89, which interacts noncompetitively with the folate-binding site of TS.

The benzoxazinoline 1843U89 is the most potent direct TS inhibitor described in the literature, with a Ki for recombi-
nant TS of 90 μM (13, 14). Furthermore, although 1843U89 has a respectable level of potency against murine tumor cells in the nanomolar range (e.g., IC_{50} against L1210 leukemia, 66 nm), IC_{50} values against numerous human cancer cell lines are in the subnanomolar range (13, 22). This difference is explained, in part, by an unusually high affinity of 1843U89 for the human reduced-folate cell membrane carrier and a high rate of cellular internalization (13). Furthermore, 1843U89 has a very high affinity for FPGS, implying that polyglutamates formation should be very rapid (13), but the affinity of the diglutamate for FPGS is poor, resulting in limited polyglutamate chain elongation (15). Unlike other folate-based TS inhibitors, such as Tomudex (AstraZeneca, Manchester, United Kingdom) and MTA (LY231514; Eli Lilly, Indianapolis, IN), polyglutamates of 1843U89, including its diglutamate, are not potent inhibitors of TS (13). Taken together, these observations suggest that polyglutamation may contribute less to the cytotoxicity of 1843U89 compared with other TS inhibitors, and it is interesting to speculate that 1843U89 may be less susceptible to cellular resistance conferred by low FPGS activity and/or high folylpolyglutamyl hydrolase activity (16).

Both myelosuppression and mucositis were the principal toxicities of 1843U89 in the present study. On the basis of the rates of severe hematological and nonhematological toxicities observed in all courses, recommended 1843U89 doses for subsequent disease-directed studies are 2 and 5 mg/m²/day without and with folic acid, respectively, in patients with normal renal and hepatic excretory functions similar to those in the present study; repetitive treatment at higher doses is not feasible. Overall, severe toxicity occurred sporadically, and patients who experienced severe neutropenia and mucositis were more prone to developing thrombocytopenia, diarrhea, and rash. Neither demographic nor biochemical determinants of severe toxicity were readily apparent in the present study; however, heavily pretreated patients were generally more susceptible to severe toxicity than their minimally pretreated counterparts. With regard to pharmacokinetic determinants of toxicity, the only pharmacodynamic relationship evident was a rough sigmoidal fit of percentage decrements in ANC values to AUC values on day 1 (R² = 0.3587). Similarly, pharmacokinetic determinants of toxicity have not been consistently delineated in clinical studies of other folate-based TS inhibitors. In contrast, plasma concentrations of vitamin metabolites, which may more accurately reflect intracellular folic acid stores than plasma folic acid and B12 concentrations, have been demonstrated to significantly predict for severe hematological and nonhematological effects of MTA (23). A multivariate analysis of potential demographic (age, gender, and prior therapy), biochemical (pretreatment albumin, liver functions, homocysteine, cystathionine, and methylenomalonic acid), and pharmacokinetic (AUC) determinants of toxicity in 139 patients treated with MTA in Phase II studies showed that homocysteine was superior to the other potential determinants in predicting for severe neutropenia, thrombocytopenia, mucositis, rash, and fatigue. In contrast, AUC was not found to predict for relevant toxicities. Plasma homocysteine concentrations ≥ 10 μM predicted for the occurrence of grade 4 neutropenia 75% of the time. Because methionine synthase, which catalyzes the production of methionine from homocysteine, is highly dependent on folic acid and vitamin B12 as cofactors, elevated levels of homocysteine can result from deficiencies of folate and/or vitamin B12. These observations have led to prospective studies of homocysteine as a predictor of MTA toxicity and, subsequently, pretreatment and concurrent supplementation of patients receiving MTA with recommended daily nutritional quantities of folic acid and vitamin B12, which have been shown to substantially reduce plasma homocysteine within 2 weeks (24).

In the present study, treatment of patients with high-dose (1000 mg) of folic acid before 1843U89 on days 1–5 was associated with fewer and less severe nonhematological and hematological toxicities than observed with identical doses of 1843U89 in the absence of folic acid. In addition, pretreatment with folic acid permitted escalation of 1843U89 doses by 2.5- to 3-fold. Furthermore, toxicities were fewer and less severe when patients who developed intolerable toxicity with 1843U89 alone were retreated with folic acid before 1843U89. In preclinical studies, treatment of dogs with 50 mg/kg of folic acid 30 min before 1843U89 on a daily ×5 schedule completely prevented weight loss and toxicity (14). However, the protection afforded by folic acid was progressively less at higher 1843U89 doses and failed to protect against the lethal effects of 1843U89 when administered at three times its lethal dose. Folic acid, at doses ≥500 mg/kg, could be safely given along with 10 mg/kg/day of 1843U89 for 5 days, and the minimal daily dose of folic acid that provided complete protection from a lethal 10 mg/kg dose of 1843U89 ranged from 10 to 50 mg/kg/day, which served, in part, as the basis for selecting the folic acid dose schedule used in the present study (14). Additionally, high-dose folic acid did not reverse the cytotoxicity of 1843U89 nor related benzoquinazolines against tumor cell lines in vitro or thymine kinase-deficient human tumor xenografts in mice. In contrast, the cytotoxic effects of methotrexate, Tomudex, and lometrexol (Tularik, South San Francisco, CA), a folate-based inhibitor of glycinamide ribonucleotide formyl transferase, have been demonstrated to be partially reversed by low doses of folic acid (14, 25–27). Additionally, high doses of folic acid, comparable with those used with 1843U89, do not reverse the toxicities of the competitive TS inhibitor ZD9331 (AstraZeneca), which is in early clinical development (14, 25–27).

The mechanisms for the selective protection of normal tissues, but not malignant tumors, from the cytotoxic effects of 1843U89 have not been investigated in detail. Nonetheless, the results are consistent with a mechanism involving selective inhibition of drug transport into sensitive normal cells combined with noncompetitive inhibition of TS by 1843U89 or its diglutamate metabolite. The mechanism assumes the presence of a folic-acid binding transporter for 1843U89 in the sensitive normal cells and a folic acid-insensitive transporter for 1843U89 in tumor cells, which is consistent with 1843U89 transport described in MOLT-4, MCF-7, WiDr, and SW480 tumors (28). Systems analogous to those proposed for normal cells have been described in intestinal and liver cells (28–31). An additional feature of the protection of normal cells by folic acid may be an effect of folic acid on the retention of 1843U89 by normal cells via competition for polyglutamation. Clearly, further investigations are required to sort out the mechanism of this very selective and unique characteristic of 1843U89, but the cumulative results of preclinical studies suggest that the efficacy of
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1843U89 would be maintained, its toxicity decreased, and its overall therapeutic index increased if the agent is administered after treatment with folic acid. In the present study, there were fewer and less severe 1843U89 toxicities at the 2 mg/m²/day dose level in the presence of high-dose folic acid than observed with 1843U89 alone, and repetitive treatment with 1843U89 at doses as high as 5 mg/m²/day with folic acid were associated with acceptable toxicity profiles.

The pharmacokinetics of 1843U89 were dose independent, similar on days 1 and 5, and not altered by the administration of high-dose folic acid. However, the CL rates of 1843U89 in dogs and monkeys were higher than those in cancer patients, resulting in the selection of an unacceptably high starting dose (6 mg/m²/day: Ref. 16). Although the starting dose was equivalent to one-sixth of the TDL in dogs, it was eventually found to be three times higher than the recommended dose of 1843U89 without folic acid. The CL rates of 1843U89 at the starting dose (mean values, 47.1 ± 21.7 and 50.5 ± 31.7 on days 1 and 5, respectively) were ~8–20 times lower than predicted from preclinical studies in dogs and monkeys, respectively (14), and t½ values on days 1 and 5 were long, averaging 7.72 ± 4.09 and 9.56 ± 4.0 h, respectively. These pharmacological differences may partially explain the toxicokinetic differences between various species, as well as the unexpected toxicity of the starting dose in the present study. Neither dogs nor monkeys evaluated in preclinical toxicology studies have high circulating thymidine levels as seen in rodents.

Plasma 1843U89 concentrations in the present study exceeded those required to inhibit TS and induce prominent antitumor effects in human tumor xenografts. At the 2 mg/m²/day dose level, minimum (pretreatment) 1843U89 concentrations in plasma on day 5 averaged 17.3 ± 14.53 ng/ml (34.6 ± 29.1 nM), which were several orders of magnitude higher than 1843U89 Kᵢₑ values for TS (=90 nm) and the range of IC₅₀ values in human tumor xenografts (0.2–0.7 nm). The low CL of 1843U89 in humans and the prolonged maintenance of plasma concentrations several orders of magnitude above biologically relevant levels after administration of 1843U89 on a daily times-5-day schedule implies that alternate 1843U89 dose schedules (e.g., weekly or once every 3 weeks) may be equivalent or superior at achieving optimal pharmacodynamics and toxicokinetics, resulting in a high therapeutic index. However, such comparisons must be tempered by the fact that tissue and tumor concentrations may be more relevant.

Although objective evidence of antitumor activity was not noted in the present study, the heavily pretreated nature of the majority of patients in the present study must be considered. Additionally, 22 (69%) of the 32 patients had fluoropyrimidine-resistant or recurrent colorectal carcinoma, which has largely been unresponsive or minimally responsive to folate-based competitive TS inhibitors. The novel structural, mechanistic, biochemical, and pharmacological features of 1843U89 relative to other TS inhibitors, particularly its noncompetitive mode of TS inhibition, high potency, low CL rate, and the selective protection of normal tissues versus tumor by folic acid pretreatment, warrant further clinical development. On the basis of the results of preclinical studies and the present trial, it would seem prudent to administer folic acid before 1843U89 in further clinical evaluations of 1843U89; however, the relative protective effects of folic acid in human tumors and normal tissues are not fully known despite the maintenance of antitumor activity in select preclinical studies. Furthermore, the relative merits of administering 1843U89 at the 2 mg/m²/day dose level with folic acid, which results in minimal toxicity, versus escalating 1843U89 with folic acid to a higher, more toxic, albeit tolerable, dose level (5 mg/m²/day) are not known. Randomized clinical trials, perhaps in tumor types found to be sensitive in early disease-directed evaluations, may clarify issues of 1843U89 dose optimization with folic acid, and studies evaluating the relative merits of alternative 1843U89 administration schedules should be designed to maximize the therapeutic index of this unique antitumor agent.

REFERENCES


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A Phase I and Pharmacokinetic Study of 1843U89, a Noncompetitive Inhibitor of Thymidylate Synthase, in Patients with Advanced Solid Malignancies


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