Phase I, Pharmacokinetic and Biological Correlative Study of OSI-7904L, a Novel Liposomal Thymidylate Synthase Inhibitor, and Cisplatin in Patients with Solid Tumors

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Abstract

Purpose: To evaluate the safety and describe the pharmacokinetic profile of OSI-7904L, a novel liposomal thymidylate synthase inhibitor, in combination with cisplatin (CDDP) in adults with advanced solid tumors.

Experimental Design: CDDP was administered as a 2-h intravenous infusion followed by OSI-7904L intravenously over 30 min, both given every 3 weeks. Doses of each drug were escalated in separate cohorts of patients. Five dose levels of CDDP/OSI-7904L were explored: 60/6, 60/9, 60/12, 60/7.5, and 75/7.5 mg/m^2. Pharmacokinetic samples, baseline plasma homocysteine, and genotype polymorphisms were evaluated.

Results: Twenty-seven patients were treated with 101 total courses of CDDP/OSI-7904L. Dose-limiting toxicity was observed in 2 patients in the CDDP/OSI-7904L 60/12 mg/m^2 cohort. One patient experienced rash, stomatitis, dehydration, renal failure, hyperbilirubinemia, and fatal neutropenic sepsis, whereas the other patient experienced grade 3 nausea, vomiting, and ileus. Therefore, the CDDP/OSI-7904L 60/9 mg/m^2 cohort was expanded, with 2 of 6 patients reporting significant fatigue. Other toxicities were mild or moderate. Intermediate dose levels of 60/7.5 and 75/7.5 mg/m^2 were evaluated, and the latter was identified as the recommended dose for phase II studies. No major pharmacokinetic interactions between CDDP and OSI-7904L were observed. Three patients had partial responses (gastric adenocarcinoma and heavily pretreated breast cancer). There was no significant relationship between baseline homocysteine and toxicity.

Conclusions: The recommended doses for CDDP and OSI-7904L administered once every 3 weeks are 75 and 7.5 mg/m^2, respectively. Pharmacokinetic interaction between the agents was not apparent. Preliminary clinical activity was observed in breast and gastric cancer.

Thymidylate synthase (TS) is a folate-dependent enzyme that catalyzes the reductive methylation of dUMP to dTMP (1). As thymidine nucleotides are used exclusively for synthesis of DNA, TS has been a validated target for anticancer therapy for >40 years (2). Drug resistance is often a limiting factor in successful chemotherapy. A variety of mechanisms of resistance to TS inhibitors have been described, including (*a*) reduced intracellular activation or increased inactivation, (*b*) relative deficiency of the reduced folate cofactor 5,10-methylenetetrahydrofolate, (*c*) reduced polyglutamation due to decreased activity or lower levels of polyglutamate synthase, (*d*) increased TS gene expression resulting in elevated enzyme levels, and (*e*) alterations in TS [which represent the most commonly described mechanism of resistance to 5-fluorouracil (5-FU); refs. 3–5]. Although the relative contribution of these mechanisms in the clinical setting is not entirely clear, much effort has focused on designing new, more effective TS inhibitors.

OSI-7904 (*S*-2-[-5-[[[1,2-dihydro-3-methyl-1-oxobenzo[*f*]-quinazolin-9-yl] methyl] amino]-1-oxo-2-isoinso-linyl] glutaric acid) is a noncompetitive inhibitor of TS, and its binding is unaffected by 5,10-methylenetetrahydrofolate (6, 7). Moreover, it differs from other folate-based inhibitors in that the parent compound is as potent as its polyglutamated analogues, suggesting that polyglutamation is not essential for its antitumor activity (8). OSI-7904 entered clinical testing as BW1843U89 on a days 1 to 5 schedule repeated every 21 days, with and without folic acid supplementation. Dose-limiting

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Translational Relevance

OSI-7904L is a liposomal formulation of a potent inhibitor of the enzyme TS. Based on preclinical and clinical data suggesting beneficial effects of TS inhibitors and DNA-platinating agents, this phase I trial was done to describe the clinical and pharmacologic characteristics of OSI-7904L in combination with CDDP. Fatigue, nausea, and vomiting were the DLTs. The recommended phase II dose for this combination is 75 mg/m^2 CDDP and 7.5 mg/m^2 OSI-7904L administered once every 3 weeks. No pharmacokinetic interactions were observed and baseline homocysteine levels did not predict for toxicity. This combination showed clinical activity with partial responses observed in patients with gastric and breast cancers and should be considered for phase II evaluation in these cancers.

toxicities (DLT) included neutropenia, pancytopenia, fever, mucositis, and rash (9). In further development, OSI-7904 was encapsulated within a liposome dispersion consisting of small, unilamellar vesicles (ranging in diameter from 20 to 80 nm) of hydrogenated soy phosphatidylcholine and cholesterol. The liposome contains the drug within its aqueous core and has been designated OSI-7904L. OSI-7904 encapsulation within liposomes appears to enhance tumor localization of active drug and potentially simplifies the schedule of administration in preclinical models (10, 11). Compared with either parent drug or 5-FU, OSI-7904L has shown enhanced efficacy in a variety of murine xenograft models (10, 12, 13).

In the phase I study of OSI-7904L, a total of 31 patients were treated at 8 dose levels starting at 0.4 mg/m^2. Minimal toxicity was reported up to 9.6 mg/m^2, but DLT occurred in both patients treated at 15 mg/m^2. One patient experienced fatigue and severe dermatologic and gastrointestinal toxicities, with fatal neutropenic sepsis. The second patient reported grade 3 fatigue. An intermediate dose level of 12 mg/m^2 was identified as the recommended dose for phase II studies. The principal toxicities were rash, pruritus, fatigue, stomatitis, and myelosuppression. *In vivo* pharmacokinetic data indicated that the liposomal formulation alters the disposition of the parent drug resulting in a prolonged plasma residence time (14). A phase II study of OSI-7904L in previously untreated patients with advanced gastric or gastroesophageal cancer reported a response rate of 17%, with a similar toxicity profile to the phase I evaluation (15).

Most of the TS inhibitors studied to date have shown significant interpatient toxicity variability. Several approaches have been investigated to better define specific patient populations that may benefit from these drugs or that may be susceptible to increased toxicity. Assessment of pretreatment homocysteine concentrations accurately identified those patients at risk of increased toxicity with the antifolate pemetrexed (16). In contrast, preclinical studies with OSI-7904L have shown that toxicity was not adversely affected by a 50% reduction in serum folate concentration or elevation in plasma homocysteine concentrations. Over the past several years, there has been an increasing appreciation of the association of cancer therapy with pharmacogenetics (17, 18). The human TS gene promoter is polymorphic, having either double or triple repeats of a 28-bp sequence. A small retrospective pilot study has suggested that genotyping patients for the TS polymorphism could be useful in identifying those patients more likely to respond to TS inhibitors therapy (19).

Preclinical studies have evaluated the efficacy of OSI-7904L in combination with oxaliplatin or cisplatin (CDDP) in human colon tumor xenografts. In general, supra-additive interactions were seen, and the combination of CDDP followed by OSI-7904L within 30 min in COLO 205 xenografts produced the greatest efficacy (20). Moreover, the significant clinical activity of TS inhibitors and CDDP combinations in head and neck, esophageal, gastric cancer and mesothelioma and the lack of overlapping principal toxicities support a rationale for combining OSI-7904L and CDDP (21–25).

The principal objectives of the present study were to identify DLT and to determine the maximum tolerated dose and recommended phase II dose of CDDP with OSI-7904L administered every 3 weeks. The secondary objectives were to determine the toxicities of this schedule, characterize the pharmacokinetic behavior of OSI-7904L in combination with CDDP, describe possible pharmacokinetic interactions, and seek preliminary evidence of antitumor activity in patients with advanced solid malignancies. An exploratory analysis of homocysteine levels and relevant gene polymorphisms, including TS, were also done.

Patients and Methods

Patient selection. Patients with histologically or cytologically confirmed solid malignancies refractory to standard therapy or for whom no standard therapy exists were eligible. Patient entry criteria also included age ≥18 years; life expectancy of ≥12 weeks; an Eastern Cooperative Oncology Group performance status of ≤2; no prior chemotherapy within 3 weeks (4 weeks for alkylating agents or carboplatin and 6 weeks for prior mitomycin C or a nitrosourea); adequate hematologic (hemoglobin ≥8.5 g/dL, absolute neutrophil count ≥1,500/μL, platelet count ≥ 100,000/μL), hepatic (bilirubin ≤1.5 times upper limit of normal, serum aspartate aminotransferase and alanine aminotransferase ≤2.5 times upper limit of normal), and renal (serum creatinine ≤1.5 times upper limit of normal) functions; measurable or evaluable disease; no peripheral neuropathy that interfered with function; and no coexisting medical problem of sufficient severity to limit compliance with the study. Concomitant administration of folic acid or vitamin supplementation was prohibited. Patients with symptomatic or unstable brain metastases, a history of any disease significantly affecting gastrointestinal function, or known hypersensitivity to liposomal formulations were not enrolled. All patients gave informed written consent before treatment according to federal and local institutional guidelines.

Treatment and dose escalation. The starting doses of CDDP and OSI-7904L were 60 and 6 mg/m^2, respectively. These doses produce minimal toxicity when each drug is administered as a single agent (14). Stepwise dose escalation of OSI-7904L and then CDDP were done in successive patient cohorts. The goal was to focus primarily on the dose escalation of OSI-7904L, whereas CDDP was dosed in the conventional range of 60 to 75 mg/m^2. At least 3 new patients were treated at each dose level to evaluate the toxicity associated with the drug combination. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. DLT was defined during the first course of therapy as neutropenia associated with fever (absolute neutrophil count <1,000/μL, fever ≥38.5°C), absolute neutrophil count <500/μL for >5 days, platelets <25,000/μL, nonhematologic

toxicity considered related to the study drug combination grade ≥3 (excluding inadequately treated nausea, vomiting, diarrhea, rash, or infusion-related reaction), and delay in the administration of subsequent courses due to unresolved toxicities lasting >21 days. In addition, the occurrence of any grade 3 or 4 nonhematologic toxicity had to represent at least a two grade increase from baseline. If one DLT occurred, a maximum of 6 patients were treated at that dose level. The maximum tolerated dose was defined as the highest dose at which <2 of 6 cohort assigned patients experienced DLT. Intrasubject dose escalation was not allowed. The dose was reduced for patients who developed DLT or other significant toxicity.

CDDP (Platinol; Bristol-Myers Squibb) was obtained from commercial supply in 10 and 50 mg vials that were reconstituted with 500 mL of 0.9% NaCl solution and given as a 120-min intravenous infusion. Patients were hydrated with 1 L of 0.9% NaCl solution with 20 mEq KCl and 2 g/L $MgSO_4$ over 2 h before the CDDP infusion and after the OSI-7904L infusion. OSI-7904L was supplied by OSI Pharmaceuticals in 5 mg vials as a sterile, translucent, liposomal dispersion of OSI-7904 in a solution composed of 9% sucrose in water for injection. Drug was diluted in 5% dextrose in water to the appropriate concentration and given as a 30-min intravenous infusion. The infusion started 2.5 h following the start of the CDDP infusion. The treatment was administered intravenously every 21 days on an outpatient basis. Antiemetic premedication included dolasetron (or equivalent) and dexamethasone, which were also administered orally for 3 days following CDDP. Lorazepam and metoclopramide were used at the discretion of the investigator.

Study investigations. The pretreatment assessment included a complete medical history, physical examination, vital signs, Eastern Cooperative Oncology Group performance status, CBC, serum electrolytes and chemistries, electrocardiogram, and urinalysis. Computed tomography was done to assess malignant disease before treatment.

Table 1. Patient characteristics

Characteristics	
No. patients	30*
Median (range) age, y	53 (38-83)
Sex (M/F)	19/11
Median (range) no. courses/patient	3 (1-9)
Performance status (Eastern Cooperative Oncology Group)	
0	6
1	18
2	4
Missing	2
Previous therapy	
Chemotherapy	27
Median (range) no. prior chemotherapy regimens	3 (0-12)
Prior TS inhibitors † therapy	18
Radiation therapy	13
Tumor types	
Colorectal	7
Breast	4
Pancreatic	3
Gastric/gastroesophageal	3
Cholangiocarcinoma	3
Renal cell	2
Esophageal, head and neck, mesothelioma, NSCLC, prostate, rectal/small bowel, retroperitoneal PDC, adenocarcinoma of unknown primary site	1 each

Abbreviations: NSCLC, non-small cell lung cancer; PDC, poorly differentiated carcinoma.
*Three of these 30 patients were registered but did not receive CDDP/OSI-7904L due to clinical deterioration before dosing.
† Includes 5-FU, capecitabine, pemetrexed, and methotrexate.

Thereafter, assessments were made within 72 h of the day of dosing and CBC was done weekly. Response was assessed using computed tomography every three cycles (9 weeks) according to the Response Evaluation Criteria in Solid Tumor. Patients with disease progression or intolerable toxicity were taken off study.

Sample collection and analytical analysis: plasma pharmacokinetics. Plasma samples were collected during course 1. Plasma pharmacokinetics of total OSI-7904 (including liposome encapsulated and nonencapsulated drug) of ultrafilterable OSI-7904 (neither liposome encapsulated nor plasma protein-bound OSI-7904) and unbound platinum were evaluated. A total of 12 blood samples for OSI-7904L were collected at the following time points: predose, 3 (at the end of OSI-7904L infusion), 4, 6, 10, 24, 48, 72, 96, and 168 h, and 14 and 21 days after the start of the CDDP infusion.

OSI-7904L blood samples were collected in vials containing EDTA via an indwelling venous catheter placed in the contralateral arm from the infusions and cooled immediately on ice and then centrifuged at 1,500 to 2,000 × *g* for 10 min. Plasma ultrafiltrate was obtained from plasma by centrifugation at 1,500 × *g* for 30 min through an Amicon Centrifree micropartition device (Millipore). Samples were shipped to MDS Pharma Services St-Laurent, and total and ultrafilterable concentrations of OSI-7904 were quantified by validated liquid chromatography-tandem mass spectrometry methods. Briefly, plasma samples (150 μL) were mixed with methanol (750 μL) containing internal standard (methylated OSI-7904) and the protein was pelleted by centrifugation. Supernatants were transferred to 13 × 100 mm culture tubes and evaporated to dryness. Samples were reconstituted with 300 μL water and injected onto a Zorbax XBD C18 (15 × 2.1 mm) analytic column (Agilent Technologies) using a mobile phase of 75:25 (v/v) methanol/0.088% formic acid in water. Mass spectral detection was done with a Perkin-Elmer Sciex API III with a Turbo ion-spray source. OSI-7904 was detected using selected reaction monitoring in positive ion mode and the mass transition monitored was *m/z* 501.2 to 223.1. Analyte quantification was done by peak area ratio using a quadratic equation weighed 1/concentration^2. The assay range for total OSI-7904 was 1 to 250 ng/mL. Relative error was ≤15%, except at the lower limit of quantification (≤20%). The lower limit of quantification for ultrafilterable OSI-7904 was 0.2 ng/mL. Noncompartmental analysis was used to determine the pharmacokinetic variables.

Eight plasma samples (4 mL each) were collected during course 1, to determine unbound (free) platinum concentrations, at the following times: day 1 before CDDP infusion and at ~2.0 (or at end of infusion), 2.5 (before starting OSI-7904L infusion), 3 (at the end of OSI-7904L infusion), 4, 6, 10, and 24 h after the start of the CDDP infusion. Blood was collected into tubes containing heparin and the sample was cooled immediately on wet ice until processed to separate plasma. Following centrifugation at ~1,500 × *g* for 10 min, the plasma was recovered and transferred into the reservoir of two unused, labeled Centrifree micropartition devices. Plasma ultrafiltrate was obtained by centrifugation at 1,500 × *g* for 30 min. Ultrafiltrate samples were frozen at -20°C or below until shipment on dry ice to MDS Pharma Services for analysis of unbound platinum by use of a validated atomic absorption method.

Plasma homocysteine concentrations. A blood sample (~5 mL) was drawn from all patients before the OSI-7904L infusion on day 1 of course 1. In addition, further samples could be collected every other cycle in patients treated at the recommended phase II dose level. Each blood sample was collected into an EDTA tube, immediately cooled on ice, and centrifuged at 1,500 to 2,000 × *g* for 10 min. Equal aliquots of separated plasma were transferred into two labeled cryovials and frozen at -70°C. Homocysteine concentrations in plasma were determined using a fully automated Abbott Imx fluorescence polarization immunoassay (26). Spearman's rank correlation tests were used to evaluate correlations between baseline homocysteine concentrations and patient demographics, response to therapy, or toxicity.

TS genotype. A blood sample of 7 to 10 mL was collected into an EDTA tube. TS genotype samples were stored at 4°C until DNA

Table 2. CDDP/OSI-7904L treatment by dose level

Dose level CDDP/OSI-7904L (mg/m^2)	No. patients treated	No. courses	Courses range/patient	No. patients with DLT in course 1
60/6	4	18*	2-6	0/4
60/9	3 (+3) †	37 ‡	2-16	2/6§
60/12	4	6	1-3	2/4
60/7.5	3	7	2-3	0/3
75/7.5	3(+7) †	28	1-9	0/10

*Includes 5 courses given at a reduced dose due to DLT at their initial dose of 60/9 mg/m^2 for 2 patients and (non-DLT) toxicity at the initial dose of 7.5/75 mg/m^2 for 1 patient.
† Number in parentheses denotes additional patients treated at that dose level during expansion of the cohort.
‡ Includes 2 courses given at a reduced dose to 1 patient due to DLT at the initial dose of 60/12 mg/m^2.
§ Both DLTs in this dose cohort occurred during expansion of the cohort.

extraction and analysis at the University of Chicago. DNA was isolated using the Puregene kit from Gentra Systems and genotyping was done by PCR amplification using primers flanking the repeat region. Amplified products were then sized on an agarose gel (27). Samples were processed to assess TS genotype (promoter polymorphism) and the single nucleotide polymorphism G→C within the promoter (28, 29). Similar methods were used to assess the MTHFR 677 C→T polymorphism (30).

Results

Thirty patients were registered in the study (Table 1). Three patients did not receive treatment due to clinical deterioration before dosing. Therefore, 27 patients were treated with 101 courses of CDDP/OSI-7904L through 5 dose levels. Twenty-three patients had received previous treatment with a TS inhibitor and/or a platinum compound. The numbers of patients and total courses administered at each dose level are shown in Table 2. The median number of courses administered per patient was 3. Four patients, 3 of whom experiencing DLT, required dose reduction for significant toxicity (Table 2).

Toxicity. Mild to moderate toxicity was reported up to CDDP/OSI-7904L doses of 60/9 mg/m^2 and dose escalation proceeded until DLT was observed in 2 of 3 patients in the CDDP/OSI-7904L 60/12 mg/m^2 cohort. One patient experienced rash, stomatitis, dehydration, renal failure, hyperbilirubinemia, and fatal neutropenic sepsis, whereas the other patient experienced grade 3 ileus. The intestinal transit impairment presented on day 4 of first course was accompanied by grade 3 nausea and vomiting and resolved in 4 days. As a result, the CDDP/OSI-7904L 60/9 mg/m^2 cohort was expanded to 6 patients, with 2 of the 3 additional patients reporting grade 3 fatigue, which met DLT criteria. Thus, an intermediate OSI-7904L dose of 7.5 mg/m^2 was evaluated, first combined with 60 mg/m^2 CDDP (3 patients) and then with 75 mg/m^2. At CDDP/OSI-7904L doses of 75/7.5 mg/m^2, the occurrence and intensity of fatigue were acceptable, although it was deemed to prevent further dose escalation attempts. CDDP/OSI-7904L 75/7.5 mg/m^2 was identified as the recommended dose, because no DLT was observed in any of 10 patients treated at this dose level.

Myelosuppression was generally mild or moderate (grade 1 or 2), except at the highest dose levels. Table 3 details the numbers of courses associated with grades 3 and 4 hematologic toxicity as a function of dose level. Myelosuppression was rarely associated with clinical sequelae. Besides the one incidence of fatal neutropenic sepsis described above, there was only one episode of neutropenia associated with fever. There was no significant bleeding related to thrombocytopenia. Overall, the effect on erythropoiesis was mild or moderate, without significant cumulative anemia with repetitive treatment. However, 7 patients required RBC transfusions on at least one occasion.

The nonhematologic toxicities of the CDDP/OSI-7904L regimen were consistent with the toxicities observed with each

Table 3. Hematologic toxicity

Laboratory analyte-grade	Dose level CDDP/OSI-7904L (mg/m^2)									
	No. patients with toxicity (first course/all courses)									
	60/6 (*n* = 4)		60/9 (*n* = 6)		60/12 (*n* = 4)		60/7.5 (*n* = 3)		75/7.5 (*n* = 10)	
	Any	3 and 4	Any	3 and 4	Any	3 and 4	Any	3 and 4	Any	3 and 4
Leukocytes (10^9/L)	2/3	0/1	4/6	0/1	4/4	0/2	2/2	0/0	4/6	1/1
Neutrophils (10^9/L)	0/2	0/1*	2/6	1/1	3/3	3/3 †	2/2	1/1	2/3	1/1
Platelet count (10^9/L)	0/3	0/0	1/4	0/1	3/4	1/1	2/2	0/1	1/1	1/1
Hemoglobin (g/dL)	4/4	0/0	5/6	0/1	4/4	1/1	3/3	1/2	8/9	0/0

*One episode of fever and neutropenia.
† One episode of fatal neutropenic sepsis.

Table 4. Nonhematologic toxicity

Grade	Dose level CDDP/OSI-7904L (mg/m²)									
	No. patients with toxicity (first course/all courses)									
	60/6 (*n* = 4)		60/9 (*n* = 6)		60/12 (*n* = 4)		60/7.5 (*n* = 3)		75/7.5 (*n* = 10)	
	Any	3 and 4	Any	3 and 4	Any	3 and 4	Any	3 and 4	Any	3 and 4
Fatigue	2/2	0/1	5/6	2*/3	3/3	2†,‡/2	2/3	1‡/2	5/6	1‡/2
Nausea	2/2	1§/1	4/5	0/0	3/3	2†/2	2/2	0/0	5/6	0/2
Vomiting	1/2	0/0	1/4	0/0	3/3	1/1	1/2	0/0	3/7	0/2
Anorexia	3/3	0/0	1/3	0/0	1/1	1/1	1/2	0/1	3/4	1/1‖
Diarrhea	1/2	0/0	2/3	0/0	2/2	0/0	0/0	0/0	1/2	0/0
Stomatitis	2/2	0/0	2/3	0/0	3/3	1/1	0/0	0/0	1/1	0/0
Dysgeusia	1/2	0/0	3/3	0/0	0/0	0/0	0/0	0/0	1/2	0/0
Constipation	1/1	0/0	2/3	0/0	0/0	0/0	0/0	0/0	1/1	1/1‖
Ileus	0/0	0/0	0/0	0/0	1/1	1/1	0/0	0/0	0/0	0/0
Dizziness	0/0	0/0	0/3	0/0	0/0	0/0	0/0	0/0	1/2	0/0
Skin (e.g., rash)	1/1	0/0	3/5	1§/3	2/2	1/1	0/0	0/0	0/2	0/0
Pruritus	0/0	0/0	2/2	1§/1	0/0	0/0	0/0	0/0	0/0	0/0
Dehydration	0/0	0/0	0/0	0/0	1/2	1‖/2	0/0	0/0	0/2	0/0

*Both DLTs in this cohort were experienced during expansion of the cohort.
†One episode of fatigue and 1 episode of nausea not considered DLTs due to inadequate antiemetic therapy.
‡This episode of fatigue did not represent a 2 grade increase from baseline (patient had grade 2 fatigue at baseline).
§Not considered a DLT due to inadequate prophylactic therapy.
‖Disease-related.

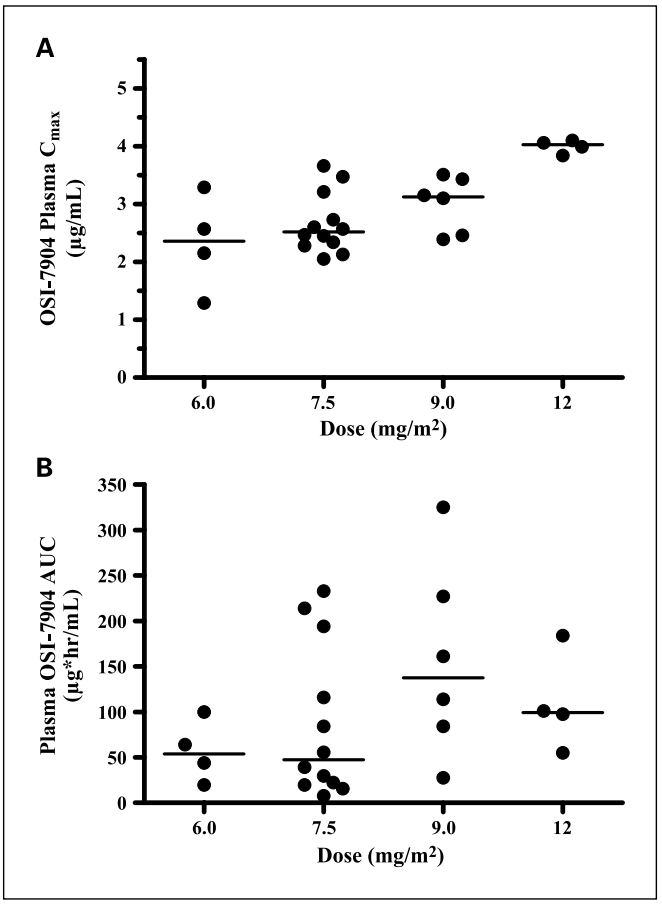

Fig. 1. Scatterplots showing the distributions of the following noncompartmental pharmacokinetic variable values reflecting OSI-7904 exposure: (*A*) C_{max} and (*B*) $AUC_{0-\infty}$ as a function of OSI-7904L dose.

individual agent (Table 4). The most common side effects were fatigue, nausea, vomiting, anorexia, and dermatologic toxicity. Particularly, grade 3 fatigue was a consistent DLT at CDDP/OSI-7904L 60/9 mg/m^2 but reversible by the fourth week following drug administration. Both patients who experienced this toxicity were able to continue CDDP/OSI-7904L treatment at 60/6 mg/m^2. Only 3 patients experienced skin-related toxicity of grade 3 severity and these occurred at doses of OSI-7904L ≥9 mg/m^2 (2 patients at 60/9 mg/m^2 and 1 at 60/12 mg/m^2). Stomatitis was experienced in the majority of cohorts but was of grade 3/4 severity in only 1 patient at 60/12 mg/m^2. Five patients received ≥6 courses of therapy without significant evidence of cumulative nonhematologic toxicity.

Pharmacokinetics. An analysis of CDDP and OSI-7904L pharmacokinetics was done to determine the effect of CDDP on the disposition of OSI-7904L. Plasma pharmacokinetics of OSI-7904 and unbound platinum were evaluated during course 1 in 26 and 24 patients, respectively. There was significant intersubject variability in total OSI-7904 C_{max} and $AUC_{0-\infty}$ (Fig. 1). Following C_{max}, the OSI-7904 plasma concentration generally declined in a multiexponential manner, with a median (range) terminal half-life ($t_{1/2}$) of 53.6 (33.1-90.4) h across all dose groups (Fig. 2). The mean noncompartmental pharmacokinetic variable estimates at each dose level are listed in Table 5. No deviation from dose proportionality was detected for C_{max} (P = 0.4624) or $AUC_{0-\infty}$ (P = 0.7128), although the small sample size and high interpatient variability limits power to detect differences. The median (range) clearance values following the 6, 7.5, 9, and 12 mg/m^2 dose were 115 (60-307), 163 (32.2-962), 67.5 (27.7-324), and 121 (65.2-218) mL/h/m^2, respectively. As was expected, due to liposome encapsulation and the high degree of protein binding of OSI-7904 (~96%), ultrafilterable OSI-7904 concentrations

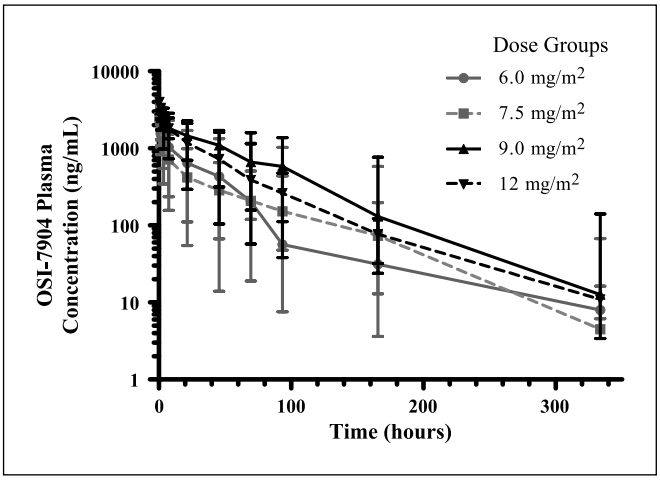

Fig. 2. Median (range) total OSI-7904 plasma concentration-time plots following a 6, 7.5, 9, or 12 mg/m^2 dose of OSI-7904L administered by a 30-min intravenous infusion.

were very low. The median (range) plasma C_{max} for ultrafilterable OSI-7904 following the 6, 7.5, 9, and 12 mg/m^2 dose was 3.19 (2.60-40.0), 1.71 (0.880-4.72), 1.59 (1.15-81.7), and 5.40 (2.30-13.7) ng/mL, respectively. These maximum ultrafilterable OSI-7904 plasma concentrations are 746- to 1,969-fold lower than the maximum total OSI-7904 plasma concentrations. Assuming an average 4% unbound fraction in plasma, the amount of extraliposomal drug is ~25-fold greater than the ultrafilterable drug. Even with this adjustment, these data suggest that the maximum extraliposomal plasma concentration is considerably less than the maximum encapsulated drug concentration.

Unbound platinum C_{max} increased in a dose-proportional manner. Following C_{max}, the plasma concentrations generally declined with time in a multiexponential manner (Fig. 3). The mean noncompartmental pharmacokinetic variable estimates at each dose level are listed in Table 6. The median (range) plasma $AUC_{0\text{-last}}$ for the 60 and 75 mg/m^2 dose cohorts was 2,794 (1,511-4,405) and 2,996 (1,935-5,152) ng/mL h, respectively, whereas the corresponding values for $AUC_{0\text{-}\infty}$ were 3,544 (1,569-32,459) and 3,595 (1,999-17,586) ng/mL h. The median plasma clearances for unbound platinum observed in this study of 11.0 L/h/m^2 (183 mL/min/m^2) and 13.6 L/h/m^2 (227 mL/min/m^2) following a 60 or 75 mg/m^2 dose of CDDP, respectively, are well within the reported range (31).

Tests for correlations between OSI-7904 pharmacokinetic variables $AUC_{0\text{-}\infty}$ and C_{max} with patient demographics, best response, and toxicity were done. No statistically significant relationships were observed between these pharmacokinetic variables and patient demographics, response, and maximum grade of any toxicity observed during the first course of therapy. Notably, no gender difference was observed for $AUC_{0\text{-}\infty}$ or C_{max}. Similarly, no statistically significant relationships were observed between these same pharmacokinetic variables and plasma homocysteine concentration or various laboratory variables. However, given the small number of patients studied and the fact that OSI-7904L was administered in combination with CDDP, results of these analyses should be viewed with caution.

Homocysteine and genotype biomarkers. Baseline homocysteine values were determined in 23 of the 27 patients who received therapy. Three of these patients had values above the upper limit of normal but did not report any grade 3 or 4 toxicity related to study therapy during course 1. There was no significant relationship between baseline homocysteine concentration and maximum grade of toxicity during course 1 (Spearman's rank correlation test, $P = 0.2390$, $R = 0.2557$). Although no relationship was observed between baseline homocysteine concentration and response or laboratory variables, there was a significant correlation between baseline homocysteine concentration and age (Spearman's rank correlation test, $P = 0.0207$, $R = 0.4791$).

A total of 12 patients provided samples for TS genotype analysis, with the majority having the 2/3 repeat pattern. Assessment of TS single nucleotide polymorphism was only possible in 5 patients, 2 of whom exhibited the G →C mutation in their triple repeat. Twelve patients provided samples for assessment of MTHFR gene polymorphism and 75% were heterozygous for the C→T transition at codon 677, with the other 25% being homozygous C/C. Five patients had DNA analyzed for the xeroderma pigmentosum group D polymorphism. Two patients were homozygous, one mutant (*Gln/Gln*) and one wild-type (*Lys/Lys*). Unfortunately, the limited data and range of dose levels tested preclude any useful assessment of relationship between these markers and outcome.

Antitumor activity. Three patients had confirmed partial responses. The first, a 45-year-old female with breast cancer, received 6 courses of CDDP/OSI-7904L at the 60/6 mg/m^2 dose

Table 5. Total OSI-7904: noncompartmental pharmacokinetic variables

Dose (mg/m^2)	No. patients	Median (range)						
		$t_{1/2}$ (h)	C_{max} (μg/mL)	$AUC_{0\text{-last}}$ (h μg/mL)	$AUC_{0\text{-}\infty}$ (h μg/mL)	$MRT_{0\text{-}\infty}$ (h)	Cl (mL/h/m^2)	Vd_{ss} (L/m^2)
6.0	4	65.1 (53.1-90.4)	2.36 (1.29-3.29)	53.5 (15.4-99.7)	54.0 (19.6-99.9)	65.1 (42.8-101)	115 (60.0-307)	5.45 (4.32-30.9)
7.5	12	54.2 (44.9-70.5)	2.52 (2.05-3.66)	47.3 (7.52-227)	47.5 (7.79-233)	62.3 (27.0-102)	163 (32.2-962)	10.1 (3.11-26.0)
9.0	6	55.7 (45.0-80.6)	3.13 (2.39-3.51)	137.0 (27.2-310)	137.0 (27.7-325)	65.5 (51.8-115)	67.5 (27.7-324)	4.39 (3.18-17.4)
12.0	4	39.9 (33.1-49.7)	4.03 (3.48-4.10)	91.5 (53.2-183)	99.4 (54.9-184)	48.7 (39.0-53.8)	121 (65.2-218)	6.01 (3.40-8.52)

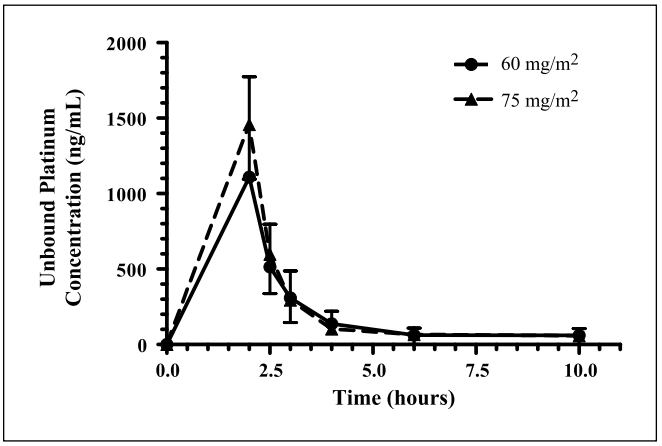

Fig. 3. Median (range) unbound platinum plasma concentration-time plots following a 60 or 75 mg/m^2 dose of CDDP administered by a 2-h intravenous infusion.

level. She was heavily pretreated, with prior CMF and AC regimens, epirubucin/docetaxel, capecitabine, and vinorelbine. The second patient, also a 45-year-old female with breast cancer, had a confirmed partial response following 5 courses of CDDP/OSI-7904L at 60/9 mg/m^2 and completed 12 courses of OSI-7904L (CDDP was discontinued after 9 courses). Her prior systemic treatment consisted of neoadjuvant AC/paclitaxel regimen and then docetaxel/capecitabine and epirubicin. This patient had also received radiotherapy. The third patient, a previously untreated 40-year-old male with metastatic gastric adenocarcinoma, received a total of 9 courses of CDDP/OSI-7904L at the recommended dose of 75/7.5 mg/m^2. In addition, 2 of 9 patients with stable disease after 3 courses of therapy, both with cholangiocarcinoma treated at CDDP/OSI-7904L 60/9 mg/m^2, had prolonged disease stabilization for 5 and 12 months. Both of these patients have been treated previously with antimetabolites (capecitabine and gemcitabine, respectively). The patient with the longest progression-free survival had symptomatic improvement and a 25% reduction in target lesions along with 70% CA 19.9 decline.

Discussion

TS is the rate-limiting enzyme in the biosynthesis of DNA, catalyzing the methylation of dUMP to dTMP, an essential precursor for DNA synthesis (1). The efficacy of inhibiting TS as anticancer therapy is well illustrated by 5-FU, originally designed almost 50 years ago as a fluorinated analogue of the naturally occurring base uracil (2). It remains widely used for the treatment of colorectal, pancreatic, breast, head and neck, and esophagogastric cancers. Nonetheless, 5-FU is far from being the ideal TS inhibitor because it is inefficiently converted to 5-fluoro-dUMP, whereas the remainder of the administered dose is converted to toxic metabolites (32). Therefore, much effort has focused on designing more effective TS inhibitors. In recent years, novel TS inhibitors of the fluoropyrimidine family (capecitabine, UFT, and S-1) or newer antifolates (raltitrexed and pemetrexed) have shown clinical activity in colon, breast, esophagogastric, and non-small cell lung carcinomas and mesothelioma (22, 24, 33–42).

TS expression is cell cycle dependent (43, 44). TS protein and TS activity levels are higher in proliferating cells than in nonproliferating cells and vary 14- to 24-fold between exponential and confluent cell populations (45–47). Accordingly, duration of exposure may be more critical than the peak concentration for effective TS inhibition (48). Clinical studies have indeed shown that continuous infusion allows for a higher total dose of 5-FU than can otherwise be safely administered by bolus, resulting in a more favorable toxicity profile, higher response rates, and a marginal but significant survival benefit (49–51). Similarly, OSI-7904 (GW1843U89) was more effective against the colon cancer cell line WiDr after long-term exposure (52). This evidence supported development of an encapsulated form of OSI-7904 with the potential to increase AUC and mean residence time (MRT).

The activity of OSI-7904L monotherapy has been evaluated in patients with adenocarcinoma of the stomach or gastroesophageal junction (15). CDDP is among the most active agents used to treat this malignancy, and this feasibility study of OSI-7904L combined with CDDP is a logical step in the clinical development of OSI-7904L, particularly in light of potential synergy suggested by preclinical studies. Specifically, this study sought to determine the maximum tolerated doses and toxicities of the combination, with an exploration of the effect of CDDP on OSI-7904L pharmacokinetics.

Although toxicity at the initial CDDP/OSI-7904L dose levels of 60/6 and 60/9 mg/m^2 was tolerable, unacceptable rates of severe toxicity occurred when the dose of OSI-7904L was increased to 12 mg/m^2. Particularly, 1 patient experienced rash, stomatitis, and neutropenic sepsis with multiple organ system failure and died despite aggressive supportive care. The second patient had grade 3 nausea, vomiting, and ileus. The CDDP/OSI-7904L 60/9 mg/m^2 cohort was thus expanded to 6 patients in total, with 2 of the additional patients reporting significant fatigue. Aside from fatigue, severe nonhematologic and hematologic toxicity were uncommon at this dose level. Due to the appearance of grade 3 fatigue as a DLT at 9 mg/m^2, dose escalation of OSI-7904L was discontinued; instead, CDDP was

Table 6. Unbound platinum: noncompartmental pharmacokinetic variables

Dose (mg/m^2)	No. patients	Median (range)						
		$t_{1/2}$ (h)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (h ng/mL)	$AUC_{0\text{-}\infty}$ (h ng/mL)	$MRT_{0\text{-}\infty}$ (h)	Cl (L/h/m^2)	Vd_{ss} (L/m^2)
60	14	8.22 (0.6-366)	1,184 (947-2,045)	2,794 (1,511-4,405)	3,543.5 (1,569-32,459)	7.16 (1.34-504)	11.0 (1.20-24.9)	79.6 (27.6-605)
75	10	7.88 (0.58-173)	1,458 (1,096-1,774)	2,996 (1,935-5,152)	3,595 (1,999-17,586)	5.52 (1.28-217)	13.6 (2.77-24.4)	75.6 (25.8-663)

escalated to 75 mg/m^2 after declaring the intermediate dose of CDDP/OSI-7904L 60/7.5 mg/m^2 safe. The dose level of CDDP/OSI-7904L 75/7.5 mg/m^2 was well tolerated without DLT in 10 patients. Of note, fatigue was present in 6 patients, but it was mild or moderate and manageable without dose reductions or interruptions. It was agreed that the toxicity profile and the evidence of cytotoxic effect at these doses excluded the need of exploring additional dose levels. Therefore, CDDP/OSI-7904L 75/7.5 mg/m^2 dose combination is the maximum tolerated dose and the recommended dose.

Noteworthy is that although skin-related toxicity occurred across most dose cohorts, this was at a lower incidence than that seen with single-agent studies of OSI-7904L (14, 15). The lower incidence might be attributed to the prophylactic effects of dexamethasone routinely administered as part of the antiemetic premedication regimen for CDDP as well as the fact that OSI-7904L was dosed below 9.0 mg/m^2 in the majority of patients (15).

As in the previous phase I trial, the current OSI-7904L pharmacokinetic data exhibit dose-proportional kinetics over the dose range tested, with substantial interpatient variability. Beutel et al. noted that the cause of this variability was differential clearance of OSI-7904L during the initial α phase and subdivided patients into two subsets (+α and -α). This might be related to differences in liposomal uptake by the reticuloendothelial system (14). The OSI-7904 drug carrier seems to perform appropriately with a good retention within the central compartment (Table 5, $MRT_{0-\infty}$), and minimal loss of drug as evidenced by the very low ultrafilterable OSI-7904 concentrations. The effect of the CDDP infusion on the pharmacokinetic variables for OSI-7904 is difficult to determine in this study due to the high interpatient variability seen with OSI-7904 and the fact that there was no OSI-7904L only component. However, the overall median (range) $t_{1/2}$ in this study for OSI-7904L of 53.6 (33.1-90.4) h is similar to the 53.7 (7.63-135) h in the study reported by Falk et al., in which OSI-7904L was administered as a single agent at 12 mg/m^2. Also, the median (range) plasma clearance of 121 (65.2-218) mL/h/m^2 (n = 4) for the 12 mg/m^2 group in our study is similar to the 98.8 (31.3-1244) mL/h/m^2 plasma clearance (n = 48) observed by Falk et al. (15). These data suggest prior administration of CDDP has little effect on OSI-7904L pharmacokinetics. Again, the effect of the OSI-7904L infusion on the pharmacokinetic variables of CDDP is difficult to determine in this study due to the high interpatient variability seen with both OSI-7904 and CDDP pharmacokinetics.

It is well known that toxicity is incompletely predicted by dose alone for antimetabolites (53). Molecular markers and pharmacogenomic profiling may help improve prediction of patients who will experience significant toxicity or clinical benefit from particular anticancer agents. For example, during the phase II development of pemetrexed, the plasma levels of homocysteine were found to correlate with serious toxicity, suggesting that some cancer patients with relative folate deficiency were at higher risk of toxicity. The introduction of nutritional supplementation led to a reduction in toxicity with apparent preservation of the anticancer activity (54). In our study, we included potentially relevant biomarker studies that might help identify patients at risk for increased toxicity or those most likely to respond to therapy. Biomarker information from a phase I study like the present must, however, be taken with caution. Certainly, the low expectations of antitumor activity as well as the heterogeneous patient population impose restrictions, but biomarkers can still provide us with hints to better develop this new drug combination. Patient numbers were limited in this study and the majority had homocysteine levels within normal limits. In contrast to pemetrexed, data from this and other studies of OSI-7904L suggest that baseline homocysteine is not a predictive factor in identifying those patients at risk of severe toxicity (15). The number of patients in whom TS genotyping was done was too small to assess the potential effect of TS genotype on outcome with CDDP/OSI-7904L, but the collection and assessment of the promoter polymorphism via a single blood sample seem to be feasible in a prospective phase I setting.

Antitumor response to CDDP/OSI-7904L was observed in patients with gastric adenocarcinoma and heavily pretreated breast cancer (including TS inhibitors). Additionally, 2 patients with previously treated cholangiocarcinoma had prolonged stable disease. In other studies, OSI-7904L monotherapy showed antitumor activity as first-line treatment in advanced esophagogastric cancer (15). Partial responses were also documented in a phase I study of oxaliplatin/OSI-7904L in patients with advanced colorectal cancer progressed to first-line therapy (including TS inhibitors; ref. 55). These findings suggest that platinum agent/OSI-7904L combinations might have activity in patients previously exposed to TS inhibitors and antimetabolites.

In conclusion, the recommended phase II doses of CDDP and OSI-7904L are 75 and 7.5 mg/m^2, respectively, once every 3 weeks. Pharmacokinetic interaction between the agents was not apparent. The observation of antitumor activity with this combination in patients with various malignancies is encouraging and provides a rationale for phase II disease-directed evaluation of the regimen.

Disclosure of Potential Conflicts of Interest

A.D. Ricart is employed by Pfizer. D.W. Drolet is employed by OSI Pharmaceuticals. C. Quaratino-Baker and J. Chick are employed by and have an ownership interest in OSI Pharmaceuticals. M.L. Rothenberg has received a commercial research grant from and has participated in expert testimony for OSI Pharmaceuticals. E.K. Rowinsky is employed by Imclone Systems.

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References

1. Carreras CW, Santi DV. The catalytic mechanism and structure of thymidylate synthase. Annu Rev Biochem 1995;64:721–62.
2. Heidelberger C, Chaudhuri NK, Danneberg P, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature 1957;179:663–6.
3. Banerjee D, Mayer-Kuckuk P, Capiaux G, Budak-Alpdogan T, Gorlick R, Bertino JR. Novel aspects of resistance to drugs targeted to dihydrofolate reductase and thymidylate synthase. Biochim Biophys Acta 2002;1587:164–73.
4. Berg RW, Ferguso PJ, DeMoor JM, Vincen MD,

Koropatnick J. The means to an end of tumor cell resistance to chemotherapeutic drugs targeting thymidylate synthase: shoot the messenger. Curr Drug Targets 2002;3:297–309.

5. Grem J. 5-Fluoropyrimidines. In: Chabner B, Longo D, editors. Cancer chemotherapy and biotherapy: principles and practice. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 185–254.

6. Duch DS, Banks S, Dev IK, et al. Biochemical and cellular pharmacology of 1843U89, a novel benzoquinazoline inhibitor of thymidylate synthase. Cancer Res 1993;53:810–8.

7. Dev IK, Dallas WS, Ferone R, Hanlon M, McKee DD, Yates BB. Mode of binding of folate analogs to thymidylate synthase. Evidence for two asymmetric but interactive substrate binding sites. J Biol Chem 1994; 269:1873–82.

8. Hanlon MH, Ferone R. *In vitro* uptake, anabolism, and cellular retention of 1843U89 and other benzoquinazoline inhibitors of thymidylate synthase. Cancer Res 1996;56:3301–6.

9. Schwartz G, Johnson TR, Goetz A, et al. A phase I and pharmacokinetic study of 1843U89, a noncompetitive inhibitor of thymidylate synthase, in patients with advanced solid malignancies. Clin Cancer Res 2001;7:1901–11.

10. Desjardins J, Emerson DL, Colagiovanni DB, Abbott E, Brown EN, Drolet DW. Pharmacokinetics, safety, and efficacy of a liposome encapsulated thymidylate synthase inhibitor, OSI-7904L [(*S*)-2-[5-[(1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl]amino-1-oxo-2-isoindolynl]-glutaric acid] in mice. J Pharmacol Exp Ther 2004;309:894–902.

11. Desjardins JP, Abbott EA, Emerson DL, et al. Biodistribution of NX211, liposomal lurtotecan, in tumor-bearing mice. Anticancer Drugs 2001;12:235–45.

12. Emerson D, Abbott B, Brown E., et al. *In vivo* antitumor activity and biodistribution of GS 7904L, a low clearance liposomal formulation of a novel thymidylate synthase inhibitor [abstract 1060]. Proc Am Assoc Cancer Res 2002;43:211–2.

13. Emerson D, Abbott B, Ashvar C, et al. GS 7904L, a low clearance, liposomal formulation of the thymidylate synthase inhibitor GS 7904 demonstrates enhanced pharmacokinetics and improved antitumor efficacy [abstract 448]. Proc AACR NCI EORTC Conference 7 2001;92.

14. Beutel G, Glen H, Schoffski P, et al. Phase I study of OSI-7904L, a novel liposomal thymidylate synthase inhibitor in patients with refractory solid tumors. Clin Cancer Res 2005;11:5487–95.

15. Falk S, Anthoney A, Eatock M, et al. Multicentre phase II pharmacokinetic and pharmacodynamic study of OSI-7904L in previously untreated patients with advanced gastric or gastroesophageal junction adenocarcinoma. Br J Cancer 2006;95:450–6.

16. Niyikiza C, Baker SD, Seitz DE, et al. Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. Mol Cancer Ther 2002;1:545–52.

17. Diasio R. Pharmacogenetics. In: Chabner B, Longo D, editors. Cancer chemotherapy and biotherapy: principles and practice. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 998–1011.

18. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999;286:487–91.

19. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Lenz HJ. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis 2002; 17:46–9.

20. Winski S, Srebernak M, Tomkinson B, Emerson D. *In vivo* efficacy of the novel thymidylate synthase inhibitor, OSI-7904L, in combination with cisplatin or oxaliplatin in xenograft models [abstract 5381]. Proc Am Assoc Cancer Res 2004;45.

21. Al Sarraf M, LeBlanc M, Giri PG, et al. Chemoradiotherapy versus radiotherapy in patients with advanced nasopharyngeal cancer: phase III randomized Intergroup Study 0099. J Clin Oncol 1998;16:1310–7.

22. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003; 21: 2636–44.

23. Ichinose Y, Yoshimori K, Sakai H, et al. S-1 plus cisplatin combination chemotherapy in patients with advanced non-small cell lung cancer: a multi-institutional phase II trial. Clin Cancer Res 2004;10: 7860–4.

24. van Meerbeeck JP, Gaafar R, Manegold C, et al. Randomized phase III study of cisplatin with or without raltitrexed in patients with malignant pleural mesothelioma: an intergroup study of the European Organisation for Research and Treatment of Cancer Lung Cancer Group and the National Cancer Institute of Canada. J Clin Oncol 2005;23:6881–9.

25. Ajani JA, Lee FC, Singh DA, et al. Multicenter phase II trial of S-1 plus cisplatin in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma. J Clin Oncol 2006;24:663–7.

26. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.

27. Horie N, Aiba H, Oguro K, Hojo H, Takeishi K. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 1995;20:191–7.

28. Kawakami K, Watanabe G. Identification and functional analysis of single nucleotide polymorphism in the tandem repeat sequence of thymidylate synthase gene. Cancer Res 2003;63:6004–7.

29. Mandola MV, Stoehlmacher J, Muller-Weeks S, et al. A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res 2003;63:2898–904.

30. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A 1999;96:12810–5.

31. Noe D. Noncompartmental pharmacokinetic analysis. In: Grochow L, Ames M, editors. A clinician's guide to chemotherapy: pharmacokinetics and pharmacodynamics. Baltimore: Williams & Wilkins; 1998. p. 515–30.

32. Gmeiner WH. Novel chemical strategies for thymidylate synthase inhibition. Curr Med Chem 2005;12: 191–202.

33. Blum JL, Jones SE, Buzdar AU, et al. Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. J Clin Oncol 1999; 17: 485–93.

34. O'Shaughnessy J, Miles D, Vukelja S, et al. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol 2002;20:2812–23.

35. Van Cutsem E, Twelves C, Cassidy J, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. J Clin Oncol 2001;19:4097–106.

36. Douillard JY, Hoff PM, Skillings JR, et al. Multicenter phase III study of uracil/tegafur and oral leucovorin versus fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2002;20:3605–16.

37. Carmichael J, Popiela T, Radstone D, et al. Randomized comparative study of tegafur/uracil and oral leucovorin versus parenteral fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2002;20:3617–27.

38. Schoffski P. The modulated oral fluoropyrimidine prodrug S-1, and its use in gastrointestinal cancer and other solid tumors. Anticancer Drugs 2004;15: 85–106.

39. Cunningham D, Zalcberg JR, Rath U, et al. "Tomudex" Colorectal Cancer Study Group. Final results of a randomised trial comparing "Tomudex" (raltitrexed) with 5-fluorouracil plus leucovorin in advanced colorectal cancer. Ann Oncol 1996;7:961–5.

40. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22: 1589–97.

41. Cunningham D, Starling N, Rao S, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2008;358:36–46.

42. Sakuramoto S, Sasako M, Yamaguchi T, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 2007;357: 1810–20.

43. Johnson LF. Posttranscriptional regulation of thymidylate synthase gene expression. J Cell Biochem 1994;54:387–92.

44. Keyomarsi K, Samet J, Molnar G, Pardee AB. The thymidylate synthase inhibitor, ICI D1694, overcomes translational detainment of the enzyme. J Biol Chem 1993;268:15142–9.

45. Pestalozzi BC, McGinn CJ, Kinsella TJ, et al. Increased thymidylate synthase protein levels are principally associated with proliferation but not cell cycle phase in asynchronous human cancer cells. Br J Cancer 1995;71:1151–7.

46. Peters GJ, Smitskamp-Wilms E, Smid K, Pinedo HM, Jansen G. Determinants of activity of the antifolate thymidylate synthase inhibitors Tomudex (ZD1694) and GW1843U89 against mono- and multilayered colon cancer cell lines under folate-restricted conditions. Cancer Res 1999;59:5529–35.

47. Derenzini M, Montanaro L, Trere D, et al. Thymidylate synthase protein expression and activity are related to the cell proliferation rate in human cancer cell lines. Mol Pathol 2002;55:310–4.

48. Kaye SB. Clinical drug resistance: the role of factors other than P-glycoprotein. Am J Med 1995;99: 40–4S.

49. Schmoll HJ, Buchele T, Grothey A, Dempke W. Where do we stand with 5-fluorouracil? Semin Oncol 1999;26:589–605.

50. Meta-Analysis Group in Cancer. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. J Clin Oncol 1998;16:301–8.

51. Meta-Analysis Group in Cancer. Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. J Clin Oncol 1998;16:3537–41.

52. Banks SD, Waters KA, Barrett LL, Dickerson S, Pendergast W, Smith GK. Destruction of WiDr multicellular tumor spheroids with the novel thymidylate synthase inhibitor 1843U89 at physiological thymidine concentrations. Cancer Chemother Pharmacol 1994;33:455–9.

53. Seymour L, Eisenhauer E. A review of dose-limiting events in phase I trials: antimetabolites show unpredictable relationships between dose and toxicity. Cancer Chemother Pharmacol 2001;47:2–10.

54. Calvert H. Folate status and the safety profile of antifolates. Semin Oncol 2002;29:3–7.

55. Clamp AR, Schoffski P, Valle JW, et al. A phase I and pharmacokinetic study of OSI-7904L, a liposomal thymidylate synthase inhibitor in combination with oxaliplatin in patients with advanced colorectal cancer. Cancer Chemother Pharmacol 2008;61: 579–85.

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Phase I, Pharmacokinetic and Biological Correlative Study of OSI-7904L, a Novel Liposomal Thymidylate Synthase Inhibitor, and Cisplatin in Patients with Solid Tumors

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