Phase I Study of OSI-7904L, a Novel Liposomal Thymidylate Synthase Inhibitor in Patients with Refractory Solid Tumors

Gernot Beutel,1 Hilary Glen,2 Patrick Schoffski,1 Jon Chick,3 Stan Gill,4 James Cassidy,2 and Chris Twelves2,5

Abstract

Purpose: OSI-7904L is a liposomal formulation of a potent noncompetitive thymidylate synthase inhibitor (TSI) that does not require polyglutamation for activity. This phase I study was done to establish the safety, tolerability, maximum tolerated dose, recommended dose, and pharmacokinetics of OSI-7904L in patients with advanced solid tumors refractory to standard therapy.

Design: OSI-7904L was given as a 30-minute i.v. infusion every 21 days to 31 patients at eight dose levels from 0.4 to 15.0 mg/m², using three patients per dose level, up to 10 patients at the recommended dose. Baseline plasma homocysteine and 2′-deoxyuridine and genotype polymorphism were measured as potential predictors of biological activity.

Results: Minimal toxicity was reported up to 9.6 mg/m², but dose-limiting toxicity was seen in both patients at 15 mg/m² including stomatitis, fatigue, tachyarrhythmia, rash and hand-foot syndrome, diarrhea, and fatal neutropenic sepsis. Other toxicity such as nausea and vomiting was mild or moderate. This resulted in the investigation of an intermediate dose level of 12 mg/m², identified as the recommended dose for phase II studies. Prolonged disease stabilization was reported in 11 of 31 heavily pretreated patients. Pharmacokinetic data indicate that this liposomal formulation alters the disposition properties of the parent drug resulting in a prolonged plasma residence time.

Conclusions: OSI-7904L given as a 30-minute i.v. infusion every 21 days is feasible and well tolerated at the recommended phase II dose of 12 mg/m². The main toxicities are rash, pruritus, lethargy, stomatitis, and myelosuppression. Observed toxicities were predictable and characteristic for TSIs.

Thymidylate synthase is the rate-limiting enzyme in the biosynthesis of DNA catalyzing the methylation of dUMP to TMP, an essential precursor for DNA synthesis (1). Because thymidine nucleotides are used exclusively for synthesis of DNA, thymidylate synthase is an important target for anticancer therapy. Thymidylate synthase inhibitors (TSI) such as members of the fluoropyrimidine family [5-fluorouracil (5-FU), capcitabine, UFT, and S-1 or newer antifolates such as raltitrexed and pemetrexed] have shown efficacy in colon, breast, mesothelioma, and non–small cell lung carcinomas (2–10). Resistance to available TSIs occurs via several mechanisms, including (i) increased gene expression resulting in elevated enzyme levels, (ii) reduced polyglutamation due to decreased activity or lower levels of folypolyglutamate synthase, or (iii) reduction of fluoropyrimidine drug levels by increased metabolism by dihydropyrimidine dehydrogenase. There is therefore a strong rationale to develop new potent TSIs that circumvent these mechanisms of resistance.

OSI-7904L is a liposomal formulation of OSI-7904 with a high drug-to-lipid ratio resulting in a prolonged plasma residence time. OSI-7904L was given as a 30-minute i.v. infusion every 21 days is feasible and well tolerated at the recommended phase II dose of 12 mg/m². The main toxicities are rash, pruritus, lethargy, stomatitis, and myelosuppression. Observed toxicities were predictable and characteristic for TSIs.
with folic acid. No unexpected organ toxicity was observed and dose-limiting toxicities (DLT) included neutropenia, pancytopenia, fever, mucositis, and rash. There were no objective responses (14).

OSI-7904 has since been encapsulated within a liposome and designated OSI-7904L. The liposome dispersion consists of small, unilamellar vesicles ranging in diameter from 20 to 80 nm containing the drug substance OSI-7904 within their aqueous cores. The vesicle membranes consist of hydrogenated soy phosphatidylcholine and cholesterol. OSI-7904L has shown enhanced efficacy, compared with either parent drug or 5-FU, in a variety of murine xenograft models and exhibited dose-dependent antitumor activity and durable cures in tumor-bearing mice (15–17). Liposomal encapsulation also altered the pharmacokinetics compared with OSI-7904 in mice (17). Decreased clearance and volume of distribution for OSI-7904L caused substantial increases in plasma exposure as measured by increased maximum serum concentration (Cmax), area under the concentration-time curve, and mean residence time (15, 17). As has been shown with other liposome encapsulated drugs, OSI-7904L may enhance the tumor localization of active drug and simplify the schedule of administration (17–19).

A number of laboratory approaches have been investigated to better define specific patient populations that may benefit from TSI therapy or those that may be predisposed to increased toxicity. First, the promoter polymorphism in the thymidylate synthase gene is a 28-bp tandem repeat DNA present in two or three copies. The frequency of the 2/2, 2/3, and 3/3 alleles is ~19%, 43%, and 38% respectively, in the Caucasian population (20). Park et al. have studied the relationship of thymidylate synthase genotype with outcome in patients with colorectal cancer who received capcitabine therapy (21). In a small retrospective pilot study, they showed that patients with a 2/2 polymorphism may have superior outcome to those patients with 2/3 or 3/3 genotype. Second, assessment of baseline homocysteine accurately identifies those patients at risk of increased toxicity with the multitargeted antifolate, pemetrexed (22). Although supplementation with folic acid and vitamin B12 has become routine with pemetrexed, preclinical studies with OSI-7904L have shown that toxicity was not adversely affected by a 50% reduction in serum folate concentration or an elevation in plasma homocysteine concentrations. Third, inhibition of thymidylate synthase increases intracellular levels of dUMP, which can be dephosphorylated to 2'-deoxyuridine (2'-dU). The efflux of intracellular 2'-dU leads to an increase in plasma 2'-dU (23). Thus, elevation of plasma 2'-deoxyuridine has been used as a surrogate marker of thymidylate synthase inhibition with a range of 5-FU schedules as well as the antifolates raltitrexed and ZD9331 (24, 25). Taken together these studies have the potential to optimize outcomes with TSIs.

The objectives of this phase I study were to evaluate the safety and tolerability of OSI-7904L by establishing the maximum tolerated dose (MTD) and recommended phase II dose when given on day 1 every 21 days. Secondary objectives were to determine the pharmacokinetic profile of this liposomally encapsulated agent and to evaluate preliminary evidence of antitumor activity in patients with advanced solid tumors refractory to standard therapy.

### Patients and Methods

#### Study design.

This was an open-label, nonrandomized, non-comparative, dose escalation study and complied with the ethical principles of Good Clinical Practice in accordance with the Declaration of Helsinki. The study was approved by medical ethics committees in both institutions; all patients gave written informed consent for the clinical study and separately for the genotype study before trial entry.

#### Patient selection.

Patients with documented advanced solid tumors refractory to standard systemic therapy or for which no effective treatment was available were eligible for the trial. Other eligibility criteria included age ≥18 years, Eastern Cooperative Oncology Group performance status ≤2, estimated life expectancy ≥3 months, no prior hormonal or radiation therapy 28 days before study entry, no other anticancer therapy within 21 days before study entry, adequate hematopoietic (absolute peripheral granulocyte count ≥1.5 x 10^9/L and platelet count ≥100 x 10^9/L), hepatic (bilirubin ≤1.25 times upper limit of normal) and serum aspartate aminotransferase and alanine aminotransferase ≤2.5 times upper limit of normal), and renal (serum creatinine ≤1.5 times upper limit of normal) function. Concomitant administration of folic acid or vitamin supplementation was prohibited. Patients with symptomatic or unstable brain metastases or a history of any disease significantly affecting gastrointestinal function were not enrolled.

#### Treatment and dose escalation.

OSI-7904L was supplied by OSI Pharmaceuticals, Inc. (Oxford, United Kingdom) 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-minute i.v. infusion every 3 weeks via peripheral line or venous port catheter on an outpatient basis. Given the increased potency observed with the liposomal formulation, a conservative starting dose of 0.4 mg/m^2, equivalent to 1:100 of the MTD observed in dogs, was selected. Dose escalation was based on the safety assessment of the previous treatment cohort. Toxicities were assessed according to the National Cancer Institute Common Toxicity Criteria version 2.0. If toxicity was limited to grade 0 or 1 then escalating doses up to 100% were considered, whereas the occurrence of a grade 2 event would permit increases only from 30% to 50%; the occurrence of grade 3 toxicity would limit any increase to 20% to 29%. A minimum of three patients were enrolled per dose level. Once DLT was observed in one out of three patients, up to three additional patients were treated at that dose level. The MTD was defined as the dose at which at least two of six patients experienced a DLT. The recommended dose was then determined to be the highest safe dose below the MTD. Once the recommended dose had been identified, a total of up to 10 patients were to be treated to more accurately assess potential interpatient variability in safety and tolerability.

DLTs were considered during the first treatment cycle and defined as absolute neutrophil count < 0.5 x 10^9/L for at least 7 days, febrile neutropenia ≥ grade 3 (absolute neutrophil count < 1.0 x 10^9/L associated with fever ≥38.5°C), platelet count ≤25 x 10^9/L or a bleeding episode requiring platelet transfusion. Nonhematologic DLTs comprised aspartate aminotransferase and/or alanine aminotransferase ≥ grade 3 for at least 7 days or grade 4 aspartate aminotransferase and/or alanine aminotransferase, cardiac toxicity ≥ grade 2, and any other grade 3 or 4 toxicity representing at least a two-grade increase from baseline (excluding the above, alopecia or unpremedicated or inadequately treated nausea, vomiting, diarrhea, rash, or infusion-related reaction). Intrapatient dose escalation was not permitted. Treatment was repeated every 21 days, provided toxicities had resolved satisfactorily.

#### Study investigations.

The baseline assessment included a complete medical history, physical examination, vital signs, Eastern Cooperative Oncology Group performance status, differential blood count, serum biochemistry (sodium, potassium, creatinine, urea, total bilirubin,
alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, γ-GT, uric acid, lactate dehydrogenase), and urinalysis. CT scans were used for assessment of the baseline tumor burden. During the first cycle, weekly evaluations included physical exam, vital signs, performance status, serum biochemistry, and urinalysis, a complete blood count was done twice weekly. Response assessment was carried out every three cycles according to the Response Evaluation Criteria in Solid Tumor (26). Patients showing disease progression were taken off study.

Sample collection and analysis: Plasma pharmacokinetics. A total of 15 blood samples were collected during cycle 1 at the following time points: predose, 0.5, 1, 1.5, 2.5, 4, 6, 8, 24, 48, 72, 96 and 168 hours, then 15 and 22 days after the start of the infusion. Following an amendment during the expansion of the recommended dose, an optional, limited sampling schedule was added in cycle 2 with time points at predose, 0.5, 4, 8, 24, 48, 72, 96 and 168 hours, then 15 and 22 days after the start of the infusion. Blood samples were collected in vials containing EDTA and cooled immediately on ice then centrifuged at 1500-2000 × g for 10 minutes under refrigeration within 30 minutes of collection. The supernatant was split into two equal aliquots, transferred into labeled cryovials and stored at −20°C. Samples were shipped to MDS Pharma Services St-Laurent (Montreal, Quebec, Canada) and total concentrations of OSI-7904 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 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 using a mobile phase of 75:25 (v/v) methanol/0.088% formic acid in water. Mass spectral detection was done with a selective reaction-monitoring mode. For analysis, relative 2'-dU levels compared with baseline 2'-dU levels were plotted versus time and maximum relative plasma concentration and length of time of elevation were determined.

Plasma homocysteine levels. A blood sample (~5 mL) was drawn from all patients before the OSI-7904L infusion on day 1 of cycle 1. In addition, further samples could be collected every other cycle in patients at the recommended dose. Each blood sample was collected into an EDTA tube, immediately cooled on ice, and centrifuged at 1,500 × g for 10 minutes under refrigeration within 30 minutes of collection. 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 Immunoassay and a fluorescence polarization immunoassay (27).

Thymidylate synthase genotype. A blood sample of 7 to 10 mL was collected into an EDTA tube and inverted several times to mix with the anticoagulant immediately after collection. Thymidylate synthase genotype samples were stored at 4°C until DNA extraction and analysis at the Department of Human Genetics at the University of Chicago. DNA was isolated using the Puregene kit from Gentra systems and genotyping done using PCR amplification, using primers flanking the repeat region. Amplified products were then sized on an agarose gel (28).

Results

A total of 32 patients were enrolled into the study at two institutions. Thirty-one patients were evaluable for toxicity and response; the remaining patient was not treated due to clinical deterioration. All patients had received extensive prior therapy with a median of three prior chemotherapy regimens per patient (range, 1-7). The majority of patients had colorectal cancer (69%) and were either asymptomatic or had only mild symptoms. Patient characteristics are listed in Table 1.

A total of 104 cycles of OSI-7904L were given, with a median of three per patient (range, 1-14). Eight dose levels were investigated, ranging from 0.4 to 15.0 mg/m² and the dose increments ranged from 50% to 100% (Table 2).

Toxicity. Minimal toxicity was reported up to 9.6 mg/m² but DLTs were seen in both patients treated at 15 mg/m². One patient experienced grade 4 stomatitis, fatigue, tachycardia, with grade 3 rash and hand-foot syndrome, diarrhea, and neutropenic sepsis. Despite extensive supportive care including i.v. antibiotics, antifungals, granulocyte colony-stimulating factor, blood and platelet transfusions, and parental opiates, this proved fatal on day 14 of cycle 1 due to a combination of neutropenic sepsis (group D streptococcus isolated from blood cultures) and atrial fibrillation. The second patient reported grade 3 fatigue and went on to receive two further cycles of OSI-7904L at a reduced dose of 9.6 mg/m².

Dose escalation was halted at 15 mg/m² and a total of 10 patients were treated at the intermediate dose level of 12 mg/m². A single patient reported DLT at this dose level, experiencing fatigue, rash, and pruritus (all grade 3). This
patient received two further cycles at a reduced dose of 9.6 mg/m². Other nonhematologic toxicities were mild or moderate and included rash, stomatitis, fatigue, diarrhea, nausea, and vomiting. Although none of these precluded drug administration, the incidence of fatigue increased from mild to moderate in some patients who received five or six cycles of OSI-7904L. Comparison of patients receiving 12 mg/m² of OSI-7904L. Comparison of Table 2. Dose levels, number of patients entered, and DLTs

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. patients treated</th>
<th>Total no. cycles</th>
<th>Range per patient</th>
<th>No. patients with DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>3</td>
<td>7</td>
<td>2-3</td>
<td>0/3</td>
</tr>
<tr>
<td>0.8</td>
<td>3</td>
<td>8</td>
<td>2-3</td>
<td>0/3</td>
</tr>
<tr>
<td>1.6</td>
<td>3</td>
<td>8</td>
<td>2-3</td>
<td>0/3</td>
</tr>
<tr>
<td>3.2</td>
<td>3</td>
<td>8</td>
<td>2-3</td>
<td>0/3</td>
</tr>
<tr>
<td>6.4</td>
<td>4</td>
<td>26</td>
<td>2-14</td>
<td>0/4</td>
</tr>
<tr>
<td>9.6</td>
<td>3</td>
<td>13*</td>
<td>3 (2*)</td>
<td>0/3</td>
</tr>
<tr>
<td>15.0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2/2</td>
</tr>
<tr>
<td>12.0</td>
<td>10</td>
<td>32</td>
<td>1-6</td>
<td>1/10</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>104</td>
<td>1-14</td>
<td>—</td>
</tr>
</tbody>
</table>

* Includes four cycles (two each) given to patients 22 and 25 at reduced dose of 9.6 due to DLT at their initial dose levels of 15 and 12, respectively.
between 2- and 18-fold. The 18-fold range in plasma clearance was observed in the 12 mg/m² dose cohort (n = 10). Comparison of terminal half-lives across all dose groups showed relatively little variability with a mean (SD) of 74.3 (28.9) hours.

Differential clearance of OSI-7904 from the plasma during the initial α phase was noted. Patients could be subdivided into two subpopulations, those who eliminated more (≥60%) or less (<60%) of the dose in the α phase (+α and −α, respectively). C_{max} was dose proportional across both subpopulations, whereas areas under the concentration-time curve were dose proportional within each subpopulation (Fig. 3A). Across all dose cohorts, the percentage of OSI-7904 cleared in the α phase showed a bimodal distribution (Fig. 3B). The constants of proportionality were however markedly different from one another.

### Table 3. OSI-7904L hematologic and nonhematologic toxicity (includes those events occurring in ≥10% of patients or >CTC grade 2 across all cycles)

<table>
<thead>
<tr>
<th>Worst CTC grade*/patient/dose level</th>
<th>6.4 mg/m² (n = 4)</th>
<th>9.6 mg/m² (n = 3)</th>
<th>15.0 mg/m² (n = 2)</th>
<th>12.0 mg/m² (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neutropenic sepsis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue/lethargy</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Skin related †</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Tachycardia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exertional dyspnea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrexia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Numbers in bold denote DLTs (i.e., occurred in cycle 1).
Abbreviation: CTC, Common Toxicity Criteria.
† National Cancer Institute CTC version 2.0.
‡ No significant hematologic toxicity and only limited nonhematologic toxicity reported at 0.4, 0.8, 1.6, or 3.2 mg/m².
§ Dermatologic toxicity included (patients may have reported a combination of symptoms) pruritus, rash, exanthem, erythema, Palmar-plantar erythrodysesthesiasyndrome, skin desquamation, skin lesion, alopecia, dry skin, pain of skin, folliculitis, cellulitis, and vasculitis.

Fig. 1. Skin rash on lower leg on day 12 after receiving 12 mg/m² OSI-7904L.
Intrapatient variability in OSI-7904 plasma pharmacokinetics was evaluated by comparison of cycle 1 and cycle 2 data obtained from two patients in the 12 mg/m² dose cohort. Comparison of plasma concentrations and other kinetic variables showed no substantial differences, although limited conclusions should be drawn due to the small sample size (data not shown). Complete 72-hour urine data were available from 26 patients. The mean (SD) percentage of the dose recovered in urine over 72 hours was 4.26% (3.47%).

Generally, plasma 2'-dU levels increased compared with baseline concentration. Plasma levels were elevated 2- to 4-fold for between 4 and 7 days, indicating inhibition of the target enzyme by OSI-7904L (Fig. 4). However, over the 3.2 to 15 mg/m² dose range neither the magnitude nor duration of elevation seemed proportional to dose. Baseline homocysteine was measured in all patients as a marker of folate status. However, the majority of patients had levels within normal limits and no correlation with toxicity was apparent (data not shown). Analysis of thymidylate synthase genotype was done in 31 patients: nine patients were 2/2, 17 patients 2/3, and five patients 3/3. No correlation with either toxicity or disease stabilization was detected.

### Discussion

This is the first phase I, dose escalation study of the liposomal formulation of the potent TSI OSI-7904. Administered to 31 patients across eight dose levels on an every 3-week schedule, OSI-7904L was generally well tolerated and the recommended dose was established as 12 mg/m². Toxicities were those characteristic of TSIs, and even with several patients receiving in excess of three cycles, there was little evidence of cumulative toxicity. The liposomal encapsulation increased exposure compared with parent drug. Whereas there were no obvious pharmacokinetic/pharmacodynamic correlations in the biomarkers used in this study, their effect will be assessed further in future studies with OSI-7904L.

The original TSI, 5-FU, is among the most widely used cytotoxic agents currently available although its effectiveness is limited by acquired and/or intrinsic resistance due to elevation of thymidylate synthase levels. In addition, there are several practical issues because longer, inconvenient infusion schedules seem to offer improved efficacy with reduced toxicity (29, 30).

Although oral fluoropyrimidines such as capecitabine, UFT, and S-1 achieve prolonged exposure of 5-FU without the need for infusion pumps, continuous oral dosing may not always be possible and elevation of thymidylate synthase remains a potential issue in terms of resistance (2–7). In addition to oral TSIs, two i.v. antifolates (ralitrexed and pemetrexed) have been developed. However, their dependence on polyglutamation for activity may also be a mechanism for resistance in tumors that have down regulated folypolyglutamate synthase (31). Whereas pemetrexed (in combination with cisplatin) is set to become the standard of care for patients with mesothelioma, the clinical utility of raltitrexed has been limited by concerns over toxicity (10, 32). Thus, a potent TSI with prolonged exposure and activity independent of polyglutamation status remains an attractive target.

OSI-7904L is the liposomal formulation of the TSI previously investigated as 1843U89 (14). Whereas a conservative starting dose of 1:100 the MTD in dogs meant that eight dose levels were required to reach MTD and recommended dose, the flexible escalation scheme permitting multiple 100% dose increments in the absence of toxicity and the introduction of smaller increases on identification of toxicity, meant that the early dose levels were completed rapidly without affecting patient safety. The toxicity profile was predictable, manageable and characteristic of other TSIs, including dermatologic

### Table 4. OSI-7904 median (minimum, maximum) pharmacokinetic variables following the cycle 1 administration of OSI-7904L (noncompartmental analysis)

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>n</th>
<th>C_{max} (ng/mL)</th>
<th>T_{max} (h)</th>
<th>AUC_{0-1} (h*ng/mL)</th>
<th>Cl (mL/h/m²)</th>
<th>t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>3</td>
<td>159 (118, 160)</td>
<td>0.65 (0.53, 1.58)</td>
<td>3,490 (1,610, 13,900)</td>
<td>115 (28.8, 248)</td>
<td>82.8 (59.7, 86.4)</td>
</tr>
<tr>
<td>0.8</td>
<td>3</td>
<td>488 (251, 634)</td>
<td>0.53 (0.53, 0.55)</td>
<td>6,540 (5,600, 20,100)</td>
<td>122 (39.9, 143)</td>
<td>40.6 (35.3, 118)</td>
</tr>
<tr>
<td>1.6</td>
<td>3</td>
<td>792 (613, 968)</td>
<td>0.53 (0.47, 1.6)</td>
<td>13,300 (6,530, 48,800)</td>
<td>121 (32.1, 245)</td>
<td>49.9 (44.7, 160)</td>
</tr>
<tr>
<td>3.2</td>
<td>3</td>
<td>1,280 (1,140, 1,550)</td>
<td>0.50 (0.50, 0.57)</td>
<td>10,200 (10,000, 88,300)</td>
<td>314 (36.2, 319)</td>
<td>95.4 (62.3, 107)</td>
</tr>
<tr>
<td>6.4</td>
<td>3</td>
<td>2,770 (2,670, 3,150)</td>
<td>0.55 (0.52, 0.55)</td>
<td>135,000 (37,500, 194,000)</td>
<td>47.5 (33.0, 171)</td>
<td>53.5 (53.2, 57.7)</td>
</tr>
<tr>
<td>9.6</td>
<td>3</td>
<td>2,470 (2,310, 3,000)</td>
<td>0.62 (0.48, 1.0)</td>
<td>91,100 (25,900, 97,600)</td>
<td>105 (98.3, 370)</td>
<td>50.6 (49.7, 71.9)</td>
</tr>
<tr>
<td>12.0</td>
<td>10</td>
<td>3,600 (2,130, 4,500)</td>
<td>0.53 (0.43, 0.65)</td>
<td>26,600 (13,900, 256,000)</td>
<td>467 (46.9, 865)</td>
<td>76.8 (49.8, 136)</td>
</tr>
<tr>
<td>15.0</td>
<td>2</td>
<td>6,770 (6,420, 7,110)</td>
<td>0.54 (0.50, 0.58)</td>
<td>259,000 (183,000, 334,000)</td>
<td>63.4 (45.0, 81.8)</td>
<td>65.4 (57.2, 73.5)</td>
</tr>
</tbody>
</table>

Abbreviation: AUC, area under the concentration-time curve.
manifestations, fatigue, gastrointestinal toxicity (stomatitis, diarrhea, nausea and vomiting), and myelosuppression (neutropenia and thrombocytopenia). Whereas some of these toxicities were severe at the MTD, the majority were mild or moderate, especially at 9.6 and 12 mg/m². Indeed, two of the three patients experiencing DLT were able to tolerate further OSI-7904L following dose reduction to 9.6 mg/m².

Rash and/or pruritus were the major skin toxicities observed with OSI-7904L. The majority of cases were of mild or moderate severity, usually occurring on first exposure to OSI-7904L and predictive of similar symptoms in subsequent cycles without precluding administration of multiple cycles in several patients. The more severe occurrences of rash did not respond well to acute treatment with corticosteroids or antihistamines but symptoms did resolve post study or with treatment delays. Similar toxicities with pemetrexed and ZD9331 are alleviated by a steroid premedication regimen (e.g., oral dexamethasone 3 or 4 mg twice a day around the day of cytotoxic administration; refs. 33, 34). Prophylaxis with a similar regimen may be considered in future studies with OSI-7904L. There was, however, only a low incidence (1 of 31) of the palmar-plantar erythrodysesthesia that has been observed with other TSIs with prolonged activity, especially continuous infusional 5-FU and oral capecitabine (2–4).

Initial pharmacokinetic data from this trial show that in comparison with the nonliposomal drug, liposomally encapsulated OSI-7904 exhibits a long plasma circulation time and an almost 10-fold increase in terminal half-life. Comparison of the pharmacokinetics of nonliposomal OSI-7904 with OSI-7904L showed a substantial decrease in clearance associated with the liposome (14). On average, the patients in the +α phase subgroup displayed an 8.5-fold slower rate of clearance compared with patients receiving a nonliposomal 2-minute i.v. infusion. Patients in the subgroup without an α phase showed a 52-fold slower rate than those who received nonliposomal OSI-7904. These data are consistent with a significant increase in exposure and altered disposition of OSI-7904 when given as a liposomal formulation.

Whereas preliminary data suggest low intrapatient variability, there is considerable interpatient variability, largely attributed to differences in the extent of the α phase which seemed to follow a biomodal distribution. The cause of this variability in drug disposition during the α phase is unclear, but it might be explained by differences in the uptake of liposomes by tissues such as those of the reticuloendothelial system, or by a relatively rapid drug release phase from the liposome, or a combination of both processes (35).

Differences in terminal half-life do not seem the primary cause of the interpatient variability and regardless of the extent of the α phase, all patients had a terminal half-life that was much greater than the 7.72 (4.09) hour half-life described for the unencapsulated drug (14). Whereas the terminal half-life observed for OSI-7904L may reflect the rate of drug release from the remaining circulating liposomes, it is possible that it could reflect the rate of release of unencapsulated drug from a tissue compartment.

Work is ongoing to develop an assay to quantify ultrafilterable OSI-7904L in plasma to distinguish between liposome-encapsulated and extraliposomal drug. Nevertheless, because OSI-7904 is 97% protein bound, it is not certain whether such an assay will have a limit of quantitation low enough to distinguish between the alternative proposed mechanisms for the α and terminal phases.

**Fig. 3.** A, area under the concentration-time curve (AUC) versus dose for OSI-7904L dosed at 0.4 to 15.0 mg/m². Two subgroups (α phase, no α phase) are shown; patients with DLT (▲). Weighed (1/x²) linear regression resulted in a coefficient of determination (r²) of 0.3126 and 0.6162 for the α phase and no α phase groups, respectively. Runs tests showed no significant deviation from the linear model (P = 0.2956 and P = 0.1753 for the α phase and no α phase fits, respectively). However, the 95% confidence intervals for the y-intercepts did not include zero. B, histogram of the bimodal distribution of percent α phase across all dose cohorts.
Preliminary assessment was also made in this study of several biomarkers as potential pharmacodynamic markers to correlate with outcome. First, elevations of plasma 2'-dU to 2- to 4-fold above baseline suggested that OSI-7904L was inhibiting the target enzyme and elevated levels were maintained for between 4 and 7 days after drug administration. The absence of any dose response effect between elevation of 2'-dU and increasing OSI-7904L dose may be explained by the fact that 2'-dU levels were already increased almost 4-fold at the 3.2 mg/m² dose level (i.e., 25% of the recommended dose), which seems the maximal level of 2'-dU elevation that is achieved with other TSIs (25). In addition, Ford et al. have reported that the duration of 2'-dU elevation may be more relevant than the magnitude of any increase, although there are no prospective data correlating elevation of 2'-dU with clinical efficacy (25). In the current trial there were no objective responses according to the Response Evaluation Criteria in Solid Tumor, but 11 of 31 heavily pretreated patients with a range of tumor types had stable disease, including two with clear reductions in tumor bulk of <30% (one metastatic colorectal and one ovarian cancer). Given that 4 of the 11 patients with stable disease received OSI-7904L at doses of approximately ≤50% of the recommended dose, it might be hypothesized that the optimal dose of OSI-7904L is <12 mg/m². Thus, levels of 2'-dU elevation may be a more appropriate guide to dosing than toxicity, as suggested previously in relation to repeat dosing of the antifolate ZD9331 (36). With regard to predicting toxicity, baseline homocysteine was measured as a marker of folate status. Whereas patient numbers were limited, the majority had levels within normal limits; in contrast to pemetrexed, no correlation between homocysteine levels and OSI-7904L toxicity was apparent (22). The number of patients in whom thymidylate synthase genotyping was done was too small to assess the potential effect of thymidylate synthase genotype on outcome with OSI-7904L, but we have shown that collection and assessment of the promoter polymorphism via a single blood sample is feasible in a prospective clinical setting.

In summary, the first phase I study with the liposomally encapsulated TSI, OSI-7904L, using a day 1 every 21 days schedule, has shown a toxicity profile that is predictable, manageable and characteristic of other TSIs. The recommended dose for future phase II studies is 12 mg/m². Initial pharmacokinetic data suggest a level of interpatient variability and show that in comparison with the nonliposomal drug, the liposomal formulation OSI-7904L exhibits a long plasma circulation time and altered disposition properties. Work is ongoing to elucidate potential pharmacokinetic/pharmacodynamic relationships, especially with respect to the difference in α phase. Ongoing studies are investigating OSI-7904L in patients with cancer of the stomach and gastroesophageal junction as well as combination studies with cisplatin and oxaliplatin.

Acknowledgments

We thank the patients who consented and took part in this study, and the various contributions made by B. Wawziz, N. Waldt, J. McDonald, R. Damji, M. Hughes, L. Adams, D. Drolet, and J. Horan in the design and conduct of the study.

References

25. Ford HER, Mitchell F, Cunningham D, et al. Patterns of elevation of plasma 2'-deoxyuridine, a surrogate marker of thymidylate synthase (TS) inhibition, 5494 www.aacrjournals.org

Downloaded from clincancerres.aacrjournals.org on April 12, 2017. © 2005 American Association for Cancer Research.
after administration of two different schedules of 5-fluorouracil and the specific TS inhibitors raltitrexed (Tomudex) and ZD9331. Clin Cancer Res 2002;8:103–9.


Phase I Study of OSI-7904L, a Novel Liposomal Thymidylate Synthase Inhibitor in Patients with Refractory Solid Tumors

Gernot Beutel, Hilary Glen, Patrick Schöffski, et al.