Pharmacokinetics, Safety, and Efficacy of a Liposome Encapsulated Thymidylate Synthase Inhibitor, OSI-7904L [(S)-2-[5-[(1,2-Dihydro-3-methyl-1-oxobenzof[f]quinazolin-9-yl)methyl]amino-1-oxo-2-isindolyl]-glutaric Acid] in Mice

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ABSTRACT

OSI-7904L [(S)-2-[5-[(1,2-dihydro-3-methyl-1-oxobenzof[f]quinazolin-9-yl)methyl]amino-1-oxo-2-isindolyl]-glutaric acid] is a liposomal formulation of the highly specific, noncompetitive, thymidylate synthase inhibitor OSI-7904 (also known as GW1843, 1843U89, and GS7904). The liposome formulation was developed to enhance the therapeutic index and dose schedule convenience of this potent antifolate compound. The studies presented here were conducted to determine the antitumor efficacy, distribution, pharmacokinetics, and safety of OSI-7904L in mice. In a human colon adenocarcinoma xenograft model in mice, OSI-7904L demonstrated superior antitumor efficacy compared with OSI-7904 or 5-fluorouracil. Furthermore, OSI-7904L could be administered less frequently than OSI-7904 although still generating greater tumor growth inhibition. Distribution studies confirmed that OSI-7904L-treated animals had much greater plasma, tissue, and tumor exposure than did OSI-7904-treated animals. Tumor exposures, based on area under the curve, in OSI-7904L-treated mice were increased over 100-fold compared with tumor exposures in OSI-7904-treated mice. Plasma exposures following OSI-7904L administration were greater than dose proportional consistent with saturation of plasma clearance mechanisms. OSI-7904L was much more toxic than OSI-7904 in the mouse with primary toxicities to the intestines, bone marrow, and thymus. Minimal toxicity to the lungs and liver was noted. These data clearly demonstrated that in mice, OSI-7904L has an increased plasma residence time as well as increased tissue and tumor exposure compared with OSI-7904, thus resulting in increased potency and toxicity. Potential benefits of OSI-7904L include improved efficacy and a more convenient schedule of administration.

Thymidylate synthase (TS) catalyzes the synthesis of dTMP by the transfer of a methyl group from 5,10-methylene-tetrahydrofolate to the 5-position of the pyrimidine ring of dUMP. Inhibition of TS removes the only source of de novo synthesis of thymine nucleotides and depletes thymine pools (Danenberg et al., 1999) leading to an inhibition of DNA synthesis and eventually cell death. Therefore, TS has been a target of cancer chemotherapy since the advent of 5-fluorouracil (5-FU) and 5-fluorodeoxyuridine over 40 years ago (Heidelberger et al., 1957, 1958). 5-FU is a prodrug that requires conversion to the active fluoropyrimidine nucleotide. Thus, resistance mechanisms can arise through the activation and elimination pathways (for review, see Peters and Köhne, 1999). The objective response rate for 5-FU in colorectal cancer is between 20 and 40% indicating that many patients have or will develop resistance to the drug (Peters and Köhne, 1999). The search for improved TS inhibitors has lately focused upon folate, rather than pyrimidine analogs, in the hope of improving TS selectivity and efficacy.

A benzoquinazoline derivative of folate, (S)-2-[5-[(1,2-dihydro-3-methyl-1-oxobenzof[f]quinazolin-9-yl)methyl]amino-1-oxo-2-isindolyl]-glutaric acid (OSI-7904) (also known as GW1843, 1843U89, and GS7904), is a selective, noncompetitive

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ABBREVIATIONS: TS, thymidylate synthase; 5-FU, 5-fluorouracil; OSI-7904, (S)-2-[5-[(1,2-dihydro-3-methyl-1-oxobenzof[f]quinazolin-9-yl)methyl]amino-1-oxo-2-isindolyl]-glutaric acid; OSI-7904L, liposome encapsulated OSI-7904; ADME, absorption, distribution, metabolism, and excretion; TK, thymidine kinase; FPGS, folylpolyglutamate synthase; MRP, multidrug resistance protein; CL, clearance; AUC, area under the plasma concentration-time curve; AUC_{last}, AUC until the last plasma time point measured; AUC_{0→t}, AUC extrapolated to infinite time; LCK, log cell kill; MRT, mean residence time; TGI, tumor growth inhibition.
TS inhibitor with a $K_i$ for the human enzyme of 90 pM (Duch et al., 1993; Dev et al., 1994). Following transport into human tumor cells, OSI-7904 is an efficient substrate for folylpolyglutamate synthase (FPGS). The addition of a single glutamate residue to OSI-7904, however, creates a poor FPGS substrate so that the drug is retained primarily as the diglutamate moiety OSI-7904glu1 (Duch et al., 1993; Hanlon and Ferone 1996). Unlike other folate-analog inhibitors of TS, polyglutamylation of OSI-7904 is not required for activity and OSI-7904, and OSI-7904glu1 are approximately equipotent TS inhibitors (Dev et al., 1994). Thus, OSI-7904 may retain activity against tumors with low FPGS or high folylpolyglutamyl hydrolase activity. On the other hand, because of the limited polyglutamylation of OSI-7904, intracellular drug concentrations decline more rapidly than more heavily polyglutamylated TS inhibitors following removal of extracellular drug (Hanlon and Ferone, 1996). Moreover, it has been demonstrated that overexpression of the multidrug resistance proteins, MRP1 and MRP2, can confer resistance to short term (4 h), but not long term (72 h), exposure of antifolate drugs including OSI-7904 (Hooijberg et al., 1999).

OSI-7904 has demonstrated antitumor efficacy in mice implanted with human tumor xenografts (Duch et al., 1993; Banks et al., 1994; Boytos et al., 1995; Smith et al., 1995, 1999). Such studies are complicated by the relatively high circulating levels of thymidine in rodents (Jackman et al., 1999), which can ameliorate the cytotoxicity of TS inhibitors. Thymidine plasma levels in mice (~1 μM) are at least 10-fold higher than observed in human plasma (Smith et al., 1999). To bypass the thymidine salvage pathway, efficacy studies in rodents are often performed with thymidine kinase (TK)-deficient xenografts (TK−/−) or by intraperitoneal administration of thymidine phosphorylase, which lowers circulating thymidine levels by metabolizing thymidine to thymine and deoxyribose-5-phosphate. Under the latter conditions, OSI-7904 displayed efficacy against a variety of human tumor xenografts including the colon adenocarcinoma line HT-29 and the ileocecal adenocarcinoma lines HCT-8. OSI-7904 also displayed efficacy against the HCT-8 TK−/− cell line (for review, see Smith et al., 1999).

OSI-7904 (as 1843U89) was tested in an open label, Phase I dose escalation study using a daily ×5 dose regimen (2-min i.v. infusion) every 3 weeks (Schwartz et al., 2001). The maximally tolerated dose without oral folic acid was established at 2 mg/m²/day. Neutropenia, mucositis, and malaise were the primary dose-limiting toxicities. Other toxicities included thrombocytopenia, rash, and fever. There was no objective evidence of antitumor efficacy, although this result was not surprising considering the heavy prior chemotherapy regimens of patients enrolled in the study. In an attempt to improve efficacy and convenience of dose schedule, OSI-7904 was encapsulated into small unilamellar liposomes (OSI-7904L). Encapsulation of drugs into liposome formulations has been shown to enhance drug delivery and efficacy in a variety of model systems (Gabizon, 1989; Ostro and Cullis 1989; Zee-Cheng and Cheng 1989; Mayer et al., 1995; Webb et al., 1998; Newman et al., 1999). Because of the restricted polyglutamylation of OSI-7904 and the short-term resistance conferred by MRPs, it was hypothesized that liposomal encapsulation could enhance efficacy by maintaining high tissue concentrations of drug while at the same time simplifying the dose schedule. The research presented here describes the disposition, elimination, and safety of OSI-7904L in mice. Furthermore, the relative efficacy of OSI-7904L and OSI-7904 against human tumor xenografts in mice was compared as well as the antitumor efficacy versus dose schedule of OSI-7904L.

Materials and Methods

Chemotherapeutics. OSI-7904 was synthesized and liposome encapsulated OSI-7904L, was prepared by Gilead Sciences, Inc. (San Dimas, CA). The methodology for liposome preparation yielded unilamellar liposomes 30 to 80 nm in diameter with a 30:1 lipid/drug ratio and a lipid composition of 4:1 fully hydrogenated soy phosphatidylcholine (HSPC)/cholesterol. OSI-7904 was passively entrapped with an efficiency of approximately 7%. This formulation was chosen for the reproducibility of manufacture and stability. Radiolabeled [14C]OSI-7904 (Fig. 1) was obtained from Moravek Biochemicals, Inc. (Brea, CA) and used in the preparation of [14C]OSI-7904L (Gilead Sciences, Inc.). [14C]OSI-7904L was mixed with OSI-7904L to achieve total doses of 25 mg/kg with a final specific activity of approximately 2 μCi/mg and a concentration of 2.5 mg/ml OSI-7904. [14C]OSI-7904 was mixed with OSI-7904L to achieve total doses of 50 mg/kg with a final specific activity of approximately 1 μCi/mg and a concentration of 5 mg/ml OSI-7904. Appropriate dilutions were made with sterile 9% w/v sucrose (McGaw, Inc., Irvine, CA), and all dosages and concentrations were based on the free base. 5-Fluorouracil was obtained from Sigma-Aldrich (St. Louis, MO) and formulated in sterile 0.9% saline.

Mice. Mice were obtained and cared for in accordance with all applicable state and federal guidelines and the Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) were adhered to. For the pharmacokinetic and safety studies, healthy BALB/c mice were obtained from Charles River.
Breeding Laboratories (Portidge, MI). Male and female mice were used for the pharmacokinetic study, whereas the safety studies used only female mice. On study day 1, the animals were approximately 3 to 11 weeks old. For the biodistribution studies and efficacy studies, female Nu/Nu mice (18–25 g) were obtained from Charles River Laboratories (Raleigh, NC), housed in microisolator filtration racks, and maintained on filtered acidified water and sterile lab chow ad libitum.

For all studies, environmental controls were set to maintain temperatures of 18 to 26°C with a relative humidity of 30 to 70%. A 12 h light/dark cycle was maintained in the animal room. Animals were allowed to acclimate to their new environment for 1 week before study day 1. Body weights were measured immediately before randomization of dosing groups.

**Animal Procedures.** For the pharmacokinetic study, dosages (1.0, 10.0, or 50.0 mg/kg) were administered as an i.v. bolus into the lateral tail vein. Blood samples were obtained by cardiac puncture (three animals/gender/time point), collected into tubes containing EDTA, processed into plasma, and frozen (−70°C) within 30 min of collection.

For the subchronic toxicity study, 9% sucrose vehicle or OSI-7904L was administered i.v. for 6 consecutive days. Mice received 30.25 mg/kg/day of OSI-7904L by i.v. bolus injection for a total dose of 181.5 mg/kg. Animals were observed daily for adverse clinical signs (for example, ruffled fur, hunched posture, body temperature, anal staining, discharges), and body weights were recorded. All animals were anesthetized with isoflurane and exsanguinated 24 h after the sixth dose of OSI-7904L. Whole blood was harvested into EDTA-containing tubes for complete blood count (CBC) evaluation. Animals were then humanely euthanized, subjected to a gross necropsy, and selected tissues were collected for histopathological assessment.

For biodistribution studies, animals received doses of 50 mg/kg [14C]OSI-7904 or 25 mg/kg [14C]OSI-7904L. The total amount of radioactivity administered to each animal for each experiment was approximately 0.5 μCi [14C]G/g b.wt. The compounds were administered by bolus i.v. injection into the lateral tail vein. Animals treated with [14C]OSI-7904 were euthanized at 0.5, 1, 2, 4, 8, 12, or 24 h postdose. Animals treated with [14C]OSI-7904L were euthanized at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, or 144 h postdosing. Animals scheduled to be euthanized at 72 h postdosing were housed in metabolic cages for urine and feces collection. The feces were homogenized in water at a ratio of 3:1 (ml/g) before analysis. The animals were euthanized by exsanguinations via cardiac puncture under anesthetic, and selected tissues were removed and weighed. Blood was collected into 1-cc syringes containing 0.1 units of heparin (Sigma-Aldrich) and processed to collect plasma.

For biodistribution studies, animals were implanted with either HCT-8 TK−/− (a kind gift of Dr. Y. Rustum, Rosewell Park Memorial Cancer Institute, Buffalo, NY) or HT-29 tumor cells (obtained from American Type Culture Collection, Manassas, VA). For efficacy studies, animals were implanted with HCT-8 TK−/− cells. Tumors were established by injecting harvested tumor cells in a single subcutaneous site on the flank of the mice in the axillary region. The tumors were grown until approximately 200 ± 50 mm3 in size. Animals were then sorted according to body weight, grouped four animals per cage, and tattooed on the tail for permanent identification. Within a treatment group, a narrow range in body weight (±1 g) and tumor size was required. Each group, consisting of eight tumor-bearing mice, was administered test article by i.v. bolus injection through the tail vein.

Treated animals were monitored daily for signs of morbidity and mortality. Tumor volumes and body weights were determined twice weekly. The tumor volume for each mouse was determined by measuring in two directions with vernier calipers and calculated using the formula: tumor volume = length × (width)2/2. The data were plotted as the percent change in mean values of tumor volume and body weight for each group. The percent tumor growth inhibition was determined as the tumor growth inhibition rate, TGI = (1 − T/C) × 100%, where T is the mean tumor weight in the treated group, and C is the mean tumor weight in the vehicle control group on a given day.

**Determination of Drug Concentrations in Plasma.** Total (liposome encapsulated plus plasma bound and free drug) OSI-7904 concentrations in plasma were quantified using a validated liquid chromatography/mass spectrometry/mass spectrometry assay performed by MDS Pharma Services (Montreal, Quebec, Canada). Briefly, 100 μl of plasma and 200 μl of internal standard working solution in methanol (N-methyl analog of OSI-7904 at 120 ng/ml) or 200 μl of methanol for blanks were added to 1.5-ml Eppendorf tubes. Samples were mixed and centrifuged at 13,000 rpm for 5 min. Supernatant was transferred to 13 × 100-mm culture tubes and evaporated to dryness in a Turbobuv (GenTech Scientific Inc., Arcade, NY) (50°C). Samples were reconstituted with 300 μl of water, transferred to injection vials, and 5 μl injected onto an API III Plus (GenTech Scientific Inc.) equipped with a BDS Hypersil Phenyl column (Thermo Electron Corp., Bellefonte, PA) (50 × 2.0 mm, 3 μm). The mobile phase consisted of 55:45 (v/v) methanol/0.1% formic acid in water. The flow rate was 0.3 ml/min. A Perkin Elmer Sciex API III (GenTech Scientific Inc.) with a Turbo Ion spray source was used to analyze the eluant from the chromatographic column. Ions were detected using selected reaction monitoring in positive ion mode. Fragmentation of the precursor ions was achieved using argon as collision gas with collision energy of 25 electron volts. Mass transitions monitored were m/z 501 → 223 for OSI-7904 and 515 → 223 for the internal standard. Quantification was performed by peak area ratio using a quadratic equation with 1/x2 weighting.

**Determination of [14C]OSI-7904 in Tissues.** Whole tissue samples and aliquots of plasma, urine, or feces homogenate were burned in a Packard tissue oxidizer model 307 (PerkinElmer Life and Analytical Sciences, Boston, MA). The resulting vials contained 8 ml of Carbosorb E (PerkinElmer Life and Analytical Sciences) and 12 ml of Permafluor E (PerkinElmer Life and Analytical Sciences). The vials then were counted for radioactivity on a Packard Tri-Carb 2100RT scintillation counter. Raw cpm data were divided by efficiency of counter and oxidizer to generate data as dpm. The concentration of radioactive material (micrograms per gram or micrograms per milliliter) was calculated by dividing dpm by the specific activity and sample size.

**Pharmacokinetic Analyses.** Pharmacokinetic parameters for total OSI-7904 after i.v. administration of OSI-7904L were determined from median plasma concentration-time data using WinNonlin version 3.1 (Pharsight Corp., Mountain View, CA). Uncompartmental analysis model 201 (i.v. bolus dose) was used with the log/linear trapezoidal rule. Cmax values were estimated by a back extrapolation to zero time. Pharmacokinetic parameters associated with the terminal phase were calculated using the median plasma values of the last two measured time points to estimate the terminal half-lives. Biodistribution data were dose normalized to the 25 mg/kg area under the curve (AUC0−∞) of the concentration of drug (micrograms per gram or micrograms per milliliter) over time (0.5 h to last quantifiable sample) for the tissues was calculated using the Prism software version 3.02 (GraphPad Software, San Diego, CA).

**Results**

**OSI-7904L Plasma Pharmacokinetics.** Following a single 1.0, 10, or 50 mg/kg i.v. bolus dose of OSI-7904L, total OSI-7904 plasma concentrations declined in a multieponent-
tial manner (Fig. 2). Following a brief distribution phase, a plateau phase occurred that lasted for approximately 4 h. There was no discernible terminal elimination phase as the concentration-time curve following the plateau phase appeared convex on a log plot. Results for males and females were similar, and the pharmacokinetic parameters across genders are shown in Table 1.

Plasma $C_{\text{max}}$ increased proportionally with dose; however, a greater than dose-proportional increase of plasma AUC with dose was observed (Table 1). The greater than dose-proportional increase in plasma AUC was reflected in the decreasing plasma clearance with dose and in the increase in plasma mean residence time with dose. Plasma clearance decreased from 7.69 ml/(h kg) for the 1.0 mg/kg dose group to 2.97 ml/(h kg) for the 50 mg/kg dose group, whereas $MRT_{\text{to-inf}}$ increased from 10.8 h for the 1.0 mg/kg dose group to 25.8 h for the 50 mg/kg dose group.

For the dose range tested, the steady-state volume of distribution did not vary with dose. Values ranged from 67.1 ml/kg for the 10 mg/kg dose group to 81.4 ml/kg for the 1.0 mg/kg dose group. These values were similar to the estimated plasma volume of the mouse (50 ml/kg) as would be expected for a liposome encapsulated polar compound.

### Tissue and Tumor Distribution after Treatment with Either $[^{14}\text{C}]$OSI-7904 or $[^{14}\text{C}]$OSI-7904L

Following a 25 mg/kg i.v. bolus dose, most tissues from $[^{14}\text{C}]$OSI-7904L-treated animals had greater exposure to OSI-7904 than the tissues of animals treated with 50 mg/kg $[^{14}\text{C}]$OSI-7904 (Fig. 3 and Table 2). Primary distribution compartments after treatment with $[^{14}\text{C}]$OSI-7904L were the spleen, kidneys, liver, and lungs, whereas, after treatment with $[^{14}\text{C}]$OSI-7904, the primary distribution compartments were the components of the intestinal tract such as the cecum, colon, jejunum, and ileum. Based upon AUC$_{\text{last}}$ values, nonintestinal tissues such as the liver (38-fold) and heart (1620-fold) had significantly greater exposure after treatment with $[^{14}\text{C}]$OSI-7904L than after treatment with $[^{14}\text{C}]$OSI-7904. Differences in exposure to the sections of the intestinal tract were less pronounced and ranged from 0.934-fold (cecum) to 8.99-fold (duodenum) different between $[^{14}\text{C}]$OSI-7904L-treated mice and $[^{14}\text{C}]$OSI-7904-treated mice.

Tumors of mice administered $[^{14}\text{C}]$OSI-7904L also had increased exposure to OSI-7904 relative to the tumors of mice treated with $[^{14}\text{C}]$OSI-7904 (Fig. 3 and Table 3). Maximal concentrations for the HCT-8 TK$^{-/-}$ and HT-29 tumors of $[^{14}\text{C}]$OSI-7904L-treated animals were 10.7 μg/g and 13.9 μg/g.
whereas the corresponding values for 
[14C]OSI-7904-treated animals were 2.24/ 
H9262 g/g and 6.98/ 
H9262 g/g, respectively. Therefore, OSI-7904L demonstrated approxi-
mately a 2- to 4-fold increase in the maximal tumor drug 
concentrations. Exposure of the tumors, based on AUC0-
T_H11009 3/ 
H11009, was increased over 100-fold for both tumor types after ad-
ministration of [14C]OSI-7904L compared with [ 14C]OSI-
7904 administration. However, the tumor to plasma concen-
tration ratio was reduced. The tumor to plasma AUC0-
T_H11009 3/ 
H11009 ratio for OSI-7904L was 0.0602 and 0.0850 for HT-29 and 
HCT-8 TK/TK tumors, respectively, whereas the correspond-
ning values for OSI-7904 were 0.202 and 0.297.

Elimination of [14C]OSI-7904L. The total amounts of 
radioactive material recovered in the urine and feces are 
listed in Table 4. Previous work demonstrated that the elim-
ation of OSI-7904 was predominantly in the feces via bili-
ary excretion (data not shown). Overall recovery at 72 h 
postdose in the excreta was approximately 69%. Feces of 
[14C]OSI-7904L-treated mice contained approximately 60% 
of the administered dose by 72 h postdose with approxi-
mately 31% recovered within the first 24 h. Urine from

TABLE 2
Dose normalized AUC based on concentration of radioactivity in tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>OSI-7904</th>
<th>OSI-7904L</th>
<th>Ratio (L/F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>18.4</td>
<td>699</td>
<td>38.0</td>
</tr>
<tr>
<td>Duodenum</td>
<td>36.8</td>
<td>331</td>
<td>8.99</td>
</tr>
<tr>
<td>Jejunum</td>
<td>190</td>
<td>356</td>
<td>1.87</td>
</tr>
<tr>
<td>Ileum</td>
<td>78.4</td>
<td>412</td>
<td>5.26</td>
</tr>
<tr>
<td>Cecum</td>
<td>558</td>
<td>521</td>
<td>0.934</td>
</tr>
<tr>
<td>Colon</td>
<td>190</td>
<td>266</td>
<td>1.40</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.78</td>
<td>1480</td>
<td>392</td>
</tr>
<tr>
<td>Kidneys</td>
<td>34.4</td>
<td>804</td>
<td>55.8</td>
</tr>
<tr>
<td>Ovaries</td>
<td>1.34</td>
<td>204</td>
<td>152</td>
</tr>
<tr>
<td>Heart</td>
<td>0.298</td>
<td>482</td>
<td>1620</td>
</tr>
<tr>
<td>Lungs</td>
<td>8.53</td>
<td>606</td>
<td>71.0</td>
</tr>
<tr>
<td>Skin</td>
<td>1.55</td>
<td>263</td>
<td>170</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.960</td>
<td>71.8</td>
<td>74.8</td>
</tr>
<tr>
<td>Brain</td>
<td>NC</td>
<td>62.5</td>
<td>NC</td>
</tr>
<tr>
<td>Plasma</td>
<td>12.5</td>
<td>4670</td>
<td>374</td>
</tr>
</tbody>
</table>

NC, not calculable.

was increased over 100-fold for both tumor types after ad-
ministration of [14C]OSI-7904L compared with [14C]OSI-
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of the administered dose by 72 h postdose with approxi-
mately 31% recovered within the first 24 h. Urine from

TABLE 3
Dose-normalized pharmacokinetic parameters for tumor tissue

<table>
<thead>
<tr>
<th>Formulation</th>
<th>HT-29</th>
<th>HCT-8 TK/TK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0-3/</td>
<td>Cmax</td>
</tr>
<tr>
<td></td>
<td>μg/h/g</td>
<td>μg/g</td>
</tr>
<tr>
<td>OSI-7904L</td>
<td>281</td>
<td>6.98</td>
</tr>
<tr>
<td>OSI-7904</td>
<td>2.52</td>
<td>6.98</td>
</tr>
</tbody>
</table>

Fig. 3. Concentration of [14C]OSI-7904 versus time in liver (A), kidney (B), HT-29 tumor (C), or HCT-8 TK/TK tumor (D) following a 25 mg/kg dose of [14C]OSI-7904L (solid circles) or following a 50 mg/kg dose of [14C]OSI-7904 (open circles).
([14C]OSI-7904L-treated mice contained approximately 9% of the administered dose by 72 h. High-pressure liquid chromatography analysis of urine and fecal extracts from OSI-7904L-treated rats indicated that the OSI-7904 was predominantly (\( \geq 95\% \) of the total peak areas) excreted as the parent molecule (data not shown). The remaining peak area from the extracts of rat excreta did demonstrate a slightly more polar metabolite, and previous work in vitro suggested that the metabolite is likely the glutamated form of OSI-7904 (data not shown); however, this metabolite has not been conclusively identified in mice.

**Safety of OSI-7904L.** In a subchronic study, 9% sucrose vehicle or OSI-7904L was administered to female BALB/c mice intravenously for 6 consecutive days. Mice received 30.25 mg/kg/day of OSI-7904L for a total dose of 181.5 mg/kg. Animals were gang-housed (four or five per cage), and no changes were noted for food or water intake. No adverse clinical signs or morbidity were noted throughout the study, and no gross lesions were observed at necropsy. In OSI-7904L-treated mice, a reduction of 19% in mean body weight correlated with reductions in the mean absolute weights of the brain, liver, heart, and paired kidneys (\( p < 0.05 \)). Marked thymic atrophy and mild to moderate epithelial necrosis of the small intestine with associated mucosal atrophy of the duodenum were observed in all treated mice but not control mice. Effects seen in some OSI-7904L-treated animals and not observed in control animals included: hypocellularity of the sternal bone marrow (56% of mice), minimal focal to multifocal subacute inflammation of the lungs (56%), and minimal hepatocellular atrophy with mild hepatocellular vacuolation (22% of mice).

**Efficacy of OSI-7904L.** An initial human HCT-8 TK\( ^{-/-}\) xenograft study compared the antitumor efficacy of the liposomal OSI-7904L dosed at 7.5 mg/kg every other day for 14 days to free drug (OSI-7904) dosed every day at 25 or 50 mg/kg for 14 days. Therefore, the total amount of OSI-7904L administered was 52.5 mg/kg, whereas the total amount of OSI-7904 administered was 350 mg/kg or 700 mg/kg. Table 5 summarizes these results, which clearly demonstrated that the liposome formulation was more efficacious than the OSI-7904 formulation. OSI-7904L produced 83% tumor regression with a LCK of 4.6 compared with results in the 25 and 50 mg/kg OSI-7904 drug groups where no tumor regression occurred and the LCK values were 1.5 and 3.5, respectively. Figure 4 depicts the tumor growth curves, which demonstrate a dose-response effect with the free drug groups and a more delayed tumor outgrowth with the OSI-7904L group. The body weight loss in all treatment groups was transient and reversible and never exceeded 10%.

A dose schedule study was performed comparing the efficacy against human HCT-8 TK\( ^{-/-}\) tumor xenografts of three different OSI-7904L schedules to a single OSI-7904 and a single 5-FU schedule. OSI-7904L was administered on days 1 to 5 at 7.5 mg/kg for 2 consecutive weeks, on days 1, 3, and 5 at 15 mg/kg for 2 consecutive weeks, or on days 1 and 8 at 25 mg/kg. OSI-7904 was administered on days 1 to 5 at 100 mg/kg for 2 consecutive weeks. 5-FU was administered on days 1 and 8 at 100 mg/kg. Animals were followed for up to 87 days. All three of the OSI-7904L dose groups demonstrated equivalent efficacy with LCK values ranging between 3.2 to 4.2 with 1/8 durable cures in each group (Table 6). The 5-FU and OSI-7904 groups were less effective in inhibiting tumor growth with LCK values of 1.5

**TABLE 4**
Percentage of radiolabeled OSI-7904 dose excreted following a single 25 mg/kg intravenous dose of OSI-7904L (mean ± S.D.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Urine Means</th>
<th>Feces Means</th>
<th>Sum of Means</th>
</tr>
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<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 24</td>
<td>7.51 ± 0.849</td>
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</tr>
<tr>
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<td>1.26 ± 0.430</td>
<td>20.8 ± 2.40</td>
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<tr>
<td>48 to 72</td>
<td>0.565 ± 0.0290</td>
<td>7.22 ± 3.22</td>
<td>7.79</td>
</tr>
<tr>
<td>sum total</td>
<td></td>
<td></td>
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Fig. 4. Growth inhibition of HCT-8 TK\( ^{-/-}\) tumor xenografts by OSI-7904 and OSI-7904L. Human adenocarcinoma tumors were implanted subcutaneously in nude mice and allowed to grow to approximately 200 mm\(^3\) before treatment was initiated. Control mice were dosed with intravenous 9% sucrose in water every other day for 2 weeks. Treated mice were dosed intravenously with 25 or 50 mg/kg OSI-7904 every day for 2 weeks or with 7.5 mg/kg liposome encapsulated OSI-7904 (OSI-7904L) every other day for 2 weeks. Data are presented as the mean ± S.E.M. (n = 8).
and 2.4, respectively. Figure 5 shows the tumor growth curves for each dose group and illustrates the differences in antitumor activity, as summarized above. The body weight loss greatest in the 5-FU group was transient and reversible and never exceeded 20%.

A dose-response study with OSI-7904L was performed with HCT-8 TK−/− tumor-bearing mice. Mice received 5, 10, 15, 20, or 25 mg/kg OSI-7904L on days 1 and 8. Initial tumor volume reduction was similar in all dose groups except the lowest where tumor growth was inhibited by 80% compared with the other four groups where growth was inhibited from 92 to 99%. There was no appreciable effect on body weight in any of the dose groups, and 7/32 cures were generated. As shown in Table 7 and Fig. 6, a dose-dependent tumor response was evident. Durable cures were observed in the 15, 20, and 25 mg/kg dose groups.

Discussion

OSI-7904L demonstrated significant antitumor activity as determined by inhibition of tumor growth, regression of established tumors, and in some experiments, durable cures. In studies comparing the activity of OSI-7904 to OSI-7904L, it was evident that OSI-7904L was more potent than OSI-7904 and could be administered less frequently generating greater tumor growth inhibition. Little difference in OSI-7904L antitumor efficacy was observed upon dose schedule modifications. When once weekly to three or five times weekly dose schedules were compared, little difference was observed in tumor growth inhibition, LCK, or cure rate. In a dose-ranging study, dose-response effects were seen in the once weekly dosing schedule over the range of 5 to 25 mg/kg/week. These findings suggest that a convenient weekly schedule is optimal in mice and support the investigation in human clinical trials of a single dose regimen with an infrequent dosing interval.

The pharmacokinetic profile of OSI-7904L was consistent with saturation of the plasma clearance. Thus, the continuously changing half-life observed following the plateau phase likely resulted from the continuously increasing plasma clearance as the plasma concentration declined and not from changes in the volume of distribution. This type of plasma profile has been described for liposomal formulations of other drugs such as cisplatin and amakacin (Fielding et al., 1999; Newman et al., 1999; Fielding, 2001). The rate of elimination from plasma at any plasma concentration (Cp) can be described as CL = (CLint × (Km + Cp) / Km) where Km is the Michaelis-Menten constant and CLint is the intrinsic clearance. When Cp is far below the Km, the rate of elimination approximates linear pharmacokinetics. When Cp is greater

![Graph showing growth inhibition of HCT-8 TK−/− tumor xenografts by 5-FU, OSI-7904, and OSI-7904L.](image)

**Fig. 5.** Growth inhibition of HCT-8 TK−/− tumor xenografts by 5-FU, OSI-7904, and OSI-7904L. Human adenocarcinoma tumors were implanted subcutaneously in nude mice and allowed to grow to approximately 200 mm³ before treatment was initiated. Control mice were dosed with intravenous 9% sucrose in water every other day for 2 weeks. Treated mice were dosed intravenously with 100 mg/kg 5-FU on study days 1 and 8 or with 100 mg/kg OSI-7904 on study days 1 to 5 and 8 to 12 or with 7.5 mg/kg liposome encapsulated OSI-7904 (OSI-7904L) every other day for 2 weeks. Data are presented as the mean ± S.E.M. (n = 8).
The normal mouse is very resistant to OSI-7904-induced toxicity; however, in the BALB/c female mouse under conditions of thymidine depletion, gastrointestinal toxicity was observed to be dose limiting (Smith et al., 1999). In contrast, OSI-7904L was more toxic in the BALB/c mouse without thymidine depletion. Following 6 consecutive days of treatment, primary toxicities to the intestines, bone marrow, and thymus were observed. Minimal toxicity to the lungs and the liver was also noted.

These data demonstrate that OSI-7904L has an increased plasma residence time as well as increased tissue and tumor exposure in mice compared with OSI-7904. These differences resulted in increased potency and toxicity with OSI-7904L. The major potential clinical benefits are enhanced efficacy and a more convenient schedule of administration. A recently completed Phase I dose escalation study has set the recommended dose at 12 mg/m² when OSI-7904L is administered by a 30-min infusion once every 21 days (Beutel et al., 2003). This is a much more convenient schedule than the original 5 consecutive day dose schedule with OSI-7904 (Schwartz et al., 2001). A Phase II trial of OSI-7904L in adults with gastrointestinal malignancies is ongoing.

Acknowledgments

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References


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table:TABLE 7

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>TGI</th>
<th>Regression</th>
<th>Cures</th>
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<tr>
<td>D5W control</td>
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</tr>
<tr>
<td>OSI-7904L</td>
<td>5 mg/kg</td>
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</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
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<tr>
<td></td>
<td>25 mg/kg</td>
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than $K_m$, the rate of elimination becomes saturated (i.e., approaches $V_{max}$) (Newman et al., 1999). The plasma pharmacokinetic properties of OSI-7904L observed in tumor bearing mice at 25 mg/kg were consistent with those observed in healthy mice dosed a 1, 10, or 50 mg/kg (Table 1 and 2, data not shown).

Compared with $[^14C]$OSI-7904-treated animals, $[^14C]$OSI-7904L-treated animals had much greater tissue, plasma, and tumor xenograft exposures. The spleen had the largest concentrations and overall exposure following $[^14C]$OSI-7904L treatment in agreement with previous work demonstrating a propensity of the spleen to accumulate liposomal encapsulated drugs (Gabizon et al., 1990; Maruyama et al., 1994). Exposure of tumor xenografts to $[^14C]$OSI-7904 was increased over 100-fold after administration of $[^14C]$OSI-7904L compared with $[^14C]$OSI-7904 administration. Comparison of the ratios for the tumors to plasma after treatment with $[^14C]$OSI-7904 demonstrated that the liposomal formulation actually had a reduced tumor to plasma AUC ratio. Therefore, these data suggested that, although maximal concentration and exposure increased greatly, the liposomal formulation of OSI-7904 did not increase the selectivity of OSI-7904 to the tumor.

The primary route of elimination of OSI-7904L was via feces. Nearly 60% of the OSI-7904L dose was eliminated into the feces by 72 h consistent with the observation that OSI-7904 is eliminated primarily by the liver via biliary excretion (unpublished data). Approximately 9% of the OSI-7904L dose was excreted into the urine. Therefore, the primary route of excretion of OSI-7904 was not significantly altered by the liposomal formulation.


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