Population Pharmacokinetics of Pegylated Liposomal CKD-602 (S-CKD602) in Patients With Advanced Malignancies

Huali Wu, MS, Ramesh K. Ramanathan, MD, Beth A. Zamboni, MS, Sandra Strychor, BS, Suresh Ramalingam, MD, Robert P. Edwards, MD, David M. Friedland, MD, Ronald G. Stoller, MD, Chandra P. Belani, MD, Lauren J. Maruca, MD, Yung-Jue Bang, PhD, and William C. Zamboni, PharmD, PhD

S-CKD602 is a pegylated long-circulating liposomal formulation of CKD-602, a potent topoisomerase I inhibitor. A population pharmacokinetic (PK) model for encapsulated and released CKD-602 following administration of S-CKD602 was developed to assess factors that may influence S-CKD602 PK. Plasma samples from 45 patients with solid tumors were collected in a phase 1 study. S-CKD602 was administered as a 1-hour intravenous infusion with doses ranging from 0.1 to 2.5 mg/m^2. Plasma concentrations of encapsulated and released CKD-602 were used to develop a population PK model using NONMEM. PK of encapsulated CKD-602 was described by a 1-compartment model with nonlinear clearance, and PK of released CKD-602 was described by a 2-compartment model with linear clearance for all patients. Covariate analysis revealed that tumor in the liver was a significant covariate for clearance of encapsulated CKD-602 and that age significantly influenced the release rate of CKD-602 from S-CKD602. Maximum elimination rate in patients with liver tumor is 1.5-fold higher compared with patients without liver tumor. Release rate of CKD-602 from S-CKD602 in patients less than 60 years old was 2.7-fold higher compared with patients 60 years old or older. These observations have potential implications in the optimal dosing of liposomal agents.

Keywords: *S-CKD602; population pharmacokinetics; pegylated liposome; nonlinear kinetics; liver metastasis*

Journal of Clinical Pharmacology, 2012;52:180-194

S-CKD602 is a STEALTH liposomal formulation of CKD-602, a camptothecin analogue that inhibits topoisomerase I.[1-3] The STEALTH liposomal formulation consists of phospholipids covalently bound to methoxypolyethylene glycol (mPEG) on the outside of the lipid bilayer. Nonliposomal CKD-602 administered intravenously (IV) at 0.5 mg/m^2/d for 5 consecutive days repeated every 21 days is approved in Korea for the treatment of newly diagnosed small cell lung cancer and relapsed ovarian cancer.[4-7]

The development of STEALTH liposomes was based on the discovery that incorporation of mPEG lipids into liposomes yields preparations with prolonged plasma exposure and superior tumor delivery compared with conventional liposomes composed of natural phospholipids.[3,8,9] STEALTH liposomal doxorubicin (Doxil) is approved for the treatment of refractory ovarian cancer, Kaposi sarcoma, and multiple myeloma.[10,11] The generation of a liposomal formulation of a camptothecin analogue, such as CKD-602, has specific advantages such as protecting the lactone form and providing prolonged exposure in tumors, which is associated with greater antitumor effect of camptothecines.[3,12-16] The STEALTH liposomal

From the University of North Carolina (UNC) Eshelman School of Pharmacy (Ms Wu, Dr Zamboni), UNC Lineberger Comprehensive Cancer Center (Dr Zamboni), UNC Institute for Pharmacogenomics and Individualized Therapy (Dr Zamboni), and Carolina Center for Cancer Nanotechology Excellence (Dr Zamboni), UNC, Chapel Hill, North Carolina; University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania (Dr Ramanathan, Ms Strychor, Dr Ramalingam, Dr Friedland, Dr Stoller, Dr Belani, Dr Maruca); School of Medicine, University of Pittsburgh, Pittsburgh (Dr Edwards); Department of Mathematics, Carlow University, Pittsburgh (Ms Zamboni); CKD Research Institute, Chonan, Korea (Dr Bang); and Seoul National University Hospital, Seoul, Korea (Dr Bang). Submitted for publication August 24, 2010; revised version accepted November 18, 2010. Address for correspondence: William C. Zamboni, Division of Pharmacotherapy and Experimental Therapeutics, School of Pharmacy, University of North Carolina, Genetic Medicine Building, Room 1013, CB# 7361, 120 Mason Farm Road, Chapel Hill, NC 27599; e-mail: zamboni@unc.edu. DOI: 10.1177/0091270010394851

formulation is expected to have an enhanced therapeutic ratio compared with free CKD-602 (nonliposomal) as well as a more convenient schedule of administration.

In mice, the plasma exposure of S-CKD602 at 1 mg/kg IV × 1 was ~25-fold greater than the plasma exposure of nonliposomal CKD-602 at 30 mg/kg IV × 1.[6,17] In the plasma of mice, ~82% of CKD-602 was encapsulated inside the liposome after administration of S-CKD602.[17] The duration of exposure of CKD-602 in the tumor was 3-fold longer with S-CKD602 than with nonliposomal CKD-602 in mice bearing human tumor xenografts.[17] The improved pharmacokinetics of S-CKD602 resulted in greater therapeutic index compared with free CKD-602. In human tumor xenograft models, the therapeutic index (TI) of S-CKD602 was estimated to be approximately 6-fold greater than that of free CKD-602 in ES-2 ovarian and approximately 3-fold greater in H82 SCLC tumors.[6] These results are consistent with the antitumor response to camptothecin analogs, which is related to the duration of time for which the drug concentration is above a critical threshold.[6,15-18] The pharmacokinetic disposition of carrier-mediated agents, such as nanoparticles, nanosomes, and conjugated agents, is dependent upon the carrier until the drug is released from the carrier. Unlike traditional anticancer agents that are cleared by the liver and kidneys, the clearance of nonpegylated and pegylated liposomes occurs via the reticuloendothelial system (RES), which includes monocytes, macrophages, and dendritic cells located primarily in the liver and spleen.[19] Uptake by the RES usually results in sequestering of the encapsulated drug in the RES, where it can be degraded. In addition, the uptake of the liposomes by the RES may result in acute impairment of the RES and toxicity. Pegylated liposomes are cleared much slower via RES compared with nonpegylated liposomes.[8,20] Once the drug is released from the carrier, the pharmacokinetic disposition of the drug will be the same as after administration of the noncarrier form of the drug.[19,21] Thus, the pharmacokinetics of liposomes are complex.

Nanoparticle agents have higher variability in pharmacokinetic (drug clearance, systemic exposure, distribution, etc) disposition with potentially higher variability in pharmacodynamic (antitumor response and toxicity) disposition compared with traditional small-molecule chemotherapy. The factors affecting the pharmacokinetic and pharmacodynamic variability of encapsulated and released forms of conventional and pegylated liposomes remain unclear but most likely include the RES.[12] We have evaluated factors affecting the pharmacokinetics and pharmacodynamics of liposomal anticancer agents. We were the first to report a reduced clearance of the liposomal encapsulated forms of Doxil and S-CKD602 in patients 60 years of age and older.[22,23] We have also reported that patients with a lean body composition may have a reduced tissue distribution and an increased plasma exposure of S-CKD602. In addition, we have reported an age-related decrease in the function of monocytes that may be associated with a reduced clearance of liposomes and reduced cytotoxicity to the monocytes.[20,24,25]

S-CKD602 was associated with high interpatient variability in the pharmacokinetic disposition of encapsulated and released CKD-602.[26] Population pharmacokinetic analysis is a useful tool to identify sources of pharmacokinetic variability and can aid in the design of alternative dosing regimens to enhance efficacy and safety. The objectives of this study were to describe the population pharmacokinetics of both encapsulated CKD-602 and released CKD-602 after administration of the pegylated liposomal form of the drug and to characterize clinical covariates that influence S-CKD602 pharmacokinetics.

METHODS

Patients

Patients 18 years of age and older with a histologically or cytologically confirmed malignancy for which no effective therapy was available were eligible for this study. Pertinent eligibility criteria included an Eastern Cooperative Oncology Group performance status of 0 to 2 and adequate bone marrow, hepatic, and renal function as evidenced by the following: absolute neutrophil count (ANC) 1500/μL or greater, platelets 100 000/μL or greater, total bilirubin 1.5× or less of the upper limit of the institutional normal range (ULN), aspartate aminotransferase (AST) 1.5× or less than ULN if liver metastases were not present and 4× or less than ULN if liver metastases were present, and absence of microscopic hematuria.[18] Prior treatment with camptothecin analogues other than S-CKD602 or nonliposomal CKD-602 was permitted. Written informed consent, approved by the institutional review board of the University of Pittsburgh Medical Center, was obtained from all patients prior to study entry.

Study Design

This was a phase 1, dose escalation study in patients with advanced solid tumors.[26,27] The study design

and clinical results have been reported elsewhere.[26,27] This phase 1 study followed a standard dose escalation design with patients enrolled in cohorts of 3 initially, with the possibility of extending the cohort up to 6 patients depending on the number of dose-limiting toxicities (DLTs).[18] No intrapatient dose escalation was permitted. The MTD was defined as the dose below the dose at which 2 out of up to 6 patients experienced a DLT. At the 2.5 mg/m^2 dose level, 2 of 3 patients experienced a DLT. Because the next lower dose level (1.7 mg/m^2) was associated with minimal toxicity, an additional intermediate dose level of 2.1 mg/m^2 was investigated.

Dosage and Administration

S-CKD602 is a formulation of CKD-602 encapsulated in long-circulating STEALTH liposomes. In S-CKD602, the STEALTH liposome bilayer is composed of N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-phosphoethanolamine (MPEG-DSPE) and 1,2-distearoyl-*sn*-glycero-phosphocholine (DSPC) in a molar ratio of approximately 5:95. The mean particle diameter is approximately 100 nm, and CKD-602 encapsulation inside the liposomes exceeds 85%. S-CKD602 was supplied by ALZA Corporation (Mountain View, California) in sterile 10-mL single-use amber vials as a clear to slightly opalescent suspension with a nominal total CKD-602 concentration of 0.1 mg/mL. S-CKD602 was diluted 3-fold in 5% dextrose prior to administration. No medications were administered prior to S-CKD602.

S-CKD602 was administered IV over approximately 1 hour every 3 weeks. Doses administered, expressed in milligrams of CKD-602 per square meter, were 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.65, 0.85, 1.1, 1.7, 2.1, and 2.5 mg/m^2.

Sample Collection, Processing, and Analytical Studies

Plasma samples for pharmacokinetic assessment were obtained from all patients. On cycle 1, blood (7 mL) was collected in EDTA (purple top) tubes prior to administration; at the end of the infusion (approximately 1 hour); and at 3, 5, 7, 24, 48, 72, 96, 168 (day 8), and 336 hours (day 15) after the start of the infusion. The blood samples were centrifuged at 1380*g* for 6 minutes. The plasma for the determination of the encapsulated and released CKD-602 was immediately placed in a refrigerator at 4°C and kept refrigerated until solid-phase separation (SPS).[17,27] The encapsulated CKD-602 and released CKD-602 were fully eluted and separated by SPS as previously described.[17,27] The encapsulated and released CKD-602 concentrations were then measured by a specific liquid chromatographic tandem mass spectrometric assay (LC-MS/MS) as previously described.[17,27] The total (lactone + hydroxy acid) form of CKD-602 was measured for encapsulated and released samples. The lower limits of quantitation (LLQ) of the total form encapsulated and released CKD-602 were 2 and 0.05 ng/mL, respectively.[17,27]

Population Pharmacokinetic Analysis

Encapsulated CKD-602 and released CKD-602 concentration–time data were analyzed using the nonlinear mixed-effects modeling approach as implemented in NONMEM (version 6; University of California, San Francisco). Both the first-order approximation method and first-order conditional estimation (FOCE) method were used in analyses. S-PLUS 8.0 (version 8.0, Insightful Corporation, Seattle, Washington) was used for graphic diagnostics and covariate screen. The population pharmacokinetic model of S-CKD602 was developed in 2 steps: *(a)* basic (structural) model development and *(b)* covariate model development.

Mean population pharmacokinetic variables, interindividual variability (IIV), and residual error were assessed in the model development. The IIV for each pharmacokinetic variable was modeled with an exponential function. Residual error models of the additive, proportional, exponential, and combination methods were evaluated for the best structural pharmacokinetic model. Individual pharmacokinetic variables were obtained by posterior Bayesian estimation.

Model selection for nonhierarchical models (ie, linear and nonlinear elimination models) was guided by goodness-of-fit plots (eg, observed vs predicted plasma concentrations, weighted residuals vs predicted concentrations, and weighted residuals vs time), Akaike information criterion (AIC), and precision of parameter estimates. The AIC was calculated as AIC = OFV + 2 × p, where OFV is the NONMEM objective function value and p is the number of pharmacokinetic variables. The model was chosen on the basis of smaller values of AIC, better precision of estimates, and superior goodness-of-fit plots.

Model Development

The structural model of encapsulated CKD-602 and released CKD-602 was built sequentially. First, the best model for encapsulated CKD-602 was selected from all the possible models. Then, based on the best model for encapsulated CKD-602, simultaneous

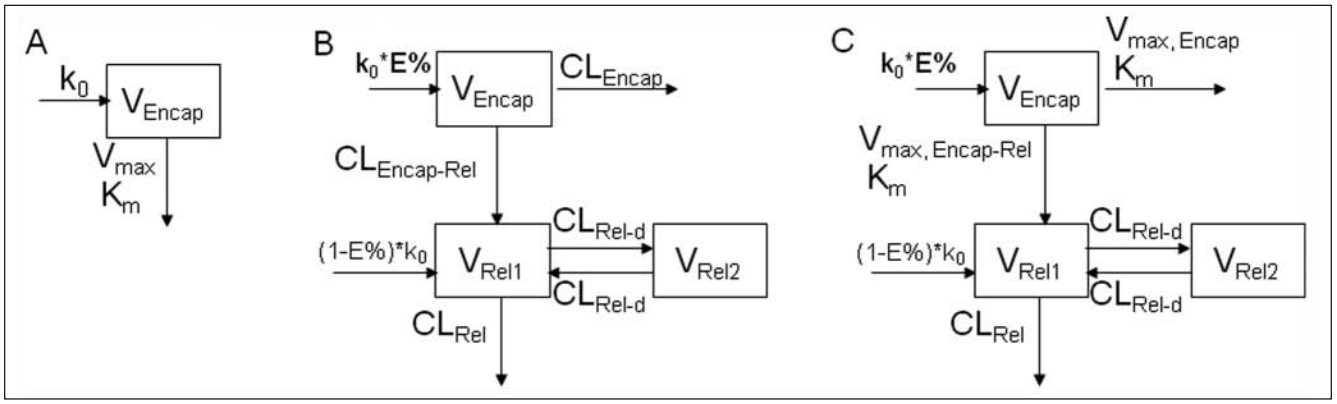

Figure 1. The final structural pharmacokinetic model for (A) CKD-602 encapsulated alone (1 compartment with nonlinear clearance) in all patients, (B) encapsulated (1 compartment with linear clearance) and released CKD-602 (2 compartments with linear clearance) in patients with linear clearance of encapsulated CKD-602, and (C) encapsulated (1 compartment with nonlinear clearance) and released CKD-602 (2 compartments with linear clearance) in patients with nonlinear clearance of encapsulated CKD-602. A fraction of dose that was administered in the form of nonliposomal CKD-602 was included. E% is encapsulation percentage of CKD-602 in the formulation. Volume of distribution for central compartment (V_{Rel1}), volume of distribution for peripheral compartment (V_{Rel2}), systemic clearance (CL_{Rel}), and distribution clearance (CL_{Rel-d}) in Figures 1B and 1C were fixed based on nonliposomal CKD-602 pharmacokinetic data.

modeling of the encapsulated and the released CKD-602 was attempted for data from all patients. In this modeling process, 1-compartment, 2-compartment, and 3-compartment models with first-order elimination were tested to fit released plasma concentration data. However, the estimation of pharmacokinetic parameters for released drug proved to be unsatisfactory because no successful minimization with successful covariance step could be achieved. Therefore, the mean values of pharmacokinetic parameters associated with distribution and elimination of released CKD-602 were determined from a CKD-602 pharmacokinetic model after administration of nonliposomal CKD-602 and were fixed in the final population pharmacokinetic model of encapsulated and released CKD-602. The IIVs of pharmacokinetic parameters associated with distribution and elimination of released CKD-602 were not fixed and were estimated in the final population pharmacokinetic model of encapsulated and released CKD-602. The ability to fix the mean value of pharmacokinetic parameters of the released drug using the estimated mean value of pharmacokinetic parameters from nonliposomal drugs is based on our studies reporting that the pharmacokinetic disposition of released drug is the same as nonliposomal drugs.[28]

Encapsulated Drug Model

The structural model was built to fit encapsulated CKD-602 plasma concentration–time profiles from all 45 patients. One-compartment and 2-compartment models with first-order elimination or nonlinear elimination characterized by Michaelis-Menten kinetics described as below were tested to fit encapsulated plasma concentration data.

The pharmacokinetic model used to characterize the disposition of encapsulated CKD-602 in all patients is shown in Figure 1A. The differential equation describing the pharmacokinetic model of encapsulated CKD-602 is as follows:

$$\frac{dA_{Encap}}{dt} = k_0 - \frac{V_{max} \bullet A_{Encap}}{K_m \bullet V_{Encap} + A_{Encap}},\ A_{Encap}(0) = 0,$$

$$C_{Encap} = \frac{A_{Encap}}{V_{Encap}}$$

where dA_{Encap}/dt is the elimination rate, V_{max} is the maximum elimination rate or maximum velocity, K_m is the concentration at which half-maximum elimination rate is achieved, V_{Encap} is the volume of distribution, A_{Encap} is the encapsulated CKD-602 amount in plasma, C_{Encap} is the plasma concentration of encapsulated CKD-602, k_0 is the infusion rate, and k_0 is 0 after end of infusion.

CKD-602 Model After Administration of Nonliposomal CKD-602

CKD-602 pharmacokinetic data were from a phase 1 and pharmacokinetic study of CKD-602 in patients with advanced solid malignancies.[5] The study design and clinical results have been reported elsewhere.[5]

Sixteen patients received CKD-602 (0.5-0.9 mg/m^2/d) IV × 1 over 30 minutes daily for 5 consecutive days. Frequent plasma sampling was performed prior to administration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the start of the infusion for all the patients. The structural model was built to fit CKD-602 plasma concentration–time profiles from all 16 patients. Two-compartment and 3-compartment models with first-order elimination were tested to fit CKD-602 pharmacokinetic profiles.

Encapsulated and Released Drug Model

Based on the best model for encapsulated CKD-602 and the best model for CKD-602 after administration of nonliposomal CKD-602, a 1-compartment model with Michaelis-Menten kinetics for encapsulated drug and a 2-compartment model with first-order elimination for released drug were used to fit combined data of encapsulated and released CKD-602 after administration of S-CKD602. In this model development, in addition to fixing mean value of pharmacokinetic parameters (volume of distribution for central compartment [V_{Rel1}], volume of distribution for peripheral compartment [V_{Rel2}], systemic clearance [CL_{Rel}], and distribution clearance [$CL_{Rel\text{-}d}$]) of released drug, we further classified patients as linear and nonlinear according to the clearance of encapsulated drug from our previous pharmacokinetic analysis.[26] As most of the CKD-602 remains encapsulated in the plasma after administration of S-CKD602 and the plasma concentration of released CKD-602 is only 1% of encapsulated CKD-602, the amount or concentration of CKD-602 existing in the nonencapsulated form in the dosage (infusion bag) is important in order to capture the relatively high concentration of released CKD-602 at earlier time points. Thus, estimates of the nonencapsulated CKD-602 in the formulation were included in models for encapsulated and released CKD-602 after administration of S-CKD602.

The pharmacokinetic model used to characterize the disposition of encapsulated and released CKD-602 in patients with linear clearance of encapsulated CKD-602 is shown in Figure 1B. The differential equations describing the pharmacokinetic model of encapsulated and released CKD-602 are as follows:

$$\frac{dA_{Encap}}{dt} = k_0 \bullet E\% - \frac{CL_{Encap}}{V_{Encap}} \bullet A_{Encap} - \frac{CL_{Encap-Rel}}{V_{Encap}} \bullet A_{Encap},\ A_{Encap}(0) = 0$$

$$\frac{dA_{Rel1}}{dt} = k_0 \bullet (1 - E\%) + \frac{CL_{Encap-Rel}}{V_{Encap}} \bullet A_{Encap} + \frac{CL_{Rel-d}}{V_{Rel2}} \bullet A_{Rel2} - \frac{CL_{Rel}}{V_{Rel1}} \bullet A_{Rel1} - \frac{CL_{Rel-d}}{V_{Rel1}} \bullet A_{Rel1},\ A_{Rel1}(0) = 0$$

$$\frac{dA_{Rel2}}{dt} = -\frac{CL_{Rel-d}}{V_{Rel2}} \bullet A_{Rel2} + \frac{CL_{Rel-d}}{V_{Rel1}} \bullet A_{Rel1},\ A_{Rel2}(0) = 0$$

$$C_{Rel} = \frac{A_{Rel1}}{V_{Rel1}}$$

The CL_{Encap} is the clearance of encapsulated CKD-602, $CL_{Encap\text{-}Rel}$ is the clearance of release CKD-602 from S-CKD602, and E% is the encapsulation percent of CKD602 in the formulation. A_{Rel1} is the released CKD-602 amount in central compartment, A_{Rel2} is the released CKD-602 amount in peripheral compartment, and C_{Rel} is the plasma concentration of released CKD-602.

The pharmacokinetic model used to characterize the disposition of encapsulated and released CKD-602 in patients with nonlinear clearance of encapsulated CKD-602 is shown in Figure 1C. The differential equations describing the pharmacokinetic model of encapsulated and released CKD-602 are as follows:

$$\frac{dA_{Encap}}{dt} = k_0 \bullet E\% - \frac{V_{max,Encap} \bullet A_{Encap}}{K_m \bullet V_{Encap} + A_{Encap}} - \frac{V_{max,Encap-Rel} \bullet A_{Encap}}{K_m \bullet V_{Encap} + A_{Encap}},\ A_{Encap}(0) = 0$$

$$\frac{dA_{Rel1}}{dt} = k_0 \bullet (1 - E\%) + \frac{V_{max,Encap-Rel} \bullet A_{Encap}}{K_m \bullet V_{Encap} + A_{Encap}} + \frac{CL_{Rel-d}}{V_{Rel2}} \bullet A_{Rel2} - \frac{CL_{Rel}}{V_{Rel1}} \bullet A_{Rel1} - \frac{CL_{Rel-d}}{V_{Rel1}} \bullet A_{Rel1},\ A_{Rel1}(0) = 0$$

$$\frac{dA_{Rel2}}{dt} = -\frac{CL_{Rel-d}}{V_{Rel2}} \bullet A_{Rel2} + \frac{CL_{Rel-d}}{V_{Rel1}} \bullet A_{Rel1},\ A_{Rel2}(0) = 0$$

$$C_{Rel} = \frac{A_{Rel1}}{V_{Rel1}}$$

The $V_{max,\ Encap}$ is the maximum velocity of encapsulated CKD-602, $V_{max,\ Encap\text{-}Rel}$ is the maximum velocity of release CKD-602 from S-CKD602, and K_m is the concentration at which half-maximum elimination rate is achieved.

Covariate Analysis

The covariate model building was a stepwise process. A screen for potential significant covariates was conducted using S-PLUS 8.0 (version 8.0, Insightful Corporation, Seattle, Washington). The potential covariates as listed in Table I were tested for influence on the structural pharmacokinetic variables

Table I Summary of Patient Demographics and Covariates Included in the Analysis

Characteristics	No. of Patients	Mean (SD)	Median (Range)
Age, y		60.6 (12.2)	62 (33-79)
Body surface area, m^2		1.91 (0.30)	1.86 (1.36-2.76)
Body weight, kg		78.7 (21.4)	75.5 (44.0-148)
Body mass index, kg/m^2		27.4 (5.60)	26.7 (18.8-45.7)
Creatinine clearance, mL/min		98.4 (46.6)	84.7 (33.2-277)
Height, cm		169 (11.9)	170 (142-196)
Ratio of body weight to ideal body weight		1.27 (0.28)	1.22 (0.83-2.13)
Sex			
Male	24		
Female	21		
Primary tumor type			
Colorectal adenocarcinoma	17		
Ovarian cancer	5		
Sarcoma	5		
Non–small cell lung cancer	4		
Pancreatic adenocarcinoma	3		
Hepatocellular carcinoma	2		
Prostate carcinoma	2		
Esophageal, metastatic breast, mesothelioma, renal cell carcinoma, thyroid, appendix, unknown primary	1 patient for each type		
Patients with tumors in liver	26		
Patients without tumors in liver	19		

that describe the pharmacokinetics of encapsulated and released CKD-602. The potential significant covariates selected from screen were introduced into the covariate model as linear, exponential, or power function and were assessed in the population pharmacokinetic models. A significant covariate was selected to be retained in the final model if addition of the covariate resulted in a decrease in OFV greater than 3.875 ($P < .05$) during the forward full covariate model building and removal of the covariate resulted in an increase in OFV greater than 10.828 ($P < .001$) during the stepwise backward model reduction. In addition, the increase in precision of the variable estimate (% relative SE of prediction) and reduction in IIV were used as indicators of improvement of the goodness of fit.

Model Evaluation

Bootstrap analyses were performed to evaluate the stability of the final models and estimate the confidence interval (CI) of the parameters using the bootstrap option in the software package Perl-speaks-NONMEM (M. Karlsson and A. Hooker, version 3.1.0, December 2009). One thousand replicate bootstrap data sets were obtained by resampling with the replacement from the original data set and fitted with the same model to obtain parameter estimates for each replicate. The median and 2.5th and 97.5th values for the population parameters were obtained.

RESULTS

Patient Demographics

Forty-five patients were enrolled on this study from September 29, 2003, to October 17, 2005, at the University of Pittsburgh Cancer Institute. Pharmacokinetic studies of encapsulated and released CKD-602 were performed in all 45 patients. Patient characteristics are listed in Table I. The numbers of male and female patients evaluated in the phase 1 study were 21 and 24, respectively. The mean (median, range) age of the patients was 60.6 years (62 years, 33-79 years). Twenty-six patients had liver tumor and 19 patients did not have liver tumor. A total of 292 plasma concentrations of encapsulated CKD-602 and 268 plasma concentrations of released CKD-602 were used to develop the population pharmacokinetic model.

Table II Population Pharmacokinetic Parameters Obtained From the Final Covariate Model for Encapsulated CKD-602

Parameter	Definition	Population Mean, (RSE%)	Bootstrap Median (95% CI[a])	IIV, CV% (RSE%)
V_{Encap}, L	Volume of distribution	3.63 (9)	3.78 (3.32-6.00)	72 (48)
V_{max}, µg/h	Maximum velocity			205 (51)
With liver tumor[b]		150 (19)	163 (102-345)	
Without liver tumor[c]		97.4 (32)	99.7 (52.3-186)	
K_m, µg/L	Michaelis-Menten constant	992 (17)	985 (558-1250)	Neg
Residual variability				
Proportional error (variability as %)		14.4 (57)	14.8 (9.4-31.9)	NE
Additive error, µg/L		10.9 (65)	10.3 (1.65-28.5)	

CI, confidence interval; CV, coefficient of variation; IIV, interindividual variability; NE, not estimated; Neg, negligible; RSE, relative standard error for estimate.
a. Confidence interval calculated from 910 bootstrap resamplings.
b. Estimated maximum velocity for patients with liver tumor.
d. Estimated maximum velocity for patients without liver tumor.

Population Pharmacokinetic Model of S-CKD602 Encapsulated Drug Model

Both linear and nonlinear pharmacokinetic models were evaluated for encapsulated CKD-602. A 1-compartment model with Michaelis-Menten kinetics (AIC = 2246) better described the data than either nonlinear plus linear (AIC = 2257) or linear kinetics (AIC = 2313). The distribution of residual variability was best described by a proportional plus additive error model. During the covariate screen, liver tumor was identified as a significant covariate for maximum velocity of encapsulated CKD-602. The pharmacokinetic parameter estimates obtained from the final covariate model and the 95% CI from bootstrap analysis are provided in Table II. The observed bootstrap medians were consistent with the population mean estimates in general. In the final model, the mean IIV (percentage coefficient of variance [CV%]) value for the distribution volume was 3.63 L (72%) and was very close to plasma volume in humans. The mean Michaelis-Menten constant (Km) was estimated to be 992 µg/L. The inclusion of liver tumor as a covariate in the final model decreased the IIV of the Vmax of encapsulated CKD-602 by 29%. The Vmax in patients with liver tumor and patients without liver tumor as estimated to be 150 (IIV 205%) and 97.4 (IIV 205%) µg/h, respectively ($P < .001$). The Vmax showed the most IIV, with IIV for Vmax estimated to be 205% even after the incorporation of liver tumor as a covariate.

Selected individual pharmacokinetic time profiles of encapsulated CKD-602 in patients with and without tumors in their livers are shown in Figure 2. The final pharmacokinetic model well characterized the nonlinear pharmacokinetic of encapsulated CKD-602. Goodness-of-fit plots from the final pharmacokinetic model are given in Figure 3. The plots indicated a reasonable fit of the model to the data.

CKD-602 Model After Administration of Nonliposomal CKD-602

Two-compartment and 3-compartment models with first-order elimination were tested to fit CKD-602 pharmacokinetic profiles. A 2-compartment model with first-order elimination resulted in a similar model fit but better precision of parameter estimates compared with a 3-compartment model with first-order elimination. The distribution of residual variability was best described by a proportional error model. No significant covariates were found. The pharmacokinetic parameter estimates from the final model are provided in Table III. The population mean IIV (CV%) values of volume of central compartment (Vc), elimination clearance (CL), volume of peripheral compartment (Vp), and distribution clearance (CLd) for nonliposomal CKD-602 were 9.72 L, 7.9 L/h (IIV 27%), 27.7 L (IIV 21.8%), and 5.07 L/h. Goodness-of-fit plots from the final pharmacokinetic model are given in Figure 4. The plots indicated a reasonable fit of the model to the data.

Encapsulated and Released Drug Model

Combined data of the encapsulated and released CKD-602 were modeled separately for patients with

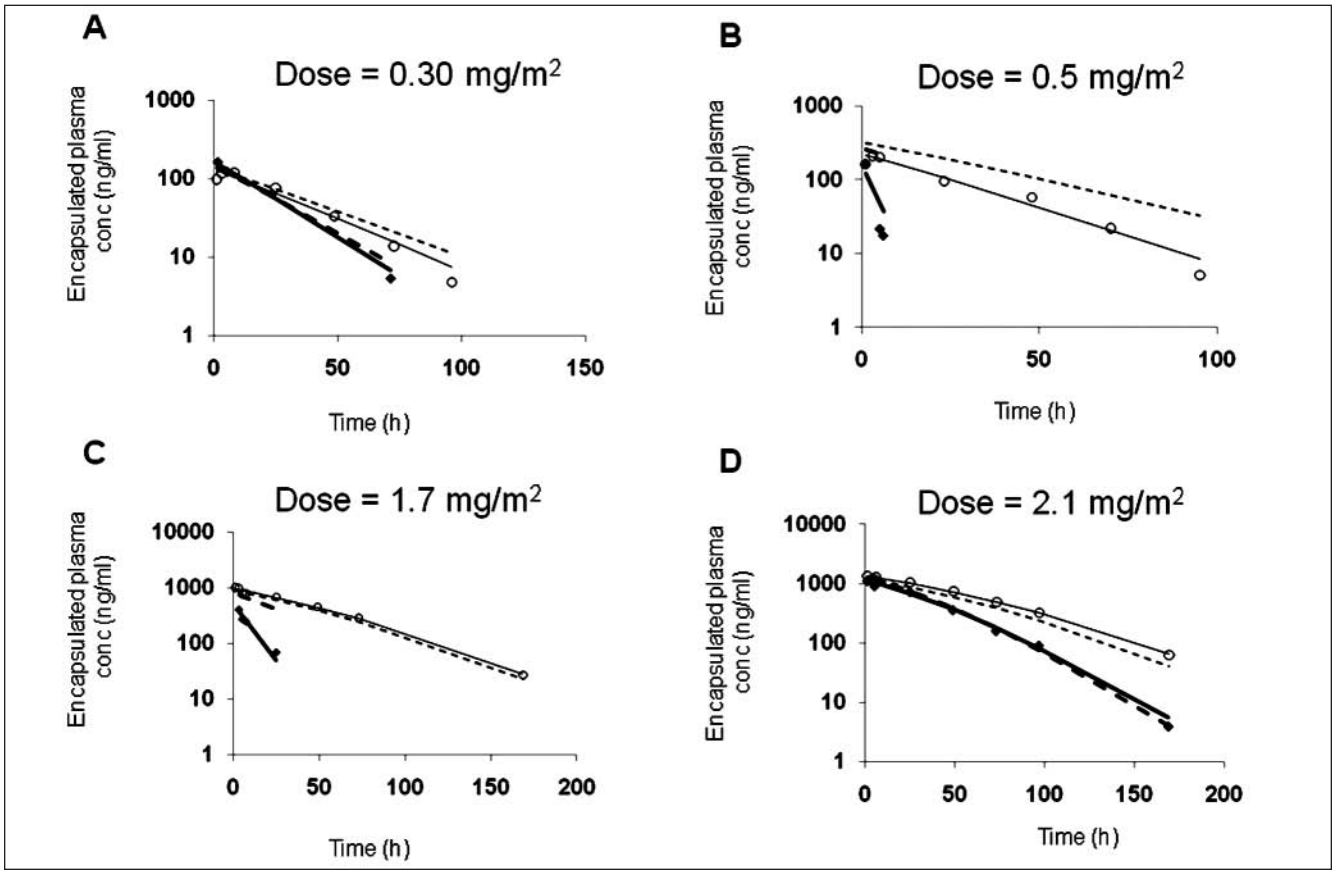

Figure 2. Representative individual plots of observed, population predicted, and individual predicted values of plasma concentrations of encapsulated CKD-602. (A, B, C, D) Concentration–time profile in patients treated with doses of 0.30, 0.5, 1.7, and 2.1 mg/m², respectively. The observed (open circles), population predicted (nonboldface dashed lines), and individual predicted (nonboldface solid lines) values of encapsulated CKD-602 in patients without liver tumor are presented. The observed (diamonds), population predicted (boldface dashed lines), and individual predicted (boldface solid lines) values of encapsulated CKD-602 in patients with liver tumor are presented.

linear clearance and nonlinear clearance of encapsulated CKD-602. Pharmacokinetic parameters (V_{Rel1}, V_{Rel2}, CL_{Rel}, and CL_{Rel-d}) of released CKD-602 were determined from CKD-602 pharmacokinetic model after administration of nonliposomal CKD-602 and fixed in the final population pharmacokinetic model of encapsulated and released CKD-602. Results of the final covariate model for patients with linear clearance of encapsulated CKD-602 are summarized in Table IV. V_{Encap} and CL_{Encap} of encapsulated CKD-602 for patients with linear clearance of encapsulated CKD-602 were estimated to be 4.69 L (IIV 53%) and 0.089 L/h (IIV 151%), respectively. The population mean of encapsulation of CKD-602 in the formulation was estimated to be 96%. The inclusion of age decreased IIV in the release of CKD-602 from S-CKD602 by 33%. The population means of release rate of CKD-602 from S-CKD602 in patients less than 60 years old and patients 60 and older were 0.130 and 0.048 L/h, respectively ($P < .001$).

The results of final model for patients with nonlinear clearance of encapsulated CKD-602 are summarized in Table V. No covariates were identified for these patients. V_{Encap}, $V_{max, Encap}$ and Km of encapsulated CKD-602 were estimated to be 3.36 L (IIV 12.6%), 36.1 µg/h (IIV 28.8%), and 1450 µg/L, respectively. The ratio of $V_{max, Encap-Rel}$ to Km for the release of CKD-602 from S-CKD602 was 0.063 L/h. The population mean of encapsulation of CKD-602 in the formulation was estimated to be 96%.

Selected individual pharmacokinetic time profiles of encapsulated and released CKD-602 in patients with linear clearance and nonlinear clearance of encapsulated CKD-602 are shown in Figure 5, a and b, respectively. In general, the observed data of encapsulated and released CKD-602 were well

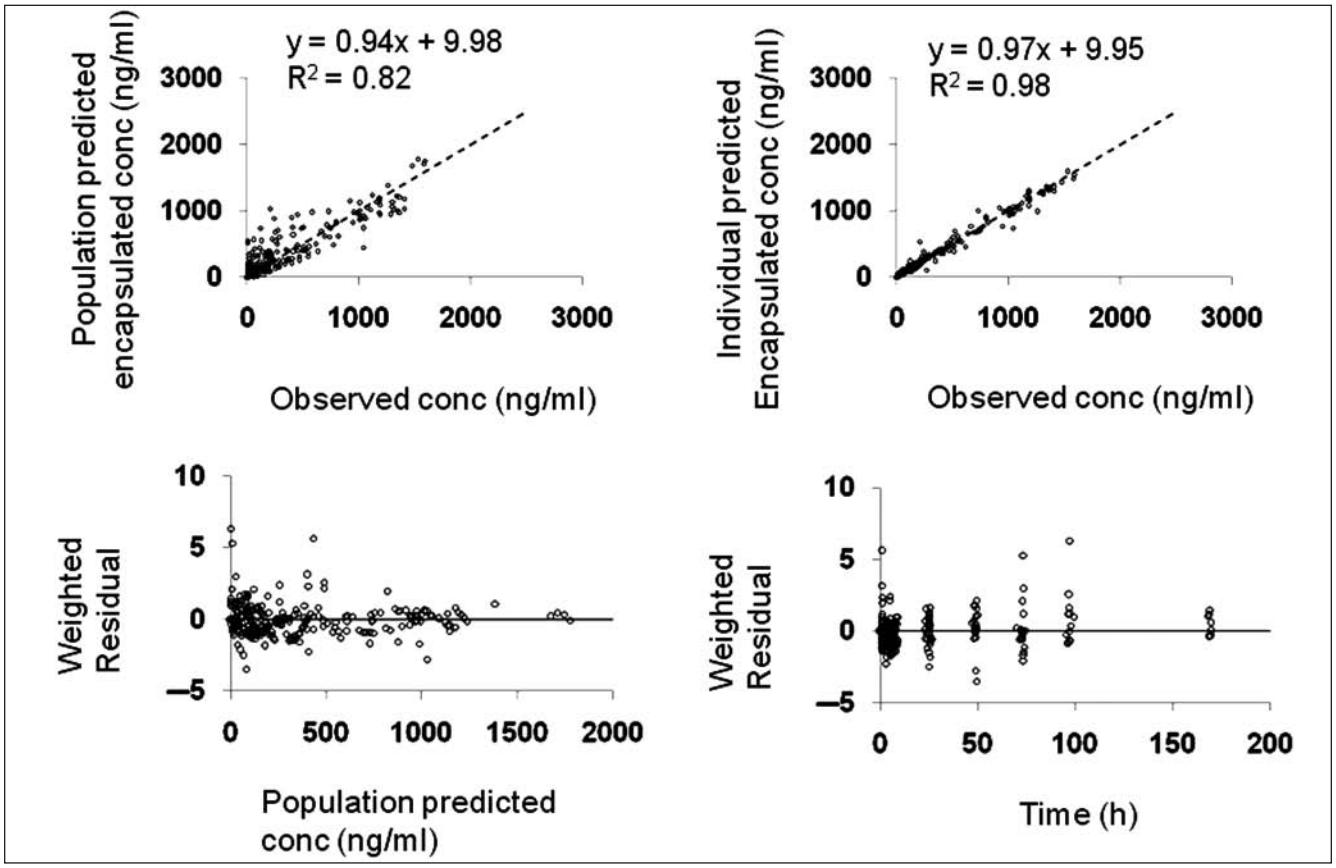

Figure 3. Goodness-of-fit plots for the final model of encapsulated CKD-602. The dashed lines in the upper left and right panels are lines of identity. The solid lines in the lower left and right panels represent the line y = 0.

Table III Population Pharmacokinetic Parameters Obtained From the Final Model for CKD-602 After Administration of Nonliposomal CKD-602

Parameter	Definition	Population Mean (RSE%)	Bootstrap Median (95% CI)	IIV, CV% (RSE%)
V_c, L	Volume of distribution for central compartment	9.72 (9)	9.57 (7.62-11.84)	NE
CL, L/h	Systemic clearance	7.90 (13)	7.84 (5.77-10.05)	27.0 (38)
V_p, L	Volume of distribution for peripheral compartment	27.7 (15)	27.8 (20.2-35.2)	21.8 (39)
CL_d, L/h	Distribution clearance	5.07 (17)	5.17 (2.90-7.26)	Neg
Residual variability				
Proportional error (variability as %)		31.3 (9.5)	40.1 (24.3-54.8)	NE

CI, confidence interval; CV, coefficient of variation; IIV, interindividual variability; NE, not estimated; Neg, negligible; RSE, relative standard error for estimate.

described by the final model. Goodness-of-fit plots from the final pharmacokinetic model in patients with linear clearance and nonlinear clearance of encapsulated CKD-602 are given in Figure 6, a and b, respectively. The model adequately describes the pharmacokinetic profile of encapsulated CKD-602 in both groups of patients. Although the pharmacokinetic data of released CKD-602 were variable, the

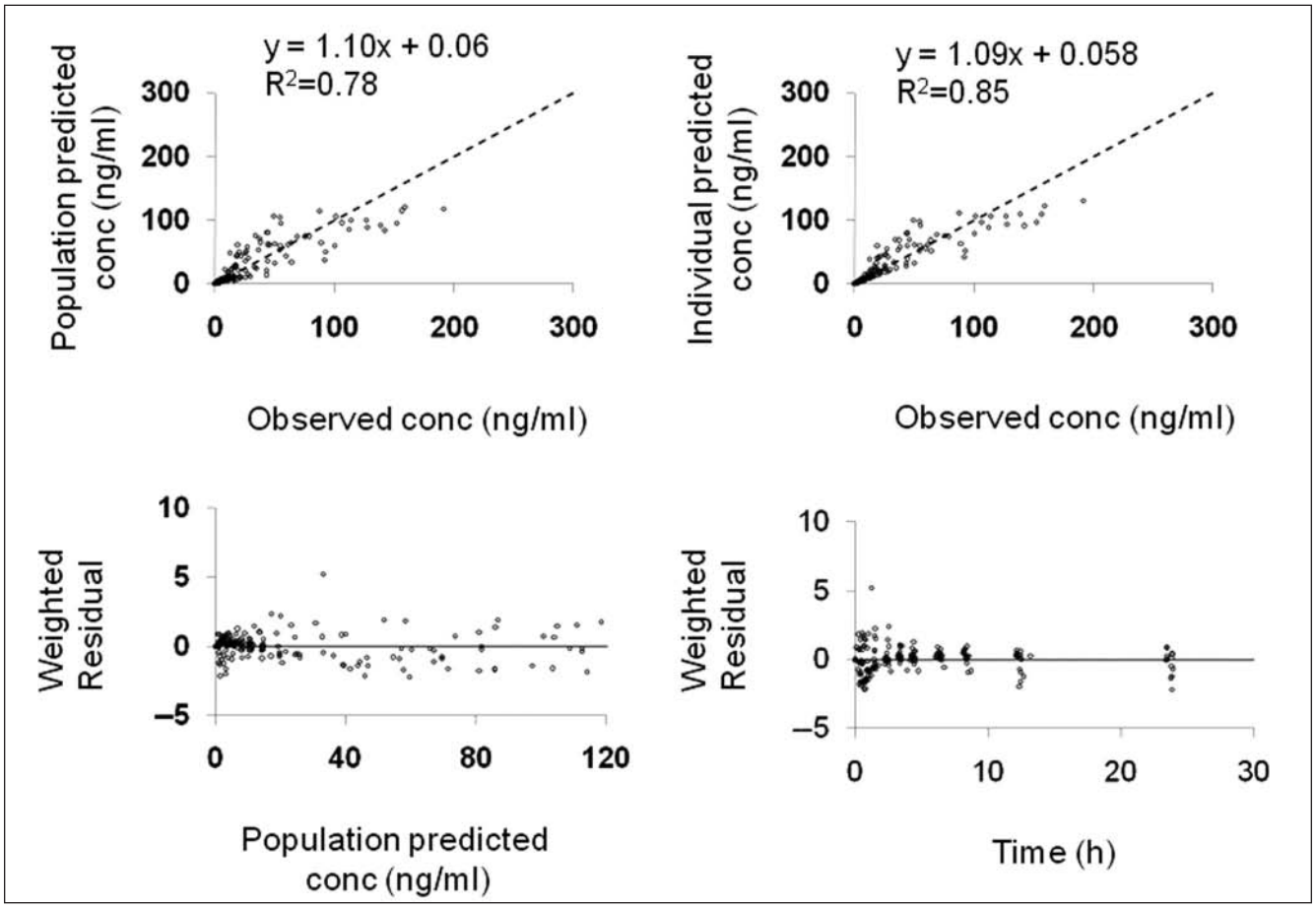

Figure 4. Goodness-of-fit plots for the final model of CKD-602 after administration of nonliposomal CKD-602. The dashed lines in the upper left and right panels are lines of identity. The solid lines in the lower left and right panels represent the line y = 0.

observed and model predicted data agreed relatively well. Bootstrap CIs for pharmacokinetic parameters were not obtained because of the computational intensity of the parameter estimation.

DISCUSSION

Major advances in the use of liposomes, conjugates, and nanoparticles as vehicles to deliver drugs have occurred the past 10 years.[3,8,9] Doxil- and albumin-stabilized nanoparticle formulations of paclitaxel (Abraxane) are now approved by the US Food and Drug Administration.[10,11,29] In addition, more than 200 liposomal and nanoparticle formulations of anticancer agents are in development.[3] This is the first study in which population pharmacokinetic modeling was applied to assess the pharmacokinetics of the encapsulated and released drug after administration of a pegylated liposomal formulation of a camptothecin analogue.[30,31] This is also the first study to identify liver tumor as a factor associated with the pharmacokinetic variability of liposomal agents. Evaluation of the pharmacokinetic disposition of the liposomal encapsulated verses released drug is of the utmost importance because the liposomal encapsulated drug is an inactive prodrug and thus only the released drug is active.[3,17]

Pegylated liposomal CKD-602 displayed nonlinear pharmacokinetics best described by a 1-compartment structural model. The volume of distribution for encapsulated CKD-602 was 3.46 L (70.9%) and is very close to plasma volume in humans. The limited volume of distribution of encapsulated-CKD602 is consistent with other liposomal anticancer agents since the size of liposome limited their distribution to the normal tissue.[32] Saturation of clearance has been reported for both Doxil and S-CKD602, and the nonlinear pharmacokinetics of

Table IV Population Pharmacokinetic Parameters Obtained From the Final Covariate Model for Encapsulated and Released CKD-602 in Patients With Linear Clearance of Encapsulated CKD-602

Parameter	Definition	Population Mean (RSE%)	IIV, CV% (RSE%)
V_{Encap}, L	Volume of distribution for encapsulated	4.69 (2.2)	53 (26)
V_{Rel1}, L	Volume of distribution for central compartment of released	9.72 (NE)	369 (46)
V_{Rel2}, L	Volume of distribution for peripheral compartment of released	27.7 (NE)	180 (103)
CL_{Encap}, L/h	Clearance of encapsulated	0.089 (6.5)	151 (27)
$CL_{Encap-Rel}$, L/h	Clearance of release CKD-602 from S-CKD602		
Age <60 years[a]		0.130 (17)	58 (65)
Age ≥60 years[b]		0.048 (14)	
E%, %	Encapsulation percentage of CKD-602 in the formulation	96.3 (28)	NE
CL_{Rel-d}, L/h	Distribution clearance for released	5.07 (NE)	126 (55)
CL_{Rel}, L/h	Systemic clearance for released	7.90 (NE)	NE
Residual variability			
Proportional error (variability as %)		19.8 (26)	NE
Additive error for encapsulated, μg/L		7.82 (31)	NE
Additive error for released, μg/L		0.019 (38)	NE

CV, coefficient of variation; IIV, interindividual variability; NE, not estimated; RSE, relative standard error for estimate.
a. Clearance of release CKD-602 from S- CKD602 for patients younger than 60 years.
b. Clearance of release CKD-602 from S- CKD602 for patients older than 60 years.

Table V Population Pharmacokinetic Parameters Obtained From the Final Model for Encapsulated and Released CKD-602 in Patients With Nonlinear Clearance of Encapsulated CKD-602

Parameter	Definition	Population Mean (RSE%)	IIV, CV% (RSE%)
V_{Encap}, L	Volume of distribution for encapsulated	3.36 (4.6)	12.6 (44)
V_{Rel1}, L	Volume of distribution for central compartment of released	9.72 (NA)	115 (104)
V_{Rel2}, L	Volume of distribution for peripheral compartment of released	27.7 (NA)	130 (89)
$V_{max, Encap}$, μg/h	Maximum velocity of encapsulated	36.1 (81)	28.8 (32)
$V_{max, Encap-Rel}$, μg/h	Maximum velocity of release CKD-602 from S-CKD602	90.9 (81)	28.8 (32)
K_m, μg/L	Michaelis-Menten constant	1450 (16)	NE
E%, %	Encapsulation percent of CKD-602 in the formulation	96.3 (31)	NE
CL_{Rel-d}, L/h	Distribution clearance for released	5.07 (NA)	NE
CL_{Rel}, L/h	Systemic clearance for released	7.90 (NA)	89 (59)
Residual variability			
Proportional error for encapsulated (variability as %)		11.7 % (43)	
Proportional error for released (variability as %)		26.2 % (29)	NE
Additive error for released, μg/L		0.632 (69)	

CV, coefficient of variation; IIV, interindividual variability; NE, not estimated; RSE, relative standard error for estimate.

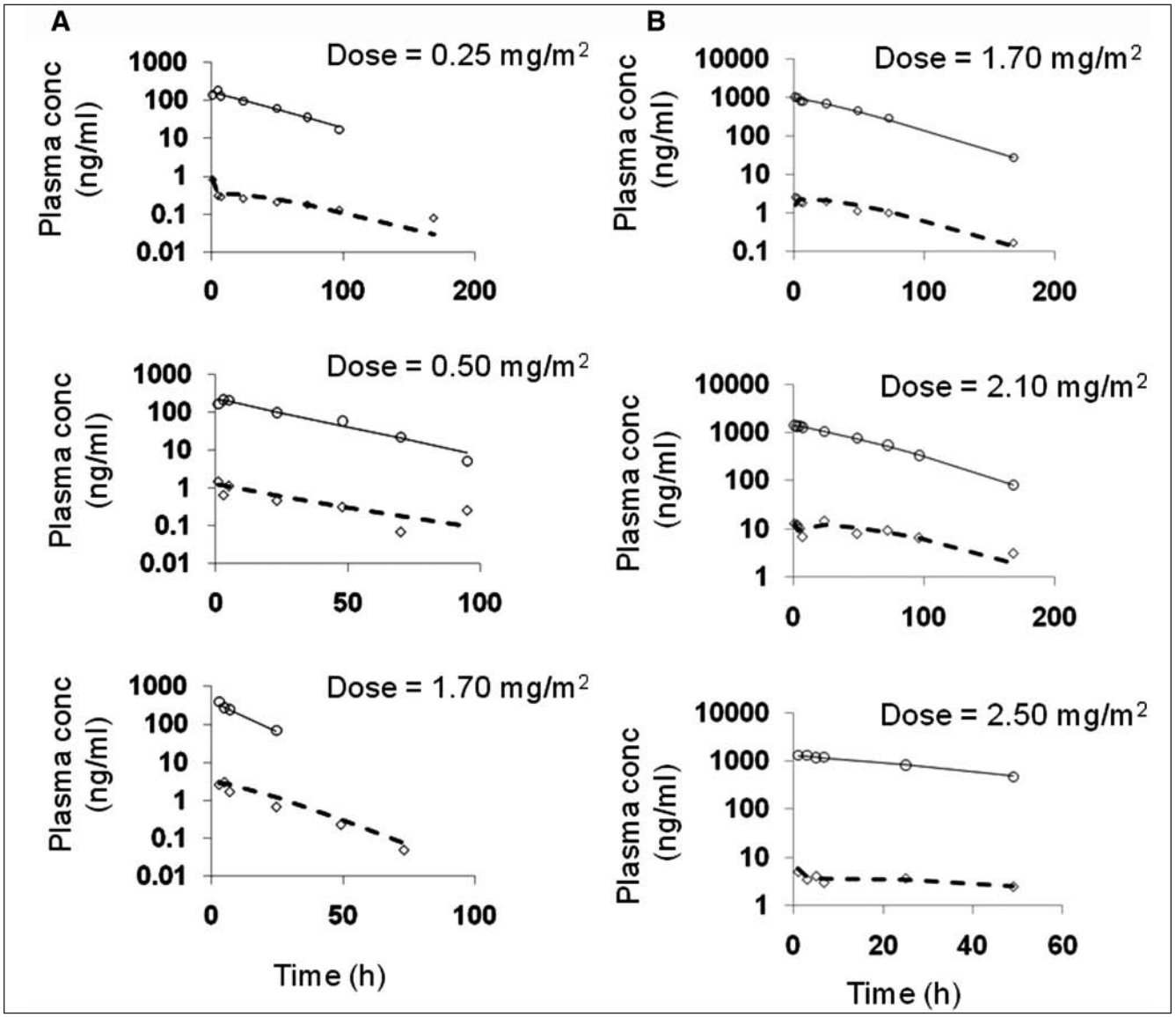

Figure 5. Representative individual plots of observed (circles) and individual predicted (sold lines) values of plasma concentrations of encapsulated and the observed (diamonds) and individual predicted (dashed lines) values of plasma concentrations of released CKD-602 in (a) patients with linear clearance of encapsulated CKD-602 and (b) patients with nonlinear clearance of encapsulated CKD-602.

these 2 drugs have been modeled using Michaelis-Menten kinetics.[26,33-35]

The interpatient variability in the disposition of S-CKD602 can be explained in part by the presence of primary or metastatic tumors located in the liver. The Vmax in patients with liver tumor is 1.5-fold higher compared with patients without liver tumor. These data suggest that patients with liver tumor may have 35% lower plasma exposure and are at risk of having a lower response potential. Most studies show a decrease in clearance of small-molecule drugs in patients with liver tumor.[36-38] This is the first study reporting an increased clearance of drug in patients with tumor involvement in the liver. The exact mechanism of this phenomenon is unknown. Recruitment of various populations of phagocytic cells (monocytes, macrophages, and dendritic cells) of the RES is involved in the immune response against tumor cell deposits in liver.[39,40] Since liposomes are mainly cleared by RES and liver is an important functional center of RES, the increased clearance of encapsulated CKD-602 may

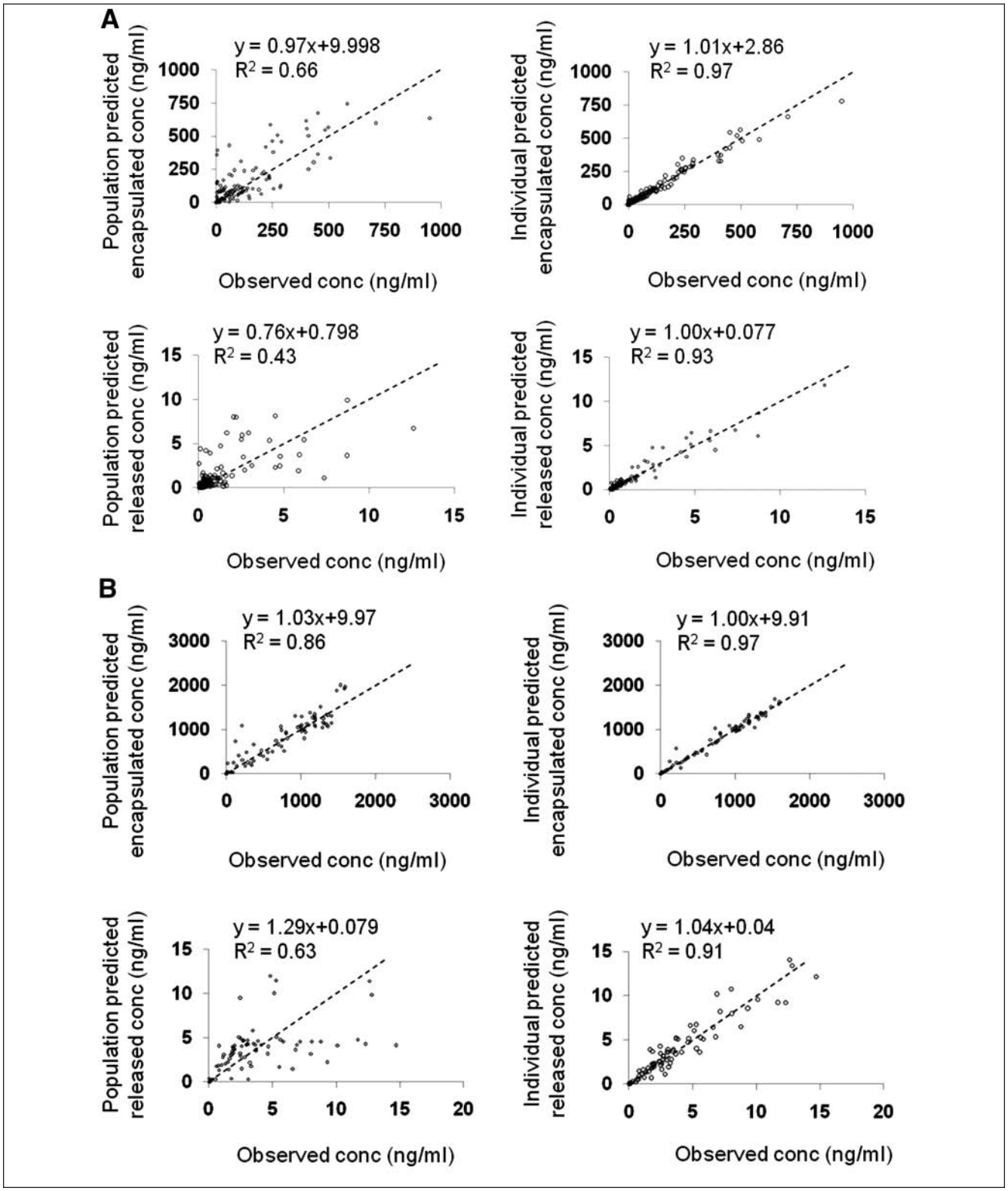

Figure 6. Observed versus population model-predicted encapsulated and released plasma concentrations for the final models in (a) patients with linear clearance of encapsulated CKD-602 and (b) patients with nonlinear clearance of encapsulated CKD-602. The dashed lines are lines of identity.

be due to enhanced RES activity in patients with liver tumor.

In patients with linear clearance of encapsulated CKD-602, patients 60 years of age and older have a reduced release rate of CKD-602 from S-CKD602 compared with patients younger than 60 years. This is consistent with our prior studies which showed that a reduced clearance of the liposomal encapsulated form of S-CKD-602 in patients 60 years of age and older and the release of drug from liposome are related to clearance of liposome.[22,23] Aging-related decrease in the function of monocytes may account for the reduced clearance of liposomal agents.[24,25] Liver tumor is not significantly associated with the pharmacokinetic variability of patients with linear clearance of encapsulated CKD-602. This may due to the lower number of patients in the group of patients with linear clearance of encapsulated CKD-602 (n = 20) compared with the patients included in the encapsulated CKD-602 pharmacokinetic analysis (n = 45). Age is not associated with the pharmacokinetic variability of patients with nonlinear clearance of encapsulated CKD-602. The results in patients with nonlinear clearance may be associated with the overall low number of patients in this group and the number of patients with complete concentration–time profiles.

The developed model did not take into account the lactone and carboxylate forms of CKD-602 since only total (lactone + hydroxy acid) drug concentrations were available on our study. Only the lactone form of CKD-602 has antitumor activity, and it undergoes a pH-dependent equilibrium with carboxylate forms. Thus, the model of encapsulated and released CKD-602 can be used only to predict total CKD-602 exposure in the encapsulated and released forms. However, the pharmacokinetics of encapsulated CKD-602 are not affected by this limitation as the drug that remains encapsulated is all in the lactone form because the pH of the core solution is around 5.4.

In conclusion, a population pharmacokinetic model was developed for encapsulated and released CKD-602 in patients with advanced solid tumors. The release rate of CKD-602 from S-CKD602 was influenced by age, and clearance of encapsulated CKD-602 was influenced by presence of liver tumor. The development of phenotypic probes of RES function in the liver of patients with and without liver tumor is needed to further evaluate these effects. The application of the population pharmacokinetic model in optimal dosing of pegylated liposomal agents needs to be further investigated to achieve a target exposure for each patient with malignant diseases.

Presented in part at the annual meeting of ASCO 2009.

Financial disclosure: Funding was provided by ALZA Corporation, Mountain View, California, and NIH/NCCR/GCRC grant 5M01 RR 00056.

REFERENCES

1. Zamboni WC, Friedland DM, Ramalingam S, et al. Relationship between the plasma and tumor disposition of STEALTH liposomal CKD-602 and macrophages/dendritic cells (MDC) in mice bearing human tumor xenografts. Paper presented at: the American Association for Cancer Research; 2006. April 1-5, 2006; Washington, DC.

2. Zamboni WC, Strychor S, Joseph E, et al. Plasma and tumor disposition of STEALTH Liposomal CKD-602 (S-CKD602) and non-liposomal CKD-602, a camptothecin analogue, in mice bearing A375 human melanoma xenograft. Paper presented at: the American Association for Cancer Research; April 1-5, 2006; Washington, DC.

3. Zamboni WC. Liposomal, nanoparticle, and conjugated formulations of anticancer agents. *Clin Cancer Res*. 2005;11:8230-8234.

4. Crul M. CKD-602. Chong Kun Dang. *Curr Opin Investig Drugs*. 2003;4:1455-1459.

5. Lee JH, Lee JM, Lim KH, et al. Preclinical and phase 1 clinical studies with Ckd-602, a novel camptothecin derivative. *Ann N Y Acad Sci*. 2000;922:324-325.

6. Yu NY, Conway C, Pena RL, Chen JY. STEALTH liposomal CKD-602, a topoisomerase I inhibitor, improves the therapeutic index in human tumor xenograft models. *Anticancer Res*. 2007; 27(4B):2541-2545.

7. Lee DH, Kim SW, Suh C, et al. Belotecan, new camptothecin analogue, is active in patients with small-cell lung cancer: results of a multicenter early phase 2 study. *Ann Oncol*. 2008;19:123-127.

8. Papahadjopoulos D, Allen TM, Gabizon A, et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci U S A*. 1991;88:11460-11464.

9. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release*. 2000;65:271-284.

10. Markman M, Gordon AN, McGuire WP, Muggia FM. Liposomal anthracycline treatment for ovarian cancer. *Semin Oncol*. 2004;31(6 suppl 13):91-105.

11. Krown SE, Northfelt DW, Osoba D, Stewart JS. Use of liposomal anthracyclines in Kaposi's sarcoma. *Semin Oncol*. 2004;31(6 suppl 13):36-52.

12. Zamboni WC. Concept and clinical evaluation of carrier-mediated anticancer agents. *Oncologist*. 2008;13:248-260.

13. Innocenti F, Kroetz DL, Schuetz E, et al. Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. *J Clin Oncol*. 2009;27:2604-2614.

14. Slatter JG, Schaaf LJ, Sams JP, et al. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [(14)C] CPT-11 in cancer patients. *Drug Metab Dispos*. 2000;28:423-433.

15. Zamboni WC, Stewart CF, Thompson J, et al. Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. *J Natl Cancer Inst*. 1998;90:505-511.

16. Stewart CF, Zamboni WC, Crom WR, et al. Topoisomerase I interactive drugs in children with cancer. *Invest New Drugs*. 1996;14:37-47.

17. Zamboni WC, Strychor S, Joseph E, et al. Plasma, tumor, and tissue disposition of STEALTH liposomal CKD-602 (S-CKD602) and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts. *Clin Cancer Res*. 2007;13:7217-7223.

18. Zamboni WC, Gajjar AJ, Mandrell TD, et al. A four-hour topotecan infusion achieves cytotoxic exposure throughout the neuraxis in the nonhuman primate model: implications for treatment of children with metastatic medulloblastoma. *Clin Cancer Res*. 1998;4:2537-2544.

19. Allen TM, Hansen C. Pharmacokinetics of stealth versus conventional liposomes: effect of dose. *Biochim Biophys Acta*. 1991; 1068:133-141.

20. Zamboni WC, Maruca LJ, Strychor S, et al. Bidirectional pharmacodynamic interaction between pegylated liposomal CKD-602 (S-CKD602) and monocytes in patients with refractory solid tumors [published online ahead of print July 14, 2010]. *J Liposome Res*.

21. Zamboni WC. Liposomal, nanoparticle, and conjugated formulations of anticancer agents. *Clin Cancer Res*. 2005;11:8230-8234.

22. Zamboni WC, Maruca LJ, Strychor S, et al. Age and body composition related-effects on the pharmacokinetic disposition of STEALH liposomal CKD-602 (S-CKD602) in patients with advanced solid tumors. Paper presented at: American Society of Clinical Oncology, June 1-5, 2007; Chicago, IL.

23. Sidone BJ, Edwards RP, Zamboni BA, Strychor S, Maruca LJ, Zamboni WC. Evaluation of body surface area (BSA) based dosing, age, and body composition as factors affecting the pharmacokinetic (PK) variability of STEALTH liposomal doxorubicin (Doxil). Paper presented at: American Association for Cancer Research, National Cancer Institute, European Organization for Research and Treatment of Cancer; October 22-26, 2007, San Francisco, CA.

24. Zamboni WC, Strychor S, Maruca L, et al. Pharmacokinetic Study of pegylated liposomal CKD-602 (S-CKD602) in patients with advanced malignancies [published online ahead of print August 12, 2009]. *Clin Pharmacol Ther*. 2009;86:519-526.

25. De Martinis M, Modesti M, Ginaldi L. Phenotypic and functional changes of circulating monocytes and polymorphonuclear leucocytes from elderly persons. *Immunol Cell Biol*. 2004;82:415-420.

26. Zamboni WC, Strychor S, Maruca L, et al. Pharmacokinetic study of pegylated liposomal CKD-602 (S-CKD602) in patients with advanced malignancies. *Clin Pharmacol Ther*. 2009;86:519-526.

27. Zamboni WC, Ramalingam S, Friedland DM, et al. Phase 1 and pharmacokinetic study of pegylated liposomal CKD-602 in patients with advanced malignancies. *Clin Cancer Res*. 2009;15:1466-1472.

28. Jones SF, Zamboni WC, Burris III HA, et al. Phase 1 and pharmacokinetic (PK) study of IHL-305 (pegylated liposomal irinotecan) in patients with advanced solid tumors. Paper presented at: ASCO; June 2009, 2009; Orlando, FL.

29. Roy V, LaPlant BR, Gross GG, Bane CL, Palmieri FM. Phase 2 trial of weekly nab (nanoparticle albumin-bound)-paclitaxel (nab-paclitaxel) (Abraxane) in combination with gemcitabine in patients with metastatic breast cancer (N0531). *Ann Oncol*. 2009;20:449-453.

30. Giles FJ, Tallman MS, Garcia-Manero G, et al. Phase 1 and pharmacokinetic study of a low-clearance, unilamellar liposomal formulation of lurtotecan, a topoisomerase 1 inhibitor, in patients with advanced leukemia. *Cancer*. 2004;100:1449-1458.

31. Gelmon K, Hirte H, Fisher B, et al. A phase 1 study of OSI-211 given as an intravenous infusion days 1, 2, and 3 every three weeks in patients with solid cancers. *Invest New Drugs*. 2004;22:263-275.

32. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science*. 2004;303:1818-1822.

33. Gabizon A, Isacson R, Rosengarten O, Tzemach D, Shmeeda H, Sapir R. An open-label study to evaluate dose and cycle dependence of the pharmacokinetics of pegylated liposomal doxorubicin. *Cancer Chemother Pharmacol*. 2008;61:695-702.

34. Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal doxorubicin: review of animal and human studies. *Clin Pharmacokinet*. 2003;42:419-436.

35. Gabizon A, Tzemach D, Mak L, Bronstein M, Horowitz AT. Dose dependency of pharmacokinetics and therapeutic efficacy of pegylated liposomal doxorubicin (DOXIL) in murine models. *J Drug Target*. 2002;10:539-548.

36. Robieux I, Sorio R, Borsatti E, et al. Pharmacokinetics of vinorelbine in patients with liver metastases. *Clin Pharmacol Ther*. 1996;59:32-40.

37. Twelves CJ, O'Reilly SM, Coleman RE, Richards MA, Rubens RD. Weekly epirubicin for breast cancer with liver metastases and abnormal liver biochemistry. *Br J Cancer*. 1989;60:938-941.

38. Wilson WH, Berg SL, Bryant G, et al. Paclitaxel in doxorubicin-refractory or mitoxantrone-refractory breast cancer: a phase 1/II trial of 96-hour infusion. *J Clin Oncol*. 1994;12:1621-1629.

39. Heuff G, van der Ende MB, Boutkan H, et al. Macrophage populations in different stages of induced hepatic metastases in rats: an immunohistochemical analysis. *Scand J Immunol*. 1993;38:10-16.

40. Gulubova M, Manolova I, Cirovski G, Sivrev D. Recruitment of dendritic cells in human liver with metastases. *Clin Exp Metastasis*. 2008;25:777-785.