

Pharmacodynamic Model of Topotecan-induced Time Course of Neutropenia¹

William C. Zamboni,² David Z. D'Argenio,
Clinton F. Stewart, Tom MacVittie,
Brian J. Delauter, Ann M. Farese,
Douglas M. Potter, Nacole M. Kubat,
David Tubergen, and Merrill J. Egorin

Program of Molecular Therapeutics and Drug Discovery [W. C. Z., B. J. D., N. M. K., M. J. E.] and Biostatistics Facility [D. M. P.], University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15213; Department of Pharmaceutical Sciences, School of Pharmacy [W. C. Z.], University of Pittsburgh, Pittsburgh, Pennsylvania 15213; Departments of Medicine [W. C. Z., M. J. E.] and Pharmacology [M. J. E.], School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213; Department of Biomedical Engineering, University of Southern California, Los Angeles, California 90089 [D. Z. D.]; Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee 38105 [C. F. S.]; Center for Pediatric Pharmacokinetics and Therapeutics, University of Tennessee, Memphis, Tennessee 38105 [C. F. S.]; Department of Pathology, University of Maryland, Baltimore, Maryland 21201 [T. M., A. M. F.]; and Department of Hematology Oncology, MD Anderson Cancer Center, Houston, Texas 77030 [D. T.]

ABSTRACT

Pharmacodynamic measures of neutropenia, such as absolute neutrophil count at nadir and neutrophil survival fraction, may not reflect the overall time course of neutropenia. We developed a pharmacokinetic-pharmacodynamic model to describe and quantify the time course of neutropenia after administration of topotecan to children and to compare this with nonhuman primates (NHPs) as a potential preclinical model of neutropenia. Topotecan was administered as a 30-min infusion daily for 5 days, repeated every 21 days. As part of a Phase I Pediatric Oncology Group study, topotecan was administered at 1.4 and 1.7 mg/m²/day without filgrastim (POG), and at 1.7, 2, and 2.4 mg/m²/day with filgrastim (POG+G). In NHPs, topotecan was administered at 5, 10, and 20 mg/m²/day without filgrastim. A pharmacokinetic-pharmacodynamic model was fit to profiles of topotecan lactone plasma concentrations and neutrophil survival fraction from cycle 1 and used to calculate topotecan lactone area under the plasma concentration-*versus*-time curve from 0 to 120 h (AUC_{LAC}) and the area between the

baseline and treatment-related neutrophil survival fraction (ABC) from 0 to 700 h. The mean ± SD neutrophil survival fraction at nadir for the POG, POG+G, and NHP groups was 0.12 ± 0.09, 0.11 ± 0.17, and 0.09 ± 0.08, respectively (*P* > 0.05). The mean ± SD for the ratio of ABC to AUC_{LAC} for the POG and NHP groups was 1.02 ± 0.38 and 0.16 ± 0.09, respectively (*P* < 0.05). The model estimate of ABC and the ratio of ABC to AUC_{LAC} in children and NHPs may better reflect sensitivity to chemotherapy-induced neutropenia.

INTRODUCTION

Topotecan is a camptothecin analogue and topoisomerase I-interactive agent that has antitumor activity against a wide range of malignancies (1, 2). Topotecan is approved for the treatment of refractory ovarian cancer, where it is administered at 1.5 mg/m²/day as a 30-min infusion for 5 days repeated every 21 days (3, 4). The primary dose-limiting toxicity associated with topotecan on this schedule is neutropenia (3–5). Moreover, the primary dose-limiting toxicity of many anticancer agents is bone marrow suppression (6–8). At present there are no reliable *in vivo* preclinical models of chemotherapy-induced bone marrow suppression (9–11). However, NHPs³ are a good model of radiation-induced bone marrow suppression and may be an appropriate model of chemotherapy-induced neutropenia and thrombocytopenia (10). Thus, we evaluated pharmacodynamic factors associated with topotecan-induced neutropenia in a NHP model and compared those data with data from a Pediatric Oncology Group Phase I study of topotecan (12).

Patients with prolonged neutropenia have a greater risk of infection than do patients who develop the same neutrophil nadir but rapidly recover their neutrophil count (6–8). Previous pharmacodynamic analyses of anticancer agents have used a linear, log-linear, or Emax model to describe the relationship between drug peak concentration or exposure (measured as the AUC or steady-state concentration), the number of neutrophils at nadir, and the percentage decrease in absolute neutrophil count (11, 13, 14). Previously, we reported a steep relationship between topotecan systemic exposure and toxicity (depicted as the percentage decrease in absolute neutrophil count; Refs. 12, 15). However, the percentage decrease in neutrophils may not

Received 12/8/00; revised 4/25/01; accepted 5/9/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by NIH Grant P41-RR01861.

² To whom requests for reprints should be addressed, at University of Pittsburgh Cancer Institute, Biomedical Science Tower, E-1040, 200 Lothrop Street, Pittsburgh, PA 15213. Phone: (412) 383-7093; Fax: (412) 624-7737; E-mail: zamboniwc@msx.upmc.edu.

³ The abbreviations used are: NHP, nonhuman primate; AUC, area under the concentration-*versus*-time curve; *k*_{pp}, rate constant describing the maturation and movement of neutrophils from the bone marrow to the blood; *k*_{ec}, rate constant describing the distribution of drug to and from the bone marrow; *k*_{in}, rate constant describing stem cell production; *k*_{out}, rate constant describing the half-life of neutrophils in peripheral blood; ABC, area between the neutrophil survival fraction-*versus*-time profile that would occur without treatment and that measured after treatment with topotecan; MPE, mean predictive error; RMSE, root mean square error.

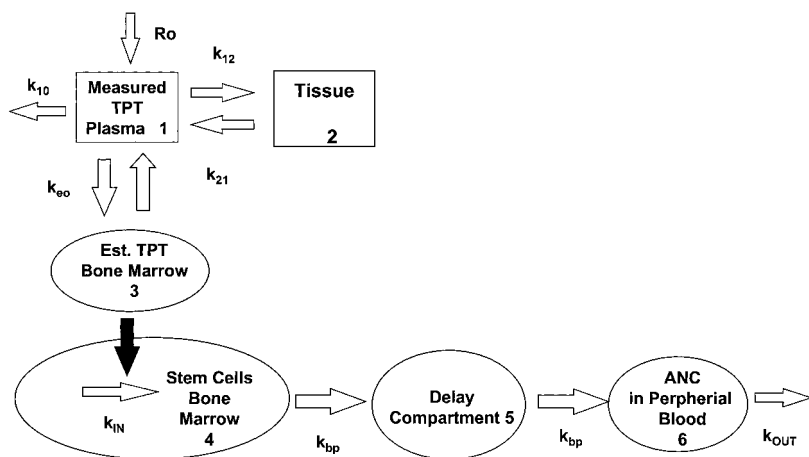


Fig. 1 Pharmacokinetic-pharmacodynamic model describing the profiles of topotecan lactone concentration *versus* time and the neutrophil survival fraction *versus* time. The parameters describing the topotecan plasma concentration-*versus*-time profile were volume of the central compartment (V_c), intercompartmental rate constants (k_{12} and k_{21}), and elimination rate constant from the central compartment (k_{10}). The parameters describing the neutrophil survival fraction-*versus*-time profile were k_{eo} , k_{IN} , k_{bp} , and k_{OUT} . Ro , dose; TPT , topotecan; ANC , absolute neutrophil count.

reflect the duration of toxicity or incorporate the time delay between drug administration and the occurrence of toxicity. Compared with the pharmacodynamic modeling of other drugs with immediate effects, modeling the myelosuppressive effects of anticancer agents must include parameters that describe the production as well as the destruction of the target cells. Pharmacodynamic measures of chemotherapy-induced neutropenia over the entire treatment cycle may provide important information. Thus, we developed a pharmacodynamic model to describe the time course of the neutrophil survival fraction in children compared with NHPs as a potential preclinical model of neutropenia after administration of topotecan daily for 5 days, repeated every 21 days.

PATIENTS AND METHODS

Patient and Subject Population. The topotecan lactone plasma concentrations and absolute neutrophil count for children were obtained from a Phase I study of topotecan in children with refractory solid tumors (POG9275; Ref. 12). The POG study was approved by the institutional review board at each of the collaborating institutions. The pharmacokinetic and pharmacodynamic studies were performed on cycle 1 only. In this Phase I study, topotecan was administered as a 30-min infusion daily for 5 consecutive days, repeated every 21 days. The dosages of topotecan evaluated were 1.4 and 1.7 mg/m²/day without subsequent filgrastim and 1.7, 2.0, and 2.4 mg/m²/day with subsequent filgrastim. Filgrastim was administered at 5 μg/kg/day, starting 24 h after the last day of topotecan and continued until the neutrophil count exceeded 10,000/μl for a minimum of 10 days. In heavily pretreated children, the maximum tolerated dosage of topotecan administered with or without subsequent filgrastim was 2.0 and 1.4 mg/m²/day, respectively. In both cases, the dose-limiting toxicity was bone marrow suppression, involving both platelets and neutrophils. The number of patients evaluated after administration of topotecan with and without subsequent filgrastim was 10 and 11, respectively.

In the NHP study, adult male rhesus monkeys (*Macaca mulatta*), weighing 5–10 kg, were given topotecan without subsequent filgrastim. This study was approved by the Institutional Animal Care and Use Committee at the University of

Maryland, Baltimore, Maryland. The pharmacokinetic and pharmacodynamic studies were performed on cycle 1 only. In a previous NHP study (16), standard dosages of topotecan did not induce significant myelosuppression. Therefore, higher dosages of topotecan were administered to NHPs in the present study. Specifically, topotecan was administered at 5 ($n = 3$), 10 ($n = 3$), and 20 ($n = 3$) mg/m²/day as a 30-min infusion for 5 consecutive days, repeated every 21 days. Pharmacokinetic studies were performed on three NHPs per dosage group. However, because of logistical issues, we were only able to perform pharmacodynamic studies on two NHPs per dosage group.

Blood Counts. Absolute neutrophil counts were measured at least twice per week and every other day in the pediatric and NHP studies, respectively. The absolute neutrophil survival fraction was calculated as the ratio of the lowest absolute neutrophil count measured on days following treatment to the absolute neutrophil count on the day prior to the start of treatment.

Pharmacokinetic Sampling and Processing. Blood samples for pharmacokinetic analysis were obtained on day 1 of cycle 1 in the pediatric and NHP studies. For each study, blood samples were obtained before and at 0.25, 0.5, 1, 1.5, 3, and 6 h after the end of the topotecan infusion. At each time, 3 ml of blood were obtained from a site contralateral to that of i.v. administration and placed into heparinized tubes. Within 2 min, blood samples were centrifuged at 2000 × *g* for 2 min, after which 200 μl of plasma were added to 800 μl of cold methanol (−20°C), vortexed for 10 s, and centrifuged at 2000 × *g* for 2 min. The resulting methanolic supernatant was decanted and stored at −70°C until analyzed.

Plasma topotecan concentrations were measured by a previously described isocratic high-performance liquid chromatography assay using fluorescence detection (12, 17, 18). The methanolic supernatant was used to measure topotecan lactone concentration. The total topotecan total (sum of lactone and hydroxy acid) concentration was measured by adding 20 μl of 20% phosphoric acid to 400 μl of the methanolic supernatant.

Pharmacokinetic and Pharmacodynamic Analysis. A pharmacokinetic-pharmacodynamic model was fit to the profiles of topotecan lactone plasma concentration *versus* time and

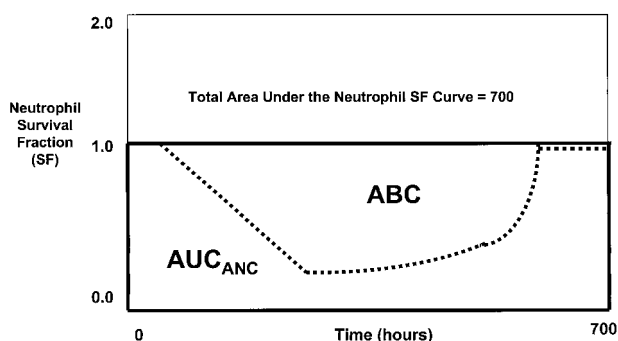


Fig. 2 Calculation of area (ABC) between the neutrophil survival fraction curve after administration of topotecan (AUC_{ANC}) and without treatment ($AUC_{Base} = 700$). The calculation was based on the following equation: $ABC = 700 - AUC_{ANC}$.

the neutrophil survival fraction *versus* time (Fig. 1). This was accomplished using maximum likelihood estimation in the ADAPT II modeling program (19). Individual parameters estimated in the two-compartment pharmacokinetic section of the model included the volume of the central compartment (V_{c1}), the intercompartmental rate constants (k_{12} and k_{21}), and the elimination rate constant from the central compartment (k_{10}). Individual parameters and standard equations were used to calculate systemic clearance (CL_{sys}) and elimination half-life ($t_{1/2}$; Refs. 19, 20). Individual parameter estimates were used to calculate the area under the topotecan lactone (AUC_{LAC}) and total (AUC_{TOT}) plasma concentration-*versus*-time curves from zero to infinity, using the log trapezoidal method (19). The ratio of topotecan lactone to total form was calculated as the ratio of AUC_{LAC} to AUC_{TOT} . For the pharmacodynamic analysis, the topotecan lactone AUC from 0 to 120 h was calculated from the simulated concentration-*versus*-time profile from 0 to 120 h (*i.e.*, after five consecutive daily doses) based on the day 1 pharmacokinetic parameters.

The pharmacodynamic section of the model was modified from an Indirect Response Model with production linked to an effect compartment (IRMLINK) in the ADAPT Model Library (19, 21). In our model, the concentration of topotecan in the bone marrow was defined as compartment-3, the effect compartment in which topotecan inhibits stem cell production was defined as compartment-4, and the measured reduction in absolute neutrophil count in the blood was defined as compartment-6. In addition, a delay compartment (compartment-5) and the rate constant describing the maturation and movement of neutrophils from the bone marrow to the blood (k_{bp}) were used to represent the time delay between the inhibition of stem cell production by topotecan and the measured effect on neutrophil count in the blood. The delay compartment and rate constant used to describe time delay were modified from the delay-chain model used to describe oral absorption of phosphates in rats (22). In the model development, the number of delay compartments varied from 1 to 10; however, a single delay compartment produced the best fit of the data.

Additional individual parameters estimated in the pharmacodynamic section of the model included the rate constant describing the distribution of drug to and from the bone marrow

Table 1 Topotecan lactone pharmacokinetic parameters in a NHP model

Topotecan lactone pharmacokinetic parameters in the NHPs after administration of topotecan at 5, 10, and 20 mg/m²/day. Mean \pm SD and median (range) are presented for area under the plasma concentration-*versus*-time curve from 0 to infinity (AUC), systemic clearance (CL), and volume of the central compartment (V_c).

Parameter	Topotecan lactone (mg/m ² /day)		
	5	10	20
	Mean \pm SD	Mean \pm SD	Mean \pm SD
AUC (ng/ml-h)	138.5 \pm 63.4 123.5 (92.5–260.4)	283.9 \pm 48.5 259.4 (240.8–351.6)	542.5 \pm 172.9 650.2 (298.6–678.8)
CL (liters/h/m ²)	30.4 \pm 13.0 21.7 (17.6–51.6)	23.2 \pm 13.7 27.2 (4.8–37.6)	21.8 \pm 9.5 18.5 (9.6–28.4)
V_c (liters)	20.7 \pm 13.4 17.0 (9.3–42.3)	39.9 \pm 14.4 46.1 (20.0–53.9)	51.5 \pm 25.8 37.0 (29.7–87.7)

(k_{co}), the rate constant describing stem cell production (k_{IN}), the concentration of topotecan that inhibits k_{IN} by 50% (IC_{50}), and the rate constant describing the half-life of neutrophils in peripheral blood (k_{OUT}). The half-life of neutrophils in peripheral blood corresponds to a k_{OUT} of 0.099 h⁻¹ (22). Chemotherapy does not affect the half-life of neutrophils; thus, in our model the k_{OUT} was fixed at 0.099 h⁻¹ for each subject. At baseline the $k_{IN} = k_{OUT}$; thus, the initial estimate of k_{IN} was 0.099 h⁻¹, and a final parameter value was estimated for each subject.

The area (ABC) between the neutrophil survival fraction-*versus*-time profile that would occur without treatment and that measured after treatment with topotecan from 0 to 700 h is depicted in Fig. 2. The ABC was calculated by the log-trapezoidal method. The degree of neutropenia associated with plasma drug exposure was calculated as the ratio of ABC to AUC_{LAC} from 0 to 120 h.

Model Evaluation and Statistical Analysis. The predictive performance of the model was determined by the MPE, RMSE, and residuals. The Mann-Whitney test was performed on k_{IN} , IC_{50} , neutrophil nadir, ABC, and the ratio of ABC to AUC_{LAC} , using StatXact-3 software (Version 3.1; Cytel Software Corp., Cambridge, MA).

RESULTS

Topotecan Pharmacokinetics in NHPs. The mean \pm SD model fits (*i.e.*, R^2) to the topotecan lactone and total plasma concentration-*versus*-time profiles were 0.98 \pm 0.01 and 0.97 \pm 0.02, respectively. The pharmacokinetic parameters for topotecan lactone and total topotecan are summarized in Tables 1 and 2, respectively. The ratios of AUC_{LAC} to AUC_{TOT} at 5, 10, and 20 mg/m² were 0.57 \pm 0.06, 0.52 \pm 0.05, and 0.37 \pm 0.06, respectively. The \sim 2-fold reductions in topotecan total clearance and the ratio of AUC_{LAC} to AUC_{TOT} suggest nonlinear clearance of the inactive hydroxy acid between topotecan doses of 10 and 20 mg/m².

Absolute Neutrophil Count Survival Fraction at Nadir. The neutrophil survival fractions at nadir for children administered topotecan with and without subsequent filgrastim and in

Table 2 Topotecan total pharmacokinetic parameters in a NHP model

Topotecan total pharmacokinetic parameters in the NHPs after administration of topotecan at 5, 10, and 20 mg/m²/day. Mean \pm SD and median (range) are presented for area under the plasma concentration-versus-time curve from 0 to infinity (AUC), systemic clearance (CL), and volume of the central compartment (V_c).

Parameter	Topotecan lactone (mg/m ² /day)		
	5	10	20
	Mean \pm SD Median (Range)	Mean \pm SD Median (Range)	Mean \pm SD Median (Range)
AUC (ng/ml·h)	324.7 \pm 120.1 222.34 (179.0–505.0)	562.0 \pm 162.2 470.0 (426.1–790.0)	1808.0 \pm 68.0 1810.4 (1740.0–1876.0)
CL (liters/h·m ²)	16.4 \pm 6.3 11.3 (8.3–25.7)	17.5 \pm 4.3 19.9 (11.5–21.2)	6.2 \pm 3.9 6.2 (2.4–10.1)
V _c (liters)	26.0 \pm 4.0 15.0 (20.9–30.6)	22.5 \pm 11.3 20.0 (10.0–37.4)	10.7 \pm 2.9 10.7 (7.8–13.6)

NHPs administered topotecan without filgrastim are represented in Fig. 3. The mean \pm SD (median; range) neutrophil survival fraction at nadir in children administered topotecan with and without subsequent filgrastim and in NHPs administered topotecan without subsequent filgrastim were 0.11 \pm 0.17 (0.05; 0.0–0.56), 0.12 \pm 0.09 (0.12; 0.01–0.30), and 0.09 \pm 0.08 (0.10; 0.01–0.25), respectively. The differences for neutrophil survival fraction at nadir among all groups were not statistically significant ($P > 0.05$).

Pharmacodynamic Model of the Time Course of Neutrophil Survival Fraction. The pharmacodynamic analysis was performed on 10, 11, and 6 subjects from children administered topotecan with and without subsequent filgrastim and in NHPs administered topotecan without subsequent filgrastim, respectively. The mean \pm SD (median; range) number of pharmacodynamic data points (*i.e.*, survival fraction of neutrophils) per subject from children administered topotecan with and without subsequent filgrastim and from NHPs administered topotecan without subsequent filgrastim were and 9.3 \pm 2.6 (10; 6–15), 7.9 \pm 2.9 (7; 5–14), and 8.9 \pm 0.7 (9.0; 8–10), respectively. The greater number (*i.e.*, >10) of pharmacodynamic data points for some patients administered topotecan with and without filgrastim was attributable to prolonged neutropenia. However, the model fit of the data was similar for subjects with a short duration of neutropenia and few neutrophil measurements (*e.g.*, $n = 5$) compared with subjects with a prolonged duration of neutropenia and a greater number of neutrophils (*e.g.*, $n > 10$). In addition, patients with neutropenia lasting longer than the 21st day (*i.e.*, 504 h after the start of treatment) of cycle 1 were delayed in receiving cycle 2. Thus, the ABC was calculated from 0 to 700 h in all patients and NHPs.

A representative time course and model fit of the neutrophil survival fraction in a patient administered topotecan without subsequent filgrastim are represented in Fig. 4. A representative time course and model fit of the neutrophil survival fraction in a NHP administered topotecan without subsequent filgrastim are represented in Fig. 5. The mean \pm SD model fits (*i.e.*, R^2) of the neutrophil survival fraction in children admin-

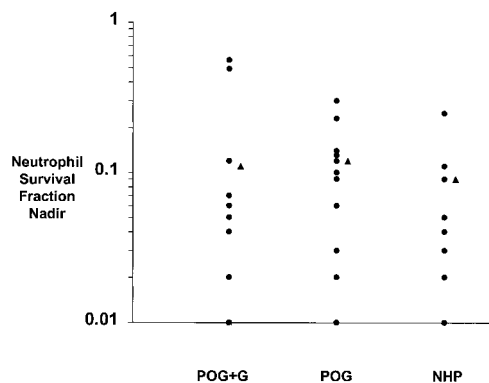


Fig. 3 Measured neutrophil survival fraction at nadir for children. Individual data points (●) and average (▲) neutrophil survival fraction at nadir are presented for NHPs administered topotecan without subsequent filgrastim (NHP) and for children administered topotecan without subsequent filgrastim (POG) and with subsequent filgrastim (POG+G).

istered topotecan with and without subsequent filgrastim and in NHPs administered topotecan without filgrastim were 0.85 \pm 0.08, 0.85 \pm 0.18, and 0.80 \pm 0.21, respectively. The MPEs for all data, for children administered topotecan with and without subsequent filgrastim, and for NHPs administered topotecan without filgrastim were -0.08 ± 0.15 , -0.10 ± 0.12 , -0.06 ± 0.15 , and -0.07 ± 0.10 , respectively. The RMSE for all data, for children administered topotecan with and without subsequent filgrastim, and for NHPs administered topotecan without filgrastim was 0.28, 0.32, 0.24, and 0.26, respectively. Residuals of prediction (predicted surviving fraction – observed surviving fraction) plotted against time for all patients after the start of topotecan are presented in Fig. 6. Negative residuals from 0 to 96 h were explained by the inherent variability in neutrophil counts at baseline and immediately after administration and by transient increases in neutrophils after topotecan administration. Negative residuals occurring ≥ 384 h after the start of treatment were explained by transient increases in neutrophils during recovery and an overshoot of neutrophil counts observed in some patients.

Parameters describing the pharmacodynamic model of topotecan-induced neutropenia are presented in Table 3. The k_{co} , ABC, and k_{bp} between the three groups were not statistically different ($P > 0.05$). However, the difference between the ratio of ABC to AUC_{LAC} for NHPs administered topotecan without filgrastim was statistically significantly different ($P < 0.05$) from those for children administered topotecan with or without filgrastim. The ratio of ABC to AUC_{LAC} is represented in Fig. 7. In addition, the difference between k_{IN} for NHPs administered topotecan without filgrastim was statistically significantly different ($P < 0.05$) from those for children administered topotecan with or without filgrastim. The differences between the ratio of ABC to AUC_{LAC} and k_{IN} for children administered topotecan with and without filgrastim were not statistically significant ($P > 0.05$). The IC₅₀ for children administered topotecan without filgrastim was significantly ($P < 0.05$) lower than those for children administered topotecan with filgrastim and the NHP group. Ultimately, the lack of an impact of filgrastim on the degree of neutropenia and ABC may be attrib-

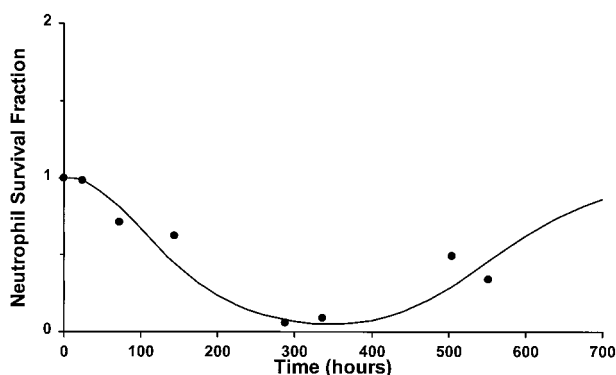


Fig. 4 Representative time course of the neutrophil survival fraction in a child administered topotecan without subsequent filgrastim. The patient was administered topotecan at $1.4 \text{ mg/m}^2/\text{day}$ daily for 5 days. Individual neutrophil survival fraction points (●) and the best-fit line (—) of the data are presented.

unable to generation of data from cycle 1 only, whereas filgrastim effects on neutrophil recovery are often greater on cycle 2 and beyond compared with cycle 1.

DISCUSSION

Several investigators have developed pharmacodynamic models of neutropenia (23–25). Our model describes the time course of neutropenia and quantifies the extent and duration of toxicity depicted by the time course of neutropenia. The present study is the first to compare the relationship between topotecan exposure and the severity and duration of neutropenia in children and NHPs, with the goal of evaluating NHPs as a model of topotecan-induced neutropenia in humans. The model estimate of ABC at similar topotecan dosages and the ratio of ABC to AUC_{LAC} depicted the differences in the degree and severity of neutropenia in children administered topotecan with or without subsequent filgrastim and in NHPs receiving topotecan without subsequent filgrastim. Application of this approach may provide a mathematical means to describe the differences in the severity and duration of neutropenia induced by anticancer agents. Moreover, the clinical relevance of this model is underscored by the need to correlate drug exposure of anticancer drugs and the degree and duration of neutropenia and to ideally predict which patients may be at increased risk of neutropenia-related complications (6–8, 25, 26).

Minami *et al.* (23) previously developed an indirect response model to describe the time course of leukopenia associated with anticancer agents in patients not receiving colony stimulating factors. In their model, a three-compartment pharmacokinetic model describing the systemic disposition of paclitaxel was used as input to the pharmacodynamic portion of the model describing the time course of leukocytes in peripheral blood. This is similar to our model; however, in addition to the two-compartment model describing the systemic disposition of topotecan, we added an additional uncoupled compartment (compartment-3) and rate constant (k_{co}) describing the concentration of topotecan in the bone marrow and distribution of drug to and from the bone marrow, respectively. The uncoupled compartment may make our model more physiologically relevant

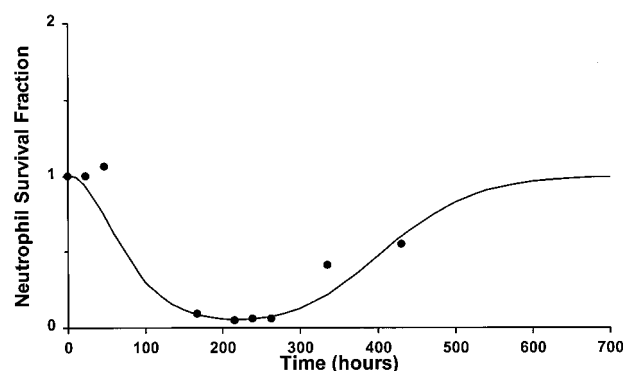


Fig. 5 Representative time course of the neutrophil survival fraction in a NHP administered topotecan without subsequent filgrastim. The NHP was administered topotecan at $20 \text{ mg/m}^2/\text{day}$ daily for 5 days. Individual neutrophil survival fraction points (●) and the best-fit line (—) of the data are presented.

based on the assumption that only a fraction of the drug exposed in the plasma reaches peripheral tissue, such as the bone marrow (27, 28). Pharmacokinetic modeling using uncoupled compartments to describe drug concentrations in peripheral tissues, where it is difficult to determine an accurate estimate of the tissue volume, has been used to model drug concentrations in tumors and cerebrospinal fluid (27, 28).

The basic structure of the pharmacodynamic model by Minami *et al.* (23) was a physiological indirect-response model. In addition, the model assumed differentiation stages of sensitivity for myeloid cells to anticancer drugs, where exposure to a drug during the sensitive period as a function of time was used to inhibit the production of leukocytes in bone marrow. Our model was modified from an indirect response model with production linked to an effect compartment (19, 21) and did not require the factor of fractional inhibition used in the model by Minami *et al.* (23). Minami *et al.* used a lag time to describe the delay between effect of paclitaxel on leukocytes in bone marrow and the measured response in the peripheral blood. The ADAPT modeling program that was used to generate our model does not allow for the incorporation of a lag time within the structure of a model; however, the use of the rate constant (k_{bp}) and delay compartment (compartment-5), modified from the delay-chain model used to describe oral absorption, produced the same effect as a lag time (22). The use of the delay chain in our pharmacodynamic model may be more physiologically relevant because the maturation and movement of neutrophils from the bone marrow to the blood is a gradual process and not an absolute time delay as would be depicted by a lag time. The measures of predictive performance (*i.e.*, MPE and RMSE) were similar for our model and the model of Minami *et al.* (23). In addition, the relatively high coefficient of variation for the parameters estimated in our model were similar to those reported by Minami *et al.* Thus, the high interpatient variability in pharmacodynamic parameters may be a result of clinically relevant patient-specific characteristics, such as prior treatment, bone marrow reserve, and the coadministration of colony-stimulating factors.

Previous studies have used percentage decrease in neutro-

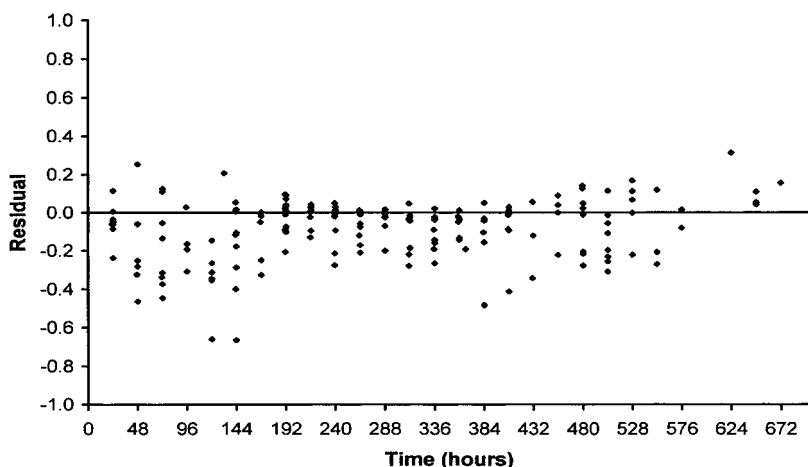


Fig. 6 Residuals (predicted value - observed value) of prediction for surviving fraction of neutrophils versus time after treatment of topotecan in NHPs administered topotecan without subsequent filgrastim and in children administered topotecan without and with subsequent filgrastim.

Table 3 Parameters describing topotecan-induced time course of neutropenia

Pharmacodynamic parameters describing topotecan-induced time course of neutropenia in NHPs administered topotecan without filgrastim (NHP group) and children administered topotecan without filgrastim (POG group) and with filgrastim (POG+G group). Mean \pm SD and median (range) are presented for area (ABC) between the neutrophil survival fraction-versus-time curve that would occur without treatment and after administration of topotecan; the ratio of ABC to topotecan lactone plasma AUC from 0 to 700 hours (ABC/AUC_{LAC}); the rate of stem cell production (k_{IN}); the concentration of topotecan that inhibits k_{IN} by 50% (IC_{50}); the rate constant describing the delay in effect between the bone marrow and blood (k_{pb}); and the rate constant describing distribution of topotecan from the plasma into the bone marrow (k_{eo}).

Parameter	Group		
	NHP Mean \pm SD Median (Range)	POG (Mean \pm SD Median (Range))	POG + G Mean \pm SD Median (Range)
ABC	379 \pm 127 350 (236-624)	409 \pm 110 382 (213-564)	335 \pm 126 347 (93-522)
ABC/AUC_{LAC}	0.16 \pm 0.09 ^a 0.11 (0.09-0.33)	1.02 \pm 0.38 0.95 (0.58-1.85)	0.82 \pm 0.39 0.70 (0.27-1.49)
k_{IN}	0.13 \pm 0.21 ^a 0.03 (0.01-0.59)	0.04 \pm 0.02 0.03 (0.01-0.09)	0.05 \pm 0.04 0.03 (0.02-0.15)
IC_{50}	0.35 \pm 0.31 0.25 (0.01-0.94)	0.04 \pm 0.07 ^a 0.007 (0.001-0.20)	0.33 \pm 0.67 0.024 (0.001-2.02)
k_{pb}	0.05 \pm 0.03 0.04 (0.01-0.11)	0.03 \pm 0.03 0.02 (0.01-0.015)	0.04 \pm 0.01 0.04 (0.03-0.06)
k_{eo}	0.04 \pm 0.03 0.05 (0.004-0.09)	0.02 \pm 0.03 0.01 (0.004-0.1)	0.08 \pm 0.04 0.08 (0.01-0.15)

^a Parameter values that are statistically ($P < 0.05$) different from the other groups.

phils at the nadir and the fraction of neutrophils at nadir compared with baseline as pharmacodynamic measures of chemotherapy-induced neutropenia (13, 14, 26). However, these measures may not adequately characterize the pharmacody-

dynamic differences between drugs and patients with regard to chemotherapy-induced neutropenia. For example, as depicted in Fig. 3, the topotecan-induced neutrophil survival fractions at nadir were similar ($P > 0.05$) in children administered topotecan with filgrastim (0.11 ± 0.17) and without filgrastim (0.12 ± 0.09) and in NHPs administered topotecan without filgrastim (0.09 ± 0.08). However, the time courses of neutropenia were significantly different among the groups at similar topotecan dosages (Figs. 4 and 5). The ratio of ABC to AUC_{LAC} represented the differences in sensitivities of topotecan-induced neutropenia among the groups.

There is a need to develop preclinical models of chemotherapy-induced neutropenia and to evaluate the utility of colony-stimulating factors in reducing the severity of this toxicity. NHPs are used as models for radiation-induced bone marrow suppression (10, 29, 30); however, it is unclear whether a NHP is a good model of chemotherapy-induced bone marrow suppression. The standard regimen of topotecan in the treatment of solid tumors is 1.5 mg/m²/day in adults (31, 32) and 2.0-2.4 mg/m²/day in children (11, 12), with dosages administered daily for 5 days and repeated every 21 days. Rowinsky *et al.* (33) achieved a maximum tolerated dosage of 4.5 mg/m²/day daily for 5 days, repeated every 21 days, in adults with refractory or relapsed acute leukemia. In a previous study using NHPs, topotecan produced very limited neutropenia (16). Thus, the starting dosage evaluated in our NHP study was 5 mg/m²/day for 5 consecutive days administered without subsequent filgrastim. The starting and two subsequent dosages (10 and 20 mg/m²/day) of topotecan administered without subsequent filgrastim did not produce significant neutropenia or thrombocytopenia in the NHPs. The resistance of NHPs to topotecan-induced neutropenia is represented by the 6.4-fold lower ratio of ABC to AUC_{LAC} in NHPs administered topotecan without subsequent filgrastim compared with children administered topotecan without subsequent filgrastim ($P < 0.05$).

The topotecan lactone clearances in the NHP model after administration of 5 mg/m² (30.4 ± 13.0 liters/h/m²), 10 mg/m² (23.2 ± 13.7 liters/h/m²), and 20 mg/m² (21.8 ± 9.5 liters/h/m²) are similar to those reported in the POG study (16.7 ± 6.5 liters/h/m²), from which we analyzed the absolute neutrophil

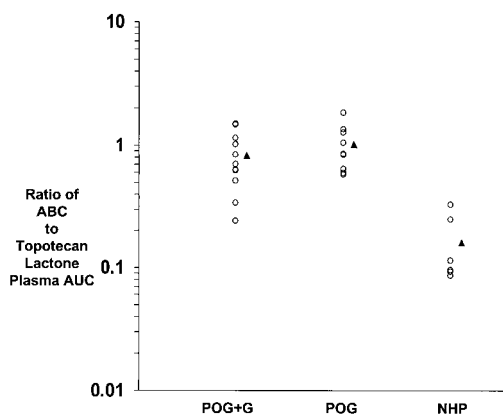


Fig. 7 Ratio of ABC to AUC_{LAC} from 0 to 700 h. Individual data points (○) and average (▲) ratio of ABC to AUC_{LAC} are presented for NHPs administered topotecan without subsequent filgrastim (NHP) and children administered topotecan without (POG) and with (POG+G) subsequent filgrastim.

count data, and in a study at St. Jude Children's Research Hospital (29.2 ± 6.2 liters/h/m²) in which topotecan was administered as a 30-min infusion (12, 34). In addition, the ratio of AUC_{LAC} to AUC_{TOT} in the NHP model is similar to those reported in the POG and St. Jude studies (12, 34). Thus, the resistance of the NHP model to topotecan-induced neutropenia is not attributable to altered systemic pharmacokinetics. In addition, the similar ($P > 0.05$) k_{eo} and k_{bp} values suggest that the difference in the severity of neutropenia between NHPs and children is not related to topotecan exposure in the bone marrow or to a delay in maturity or movement of cells from the bone marrow to the peripheral blood. The statistical difference in k_{IN} between the NHPs administered topotecan without subsequent filgrastim and the children administered topotecan with and without filgrastim suggests that the sensitivity difference is related to inhibition of stem cells. Erickson-Miller *et al.* (11) reported the differential toxicity of camptothecin, topotecan, and 9-aminocamptothecin to human, canine, and murine myeloid progenitors *in vitro*. The murine myeloid progenitors were 21-fold less sensitive to topotecan than human myeloid progenitors. However, canine myeloid progenitors were more sensitive to camptothecins than human myeloid progenitors. At present, there are no *in vitro* studies evaluating the sensitivity to camptothecin-induced neutropenia in NHP stem cells.

We developed a pharmacokinetic-pharmacodynamic model that describes the plasma concentration-*versus*-time profile and the time course of the neutrophil survival fraction after administration of topotecan. The model estimated the area (ABC) between the neutrophil survival fraction curve without treatment and after administration of topotecan as a measure of the severity and duration of neutropenia. The ABC in NHPs administered topotecan at dosages similar to those administered to children depicted the differences in the degree and duration of neutropenia in children. The ratio of ABC to AUC_{LAC} depicted the differences in topotecan-induced neutropenia in children administered topotecan with and without filgrastim and in NHPs administered topotecan without filgrastim. Application of this model may provide a way to quantitate the differences in se-

verity and duration of neutropenia induced by anticancer agents. In addition, this model can be used to evaluate the effects of colony-stimulating factors on the rate of recovery and the duration of neutropenia (34, 35, 36, 37). The clinical significance of this study is underscored by the need to develop clinically relevant pharmacodynamic measures of chemotherapy-induced neutropenia. This information could be used in the design of future studies of topotecan, and the pharmacodynamic modeling concepts might be generalizable to other drugs that induce neutropenia. Future studies will validate our pharmacokinetic-pharmacodynamic model in patients receiving standard dosages of topotecan and in patients receiving dosages of 7.5 mg/m²/day as part of bone marrow transplant regimens. In addition, future prospective studies are required to determine the relationship between ABC and clinically significant outcomes such as neutropenic fever.

REFERENCES

- Pommier, Y. DNA topoisomerase I and II in cancer chemotherapy: update and perspectives. *Cancer Chemother. Pharmacol.*, 32: 103–108, 1993.
- Pommier, Y., Leteurtre, F., Fesen, M. R., Fujimori, A., Bertrand, R., Solary, E., Kohlhagen, G., and Kohn, K. W. Cellular determinants of sensitivity and resistance to DNA topoisomerase inhibitors. *Cancer Invest.*, 12: 530–542, 1994.
- Belinson, J., Kennedy, A., and Webster, K. Preliminary results of a Cleveland Clinic Cancer Center Gynecologic Oncology Program phase 2 trial of topotecan administered on a 3-day schedule as salvage therapy of platinum and paclitaxel refractory ovarian cancer. *J. Clin. Oncol.*, 18: 369a, 1999.
- Swisher, E. M., Mutch, D. G., Rader, J. S., Elbendary, A., and Herzog, T. J. Topotecan in platinum- and paclitaxel-resistant ovarian cancer. *Gyn. Oncol.*, 66: 480–486, 1997.
- O'Reilly, S., Rowinsky, E., Slichenmyer, W., Donehower, R. C., Forastiere, A., Ettinger, D., Chen, T. L., Sartorius, S., Bowling, K., Smith, J., Brubaker, A., Lubejko, B., Ignacio, V., and Grochow, L. Phase I and pharmacologic studies of topotecan in patients with impaired hepatic function. *J. Natl. Cancer Inst. (Bethesda)*, 88: 817–824, 1996.
- Pizzo, P. A. Fever in immunocompromised patients. *N. Engl. J. Med.*, 34: 893–900, 1999.
- Pizzo, P. A. Management of fever in patients with cancer and treatment-induced neutropenia. *N. Engl. J. Med.*, 328: 1323–1332, 1993.
- Pizzo, P. A. Granulocytopenia and cancer therapy: past problems, current solutions, future challenges. *Cancer (Phila.)*, 54: 2649–2661, 1984.
- Zamboni, W. C., Stewart, C. F., Thompson, J., Santana, V. M., Cheshire, P. J., Richmond, L. B., Luo, X., Poquette, C., Houghton, J. A., and Houghton, P. J. Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. *J. Natl. Cancer Inst. (Bethesda)*, 90: 505–511, 1998.
- MacVittie, T. J., Farese, A. M., Smith, W. G., Baum, C. M., Burton, E., and McKearn, J. P. Myelopoietin, an engineered chimeric IL-3 and G-CSF receptor agonist, stimulates multilineage hematopoietic recovery in a nonhuman primate model of radiation-induced myelosuppression. *Blood*, 95: 837–845, 2000.
- Erickson-Miller, C. L., May, R. D., Tomaszewski, J., Osborn, B., Murphy, M. J., Page, J. G., and Parchment, R. E. Differential toxicity of camptothecin, topotecan, and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) *in vitro*. *Cancer Chemother. Pharmacol.*, 39: 467–472, 1997.
- Tubergen, D. G., Stewart, C. F., Pratt, C. B., Zamboni, W. C., Winick, N., Santana, V. M., Dryer, Z. A., Kurtzberg, J., Bell, B., Grier, H., and Vietti, T. J. Phase I trial and pharmacokinetic (PK) and phar-

macrodynamics (PD) study of topotecan using a five-day course in children with refractory solid tumors: a Pediatric Oncology Group Study. *J. Pediatr. Hematol. Oncol.*, *18*: 352–361, 1996.

13. Jodrell, D. I., Egorin, M. J., Canetta, R. M., Langenberg, P., Goldbloom, E. P., Burroughs, J. N., Goodlow, J. L., Tan, S., and Wiltshaw, E. Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. *J. Clin. Oncol.*, *10*: 520–528, 1992.
14. Gianni, L., Kearns, C. M., Giani, A., Capri, G., Vigano, L., Lacatelli, A., Bonadonna, G., and Egorin, M. J. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. *J. Clin. Oncol.*, *13*: 180–190, 1995.
15. Stewart, C. F., Zamboni, W. C., Crom, W. R., Gajjar, A. J., Heideman, R. L., Furman, W. L., Meyer, W. H., Houghton, P. J., and Pratt, C. B. Topoisomerase I interactive drugs in children with cancer. *Investig. New Drugs*, *14*: 37–47, 1996.
16. Zamboni, W. C., Gajjar, A. J., Mandrell, T. D., Einhaus, S. I., Danks, M. K., Rogers, W. P., Heideman, R. L., Houghton, P. J., and Stewart, C. F. 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.*, *4*: 2537–2544, 1998.
17. Stewart, C. F., Baker, S. D., Crom, W. R., and Pratt, C. B. Clinical pharmacokinetics of topotecan (T) in children with cancer. *Proc. Am. Assoc. Cancer Res.*, *34*: 395, 1993.
18. Rosing, H., Doyle, E., Davies, B. E., and Beijnen, J. High-performance liquid chromatographic determination of the novel antitumor drug topotecan and topotecan as the total of the lactone plus carboxylate forms in human plasma. *J. Chromatogr. Biol. Biomed. Appl.*, *668*: 107–111, 1997.
19. D'Argenio, D. Z., and Schumitzky, A. ADAPT II User's Guide: Pharmacokinetic and Pharmacodynamic Systems Analysis Software. Los Angeles: Biomedical Simulations Resource, 1997.
20. Gibaldi, M., Perrier D. *Pharmacokinetics*, Ed. 2. New York: Dekker, 1982.
21. Dayneka, N. L., Garg, V., Jusko, W. J. Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokinet. Biopharm.*, *21*: 457–478, 1993.
22. Kayne, L. H., D'Argenio, D. Z., Meyer, J. H., Jamgotchian, N., and Lee, D. B. N. Analysis of segmental phosphate absorption in intact rats: a compartmental analysis approach. *J. Clin. Investig.*, *91*: 915–922, 1993.
23. Minami, H., Sasaki, Y., Saijo, N., Ohtsu, T., Fujii, H., Igarashi, T., and Itoh, K. Indirect-response model for the time course of leukopenia with anticancer drugs. *Clin. Pharmacol. Ther.*, *64*: 511–521, 1998.
24. Haurie, C., Dale, D. C., and Mackey, M. C. Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. *Blood*, *92*: 2629–2640, 1998.
25. Mick, R., and Ratain, M. J. Statistical approaches to pharmacodynamic modeling: motivations, methods, and misperceptions. *Cancer Chemother. Pharmacol.*, *33*: 1–9, 1993.
26. Ratain, M. J. Therapeutic relevance of pharmacokinetics and pharmacodynamics. *Semin. Oncol.*, *19*: 8–13, 1992.
27. Zamboni, W. C., Lufter, D. I., Egorin, M. J., Schweigert, M., Sezer, O., Richter, T., Natale, J. J., and Possinger, K. The effect of increasing topotecan infusion from 30 minutes to 4 hours on the duration of exposure in cerebrospinal fluid. *Ann. Oncol.*, *12*: 119–122, 2001.
28. Zamboni, W., Egorin, M., Grimm, A., Stewart, J., Sentz, D., Zuhowski, E., and Eiseman, J. Disposition of unbound- and total-platinum in B16 murine melanoma tumors after cisplatin administration. *Proc. Am. Assoc. Cancer Res.*, *40*: 392, 1999.
29. MacVittie, T. J., Farese, A. M., Smith, W. G., Baum, C. M., Burton, E., and McKearn, J. P. Myelopoietin, an engineered chimeric IL-3 and G-CSF receptor agonist, stimulated hematopoietic recovery in a nonhuman primate model of radiation-induced neutropenia. *Blood*, *95*: 837–845, 2000.
30. MacVittie, T. J., Farese, A. M., Davis, T. A., Lind, L. B., and McKearn, J. P. Myelopoietin, a chimeric agonist of human interleukin-3 and granulocyte c factor receptors, mobilizes CD34+ cells that rapidly engraft lethally x-irradiated nonhuman primates. *Exp. Hematol.*, *27*: 1557–1568, 1999.
31. Rowinsky, E. K., Grochow, L. B., Hendricks, C. B., Ettinger, D. S., Forastiere, A. A., Hurowitz, L. A., McGiure, W. P., Sartorius, S. E., Lubejko, B. G., and Kaufmann, S. H. Phase I and pharmacologic study of topotecan: a novel topoisomerase I inhibitor. *J. Clin. Oncol.*, *10*: 647–656, 1992.
32. Vereij, J., Lund, B., Beijnen J., Planting, A., de Boer-Dennert, M., Koier, I., Rosing, H., and Hnasen, H. Phase I and pharmacokinetics study of topotecan, a new topoisomerase I inhibitor. *Ann. Oncol.*, *4*: 673–678, 1993.
33. Rowinsky, E. K., Kaufmann, S. H., Baker, S. D., Miller, C. B., Sartorius, S. E., Bowling, M. K., Chen, T. L., Donehower, R. C., and Gore, S. D. A phase I and pharmacological study of topotecan infused over 30 minutes for five days in patients with refractory acute leukemia. *J. Clin. Oncol.*, *2*: 1921–1930, 1996.
34. Zamboni, W. C., Santana, V. M., Gajjar, A. J., Meyer, W. H., Pappo, A. S., Houghton, P. J., and Stewart, C. F. Pharmacokinetically guided dose adjustment reduces variability in topotecan (TPT) systemic exposure in children with solid tumors. *Proc. Am. Soc. Clin. Oncol.*, *16*: 205, 1997.
35. Leischke, G. J., and Burgess, A. W. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (second of two parts). *N. Engl. J. Med.*, *327*: 99–106, 1992.
36. Crawford, J., Ozer, H., Stroller, R., Johnson, D., Lyman, G., Tabbara, I., Kris M. Grous, J., Picozzi, V., and Rausch, G. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. *N. Engl. J. Med.*, *325*: 164–170, 1991.
37. Kotake, T., Miki, T., Akaza, H., Kubota, Y., Nishio, Y., Matsumura, Y., Ota, K., and Ogawa, N. Effect of recombinant granulocyte colony-stimulating factor on chemotherapy-induced neutropenia in patients with urogenital cancer. *Cancer Chemother. Pharmacol.*, *27*: 253–257, 1991.