

Paclitaxel Steady-State Plasma Concentration as a Determinant of Disease Outcome and Toxicity in Lung Cancer Patients Treated with Paclitaxel and Cisplatin¹

Eric K. Rowinsky,² Micheal Jiroutek,
Philip Bonomi, David Johnson, and
Sharyn D. Baker

The Institute for Drug Development, Cancer Therapy and Research Center, San Antonio Texas and The University of Texas Health Science Center, San Antonio, Texas 78229 [E. K. R., S. D. B.]; Dana Farber Cancer Institute, Boston, Massachusetts 02115 [M. J.]; Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612 [P. B.]; and Vanderbilt University Medical Center, Nashville, Tennessee 37232 [D. J.]

ABSTRACT

The principal purpose of this study was to evaluate relationships between paclitaxel plasma steady-state concentration (C_{ss}) and both disease outcome and toxicity in patients with non-small cell lung cancer (NSCLC) treated with paclitaxel and cisplatin in an Eastern Cooperative Oncology Group (ECOG) Phase III study E5592. Chemotherapy-naïve patients with stage IIIb and IV NSCLC were randomized to treatment with either 75 mg/m² cisplatin i.v. on day 1 and 100 mg/m² etoposide i.v. on days 1–3 (EC arm) or 75 mg/m² cisplatin i.v. combined with either a low dose of paclitaxel (135 mg/m², 24-h i.v. infusion; PC arm) or a higher dose of paclitaxel (250 mg/m² i.v., 24-h i.v. infusion) with granulocyte colony-stimulating factor (PCG arm). End-of-24-h-infusion paclitaxel concentrations, which have been demonstrated to be nearly equal to C_{ss} s on this schedule, were obtained during the first and second courses in patients on the PC and PCG arms. Relationships between the average paclitaxel C_{ss} ($C_{ss,avg}$) and the best response to treatment, time to treatment failure (TTF), survival, and worst grade of leukopenia and neurotoxicity were evaluated by univariate analysis. A multivariate model was used to assess the influence of paclitaxel C_{ss} in conjunction with other potentially relevant patient variables that may affect disease

outcome, including the paclitaxel treatment arm, age, sex, performance status, weight loss during the previous 6 months, and disease stage. Paclitaxel C_{ss} in both courses 1 and 2 were obtained in 71 patients treated with PC and 75 patients treated with PCG. Although $C_{ss,avg}$ s in patients treated with PC and PCG were significantly different (median, 0.32 versus 0.81 $\mu\text{mol/liter}$; $P < 0.0001$), response rates were not (33.8 versus 26.7%; $P = 0.3719$). In addition, there were no differences between the PC and PCG arms in TTF (median, 5.1 versus 5.5 months, $P = 0.6201$) or survival (median, 11.6 versus 11.3 months, $P = 0.7173$). Combined analysis of paclitaxel concentrations from both treatment arms revealed no significant difference in paclitaxel $C_{ss,avg}$ between responders and nonresponders [median, 0.40 (range, 0.16–1.6) $\mu\text{mol/liter}$ versus 0.55 (range, 0.11–3.6)], and $C_{ss,avg}$ s were similar in patients segregated according to whether they had a complete response, partial response, stable disease, or progressive disease as their best response to treatment ($P = 0.7612$). In addition, the relationship between $C_{ss,avg}$ and TTF was weak ($r^2 = 0.00003$, $P = 0.94$), as was the relationship between $C_{ss,avg}$ and survival ($P = 0.1267$). With regard to the principal toxicities, neither the propensity to develop neuromuscular and neurosensory toxicity nor the worst grade of these adverse effects were related to $C_{ss,avg}$ ($P = 0.5000$ and 0.2033 , respectively); however, the relationship between $C_{ss,avg}$ and the worst grade of leukopenia experienced was marginally significant ($P = 0.0796$). In a multivariate model, neither the combined effect of relevant demographic and stratification variables nor paclitaxel $C_{ss,avg}$ predicted for either response ($P = 0.1544$) or TTF ($P = 0.2574$), whereas the combined effect of all covariates predicted for survival ($P = 0.0249$). With regard to individual covariates, a lower disease stage (stage IIIb) was the only significant positive determinant of response ($P = 0.0173$), female sex was the only significant favorable predictor for TTF ($P = 0.0195$), and a lower ECOG performance status (= 0) was the only significant positive determinant of survival ($P = 0.0121$) in the multivariate model. In summary, paclitaxel $C_{ss,avg}$ was not a determinant of response, TTF, or survival in patients with advanced NSCLC treated with paclitaxel as a 24-h i.v. infusion combined with cisplatin. On the basis of both the clinical and pharmacodynamic results of E5592, there is no compelling reason to treat patients with advanced NSCLC with paclitaxel on a 24-h i.v. schedule at doses of >135 mg/m² in combination with cisplatin, although higher doses are associated with higher paclitaxel C_{ss} s.

INTRODUCTION

Paclitaxel has become one of the most commonly used anticancer agents due to its broad spectrum of antitumor activity and high therapeutic indices in many clinical settings (1). Still, as with many other anticancer agents, clinical and pharmacoki-

Received 11/17/98; accepted 1/22/99.

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 This study was conducted by the Eastern Cooperative Oncology Group (Dr. Robert L. Comis, Chair) and was supported in part by USPHS Grants CA25988, CA23318, CA49957, CA66636, and CA2115 from the National Cancer Institute, NIH, and Department of Health and Human Services. Its contents are solely the responsibilities of the authors and do not necessarily represent the official views of the National Cancer Institute.

² To whom requests for reprints should be addressed, at The Institute for Drug Development, Cancer Therapy and Research Center, 8122 Data-point Drive, Suite 700, San Antonio, TX 78229. Phone: (210) 616-5945; Fax: (210) 616-5865; E-mail: erowinsk@saci.org.

netic determinants of therapeutic outcome with paclitaxel have not been identified. In addition, there has been substantial interindividual variability in therapeutic outcome in practically all clinical settings in which paclitaxel has demonstrated utility, despite the fact that patients have been similar with respect to tumor type, extent of prior therapy, and sensitivity to prior chemotherapy.

The identification of pharmacological determinants of drug effect may enhance the therapeutic index of any anticancer agent. For example, the clearance rates of methotrexate, teniposide, and cytarabine appear to be principal determinants of therapeutic outcome in childhood acute lymphocytic leukemia (2). Furthermore, the results of prospective evaluation suggests that therapeutic monitoring of the plasma concentrations of these agents and "real time" adaptive dosing may lead to improved outcome (2). For carboplatin, retrospective analyses of therapeutic and toxicological results in both untreated and previously treated women with ovarian cancer have indicated that plasma AUC³ may be an accurate determinant of both therapeutic outcome and toxicity (3, 4). Accordingly, the use of this determinant for carboplatin dosing has been incorporated into general clinical practice. Identifying pharmacokinetic determinants of drug effect may also lead to a greater understanding of the influence of dose and schedule on both disease outcome and toxicity in the clinic.

For paclitaxel, treatment duration appears to be the most important pharmacological determinant of drug effect *in vitro* (5–15). Prolonging the duration of drug exposure *in vitro* generally produces much greater cytotoxicity than increasing drug concentration, although paclitaxel concentration is also an important variable until a threshold level is exceeded (5–15). In clinical practice, the principal pharmacokinetic determinants of paclitaxel effect have not been determined prospectively; however, the influences of paclitaxel dose and schedule on clinical efficacy are being addressed in Phase III evaluations in many disease settings (16–22). In early clinical trials, the principal toxicities of the taxanes have been related to several pharmacokinetic parameters, including peak plasma concentration, plasma C_{ss}, AUC, and the duration that plasma concentrations exceed relevant threshold levels (23–30). For example, the results of several studies of paclitaxel on 3- and 24-h schedules have indicated that the severity of myelosuppression is related to the duration that plasma concentrations exceed 0.05–0.1 μmol/liter (23–27). In other studies, the severity of neuromuscular effects and mucositis has been demonstrated to correlate with AUC or C_{ss} (24, 25, 29–31).

Encouraged by the 1-year survival rates observed in single-agent trials of paclitaxel in patients with stage IIIb–IV NSCLC, ECOG designed a Phase III study (E5592) to evaluate the effect of paclitaxel on survival (19, 31). Chemotherapy-naive stage IIIb–IV NSCLC patients were randomized to treatment with 75

Table 1 Patient demographic characteristics, stratification variables, paclitaxel C_{ss,avg}, and disease outcome by treatment arm

| | Paclitaxel treatment arms ^a | | P |
|---|--|------------------|---------|
| | PC | PCG | |
| No. of patients | 75 | 71 | |
| Median age (range) | 60.9 (37.9–75.6) | 59.4 (39.8–76.3) | 0.9594 |
| ECOG performance status (0:1) | 28:43 | 24:51 | 0.3897 |
| Sex (male:female) | 47:24 | 47:28 | 0.7304 |
| Weight loss (<5%:≥5%) | 56:15 | 54:21 | 0.3457 |
| Disease stage (IIIb:IV) | 11:60 | 12:63 | 1.0000 |
| C _{ss,avg} (μmol/liter) ^b | 0.32 (0.12–2.7) | 0.81 (0.11–3.6) | <0.0001 |
| Response (CR/PR): (SID/PD) | 24:47 | 20:55 | 0.3719 |
| TTF (months) ^b | 5.5 (1.4–27.0) | 5.1 (0.62–31.6) | 0.6201 |
| Survival (months) ^b | 11.3 (2.4–32) | 11.6 (1.6–33) | 0.7173 |

^a Treatment arms: PC, 75 mg/m² cisplatin i.v. on day 2 preceded by 135 mg/m² paclitaxel over 24 h i.v. on day 1; PCG, 75 mg/m² i.v. cisplatin on day 2 preceded by 250 mg/m² paclitaxel i.v. over 24 h on day 1 plus 5 μg/kg G-CSF starting on day 3.

^b Values represent median (range).

mg/m² cisplatin i.v. on day 1 and 100 mg/m² etoposide i.v. on days 1–3 (EC arm), which was selected as the reference arm because it had produced the highest 1-year survival in previous ECOG trials, or 75 mg/m² i.v. cisplatin combined with either a low dose of paclitaxel (135 mg/m², 24-h i.v. infusion; PC arm) or a higher dose of paclitaxel (250 mg/m² i.v.) with G-CSF (PCG arm). The primary objective of this trial was to compare survival in patients treated with paclitaxel-cisplatin *versus* etoposide-cisplatin. Secondary clinical objectives included comparisons of serial quality of life measurements, response rates, and toxicity profiles as well as comparisons of the same parameters for two dose levels of paclitaxel (135 *versus* 250 mg/m²). Patients randomized to the paclitaxel-containing regimens experienced superior response rates and improved survival compared to patients randomized to treatment with EC (median survival, 9.9 *versus* 7.6 months; 1-year survival, 39.9 *versus* 31.8%, *P* = 0.048; Ref. 19). However, there were no differences in response or survival relative to paclitaxel dose (19). With the exception of increased myalgia with paclitaxel and increased neurotoxicity with high-dose paclitaxel, toxicity and quality of life in the three treatment arms were similar.

E5592 provided a unique opportunity to prospectively perform population pharmacodynamic studies in patients receiving paclitaxel to determine whether disease outcome or toxicity or both are related to the plasma paclitaxel C_{ss}. The study also provided an opportunity to gauge the importance of paclitaxel pharmacokinetics relative to other treatment, demographic, and stratification variables, such as the paclitaxel treatment arm (paclitaxel dose), age, sex, performance status, weight loss in the previous 6 months, and disease stage. The results of these studies are detailed here.

PATIENTS AND METHODS

Clinical Study Design. This study was performed in conjunction with an ECOG-sponsored Phase III trial in which the following combination chemotherapy regimens in patients

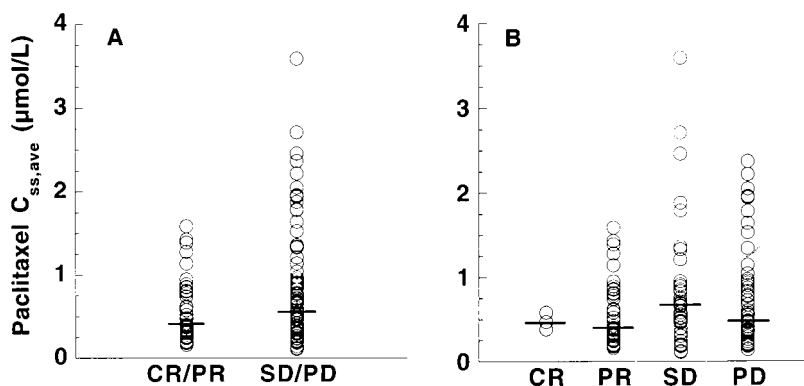
³ The abbreviations used are: AUC, area under the time *versus* concentration curve; NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; G-CSF, granulocyte colony-stimulating factor; TTF, time to treatment failure; STD, stable disease; PD, progressive disease; CR, complete response; PR, partial response.

Table 2 Numbers of patients and paclitaxel C_{ss} according to paclitaxel treatment arm^a

| | Paclitaxel treatment arm ^a | | P |
|--|---------------------------------------|------------------|---------|
| | PC | PCG | |
| No. of patients randomized in ECOG study 5592 (eligible and treated) | 201 (187) | 198 (190) | |
| No. of patients with plasma samples in course 1 | 101 | 102 | |
| Mean (SD) paclitaxel C_{ss} ($\mu\text{mol/liter}$) | 0.44 ± 0.55 | 1.03 ± 0.69 | |
| Median (range) | 0.32 (0.10–4.63) | 0.85 (0.15–4.53) | <0.0001 |
| No. of patients with plasma samples in course 2 | 78 | 87 | |
| Mean (SD) paclitaxel C_{ss} ($\mu\text{mol/liter}$) | 0.41 ± 0.56 | 0.91 ± 0.75 | |
| Median (range) | 0.25 (0.04–3.64) | 0.75 (0.07–4.97) | <0.0001 |
| No. of patients with plasma samples in courses 1 and 2 | 71 | 75 | |
| Mean (SD) paclitaxel $C_{ss,avg}$ ($\mu\text{mol/liter}$) | 0.44 ± 0.45 | 0.94 ± 0.59 | |
| Median (range) | 0.32 (0.12–3.70) | 0.81 (0.11–3.60) | <0.0001 |

^a Treatment arms: PC, 75 mg/m² i.v. cisplatin on day 2 preceded by 135 mg/m² paclitaxel over 24 h i.v. on day 1; PCG, 75 mg/m² i.v. cisplatin on day 2 preceded by 250 mg/m² i.v. paclitaxel over 24 h on day 1 plus G-CSF 5 $\mu\text{g/kg}$ starting on day 3.

Fig. 1 Scatterplots depicting paclitaxel $C_{ss,avg}$ as a function of best response. A, paclitaxel $C_{ss,avg}$ in responders (CR/PR as their best response) versus nonresponders (STD/PD as their best response). B, paclitaxel $C_{ss,avg}$ in patients with CR, PR, STD, and PD as their best response. —, medians.



with stage IIIb and IV NSCLC were used: (a) 75 mg/m² cisplatin i.v. on day 2 preceded by 135 mg/m² paclitaxel as a 24-h i.v. infusion on day 1 (PC arm); (b) 75 mg/m² cisplatin i.v. on day 2 preceded by 250 mg/m² paclitaxel as a 24-h i.v. infusion on day 1 plus 5 $\mu\text{g/kg}$ Filgrastim (G-CSF; Amgen, Thousand Oaks, CA) s.c. beginning on day 3 and continuing until the absolute neutrophil count was at least 10,000/ μl (PCG arm); and (c) 75 mg/m² cisplatin i.v. on day 1 plus 100 mg/m² etoposide i.v. on days 1, 2, and 3 (EC arm). Each of the regimens was repeated every 3 weeks, providing toxicity was acceptable and there was no disease progression. Patients were stratified according to the following parameters: (a) ECOG performance status 0 versus 1; (b) <5% versus \geq 5% weight loss during the previous 6 months; (c) stage IIIb versus stage IV disease; and (d) bidimensional measurable disease versus evaluable disease. Medical or interval histories, physical examinations, tumor measurements, and routine chemistry and electrolyte studies were performed before each course of treatment. Complete blood counts and differential WBC counts were performed at least weekly. Grading and tumor response were according to ECOG criteria (32).

Patient demographic, toxicity, response, and survival data were obtained from the ECOG statistical office using the central study database used for the principal analysis of the clinical study results (19).

Paclitaxel Plasma Sampling and Analytical Assay.

Blood samples (7–10 ml) were collected in heparinized tubes from patients who were treated with paclitaxel during the last hour of the 24-h i.v. infusion of paclitaxel during the first and second courses of treatment. Treating physicians and their staff were instructed to centrifuge the samples immediately, store the plasma at -20°C , and ship the sample on dry ice to the central analytical pharmacology laboratory. Paclitaxel concentrations were measured by a high-performance liquid chromatography assay, as described previously (25). Using the mean kinetic parameters for paclitaxel disposition on the 24-h schedule, end-of-infusion paclitaxel concentrations have been demonstrated to be nearly equal, on average, to C_{ss} s, using the formula derived by Weiss (33, 34). Paclitaxel C_{ss} s exceeding 5 $\mu\text{mol/liter}$ (five patients) were excluded from the analyses because they were mostly due to plasma sampling proximal to the drug infusion and represented more than three SDs above the mean C_{ss} s in this and other studies that used identical paclitaxel dose schedules (23, 25, 27). In all of these subjects, paclitaxel C_{ss} s were at least 8–596-fold higher than the paired sample.

Demographic and Outcome Comparisons: Univariate Analysis. Both nonparametric and parametric statistical tests were used to assess whether there were differences in relevant demographic characteristics or indices of outcome between patients in the low- and high-dose paclitaxel treatment arms (PC

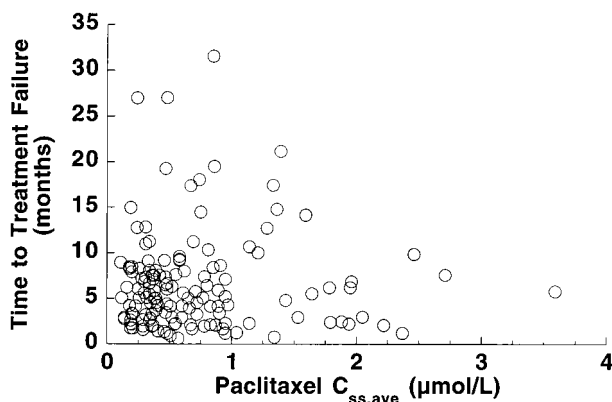


Fig. 2 Scatterplot depicting TTF (months) as a function of paclitaxel $C_{ss,ave}$ in patients in the PC and PCG treatment arms.

versus PCG arm). Descriptive statistics were used to describe relevant demographic characteristics, stratification variables, paclitaxel C_{ss} , and disease outcome parameters (mean, median, SD, and range). The paired t test was used to compare C_{ss} between courses 1 and 2 in those patients whom had matched pairs. The Fisher's exact test was used to compare each of the following categorical parameters between the treatment arms: ECOG performance status (0 or 1), sex (male or female), weight loss (<5% or \geq 5%) in the previous 6 months, disease stage (IIIb or IV), disease measurability (bidimensional or evaluable), response (STD/PD or CR/PR), and survival status (alive or dead). The Wilcoxon rank sums test was used to compare median values for the following data between the PC and PCG treatment arms: C_{ss} during course 1, C_{ss} during course 2, average C_{ss} for courses 1 and 2 ($C_{ss,ave}$), age, TTF [time from study arm randomization to the first evidence of treatment failure, death, or last follow-up evaluation (censored)], and survival (time from study arm randomization to the time of death or last follow-up, which was censored).

The Kruskal-Wallis Rank Sums test was used evaluate differences in paclitaxel C_{ss} during course 1, C_{ss} during course 2, and $C_{ss,ave}$ s in patients who achieved the following as their best responses to treatment with PC or PCG: CR, PR, STD, or PD. Similarly, the Kruskal-Wallis Rank Sums test was used to evaluate differences in paclitaxel C_{ss} among patients who experienced a range of ECOG toxicity grades (leukopenia, neurosensory toxicity, or neuromotor toxicity) as their worst toxicity during treatment with PC or PCG. There were insufficient data available in the database to assess neutropenia. The Wilcoxon rank sums test was used to assess differences in paclitaxel C_{ss} between responders (CR/PR as their best response) and nonresponders (STD/PD as their best response) and as a function of survival status. Associations between paclitaxel C_{ss} and TTF were evaluated using linear regression analysis. The Tukey-Kramer honestly significant difference method was used to test for type I error when multiple pairs of means were compared.

Multivariate Analysis. The influence of the paclitaxel $C_{ss,ave}$ and other potentially relevant patient characteristics on response, TTF, and survival were evaluated in multiple logistic regression analyses. The logistic and phreg procedures in SAS

Table 3 Multivariate analysis of the effects of patient demographics, stratification variables, and paclitaxel $C_{ss,ave}$ on disease outcome

| Covariate | P | | |
|-------------------------|----------|--------|----------|
| | Response | TTF | Survival |
| Age | 0.7051 | 0.4401 | 0.1791 |
| Performance status | 0.6564 | 0.8735 | 0.0121 |
| Female sex | 0.5091 | 0.0195 | 0.0798 |
| Weight loss | 0.9711 | 0.8906 | 0.5811 |
| Disease stage | 0.0173 | 0.0544 | 0.6016 |
| Paclitaxel $C_{ss,ave}$ | 0.0971 | 0.9103 | 0.0971 |
| Treatment arm | 0.9288 | 0.7778 | 0.6416 |
| All covariates combined | 0.1544 | 0.2574 | 0.0249 |

(SAS Institute, Cary, NC) were used to assess the degree of association of paclitaxel treatment arm (PC versus PCG), $C_{ss,ave}$, age, ECOG performance status (0 versus 1), sex (male versus female), weight loss (<5% versus \geq 5%), and stage (IIIb versus IV) with response, TTF, and survival (35, 36).

RESULTS

Plasma Sampling. The total numbers of patients who were randomized and received treatment in the PC, PCG, and EC arms of this study were 187, 190, and 194, respectively. Plasma sampling was performed and paclitaxel C_{ss} was determined in at least one course in 101 and 102 patients treated with PC and PCG, respectively, and in 71 and 75 patients treated with PC and PCG, respectively, during both courses 1 and 2. A paired analysis revealed no significant difference in C_{ss} between courses 1 and 2 ($P = 0.6509$).

Patient Demographics and Paclitaxel C_{ss} . Demographic characteristics and relevant parameters of disease outcome in patients who were treated with PC and PCG and had plasma sampling during both courses are depicted in Table 1. There were no significant differences between the PC and PCG arms with regard to age, ECOG performance status, disease stage, or weight loss. However, paclitaxel C_{ss} s in courses 1 and 2 as well as $C_{ss,ave}$ differed significantly between patients treated with the low- and high-dose paclitaxel regimens, as shown in Tables 1 and 2. The median (range) paclitaxel C_{ss} s in course 1 were 0.32 $\mu\text{mol/liter}$ (0.10–4.63 $\mu\text{mol/liter}$) and 0.85 $\mu\text{mol/liter}$ (0.15–4.53 $\mu\text{mol/liter}$) in patients in the PC and PCG treatment arms, respectively ($P < 0.0001$), and corresponding values in course 2 were 0.25 $\mu\text{mol/liter}$ (0.04–3.64 $\mu\text{mol/liter}$) and 0.75 $\mu\text{mol/liter}$ (0.07–4.97 $\mu\text{mol/liter}$), respectively ($P < 0.0001$). Similarly, median (range) paclitaxel $C_{ss,ave}$ s in patients with plasma sampling performed during both courses 1 and 2 differed significantly between patients in the PC and PCG treatment arms [0.32 $\mu\text{mol/liter}$ (0.12–3.70 $\mu\text{mol/liter}$) versus 0.81 $\mu\text{mol/liter}$ (0.11–3.60 $\mu\text{mol/liter}$), $P < 0.0001$].

Relevant Disease Outcome Parameters: PC versus PCG. The major response (PR/CR) rates in patients treated in the PC and PCG arms in whom paclitaxel C_{ss} were measured during both courses and paclitaxel $C_{ss,ave}$ could be calculated were 33.8 and 26.7% respectively. Although paclitaxel $C_{ss,ave}$ s differed significantly between the PC and PCG arms, the difference in response rates was not significant ($P = 0.3719$). Similarly, there were no differences between the PC and PCG

arms with regard to TTF (median, 5.5 versus 5.1 months, $P = 0.6201$) or survival (median, 11.3 versus 11.6 months, $P = 0.7173$).

Relationship between Paclitaxel C_{ss} and Disease Outcome. Relationships between paclitaxel C_{ss} and disease outcome were evaluated using univariate methods. There was no difference between responders (*i.e.*, patients who had either CR or PR as their best response) and nonresponders (*i.e.*, patients who had either STD or PD as their best response) in the magnitude of paclitaxel $C_{ss,avg}$ [median, 0.40 $\mu\text{mol/liter}$ (range, 0.16–1.6 $\mu\text{mol/liter}$) versus 0.55 $\mu\text{mol/liter}$ (range, 0.11–3.6 $\mu\text{mol/liter}$), $P = 0.15$]. Median paclitaxel $C_{ss,avg}$ s were also similar in patients segregated according to whether they experienced CR, PR, STD, or PD as their best response (0.47, 0.39, 0.66, and 0.48 $\mu\text{mol/liter}$, respectively). Scatterplots depicting individual paclitaxel $C_{ss,avg}$ s according to categorical response are depicted in Fig. 1. The relationship between paclitaxel $C_{ss,avg}$ and TTF was also evaluated. As shown in Fig. 2, this relationship was weak ($r^2 = 0.00003$, $P = 0.94$). The results are nearly identical if categorical response and TTF are related to paclitaxel C_{ss} achieved during either course 1 or course 2 (results not shown).

A multivariate analysis was performed to assess the effect of relevant demographic (age, performance status, and sex) and stratification variables (*e.g.*, weight loss, disease stage, and paclitaxel-cisplatin treatment arm), and paclitaxel $C_{ss,avg}$ on disease outcome (Table 3). The overall combined effect of all covariates in the model did not predict significantly for either response ($P = 0.1544$) or TTF ($P = 0.2574$), whereas the combined effect of all covariates predicted significantly for survival ($P = 0.0249$). Analysis of individual variables demonstrated that a lower disease stage (stage IIIb) was the only significant positive determinant of response ($P = 0.0173$), female sex was the only significant favorable predictor of TTF ($P = 0.0195$), and a lower ECOG performance status ($= 0$) was the only significant positive determinant of survival ($P = 0.0121$). Several of the covariates predicted marginally, albeit not significantly, for response [$C_{ss,avg}$ ($P = 0.0971$)], longer TTF [stage IIIb ($P = 0.0544$)], and longer survival [female sex ($P = 0.0798$)].

Relationships between Paclitaxel C_{ss} and Toxicity. Scatterplots of paclitaxel $C_{ss,avg}$ as a function of the highest grade of neuromotor toxicity, neurosensory toxicity, and leukopenia experienced by each patient are shown in Fig. 3. The magnitude of the paclitaxel $C_{ss,avg}$ was not related to the propensity to experience neuromotor or neurosensory toxicities nor to the toxicity grade ($P = 0.2033$ and 0.5000, respectively). Although the relationship between paclitaxel $C_{ss,avg}$ and grade of leukopenia approached a level of marginal statistical significance ($P = 0.0796$), further analysis using the Tukey-Kramer honestly significant difference test revealed a very low likelihood that any single one of the multiple paired comparisons was significant. Similar results were achieved when analyses were performed using paclitaxel C_{ss} achieved during either course 1 or course 2 (results not shown).

DISCUSSION

Paclitaxel is one of the most commonly used anticancer drugs due to its broad antitumor spectrum; however, similar to

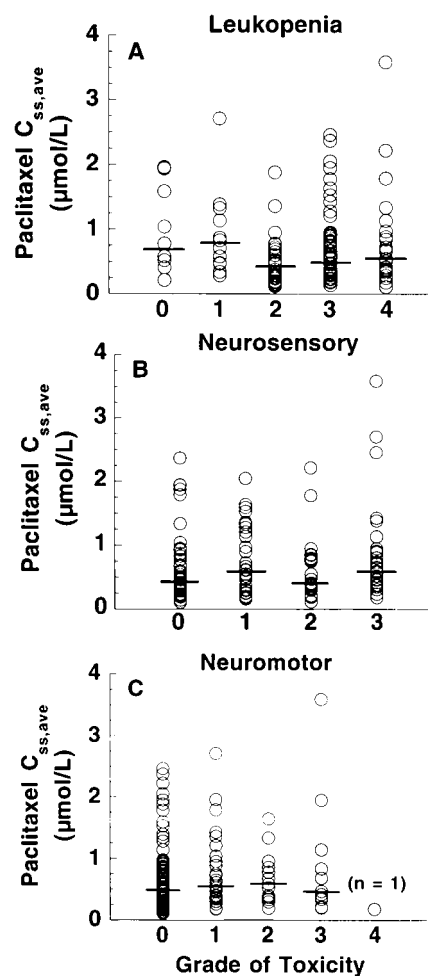


Fig. 3 Scatterplots depicting worst grades of toxicity as a function of paclitaxel $C_{ss,avg}$. A, leukopenia; B, neurosensory toxicity; C, neuromotor toxicity. —, medians.

the situation with many other anticancer agents, patients with similar tumor types have vastly different disease outcomes and toxicity, and pharmacokinetic determinants of disease outcome have not been identified. E5592 has addressed several relevant issues regarding the role of paclitaxel as a component of first-line therapy in patients with advanced NSCLC (19). The study demonstrated that both 1-year and overall survival were improved significantly and response rates were significantly higher in patients treated with paclitaxel and cisplatin than the reference regimen of etoposide and cisplatin. E5592 also addressed whether there was a paclitaxel dose-response effect in patients treated with two dose levels of paclitaxel (135 and 250 mg/m^2) administered as a 24-h i.v. infusion followed by 75 mg/m^2 cisplatin i.v. and demonstrated no differences in disease outcome. In addition, paclitaxel C_{ss} was measured to evaluate relationships between paclitaxel dose and C_{ss} and, more importantly, between paclitaxel C_{ss} and outcome.

Although the 146 patients in whom paclitaxel $C_{ss,avg}$ s were calculated represent a subset of the 398 patients entered on both paclitaxel arms, this subset was representative of the entire

group of patients. The sole requirement for including patients in the pharmacodynamic component of the study was plasma sampling at the end of both courses 1 and 2, which should not be influenced by either demographic or disease-related factors. In fact, comparisons of the distribution of demographic and disease-related variables (age, sex, weight loss, stage, and performance status) and principal study end points (response rate, TTF, and survival) revealed no differences between the 398 total patients entered on the PC and PCG arms and the subset of patients participating in the pharmacodynamic component of the study.⁴

The questions of whether plasma C_{ss} is an appropriate parameter to use for the pharmacodynamic analyses performed in this study and whether alternate pharmacokinetic parameters would yield disparate results must also be considered. Because the paclitaxel plasma concentration at the end of infusion has been demonstrated to be nearly equivalent to C_{ss} when paclitaxel is administered over 24 h, relationships between it and other pharmacokinetic parameters, such as AUC and duration of exposure to drugs at concentrations of >0.05 – $0.1 \mu\text{mol/liter}$, become stronger with more prolonged infusions (25–30, 33, 34). Another consideration in using paclitaxel C_{ss} for these studies was the feasibility of obtaining plasma samples during both courses 1 and 2 in a sufficiently large number of patients in a Phase III multicenter study. However, one potential pitfall in using $C_{ss,avg}$ is that it is derived from the first two courses only, whereas disease outcome and toxicity represent summations of effects overall courses. This concern is perhaps most applicable to neurotoxicity, which, in contrast to myelosuppression, is clearly a cumulative effect of paclitaxel and cisplatin (30, 34, 37). In this study, neither the total number of courses nor the cumulative dose of paclitaxel was considered in the pharmacodynamic analyses relating C_{ss} to toxicity. However, minimal intrasubject course-to-course variability in pharmacokinetics has been reported in previous studies of paclitaxel. In addition, there was good concordance in paclitaxel C_{ss} between courses 1 and 2 in this study, suggesting that the pharmacokinetic behavior of paclitaxel during the first two courses is a satisfactory representation of paclitaxel pharmacokinetics during subsequent courses (23, 24, 29, 38).

Even in situations in which there are true dose-, concentration-, or duration of exposure-response relationships, small, albeit significant, differences between patients treated at various dose levels may not be appreciated if there is large interindividual pharmacokinetic variability and an insufficient sample size. Because these factors may have, in part, accounted for the apparent lack of a significant differences in disease outcome between patients receiving low- and high-dose paclitaxel in combination with cisplatin in this study, population pharmacokinetic and pharmacodynamic assessments across both dose levels were undertaken to better identify relationships between paclitaxel C_{ss} and disease outcome. Indeed, there was substantial interindividual variability in paclitaxel C_{ss} in patients treated with paclitaxel doses of 135 mg/m^2 (PC arm) and 250 mg/m^2 (PCG arm), but there was a clear difference between patients in

the PC and PCG treatment arms [median $C_{ss,avg}$ s, 0.32 and $0.81 \mu\text{mol/liter}$, respectively ($P < 0.0001$)]. However, no relationships between paclitaxel $C_{ss,avg}$ and either response, TTF, survival, or worst grade of leukopenia, neurosensory toxicity, or neuromotor toxicity were apparent. In the multivariate models that included potential determinants of outcome, paclitaxel $C_{ss,avg}$ was not a determinant of response, TTF, or survival. In fact, a lower disease stage (stage IIb) was the only significant positive determinant of response ($P = 0.0173$), female sex was the only significant predictor of time to progression ($P = 0.0195$), and a lower ECOG performance status ($= 0$) was the only significant positive determinant of survival ($P = 0.0121$) in the models.

The results of these pharmacodynamic studies support the initial clinical results indicating no differences between the PC and PCG arms in disease outcome despite ~ 2 -fold differences in paclitaxel dose and median $C_{ss,avg}$ s. At first glance, these results may not appear to be congruent with those of *in vitro* studies (5–15). Although the duration of paclitaxel treatment appears to be a more important determinant of drug effect than drug concentration *in vitro*, the magnitude of most biological effects of paclitaxel (*i.e.*, cytotoxicity, formation of microtubule bundles and mitotic asters, increase in tubulin polymer mass, stabilization of microtubules against depolymerization, apoptosis, radiosensitization, antiangiogenesis, and inhibition of chemotaxis and motility) are also related to drug concentration (5–15). However, a plateauing of dose response or a situation of diminishing returns is typically noted as paclitaxel concentrations increase, and the drug concentration at which the plateauing of effect occurs varies from cell line to cell line (5–7, 18). The broad clinical implications of these results is that there may be a critical “plateau” paclitaxel concentration or dose above which toxicity but not efficacy increases, but there also appears to be a critical concentration, below which drug effects do not usually occur. The cumulative results of *in vitro* studies suggest that the precise concentration at which plateauing occurs depends upon the specific treatment schedule, and the “threshold” concentration is inversely related to the duration of treatment.

In essence, clinical observations to date resemble those of *in vitro* studies. On the basis of the results of both nonrandomized and randomized clinical trials that have evaluated relationships between paclitaxel dose and disease outcome, the flat or plateau portion of the dose- or concentration-response curve *in vitro* is noted in the clinic with paclitaxel at doses of $>135 \text{ mg/m}^2$ on a 24-h schedule (18–20). For example, a pooled analysis of 191 ovarian cancer patients treated with paclitaxel doses ranging from 110 to 300 mg/m^2 as a 24-h infusion in the first five disease-directed studies used for registration in the United States that controlled for the effects of individual study, performance status, number of prior regimens, platinum sensitivity, and age, demonstrated that the probability of responding and longer progression-free and overall survival were not related to paclitaxel dose in the range of 110 – 300 mg/m^2 (24-h infusion; Ref. 39). In addition, a Phase III intergroup study in which patients with recurrent and refractory ovarian cancer were treated with 24-h infusions of paclitaxel at 175 or 250 mg/m^2 plus G-CSF demonstrated only modest a difference in overall response rates, 36 *versus* 28%, respectively (18). This modest difference was even nullified by a higher response rate in

⁴ P. Bonomi, unpublished results.

platinum-sensitive patients treated with the lower dose and no differences in both progression-free and overall survival between the high- and low-dose groups. The dose-response issue has also been evaluated in ECOG study E1393 in which patients with metastatic or locally advanced head and neck cancer who had not previously received chemotherapy for recurrent disease were randomized to treatment with 75 mg/m² cisplatin following either low-dose paclitaxel (135 mg/m², 24-h schedule) or high-dose 200 mg/m² paclitaxel (24-h schedule) plus G-CSF. Response rates were identical (35%) with both treatments, and there was no difference in survival (20). Although the results of these studies indicate that paclitaxel doses of >135 mg/m² on a 24-h schedule produces either no or little, if any, further benefit, this generalization is applicable to the 24-h paclitaxel schedule only, and the precise dose with other schedules at which plateauing of the dose- or concentration-response curve occurs must be determined prospectively. However, the results of randomized trials in ovarian, breast, and lung cancer to date suggest that plateauing occurs at paclitaxel doses of at least 175 mg/m² on shorter (3-h) schedules (16, 17, 22).

The lack of relationships between disease outcome and both paclitaxel dose and C_{ss} may be explained by saturation of paclitaxel receptors on β -tubulin at C_{ss} achieved with paclitaxel doses at or above 135 mg/m² on a 24-h schedule. In addition, plasma paclitaxel C_{ss} may not reflect drug effects in peripheral tissues. The pharmacokinetic behavior of a drug in plasma cannot always be generalized to actions at the cellular level, particularly when drug concentrations in plasma and peripheral tissues are disparate. For the taxanes, plasma concentrations achieved with almost any dose schedule are capable of inducing relevant biological effects *in vitro*, but the degree of tissue distribution for the taxanes is very large, most likely due to avid drug binding to tubulin, plasma proteins, and high tissue:plasma concentration ratios, have been noted in virtually all tissues except testes and brain in animal studies (23, 24, 40). Not only are high paclitaxel concentrations achieved in almost all peripheral tissues, but biologically relevant drug concentrations are sequestered in peripheral tissues and tumors for relatively long periods, which may not be accurately estimated from plasma concentration data (40, 41). Therefore, paclitaxel C_{ss} , which is seemingly a relevant parameter based on the results of *in vitro* studies, may correlate poorly with drug actions in peripheral tissues. In addition, $C_{ss,avg}$ achieved following a 24-h infusion may not reflect the duration that paclitaxel concentrations are above the threshold levels of 0.05–0.1 μ mol/liter, which have been determined to be both biologically and clinically relevant (8–13, 25–27).

These results indicate that the plasma paclitaxel C_{ss} alone is not a determinant of disease outcome or principal toxicities in patients with advanced NSCLC receiving treatment with paclitaxel as a 24-h infusion combined with cisplatin. On the basis of both the clinical and pharmacodynamic results of E5592, there is also no compelling reason to administer paclitaxel on a 24-h treatment schedule at doses of >135 mg/m² in combination with cisplatin in patients with advanced NSCLC, although higher doses are associated with higher paclitaxel C_{ss} s, on average. Although it is likely that similar dose and concentration effects occur with other paclitaxel schedules and in other disease settings, the generalizability of these results is not known, and the

precise paclitaxel dose range or concentrations at which the plateauing of drug effects occurs should be based on the results of randomized prospective trials similar to the study described here.

REFERENCES

- Rowinsky, E. K., and Donehower, R. C. Drug therapy: paclitaxel (Taxol). *N. Engl. J. Med.*, 332: 1004–1114, 1995.
- Evans, W. E., Relling, M. V., Rodman, J. H., Crom, W. R., Boyett, J. M., and Pui, C. H. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N. Engl. J. Med.*, 338: 499–505, 1998.
- Calvert, A. H., Newell, D. R., Gumbrell, L. A., O'Reilly S., Bunell, M., Boxall, F. E., Siddik, Z. H., Judson, I. R., and Gore, M. E. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J. Clin. Oncol.*, 7: 1748–1756, 1989.
- Jodrell, D. I., Egorin, M. J., Canetta, R. M., Langenberg, P., Goldbloom, E. P., Burroughs J. N., Goodlow, J. N., Tan, S., and Wiltshaw, E. Relationships between carboplatin exposure and tumour response in patients with ovarian cancer. *J. Clin. Oncol.*, 10: 520–528, 1992.
- Rowinsky, E. K. The taxanes: dosing and scheduling considerations. *Oncology*, 11 (Suppl. 2): 1–13, 1997.
- Arbuck, S. G., Canetta, R., Onetto, N., and Christian, M. Current dosage and scheduling issues in the development of paclitaxel (Taxol). *Semin. Oncol.*, 4 (Suppl. 3): 31–39, 1993.
- Arbuck, S. A., and Blaylock, B. Dose and schedule issues. *In: W. P. McGuire and E. K. Rowinsky (eds.), Paclitaxel in Cancer Treatment*, pp. 151–173. New York: Marcel Dekker, 1995.
- Rowinsky, E. K., Donehower, R. C., Jones, R. J., and Tucker, R. W. Microtubule changes and cytotoxicity in leukemic cell lines treated with Taxol. *Cancer Res.*, 48: 4093–4100, 1988.
- Schiff, P. B., and Horwitz, S. B. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA*, 77: 1561–1565, 1970.
- Bhalla, K., Ibrado, A. M., Tourkina, E., Tang, C., Mahoney, M. E., and Huang, Y. Taxol induces intranucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. *Leukemia (Baltimore)*, 7: 563–568, 1993.
- Lopes, N. M., Adams, E. G., Pitts, T. W., and Bhuyan, B. K. Cell kill kinetics and cell cycle effects of Taxol on human and hamster ovarian cell lines. *Cancer Chemother. Pharmacol.*, 32: 235–242, 1993.
- Helson, L., Helson, C., Malik, S., Ainsworth, S., and Mangiardi, J. A saturation threshold for Taxol cytotoxicity in human glial and neuroblastoma cells. *Anti-Cancer Drugs*, 4: 487–490, 1993.
- Liebmann, J. E., Cook, J. A., Lipschultz, C., Teague, D., Fisher, J., and Mitchell, J. B. Cytotoxic studies of paclitaxel (Taxol) in human tumor cell lines. *Br. J. Cancer*, 68: 1104–1109, 1993.
- Georgiadis, M. S., Russell, E., and Johnson, B. E. Prolonging the exposure of human lung cancer cell lines to paclitaxel increases the cytotoxicity. *Proc. Int. Assoc. Study Lung Cancer*, 11: 95, 1994.
- Figg, W. D., Thibault, A., McCall, N. A., Cooper, M. R., Kohn, E. C., and Myers, C. E. The *in vitro* activity of Taxol on three hormone refractory prostate cancer cell lines, PC3, DU145, and PC3M. *Proc. Am. Assoc. Cancer Res.*, 35: 431, 1994.
- Nabholtz, J.-M., Gelmon, K., Bontenbal, M., Spielmann, M., Catimel, G., Conte, P., Klaassen, U., Namer, M., Bonnetterre, J., Fumoleau, P., and Winograd, B. Multicenter, randomized comparative study of two doses of paclitaxel in patients with metastatic breast cancer. *J. Clin. Oncol.*, 14: 1858–1867, 1996.
- Eisenhauer, E., ten Bokkel Huinink, W., Swenerton, K. D., Gianni, L., Myles, J., van der Burg, M. E. L., Kerr, I., Vermorken, J. B., Buser, K., Colombo, N., Bacon, M., Santabarbara, P., Onetto, N., Winograd, B., and Canetta, R. European-Canadian randomized trial of Taxol in relapsed ovarian cancer: high *versus* low dose and long *versus* short infusion. *J. Clin. Oncol.*, 12: 2654–2666, 1994.

18. Omura, G. A., Brady, M. F., Delmore, J. E., Long, H. J., Long, H. J., Look, K. Y., Averette, H. E., Wadler, S., and Spiegel, G. A randomized trial of paclitaxel at 2 dose levels and Filgrastim (G; G-CSF) at 2 dose levels in platinum pretreated epithelial ovarian cancer (OVCA): a Gynecologic Oncology Group, SWOG, NCTTG and ECOG study. *Proc. Am. Soc. Clin. Oncol.*, *15*: 280, 1996.
19. Bonomi, P., Kim, K., Chang, A., and Johnson, D. Phase III trial comparing etoposide-cisplatin *versus* Taxol with cisplatin-granulocyte-colony-stimulating factor *versus* Taxol-cisplatin in advanced non-small cell lung cancer. An Eastern Cooperative Group trial. *Proc. Am. Soc. Clin. Oncol.*, *15*: 382, 1996.
20. Forastiere, A. A., Leong, T., Murphy, B., Rowinsky, E., DeConti, R., Karp, D., and Adams, G. A Phase III trial of high dose paclitaxel + cisplatin + G-CSF *versus* low-dose paclitaxel + cisplatin in patients with advanced squamous cell carcinoma of the head and neck: an Eastern Cooperative Oncology Group trial. *Proc. Am. Soc. Clin. Oncol.*, *16*: 384, 1997.
21. Mamounas, E., Brown, A., Smith, R., Lembersky, B., Fisher, B., Wickerham, D. L., Wolmark, N., Atkins, J., Shibata, H., Baez, L., DeFusco, P., Davila, E., Thirlwell, M., Bearden, J., Tipping, S., and Scholnik, A. Effect of Taxol duration of infusion in advanced breast cancer (ABC): results from NSABP B-26 trial comparing 3- to 24-hour infusion of high-dose Taxol. *Proc. Am. Soc. Clin. Oncol.*, *17*: 101a, 1998.
22. Winer, E., Berry, D., Duggan, D., Henderson, I. C., Cirrincione, C., Cooper, R., and Norton, L. Failure of higher dose paclitaxel to improve outcome in patients with metastatic breast cancer: results from CALGB 9342. *Proc. Am. Soc. Clin. Oncol.*, *17*: 101a, 1998.
23. Rowinsky, E. K. Pharmacology and metabolism. *In*: W. G. McGuire and E. K. Rowinsky (eds.), *Paclitaxel in Cancer Treatment*, pp. 91–120. New York: Marcel Dekker, 1995.
24. Rowinsky, E. K., Wright, M., Monsarrat, B., Lesser, G. J., and Donehower, R. C. Taxol: pharmacology, metabolism, and clinical implications. *Cancer Surv.*, *17*: 283–304, 1993.
25. Huizing, M. T., Keung, A. C. F., Rosing, H., van der Kuij, V., ten Bokkel Huinink, W. W., Mangjes, I. M., Dubbelman, A. C., Pinedo, H. M., and Beijnen, J. H. Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum pretreated ovarian cancer patients. *J. Clin. Oncol.*, *11*: 2127–2135, 1993.
26. Ohtsu, T., Sasaki, Y., Tamura, T., Miyata, Y., Nakanomyo, H., Nishiwaki, Y., and Saijo, N. Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion *versus* a 24-hour infusion. *Clin. Cancer Res.*, *1*: 599–606, 1995.
27. Gianni, L., Kearns, C., Gianni, A., Capri, G., Vigano, L., Lacatell, A., Bonnadonna, G., and Egorin, M. J. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. *J. Clin. Oncol.*, *13*: 180–189, 1995.
28. Sonnichsen, D., Hurwitz, C., Pratt, C., and Relling, M. C. Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. *J. Clin. Oncol.*, *12*: 532–538, 1994.
29. Longnecker, S. M., Donehower, R. C., Cates, A., Chen, T., Brundrett, R. B., Grochow, L. B., Ettinger, D. S., and Colvin, M. High performance liquid chromatographic assay for paclitaxel (NSC 125973) in human plasma and urine pharmacokinetics in a Phase I trial. *Cancer Treat. Rep.*, *71*: 53–59, 1981.
30. Rowinsky, E. K., Chaudhry, V., Forastiere, A. A., Sartorius, S. E., Ettinger, D. S., Grochow, L. B., Lubejko, B. G., Cornblath, D. R., and Donehower, R. C. A Phase I and pharmacologic study of Taxol and cisplatin with granulocyte colony-stimulating factor: neuromuscular toxicity is dose-limiting. *J. Clin. Oncol.*, *11*: 2010–2020, 1993.
31. Chang, A. T., Kim, L., Glick, J., Karp, D., and Johnson, D. Phase II study of Taxol, merbaroaone, and piroxantrone in stage IV non-small cell lung cancer. The Eastern Cooperative Group results. *J. Natl. Cancer Inst. (Bethesda)*, *85*: 388–394, 1993.
32. Oken, M. M., Creech, R. H., Tormey, D. C., Horton, J., Davis, T. E., McFadden, E. T., and Carbone, P. P. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am. J. Clin. Oncol.*, *5*: 649–655, 1982.
33. Weiss, M. Model-independent assessment of accumulation kinetics based on moments of drug disposition waves. *Eur. J. Clin. Pharmacol.*, *27*: 355–358, 1984.
34. Rowinsky, E. K., Gilbert, M., McGuire, W. P., Ettinger, D. S., Forastiere, A., Grochow, L. B., Clark, B., Sartorius, S. E., Cornblath, D. R., Hendricks, C. B., and Donehower, R. C. Sequences of Taxol and cisplatin: a Phase I and pharmacologic study. *J. Clin. Oncol.*, *9*: 1692–1703, 1991.
35. SAS Institute. *SAS/STAT Users Guide, Version 6, Ed. 4, Vol. 2*. Cary, NC: SAS Institute Inc., 1992.
36. SAS Institute. *Changes and Enhancements, Release 6.07, SAS Technical Report P-229, SAS/STAT Software*. Cary, NC: SAS Institute Inc., 1992.
37. Rowinsky, E. K., Chaudhry, V., Cornblath, D. R., and Donehower, R. C. The neurotoxicity of Taxol. *Monogr. Natl. Cancer Inst.*, *15*: 107–115, 1993.
38. Rowinsky, E. K., Burke, P. J., Karpe, J. E., and Tucker, R. W. Phase I and pharmacodynamic study of Taxol in refractory acute leukemia. *Cancer Res.*, *49*: 4640–4647, 1989.
39. Rowinsky, E. K., Mackey, M. K., and Goodman, S. N. Meta analysis of paclitaxel dose-response and dose-intensity in recurrent or refractory ovarian cancer. *Proc. Am. Soc. Clin. Oncol.*, *15*: 284, 1996.
40. Lesser, G., Grossman, S. A., Eller, S., and Rowinsky E. K. The neural and extraneural distribution of systemically administered [³H]paclitaxel in rats: a quantitative autoradiographic study. *Cancer Chemother. Pharmacol.*, *34*: 173–178, 1995.
41. Walle, T., Walle, U. K., Kumar, G. N., and Bhalla, K. N. Taxol metabolism and disposition in cancer patients. *Drug Metab. Disp.*, *23*: 506–512, 1995.

Clinical Cancer Research

Paclitaxel Steady-State Plasma Concentration as a Determinant of Disease Outcome and Toxicity in Lung Cancer Patients Treated with Paclitaxel and Cisplatin

Eric K. Rowinsky, Micheal Jiroutek, Philip Bonomi, et al.

Clin Cancer Res 1999;5:767-774.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/5/4/767>

Cited articles This article cites 30 articles, 13 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/5/4/767.full#ref-list-1>

Citing articles This article has been cited by 8 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/5/4/767.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/5/4/767>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.