Cancer Chemother Pharmacol (1994) 33: 460–464

Cancer Chemotherapy and Pharmacology

Taxol and taxotere in bladder cancer: in vitro activity and urine stability

Cristina Rangel[1], Harvey Niell[2], Antonius Miller[2], Clair Cox[1]

[1] Department of Urology, University of Tennessee, Memphis, TN 38163, USA
[2] Department of Internal Medicine, University of Tennessee, Memphis, TN 38163, USA

Received: 10 August 1993 / Accepted: 27 October 1993

Abstract. In this study the antimicrotubular agents taxol, taxotere, and vinblastine were compared for their ability to inhibit the clonal growth of human bladder tumor cell lines using a soft-agar clonogenic assay. The stability of taxol and taxotere was evaluated by high-performance liquid chromatography over a range of pH in human urine. Both taxol and taxotere were shown to maximally inhibit the clonal growth of human bladder cell lines within 1 h of drug incubation. The most active agent in the panel of tumor lines was taxotere, with 6 of 12 lines being sensitive to the agent at 0.01 μ*M* and all cell lines being sensitive at 0.1 μ*M*. Taxol was active in 1 of 12 lines at 0.01 μ*M* and in 11 of 12 at 0.1 μ*M*. Only 2 of 12 cell lines were sensitive to vinblastine over the 0.01- to 0.1-μ*M* dose range. Taxol and taxotere were found to be stable in human urine for 4 h over a pH range of 5–7. At least 85% of both drugs were present during this period of drug incubation. Our findings suggest that both taxol and taxotere may be clinically useful agents for systemic and intravesical use in bladder cancer.

Introduction

Chemotherapy is an important therapeutic modality in a variety of clinical settings in bladder cancer. Patients with superficial bladder cancer who experience a true recurrence or new occurrence of tumor after initial resection are often given intravesical chemotherapy for significant long-term control of disease [23]. There is mounting evidence that neoadjuvant chemotherapy will positively impact on the local recurrence rate, metastatic spread, and the survival of patients with muscle-invasive bladder cancer [15]. In metastatic bladder cancer, combination chemotherapy regimens produce 60%–80% response rates with 15%–20% long-term survivors. In spite of these advances, tumor progression is common after chemotherapy, pointing to the importance of evaluating new anticancer agents with potential activity in bladder cancer.

Antimicrotubular agents have proven to be extremely active and useful in the treatment of human neoplasms. The vinca alkaloids have been important agents in the curative regimens for Hodgkin's and non-Hodgkin's lymphomas, germ cell neoplasms, and childhood leukemias. Two new naturally occurring antimicrotubular agents have now become available for clinical trials. Taxol, which was isolated from the stem bark of the western yew, *Taxus brevifolia*, and taxotere, a semisynthetic agent derived from the leaves of *T. baccata*, have been found to display a novel mechanism of action and a broad range of antineoplastic activity. Both agents bind preferentially to microtubules and promote their assembly and stabilization in vitro [17, 22]. Both taxol and taxotere have demonstrated a broad range of preclinical activity [2, 5] and appear to have manageable toxicities in phase I clinical trials [13, 24]. Phase II trials of taxol have demonstrated significant activity in ovarian cancer [12, 20], breast cancer [8], and non-small-cell lung cancer [4]. Phase II trials of taxotere are now in progress.

Therefore, activities of taxol or taxotere in bladder cancer are unknown. The aim of the present study was to determine the in vitro activity of taxol and taxotere in a panel of human bladder-tumor cell lines and to identify characteristics of these agents that might prove useful for intravesical or systemic therapy of bladder cancer.

Materials and methods

Cell lines. All human bladder-tumor cell lines (HBTCL) used in this study have been well characterized and published in the literature [7, 11]. The panel of HBTCL consists of MGH-U1, CUB-2, COLO 232, SD, HU549, A1698, HT1376, UM-UC-3, SCaBER, J82, and TCCSUP. The cell lines were either purchased from the American Type Culture Collection (Rockville, MD.) or provided by the originating laboratory. All tumor cell lines were maintained in monolayer culture grown in

This work was supported in part by the Veterans Administration Research Service

Correspondence to: Cristina Rangel, M.D., University of Tennessee, 956 Court Avenue, Box 10, Memphis, TN 38163, USA

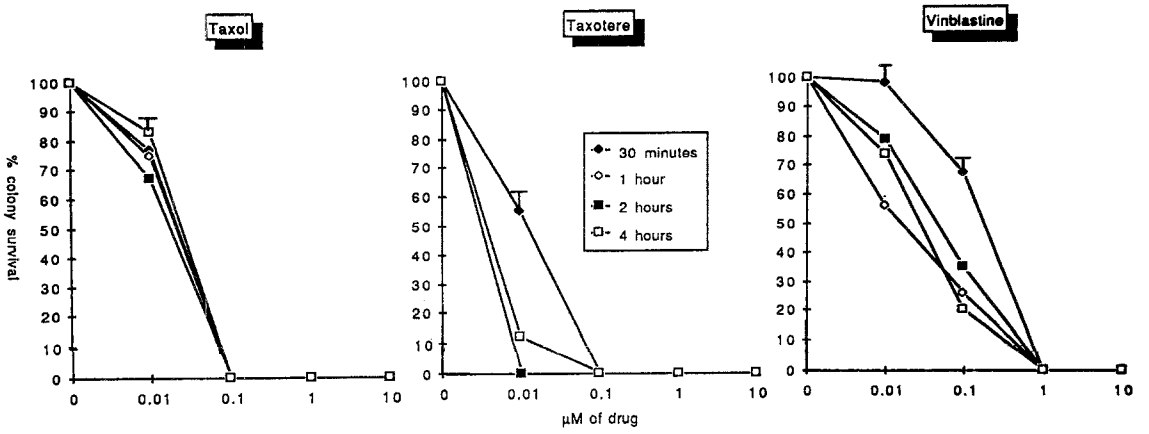

Fig. 1. Effect of the duration of exposure on the inhibition of clonal growth of UM-UC-3. Cytotoxicity was assessed using a clonogenic assay. *Points*, Mean values for four plates; *bars*, SD

100-mm plastic petri dishes (Fisher, Pittsburgh, PA) incubated at 37° C in a 100% humidified atmosphere containing 6% CO_2 with the media recomended by the American Tissue Culture Collection or originating laboratories. Cell cultures were fed biweekly with fresh medium and passaged when they had reached 80%–90% confluence. Most tumor cells were harvested from monolayer culture by incubation for 10 min at 37° C in 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) in Hanks' balanced salt solution (Sigma, St. Louis, MO). The resulting cell suspension was counted in a hemocytometer, and the viability was determined by trypan blue exclusion. Subcultures of all tumor cells were stored in a Union Carbide liquid nitrogen tank (model 35 VNC) in 90% fetal calf serum (Sigma) and 10% dimethylsulfoxide (DMSO) (Sigma) for future study.

Antimicrotubular agents. Taxol was kindly provided by Dr. M. Suffness, NHI/NCI (Bethesda, Md.) The drug, provided as a powder, was suspended in a 100-μ*M* solution of DMSO and subsequently diluted in medium. Aliquots of 1 ml were stored at –70° C for future use. The final concentration of DMSO in culture media was less than 0.2%. Preliminary experiments done to evaluate the effect of these vehicle concentrations showed no effect on tumor cell growth in culture.

Taxotere was provided by Dr. J.L. Favre, Institute Rhone-Poulenc Rorer (France). The drug was obtained in powder form, and 10 mg was suspended in 5 ml of methanol (Fisher), then diluted in medium to a concentration of 100 μ*M*, and stored at –70° C for future use.

Vinblastine was purchased as a standard pharmaceutical preparation (LyphoMed Rosemount, IL). A 100-μ*M* stock solution was diluted in sterile water, aliquoted, and stored at –70° C. Immediately prior to its use, the stock solution was diluted in DMEM to the required concentrations.

Clonogenic assay. Drug testing was performed using a modification of the Hamburger and Salmon assay [6]. The upper layer consisted of Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (Flow Lab., Newbury Park, CA), penicillin-streptomycin solution (Sigma), and 0.03% agar (Sigma). The lower layer contained DMEM, 10% fetal calf serum (Sigma), penicillin-streptomycin solution, and 0.05% agar. Tumor cells and drugs were incorporated into the upper layer. Tumor cell lines were plated in concentrations of 20,000–30,000 cells/plate. Cells were incubated at 37° C in a 100% humidified atmosphere containing 6% CO_2. Cultures were examined with an Olympus CK inverted microscope at magnificatin of ×40 and ×100. Final colony counts were made at 14–21 days after plating. Aggregates of 50 or more cells were scored as colonies. Cloning efficiencies of the HBTCL range from 0.2% to 4%. All drug testing was done at 4 dose levels using 4–6 plates/dose and the results were compared with those obtained for control plates. All drug assays were repeated at least three times to assure reproducibility. Drugs were deemed active if they produced at least 75% colony inhibition relative to control values.

High-performance liquid chromatograpy. Taxol and taxotere levels were measured by high-performance liquid chromatography (HPLC) by a modified method of Longnecker et al. [9]. Taxol and taxotere stability studies were performed using a system (Waters Chromatography, Millipore Corp., Milford, Mass.) consisting of a WISP 712 automatic injector, an M510 pump, an M490 variable-wavelength UV detector set to 227 nm, and Baseline 810 software on a Compuadd 286 computer. The mobile phase was water:acetonitrile (1:1, v/v) and the stationary phase was a Waters μ Bondapak C18 column (dimensions, 3.9 × 300 mm). Water and solvents were HPLC-grade and were degassed before their use. The flow rate was 2.5 ml/min. The injection volume was 100 μl. The retention times for taxol and taxotere for this isocratic method were 6.2 and 5.1 min, respectively.

Results

The concentration and the duration of drug exposure were evaluated using taxol, taxotere, and vinblastine. Vinblastine was used for comparison because it is an antimicrotubular agent that induces microtubule disassembly and it has know activity in bladder cancer. Tumor cells were exposed to taxol, taxotere and vinblastine at concentrations ranging from 0.01 to 10 μ*M* for varing incubation periods (range, 30 min to 4 h) in a 37° C shaking water bath. Cells were then washed three times and plated in vitro. Figure 1 depicts the results of those experiments using UM-UC-3. The maximal activity of vinblastine occurred after 1 h of drug exposure to 1 μ*M* of drug. Both taxol and taxotere produced maximal activity with exposure times as brief as 30 min and 1 h, respectively, at 0.1 μ*M* of drug.

To determine the activity of these agents, we utilized 12 HBTCL (Table 1). This panel of tumor cells represents both primary and metastatic tumors as well as a variety of grade and stage of tumor, and only the patient with COLO 232 had received therapy (radiation) prior to removal of the tumor. Taxol, taxotere, and vinblastine were exposed to the HBTCL in the clonogenic assay at concentrations of 0.01–10 μ*M* for 2 h. The 2-h drug incubation periods were chosen because previous experiments had demonstrated optimal drug activity over this exposure period and because intravesical chemotherapy is usually instilled intravesically for 2 h during the treatment of superficial bladder cancer. The results of these studies are listed in Table 2.

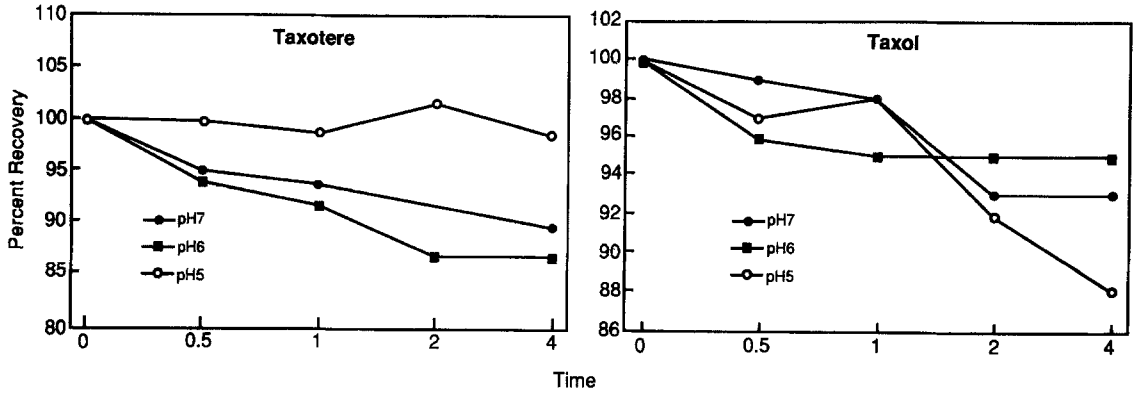

Fig. 2. Recovery of taxol and taxotere by HPLC. Drug incubations were carried out in dark in pH-adjusted human urine over a period of 0.5 to 4 h

Table 1. Human bladder tumor cell lines used for in vitro experiments

Cell line	Originator	Origin of tumor	Histology/grade
MGH-U1	Kato/1972	Bladder	TCC, invasive, poorly differentiated, grade II
CUB-2	Kodera/1975	Lymph node	Squamous-cell carcinoma, grade
COLO 232	Moore/1976	Bladder	TCC, well-differentiated, grade III
SD	Grups/1988	Bladder	TCC
HU 549	Vilien/1975	Unknown	TCC, noninvasive, grade II
T24	Bubenik/1970	Bladder recurrence	TCC, invasive, poorly differentiated, grade III
A1698	Rosen/1980	Bladder recurrence	TCC
HT 1376	Raheed/1973	Bladder	TCC, invasive, moderately pleomorphic, grade III
UM-UC-3	Grossman/1982	Bladder	TCC, high-grade
SCaBER	O'Toole/1974	Bladder	Squamous-cell carcinoma, moderately differentiated
J82	O'Toole/1972	Bladder	TCC, invasive, poorly differentiated, grade III
TCCSUP	O'Toole/1974	Bladder	TCC, undifferentiated, grade IV

TCC, Transitional-cell carcinoma

Table 2. Chemosensitivity of human bladder tumor cell lines as determined using a clonogenic assay

Cell line	Vinblastine (μ*M*)				Taxol (μ*M*)				Taxotere (μ*M*)			
	0.01	0.1	1	10	0.01	0.1	1	10	0.01	0.1	1	10
MGH-U1	110	94	92	23	56	1	0	0	2	5	0	0
CUB-2	102	28	0	0	98	0	0	0	90	4	0	0
COLO 232	84	41	0	0	41	0	0	0	4	0	0	0
SD	122	40	2.2	0	95	0	0	0	31	0	0	0
HU 549	0	0	0	0	10.5	0	0	0	27	0	0	0
T24	108	68	13.5	2	83	26	8	4	26	8	5	3
A1698	106	33	0	0	51	0	0	0	11	0	0	0
HT 1376	24	2	0	0	52	0	0	0	2	0	0	0
UM-UC-3	64	51	0	0	56	0	0	0	0	0	0	0
SCaBER	95	83	26	6	38	22	18	1	59	20	14	7
J82	69	46	17	0	89	4	15	2	57	11	2	0
TCCSUP	50	38	0	0	53	0	0	0	0	0	0	0

This table gives the percentage of colony survival at four drug concentrations for vinblastine, taxol, and taxotere as determined using the clonogenic assay. A total of 12 HBTCL was used for the studies. Data represent the mean values for four plates (colony counts) relative to four control plates. A colony survival value of 25% or less represents a significantly active drug level

Vinblastine produced significant inhibition of clonal growth in 2 of the 12 tumor cell lines (17%) within the clinically achievable concentration range of 0.01–0.1 μ*M*. Taxol produced significant clonal inhibition in 1 HBTCL at a concentration of 0.01 μ*M* (8%) but was significantly more active than vinblastine at 0.1 μ*M*, with activity being noted in 11 of 12 HBTCL (92%). At 1 μ*M*, taxol was active in all cell lines tested. Taxotere was found to be the most active agent of the three drugs with significant suppression of clonal growth occurring in 6 of 12 (50%) at a concentration

of 0.01 μ*M* HBTCL and at 0.1 μ*M* in all HBTCL. The mean 50% growth-inhibitory concentrations (IC_{50} values) were 0.06 μ*M* for vinblastine, 0.01 μ*M* for taxol, and 0.008 μ*M* for taxotere. No relationship was found with any drug between its activity in vitro and the histology, grade, or stage of the original bladder tumor.

The stability of taxol and taxotere was assessed in urine at pH 5, 6, and 7 in the dark. Urine (pH 7) from a normal volunteer was used and adjusted to pH 5 and 6 with 2 *N* HCl. The concentrations of taxol and taxotere in urine were 10 and 25 μ*M*, respectively. The samples were incubated at pH 5, 6, and 7 at 37° C for 0.5, 1, 2, and 4 h and then analyzed by HPLC. Peak areas on the chromatograms were compared with the area at 0 h and expressed as percentages. Figure 2 shows the stability over time for taxol and taxotere. The highest recoveries of taxol were found at pH 6 (95%–96%) whereas 88% of the drug remained after 4 h at pH 5. Taxotere demonstrated stabilities at 4 h of 99%, 87%, and 90% at pH 5, 6, and 7, respectively. At least 85% of either compound remained present at 4 h over the pH range tested.

Discussion

Both taxol and taxotere are highly active against the panel of HBTCL used in this study. The activity of these agents occurs within the clinically achievable range for systemic administration of the drugs. These findings suggest that both agents may have significant clinical activity in bladder cancer.

Difficulty with the production of taxol has stimulated a search for analogs of the drug. Taxotere may be easier to obtain than that of taxol since its partial synthesis from the leaves (needles) of the yew tree does not result in the death of the tree. This allows a replenishable source of taxotere without endangering the yew tree population. Mounting in vitro and in vivo data suggest that taxotere is at least as active as taxol. Microtubule assembly has been shown to be higher for taxotere than for taxol [17]. Bissery et al. [2] also noted that the concentration of taxotere required to inhibit 50% of microtubular assembly was 2 times lower than that of taxol. In vitro studies [17] have shown taxotere to be 2.5 times more potent than taxol in inhibiting replication of J774.2 and P388 cells and 5 times more potent in taxol-resistant tumor cells. Using a clonogenic assay and ovarian human tumor explants, Alberts et al. [1] found at least equivalent activity between taxol and taxotere. Bissery et al. [2] have shown taxotere to be more active than taxol in vivo in the B16 melanoma model. We have also found that taxotere is active in lower concentrations than taxol in HBTCL.

Vinblastine was found to have minimal activity in vitro at similar clinically achievable drug levels. In a previous study [14] using 1-h drug exposures, we found that a group of standard and investigational anticancer agents (including vinblastine) had low levels of activity against a panel of HBTCL and that these results paralleled the low level of clinical activity of these drugs in bladder cancer. Two HBTCL (17%) in the present study were sensitive to vinblastine. Both of these in vitro studies paralleled the low level of activity (18%) reported in clinical trials [3].

Both taxol and taxotere were found to have properties that might prove useful in treating patients with superficial bladder cancer. We found that in vitro exposures of as short as 30 min resulted in significant activity against all HBTCL. Schiff et al. [21] have reported that as little as 30 min of exposure to taxol promotes microtubule assembly and stabilization of microtubules by shifting the dynamic equilibrium toward microtubular assembly. Manfredi et al. [10] demonstrated that saturation of taxol binding sites was reached within 60 min at 0.3 μ*M* taxol, with continued treatment resulting in microtubular bundle formation and stabilization. The mechanism of drug uptake is thought to result from passive diffusion.

Results of animal experiments have demonstrated that both taxol and taxotere appear to be schedule-independent [2]. We noted that the in vitro activity of both taxol and taxotere was maximal over brief periods of drug exposure. Using a soft-agar assay system, Rowinsky et al. [19] demonstrated that inhibition of the clonogenic growth of several resistant leukemic cell lines (LC8A, HL-60, Daudi, and K562) by taxol was both time- and concentration-dependent. They noted increasing inhibition of clonal activity by taxol occurring within 2–22 h of drug exposure. The maximal activity of taxol and taxotere after brief drug exposures seen in these HBTCL could be useful in the management of patients with superficial bladder cancer since the administration of intravesical chemotherapy is technically feasible for only 1–2 h.

For taxotere and taxol to be useful for intravesical therapy, they should be stable during intravesical therapy. We show herein that both drugs are stable within a narrow pH range in human urine. We obtained a high level of recovery of drug at pH 5 –7, with 85% of the compounds being detectable at 4 h. Ringel and Horwitz [18] demonstrated that taxol was stable in the pH range used in these experiments but was unstable at a pH of 9 or higher. These authors [16] demonstrated that the effect of taxol on the stability of microtubules was constant through pH 5–7 but began to drop at a pH of greater than 7.7.

Our studies demonstrate that both taxol and taxotere are active against HBTCL. The studies suggest that both taxol and taxotere may be active agents for use in the systemic therapy of bladder cancer. The activity of these agents over short periods of drug exposure combined with their stability over several hours and at the clinical range of pH experienced in human urine suggest their potential usefulness for intravesical therapy.

References

1. Alberts DS, Garcia D, Fanta P, Liu R, Roe D, Salmon SE (1992) Comparative cytotoxicities of taxol and taxotere in vitro against fresh human ovarian cancers. Proc Am Soc Clin Oncol 11: 226
2. Bissery M-C, Guenard D, Gueritte-Voegelein F, Lavelle F (1991) Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res 51: 4845–4852
3. Blumenreich MS, Yagoda A, Natale RB, Watson RC (1982) Phase II trial of vinblastine sulfate for metastatic urothelial tract tumors. Cancer 50: 435–438

4. Chang A, Kim K, Glick J, Anerson T, Karp K, Hohnson D (1992) Phase II study of taxol in patients with stage IV non-small cell lung cancer: the Eastern Cooperative Oncology Group. Proc Am Soc Clin Oncol 11: 293
5. Gueritte-Voegelein F, Guenard D, Lavelle F, Le Goff M-T, Mangatal L, Potier P (1991) Relationships between the structure of taxol analogs and their antimitotic activity. J Med Chem 34: 992–998
6. Hamburger A, Salmon SE (1977) Primary bioassay of human myeloma stem cells. J Clin Invest 60: 846–854
7. Hepburn PJ, Masters JRW (1983) The biological characteristics of continuous cell lines derived from human bladder. In: Bryan GT, Cohen SM (eds) The pathology of bladder cancer, vol 2. CRC Press. Boca Raton, Florida, p 213–227
8. Holmes RA, Walters RS, Theriault RL, Forman AD, Newton LK (1991) Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. J Natl Cancer Inst 83: 1797–1805
9. Longnecker SM, Donehower RC, Cates AE, Chen T-L, Brundrett RB, Growchow LB, Ettinger DS, Colvin M (1987) High-performance liquid chromatographic assay for taxol in human plasma and urine and pharmacokinetics in a phase I trial. Cancer Treat Rep 71: 53–59
10. Manfredi JJ, Horwitz SB (1984) Taxol: an antimitotic agent with a new mechanism of action. Pharmacol Ther 25: 83–125
11. Masters JRW, Hepburn PJ, Walker L, Highman WJ, Trejdosiewicz LK, Povey S, Parker M, Hill BT, Riddle PR, Franks LM (1986) Tissue culture model of transitional cell carcinoma: characterization of twenty-two human urothelial cell lines. Cancer Res 46: 360–363
12. McGuire WP, Rowinsky EK, Rosenshein NB, Grumbine FC, Ettinger DS, Armstrong DK, Donehower RC (1989) Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann Intern Med 111: 273–279
13. National Cancer Institute (1983) Clinical brochure: Taxol(NSC 125973). NCI, Division of Cancer Treatment, Bethesda, Maryland, pp 6–12
14. Niell HB, Webster KC, Smith EE (1985) Anticancer drug activity in human bladder tumor cell lines. Cancer 56: 1039–1044
15. Raghavan D, Shipley W, Garnick M, Russell P, Richie JP (1990) Biology and management of bladder cancer. New Engl J Med 322: 1129–1138
16. Ringel I, Horwitz SB (1991) Effect of alkaline pH on taxol-microtubule interactions. J Pharmacol Exp Ther 259: 855–860
17. Ringel I, Horwitz SB (1991) Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83: 288–291
18. Ringel L, Horwitz SB (1987) Taxol is converted to 7-epitaxol, a biologically active isomer, in cell culture medium. J Pharmacol Exp Ther 242: 692–698
19. Rowinsky EK, Donehower RC, Jones RJ, Tucker RW (1988) Microtubule changes and cytotoxicity in leukemic cell lines treated with taxol. Cancer Res 48: 4093–4100
20. Sarosy G, Kohn E, Link C, Adamo D, Davis P, Ognibene R, Goildspiel B, Christian M, Reed E (1992) Taxol dose intensification in patients with recurrent ovarian cancer. Proc Am Soc Clin Res 11: 226
21. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277: 665–667
22. Schiff PM, Horwitz SB (1980) Taxol stabilized microtubules in mouse fibroblast cells. Proc Natl Acad Sci USA 77: 1561
23. Soloway MS (1987) Selecting initial therapy for bladder cancer. Cancer 60: 502–513
24. Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E (1987) Phase I clinical and pharmacokinetic study of taxol. Cancer Res 47: 2486–2493