Experimental Antitumor Activity of Taxotere (RP 56976, NSC 628503), a Taxol Analogue[1]

Marie-Christine Bissery,[2] Daniel Guénard, Françoise Guéritte-Voegelein, and François Lavelle

Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 94403 Vitry sur Seine [M-C. B., F. L.], and Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette [D. G., F. G-V.], France

ABSTRACT

Taxotere (RP 56976; NSC 628503; N-debenzoyl-N-tert-butoxycarbonyl-10-deacetyl taxol) is a new microtubule stabilizing agent. It is obtained by semisynthesis from a noncytotoxic precursor extracted from the needles of the tree, *Taxus baccata* L. Taxotere was evaluated for antitumor activity against a variety of transplantable tumors of mice. Taxotere had no marked schedule dependency and was found active by the i.v. and the i.p. routes. Upon i.v. administration, 9 of 11 tumor models tested responded to Taxotere. B16 melanoma was found highly sensitive to Taxotere, with a tumor growth inhibition of 0% and a 3.0 $\log_{10}$ tumor cell kill at the maximum tolerated dose. In the same trial, taxol produced only a 1.1 $\log_{10}$ tumor cell kill at the maximum tolerated dose. Taxotere cured early stage pancreatic ductal adenocarcinoma 03 (6 of 6 cures) and colon adenocarcinoma 38 (7 of 7 cures). It also effected greater than 80% complete regressions of advanced stage disease with both tumors. Taxotere was active against early and advanced stage colon adenocarcinoma 51, with 2.3 and 1.7 $\log_{10}$ cell kill, respectively. Four other tumors responded to a lesser extent: Lewis lung (5.5% tumor growth inhibition), Glasgow osteogenic sarcoma (27.2% tumor growth inhibition), L1210 and P388 leukemias (70 and 54% increase in life span, respectively). Because of its good preclinical activity and its unique mechanism of action, Taxotere has entered Phase I clinical trials.

INTRODUCTION

In the late 1960s, a crude alcohol extract of bark from the Pacific yew, *Taxus brevifolia* L., tested by the Division of Cancer Treatment at the NCI,[3] showed cytotoxicity *in vitro*, and efficacy *in vivo* against transplantable leukemias of mice (1–3). In 1971, Wani *et al.* (2) isolated and characterized taxol, the active principle of the extract. Taxol is a mitotic spindle poison (4), that stabilizes microtubules and inhibits their depolymerization to free tubulin (5). The recognition of this unique mechanism of action was decisive in pursuing taxol development despite difficulties to obtain an adequate supply.

An analog of taxol, Taxotere [N-debenzoyl-N-tert-butoxycarbonyl-10-deacetyl taxol; RP 56976; NSC 628503; (Fig. 1)], was obtained by semisynthesis at the Institut de Chimie des Substances Naturelles (Gif sur Yvette, France), through a collaborative agreement with the Centre National de la Recherche Scientifique. It was prepared from a noncytotoxic precursor extracted from the needles of *Taxus baccata* L., 10-deacetyl baccatin III, which was then condensed by esterification with the side chain prepared by chemical synthesis (6–8).

Taxotere was first selected by using a tubulin test (9) and was

---

found to inhibit microtubule depolymerization (10). It was then found cytotoxic *in vitro* against P388 leukemia cells, and initial *in vivo* experiments proved Taxotere to be more active than taxol (11).

These encouraging results, together with the development of an efficient semisynthesis process using a renewable source of natural precursor, led to broader preclinical testing. The work reported herein presents the *in vivo* antitumor activity of Taxotere against a wide variety of transplantable tumors of mice with different biological properties and chemosensitivities (12).

MATERIALS AND METHODS

Drugs

Taxotere (RP 56976, NSC 628503) was first dissolved in ethanol, then polysorbate 80 was added and the final dilution of Taxotere was obtained with 5% glucose in water (5/5/90; v/v/v). The pH of the final solution was 5. It was injected i.v., 0.4 ml/mouse. Taxol (NSC 125973) was obtained from the National Cancer Institute (Bethesda, MD). It was prepared as described above for Taxotere, and injected in the same volume. Reference compounds were obtained from various suppliers: cyclophosphamide (Laboratoire Lucien, Colombes, France); 5-fluorouracil (Laboratoire Roche, Neuilly sur Seine, France); doxorubicin (Laboratoire Roger Bellon, Neuilly sur Seine, France, and Mead Johnson, Evansville, IN); cis-platinum (Laboratoire Roger Bellon). All reference compounds were prepared in 5% glucose in water, except for cis-platinum, which was prepared in 0.9% sodium chloride solution, pH 4.5. The volume of injection for all reference compounds was 0.2 ml/mouse.

Mice

Male and female DBA/2, C57BL/6, and C57BL/6 female × DBA/2 male hereafter called B6D2F₁ mice were bred at IFFA CREDO (L'Abresie, France) from strains obtained from The Jackson Laboratory, Bar Harbor, ME. Male and female C3H/Hen, BALB/c, and BALB/c female × DBA/2 male hereafter called CD2F₁ mice, were bred at Charles River (Cléon, France) from strains obtained from Charles River Laboratories, Wilmington, MA. Mice were over 18 g at start of chemotherapy. They were supplied food (UAR Reference 113, Epinay sur Orge, France) and water *ad libitum*.

Tumor Models

The tumors used for *in vivo* evaluation were: B16 melanoma (B16) (13); colon adenocarcinomas 38 (C38), and 51 (C51); colon carcinoma 26 (C26) (14); pancreatic ductal adenocarcinoma 03 (P03) (15); mammary adenocarcinoma 16/C (MA 16/C) (16); Lewis lung carcinoma (3LL) (17); Glasgow osteogenic sarcoma (GOS) (18); M5076 histiocytosarcoma (M5076) (19); P388 lymphocytic leukemia (P388), and L1210 lymphoid leukemia (L1210). The tumors used for cross-resistance evaluation were: P388 leukemia resistant to doxorubicin (P388/Dox), P388 leukemia resistant to vincristine (P388/Vcr), L1210 resistant to cis-platinum (L1210/CisDDP), L1210 resistant to BCNU (L1210/BCNU) (20). These tumors are in the National Cancer Institute frozen tumor repository, maintained at the Frederick Cancer Research Facility (Frederick, MD), and have a code identification number, a detailed description, and a list of references.

Tumors were maintained in the mouse strain of origin, *i.e.*, C57BL/
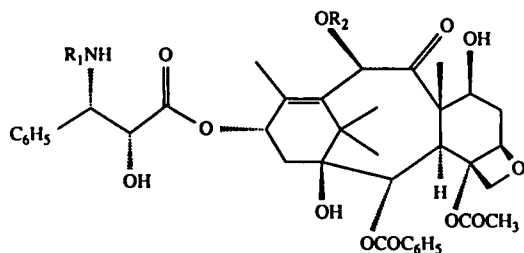
Taxotere : R_1 = -COOC(CH$_3$)$_3$; R_2 = H

Taxol : R_1 = -COC$_6$H$_5$; R_2 = -COCH$_3$

Fig. 1. Taxotere (RP 56976, NSC 628503); N-debenzoyl-N-tert-butoxycar-bonyl-10-deacetyl taxol.

6 (C38, P03, B16, 3LL, GOS, M5076), BALB/c (C51, C26), C3H/Hen (MA 16/C), DBA2 (L1210, P388). Solid tumors were transplanted as s.c. fragments. Leukemias were passaged as weekly i.p. implants. For chemotherapy trials, tumors were transplanted in the strain of origin or in the appropriate F$_1$ hybrid.

In Vivo Solid Tumor Studies

Chemotherapy. The methods of protocol design, chemotherapy techniques, and data analysis have been presented in detail (21–24). Briefly, the animals necessary to begin a given experiment were pooled and implanted s.c. bilaterally with 30- to 60-mg tumor fragments on day 0 with a 12-gauge trocar. Bilateral implants were used to ensure a more uniform tumor burden per mouse and thus reduce the requirement for a greater number of mice per group. For an early stage tumor treatment, the animals were again pooled before distribution to the various treatment and control groups. For an advanced stage treatment, tumors were allowed to grow to the desired size range (animals with tumors not in the desired range were excluded). The mice were then pooled and distributed to the various treatment and control groups. Non-tumor-bearing animals were often matched to tumor-bearing groups and given the same agents by the same route, dose, and schedules. In this way, drug-induced toxicity could be clearly separated from the toxic effects of tumors. Chemotherapy was started 3 to 22 days after tumor transplantation. Each group of mice was treated on the basis of group average weight. Mice were checked daily and adverse clinical reactions were noted. Each group of mice was weighed 3 to 5 times weekly until the weight nadir was reached. Then, groups were weighed once or twice weekly until the end of the experiment. Body weight change data were reported as the maximum treatment-related weight loss.

Tumors were measured with a caliper 2 or 3 times weekly, depending on the tumor growth rate, until the tumor reached 2000 mg or until the animal died. Solid tumor weights (mg) were estimated from two dimensional tumor measurements (mm):

$$\text{Tumor weight} = \frac{\text{Length} \times \text{width}^2}{2}$$

The day of death was recorded, and macroscopic examination of the thoracic and abdominal cavities was carried out to assess the probable cause of death. In some cases, tissues samples were submitted for histological evaluation.

A reference drug known to be active for the tumor under investigation was included at two or more dose levels in the majority of the trials to ensure proper tumor chemosensitivity.

End Points for Assessing Solid Tumor Activity. In each trial, four dose levels were evaluated, including at least one dose level that was frankly toxic. Antitumor activity was evaluated at the HNTD which is the highest dosage that can be administered without causing death or undue toxicity. A dosage producing a weight loss nadir ≥20%, or 20%

or more drug deaths, was considered as excessively toxic. Animal body weights included tumor weights.

Tumor Growth Inhibition (T/C Value). For early stage disease, this is the most widely used criterion for the determination of antitumor activity. The tumor weight was determined simultaneously for the treated and the control groups. When the median tumor weight of the control (C) reached approximately 750 to 1500 mg, the median tumor weight of each treated group (T) were determined, including zeros. The T/C value in percentage is calculated as follows:

$$\text{T/C (\%)} = \frac{\text{Median tumor weight of the treated}}{\text{Median tumor weight of the control}} \times 100$$

According to NCI standards, a T/C ≤42% is the minimum level for activity. A T/C <10% is considered as a high antitumor activity level which justifies further development (DN-2 level).

Tumor Growth Delay (T − C Value). The tumor growth delay assays are based on the median time (in days), required for the treatment group (T) and the control group (C) tumors, to reach a predetermined size (usually 750 to 1000 mg). Tumor-free survivors are excluded from these calculations and are tabulated separately. This value is very useful as it allows the quantitation of the tumor cell kill.

Determination of Tumor-doubling Time. Td is estimated from the best fit straight line from a log linear growth plot of the control group tumors in exponential growth (100- to 1000-mg range).

Calculation of Tumor Cell Kill. For s.c. growing tumors, the total log$_{10}$ cell kill is calculated from the following formula:

$$\text{Log}_{10} \text{ cell kill (gross or total)} = \frac{T - C \text{ value in days}}{3.32 \times \text{Td}}$$

where $T - C$ is the tumor growth delay in days as defined above, and Td is the tumor volume-doubling time in days. The conversion of the tumor growth delay values to log$_{10}$ cell kill is possible because the Td of tumors regrowing after treatment approximated the Td values of tumors in the control mice.

Regression of Advanced Stage Primary Site Tumor. These criteria are used for advanced stage trials. A complete regression is a regression below the limit of palpation. A partial regression is a regression greater than 50% reduction in tumor mass. In the tables, the partial regression column includes complete regressions.

In Vivo Leukemia Studies

Chemotherapy. Hemocytometer counted cells [10^6 cells/mouse for P388, P388/Dox, P388/Vcr, or 10^5 cells/mouse for L1210, L1210/CisDDP, L1210/BCNU, suspended in Hanks' medium (Gibco, Cergy-Pontoise, France)] were implanted i.p. (0.5 ml/mouse) in B6D2F$_1$ mice after randomization on day 0. The mice were again randomized into treatment cages. The test agents were injected i.v. starting on day 1, on the basis of average group weight. The treatment duration was 4 days (days 1 through 4). The body weight change was recorded daily during therapy and once a week thereafter. Cause of death was determined by examination of spleen size, liver involvement, and presence or absence of ascites. This visual examination allowed for discrimination between delayed drug deaths and tumor deaths. Non-tumor-bearing animals were often matched to tumor-bearing groups and received the same agents by the same route, dose, and schedules, to clearly separate drug-induced toxicity from leukemia effects.

End Points for Assessing Antileukemic Activity

Percentage of Increase in Life Span. The increase in life span was determined as follows:

% of ILS =

$$\frac{100 \times [(\text{MDD treated tumor-bearing mice}) - (\text{MDD control tumor-bearing mice})]}{\text{MDD control tumor-bearing mice}}$$

where MDD was the median day of death; % of ILS was only used for

leukemia trials. According to NCI standards for i.p. tumor/i.p. drug, an ILS ≥25% for L1210 and 27% for P388 is the minimum level for activity. An ILS ≥50% for L1210 and 75% for P388 is considered a high level of antileukemic activity (DN-2 level). However, the model used in this evaluation is a more difficult one (i.p. tumor, i.v. drug) and any ILS over 40% is considered good leukemia activity.

RESULTS

Schedule Dependency. To evaluate the schedule effect on the total dose that could be injected without undue toxicity, four i.v. administration schedules were tested (Table 1). The intermittent schedules (days 1 and 6, and days 1, 5, 9, 13), allowed the administration of the largest total doses of Taxotere (80.6 and 66.4 mg/kg, respectively). The daily schedule (days 1–8) and the split dose schedule (every 3 h for 3 doses, days 1–6), reduced the total dose that could be administered (49.6 and 36.8 mg/kg of Taxotere, respectively). Thus, the agent is considered schedule independent because of the relatively small difference in total dosage on the different schedules. Historically, a schedule-dependent agent (*e.g.*, amethopterin or 1-β-D-arabinofuranosylcytosine) would have a 10- to 30-fold lower total dosage on the every 3 h for 3 doses on days 1–6 compared to the day-1 and -6 schedule (25).

Evaluation of Taxotere against Solid Tumors

B16 Melanoma. Taxotere and taxol were tested by using an intermittent schedule (days 4, 6, 8, 10) against early stage s.c. B16 melanoma (Table 2). Taxotere was highly active at the HNTD (13.4 mg/kg/injection, total dose, 53.6 mg/kg), with a T/C of 0% and a total log cell kill value of 3. In comparison, the HNTD of taxol was 21.7 mg/kg/injection (total dose, 86.8 mg/kg), with a T/C of 0% but a total log cell kill value of only 1.1. In this trial, cyclophosphamide (included as a positive control) was found highly active at the highest dose tested. Additional studies were performed on mice bearing early stage B16 melanoma to evaluate the effect of the route of administration on the antitumor efficacy of Taxotere (Table 3). At the highest dosage tested, i.p. administration yielded comparable activity to i.v. administration with 3 logs of tumor cell kill. Administration p.o. led to a total loss of Taxotere activity (T/C = 53%), with a total HNTD of 270 mg/kg producing minimal weight loss (3% of the initial body weight). At higher dosages (450–750 mg/kg), the dose-limiting toxicity was gastrointestinal (data not shown).

Colon Adenocarcinoma 38. Taxotere was tested against both early and advanced stage colon adenocarcinoma 38. Mice with early stage disease were treated i.v. on days 3, 5, and 7 (Table 4). At the HNTD (23.5 mg/kg/injection; total dose, 70.5 mg/kg), the agent was found highly active with a 100% cure rate on day 270. The dosage below the HNTD (14.6 mg/kg/injection) retained activity with 2 of 7 cures.

Mice with advanced stage disease were treated i.v. with three schedules: A, every 5 days for 2 treatments; B, every other day for 3 treatments; and C, twice daily for 5 days (Table 5). Treatment started when the median tumor weight was 150 mg for schedule A, and 120 mg for schedules B and C. The treatment duration was identical for the three experiments (5 days). The HNTD ranged from 80.6 mg/kg (schedules A and B) to 50 mg/kg (schedule C) and produced a body weight loss at nadir of 6.3% for schedule A, 12.8% for schedule B, and 14.4% for schedule C (nadir reached 3–6 days after last treatment). Full host recovery occurred within 10 days for the three schedules. Overall, advanced stage colon adenocarcinoma 38

Table 1 *Evaluation of schedule of administration on Taxotere toxicity*[a]

Schedule	i.v. dose range (mg/kg/dose)	HNTD (mg/kg/dose)	Total dose (mg/kg)	% of mean wt loss (day of nadir)
Days 1, 6	15.5–65	40.3	80.6	11 (12)
Days 1, 5, 9, 13	6.2–27.6	16.6	66.4	15 (18)
Days 1–8	2.3–10	6.2	49.6	18 (10)
Days 1–6[b]	0.33–6.2	2.3	36.8	15 (7)

[a] B6D2F₁ male mice were used, average weight 22.4 g, 5 mice per group.
[b] Every 3 h for 3 doses. The volume of injection was reduced to 0.2 ml/injection due to the frequency of injection. The compound was given 3 times/day on days 1–5, and once only on day 6.

was found very sensitive to Taxotere. At equitoxic dosages, the intermittent schedules A and B were the most active with 5 of 5 and 4 of 5 complete tumor regressions, respectively. The corresponding log cell kill were 1.6 for schedule A, and 1.7 for schedule B. Schedule C was also effective with a 0.9 log cell kill, but tumor regression could not be achieved.

As an internal control, 5-fluorouracil, the best reference agent against early stage colon 38, was included in all these trials (Tables 4 and 5). As expected, it was found highly active at the HNTD against early stage disease, and moderately active against advanced stage disease.

Colon Adenocarcinoma 51/A. Mice bearing early stage colon adenocarcinoma 51/A were treated with i.v. Taxotere, using an intermittent schedule on days 3, 5, and 7 (Table 6). At the HNTD, 12.7 mg/kg/injection (total dose, 38.1 mg/kg), the T/C was 2.4%, indicating a high level of activity (2.3 log cell kill). The dosage below the HNTD retained activity.

Taxotere was also tested against advanced stage colon 51 (tumors were approximately 70 mg at start of therapy), using an intermittent administration on days 10, 12, and 14. At the HNTD (15.2 mg/kg/injection, total dose, 45.6 mg/kg), colon 51 was still sensitive to Taxotere with a 1.7 log cell kill. *cis*-Platinum, included as a positive control, was found highly active against advanced stage disease.

Colon Carcinoma 26. Taxotere was administered i.v. to early stage colon 26-bearing mice on a daily schedule on days 1–4. The highest dose tested, 5 mg/kg/injection (total dose, 20 mg/kg) was found modestly active. Although this dosage is low, it produced a 13% body weight loss, indicating that it is close to the highest nontoxic dose. The dosages below were inactive (data not shown).

Pancreatic Ductal Adenocarcinoma 03. Taxotere was administered i.v. every other day for four treatments to mice bearing early stage or advanced stage pancreatic ductal adenocarcinoma 03 (Table 7). Early stage disease (treatment starting on day 3) was found highly sensitive to Taxotere with a 100% cure rate at optimal dosage (20.5 mg/kg/injection, total dose, 82 mg/kg).

Advanced stage pancreatic adenocarcinoma 03 (median tumor range, 273–377 mg) was also sensitive to Taxotere at the HNTD (18 mg/kg/injection) with 5 of 6 complete regressions and a log cell kill value of 1.8. However, the dosage below did not retain activity. Doxorubicin, the reference compound for this tumor, was found marginally active against advanced stage disease.

Lewis Lung Carcinoma. Taxotere was injected i.v. on days 3 through 7 to mice bearing Lewis lung carcinoma. The optimal dosage (23.2 mg/kg/injection; total dose, 116 mg/kg) was found active with a 5.5% T/C and a corresponding log cell kill value of 1.2. The two dosages below the optimal dose retained activity (Table 8). Cyclophosphamide, the positive control for this tumor, was found highly active.

Table 2 *Comparison of Taxotere and taxol against early-stage B16 melanoma*[a]

i.v. agent	Dosage (mg/kg/injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body wt change [g/mouse (day of nadir)]	Median tumor wt [mg on day 11 (range)]	% of T/C, day 11	Time for median tumor to reach 1000 mg	T – C (days)	Log cell kill total	Comments
Taxotere	35	4, 6, 8, 10	140	5/5 (12, 13, 3D14)[b]							Toxic
	21.7		86.8	0/5	−6.14 (13)						Toxic[c]
	13.4		53.6	0/5	−3.99 (12)	0 (0–0)	0	23	12.3	3.0	HNTD highly active
	8.3		33.2	0/5	−1.63 (12)	0 (0–151)	0	20	9.3	2.2	Highly active
Taxol	35	4, 6, 8, 10	140	0/5	−6.31 (14)			18.5	7.8		Toxic[c]
	21.7		86.8	0/5	−0.72 (13)	0 (0–0)	0	15.5	4.8	1.1	HNTD active
	13.4		53.6	0/5	−0.86 (7)	120 (0–764)	10	14.2	3.5	0.8	Marginally active
	8.3		33.2	0/5	−0.30 (5)	738 (196–964)	63	12.5	1.8	0.4	Inactive
Cyclophosphamide	180	4, 10	360	0/5	−3.21 (16)	0 (0–0)	0	25.2	14.5	3.5	HDT[d] highly active
	108		216	0/5	−1.32 (11)	0 (0–0)		22.5	11.8	2.8	Highly active
Control				0/9		1171 (944–1599)		10.7			

[a] B16 melanoma implanted s.c., passage 66, tumor doubling time, 1.25 days; male C57BL/6; mouse average weight; Taxotere, 25.3 g; taxol, 25.3 g; cyclophosphamide, 26.4 g.
[b] 3D14, 3 deaths on day 14.
[c] Toxicity based on the total body weight loss above 20% which is considered excessively toxic by NCI standards.
[d] HDT, highest dose tested.

Table 3 *Effect of route of administration on Taxotere activity against B16 melanoma*

Schedule	Route	Dosage range (mg/kg/dose)	Optimal dose (mg/kg/dose)	Total dose (mg/kg)	Mean body wt change [g/mouse (day of nadir)]	T/C (%)	T – C (days)	Log cell kill total	Comments
Days 3, 5, 7, 9[a]	i.v.	40–8.6	14.4	57.6	−3.9 (13)	0	12.2	2.5	Highly active
Days 3–7[b]	i.v.	30–6.5	18	90	−3.7 (14)	0	17.5	3.5	Highly active
Days 3–4/5–7[b, c]	i.p.	16–3.9/32–7.8	16/32	128	−2.7 (11)	0	15.4	3.0	Highly active
Days 3–7[d]	p.o.	150–54	54	270	−0.7 (8)	53			Inactive

[a] B16 melanoma implanted s.c., passage 55, tumor-doubling time = 1.5 days; male B6D2F$_1$ mice; mouse average weight, i.v. route, 28 g.
[b] B16 melanoma implanted s.c., passage 66, tumor-doubling time = 1.5 days; female B6D2F$_1$ mice; mouse average weight, i.v. route, 20.5 g; i.p. route, 21.5 g.
[c] Dosages had to be escalated during the trial; starting dosages were doubled on days 5–7.
[d] B16 melanoma implanted s.c., passage 75, tumor-doubling time = 1.25 days; male B6D2F$_1$ mice; mouse average weight, p.o. route, 24.4 g.

Table 4 *Evaluation of Taxotere against early-stage colon adenocarcinoma 38*[a]

i.v. agent	Dosage (mg/kg/dose)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body wt change [g/mouse (day of nadir)]	Median tumor wt [mg on day 49 (range)]	T/C (%)	Time for median tumor to reach 1000 mg (day)	T – C (days)	Total log cell kill	Tumor-free survivors on day 270	Comments
Taxotere	38	3, 5, 7	114	2/5 (14, 18)							3/5	Toxic
	23.5		70.5	0/7	−2.49 (14)	0 (0–0)	0				7/7	HNTD highly active
	14.6		43.8	0/7	−2.07 (13)	172 (0–772)	15.1	60.7	15.6	1.2	2/7	Active
5-Fluorouracil	100	3, 7	200	2/5 (13, 14)							3/5	Toxic
	60		120	0/5	−0.7 (8)	0 (0–108)	0				3/5	HNTD highly active
	36		72	0/5	+0.32 (8)	288 (0–533)	25.3	64	18.9	1.4	0/5	Active
Control				0/9		1136 (100–3017)		45.1			0/13	

[a] C38 adenocarcinoma implanted s.c., passage 98, tumor-doubling time = 3 days; female B6D2F$_1$; mouse average weight: Taxotere, 20.1 g; 5-fluorouracil, 20 g.

Glasgow Osteogenic Sarcoma. Taxotere was administered i.v. on a daily schedule (days 3–7) against early stage Glasgow osteogenic sarcoma. The HNTD, 18.6 mg/kg/injection (total dose, 93 mg/kg), caused a 13% body weight loss and was found marginally active with 27.2% T/C. The dosages below (11.5 and 7.1 mg/kg/injection) were inactive (data not shown).

M5076 Histiocytosarcoma. Taxotere was tested against early stage M5076 and was given i.v. on days 3–7. The highest nontoxic dose (8.6 mg/kg/injection) produced an 11% body weight loss and was found inactive with a 51% T/C (data not shown).

Mammary Adenocarcinoma 16/C. Mice bearing early stage mammary 16/C were treated i.v. with Taxotere on days 3, 5, and 7. The HNTD (14.4 mg/kg/injection; total dose, 43.2 mg/kg) produced a 14.8% body weight loss and was found inactive with a 47.5% T/C (data not shown).

Evaluation of Taxotere against Leukemias

Taxotere was tested against P388 and L1210 leukemias (data not shown). The leukemic cells were implanted i.p. and Taxotere was administered i.v. days 1–4. The HNTD were 23.2 mg/kg/injection (total dose, 92.8 mg/kg) for P388 leukemia and 21.7 mg/kg/injection (total dose, 86.8 mg/kg) for L1210 leukemia and produced a 54 and a 70% increase in life span, respectively.

Table 5 *Evaluation of Taxotere against advanced colon adenocarcinoma 38; schedule of administration studies[a]*

i.v. agent	Dosage (mg/kg/injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body wt change [g/mouse (day of nadir)]	Regressions Partial	Regressions Complete	Cure, day 60	Time for median tumor to reach 750 mg in days	T − C (days)	Log cell kill total	Comments
Schedule A[b]												
Taxotere	65	14, 18	130	1/5 (29)								Toxic
	40.3		80.6	0/5	−1.50 (24)	4/5	4/5	0/5	38.0	15.5	1.6	HNTD active
	25		50	0/5	−0.23 (19)	0/5	0/5	0/5	31.5	9.0	0.9	Active
	15.5		31	0/5	+0.95 (19)	0/5	0/5	0/5	28.5	6.0	0.6	Inactive
5-Fluoroura-cil	90	14, 18	180	3/5 (2D25, 28)[c]								Toxic
	56		112	0/5	−0.75 (15)	3/5	2/5	0/5	32.2	9.7	1.0	HNTD active
Schedule B[b]												
Taxotere	43.3	14, 16, 18	129.9	2/5 (24, 25)								Toxic
	26.8		80.4	0/5	−3.43 (22)	5/5	5/5	0/5	39.0	16.5	1.7	HNTD active
	16.6		49.8	0/5	−2.32 (21)	0/5	0/5	0/5	32.7	10.2	1.0	Active
	10.3		30.9	0/5	−1.53 (21)	1/5	1/5	0/5	33.0	10.5	1.0	Active
Schedule C[b]												
Taxotere	13	18/22	130	5/5 (3D25, 26, 27)[d]								Toxic
	8.06	2×/day	80.6	2/5 (26, 27)								Toxic
	5		50	0/5	−3.43 (25)	0/5	0/5	0/5	36.0	9.5	0.9	HNTD active
	1.5		15	0/5	−0.35 (24)	0/5	0/5	0/5	32.5	6	0.6	Inactive

[a] C38 adenocarcinoma implanted s.c., passage 100, tumor-doubling time, 3 days; male C57BL/6; mouse average weight: Taxotere (days 14, 18), 23.7 g; Taxotere (days 14, 16, 18), 24.3 g; Taxotere (days 18–22, 2×/day), 24.0 g; 5-fluorouracil, 24.2 g.

[b] Although all mice were implanted on the same day, the experiment started at two different times in order to treat mice with similar tumor burden. Schedules A and B started on day 14: the median tumor size at start of therapy was 150 mg for schedule A, and 120 mg for schedule B; the time for the median control tumors to reach 750 mg was 22.5 days for both schedules. Schedule C started on day 18: the median tumor size at start of therapy was 120 mg for schedule C, and the time for the median control tumors to reach 750 mg was 26.5 days.

[c] 2D25, 28, 2 deaths on day 25, 1 death on day 28.

[d] 3D25, 26, 27, 3 deaths on day 25, 1 death on day 26, 1 death on day 27.

Table 6 *Evaluation of Taxotere against early and advanced stage colon adenocarcinoma 51/A*

i.v. agent	Dosage (mg/kg/injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body wt change [g/mouse (day of nadir)]	Median tumor wt (mg on day 25 (range)]	% of T/C, day 25	Time for median to reach 1000 mg (days)	T − C (days)	Log cell kill total	Comments
Early-stage tumor[a]											
Taxotere	33	3, 5, 7	99	2/5 (10, 11)							Toxic
	20.5		61.5	0/6	−4.35 (13)						Toxic[b]
	12.7		38.1	0/6	−3.28 (17)	32 (0–40)	2.4	44.0	22.5	2.3	HNTD active
	7.9		23.7	0/6	−1.84 (17)	328 (88–440)	24.5	33.0	11.5	1.2	Modest activity
Advanced-stage tumor[c]											
Taxotere	33	10, 12, 14	99	1/7 (19)							Toxic
	21.8		65.4	1/7 (19)							Toxic
	15.2		45.6	0/7	−3.59 (21)	88 (0–115)	6.7	41.9	17.2	1.7	HNTD active
	10.6		31.8	0/7	−3.19 (21)	597 (184–811)	45.12	32.8	8.1	0.8	Active
	7.4		22.2	0/7	−1.72 (20)	1071 (630–1450)	80.1	26.3	1.6	0.2	Inactive
cis-platinum	6.9	10, 12, 14	20.7	5/5 (16, 2D17,[d] 18)							Toxic
	4.6		13.8	0/7	−4.64 (20)						Toxic[b]
	3.0		9	0/7	−1.88 (17)	0 (0–75)	0	50.8	26.1	2.6	HNTD active
	2.0		6	0/7	−0.44 (17)	0 (0–108)	0	44	19.3	1.9	Active

[a] C51/A adenocarcinoma implanted s.c., passage 172, tumor-doubling time, 3 days; female BALB/c; mouse average weight: Taxotere, 19.7 g. The time for the median control tumors to reach 1000 mg was 21.5 days.

[b] Toxicity based on the total body weight loss above 20% which is considered excessively toxic by NCI standards.

[c] C51/A adenocarcinoma implanted s.c., passage 176, tumor-doubling time, 3 days; female BALB/c; mouse average weight: Taxotere, 21.2 g; cis-platinum, 20.9 g. The time for the median control tumors to reach 1000 mg was 24.7 days.

[d] 2D17, 2 deaths on day 17.

Cross-resistance of doxorubicin- and vincristine-resistant P388 leukemias, and cis-platinum- and BCNU-resistant L1210 leukemias, with Taxotere was evaluated and Taxotere was found inactive against the 4 resistant leukemia sublines at optimal dosages (data not shown).

Toxicity and Host Recovery

At the HNTD, Taxotere produced an average body weight loss of 13%. The weight loss nadir usually occurred 4 to 5 days after the last treatment. Host recovery time was in direct relation to the extent of the weight loss and ranged from 4 to 18 days with a median 8 days postnadir, which is considered moderate host recovery. Once pretreatment weight was reached,

mice gained in body weight and in skeletal size (7 g over 150 days post-tumor implantation as observed in the 2 trials with tumor-free survivors, early stage colon 38, and early stage P03). At these dosages, mice held for more than 200 days did not display any sign of delayed toxicity, as evaluated by gross and microscopic observations. The agent did not cause any tissue necrosis even on an every 3 h for 3-dose day 1–6 schedule. A review of all trials performed showed that the mean optimal total dose was 80 mg/kg (range, 38–96 mg/kg) and depended on the mouse strain used, BALB/c and C3H/Hen mice being more sensitive to Taxotere toxicity than C57BL/6 and B6D2F1 mice.

At frankly toxic individual dosages, in cases excluding death,

Table 7 Evaluation of Taxotere against early and advanced stage pancreatic ductal adenocarcinoma 03

i.v. agent	Dosage (mg/kg/injection)	Schedule (days)	Total dose (mg/kg)	Mean body wt change [g (day of nadir)]	Drug death (day of death)	Median tumor wt [g on day 32 (range)]	% of T/C	Regressions Partial	Regressions Complete	Median time to reach 1000 mg	T – C (days)	Total log cell kill	Cures, day 170	Comments
Early stage tumor[a] Taxotere	33	3, 5, 7, 9	132	Toxic	1/5 (17)									Toxic
	20.5		82	−2.87 (13)	0/6	0 (0–0)	0						6/6	HNTD highly active
	12.7		50.8	−1.61 (13)	0/6	0 (0–847)	0			44.2	15.8	1.4	0/6	Active
	7.9		31.6	−0.11 (13)	0/6	581 (100–1083)	42			34.6	6.2	0.5	0/6	Inactive
Advanced stage tumor[b] Taxotere	29	22, 24, 26, 28	116	−6.71 (36)	0/5			5/5	5/5	50.8	21.3	1.8	3/5	Toxic[c]
	18		72	−3.57 (33)	0/6			5/6	5/6	33.0	3.5	0.3	0/6	HNTD active
	11		44	−1.60 (33)	0/5			0/5	0/5				0/5	Inactive
Doxorubicin	18	22, 28	36	−4.79 (34)	0/5			5/5	4/5	38.0	8.5	0.7	0/5	Toxic[c]
	11.1		22.2	−0.89 (30)	0/5			0/5	0/5				0/5	HNTD marginal activity
	6.9		13.8	+0.26 (29)	0/5			0/5	0/5	34.0	4.5	0.4	0/5	Inactive

[a] PO3 adenocarcinoma implanted s.c., passage 57, tumor-doubling time, 3.5 days; male B6D2F1; mouse average weight: Taxotere, 22.8 g. The median time for the control tumors to reach 1000 mg was 28.4 days.
[b] PO3 adenocarcinoma implanted s.c., passage 58, tumor-doubling time, 3.75 days; female B6D2F1; mouse average weight: Taxotere, 21.2 g; doxorubicin, 21.3 g. The median time for the control tumors to reach 1000 mg was 29.5 days.
[c] Toxicity based on the total body weight loss above 20% which is considered excessively toxic by NCI standards.

ataxia, weight loss, and delayed neuromotor changes were observed (nonextension of hind legs). Using repeated dosages to reach true cumulative toxicity, drug toxicity-related death occurred 2 to 11 days after last treatment. In surviving mice bearing slow growing tumors, neurological signs could be observed as early as 3 days after last treatment; the neuropathy progressed from the hind legs, with dosages 1.6 times the cumulative LD_{10}, to the forelegs with dosages 2.6 times the cumulative LD_{10}, approximately 9 days after last treatment. However, this neuropathy was reversible, as in the few cases where quadraplagia occurred, reversal of these signs started 15 to 30 days after last therapy, with recovery of the forelegs motoricity first. Histological evaluation of mice treated with dosages 1.6 times the LD_{10}, and cured from their colon 38 tumor (Table 4), showed the presence of vacuolized Schwann cells and discrete fibrosis of peripheral nerves, but did not show central nervous system lesion. In the Taxotere-taxol comparison trial (Table 2), paraplegia occurred with both compounds but its reversal could not be observed as B16 melanoma is a fast-growing tumor and mice died from their disease.

DISCUSSION

Taxotere was tested against syngeneic transplantable tumors of mice representing a variety of tissue types and chemosensitivity patterns to evaluate its breadth of activity. Clearly, Taxotere had a good spectrum of efficacy against transplantable leukemias and solid tumors at maximum tolerated dosages, and in most cases efficacy was seen at multiple dosage levels. Nine of the 11 tumors tested responded to Taxotere. Six of them were found active at the DN-2 level, which is the level used by the NCI to justify further development (13).

Taxotere was found highly active against the fast growing B16 melanoma. A direct comparison with taxol in this tumor showed clear superiority of Taxotere, as the total log_{10} cell kill was 2.7 times greater for Taxotere than for taxol, at equitoxic dosages.

Taxotere was found active against the three colon tumors tested. The most responsive one was the 5-fluorouracil-sensitive colon 38 with 100% cure rate of early stage disease, and complete regressions of advanced stage tumors. It was also active against the slow-growing, mucin-producing, adenocarcinoma colon 51 at both an early and advanced stage. This tumor forms metastases to lymph nodes and lungs, and is mostly sensitive to alkylating agents. Modest activity was found against the undifferentiated colon adenocarcinoma 26.

Another very sensitive tumor to Taxotere was the pancreatic ductal adenocarcinoma 03. This slow-growing adenocarcinoma is mostly sensitive to doxorubicin and was cured at an early stage by Taxotere. Complete regressions of advanced stage disease could also be obtained.

Taxotere was also active to a lesser extent against a variety of other tumors: Lewis lung carcinoma, Glasgow osteogenic sarcoma, and L1210 and P388 leukemias. Taxotere was found inactive against M5076 histiocytosarcoma and mammary 16/C adenocarcinoma.

Evaluation of Taxotere against tumors with acquired resistance revealed that P388/Dox, P388/Vcr, L1210/BCNU, and L1210/CisDDP were markedly cross-resistant to Taxotere. Similar cross-resistance was reported for taxol with P388/Dox and P388/Vcr (3), and was later related to multidrug resistance (26). Interestingly, Taxotere was found more potent than taxol against a taxol-resistant cell line, J7.TAX-50, in vitro (27).

Table 8 *Evaluation of Taxotere against early stage Lewis lung carcinoma[a]*

i.v. agent	Dosage (mg/kg/ injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body wt change [g/mouse (day of nadir)]	Median tumor wt on day 10 [mg (range)]	% of T/C (day 10)	Time for median tumor to reach 1000 mg (days)	T − C (days)	Log cell kill total	Comments
Taxotere	37.5	3, 4, 5, 6, 7	187.5	4/5 (3D11,[b] 12)	Toxic	NTBA[c]					Toxic
	23.2		116	0/7	−4.15 (12)	80 (0–354)	5.6	14.9	6.1	1.2	HNTD active
	14.4		72	1/7 (11)	−3.25 (12)	111 (88–128)	7.7	14.6	5.8	1.2	Active
	8.9		44.5	0/7	−1.93 (13)	432 (266–553)	30.1	12.5	3.7	0.7	Marginal activity
Cyclophosphamide	200	3, 7	400	0/5	−1.60 (8)	NTBA[c]					
	124		248	0/5	−2.69 (8)	0 (0–0)	0	3/5 tumor-free survivors on day 40			HNTD active
	76		152	0/5	−0.90 (8)	56 (0–176)	3.9	22.5	13.7	2.8	Highly active
Control				0/10		1437 (523–2502)		8.8			

[a] Lewis lung carcinoma implanted s.c.; female B6D2F₁; tumor-doubling time, 1.5 days; mice average weight: Taxotere, 22.13 g; cyclophosphamide, 21.88 g.
[b] 3D11, 3 deaths on day 11.
[c] NTBA, non-tumor-bearing animals.

Taxotere was found active i.p. and i.v. against s.c. implanted tumors, indicating that it crosses physiological barriers well. However, it was found inactive by the p.o. route. This was not unexpected as the three esters and the oxetane ring system on the molecule could be cleaved by stomach acid.

Based on our results, Taxotere seems to belong to the schedule-independent drug category, because the schedule of administration does not influence markedly the total dosage that can be administered (22, 27). The antitumor activity correlates with the total dosage that can be administered, and dose splitting does not appreciably change efficacy. Examples of drugs belonging to this category are doxorubicin, vincristine, cyclophosphamide, and 5-fluorouracil. Although Taxotere is not markedly schedule dependent, the best host recovery occurred with the most-spaced dosage schedule.

At the HNTD, Taxotere produced only body weight loss, and there was no delayed toxicity. The host recovery time was related to the extent of the weight loss. At frankly toxic dosages, tissues with high cell turnover (hematopoietic, gastrointestinal, and testicular) or tissues where microtubules play an important functional role (peripheral nerves), were the most affected.

Although Taxotere and taxol belong to the spindle poison category, which includes the *Vinca* alkaloids and the podophyllotoxins, their mechanism of action is markedly different. The *Vinca* alkaloids inhibit the assembly of microtubules and lead to the mitotic spindle dissolution (28). The podophyllotoxins

Table 9 *Comparison of in vivo antitumor activity of Taxotere and vincristine at optimal i.v. treatment schedules*

	Activity rating[a]	
Tumor system	Taxotere	Vincristine[b]
s.c. B16 melanoma	++++	−
s.c. colon 26 adenocarcinoma	±	−
s.c. colon 38 adenocarcinoma	++++	±
s.c. colon 51/A adenocarcinoma	+++	+
s.c. pancreatic 03 adenocarcinoma	++++	++
s.c. Lewis lung carcinoma	+	−
s.c. Glasgow osteogenic sarcoma	+	−
s.c. mammary 16/C adenocarcinoma	−	++
i.p. P388 leukemia	++	+++
i.p. L1210 leukemia	++	+

[a] Activity ratings in log₁₀ cell kill total:

Rating		Leukemias	Solid tumors
Inactive	−	<1.5	<0.7
	+	1.5–2.9	0.7–1.2
	++	3.0–4.4	1.3–1.9
	+++	4.5–5.9	2.0–2.8
Highly active	++++	6–7.5	>2.8

[b] Antitumor activities as listed in Refs. 30 and 34.

also inhibit tubulin polymerization but their binding to tubulin occurs at a different site from that of the *Vinca* alkaloids (28). Taxol binds preferentially to microtubules, promotes their assembly, and stabilizes them *in vitro* (29). Taxotere mechanism of action is similar to that of taxol; *in vitro*, it inhibits cell replication, promotes the assembly of microtubules, and induces the formation of microtubule bundles (27). The concentration required to provide 50% inhibition of microtubule disassembly was found to be approximately 2 times less for Taxotere than for taxol (10). The difference between the mechanism of action of *Vinca* alkaloids and Taxotere is also observed when comparing their *in vivo* spectrum of antitumor activity (Table 9) (30). Vincristine is mostly active against leukemias and has a limited solid tumor activity spectrum, with no activity against B16 melanoma and Glasgow osteogenic sarcoma, and only marginal activity against colon adenocarcinomas 38 and 51. Taxotere has a greater spectrum of activity than vincristine, and can achieve regression of advanced stage disease.

We investigated Taxotere activity against CisDDP-sensitive and -insensitive tumors, because of an earlier clinical report showing taxol antitumor efficacy in ovarian carcinomas (31–33), some of which clearly refractory to CisDDP (a reference drug for this disease). Our results indicated that Taxotere was active against both CisDDP-sensitive (C51, GOS, Lewis lung) and CisDDP-refractory tumors (advanced stage P03 and C38). But Taxotere was also inactive on a CisDDP-sensitive tumor (M5076) and on a tumor with acquired resistance to CisDDP (L1210/CisDDP). Therefore we cannot generalize on Taxotere activity on CisDDP-sensitive and CisDDP-insensitive tumors. These results underline the well-known fact that each independently arising tumor is a unique biological entity with its own pattern of chemosensitivity (34).

In conclusion, Taxotere, obtained through the development of an efficient semisynthesis process using a renewable source of natural precursor, represents a truly new chemical entity with a unique mechanism of action, and a broad spectrum of antitumor activity different from that of other clinically available spindle poisons. This agent is currently undergoing Phase I clinical trials in Europe and in the United States of America.

ACKNOWLEDGMENTS

REFERENCES

1. Wall, M. E., and Wani, C. Recent progress in plant anti-tumor agents. Presented at the 153rd National Meeting of the American Chemical Society, Miami Beach, FL, Abstract M-006, 1967.
2. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., and McPhail, A. T. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc., *93:* 2325–2327, 1971.
3. Douros, J., and Suffness, M. New natural products under development at the National Cancer Institute. Recent Results Cancer Res., *76:* 153–175, 1981.
4. Fuchs, D. A., and Johnson, R. K. Cytologic evidence that taxol, an antineoplastic agent from *Taxus brevifolia*, acts as a mitotic spindle poison. Cancer Treat. Rep., *62:* 1219–1222, 1978.
5. Schiff, P. B., Fant, J., and Horwitz, S. B. Promotion of microtubule assembly *in vitro* by taxol. Nature (Lond.), *22:* 665–667, 1979.
6. Colin, M., Guénard, D., Guéritte-Voegelein, F., and Potier, P. Process for preparing derivatives of baccatin III and of 10-deacetyl baccatin III. US Patent 4924012; granted May 8, 1990.
7. Denis, J. N., Greene, A. E., Guénard, D., Guéritte-Voegelein, F., Mangatal, L., and Potier, P. A highly efficient, practical approach to natural taxol. J. Am. Chem. Soc., *110:* 5917–5919, 1988.
8. Mangatal, L., Adeline, M. T., Guénard, D., Guéritte-Voegelein, F., and Potier, P. Application of the vicinal oxyamination reaction with asymmetric induction to the hemisynthesis of taxol and analogues. Tetrahedron, *45:* 4177–4190, 1989.
9. Shelanski, M. L., Gaskin, F., and Cantor, C. R. Microtubule assembly in the absence of added nucleotides. Proc. Natl. Acad. Sci. USA, *70:* 765–768, 1973.
10. Gueritte-Voegelein, F., Guénard, D., Lavelle, F., Le Goff, M-T., Mangatal, L., and Potier, P. Relationships between the structure of taxol analogues and their antimitotic activity. J. Med. Chem., *34:* 992–998, 1991.
11. Lavelle, F., Fizames, C., Gueritte-Voegelein, F., Guénard, D., and Potier, P. Experimental properties of RP 56976, a taxol derivative. Proc. Am. Assoc. Cancer Res., *30:* 2254, 1989.
12. Bissery, M. C., Bayssas, M., and Lavelle, F. Preclinical evaluation of intravenous Taxotere (RP 56976, NSC 628503), a taxol analog. Proc. Am. Assoc. Cancer Res., *31:* 417, 1990.
13. Geran, R. I., Greenberg, N. H., Macdonald, M. M., Schumacher, A. M., and Abbott, B. J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (Ed. 3). Cancer Chemother. Rep., Part 3, *3:* 1–103, 1972.
14. Corbett, T. H., Griswold, D. P., Jr., Roberts, B. J., Peckham, J. C., and Schabel, F. M., Jr. Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinomas. Cancer (Phila.), *40:* 2660–2680, 1977.
15. Corbett, T. H., Roberts, B. J., Leopold, W. R., Peckham, J. C., Wilkoff, L. J., Griswold, D. P., Jr., and Schabel, F. M., Jr. Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. Cancer Res., *44:* 717–726, 1984.
16. Corbett, T. H., Griswold, D. P., Jr., Roberts, B. J., Peckham, J. C., and Schabel, F. M., Jr. Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy. Cancer Treat Rep., *62:* 1471–1499, 1978.
17. Mayo, J. G. Biologic characterization of the subcutaneously implanted Lewis lung tumor. Cancer Chemother. Rep., Part 2, *3:* 325–330, 1972.
18. Glasgow, L. A., Crane, J. L., Jr., and Kern, E. R. Antitumor activity of interferon against murine osteogenic sarcoma cells *in vitro*. J. Natl. Cancer Inst., *60:* 659–663, 1978.
19. Langdon, S. P., Gescher, A., Hickman, J. A., and Stevens, M. F. G. The chemosensitivity of a new experimental model—the M5076 reticulum cell sarcoma. Eur. J. Cancer. Clin. Oncol., *20:* 699–705, 1984.
20. Schabel, F. M., Jr., Skipper, H. E., Trader, M. W., Laster, W. R., Griswold, D. P., Jr., and Corbett, T. H. Establishment of cross-resistance profiles for new agents. Cancer Treat. Rep., *67:* 905–922, 1983.
21. Corbett, T. H., Roberts, B. J., Trader, M. W., Laster, W. R., Jr., Griswold, D. P., Jr., and Schabel, F. M., Jr. Response of transplantable tumors of mice to anthracenedione derivatives alone and in combination with clinically useful agents. Cancer Treat. Rep., *66:* 1187–1200, 1982.
22. Corbett, T. H., Leopold, W. R., Dykes, D. J., Roberts, B. J., Griswold, D. P., Jr., and Schabel, F. M., Jr. Toxicity and anticancer activity of a new triazine antifolate (NSC 127755). Cancer Res., *42:* 1701–1715, 1982.
23. Schabel, F. M., Jr., Griswold, D. P., Jr., Corbett, T. H., Laster, W. R., Jr., Mayo, J. G., and Llyod, H. H. Testing on the therapeutic activity against advanced solid tumors of man. *In:* H. Busch and V. DeVita, Jr. (eds.), Cancer Drug Development, Part B, Vol. 17, pp. 3–51. New York: Academic Press, Inc., 1979.
24. Schabel, F. M., Jr., Griswold, D. P., Jr., Corbett, T. H., Laster, W. R., Jr., Mayo, J. G., and Lloyd, H. H. Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol. Ther. Part A, *1:* 411–435, 1977.
25. Corbett, T. H., Bissery, M. C., Mucci-Lorusso, P., and Polin, L. 5-Fluorouracil containing combinations in murine tumor systems. Invest. New Drugs, *7:* 37–49, 1989.
26. Ling, V., Kartner, N., Sudo, T., Siminovitch, L., and Riordan, J. R. Multidrug-resistance phenotype in Chinese hamster ovary cells. Cancer Treat. Rep., *67:* 869–874, 1983.
27. Ringel, I., and Horwitz, S. B. Studies with RP 56976 (Taxotere): a semisynthetic analog of taxol. J. Natl. Cancer Inst., *83:* 288–291, 1991.
28. Schiff, P. B., and Horwitz, S. B. Tubulin: a target for chemotherapeutic agents. *In:* A. C. Sartorelli, J. S. Lazo, and J. R. Bertino (eds.), Molecular Actions and Targets for Cancer Chemotherapeutic Agents, Vol. 2, pp. 483–507. New York: Academic Press, Inc., 1981.
29. Manfredi, J. J., and Horwitz, S. B. Taxol: an antimitotic agent with a new mechanism of action. *In:* L. A. Dethlefsen (ed.), International Encyclopedia of Pharmacology and Therapeutics, Cell Cycle Effects of Drugs, Vol. 121, pp. 287–333. Oxford: Pergamon Press, 1986.
30. Bissery, M. C., White, K., Polin, L., Puzycki, D., Zalupski, M. M., and Corbett, T. H. Murine solid tumor activity of vinzolidine. Proc. Am. Assoc. Cancer Res., *29:* 333, 1988.
31. McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K., and Donehower, R. C. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Int. Med., *111:* 273–279, 1989.
32. Rowinsky, E. K., Cazenave, L. A., and Donehower, R. C. Taxol: a novel investigational antimicrotubule agent. J. Natl. Cancer Inst., *82:* 1247–1259, 1990.
33. Slichenmyer, W. J., and Von Hoff, D. D. New natural products in cancer chemotherapy. J. Clin. Pharmacol., *30:* 770–788, 1990.
34. Corbett, T. H., Valeriote, F. A., and Baker, L. H. Is the P388 murine tumor no longer adequate as a drug discovery model? Invest. New Drugs, *5:* 3–20, 1987.