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Phase I and Pharmacodynamic Study of Taxol in Refractory Acute Leukemias[1]

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ABSTRACT

Taxol, a novel antimicrotubule agent that enhances tubulin polymerization and microtubule stability, was administered to adults with refractory leukemias as a 24-h i.v. infusion in a Phase I study. The primary objectives were to determine the maximum tolerated dose of taxol administered on this schedule to patients with acute leukemias and describe the nonhematological toxicities which became dose limiting. The starting dose, 200 mg/m², was based on the maximum tolerated dose in solid tumor trials, in which myelosuppression precluded dose escalation. Seventeen patients received 28 evaluable courses at 200, 250, 315, and 390 mg/m². Severe mucositis limited further dose escalation. Other nonhematological effects included peripheral neuropathy, alopecia, myalgias, arthralgias, nausea, vomiting, diarrhea, and an acute pulmonary reaction that was presumptively due to taxol's Cremophor vehicle.

Mean peak taxol plasma concentrations at all dose levels were in the range of concentrations that were previously demonstrated to induce microtubule bundles, a morphological effect associated with cytotoxicity, in leukemia cells *in vitro*. Pretreatment blasts from 12 patients were incubated with taxol *ex vivo*. Taxol-induced microtubule bundles were apparent in the blasts of eight patients who also had cytoreduction of tumor, and sensitivity to bundle formation was related to the magnitude of antitumor activity. In contrast, taxol did not induce microtubule bundles *ex vivo* in the blasts of the other four total nonresponders.

Based on this study, the maximum tolerated doses and recommended Phase II doses for taxol, limited by nonhematological toxicity and administered as a 24-h i.v. infusion to patients with refractory leukemias, are 390 and 315 mg/m². Phase II trials at these myelosuppressive doses are required to determine taxol's activity in the treatment of leukemias. In addition, further evaluation of microtubule bundle formation *ex vivo* in Phase II studies is necessary to determine the ultimate utility of this assay in assessing tumor sensitivity to taxol.

INTRODUCTION

Microtubules have been demonstrated to be very strategic targets for antineoplastic agents; however, few antimicrotubule agents have been discovered and encompassed into standard chemotherapeutic regimens. Taxol, a diterpenoid plant product extracted from the bark of the western yew (*Taxus brevifolia*), has a unique mechanism of action (1). Unlike other antimicrotubule agents in clinical use (*e.g.*, colchicine, vincristine, and vinblastine) that shift the equilibrium between microtubules and tubulin subunits toward microtubule disassembly, taxol promotes assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization (1–3). These microtubules are stable even when treated with low temperatures or calcium, conditions that usually promote disassembly (2–4). This unusual stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions (5). In addition, taxol induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis (6–8). In human leukemia cell lines, sensitivity to the formation of taxol-induced microtubule bundles and the irreversibility of bundles is related to cytotoxicity (8).

Taxol demonstrated a broad spectrum of activity against murine and human solid tumors and leukemias in studies directed by the National Cancer Institute's Division of Cancer Treatment (9). It had its greatest activity against i.p. B16 murine melanoma and subrenal capsule implants of the MX-1 human breast cancer xenograft in nude mice. Taxol was also active against i.p. P388 and L1210 murine leukemias and human CX-1 colon and LX-1 lung cancer xenografts. These results provided the impetus for the further clinical development of this novel compound.

The dose-limiting toxicities and MTD[3] of taxol administered on a variety of schedules to patients with solid neoplasms were previously evaluated in Phase I trials (10–15). In these studies, taxol was infused over 1, 3, 6, and 24 h, but severe acute reactions, characterized by bronchospasm, hypotension, stridor, tachy- and bradyarrhythmias, and death, resulted in the temporary discontinuation of all trials. These reactions were attributed to taxol's Cremophor vehicle, since identical reactions were observed with other drugs formulated with it and when the vehicle alone was administered to animals (16, 17). Since a higher incidence of these acute reactions was observed with shorter durations of infusion, studies that used shorter infusions were permanently discontinued, and trials that evaluated longer infusion durations (24 h) were resumed using antiallergic premedications consisting of corticosteroids, H_1-, and H_2-histamine antagonists (10–12). These modifications were associated with a marked reduction in the incidence of acute reactions. Neutropenia was the major dose-limiting toxicity of taxol in Phase I solid tumor trials. In addition, a sensory neuropathy, characterized by a glove-and-sock distribution of numbness and paresthesias, was observed at higher doses (10–12). Nausea and vomiting, myalgias, mucositis, total-body alopecia, diarrhea, and phlebitis were also observed (10–15). The MTD and recommended Phase II doses of taxol administered as a 6-h infusion were 265 and 212 mg/m², respectively, and 275 and 250 mg/m², respectively, as a 24-h infusion (10–12).

The rationale for this Phase I evaluation of taxol in leukemia included the established efficacy of other antimicrotubule agents, such as the *Vinca* alkaloids in leukemia (18) and the sensitivity of P388 and L1210 murine leukemias to taxol (9). In addition, the relative lack of significant dose-limiting nonhematological toxicities in Phase I solid tumor trials suggested that further dose escalation above myelosuppressive doses may be possible and feasible in patients with leukemia. The major goals of this study were: (*a*) to describe and quantitate the dose-limiting nonhematological toxicities of higher myelosuppressive doses of taxol administered as a 24-h i.v. infusion to patients with refractory acute leukemias; (*b*) to determine the

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[3] The abbreviations used are: MTD, maximum tolerated dose; ANLL, acute nonlymphocytic leukemia; ALL, acute lymphocytic leukemia; CR, complete remission; ECOG, Eastern Cooperative Oncology Group; NCI, National Cancer Institute; PR, partial response; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline.

MTD and recommended Phase II doses of myelosuppressive doses of taxol in leukemia patients; (*c*) to seek preliminary evidence of activity in leukemias; and (*d*) to determine if there is a relationship between taxol-induced microtubule bundle formation *ex vivo* and clinical antitumor activity.

MATERIALS AND METHODS

Patient Selection. Patients with histologically documented ANLL, ALL, or chronic myelogenous leukemia in blast crisis who failed to respond to intensive conventional chemotherapy or relapsed after achieving a CR were candidates for entry onto the study. Diagnoses were based on French-American-British classification guidelines (19, 20). Eligibility criteria included: (*a*) age ≥ 18 yr; (*b*) ECOG performance status ≤ 3; (*c*) life expectancy enabling the completion of at least one course of therapy; (*d*) adequate hepatic (total bilirubin ≥ 2.0 mg/dl) and renal (creatinine ≤ 1.5 mg/dl) functions; and (*e*) no other coexisting medical problems of sufficient severity to prevent compliance with the study. Before entry, patients had complete histories and physical examinations performed. Height, weight, and performance status were recorded. Pretreatment evaluation included a bone marrow aspirate and biopsy, CBC, electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, prothrombin and partial thromboplastin times, fibrinogen, and fibrin split products. Chest radiographs, urinalyses, and electrocardiograms were also performed. Informed written consent was given according to federal, state, and institutional guidelines.

Dosage and Formulation. The starting dose of taxol was 200 mg/m^2. This dose was based on the MTD and recommended Phase II doses, 275 and 250 mg/m^2, respectively, that were derived from a previous Phase I trial in patients with solid tumors (12). Subsequent courses were administered at a minimum interval of 15 days, at which time recovery of hematological and nonhematological toxicities usually occurred based on previous clinical trials. Doses at successive dose levels were 25% higher than at preceding levels: 200; 250; 315; and 390 mg/m^2. At least three new patients were entered at each dose. Dose escalations were allowed in the same patient if two new patients were previously treated at the next higher dose.

Taxol was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). The drug was provided as a concentrated sterile solution with 6 mg/ml in a 5-ml ampul in 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol. Twenty-five % of the total dose was diluted in 250 ml of 5% dextrose in water and infused over 6 h to 4 treatments.

Drug Administration. Due to the association of acute reactions with taxol's Cremophor vehicle and reduction in the incidence of reactions with antiallergic premedications, the following premedications were given: (*a*) dexamethasone, 20 mg i.v. (14 and 7 h before taxol); (*b*) diphenhydramine, 50 mg i.v. (30 min before taxol); and (*c*) ranitidine, 50 mg i.v. (30 min before taxol) (10–12). Vital signs were obtained every 15 min for the first hour during the infusion and every 30 min thereafter. Patients were monitored by electrocardiographic telemetry. Acyclovir (250 mg/m^2 i.v. every 8 h) and norfloxacin (400 mg p.o. every 12 h) were administered for prophylaxis against herpetic and aerobic gram-negative bacterial infections (21, 22).

Follow-up Studies. Histories, physical examinations, blood, and urine studies, as previously noted, were performed at least weekly. Toxicities were evaluated according to the NCI common toxicity criteria (23). Bone marrow aspirates and biopsies were obtained weekly to assess antitumor activity; however, the Day 14 bone marrow examination was used to assess tumor response, gauge cellularity, predict hematopoietic recovery, and determine if the next courses should be administered on Day 15 or delayed for an additional 7 days, at which time a repeat bone marrow examination was performed. Additional courses were administered if leukemia cellularity was unchanged or reduced during the preceding course, hematopoietic recovery was felt to be maximal, and nonhematological toxicities resolved. A CR was defined as a cellular bone marrow with ≤ 5% blasts, a WBC ≥ 3000/μl, hemoglobin ≥ 10 g/dl, and platelets ≥ 100,000/μl. A PR was defined as a reduction in bone marrow blasts to ≤ 25% of cellular constituents (M1) with recovery of peripheral blood counts as described for CR.

Pharmacological Studies. Blood samples were obtained at the end of infusion (24 h) to measure peak taxol concentrations in order to complement cellular pharmacodynamic studies. Since taxol's pharmacokinetic behavior was described previously (11, 12, 24), a detailed pharmacokinetic evaluation was not a primary objective of this study. In one patient, cerebrospinal fluid was collected at the end of the taxol infusion. Taxol concentrations were measured by a high-performance liquid chromatographic assay as described previously (24).

Tubulin Immunofluorescence Studies. To assess the sensitivity of leukemia cells to taxol-induced microtubule bundle formation *ex vivo*, blasts were separated immediately from pretreatment bone marrow aspirates by a Ficoll-Hypaque density gradient (Pharmacia, Piscataway, NJ), washed twice in RPMI 1640 medium (GIBCO, Grand Island, NY), and resuspended in RPMI 1640 medium with 10% fetal bovine serum (GIBCO), penicillin, and streptomycin. Occasionally bone marrow aspirates and peripheral blood were obtained 24 h postinfusion, and blasts were separated as described.

One × 10^6 cells/ml were treated in duplicate with 0, 0.1, 1.0, and 10.0 μM taxol (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI) for 4 and 22 h in each 2.1-cm^3 well of a 24-well plate (Falcon) at 37°C in 7.5% CO_2. A glass coverslip that had been previously immersed for 24 h in 0.01% polylysine (Sigma, St. Louis, MO) was placed at the bottom of each well prior to treatment. Taxol was diluted from stock solutions (0.01 M) that were made in DMSO, so that the highest concentration of DMSO used was 0.1%. At the end of the treatment period, the plate was centrifuged at 500 rpm for 5 min. Cells attached to polylysine-coated coverslips were fixed for 30 min with 10% formalin (Baker Chemicals, Phillipsburg, NJ) in PBS at room temperature, permeabilized with cold acetone for 7 min, air dried, and incubated with rabbit antitubulin antiserum (1:30 dilution with PBS) for 30 min at 30°C. The rabbit antitubulin antibody, derived from vinblastine-induced tubulin crystals from sea urchin eggs, was characterized as described previously (25, 26). After 10-min wash in PBS, the cells were stained with a 1:60 dilution of rhodamine-conjugated goat anti-rabbit globulin (Cappel Laboratories, Cochranville, PA) and then washed in PBS for 10 min. The wet coverslips were mounted in 90% glycerol in PBS and viewed with an Ortholux II epifluorescence microscope (Leitz, West Germany) equipped with a rhodamine excitation filter set (Leitz), 100 W mercury arc lamp, and ×63 (N. A. 1.4) objective lens. At least 200 consecutive cells on each coverslip were scored as positive or negative for the presence of taxol-induced microtubule bundles, which were demonstrated to be associated with cytotoxicity when leukemia cell lines were treated *in vitro* under identical conditions (18). Cells were considered sensitive to microtubule bundle formation if bundles were observed in at least 20% of cells. The fluorescent images were photographed using 35-mm Tri-X film (Kodak, Rochester, NY).

RESULTS

Patient Population. Seventeen patients (characteristics in Table 1) were entered on this study. Patient ages ranged from 23 to 76 yr with a median of 53 yr. Diagnoses included ALL (2), ANLL (13), and biphenotypic leukemia (2). ALL subclassifications included L-2 (1) and L-3 (1). ANLL subclassifications included M-2 (2), M-4 (6), M-5 (3), and M-7 (2). One patient with biphenotypic leukemia had a Philadelphia chromosome (Ph^{1+}) with a breakpoint outside the breakpoint cluster region (bcr$^-$). All patients had extensive prior chemotherapy, and three had bone marrow transplantations. Prior chemotherapy was usually "timed-sequential" that consisted of sequential infusions of high doses of 1-β-D-arabinofuranosylcytosine, daunomycin, etoposide, and/or amsacrine, which usually produced greater than 25 days of deep aplasia (27, 28). With respect to the number of prior chemotherapy courses and preparative

Table 1 *Patient characteristics*

Characteristic	No. of Patients
No. of patients (evaluable)	17 (16)
No. of courses (evaluable)	29 (28)
Median age (range)	53 (23–76)
Sex ratio (male:female)	10:7
ECOG performance status	
0	5
1	9
2	3
3	0
Leukemia type	
ANLL	13
ALL	2
Biphenotypic	2
Prior therapy	
Chemotherapy	15
+ CNS[a] RT	2
No. of prior chemo/BMT regimens	2–6
(severe aplasia > 25 days)	3–8
	4–3

[a] CNS, central nervous system; RT, radiotherapy; BMT, bone marrow transplant.

Table 2 *Dose escalations*

Dose (mg/m²)	No. of new/total patients (evaluable)	No. of total courses (evaluable)
200	3/3 (3)	3/4 (4)
250	4/5 (4)	4/6 (5)
315	7/11 (11)	7/13 (13)
390	3/5 (5)	3/6 (6)

cytotoxic regimens for bone marrow transplantation that produced greater than 25 days of aplasia, six patients had two, eight patients had three, and three patients had four prior courses.

Twenty-eight evaluable courses were administered at four dose levels: 200; 250; 315; and 390 mg/m² (Table 2). A 54-yr-old man with refractory ANLL received one course that was unevaluable for toxicity. He had a rapidly increasing blast count and expired hours after treatment due to pulmonary leukostasis that was confirmed at postmortem examination. Seven patients received two courses at different dose levels; three patients were treated with two courses at identical dose levels; and one patient received three courses at three dose levels.

Antileukemic Activity. The antileukemic activity observed in this Phase I study is depicted in Table 3. CRs were not observed. To identify relationships between clinical antitumor activity and the sensitivity of patients' blasts to taxol-induced microtubule bundles *ex vivo*, drug-related cytoreduction was noted. Transient reductions in, but persistence of, blasts in peripheral blood and bone marrow occurred during 13 courses administered to nine patients. Complete clearance of leukemia from bone marrow and peripheral blood occurred during one course administered at 315 mg/m² and two courses administered to two patients at 390 mg/m². Tumor lysis during these courses and other responses were rapid with WBC nadirs occurring between Days 3 and 8. However, tumor recurred within a short period in all three patients (2, 2.5, and 4 wk). Recovery of normal peripheral counts with a concurrent M2 bone marrow aspirate (PR) was documented in one course.

Nonhematological Toxicity. The nonhematological toxicities of taxol and pertinent toxicity grading criteria are depicted in Table 4.

Mucositis. Although mucositis was rarely observed in previous studies that evaluated lower doses of taxol, it was the dose-limiting nonhematological toxicity in this Phase I study in refractory leukemias. Severe mucositis precluded dose escalation above 390 mg/m² and occurred during all six course administered to five patients at this dose; three patients had Grade 3 (liquid alimentation only) and three had Grade 4 mucositis (alimentation not possible). At 390 mg/m², severe mucositis occurred during initial as well as subsequent courses. At 315 mg/m², Grades 3 and 4 mucositis occurred during 2 and 3 of 13 courses, respectively. Four of these five episodes of

Table 3 *Antileukemic activity*

Patient	Diagnosis (FAB)[a]	Dose (mg/m²)	Response and comments
3	ALL (L3)	200	Transient reductions in peripheral blasts at 200 and 250 mg/m². At 313 mg/m², WBC[b] 41.4 (79% blasts) to 0.4 on Day 5. Aplasia and complete tumor clearance for 4 wk.
		315	
		390	
13	ALL (L2)	390	WBC 131 (85% blasts) to 1 (0% blasts) on Day 7. Residual blasts in bone marrow.
		390	PR. WBC 15 (65% blasts) to complete tumor clearance on Day 8. M2 on Day 17.
12	ANLL (M2)	390	WBC 3.9 (60% blasts) to complete tumor clearance on Day 8. Leukemia in bone marrow on Day 15.
14	ANLL (M4)	390	WBC 241 (97% blasts) to 5.5 (60% blasts) on Day 7.
		315	WBC 16 (95% blasts) to 1.9 (90% blasts) on Day 5.
2	ANLL (M4)	200	Transient reductions in peripheral blood and bone marrow blasts.
8	ANLL (M4)	315	
15	ANLL (M4)	315	
		315	
16	ANLL (M7)	315	
9	Bipheno	315	
		390	
17	Bipheno	315	

[a] FAB, French-American-British.
[b] White blood count × $10^3/\mu l$.

Table 4 *Nonhematological toxicity of taxol in acute leukemia*

Dose (mg/m²)	No. of evaluable courses	NCI toxicity grade[a]	Nausea and vomiting	Peripheral nerve	Mucositis	Myalgias and arthralgias
200	4	0	2	2	2	4
		1	0	2	0	0
		2	2	0	1	0
		3	0	0	1	0
250	5	0	2	2	3	4
		1	0	3	1	1
		2	3	0	0	0
		3	0	0	1	0
315	13	0	11	0	0	6
		1	0	6	3	3
		2	2	6	5	4
		3	0	1	2	0
		4	0	0	3	0
390	6	0	4	0	0	1
		1	0	2	0	0
		2	2	3	0	4
		3	0	1	3	1
		4	0	0	3	0

[a] NCI common toxicity grading criteria. Nausea/vomiting: 1, nausea; 2, transient vomiting; 3, requires therapy; 4, intractable. Peripheral nerve: 1, mild parasthesias/DTRs decreased; 2, moderate parasthesias, mild or moderate weakness and objective sensory loss; 3, parasthesias interfering with functions, severe objective sensory loss. Mucositis: 1, painless ulcers, erythema, mild soreness; 2, painful erythema, edema, ulcers (can eat); 3, painful erythema, edema, ulcers (cannot eat); 4, requires parenteral or enteral support. Myalgias and arthralgias (pain): 1, mild pain; 2, moderate pain; 3, severe pain; 4, intractable.

severe mucositis at 315 mg/m^2 occurred during second or third courses, which suggested the toxicity was, in part, cumulative. The only exception was in a patient who had received high doses of busulfan and cyclophosphamide in preparation for bone marrow transplantation and experienced Grade 4 mucositis during his first course at 315 mg/m^2. However, prior treatment with either busulfan or etoposide was not generally associated with more severe mucositis within a given dose level. At 315 and 390 mg/m^2, mucositis was generally more severe during subsequent courses if the later treatment was administered 15 days or less from the previous treatment. This also suggested that mucositis was cumulative and subclinical mucosal repair was incomplete even until 15 days following treatment at 315 and 390 mg/m^2.

Mucositis was characterized by diffuse ulcerations of the lips, oral cavity, and pharynx. Dysphagia and pain that reflected esophageal involvement were also common. In addition, three postmortem examinations revealed mucosal ulcerations in the oropharynx, esophagus, and intestines without evidence of viral, fungal, or bacterial mucosal invasion. Ultrastructural examination of ulcerated esophageal mucosa revealed an accumulation of epidermal cells with taxol-induced mitotic spindle asters that was indicative of cell cycle arrest in the mitotic phase (8). Severe mucositis was usually associated with severe pain, and continuous i.v. infusions of narcotics were often required. Oropharyngeal ulcerations usually occurred early (Days 3 to 7) and resolved within 5 to 7 days.

Neurotoxicity. A peripheral neuropathy was observed following two of four courses of taxol administered at 200 mg/m^2, two of five courses at 250 mg/m^2, and after all courses at 315 and 390 mg/m^2. A sensory neuropathy in a glove-and-stocking distribution with loss of pain, temperature, and vibratory discrimination was typical. An ongoing sensation of numbness and tingling that patients considered to be moderately uncomfortable and painful was common, but function was rarely disrupted. One patient had subjective complaints and objective findings of mild weakness in her distal lower extremities after receiving three courses of taxol at 200, 250, and 315 mg/m^2, which suggested that taxol was inducing a complex peripheral neuropathy involving motor as well as sensory nerves. Overall, the severity of neurosensory toxicity was dose related. Whereas only mild sensory changes occurred at 200 and 250 mg/m^2, moderate paresthesias and sensory loss (Grade 2) were noted after 6 of 13 courses at 315 mg/m^2, and 3 of 6 courses at 390 mg/m^2. One patient complained of severe paresthesias after each of two courses administered at 315 and 390 mg/m^2. Although the data were limited with respect to cumulative toxicity in patients who received multiple courses at the same dose, neurotoxicity progressively worsened with each successive course.

In patients who survived weeks to months following taxol, neurosensory symptoms did not worsen. Instead, paresthesias and numbness progressively improved. Although amitryptyline was reported to be effective in the symptomatic relief of some patients with taxol-induced paresthesias (12), the drug was ineffective at relieving severe paresthesias in one patient. His symptoms, though, resolved completely within 5 mo after his second course.

Hematological Toxicity. Myelosuppression was consistently observed at all dose levels. However, most of these patients with refractory leukemias had profound neutropenia and thrombocytopenia prior to taxol due to either progressive leukemia and/or previous chemotherapy, which precluded reliable quantitation of the myelotoxicity produced by taxol doses of 200 to 390 mg/m^2. The quantitation of myelotoxicity was further confounded by the extreme interpatient variability in the pretreatment proportions of leukemic blasts to normal hematopoietic elements. In 11 courses that were evaluable for hematological toxicity [no progressive leukemia during treatment; and adequate pretreatment platelet ($\geq$ 25,000/μl) or neutrophil counts ($\geq$ 500/μl)], platelet and neutrophil counts were lowest 7 to 11 days posttreatment and returned to baseline or plateaued by Days 15 to 21.

Miscellaneous Toxicities. Joint discomfort and myalgias generally occurred 2 to 3 days after taxol and resolved within 4 to 7 days. Myalgias characteristically involved shoulder and paraspinal muscles, and patients typically complained of arthralgias of the large joints of the arms and legs. Signs of inflammation were uncommon. Taxol-induced myalgias and arthralgias were dose related. Mild arthralgias and myalgias (Grade 1) occurred during 1 of 5 courses at 250 mg/m^2, and 3 of 13 courses at 315 mg/m^2. Moderate and severe (Grades 2 and 3) arthralgias and myalgias were not observed at doses below 315 mg/m^2. However, 3 of 12 courses at 315 mg/m^2, and 5 of 6 courses at 390 mg/m^2 were associated with Grades 2 and 3 arthralgias and myalgias, respectively. Narcotics were useful for pain relief; nonsteroidal antiinflammatory agents were not used because of severe thrombocytopenia in most patients.

All patients who were evaluable for drug-induced alopecia developed total loss of scalp hair after receiving 200 to 390 mg/m^2 of taxol. Hair loss was abrupt and usually occurred 10 to 14 days after treatment. In addition, a cumulative loss of facial and body hair (*e.g.*, pubic, axillary, eyebrows, and eyelashes) was observed with each successive course. Other nonhematological toxicities that were not dose dependent within the 200 to 390-mg/m^2 dose range included nausea and vomiting (9 of 28 courses) and diarrhea (4 of 28 courses). Nausea and vomiting occurred during the infusion, and diarrhea was usually noted within 1 wk after taxol.

One acute reaction, characterized by shortness of breath and bronchospasm, occurred in a 65-yr-old woman with a history of chronic obstructive pulmonary disease and congestive heart failure. Shortness of breath and wheezing began 15 min into her first infusion at 250 mg/m^2 and rapidly abated after discontinuation of taxol and treatment with dexamethasone, 20 mg i.v. every 6 h for four doses. Similar sequalae did not ensue after reinfusion of taxol 24 h later nor during her second cycle, 3 wk later at a higher dose, 315 mg/m^2. Significant tachyarrhymias and bradyarrhymias were not observed in any patient.

Pharmacological Studies. Mean peak taxol concentrations ($\pm$ SE) were 1.57 $\pm$ 0.29 μM at 250 mg/m^2, 2.93 $\pm$ 0.42 μM at 315 mg/m^2, and 3.50 $\pm$ 1.43 μM at 390 mg/m^2. Taxol was not detected (assay limit of detection, 50 nM) in the cerebrospinal fluid of one patient who had a concurrent plasma level of 2.74 μM at the end of infusion.

Blasts from 12 patients were assessed prior to treatment for taxol-induced microtubule bundle formation by indirect immunofluorescence (Table 5). Microtubule morphology was assessed after treatment of blasts *ex vivo* with 0, 0.1, 1.0, and 10.0 μM taxol for 4 and 22 h. Untreated blasts from patients with ALL, ANLL, and biphenotypic leukemias had similar microtubule patterns. Most untreated blasts had a diffuse and faint background of intracellular fluorescence. Microtubule patterns consisted of a very fine network of microtubules that emanated from a single perinuclear organizing center and extended to the cell membrane (Fig. 1*A*). Bundles were not identified in any untreated blast.

Microtubule bundles, apparent as fluorescent cytoplasmic

Table 5 *In vitro sensitivity of leukemic blasts to taxol-induced microtubule bundles*

Patient	Diagnosis (FAB)[a]	Taxol dose (mg/m²)	Response[b]	Microtubule bundles produced *ex vivo*[c]
6	ANLL (M5)	250 (1.19)[d]	Stable	−
11	ANLL (M4)	250 (1.21) 315 (5.49)	Stable	−
4	ANLL (M2)	250 (2.06)	Progression	−
7	ANLL (M4)	315 (1.95) 390 (2.54)	Progression	−
15	ANLL (M4)	315 (4.75) 315 (2.38)	Decrease in blasts	+
12	ANLL (M2)	390 (4.25)	Complete clearance	+++
16	ANLL (M7)	315 (2.58)	Decrease in blasts	++
14	ANLL (M4)	390 (9.45) 315 (2.38)	Decrease in blasts	++
9	Bipheno	315 (0.63) 390 (1.20)	Decrease in blasts	+
17	Bipheno (Ph¹)	315 (2.74)	Decrease in blasts	+++
3	ALL (L3)	200 250 (2.18) 315 (5.16)	Complete clearance	+++
13	ALL (L2)	390 (0.79) 390 (2.77)	Complete clearance	++

[a] FAB, French-American-British.

[b] Response: stable, no change in numbers of blasts; progression, increase in numbers of blasts; complete clearance, temporary complete clearance of peripheral blood and bone marrow blasts; decrease in blasts; decrease but less than complete clearance of bone marrow and peripheral blood clearance.

[c] Bundles produced *ex vivo*: −, no bundles; +, ≥20% of cells with bundles after 10 μM taxol for 4 to 22 h; ++, ≥20% of cells with bundles after 1 to 10 μM taxol for 4 to 22 h; and +++, ≥20% of cells with bundles after 0.1 to 10 μM taxol for 4 to 22 h.

[d] Numbers in parentheses, peak taxol concentration (μM).

bands, often disrupting cellular shape (Figs. 1, *B* to *D*), were easily identified in leukemic blasts from 8 of 12 patients following *ex vivo* treatment with 0.1 to 10.0 μM taxol for 4 to 24 h. In addition, various degrees of clinical antitumor activity occurred during treatment of these 8 patients, and the magnitude of taxol-induced leukemia cytoreduction approximately correlated with the sensitivity of their blasts to taxol-induced microtubule bundle formation *ex vivo*. This correlation was particularly evident in patients achieving complete clearance of tumor from their bone marrow and peripheral blood following treatment. For example, the blasts of 2 of these 3 patients (Patients 3 and 12) were demonstrated to be the most sensitive to microtubule bundle formation *ex vivo* with bundles forming following treatment with taxol concentrations as low as 0.1 μM for 4 h. The blasts of the third patient (Patient 13), who also exhibited complete clearance of tumor and obtained a PR, did not form microtubule bundles *ex vivo* following treatment with 0.1 μM taxol, but bundles were induced after treatment with higher concentrations (1.0 to 10 μM). Most interestingly, complete clearance of tumor did not occur during Patient 13's first course that was associated with a peak taxol concentration of 0.79 μM, which was slightly lower than concentrations required to induce microtubule bundles in the patient's blasts *ex vivo*. However, complete clearance of blasts from the peripheral blood and bone marrow was documented only after a second course, in which a higher peak taxol concentration (2.77 μM) was attained. This level exceeded concentrations that were capable of inducing microtubule bundles in the patient's blasts *ex vivo*.

In contrast, microtubule bundles were not induced *ex vivo* in the blasts of 4 of the 12 patients with tumors that remained stable or progressed through treatment. Peak plasma taxol concentrations in these "total" nonresponders were in the range of concentrations used during *ex vivo* treatment (mean, 2.41 ± 0.65 μM; range, 1.19 to 5.49 μM). The mean peak taxol concentration for the group of patients that demonstrated some degree of clinical response (3.17 ± 0.65 μM; range, 0.63 to 9.45 μM) was higher than the mean peak taxol concentration for the totally unresponsive patients. Although this difference could potentially account for differences in antineoplastic activity in this study, it did not approach statistical significance (Student's *t* test: $P > 0.10$).

During three courses administered to three patients, leukemic blasts from peripheral blood were examined for microtubule bundle formation 24 h posttreatment. Microtubule bundles were not identified in the blasts of two nonresponding patients who were treated with taxol at 250 (Patient 4) and 315 mg/m² (Patient 7). However, bundles were apparent posttreatment in the blasts of Patient 3 who had complete clearance of tumor after 315 mg/m² of taxol.

DISCUSSION

Taxol is a unique plant-derived antineoplastic agent on the basis of its structure and novel mechanism of action. In preliminary Phase I trials, antineoplastic activity was observed in melanomas, ovarian, non-small cell lung, gastric, colon, and head and neck carcinomas (10–15), and in limited Phase II trials, considerable antitumor activity occurred in melanomas and cisplatin-refractory ovarian carcinomas that have been traditionally refractory to conventional antineoplastic agents (29, 30). The impetus for this study included taxol's activity in experimental tumors and the established efficacy of other antimicrotubule agents in leukemia therapy. Taxol's relative lack of severe dose-limiting nonhematological toxicity in Phase I solid tumor trials also implied that further dose escalations would be possible in situations in which hematological toxicity was not a major concern, such as in the treatment of leukemias and preparative regimens for the bone marrow transplantational therapy of leukemias and solid tumors.

Mucositis was the major dose-limiting toxicity of taxol in this study. Oropharyngeal ulcerations with severe pain were consistently severe at 390 mg/m² and precluded escalation of dosage. With myelosuppression and severe mucositis as the major dose-limiting toxicities of taxol in solid tumor and leukemia patients, respectively, the MTD and recommended Phase II doses in acute leukemia, 390 and 315 mg/m², were not significantly higher than the respective values, 275 and 250 mg/m², that were previously established for solid tumor patients. In contrast to agents such as 1-β-D-arabinofuranosylcytosine and etoposide, the lack of a wide discrepancy between taxol doses associated with dose-limiting hematological and nonhematological toxicities suggested that taxol may not offer a significant dose-related advantage in the treatment of leukemias, in which the administration of agents above myelotoxic doses is acceptable and often desirable. However, most patients in this study were heavily pretreated with other mucosal toxins such as etoposide, busulfan, and 1-β-D-arabinofuranosylcytosine, which may have been a major factor in limiting dose escalation. Therefore, less heavily pretreated patients may be able to tolerate significantly higher taxol doses.

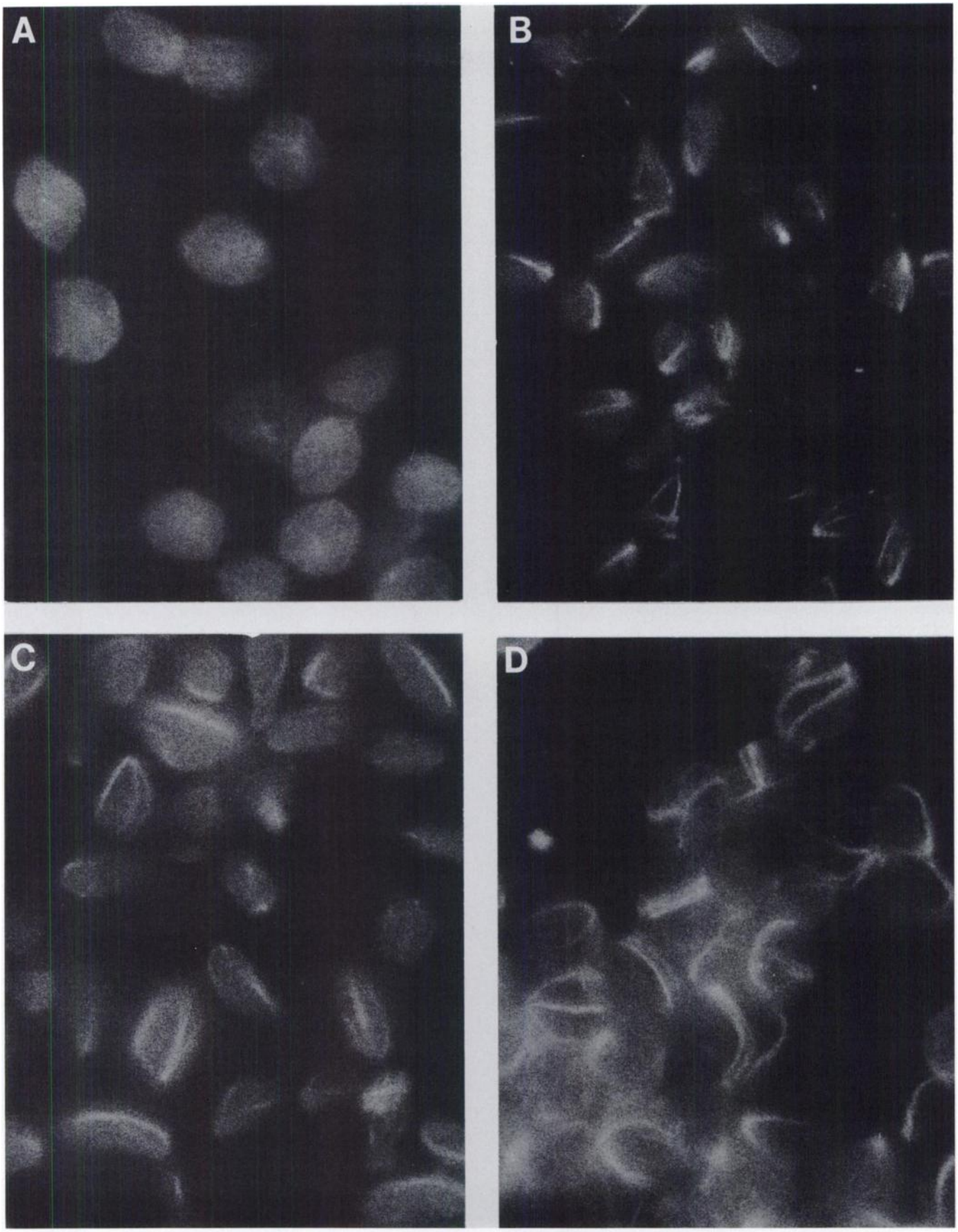

Fig. 1. Indirect immunofluorescence staining of tubulin following incubation of patient's blasts with taxol pretreatment. *A*, blasts of Patient 14, untreated; *B*, blasts of Patient 17 following *ex vivo* treatment with 10 μM taxol for 22 h; *C*, blasts of Patient 16 following *ex vivo* treatment with 1.0 μM taxol for 22 h; and *D*, blasts of Patient 3 following *ex vivo* treatment with 1.0 μM taxol for 2 h.

Reactivation of latent herpes simplex virus was proposed to explain some of the mucositis induced by high doses of several antineoplastic agents such as etoposide (31, 32). This explanation was based on the reductions in the severity of mucositis with the concurrent use of antiviral agents like acyclovir and the tolerance of higher drug doses with acyclovir. However, it did not appear that latent viral reactivation was a major contributing factor in producing the mucositis that was observed in this study, particularly since acyclovir was used prophylactically prior to and throughout taxol-induced aplasia in all patients. In addition, a viral etiology was not supported by the histological and ultrastructural appearance of mucosal tissues at postmortem examination and the clear dose-related nature of the toxicity. It was also unlikely that concurrent use of acyclovir permitted the tolerance of higher taxol doses, since doses up to 275 mg/m^2 were not associated with significant mucositis in a previous study in which patients did not receive acyclovir prophylactically (12).

Severe peripheral neurotoxicity occurred in 75% of solid tumor patients who were treated with taxol as a 24-h infusion at the MTD, 275 mg/m^2, in a previous Phase I study (12). Therefore, it was anticipated that neurotoxicity would be the dose-limiting nonhematological toxicity of taxol in this study. Instead, severe neurosensory toxicity occurred in only one patient who received two courses at 315 and 390 mg/m^2. It is possible that myalgias and arthralgias, which were moderate to severe at higher doses, were graded as neurotoxicity in previous Phase I studies. Patients who developed moderate to severe neurosensory toxicity did not have any predisposing risk factors such as diabetes mellitus nor prior treatment with *Vinca* alkaloids, which have been associated with severe taxol-induced neuropathic effects (10–12). Neurotoxicity appeared to be cumulative, and therefore, it is possible that severe neurotoxicity would have occurred more frequently if a greater number of courses were administered to each patient. Peripheral neurotoxicity was also very mild and not disabling for most patients with advanced ovarian carcinomas who received multiple courses of taxol in a Phase II study (29). In fact, most of those patients had evidence of a preexisting cisplatin-induced peripheral neuropathy prior to treatment.

The peak plasma concentrations of taxol attained in patients in this study were demonstrated to be capable of inducing morphological effects indicative of cytotoxicity in several leukemic cell lines *in vitro* (8). Taxol concentrations as low as 0.1 μM for 2 to 22 h induced irreversible microtubule bundles in a significant proportion of cells in several leukemic cell lines, which was also associated with significant reductions in the clonogenic survival. However, microtubule bundles formed to a lesser extent and did not persist in relatively resistant leukemia cells following 22 h of treatment with taxol concentrations as high as 10 μM (8). Although it would have been optimal if a full range of pharmacokinetic parameters as well as peak concentrations were obtained to compare drug exposure in patients with drug exposure during *in vitro* and *ex vivo* treatment, the achievement of peak concentrations at clinical doses that are capable of inducing effects indicative of cytotoxicity is nevertheless encouraging.

Microtubule morphology as assessed by indirect immunofluorescence was used to correlate distinct taxol-induced changes such as microtubule bundles with clinical antineoplastic activity. Transient complete clearances of leukemia cells, rapid reductions in large tumor burdens, and modest reductions in leukemic blasts were used as indices of clinical antitumor activity and compared to microtubule bundle formation *ex vivo*. Since any response that is less than a CR is probably clinically meaningless and not useful in predicting taxol's ultimate utility in the treatment of leukemia, these indices of antitumor activity were only used to preliminarily study taxol-induced microtubule effects in clinical tumor specimens. Sensitivity to taxol-induced microtubule bundle formation *ex vivo* was associated with clinical antileukemia activity within the 250- to 390-mg/m^2 dose range. Bundle formation did not occur in the blasts of four patients who had progressive or stable disease even after *ex vivo* treatment with taxol concentrations as high as 10 μM for 24 h. Clinical antitumor activity was associated with various degrees of sensitivity to microtubule bundle formation. Although these data were too limited for meaningful statistical analysis, lower taxol concentrations and exposure periods (*i.e.*, 0.1 μM for 4 h) were generally required to induce microtubule bundles *ex vivo* in the blasts of patients who had profound and rapid clinical responses. Peak taxol concentrations in these patients were significantly higher than levels that were required to induce bundles *ex vivo*.

Clinically, antimicrotubule agents have been among the most effective classes of antineoplastic drugs. However, few have been developed that possess higher therapeutic indices and broader antineoplastic spectra than the *Vinca* alkaloids. Based on taxol's novel mechanism of action, activity in a wide variety of solid tumors and leukemias in preclinical studies, and the ability to achieve plasma concentrations that can induce pertinent microtubule effects and cytotoxicity *in vitro*, taxol may find a unique niche in oncological therapeutics. This Phase I study of taxol in leukemia determined that severe mucositis limited the recommended Phase II dose and MTD for leukemia patients to 315 and 390 mg/m^2, respectively. Although the magnitudes of these safe and maximal doses were not significantly higher than similar values derived for solid tumor patients, in which myelosuppression limited dose escalation, additional Phase II studies at these myelosuppressive doses will be necessary to determine taxol's activity in leukemias, and perhaps in combination with hematopoietic growth factors or as part of a preparative regimen for the bone marrow transplantational therapy of solid neoplasms. Sensitivity to taxol-induced microtubule bundle formation *ex vivo* appeared to be related to taxol's antineoplastic activity *in vivo* and may be useful in assessing tumor sensitivity prior to therapy. However, further evaluation of this assay in Phase II studies will be necessary to determine its ultimate utility.

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