Phase I Trial of the Cyclin-Dependent Kinase Inhibitor Flavopiridol in Combination with Docetaxel in Patients with Metastatic Breast Cancer

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ABSTRACT

Purpose: **The purpose of this study was to determine the toxicities and characterize the pharmacokinetics of docetaxel and flavopiridol in patients with metastatic breast cancer.**

Experimental Design: **Docetaxel was administered at an initial dose of 60 mg/m^2 followed in 24 hours by a 72-hour infusion of flavopiridol at 50 mg/m^2/d every 3 weeks. Because dose-limiting myelosuppression occurred, the schedule was amended to docetaxel, 50 mg/m^2, followed by escalating doses of flavopiridol (starting dose, 26 mg/m^2/d) as a 1-hour infusion daily for 3 days. Pharmacokinetic studies were performed. Ki67, p53, and phosphorylated retinoblastoma protein (phospho-Rb) in paired tumor and buccal mucosa biopsies (obtained pre- and posttreatment) were examined by immunohistochemistry.**

Results: **Eleven patients were enrolled. Five patients received docetaxel and 72-hour flavopiridol. Dose-limiting toxicity was grade 4 neutropenia. Six patients received docetaxel and 1-hour flavopiridol, and the dose-limiting toxicity was grade 3 hypotension. Pharmacokinetics of flavopiridol and docetaxel were consistent with historical data. Nuclear staining with p53 increased and phospho-Rb decreased in 10 pairs of buccal mucosa biopsies posttreatment ($P = 0.002$ and $P = 0.04$, respectively). No significant changes in Ki67, p53, or phospho-Rb were detected in six paired tumors. Two patients sustained stable disease for >3 months (72-hour flavopiridol), and one partial response was observed (1-hour flavopiridol).**

Conclusions: **Docetaxel combined with 72-hour flavopiridol was not feasible because of dose-limiting neutropenia. Dose escalation of a 1-hour infusion of flavopiridol with docetaxel was also not possible. The changes in p53 and phospho-Rb in buccal mucosa suggest that a biological effect with flavopiridol was achieved.**

INTRODUCTION

Cyclin-dependent kinases (cdks) and their associated cyclins are critical in the regulation of cell cycle progression. Dysregulation of the cell cycle has been implicated as a causative factor in the majority of human cancers, either through overexpression of cyclins and cdks, loss of endogenous cdk inhibitors, or mutations in retinoblastoma protein (Rb; refs. 1 and 2). Amplification of the *cyclin D1* gene occurs in ~20% of human breast carcinomas and the overexpression of cyclin D1 protein in more than 50% of breast cancers (3, 4). Thus, inhibition of the cell cycle with a cdk inhibitor represents a novel approach to the treatment of breast cancer, especially in combination with chemotherapy.

Flavopiridol is the first cdk inhibitor to be tested in clinical trials. It causes cell cycle arrest (5, 6), induces apoptosis (7, 8), inhibits angiogenesis (9, 10), and potentiates the effects of chemotherapy (11). In preclinical studies, flavopiridol has been shown to inhibit cell proliferation (5), induce cell cycle arrest in G_1 by inhibition of cdk2 and cdk4 (6), reduce levels of cyclin D1 (12), induce erbB-2-independent apoptosis (13), and bind to DNA at high concentration of drug (14).

Several *in vitro* studies also demonstrate the ability of flavopiridol to enhance apoptosis induced by chemotherapy, including taxanes (11). A sequence-dependent induction of apoptosis in which flavopiridol must be given after paclitaxel has been shown (15). Similarly, MCF-7 breast cancer cells that were exposed to docetaxel followed by flavopiridol showed enhancement of apoptosis in a sequence-dependent manner (16, 17). A phase I study of flavopiridol given as a 24-hour infusion in combination with paclitaxel in patients with advanced solid tumors has been reported (18).

On the basis of this preclinical sequence-dependent activity, we conducted a phase I study of docetaxel followed by a 72-hour infusion of flavopiridol every 3 weeks. When dose-limiting myelosuppression occurred, the protocol was amended to administer flavopiridol as a 1-hour infusion daily for 3 days every 3 weeks. We also examined Ki67 and the expression of p53 and phosphorylated Rb (phospho-Rb; as a marker of cdk inhibition) in breast cancer tumor specimens and buccal mucosa (as a readily accessible surrogate tissue) before and after treatment.

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PATIENTS AND METHODS

Eligibility Criteria. Patients were eligible if they had a histologically confirmed diagnosis of breast adenocarcinoma, were ≥18 years of age, and had an Eastern Cooperative Oncology Group performance status of ≤2. Requirements for adequate organ function included an absolute neutrophil count ≥1,500/μL, platelets ≥100,000 μL, creatinine ≤1.5 mg/dL, total bilirubin within normal institutional limits, aspartate aminotransferase ≤1.5 times upper limit of normal, and alkaline phosphatase ≤2.5 times upper limit of normal. Other eligibility criteria included an ejection fraction ≥50% and no prior chemotherapy within 4 weeks of enrollment (2 weeks for prior hormonal therapy). There was no limit to the number of previous chemotherapies or hormonal treatments. Patients were excluded if they had symptomatic brain metastases, a grade ≥2 peripheral neuropathy, coagulopathy requiring therapeutic intervention, or history of hypersensitivity reaction to products containing polysorbate 80. The protocol was approved by the Institutional Review Board of the National Cancer Institute. All of the patients gave written informed consent before treatment.

Treatment Plan and Toxicity Evaluation. Toxicities were evaluated every 3 weeks and were graded by the National Cancer Institute Common Toxicity Criteria, version 2.0. Dose-limiting toxicity (DLT) was defined as any grade ≥3 nonhematologic toxicity, grade 2 nonhematologic toxicity persisting after 2 weeks, or grade 4 hematologic toxicity during the first cycle. Duration of the latter was initially defined at 1 day and revised to more than 5 days. This phase I trial was designed in which at least three to six patients were to be treated with increasing doses of docetaxel and a fixed dose of flavopiridol over a 72-hour period, until DLT occurred in at least two of six patients. Docetaxel (Taxotere, Aventis, Bridgewater, NJ) and flavopiridol were administered every 3 weeks. Docetaxel was given at an initial dose of 60 mg/m^2 (the minimum dose approved for breast cancer) over a period of 1 hour with standard dexamethasone premedications.

The Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD, supplied flavopiridol. It was administered as a 72-hour continuous infusion 24 hours after docetaxel at a fixed dose of 50 mg/m^2/d (the recommended phase II dose; ref. 19). Only two patients were treated at the initial dose level of 60 mg/m^2 docetaxel because both experienced grade 4 neutropenia. The subsequent three patients were treated with 60 mg/m^2 docetaxel, but a lower dose of 72-hour flavopiridol at 28 mg/m^2/d. Because grade 4 neutropenia occurred in two of these patients, the protocol was amended to docetaxel at a fixed dose of 50 mg/m^2 and flavopiridol as a 1-hour infusion daily for 3 days. Flavopiridol as a daily 1-hour infusion has been shown to achieve plasma concentrations in the micromolar range sufficient to inhibit cdks in preclinical models; therefore, this new schedule was chosen (20). It was also hypothesized that a briefer exposure time of flavopiridol in combination with docetaxel may yield a different toxicity profile but may still be sufficient for an antitumor effect.

The initial dose of 1-hour flavopiridol was 26 mg/m^2/d. Subsequent dose escalations in 25% increments were planned to a maximum of 50 mg/m^2/d or until DLT was documented. The plan was to enroll a minimum of three patients for each dose level unless DLT was observed. If one instance of DLT occurred, up to three additional patients were treated at that dose level before escalation could proceed to the next level. If DLTs were observed in at least two of six patients at a given dose level, the maximum-tolerated dose had been surpassed, and a total of up to six patients had to be treated at the previous dose level. Thus, the maximum-tolerated dose was defined as the dose level at which no more than one of six patients experienced DLT and the level below the level that produced two instances of DLT during cycle 1.

On the day of treatment, the absolute neutrophil count was required to be ≥1,500/μL, platelets ≥100,000 μl, and the total bilirubin normal. If counts were not adequate, then treatment was delayed. A dose reduction to the next lower dose level occurred for grade 4 neutropenia more than 5 days, grade 4 neutropenia with infection, grade 4 thrombocytopenia, or febrile neutropenia. For grade 2 diarrhea, treatment was withheld until resolution and then was restarted at the same dose.

Clinical Evaluation. At baseline, a history, physical examination, electrocardiogram, and laboratory tests (complete blood count with differential, electrolytes, creatinine, total bilirubin, alanine aminotransferase, aspartate aminotransferase, prothrombin time, partial thromboplastin time, and urinalysis) were obtained. The laboratory tests were repeated every 3 weeks. Imaging of involved sites was performed within 4 weeks of enrollment and after every 9 weeks. Treatment continued until disease progression or unacceptable toxicity. Response Evaluation Criteria in Solid Tumor was used to assess response (21).

Tissue Biopsies and Immunohistochemistry. Before and after one cycle of treatment, tumor and buccal mucosa biopsies were obtained. For the latter, patients underwent two 3-mm punch biopsies in the cheek mucosa under local anesthesia. For each of these sites, a sample was immediately formalin fixed and subsequently paraffin embedded. Consent from patients was obtained separately for the above procedures.

Tissue sections (4-μm thick) from each paraffin block were mounted on poly-L-lysine coated slides and evaluated for histological changes using H&E staining, proliferation using anti-Ki67 monoclonal antibody MIB-1 (Dako, Carpinteria, CA; diluted at 1:50), and expression of p53 (DO-7; Novocastra Laboratories; diluted at 1:50) and phospho-Rb (Ser780 Cell Signaling Technology, Beverly, MA; diluted at 1:300). For Ki67, a labeling percentage was reported. For Ki67, p53, and phospho-Rb, the percentage of positive nuclei was measured using an automated cellular imaging system (ChromaVision Medical Systems, Inc, San Juan Capistrano, CA).

Pharmacokinetic Studies. Docetaxel plasma concentrations were collected before initiation of the drug and at 55, 65, 75, and 90 min and 2, 3, 5, 12, and 24 hours after the beginning of the infusion during cycle 1 and were measured using a validated high-performance liquid chromatography method with tandem mass spectrometric detection (22). Flavopiridol plasma concentrations were obtained just before the beginning of the 72-hour infusion of flavopiridol, and at 6, 12, 24, 36, 48, and 60 hours during the infusion, at the end of infusion, and at 10 min, 30 min, and 1, 2, 4, 6, and 10 hours after the end of the infusion. On the 1-hour flavopiridol schedule, samples were collected before the infusion; at 10 min, 30 min, and 2, 3, 5, 9, 12, 24, 48,

and 49 hours after the start of the first flavopiridol infusion; and at the end of the infusion during cycle 1. Plasma concentrations of flavopiridol were measured using a previously described validated high-performance liquid chromatography assay (23).

Pharmacokinetic parameters for docetaxel and flavopiridol were calculated by noncompartmental analysis using WinNonlin software version 4.0 (Pharsight Corporation, Mountain View, CA). The parameters of interest included the *AUC* (area under the plasma concentration-time curve) extrapolated to infinity, clearance (defined as dose, divided by *AUC*), volume of distribution at steady-state, and the half-life of the terminal disposition phase. The latter parameter was calculated as $\ln(2)/k$, where k is the rate constant of the terminal phase estimated from log-linear regression analysis of the final three to five sampling time points.

Statistical Analysis of Marker Data. To evaluate expression of markers, we evaluated differences from baseline to assess whether they were statistically significantly different from zero using the Wilcoxon signed rank test. Because of the limited number of patients, results were obtained by using all of the available samples pooled together rather than by dose level. In view of the large number of tests performed, individual P values such that $P < 0.01$ was interpreted as statistically significant, whereas $0.01 \leq P < 0.05$ represented trends. Spearman correlation analyses were performed. The correlation coefficients were interpreted as follows: $r > 0.70$ is a strong correlation; $0.50 \leq r < 0.70$ is a moderate correlation; $0.30 \leq r < 0.50$ is a weak to moderate correlation; and $r < 0.30$ is a weak correlation. All P values are two-tailed and reported without adjustment for multiple comparisons.

RESULTS

Patient Characteristics. Eleven patients with metastatic breast cancer were enrolled between November 2000 and December 2002 (Table 1). All of the patients had previously received both anthracycline-based chemotherapy and paclitaxel as adjuvant or neoadjuvant therapy and/or as treatment for metastatic disease. None received prior docetaxel. Median time on study was 62 days (range, 22–371 days). A total of 48 cycles were administered. No patients remain on study.

Table 1 Patient characteristics

		No. of patients
Total		11
Treated with 72-h flavopiridol		5
Treated with 1-h flavopiridol		6
Median age, y (range)	55 (33–71)	
Median ECOG performance status		1
Hormone receptor status		
ER+ or PR+		3
ER− and PR−		7
HER2/neu-positive		5
Previous paclitaxel		
Adjuvant		5
Neoadjuvant		1
Metastatic		6
Prior high-dose chemotherapy		2
Median no. of prior hormonal therapies (range)		1 (0–2)
Median no. of prior chemotherapy regimens (range)		1 (0–3)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PR, progesterone receptor.

Docetaxel and 72-Hour Flavopiridol. The first two patients, who were treated with 60 mg/m^2 of docetaxel on day 1 and 50 mg/m^2/d of flavopiridol as a 72-hour infusion starting on day 2, developed grade 4 neutropenia with a mean absolute neutrophil count of 190/μL. This nadir count occurred approximately on day 12 of the cycle and resolved by day 15. Neither patient was admitted for neutropenic fever. Both patients (who had prior treatment with high-dose chemotherapy and stem cell reinfusion) were re-treated with a reduced docetaxel dose of 45 mg/m^2 and the flavopiridol maintained at 50 mg/m^2/d. With this lower dose of docetaxel, neither patient experienced further dose-limiting neutropenia, although both patients required a reduced dose of flavopiridol to 39 mg/m^2/d for subsequent cycles because of other toxicities, including grade 3 fatigue and grade 3 hypotension.

Because of the hematologic toxicity, rather than further reducing docetaxel to less than 60 mg/m^2 at this point, we elected to lower flavopiridol to 28 mg/m^2/d. Dose-limiting neutropenia (grade 4) again occurred in two of three patients, and we were unable to escalate the dose of flavopiridol.

In summary, on the 72-hour flavopiridol schedule, four of five patients experienced DLTs, which included grade 4 neutropenia, grade 3 fatigue, grade 3 hypotension, and grade 3 hypophosphatemia. Flavopiridol steady-state concentrations in these five patients ranged from 0.12 to 0.41 μM and were not greater than expected at these doses (19, 24).

Docetaxel and One-Hour Flavopiridol. Because dose-limiting myelosuppression occurred, we elected to change the infusion of flavopiridol from 72 hours to 1 hour and to administer it daily for 3 days with a docetaxel dose of 50 mg/m^2. The first three patients to enroll on this schedule were treated with 50 mg/m^2 of docetaxel on day 1 and 26 mg/m^2/d of flavopiridol as a 1-hour infusion for 3 days starting on day 2. At these doses, there were no DLTs during cycle 1. However, one patient experienced grade 4 neutropenia, grade 4 hypotension, and grade 3 typhilitis during cycle 2.

A patient treated at the next dose level of 34 mg/m^2/d of flavopiridol developed grade 3 hypotension and was unable to receive the third dose of flavopiridol during cycle 1. She was re-treated with the same dose of docetaxel, but with a reduced dose of flavopiridol (26 mg/m^2/d) and again developed grade 3 hypotension. Flavopiridol was reduced to 20 mg/m^2/d by the third cycle with no further blood pressure changes. We were able to treat an additional two patients with 20 mg/m^2/d of flavopiridol for one complete course. Neither could receive further therapy because of disease progression. Because there were considerable clinical toxicities observed with 26 mg/m^2/d and 34 mg/m^2/d of flavopiridol, we elected not to continue with further enrollment, because the numerous DLTs argued against the practical utility of this combination on these schedules (Table 2).

Nonhematologic Toxicities. Toxicities for the first cycle of therapy are summarized in Table 3. Most toxicities were grade 1 or 2, except for one episode of grade 3 fatigue, three episodes of grade 3 hypotension, and two instances of

Table 2 DLTs in the first course at each dose level

Docetaxel mg/m^2	72-h flavopiridol mg/m^2/d	1-h flavopiridol mg/m^2	No. of patients	No. of patients with DLT	DLTs
60	50		2	2	Neutropenia and hypotension*
60	28		3	2	Neutropenia and hypotension*
50		26	3	0	None†
50		34	1	1	Hypotension‡
50		20	2§	0	None

* Patients ($n = 4$) required a dose reduction of docetaxel to 45 mg/m^2 in subsequent cycles.
† One patient had grade 4 neutropenia and grade 4 hypotension during cycle 2.
‡ One patient was unable to complete the third dose of flavopiridol because of grade 3 hypotension.
§ The patient who received 34 mg/m^2 of flavopiridol was treated during cycle 3 with 20 mg/m^2.

Table 3 Cycle 1 toxicities with docetaxel and flavopiridol

Docetaxel, mg/m^2	60				60				50				50				50			
Flavopiridol, m/m^2/d	50				28				26				34				20			
Duration of infusion, h	72				72				1				1				1			
No. of patients	2				3				3				1				2			
Event	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G1	G2	G3	G4	
Neutropenia				2				2			2	1			1	1			1	
Thrombocytopenia		1			1	1														
Diarrhea	2				2	1			1								1			
Nausea		1			1				1							1				
Vomiting		1			1															
Anorexia	2															1				
Fatigue	1		1		1	2				2			1			1				
Myalgia	1				2															
Hypotension			1				1			1					1					
Tumor pain					1															
Hypoalbuminemia	1				1	1			2								1			
Hypophosphatemia			1				2													

Abbreviation: G, grade.

grade 3 hypophosphatemia that had no clinical consequences. In one patient, treated with docetaxel 50 mg/m^2 and 1-hour flavopiridol at 26 mg/m^2/d, an unsuspected gastrointestinal toxicity occurred during cycle 2. She developed typhilitis with refractory gastrointestinal bleeding and required a right hemicolectomy and ileostomy. The specimen revealed ischemic colitis. We elected not to re-treat her.

During cycle 1, 36% of patients experienced grade 2 or 3 hypotension. It generally developed on day 4 or 5, which is later in onset compared with the single agent 72- and 1-hour flavopiridol trials, and resolved within 24 to 48 hours.

Pharmacokinetics of Docetaxel. Pharmacokinetic parameter estimates are shown in Table 4. Pertinent parameters were independent of the administered dose ($P > 0.36$, Kruskal-Wallis test), suggesting a linear pharmacokinetic profile. Overall, a moderate degree of interindividual variability was observed with a coefficient of variation for clearance of 45%. The mean (±SD) value for clearance was 31.4 ± 14.2 liters/h/m^2 (range, 18.3 to 63.7 liters/h/m^2) and the half-life of the terminal disposition phase was 11.0 ± 6.23 hours (range, 3.19 to 22.7 hours), consistent with previously reported estimates (25).

Pharmacokinetics of Flavopiridol. Mean C_{ss} (concentration at steady state) of 72-hour flavopiridol were 0.41 μM ($n = 3$) and 0.20 μM ($n = 2$) at the doses of 50 mg/m^2/d and 28 mg/m^2/d, respectively. Mean C_{max} (peak plasma concentration) for the 1-hour infusion were 1.13 μM ($n = 2$), 1.4 μM ($n = 3$), and 2.92 μM ($n = 1$) at the doses of 20 mg/m^2/d, 26 mg/m^2/d, and 34 mg/m^2/d,

Table 4 Docetaxel pharmacokinetic parameters

Dose mg/m^2	No. of patients	C_{max} ng/mL	*AUC* ng/mL·h	*Cl* L/h/m^2	$t_{1/2}$ h	V_{ss} L/m^2
60	5	2140 ± 1171 (524–3730)	1999 ± 859 (943–3280)	35.4 ± 17.2 (18.3–63.7)	9.91 ± 7.5 (3.19–22.7)	204 ± 155 (62.8–461)
50	6	1688 ± 837 (455–2629)	2015 ± 705 (1017–2785)	28.1 ± 11.8 (18.0–49.2)	11.9 ± 5.52 (7.74–22.6)	247 ± 181 (137–604)

NOTE. Values represent the mean value ± SD with range in parentheses.
Abbreviations: C_{max}, peak plasma concentration; *AUC*, area under the plasma concentration-time curve; *Cl*, clearance; $t_{1/2}$, terminal half-life; V_{ss}, volume of distribution at steady state.

Table 5 Pharmacokinetic parameters for 72-hour infusion of flavopiridol

Patient no.	Dose mg/m^2/d	C_{ss} μmol/L	C_{ss}/dose μmol/L	$AUC_{(0\text{-infinity})}$ μmol/L·h	$AUC_{(adjusted)}$ μmol/L·h	*Cl* L/h/m^2
1	50	0.41	0.008	30.05	0.20	11.5
2	50	0.40	0.008	26.69	0.18	12.8
Mean	50	0.41	0.008	28.37	0.19	12.1
3	28	0.18	0.006	11.99	0.14	15.6
4	28	0.12	0.004	8.13	0.10	23.6
5	28	0.30	0.011	23.97	0.29	8.0
Mean	28	0.20	0.007	14.70	0.17	15.7
Median	28	0.18	0.006	11.99	0.14	15.6

Abbreviations: C_{ss}, concentration at steady state; $AUC_{(0\text{-infinity})}$, area under the plasma concentration-time curve from time 0 to infinity; *Cl*, clearance.

respectively, for 3 days. The *AUC* of flavopiridol appeared linear at all of the doses explored. Mean total clearance was 14.3 and 17.5 liters/h/m^2 for the 72-hour and 1-hour infusion, respectively. The dose-adjusted C_{max} and *AUC* (C_{max} or *AUC* were divided by dose) were comparable between the different doses and between the two schedules (Tables 5 and 6).

Response Data. On the 72-hour flavopiridol schedule, two patients sustained stable disease (10.5 and 4.3 months) and two patients progressed. A patient with an inflammatory breast recurrence treated initially with 60 mg/m^2 docetaxel and 28 mg/m^2/d flavopiridol as a 72-hour infusion experienced a decrease in erythema and skin thickening after four cycles and proceeded to simple mastectomy. She returned for evaluation 9 weeks from her last therapy because of prolonged healing from surgery and was discovered to have recurrent chest wall disease. On the 1-hour flavopiridol schedule, one patient achieved a partial response in the liver and was withdrawn from the study because of toxicity. The other five patients treated with 1-hour flavopiridol had progressive disease.

Effect of Docetaxel and Flavopiridol on Tumor and Buccal Mucosa Biopsies. The effect of docetaxel and flavopiridol on markers in six pairs of matched tumor biopsies and 10 pairs of matched buccal mucosa are shown in Fig. 1. In buccal mucosa, p53 protein significantly increased [pretherapy *versus* posttherapy (mean ± SD), 2.8 ± 1.0 *versus* 9.8 ± 2.0; $P = 0.002$]. There was an increase in p53 in tumor postflavopiridol (8.3 ± 5.4 *versus* 14.2 ± 6.3) that did not reach statistical significance ($P = 0.09$), but the increase was seen in five of six (83%) paired tumor samples and was not seen in one sample that had abnormal p53 expression. There was a trend toward a reduction in phospho-Rb in buccal mucosa biopsies (9.8 ± 3.0 *versus* 2.4 ± 0.5; $P = 0.04$), which was evident in 70% of paired samples, and there was no significant change in phospho-Rb in posttreatment tumor samples overall (21.9 ± 5.6 *versus* 19.0 ± 12.2; $P = 0.50$). There were no significant changes in Ki67 in buccal mucosa (13.6 ± 1.9 *versus* 12.5 ± 2.1; $P = 0.71$) and tumor (29.7 ± 7.6 *versus* 23.8 ± 7.2; $P = 0.31$) after treatment with docetaxel and flavopiridol, but an inverse correlation was observed between the percentage change in Ki67 in tumor and *AUC* of flavopiridol ($r = -0.77$; $P = 0.07$).

Interestingly, in the patient whose cutaneous lesions improved after four cycles of docetaxel and 28 mg/m^2/d of flavopiridol as a 72-hour infusion, Ki67 decreased (pretherapy *versus* posttherapy, 29 *versus* 14%) concurrent with an increase in p53 (3 *versus* 20%) and a decrease in phospho-Rb (27 *versus* 16%; Fig. 2). In her buccal mucosa specimens, there was also a decrease in Ki67 (18 *versus* 6%), increase in p53 (7 *versus* 10%), and decrease in phospho-Rb (13 *versus* 4%; Fig. 3). There were no tumor biopsies available in the patient with a partial response. Her buccal mucosa did reveal a decrease in Ki67 (25 *versus* 10%), an increase in p53 (9 *versus* 26%), and a decrease in phospho-Rb (19 *versus* 5%).

Table 6 Pharmacokinetic parameters for 1-hour infusion flavopiridol

Patient no.	Dose mg/m^2	C_{max} μmol/L	C_{max}/dose μmol/L	$AUC_{(0\text{-infinity})}$ μmol/L·h	$AUC_{(adjusted)}$ μmol/L·h	*Cl* L/h/m^2
6	26	1.71	0.066	3.31	0.13	17.6
7	26	1.74	0.067	5.55	0.21	10.6
8	26	0.75	0.029	1.97	0.08	30.3
Mean	26	1.40	0.054	3.61	0.14	19.5
Median	26	1.71	0.066	3.31	0.13	17.6
9	34	2.92	0.086	9.77	0.29	8.0
10	20	0.97	0.049	1.99	0.10	22.7
11	20	1.29	0.065	2.85	0.14	15.6
Mean	20	1.13	0.057	2.42	0.12	19.1

Abbreviations: C_{max}, peak plasma concentration; $AUC_{(0\text{-infinity})}$, area under the plasma concentration-time curve from time 0 to infinity; *Cl*, clearance.

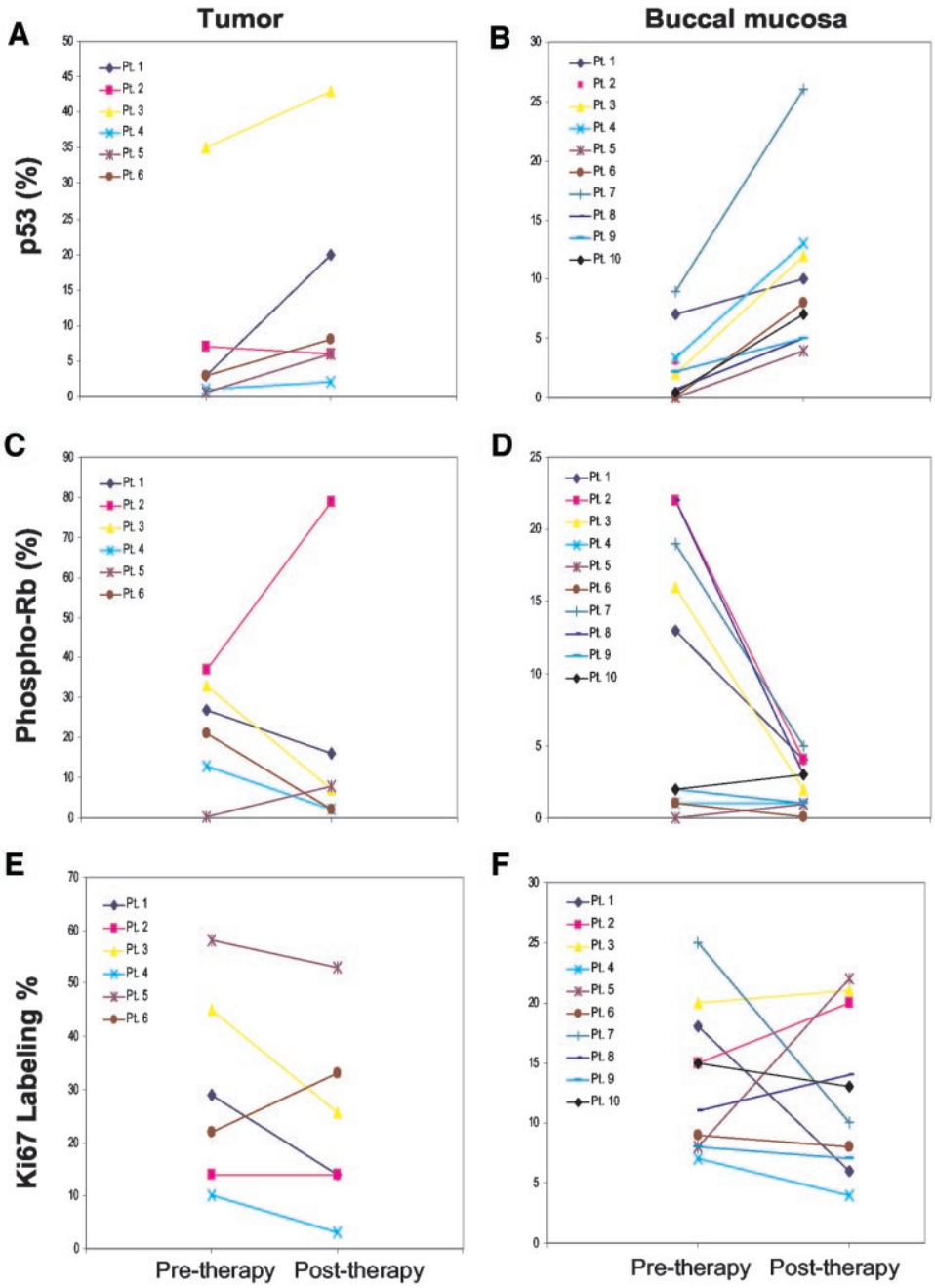

Fig. 1 Measurement of p53 and phosphorylated Rb (*phospho-Rb*; percentage of positive nuclei) and Ki67 (labeling percentage) pre- and posttherapy (after flavopiridol) in tumor (*A*, *C*, and *E*) and buccal mucosa (*B*, *D*, and *F*) biopsies. *Each line/symbol*, an individual subject.

DISCUSSION

This phase I study was conducted to determine the safe dose of docetaxel and flavopiridol in patients with metastatic breast cancer. Flavopiridol as either a 72-hour or a 1-hour infusion was difficult to combine with docetaxel. DLTs were primarily neutropenia and orthostatic hypotension. With flavopiridol as a 72-hour infusion, myelotoxicity developed even when the dose was lowered from 50 to 28 mg/m^2/d. This occurred in the two patients treated at 50 mg/m^2/d who had received previous high-dose chemotherapy and in two patients treated at 28 mg/m^2/d, one of whom had not received prior chemotherapy for metastatic disease, making it less likely to be related to prior extensive therapy. Four of five patients also required a reduced dose of docetaxel at 45 mg/m^2 in subsequent cycles. In addition, orthostatic hypotension (grade 3) was problematic, occurring in one patient treated at 50 mg/m^2/d and the other at 28 mg/m^2/d. When the schedule of flavopiridol was changed to 1 hour, DLT of hypotension still occurred at 34 mg/m^2. Neither changing the schedule nor lowering the dose of flavopiridol altered the toxicity profile, but other schedules of this drug combination were not explored.

In the first reported 72-hour flavopiridol phase I trial,

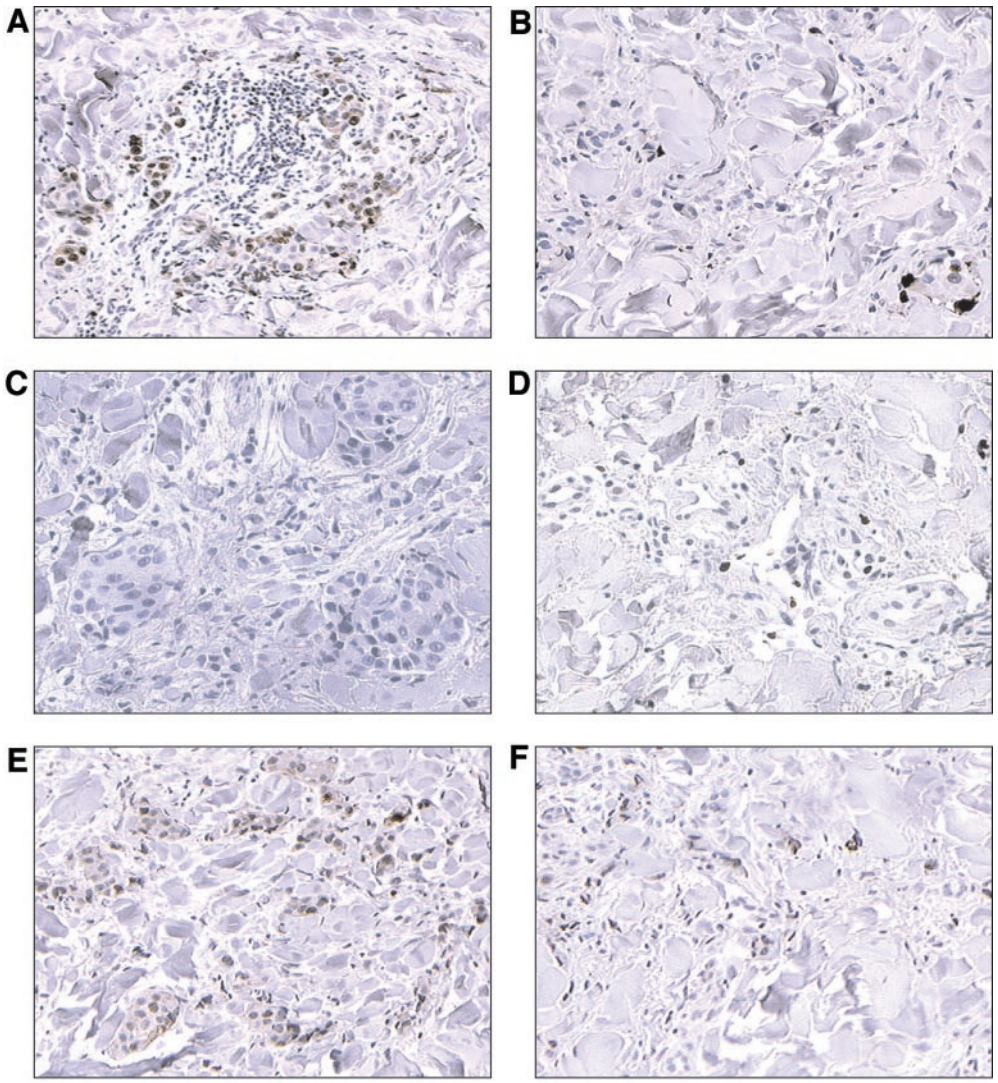

Fig. 2 Marker expression changes in paired tumor biopsies from a patient treated with docetaxel 60 mg/m^2 and 72-hour flavopiridol 28 mg/m^2/d (×200). Ki67 (*dark cells*) is decreased (*B*), p53 is increased (*D*), and phospho-Rb is decreased (*F*) after treatment with flavopiridol compared with the corresponding baseline samples (*A*, *C*, and *E*).

myelosuppression was not observed, although dose-limiting hypotension occurred at 98 mg/m^2/d (19). In a single-agent 1-hour flavopiridol phase I trial, grade 3 hypotension occurred at 62.5 mg/m^2/d daily for three days (20). These toxicities occurred at much higher doses than what was used in the present study, which suggests that these effects may be secondary to the combination rather than to flavopiridol alone. Diarrhea was a major DLT in the single-agent 72-hour flavopiridol experiences and occurred as grade 1 and 2 toxicities with docetaxel and 72-hour flavopiridol. Fatigue, a prominent symptom in the single-agent flavopiridol studies, was also a common toxicity occurring in 65% of our total administered cycles. Venous and arterial thromboses have been described in the phase II flavopiridol trials (26, 27, 28). In the present study, there was one episode of ischemic colitis in which the etiology is unknown, but it may have been secondary to a thrombotic event.

In the phase I trial of paclitaxel combined with flavopiridol (both given as 24-hour infusions), neutropenia and pulmonary toxicity (dyspnea) were dose limiting (18). Myelosuppression was predominant in our trial, too, but pulmonary events were not observed. Schwartz *et al.* (18) were able to avoid myelotoxicity by changing paclitaxel to a 3-hour infusion and were able to escalate the dose of flavopiridol to 94 mg/m^2. We elected to change flavopiridol to a 1-hour infusion and to lower docetaxel to 50 mg/m^2, but the flavopiridol dose could not be escalated beyond 34 mg/m^2 secondary to hypotension. Despite treating three patients with 1-hour flavopiridol at 20 mg/m^2/d with manageable toxicity, peak plasma concentrations of flavopiridol were at the low end necessary to achieve cdk inhibition *in vitro*, and we elected not to pursue this dosage and schedule further.

Neutropenia has also been problematic with other schedules of docetaxel and flavopiridol (29, 30). An alternate strategy of weekly administration of this combination is currently being studied, in which docetaxel (given over a period of 30 min) is being followed 4 hours later by a 1-hour flavopiridol infusion, which may circumvent the myelosuppression that we and other investigators have encountered (17).

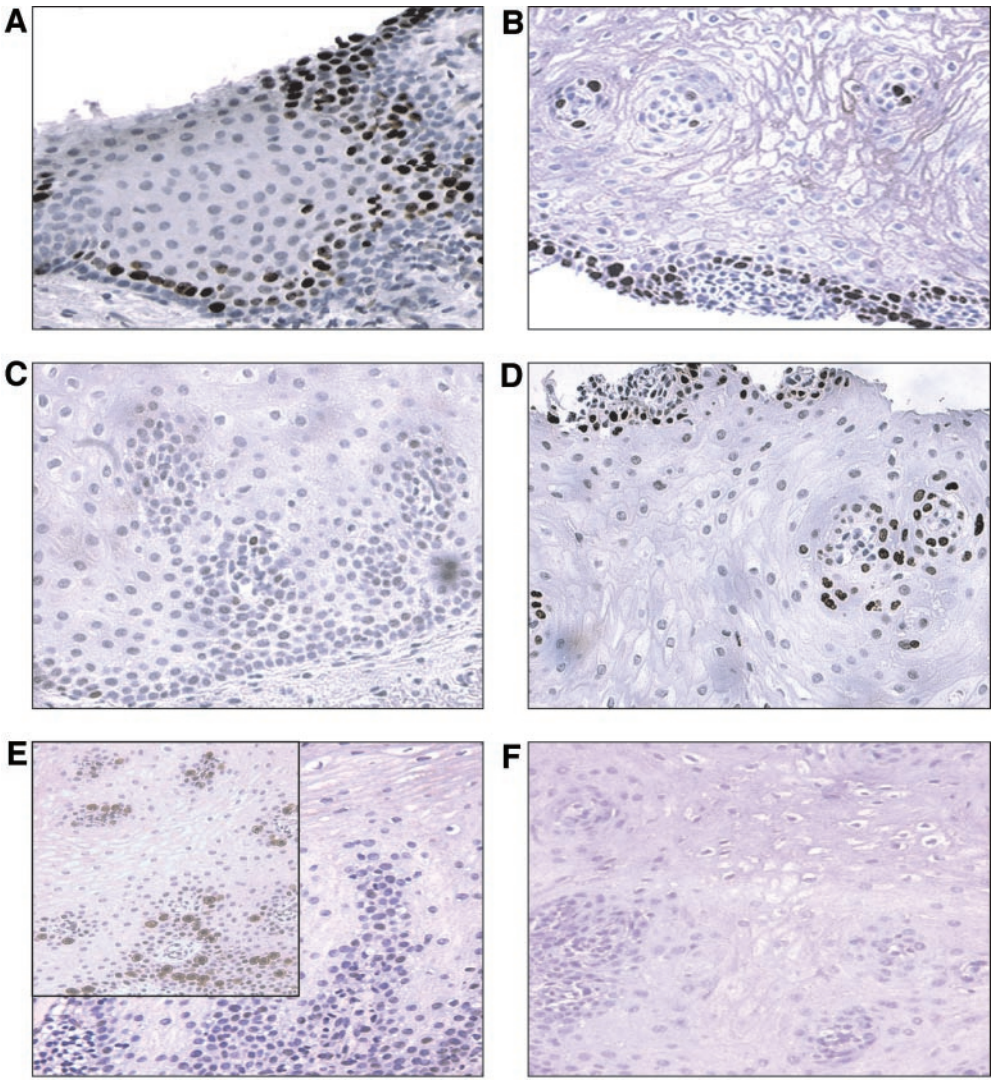

Fig. 3 Marker expression changes in paired buccal mucosa biopsies from the same patient treated with docetaxel 60 mg/m^2 and 72-hour flavopiridol 28 mg/m^2/d (×400). Ki67 (*dark cells*) is decreased (*B*), p53 is increased (*D*), and phospho-Rb is decreased (*F*) after treatment with flavopiridol compared with the corresponding baseline samples (*A*, *C*, and *E*). *Inset in E* shows an increase in phospho-Rb after docetaxel, compared with baseline.

The steady-state concentrations of 72-hour flavopiridol achieved with docetaxel were consistent with other clinical trials of flavopiridol (19, 24, 31) and sufficient for cdk inhibition *in vitro*. Our limited data suggest that docetaxel did not affect the concentration of flavopiridol. In addition, the peak concentration of 1-hour flavopiridol at 20, 26, and 34 mg/m^2 (range, 0.75–2.92 μM) with docetaxel was not higher than expected (20).

We assessed Ki67 and the expression of p53 and phospho-Rb with immunohistochemistry in serially collected tumor and buccal mucosa biopsies. There are no previous reports published on Ki67 and p53 or phospho-Rb expression after treatment with flavopiridol in tissue specimens. The significant increase in p53 in buccal mucosa could be due to the action of flavopiridol binding to DNA (14) or could be due to the inhibition of transcription and down-regulation of the MDM2 protein, which has been reported with other cdk inhibitors (32, 33). The decrease in phospho-Rb post-treatment in buccal mucosa suggests that cdk4 kinase activity was inhibited by flavopiridol because cdk4/cyclin D is required for the phosphorylation of Rb (34). The strong inverse correlation between tumor Ki67 and the *AUC* of flavopiridol suggests probable drug effect even at low doses. Although definitive conclusions cannot be drawn from our small sample of specimens, the results are encouraging in that target modulation could be achieved; alternate strategies or agents to mitigate the toxicities warrant further development.

Interestingly, for the patient with improved skin lesions, the reduced tumor proliferation was associated with a decrease in Rb phosphorylation, suggesting cdk inhibition by flavopiridol halted tumor growth. It would have been interesting to see the marker changes in the tumors of the patients with a partial response and stable disease if tissue was available. No significant changes in phospho-Rb and Ki67 after treatment were observed in patients with progressive disease, suggesting that these tumors are resistant to cdk inhibition by flavopiridol and/or resistant to docetaxel and

that not all tumors in advanced stages respond to one type of targeting or one drug combination.

Recent reports highlight the importance of appropriate target selection for cdk-directed drugs in cancer treatment. In a study using colon cancer cell lines, proliferation of cancer cells occurred despite cdk2 inhibition, which suggests that cdk2 may not be that essential in the progression of the cell cycle (35). Also, cyclin E, a key component in the progression of the cell from G_1 to S phase, on reexamination has not been found to be critical in the embryonic development of mice (36). These studies perhaps question the relevance of targeting one specific cdk or cyclin, but flavopiridol is a pan-cdk inhibitor and other cdks (*i.e.*, cdk1, cdk4, cdk6, and cdk7) besides cdk2 would still be affected by drug. In view of these findings, cdk-directed anticancer therapy should continue undergoing clinical development, and consideration should be given to alternate strategies of interrupting the cell cycle, such as modulation of the phosphorylation states of cdks or decreasing levels of cyclin proteins (37).

In summary, DLTs frequently occurred with the combination of docetaxel either with 72-hour or with dose escalation of 1-hour flavopiridol and did not appear to be related to steady-state or peak drug concentrations of flavopiridol. Also, the drug effect of flavopiridol was demonstrated by changes in p53 and phospho-Rb in normal tissues. Although flavopiridol and docetaxel were difficult to combine in our patient population, flavopiridol as a modulator of chemotherapy continues to be the focus of several ongoing phase I combination trials with standard chemotherapy. More promising approaches are currently being tested, such as combining flavopiridol with signaling agents (38, 39). In addition, future studies need to evaluate other markers of cdk modulation in tumor samples so that the effect of flavopiridol to its target sites of action is optimally determined.

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