Flavopiridol is the first cyclin-dependent kinase inhibitor to undergo widespread clinical testing (1). Although flavopiridol was initially considered to be a strictly cytostatic agent (2–7), more recent studies have shown that flavopiridol kills cycling and noncycling cells in vitro, ex vivo, and in xenograft cancer models (8–17). Additional studies have shown that flavopiridol can dramatically enhance the cytotoxicity of other antineoplastic agents in vitro, particularly when given as the second agent in the sequence (18–28). Based upon promising antitumor activity in a number of xenograft models (10, 17), as well as its unique mechanism of action, flavopiridol has undergone phase 1 testing as 72-hour, and now 24- and 1-hour continuous infusions, as well as in combination with a variety of other antineoplastic agents (29).

Mechanistically, flavopiridol is now known to affect several molecular targets in addition to cyclin-dependent kinases. It is a potent inhibitor of P-TEFb in vitro and in vivo, the kinase responsible for the phosphorylating activation of RNA polymerase II, and can globally attenuate transcription on this basis (30, 31). Flavopiridol also binds to double-stranded DNA with a similar binding constant to doxorubicin, and the National Cancer Institute COMPARE analyses suggest that its cytotoxicity may be on this basis (32–34).

The present report describes the results of a phase 1 trial of flavopiridol combined with either cisplatin or carboplatin in the treatment of patients with advanced solid tumors. Preclinical rationale for the trial include (a) evidence of in vitro synergy between the two agents (18) and (b) the realization that flavopiridol affects increased intracellular concentrations of platinum in cancer cells when combined with cisplatin in vitro (35). We sought to evaluate toxicity, establish maximum tolerated dose (MTD), and also evaluate two hypotheses: (a) that cisplatin pretreatment might not alter flavopiridol pharmacokinetics and (b) that the known in vitro effects of flavopiridol on cellular proteins [including
p53, Mcl-1, and phosphoRNA (pRNA) polymerase II] might be recapitulated in \textit{vivo} in peripheral blood mononuclear cells (PBMC) obtained from flavopiridol-treated patients.

\section*{Patients and Methods}

\textbf{Inclusion/exclusion criteria.} Requirements for enrollment in the trial included histologic proof of advanced solid tumor malignancy with no hope of curative, or clearly established life-prolonging therapy; age >18 years; performance status 0 to 2; life expectancy >3 months; ability to accomplish informed consent; WBC >3,500; ANC >1,700; PLT >100,000; normal direct bilirubin; and creatinine, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase <2.5 x ULN. Exclusion criteria included radiation to >25% bone marrow, prior cisplatin or carboplatin therapy; chemotherapy, biological, immunotherapy, or radiotherapy within the past 4 weeks (6 weeks for mitomycin C/nitrosoureas); New York Heart Association class III or IV or history of angina; central nervous system metastases or seizure disorder; pregnancy/lactation; and grade >1 peripheral neuropathy.

\textbf{Treatment cohorts and schedules.} Patients were enrolled in three sequential cohorts. In cohort/stage I, cisplatin was fixed at 30 mg/m\textsuperscript{2} with escalating flavopiridol (50-135 mg/m\textsuperscript{2}/24 hours). In cohort/stage II, 54.5 mg of flavopiridol was fixed at the stage I MTD (100 mg/m\textsuperscript{2}/24 hours) with escalation of cisplatin (45-75 mg/m\textsuperscript{2}) to attain recommended dosages for future flavopiridol/CDDP phase 2 trials. In cohort/stage III, flavopiridol was fixed at the stage I MTD (100 mg/m\textsuperscript{2}/24 hours) with (de)escalation of cisplatin (3 and 2 AUC). Therapy was given every 3 weeks, except to note that a single cycle of flavopiridol alone (cycle 0) was given to all patients enrolled in cohorts 1 and 2 followed by initiation of combination therapy 2 weeks later (this enabled the assessment of the effects of cisplatin on flavopiridol pharmacokinetics and the effects of flavopiridol alone on PBMC levels of selected polypeptides).

Cisplatin in 750 mL D5/0.45% NaCl containing 25 g mannitol was infused i.v. over 2 hours immediately before flavopiridol (cohorts 1 and 2). Carboplatin in 250 mL D5W was infused i.v. over 30 minutes immediately before flavopiridol (cohort 3). Carboplatin dosages were calculated using the Calvert formula with the Jelliffe equation.

Flavopiridol was supplied by the National Cancer Institute in 50-mg sterile vials containing 54.5 mg lyophilized flavopiridol (equivalent to 50 mg of free base) with 96 mg citric acid, 1,500 mg hydroxypropyl-\(\beta\)-cyclodextrin, and sodium hydroxide to adjust pH to 3.5 to 5.5. The 50-mg vials were reconstituted with 10 mL Sterile Water for Injection, USP; 5% Dextrose for Injection, USP; or 0.9% Sodium Chloride Injection, USP to give 4.5 mg flavopiridol, 8.6 mg citric acid, and 134 mg hydroxypropyl-\(\beta\)-cyclodextrin per mL. Flavopiridol was given over 24 hours by continuous i.v. infusion via ambulatory pump beginning immediately after completion of cisplatin or carboplatin infusion.

\textbf{Materials.} Flavopiridol was provided by the Pharmaceuticals Resources Branch of the National Cancer Institute (Bethesda, MD). Antibodies were purchased from the indicated suppliers: Mcl-1 (BD PharMingen, San Diego, CA), Bcl-2 (DAKO, Glostrup, Denmark), p53 (NeoMarkers, Fremont, CA), cyclin D1 (Calbiochem, San Diego, CA), pRNA polymerase II (Covance, Cumberland, VA), and phospho(Tyr)STAT3 (Cell Signaling, Beverly, MA). PARP antibody (loading control) was kindly provided by Dr. Scott Kaufmann (Mayo Clinic, Rochester, MN). All other reagents were obtained as described above.

\textbf{Cell culture.} A549 human non–small cell lung carcinoma cells were cultured in RPMI 1640 containing 5% heat-inactivated fetal bovine serum, 100 units/mL penicillin G, 100 \(\mu\)g/mL streptomycin, and 2 mmol/L glutamine and maintained at 37°C in an atmosphere of 95% air/5% CO\(_2\) as previously described (18). Cells were treated with either 1:1,000 DMSO (diluent) or previously prepared flavopiridol dissolved in DMSO and washed twice with PBS before direct solubilization in alkylation buffer [6 mol/L guanidine hydrochloride, 250 mmol/L Tris-HCl (pH 8.5 at 21°C), and 10 mmol/L EDTA supplemented immediately before use with 150 mmol/L \(\beta\)-mercaptoethanol and 1 mmol/L L-α-phenylmethylsulfonyl fluoride] in preparation for immunoblotting.

\textbf{Collection and processing of peripheral blood mononuclear cells: immunoblotting.} Peripheral blood mononuclear cells were collected by venipuncture pretreatment and immediately at the conclusion of 24-hour flavopiridol infusion for cycle 0 only (flavopiridol alone) from patients in cohorts 1 and 2. PBMCs were isolated from heparanized blood by ficoll-hypaque density gradient centrifugation, and harvested cells were washed twice in PBS and solubilized in alkylation buffer in preparation for immunoblotting. Resulting lysates were then processed for SDS-PAGE and subsequent immunoblotting using techniques previously described in detail (35).

\textbf{Collection of blood for pharmacokinetic studies.} Blood was collected in heparin-containing tubes from all patients enrolled in cohorts 1 and 2 during cycles 0 (flavopiridol alone) and 1 (the first cycle of CDDP combined with flavopiridol) using a percutaneously placed catheter at the following times: 1, 4, 8, 12, 22, and 24 hours following flavopiridol infusion initiation; 5, 10, 15, 30 minutes and 1, 5, 10, 24, and 48 hours subsequent to flavopiridol infusion completion. Plasma was separated by centrifugation (1,000-1,200 \(\times\) g for 10 minutes) and transferred into plastic tubes that were stored at \(-70^\circ\)C until analysis as described below.

\textbf{Determination of plasma levels of flavopiridol.} Plasma and urine concentrations of flavopiridol were determined by a modification of the reverse-phase high-performance liquid chromatography procedure of Innocenti et al. (36). Separation of flavopiridol and the internal standard flavone was achieved on a Discovery RP Amide C16 column (10 cm \(\times\) 4.6 mm, 5 \(\mu\)m) with a Discovery RP AmideC16 guard column (2 cm \(\times\) 4.0 mm, 5 \(\mu\)m) under gradient elution. The mobile phase consisted of acetonitrile and 50 mmol/L ammonium acetate with 0.1% TEA adjusted to pH 4.15 with glacial acetic acid. The gradient profile was as follows: elution with 25:75 acetonitrile/ammonium acetate (pH 4.15) with 0.15% (v/v) TEA for 5 minutes followed by a 5-minute linear gradient to 35:65 acetonitrile/ammonium acetate (pH 4.15) with 0.15% (v/v) TEA and 10-minute elution with 35:65 acetonitrile/ammonium acetate (pH 4.15) with 0.15% (v/v) TEA. After completing the gradient, the columns were equilibrated with 25:75 acetonitrile/ammonium acetate (pH 4.15) with 0.15% (v/v) TEA for 10 minutes before the next injection. The flow rate and detection wavelength were 1.0 mL/min and 263 nm, respectively. Patient and standard curve plasma samples (100 \(\mu\)L) were added to a microcentrifuge tube on ice followed by the addition of 10 \(\mu\)L flavopiridol (33.8 \(\mu\)mol/L). Plasma proteins were precipitated with 500 \(\mu\)L acetonitrile. After 10 minutes on ice, samples were centrifuged at 14,000 rpm for 2 minutes. The supernatant was dried under nitrogen and reconstituted with 100 \(\mu\)L mobile phase containing 10 \(\mu\)g/mL desipramine (internal standard) and 50 \(\mu\)L injected onto the high-performance liquid chromatography. The assay was linear over the concentration range of 0.02 to 10.0 \(\mu\)g/mL with a lower limit of detection of 0.02 \(\mu\)g/mL.

\textbf{Pharmacokinetic analyses.} Flavopiridol plasma concentration data were analyzed by noncompartmental methods using the program WINNONLIN. The apparent terminal elimination rate constants (\(k_t\)) were determined by linear least-squares regression through the 5-25 hours plasma concentration time points. The apparent elimination half-life (\(t_{1/2}\)) was calculated as 0.693/\(k_t\). Areas under the plasma concentration-time curves (AUC) were determined using the linear trapezoidal rule from time 0 to the time of the last detectable sample (\(C_{last}\). Areas under the plasma concentration-time curves through infinite time (AUC\(_{0\rightarrow\infty}\)) were calculated by adding the value \(C_{last}/k_t\) to AUC\(_{last}\). The CL of flavopiridol was calculated as dose/AUC\(_{0\rightarrow\infty}\).
Statistics. The primary end point for the study was to establish the MTD for the administration of a single i.v. bolus of cisplatin followed immediately by flavopiridol and to establish the MTD for the administration of carboplatin followed immediately by flavopiridol. Secondary goals were the assessment of flavopiridol pharmacokinetics and effects on patient PBMC levels of selected polypeptides known to be altered by flavopiridol in vitro. Data monitoring and analysis for the primary end point was carried out using an integrated system developed specifically for the Mayo Clinic Comprehensive Cancer Center Phase I Clinical Trials program. Routine reporting capabilities and standard analytic procedures have been developed and validated (37). Analyses of patient characteristics, MTD, incidence of adverse events, treatment administration, and tumor responses were descriptive using simple summary statistics and cross-tabulation by dose level. Correlations were assessed using both parametric (Pearson’s) and nonparametric (Spearman’s) procedures, as appropriate. Duration of response and time to tumor progression were calculated using Kaplan-Meier methods from the day that patients first received protocol chemotherapy until progressive disease was documented.

Results

Preclinical rationale. The used combination of flavopiridol and cisplatin was based partially upon the detection of largely sequence-independent synergy when combining the two agents in vitro (18). Whereas synergy between flavopiridol and most other agents (especially paclitaxel) was highly sensitive to sequence of administration, this was not the case when flavopiridol and cisplatin were combined (18).

Preclinical data indicating that maximal flavopiridol-induced cytotoxicity in vitro in solid tumor cells seems to require ≥24-hour flavopiridol exposure prompted the use of a 24-hour continuous infusion schedule for flavopiridol rather than shorter infusion durations (8). Although the mechanism(s) involved in the cytotoxic synergy resulting from the combination of flavopiridol and cisplatin are largely undefined, we observed that (in vitro) whole cell platinum levels increased.

Table 1. Patient characteristics

| Total number of patients enrolled | 39 (18 female, 21 male) |
| Median age at enrollment:         | 59 (range, 32-78)       |
| Baseline ECOG performance status  | 0 (19), 1 (18), 2 (2)   |
| Median number of prior chemotherapy regimens | 2 (37 prior chemotherapy, 13 prior radiotherapy) |

Tumor types
- Colorectal: 18
- Lung: 4
- Gastric: 2
- Skin: 2
- Other: 11

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. Treatment-related toxicity: flavopiridol/CDDP (cohort 1, escalating flavopiridol)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Flavopiridol (50 mg/m²)/CDDP (30 mg/m²), grade</th>
<th>Flavopiridol (75 mg/m²)/CDDP (30 mg/m²), grade</th>
<th>Flavopiridol (100 mg/m²)/CDDP (30 mg/m²), grade</th>
<th>Flavopiridol (135 mg/m²)/CDDP (30 mg/m²), grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>Nausea</td>
<td>1/4</td>
<td>1/4</td>
<td>0/4</td>
<td>2/3</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1/4</td>
<td>2/4</td>
<td>0/4</td>
<td>1/3</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>Tumor pain</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>1/3</td>
</tr>
<tr>
<td>Anemia</td>
<td>0/4</td>
<td>0/4</td>
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<td>1/3</td>
</tr>
<tr>
<td>Hypokalemia</td>
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<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>Dehydration</td>
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<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
<td>Dyspnea</td>
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<td>0/4</td>
<td>0/3</td>
</tr>
<tr>
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<td>0/4</td>
<td>0/4</td>
<td>1/3</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>0/4</td>
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<td>0/4</td>
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<tr>
<td>Hyperglycemia</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
</tr>
</tbody>
</table>

DLTs None None None grade 3 abdominal pain, grade 3 diarrhea/
N and V, grade 3 N and V/dehydration

NOTE: Includes all toxicities seen more than once. No grade 4 or 5 toxicities were seen.
Abbreviations: AST, aspartate aminotransferase; DLT, dose-limiting toxicity.
Table 3. Treatment-related toxicity: flavopiridol/CDDP (cohort 2, escalating CDDP)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Flavopiridol 100 mg/m² CDDP (30 mg/m²), grade</th>
<th>Flavopiridol 100 mg/m² CDDP (45 mg/m²), grade</th>
<th>Flavopiridol 100 mg/m² CDDP (60 mg/m²), grade</th>
<th>Flavopiridol 100 mg/m² CDDP (75 mg/m²), grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
| Nausea            | 2/3| 1/3| 0/3| 0/3| 3/3| 2/6| 2/6| 2/6| 2/5| 0/5| 1/5| 15/17
| Anorexia          | 0/3| 2/3| 0/3| 2/3| 1/3| 0/3| 3/6| 2/6| 1/6| 2/5| 0/5| 1/5| 14/17
| Diarrhea          | 2/3| 0/3| 1/3| 1/3| 0/3| 3/6| 2/6| 1/6| 3/5| 1/5| 0/5| 14/17
| Fatigue           | 2/3| 1/3| 0/3| 2/3| 1/3| 0/3| 2/6| 3/6| 0/6| 1/5| 0/5| 1/5| 13/17
| Vomiting          | 0/3| 2/3| 0/3| 0/3| 2/3| 1/3| 2/6| 3/6| 0/6| 2/5| 0/5| 1/5| 13/17
| Anemia            | 1/3| 0/3| 0/3| 2/3| 0/3| 0/3| 1/6| 2/6| 1/6| 1/5| 2/5| 1/5| 11/17
| Hypokalemia       | 0/3| 0/3| 1/3| 2/3| 0/3| 0/3| 0/6| 0/6| 0/6| 2/5| 0/5| 0/5| 4/17
| Dehydration       | 0/3| 0/3| 0/3| 0/3| 0/3| 1/3| 0/6| 2/6| 0/6| 0/5| 1/5| 1/5| 5/17
| Hyperglycemia     | 1/3| 0/3| 0/3| 0/3| 0/3| 0/3| 1/6| 1/6| 0/6| 0/5| 0/5| 0/5| 4/17
| Leukopenia        | 0/3| 1/3| 0/3| 0/3| 0/3| 0/3| 0/6| 1/6| 1/6| 0/5| 1/5| 0/5| 4/17
| Neutropenia       | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 0/6| 1/6| 1/6| 0/5| 0/5| 1/5| 3/17
| Dizziness         | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 1/6| 0/6| 0/6| 1/5| 1/5| 0/5| 3/17
| Fever (Non-neut.) | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 0/6| 1/6| 0/6| 2/5| 0/5| 0/5| 3/17
| Hyperbilirubinemia| 1/3| 0/3| 0/3| 0/3| 0/3| 0/3| 1/6| 0/6| 0/6| 0/5| 1/5| 0/5| 3/17
| Stomatitis        | 0/3| 0/3| 0/3| 1/3| 0/3| 0/3| 2/6| 0/6| 0/6| 0/5| 0/5| 0/5| 3/17
| Hypotension       | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 0/6| 0/6| 0/6| 1/5| 1/5| 1/5| 3/17
| Hypercalcemia     | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 1/6| 0/6| 0/6| 0/5| 0/5| 0/5| 1/17
| Abdominal Pain    | 0/3| 0/3| 0/3| 0/3| 0/3| 0/3| 0/6| 2/6| 0/6| 0/5| 0/5| 0/5| 2/17

DLTs None None None Grade 3 Anorexia/Fatigue Grade 3 Hypotension

NOTE: The first column of toxicity data is redundant with that shown in Table 2. Bolded data correspond to toxicities resulting from recommended phase 2 dosages. Includes all toxicities seen more than once. No grade 4 or 5 toxicities were seen. Abbreviations: AST, aspartate aminotransferase; DLT, dose-limiting toxicity.

Table 4. Treatment-related toxicity: flavopiridol/CBDCA (cohort 3)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Flavopiridol (100 mg/m²)/ CBDCA (AUC = 2), grade</th>
<th>Flavopiridol (100 mg/m²)/ CBDCA (AUC = 3), grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
| Fatigue           | 4/6| 0/6| 0/6| 0/6| 0/6| 4/6| 2/6| 0/6| 0/6| 0/6| 10/12
| Nausea            | 3/6| 0/6| 0/6| 0/6| 0/6| 4/6| 1/6| 0/6| 0/6| 0/6| 9/12
| Diarrhea          | 3/6| 0/6| 1/6| 0/6| 0/6| 2/6| 0/6| 0/6| 0/6| 8/12
| Anorexia          | 2/6| 0/6| 0/6| 0/6| 0/6| 2/6| 3/6| 0/6| 0/6| 7/12
| Vomiting          | 0/6| 0/6| 0/6| 0/6| 0/6| 3/6| 0/6| 0/6| 0/6| 3/12
| Thrombocytopenia  | 1/6| 0/6| 0/6| 0/6| 0/6| 2/6| 0/6| 0/6| 0/6| 3/12
| Alopecia          | 1/6| 0/6| 0/6| 0/6| 0/6| 1/6| 1/6| 0/6| 0/6| 3/12
| Anemia            | 0/6| 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 0/6| 1/6| 2/12
| Elevated creatinine| 0/6| 0/6| 0/6| 0/6| 0/6| 1/6| 1/6| 0/6| 0/6| 2/12
| Fever (nonneutropenia) | 1/6| 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 0/6| 0/6| 2/12
| Hypoalbuminemia   | 0/6| 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 1/6| 0/6| 2/12
| Hypocalcemia      | 0/6| 0/6| 0/6| 0/6| 0/6| 1/6| 1/6| 0/6| 0/6| 2/12
| Hypontonatria     | 0/6| 0/6| 0/6| 0/6| 0/6| 2/6| 0/6| 0/6| 0/6| 2/12
| Headache          | 1/6| 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 0/6| 0/6| 2/12
| Dehydration       | 0/6| 0/6| 0/6| 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 1/12
| Thromboembolism (PE) | 0/6| 0/6| 0/6| 1/6| 0/6| 0/6| 0/6| 0/6| 0/6| 1/12
| CNS hemorrhage    | 0/6| 0/6| 0/6| 0/6| 1/6| 0/6| 0/6| 0/6| 0/6| 1/12

DLTs Grade 4 thromboembolism (PE)/grade 3 dyspnea [death from CNS bleed after anticoagulated] Grade 4 anemia, grade 3 dehydration

NOTE: Includes all toxicities seen more than once, as well as all grade 5 toxicities. Abbreviations: AST, aspartate aminotransferase; DLT, dose-limiting toxicity; CNS, central nervous system.
when cisplatin is combined with flavopiridol in human OV202hp ovarian carcinoma cells (35). These observations, combined with the knowledge that cisplatin has high clinical activity in a wide variety of cancers, led to the proposal of the present trial. In part, we reasoned that there would be a greater chance of capitalizing on observed synergy if it was less schedule dependent, as is the case for the combination of flavopiridol and cisplatin.

**Patient characteristics.** The trial was conducted with approval from both the sanctioning National Cancer Institute and also the local Mayo Clinic Institutional Review Board. Patient characteristics are summarized in Table 1. The most common tumor type in enrolled patients was colorectal carcinoma, the average performance score was 1 and the average age was 59 years.

**Toxicity.** Toxicities for patients enrolled in all three cohorts of the trial are shown in Tables 2-4. In cohort 1 (Table 2), cisplatin was fixed at 30 mg/m², with stepwise escalating of flavopiridol 50 to 135 mg/m²/24 hours in groups of three patients per dosage level. Dose-limiting toxicities were not encountered until attaining 135 mg/m²/24 hours flavopiridol. At this level, all three patients incurred dose-limiting toxicities requiring hospitalization (intractable severe abdominal pain in one patient and vomiting/diarrhea/dehydration in two other patients). In cohort 2 (Table 3), dose-limiting toxicities were encountered at 100 mg/m²/24 hours flavopiridol and 75 mg/m² cisplatin in the form of anorexia/fatigue (grade 3) and hypotension (grade 3), to attain recommended phase 2 dosages of 100 mg/m²/24 hours flavopiridol and 60 mg/m² cisplatin.

In contrast to the relatively modest toxicities observed when combining flavopiridol and cisplatin, severe toxicities were unexpectedly seen when combining flavopiridol with carboplatin in cohort 3 (Table 4). Dose-limiting toxicities were observed in the first cohort of patients treated with 100 mg/m²/24 hours flavopiridol and 3 AUC carboplatin (grade 4 hemoglobin and grade 3 dehydration). In the six patients subsequently treated with 100 mg/m²/24 hours flavopiridol and 2 AUC carboplatin, one patient incurred treatment-related pulmonary embolism, with death resulting from intracranial bleeding from occult brain metastases upon anticoagulation with heparin. Further evaluation of this combination was deemed unattractive due to the observed unexpectedly high regimen toxicity.

**Effects of treatment on patient outcome.** The duration of therapy for treated patients ranged from 16 to 253 days (Fig. 1A). Although no patients attained objective responses on study, 34% of patients incurred disease stability lasting ≥3 months.

**Pharmacokinetics of flavopiridol in the presence and absence of cisplatin.** Flavopiridol pharmacokinetics was studied in cohorts 1 and 2 during cycle 0, when flavopiridol was give alone, and during cycle 1, when flavopiridol was given after cisplatin. A representative plasma profile for a patient given a 100 mg/m² dose (with and without cisplatin) is illustrated in Fig. 1B. Flavopiridol AUC increased linearly over the dose range 50 to 100 mg/m² but did not increase when a higher dose of 135 mg/m² was given (Fig. 1C). During flavopiridol dose escalation, administration of 30 mg/m² cisplatin before flavopiridol did not seem to alter flavopiridol pharmacokinetics (Fig. 1A and B). In particular, when flavopiridol was given alone, mean (±SD) half-life, plasma clearance, and steady-state volume of distribution values were 23.4 ± 13.5 hours, 20.4 ± 5.5 L/h, and 322 ± 143 L, respectively. When flavopiridol was given after cisplatin, mean (±SD) half-life, plasma clearance and steady-state volume of distribution values were 20.9 ±
10.8 hours, 20.6 ± 6.7 L/h, and 337 ± 220 L, respectively. Steady-state plasma flavopiridol concentrations in the range of 300 ng/mL were achieved 12 to 24 hours after beginning the infusion.

Effects of flavopiridol on levels of selected polypeptides in patient peripheral blood mononuclear cells. Differences in levels of selected polypeptides in patient PBMCs were assessed via immunoblotting. Flavopiridol-induced changes in PBMCs from patients receiving 100 mg/m² flavopiridol alone are shown in Fig. 2. Whereas flavopiridol treatment leads to increased p53 (possibly because of its interactions with DNA; refs. 32, 33) and pSTAT3 (possibly because of compensatory up-regulation of pSTAT3 in response to its ability to disrupt pSTAT3 binding to DNA; ref. 34) levels, decreased cyclin D and Mcl-1 (possibly because of its interactions with DNA; refs. 32, 33) and decreased pRNA polymerase II level (consequent to direct inhibition of its activating kinase P-TEFb; refs 30, 31) in vitro, we observed different effects in patient PCMCN polypeptide levels. As noted in vitro, flavopiridol induced increased p53 and pSTAT3 levels in most patient’s PBMCs (seven of nine and six of nine treated patients respectively; Fig. 2). In particular, p53 and pSTAT3 both increased in P15, P16, P27, and P43; whereas p53 alone increased in P18, P28, and P39, and pSTAT3 alone increased in P33 and P35. In contrast, we were unable to detect any consistent influence of flavopiridol on PBMC levels of Mcl-1, pRNA polymerase II, or cyclin D (Fig. 2). Furthermore, we noted no apparent correlations between flavopiridol-induced polypeptide alterations and pharmacokinetic variables or time on study.

Discussion

Several observations of potential importance arise from the results of this trial. First, flavopiridol and cisplatin can be safely combined in the treatment of patients with advanced solid tumors. We did not observe the high incidence of thrombotic events noted in some other flavopiridol trials, and flavopiridol-induced diarrhea was generally easily controlled except when given at levels over 100 mg/m². Recommended phase 2 dosages are 100 mg/m²/24 hours flavopiridol, following 2-hour 60 mg/m² cisplatin infusion. Second, as given, the combination of flavopiridol and carboplatin led to unexpectedly high toxicities even at the modest carboplatin AUCs of 3 or 2, with one treatment-related death. This observation leads to limited enthusiasm for the combination of flavopiridol and carboplatin, despite the fact that carboplatin is generally viewed as less toxic than cisplatin. Third, flavopiridol seemed to affect levels of selected polypeptides (p53 and pSTAT3) in patient PBMsNs, whereas not affecting others (e.g., Mcl-1, Bcl-2, pRNA polymerase II; Fig. 2). This leads to some uncertainty as to whether flavopiridol is affecting intended in vitro molecular targets in vivo. It may be, however, that consequences of flavopiridol/DNA binding (e.g., increased p53 and pSTAT3) in the absence of inhibition of P-TEFb by flavopiridol (e.g., decreased pRNA polymerase II) account for these observations in PBMCs. Fourth, the present study showed no clear indication that the combination of flavopiridol and cisplatin or carboplatin can produce clinical responses in heavily pretreated patients with advanced solid tumors, albeit that over one third of all study patients attained disease stabilization for >3 months.

At present, the explanation for the encountered high toxicity of flavopiridol/carboplatin in comparison with the lower toxicity of flavopiridol/cisplatin is uncertain. We preliminarily examined the possibility that flavopiridol might displace carboplatin from human serum proteins more facilely than cisplatin but could not show this to be the case in vitro displacement assays. In any event, we feel that the extreme toxicity encountered with the combination of flavopiridol and cisplatin is worrisome and should preclude further clinical studies of this combination.

Several polypeptides known to be altered by flavopiridol in vitro (pSTAT3 and p53) were similarly altered by flavopiridol in vivo in patient PBMCs (Fig. 2). To our knowledge, this represents the first report indicating that flavopiridol-induced alterations of cellular polypeptides in vivo might be recapitulated in vitro. On the other hand, although the antiapoptotic polypeptide Mcl-1 is consistently dramatically down-regulated by flavopiridol in vitro (thereby potentially playing a role in flavopiridol-induced cytotoxicity),

we did not observe this effect in vivo (Fig. 2). This absence of effect of flavopiridol on Mcl-1 levels may thereby provide a potential explanation for the lack of clinical responses observed in this trial. The use of patient PBMCs for similar analyses has previously been used with considerable success in conjunction with many prior clinical trials (38–40). However, because polypeptide levels were assessed in (non-cycling) PBMCs, and not cancer cells, it is uncertain whether the same effects would have been recapitulated in tumor material.

At the present time, a phase 2 clinical trial combining flavopiridol and cisplatin is planned in patients with ovarian cancer/primary peritoneal carcinoma. It is hoped that this future trial involving less heavily pretreated patients will show antitumor efficacy for this regimen.

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References

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