A Phase I Trial of Carboxyamido-triazole and Paclitaxel for Relapsed Solid Tumors: Potential Efficacy of the Combination and Demonstration of Pharmacokinetic Interaction

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ABSTRACT

Purpose: Preclinical and clinical investigation of the combination of the antiangiogenesis/anti-invasion agent carboxyamido-triazole (CAI) administered with the cytotoxic agent paclitaxel (PAX).

Experimental Design: Colony-forming assays were used to test the activity of CAI plus PAX on A2780 human ovarian cancer. The sequence of CAI followed by PAX (CAI>Pax) was modeled in nude mice to test for potential additive toxicity. The Phase I clinical dose escalation schema tested p.o. administered CAI in PEG-400 (50–100 mg/m2) or micronized CAI (250 mg/m2) for 8 days followed by a 3-h infusion of PAX (110–250 mg/m2) every 21 days. Patients were assessed for toxicity, pharmacokinetics of CAI and PAX, and disease outcome.

Results: In preclinical studies, CAI>Pax was additive in A2780 human ovarian cancer cell lines when CAI (1 or 5 μM) preceded subtherapeutic doses of PAX. CAI did not reverse PAX resistance and collateral resistance to CAI was documented in PAX-resistant cells. CAI>PAX administration had no overt additive toxicity in nude mice. Thirty-nine patients were treated on a dose-escalation Phase I trial using daily oral CAI for 8 days followed by the PAX infusion. Pharmacokinetic analysis revealed that PAX caused an acute increase in circulating CAI concentrations in a dosedependent fashion. No additive or cumulative toxicity was observed, and grade 3 nonhematological toxicity was rare. Three partial responses and two minor responses were observed.

Conclusions: The sequential combination of CAI and PAX is well tolerated, and the activity observed suggests that further study of the combination is warranted.

INTRODUCTION

Progress in understanding the signaling pathways and mechanisms of angiogenesis and tumor invasion has led to the discovery of multiple new putative therapeutic targets against which new agents have been directed. We have previously identified transmembrane calcium influx as an important signaling pathway that is activated during the physiological invasion associated with neovascularization and the malignant invasion of tumor metastasis (1–10). CAI3 was shown to be an inhibitor of transmembrane calcium influx in nonexcitable cells, such as endothelium and most epithelial tumor types (3, 11). CAI inhibits several components of invasion, including adhesion and spreading (6, 7), proteolysis (4), and migration (1, 5). Inhibition of angiogenesis and metastasis occurs at concentrations that are achievable in in vivo models (2, 3, 5, 12) and in patients receiving p.o. administered CAI (13–16). One logical new direction for the use of antiangiogenic and anti invasive agents is to combine them with cytotoxic chemotherapy or radiation therapy, or with other antiangiogenic or anti-invasive compounds (17–20). We thus hypothesized that CAI would be additive or supra-additive with selected chemotherapeutic agents.

PAX is a cytotoxic agent that promotes and stabilizes microtubule polymerization, locking the microtubules into the polymerized state and freezing the cell cytoskeleton (21–23). Intracellular calcium has been suggested as an important cofactor in the regulation of microtubular function, thus leading to the possibility that CAI may have a regulatory effect in this pathway. PAX has been reported to have antiangiogenic activity in xenografts at concentrations that translate to treatment doses at or below those that are administered therapeutically in patients (24, 25). It has been proposed but not yet demonstrated that the lower-dose weekly PAX schedule acts in an antiangiogenesis mode. PAX treatment has been demonstrated to activate apoptotic pathways at concentrations at or below those that promote

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3 The abbreviations used are: CAI, carboxyamido-triazole; PAX, paclitaxel; NCI, National Cancer Institute; ECOG, Eastern Cooperative Oncology Group; Cpmax, maximum plasma concentration; CYP 3A4, cytochrome P-450 3A4; PGP, P-glycoprotein; PEG, polyethylene glycol; AGC, absolute granulocyte count.

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microtubule polymerization (26). Our interest in the treatment of epithelial ovarian cancer led us to investigate combination therapies that incorporate the effective agent, PAX, with CAI, an agent that had demonstrated disease stabilization with minimal toxicity in Phase I clinical trials (14, 15).

We now report that the sequenced administration of CAI followed by PAX is active both in culture and in clinical trial. CAI was administered for 8 days to achieve a steady-state plasma concentration of $\geq 1 \mu M$ (0.4 $\mu g/ml$), the minimal effective concentration in vitro. The objectives of the clinical trial were to determine the maximally tolerated doses of CAI and PAX when given in combination, to evaluate the pharmacokinetics of CAI and PAX administered in combination, to identify the toxicities of the regimen, and, secondarily, to identify disease sites in which this two-drug regimen might be active.

**MATERIALS AND METHODS**

**Materials and Pharmaceuticals.** Cell culture reagents were from Life Technologies, Inc. (Gaithersburg, MD). PAX for laboratory use was purchased from ICN Pharmaceuticals Inc. (Costa Mesa, CA). CAI and carboplatin for laboratory and clinical use was obtained from the Developmental Therapeutics Program, NCI or the Pharmaceutical Management Branch, Cancer Therapy Evaluation Program, NCI, respectively. CAI for laboratory use was received in powder form and solubilized in DMSO for tissue culture use and in PEG-400 for animal use. PAX and filgrastim for the clinical trial were supplied by Pharmaceutical Management Branch, Cancer Therapy Evaluation Program, NCI. PAX for patient administration was provided as a concentrated nonaqueous solution, 6 mg/ml in cremophor EL vehicle and was diluted in 0.9% sodium chloride immediately prior to use as reported (27).

**Colony-forming Assays.** Colony-forming assays tested the growth inhibitory response of A2780 cells and their cisplatin- or PAX-resistant sublines to exposure to carboplatin, PAX, CAI, or drug combinations, as described. Cells were plated at 300–500 cells/well in 12-well tissue culture plates and allowed to attach overnight in a mixture including penicillin, streptomycin, and glutamine and containing RPMI medium with 10% FCS and added 0.2% v/v U-100 human insulin. Medium was then removed and cells were exposed to CAI for 24 h, PAX for 24 h, or CAI for 24 h followed after a washout by PAX for 24 h. CAI was used at 1 or 5 $\mu M$ and PAX was used at subtherapeutic concentrations based on dose analysis. After the indicated treatment, wells were washed twice with PBS, stained with crystal violet (0.5% w/v in 10% methanol), rinsed free of excess dye, and allowed to air-dry overnight. All of the colonies were counted, and $IC_{50}$ was calculated by nonlinear regression analysis from at least triplicate experiments using Prism software analysis (GraphPad Software, Inc, San Diego, CA).

**Mouse Safety Study.** A NCI Animal Care and Use Committee protocol was approved for the analysis of the toxicity of the sequence of CAI administration followed by PAX administration. CAI was used at the previously tested concentration of 100 mg/kg/day in PEG-400 vehicle in a gavage-administered volume that did not exceed 100 $\mu l$ daily. CAI was given for 8 consecutive days. On the 8th day, 2 h after the final CAI administration, PAX was administered i.p. in a 10% final concentration 1:1 cremophor EL:ethanol vehicle at two doses: the LD$_{10}$ (10 mg/kg) and LD$_{05}$ (~6 mg/kg). Dose recommendations were provided by the Developmental Therapeutics Program, NCI. Animals were observed for 21 days and killed humanely. At necropsy, major organs were observed for toxicity and weighed. Ten animals per treatment group were tested, and all survived until completion of the study. A previous report demonstrated no adverse effect of CAI on hematopoietic and myelopoietic lineages at therapeutically attainable doses (28).

**Patients.** Patients were eligible for participation in the clinical trial if they fulfilled the following criteria: histologically proven solid tumor or non-Hodgkin’s lymphoma; ECOG performance status of 0, 1, or 2; failed therapy of proven efficacy or have untreated metastatic melanoma, renal cell cancer, or non-small cell lung cancer (this trial was initiated prior to standard use of PAX and platinum in newly diagnosed non-small cell lung cancer patients); adequate end organ function as defined by white blood count $\geq 3000/mm^3$, hematocrit $\geq 30\%$ without transfusion support, platelet count $\geq 100,000/cm^3$, measured creatinine clearance $\geq 45 \text{ ml/min}$, liver function tests within twice the upper limit of normal with a normal bilirubin; normal PT and PTT; no concurrent infections or use of imidazole antifungal agents such as ketoconazole; no evidence of myocardial damage or ischemia before the preceding 6 months and no current cardiac conduction defects requiring antiarrhythmic therapy; no brain metastases (computed tomography or magnetic resonance imaging were required for patients with metastatic melanoma or lung cancer prior to entry); evaluable or measurable disease by physical examination or noninvasive testing or prostate-specific antigen-only disease in refractory hormone-independent prostate cancer patients; and life expectancy of at least 3 months. Patients were to be at least 4 weeks from prior chemotherapy, hormonal therapy, radiation therapy, or biological therapy with the exception of patients who had received mitomycin C, nitrosoureas, or carboplatin, for whom the restriction was 6 weeks. Patients who had progressed during or within 6 months of prior PAX administration were excluded as were patients who had received CAI previously. All of the patients of child-bearing capability were required to use contraception during therapy and for 2 months after completion of therapy, and pregnant or lactating patients were excluded. Because the safety of CAI as a single agent or the combination of CAI and PAX in combination with antiretroviral drugs or active HIV infection is unknown and the potential for undue risk was deemed excessive, all of the patients were tested for HIV prior to trial entry, and HIV-positive patients were excluded and referred elsewhere. All of the patients gave signed informed consent before participation. This study and all of the amendments were approved by the NCI Institutional Review Board and the Cancer Therapy Evaluation Program, NCI.

**Treatment Plan.** Liquid formulation CAI in PEG-400 vehicle was administered p.o. by syringe, and micronized formulation was provided as 50-mg capsules. On completion of the Phase I trial of micronized CAI and its selection as the preferred formulation, this protocol was amended to use micronized CAI; its dose was rounded down to the nearest 50 mg. Prior studies indicated a reduced bioavailability of the micronized formulation; the doses tested were based on the prior Phase I results (15). Patients took nothing p.o. for at least 3 h prior to, and 1 h...
Table 1  Drug administration schema

<table>
<thead>
<tr>
<th>Drug day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PAX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filgrastim (when indicated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

after, CAI administration. PAX was administered in the outpatient setting as a 3-h i.v. infusion beginning 2 h after the eighth daily CAI dose. Central venous access was required for doses that were \( \geq 175 \) mg/m\(^2\). Premedication consisted of hydrocortisone 100 mg p.o. at 12 h, 6 h, and immediately prior to PAX administration, with diphenhydramine (50 mg) and cimetidine (300 mg) p.o. or i.v. 30 min prior to PAX administration. Patients were eligible for self-administered s.c. filgrastim only after having had an episode of fever with neutropenia or needing a prolonged time to recover granulocytes, which necessitated a delay in the administration of a subsequent cycle of chemotherapy. The dose of filgrastim was held at 5 \( \mu \)g/kg/day. When used, filgrastim was initiated 24 h after the PAX infusion and continued until the WBC was \( \geq 30,000/\)mm\(^3\) or the absolute granulocyte count was \( \geq 1500/\)mm\(^3\) on two filgrastim determinations at least 24 h apart. Subsequent cycles of treatment were not administered until the filgrastim had been discontinued for at least 3 days. Dosing levels, treatment schema, and formulations used are shown in Tables 1 and 2.

Patients were seen in clinic every cycle for history and physical examination and assessment of toxicity. Disease reassessment was done every two cycles and consisted of a history and physical examination, the measurement of pertinent markers, and a repeat of all of the noninvasive studies on which disease was assessed. Patients were eligible to continue treatment as long as they had evidence of stable or improved disease and did not have dose-limiting toxicity. Dose-limiting toxicity was defined as grade III nonhematological toxicity and/or hematological toxicity consisting of prolonged neutropenia or thrombocytopenia while receiving filgrastim (50,000/\)mm\(^3\) or the development of transfusion-dependent anemia. Dose-limiting toxicity was defined by the occurrence of one or more of these toxicities in two or more patients in a 3–6-patient cohort treated at the index dose level. The maximally tolerated dose was defined as the dose level immediately below the level at which dose-limiting toxicity was documented. A total of at least six patients were to be treated at the maximally tolerated dose.

Sampling, Analysis, and Pharmacokinetics. Blood samples for determination of CAI and PAX concentrations were collected on study day 8 prior to CAI administration, prior to PAX administration, and at 1, 2, 3, 3.5, 4, 5, 6, 7, 24, and 26 h after the start of the PAX infusion. CAI and PAX concentrations were determined simultaneously using a modification of a previously reported reversed-phase high performance liquid chromatographic (HPLC) assay with UV detection (29). Briefly, calibration curves of 0.04–10.0 \( \mu \)g/ml (0.09–23.55 \( \mu \)M CAI and 0.05–11.71 \( \mu \)M PAX) of each analyte in plasma were prepared. Each plasma calibration standard contained both analytes. The internal standard harmine was added to each sample to a final concentration of 4.0 \( \mu \)g/ml. Samples were extracted using Varian C\(_{18}\) solid-phase extraction cartridges and eluted with 0.1% triethylamine in acetonitrile. Chromatographic separation was performed using a Waters Nova-Pak C-18 (3.9 \times 300 mm) column with a mobile phase of acetonitrile and 0.01 m aqueous ammonium acetate pumped over a gradient at a flow rate of 1.0 ml/min. Detection was accomplished with a diode array detector with wavelengths set at 264, 242, and 323 nm for CAI, PAX, and harmine, respectively. The \( C_{\text{peak}} \) observed for CAI and PAX was reported from direct observation of the data.

Definition of Response. Patients were designated complete responders (CR) if they attained complete resolution of all of the disease lasting at least 4 weeks; partial responders (PR) had 50% or greater reduction in the sum of the products of the bidirectional measurements of all of the index lesions with no new lesions identified, lasting at least 4 weeks. Minor response (MR) was at least a 25% reduction in the sum of the products of the bidirectional measurements of all of the index lesions, with no new lesions identified, lasting at least 4 weeks. Patients with stable disease (SD) had tumor measurements that were within 25% of on-study measurements and lasted at least 4 weeks without development of new lesions. Patients with new lesions or greater than a 25% increase in summated tumor products were designated as having progressive disease (PD).

RESULTS

Preclinical Studies of CAI and PAX Combinations. CAI was used at concentrations of 1 and 5 \( \mu \)M, concentrations attainable in patients receiving daily dosing for at least 1 week (14, 30, 31). Antagonism was observed when A2780 and the cisplatin-resistant A2780/Cp70 ovarian cancer cell lines were exposed to CAI and carboplatin sequentially in colony-forming assays in vitro (CAI>carboplatin and carboplatin>CAI; data not shown). These studies were followed by analysis of PAX/CAI combinations. Cells were exposed to drug in the sequence of CAI for 24 h followed by 24-h PAX exposure (CAI>PAX) and PAX prior to CAI (PAX>CAI). PAX concentrations used were as low as 0.003 \( \mu \)M, the IC\(_{10}\) PAX concentration for the A2780 and the A2780/Cp70 cells. Supra-additive colony-forming toxicity of both CAI>PAX (\( p_2 = 0.02 \) v. PAX; \( p_2 = 0.03 \) v. CAI) and PAX>CAI (\( p_2 = 0.005 \) v. PAX; \( p_2 = 0.03 \) versus CAI) was seen in the A2780/Cp70 cells at concentrations below that at which PAX alone was cytotoxic (Fig. 1). The effect was greater for the combination of CAI>PAX than for PAX followed by CAI, underlying its choice for further preclinical and clinical study. A statistically significant difference was found between single agent CAI and single agent PAX activity in the parental A2780 cells (\( p_2 = 0.05 \)) that was not seen in the A2780/Cp70 cells. Furthermore, no additive toxicity of either combination was seen in these cells, which may be attributable to the more profound effect of PAX on these cells.

A collateral resistance to CAI was demonstrated in the PAX-resistant A2780/TAX10 subline (32), for which an almost 300% increase in the IC\(_{10}\) concentration of CAI was measured. CAI treatment did not overcome resistance to PAX
in the A2780/TAX10 PAX-resistant subline. The PAX IC_{50} concentration of 49 nM was increased to 145 nM with 1 μM CAI preexposure and to 102 nM with 5 μM CAI preexposure, which suggests antagonism of the combination in PAX-resistant cells. The CAI>PAX drug-exposure schedule was modeled in nude mice using a daily-for-8-day administration of CAI at 150 mg/kg/day alone or followed on day 8 by a LD_{50} or LD_{10} dose of PAX as described. All of the animals received equivalent PEG-400 and cremophor EL/ethanol exposures. On sacrifice, 21 days after the PAX administration, no overt single-agent or combination-drug organ toxicity was identified (Fig. 2).

**Patients.** Thirty-nine patients with refractory or relapsed solid tumors were accrued for study in a dose-escalation fashion. Table 3 shows the general cohort demographics. Most of the patients were heavily pretreated with a median of 2 (range, 0–8) prior treatment regimens. All of the patients were of good performance status (median, ECOG grade 1) and maintained their performance status during treatment, with the exception of one patient who developed peripheral neuropathy (discussed below). Patients received a median of four cycles of treatment (mean, 4.8; range, 1–18; Table 2). Ten patients fulfilled the requirements for filgrastim administration with six of eight patients treated at dose level 7A, 250 mg/m² CAI and 250 mg/m² PAX, requiring filgrastim. Initiation of filgrastim occurred most commonly at cycle 2 because of an incomplete recovery of granulocyte count by day 15 after PAX administration (day 1 of the subsequent cycle), which necessitated a 1-week delay in the initiation of the next cycle.

**Pharmacokinetics.** Complete pharmacokinetic profiles were obtained from 24 of 25 patients. One patient at dose level 7 did not have blood drawn for pharmacokinetic analysis after the PAX infusion. Mean Cp_{max} for CAI and PAX at each administered dose level are presented in Table 4. No increase in the day-8 post-PAX CAI Cp_{max} was observed with the administration of CAI 50, 75, or 100 mg/m²/day for 8 days followed by the 3-h infusion of PAX (110 mg/m²; r², 0.18; P = 0.72). PAX Cp_{max} increased with increasing PAX dosage for patients receiving a CAI dose of 100 mg/m²/day (P < 0.01; Fig. 3). However, the CAI Cp_{max} in those patients receiving CAI 100 mg/m²/day also increased in a statistically significant and PAX dose-dependent manner over the range of PAX doses administered, as is shown in Fig. 4 (P = 0.03). This trend was not seen in patients receiving a fixed dose of PAX (110 mg/m²) at increasing CAI doses (50, 75, and 100 mg/m²/day; r², 0.41; P = 0.56).

**Toxicity.** Toxicity was assessed using the NCI Common Toxicity Criteria, version 1.0. All of the patients were evaluable for toxicity, which is tabulated by grade as shown in Table 5. No trends were evident in the occurrence of toxicities with two exceptions: neutropenia, for which progression of toxicity was seen with increasing micronized CAI dose; and constipation, which occurred solely in patients receiving PAX 200 mg/m² or greater. No late onset of toxicity was observed. One grade 3 neurotoxicity was observed in a heavily pretreated, advanced diabetic patient with renal cell cancer who had grade 1 neuropathy at study entry. Electromyelogram and nerve conduction studies demonstrated bilateral median nerve injury, but no evidence for classical chemotherapy-associated peripheral neuropathy. This patient had persistent median nerve injury and elected to remove himself from the study. All of the cases of grade 3 hyperglycemia occurred in patients with preexisting diabetes mellitus and may have been exacerbated by the corticosteroid premedication. Neither CAI nor PAX has been shown to cause significant hyperglycemia as single agents (14, 15, 33).

Ten patients had delay of cycle and subsequent initiation of filgrastim administration because of slowly resolving neutropenia; all but one patient received micronized formulation CAI. The one patient in the liquid CAI cohort (level 3) for whom filgrastim was indicated, began filgrastim on cycle 3. All of the other patients requiring filgrastim began on cycle 2 and had received micronized CAI 250 mg/m² with PAX at either 200 or 250 mg/m². The assessment of granulocyte recovery for the initiation of the subsequent treatment cycle was made on the 14th day post-PAX. The 1-week delay of treatment that necessitated filgrastim administration constituted a time of 21 days between PAX dose and cycle reinitiation. All of the delayed patients had recovery by that 21-day post-PAX mark. There was one occurrence of fever during neutropenia. This patient received treatment for complex and extensive abdominal sarcoma on dose level 7 (100 mg/m² CAI in liquid formulation and 250 mg/m² PAX). The febrile episode was caused by polymicrobial intra-abdominal sepsis attributable to tumor-associated bowel obstruction and responded to appropriate supportive measures and antibiotics. Treatment-associated anemia was grade 1 or 2, with only one patient receiving transfusion of packed cells on a single occasion. There was no platelet toxicity. Thus, dose-limiting toxicity was not observed within the tested dose levels. No further dose escalation was planned because of the well-documented risk of dose-limiting peripheral neuropathy.

**Table 2** Drug administration dose levels

<table>
<thead>
<tr>
<th>Dose level</th>
<th>CAI (formulation)</th>
<th>PAX</th>
<th>No. of patients</th>
<th>Median no. of cycles (range)</th>
<th>No. of patients requiring filgrastim</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 mg/m² (liquid)</td>
<td>110 mg/m²</td>
<td>3</td>
<td>14 (6–18)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>75 mg/m² (liquid)</td>
<td>110 mg/m²</td>
<td>3</td>
<td>6 (2–10)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100 mg/m² (liquid)</td>
<td>110 mg/m²</td>
<td>3</td>
<td>2 (2–6)</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>100 mg/m² (liquid)</td>
<td>135 mg/m²</td>
<td>3</td>
<td>8 (3–8)</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>100 mg/m² (liquid)</td>
<td>170 mg/m²</td>
<td>3</td>
<td>4 (3–16)</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>100 mg/m² (liquid)</td>
<td>200 mg/m²</td>
<td>4</td>
<td>2.5 (2–6)</td>
<td>0</td>
</tr>
<tr>
<td>6A</td>
<td>250 mg/m² (micronized)</td>
<td>200 mg/m²</td>
<td>6</td>
<td>4.5 (2–6)</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>100 mg/m² (liquid)</td>
<td>250 mg/m²</td>
<td>6</td>
<td>2 (1–6)</td>
<td>0</td>
</tr>
<tr>
<td>7A</td>
<td>100 mg/m² (micronized)</td>
<td>250 mg/m²</td>
<td>8</td>
<td>3 (2–8)</td>
<td>6</td>
</tr>
</tbody>
</table>
at 300 mg/m² PAX (34) and at higher doses of CAI, for which cerebellar ataxia and confusion are also dose-limiting but rare toxicities (14, 15).

Response to Therapy. Patients were evaluable for response if they completed the scheduled reassessment at the completion of two cycles of treatment, unless progression of disease was documented earlier. Three patients were not evaluable for response because of death attributable to disease prior to restaging (one patient, cycle 2, day 3), intercurrent infection caused by bowel obstruction (1), and recurrent infection from a central line (1). Three partial responses and two minor responses were documented (Table 6), with no clear dose-response effect. Partial responses were seen in one patient each with fallopian tube cancer (6+ months) and epithelial ovarian cancer (5 months), and metastatic melanoma (15 months). Both patients with gynecological malignancy had prior PAX and platinum therapy and relapsed more than 6 months after the completion of primary carboplatin-PAX therapy. One patient had a single liver metastasis on computed tomography scan, and the other had numerous pulmonary metastases. CA-125 normalized from 110 to 28 in the first patient and then rose to 43 at the time of documented progressive disease; CA-125 was uninformative in the second patient. The first patient received 8 cycles of treatment, after which she removed herself from the study because of stable grade 2 neuropathy. The patient with melanoma had pathologically confirmed skin and dermal lymphatic involvement of her breast and extensive neck adenopathy. She had a rapid resolution of the inflammatory breast disease and a gradual measurable reduction in the nodal disease over a total of 16 cycles of treatment. Progression was documented in the nodal disease with no new lesions identified. Disease stabilization was seen for as long as 1 year in a patient with metastatic melanoma and in one with non-small cell lung cancer.

DISCUSSION

Analysis of the combination of antiangiogenesis agents with cytotoxic chemotherapeutic agents is now coming into the clinic. We described previously the preclinical and clinical anti-invasive and antiangiogenic activity of CAI (2–5, 12, 14, 15) and now address the application of CAI to combination therapy with cytotoxic agents. Our preclinical results demonstrated an antagonism of CAI when administered with carboplatin but an additive activity of CAI when administered in sequence prior to PAX (CAI>PAX). CAI>PAX was not additive in end-organ toxicity when evaluated in nude mice and was, thus, evaluated in the clinic in a dose-escalation Phase I design. Eligibility was limited to patients without prior CAI exposure and to those with PAX-sensitive tumors, because of collateral resistance to CAI. The in vitro CAI>PAX sequence was translated to patient dosing using 8 daily CAI doses followed by a 3-h PAX infusion, repeated every 3 weeks. Thirty-nine patients have been accrued to 9 dose levels testing both the liquid and micronized CAI formulations concurrent with PAX doses ranging from 110 to 250 mg/m². No additive toxicity was observed, and the regimen was in general well-tolerated. Grade 3 nonhematological toxicity was rare even at the administered PAX dose of 250 mg/m², and no cumulative toxicity was observed. Pharmacokinetic analysis revealed that PAX administration caused an acute increase in circulating CAI concentrations in a dose-dependent fashion. Three partial responses and 2 minor responses were observed. These results suggest that CAI>PAX is well tolerated and that it may be effective in several different types of cancer.
Clinical trials using other antiangiogenic agents in combination with chemotherapeutics are now being developed or used increasingly with patients (18–20, 35). Selection of the cytotoxic agents for these combinations has generally been empiric. We chose a combination of CAI with carboplatin initially because we had seen disease stabilization in ovarian cancer patients receiving single-agent CAI (14, 15). However, an antagonism was observed with carboplatin and CAI. The mechanism of the antagonism of CAI with carboplatin is unclear. One hypothesis is that CAI could increase intracellular chloride concentration and thus reduce the cytotoxic activity of the carboplatin (36). PAX was chosen for its general activity in solid tumors. The CAI, PAX combination had supra-additive efficacy for the ovarian cancer cell lines tested and also in monolayer growth assays of MDA-435 human breast cancer cell lines (data not shown). This combination also is additive in culture against small cell lung cancer cell lines and more than additive in xenografts, with an increase in survival time. Identification of the mechanism of the additivity of CAI and PAX has been elusive to date. No differential proapoptotic activity of the CAI, PAX combination was seen in treated cells and no difference was seen in the quantity or electrophoretic mobility of β-tubulin. Combination studies of CAI with other antiangiogenic or anti-invasive agents and other chemotherapeutic agents are under way.

All of the doses of CAI and PAX tested in this schedule were well tolerated. Low starting doses of CAI and PAX were used in the initial treatment levels. The micronized CAI was initiated at the higher dose because of its lower relative bioavailability (15). CAI in liquid formulation was dosed at one-half the defined maximally tolerated dose (13, 14) and given with PAX 110 mg/m², a dose well below the currently recom-

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Table 3 Patient characteristics (n = 39)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>53 (31–74)</td>
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<tr>
<td>Men/Women</td>
<td>23/16</td>
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<td>Performance status on study</td>
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<tr>
<td>Stage on study</td>
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<td>Prior treatments</td>
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<td>No. of patients</td>
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<td>Malignancies accrued:</td>
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<tr>
<td>Melanoma</td>
<td>7</td>
</tr>
<tr>
<td>Renal</td>
<td>7</td>
</tr>
<tr>
<td>Soft tissue sarcomas</td>
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</tr>
<tr>
<td>Ovary/fallopian tube</td>
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</tr>
<tr>
<td>Colorectal</td>
<td>4</td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td>4</td>
</tr>
<tr>
<td>Cervix</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>1</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>1</td>
</tr>
<tr>
<td>Urothelial</td>
<td>1</td>
</tr>
</tbody>
</table>

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*Chemotherapy, hormonal therapy, radiation therapy inclusive.

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3 F. Bostick-Bruton, E. C. Kohn, and E. Reed, unpublished data.
4 T. W. Moody and E. Kohn, unpublished data.
mended standards in dose level 1 (23). The starting doses were low because of concern that both CAI and PAX can cause axonal peripheral sensory neuropathy (14, 37–39). The CAI Cpmax in patients receiving micronized CAI at 250 mg/m² was 11.2 ± 6.2 μM (n = 6) or 13.0 ± 7.3 μM (n = 6) when administered with PAX at 200 or 250 mg/m², respectively. These CAI concentrations were reasonably comparable with those in patients receiving liquid-formulation CAI at the dose of 100 mg/m² (Table 4). The relative bioavailability was not contradictory to our previous report (15). PAX also has a well-described noncumulative marrow toxicity presenting primarily as granulocytopenia with a lesser risk of thrombocytopenia and anemia (37, 40). CAI had no marrow toxicity in clinical studies nor in preclinical studies using myeloid and erythroid precursor cells (14, 15, 28). No additive toxicity to granulocyte/macrophage colony-forming unit precursor growth potential was detected with \( ex \) vivo CAI–PAX treatment. The lack of additive organ or detectable neurological toxicity in the mouse pilot study suggested that there was a window of safety for this combination of agents that was borne out in the dose levels tested. No dose-limiting toxicity was observed within the administered dose levels. Escalation was discontinued at dose levels 7 and 7A because both agents were documented to have dose-limiting peripheral neuropathy as single agents at higher administered doses.

PAX has been found to have efficacy against multiple tumor types including breast, ovary, and lung cancers, for which it is considered by most a first-line agent, and to have limited activity against other cancers including renal cell carcinoma and melanoma (40–42). It is generally felt to be inactive in the gastrointestinal malignancies and poorly active in sarcomas. Prolonged disease stabilization was observed in the first dosing cohort using suboptimal doses of PAX and CAI in one patient with melanoma and one with lung cancer. Of the five partial and minor responses documented, two were in patients with melanoma [2 (29%) of 7 melanoma patients] and one in a patient with renal cell cancer (1 of 7), and the final two were in patients

![Fig. 3](image1.png)

**Fig. 3** Cpmax increase with PAX dose. PAX Cpmax were measured on day 8 after a 3-h infusion of PAX (110, 135, 170, 200, or 250 mg/m²) initiated 2 h after the administration of the eighth daily dose of CAI 100 mg/m². **Data points**, mean; **bars**, SD.

![Fig. 4](image2.png)

**Fig. 4** CAI plasma concentration is increased by PAX administration. CAI plasma concentrations were measured at the completion of the day-8 PAX infusion. All of the patients received eight daily doses of CAI 100 mg/m². PAX doses were 110, 135, 170, 200, or 250 mg/m² infused over 3 h. **Data points**, mean; **bars**, SD.

### Table 4 Maximum observed plasma CAI and paclitaxel concentrations

<table>
<thead>
<tr>
<th>Dose level</th>
<th>CAI dose(^a) (mg/m²/day)</th>
<th>PAX dose(^b) (mg/m²)</th>
<th>No. of patients</th>
<th>CAI Cpmax(^c) (μM)</th>
<th>PAX Cpmax(^c) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>110</td>
<td>3</td>
<td>5.92 ± 1.81</td>
<td>1.77 ± 0.23</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>110</td>
<td>3</td>
<td>9.17 ± 5.47</td>
<td>1.95 ± 0.82</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>110</td>
<td>3</td>
<td>7.29 ± 3.17</td>
<td>1.44 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>135</td>
<td>3</td>
<td>11.6 ± 1.25</td>
<td>2.50 ± 0.72</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>170</td>
<td>3</td>
<td>14.4 ± 0.40</td>
<td>3.59 ± 0.86</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>200</td>
<td>4</td>
<td>13.9 ± 1.54</td>
<td>4.59 ± 0.92</td>
</tr>
<tr>
<td>6A</td>
<td>250(^d)</td>
<td>200</td>
<td>6</td>
<td>11.2 ± 6.2</td>
<td>N.A.(^e)</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>250</td>
<td>5</td>
<td>16.4 ± 4.19</td>
<td>8.41 ± 2.65</td>
</tr>
<tr>
<td>7A</td>
<td>250(^d)</td>
<td>250</td>
<td>6</td>
<td>13.0 ± 7.3</td>
<td>N.A.(^e)</td>
</tr>
</tbody>
</table>

\(^a\) CAI solution in PEG-400 administered p.o. once daily for 8 days.

\(^b\) PAX injection administered as i.v. infusion over 3 h on day 8.

\(^c\) Arithmetic mean ± SD of the Cpmax.

\(^d\) Micronized CAI administered p.o. once daily for 8 days.

\(^e\) N.A., not done.
with fallopian tube and ovarian cancer [2 (40%) of 5]. Single-agent activity for patients with PAX-naive melanoma or ovarian cancer is 14–20% and 24–36%, respectively (43–46). PAX doses for the responding patients were 110, 135, 170, and 250 mg/m² with CAI administered at the maximal tested dose, which suggested that the maximal administered or tolerable dose may not be the optimal dose for a combination such as this. These results suggest that there may be a benefit of using the combination that warrants further investigation.

Both CAI and PAX are highly protein-bound and heptatically cleared (47, 48), which suggests that there is a possibility for drug interaction. The observed PAX \( C_{\text{pmax}} \) in this study are similar to those reported previously, after a 3-h PAX infusion in which PAX doses of 135–250 mg/m² produced \( C_{\text{pmax}} \) values from 1.9–3.5 to 9.2 ± 2.0 \( \mu \text{M} \), respectively (49, 50). Circulating CAI concentrations that exceeded 5 \( \mu \text{M} \) did not affect the PAX \( C_{\text{pmax}} \) (\( P = 0.56 \)). Maximum CAI concentrations have previously been shown to increase in a less-than-dose-proportional manner. Although the elimination of CAI has been shown to be linear, \( C_{\text{pmax}} \) values did not increase in a linear fashion (15, 30): this has been postulated to be attributable to saturable absorption (31). A significant effect on the CAI \( C_{\text{pmax}} \) was observed as a function of administered PAX dose. One possible explanation for this interaction might be the inhibition of CYP 3A4 by PAX. CYP 3A4 has been identified as one of the metabolizing enzymes for both CAI (47) and PAX (51). PAX has been identified as a substrate for CYP 3A4; thus, it might also have a dual role as an inhibitor of this enzyme. Although CAI has not yet been shown to be a substrate of PGP, PAX has been long known to be a substrate of this efflux pump (23, 40, 52). Recent studies have suggested that the intestinal PGP may play a role in drug absorption (53). Thus, an alternative hypothesis is that the effect of PAX on CAI may be attributable to altered absorption because the PAX-PGP interaction may promote CAI absorption.

Development and testing of combinations of antiangiogenic and anti-invasive compounds with chemotherapy or radiation therapy is an important new direction. The results of this study suggest that both the biochemical mechanisms of action and the end point activity of angiogenesis inhibition or cytotoxicity should be incorporated into trial design. In addition, non-disease-specific accrual will provide the opportunity to observe otherwise unanticipated activity. The present study used an intermittent schedule of CAI, known from preclinical and xenograft studies to be less active than a continuous daily CAI treatment. An intermittent CAI schedule might theoretically be preferable to continuous CAI exposure if there were a pharmacokinetic interaction on PAX availability or altered PAX resistance as was seen in vitro using the PAX-resistant cell lines. However, no pharmacokinetic interaction was observed, and the collateral resistance demonstrated in vitro that mandated patients with PAX-resistant tumors be ineligible. We are currently investigating whether the schedule of daily administered CAI with three weekly PAX infusions is as well tolerated and provides the same or greater potential for benefit as was seen in the schedule currently reported. There have been no untoward toxicities in this new schedule to date, and escalation continues. Other schedules that capitalize on the augmentation of the circulating CAI concentration by PAX may yet be preferable to those under study at this time and could be considered in the design of subsequent trials. Thus, the sequence of 8 days of p.o. administered CAI 250 mg/m² in the micronized formulation followed by PAX 250 mg/m² on a three-weekly schedule is well tolerated and has activity. Additional studies of this regimen or the daily CAI/pulse PAX approach are warranted.

**ACKNOWLEDGMENTS**

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A Phase I Trial of Carboxyamido-triazole and Paclitaxel for Relapsed Solid Tumors: Potential Efficacy of the Combination and Demonstration of Pharmacokinetic Interaction

Elise C. Kohn, Eddie Reed, Gisele A. Sarosy, et al.


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