A Phase 1 Study of Pralatrexate in Combination with Paclitaxel or Docetaxel in Patients with Advanced Solid Tumors

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Abstract

Purpose: Pralatrexate is a rationally designed antifolate with greater preclinical antitumor activity than methotrexate. Pralatrexate was synergistic with paclitaxel and with docetaxel in mouse xenograft experiments. This phase 1 study was designed to determine the maximum tolerated dose and toxicity of pralatrexate plus paclitaxel or docetaxel in patients with advanced cancer.

Experimental Design: Pralatrexate was administered i.v. every 2 weeks (days 1 and 15) in a 4-week cycle. Depending on the taxane used and dose being tested, the taxane was administered on days 1 and 15; days 2 and 16; or days 1, 8, and 15. In the latter part of the study, patients in the docetaxel arm were treated with vitamin B12 and folic acid supplementation to mitigate toxicity and allow pralatrexate dose escalation.

Results: For the combination of pralatrexate plus paclitaxel without vitamin supplementation, dose-limiting stomatitis and peripheral neuropathy were encountered at the lowest dose levels tested. For pralatrexate plus docetaxel plus vitamin supplementation, pralatrexate 120 mg/m2 plus docetaxel 35 mg/m2 administered on the same day every other week was defined as the maximum tolerated dose and schedule, with dose-limiting toxicities at higher dose combinations including stomatitis and asthenia. Significant antitumor activity was observed for this combination in patients with non–small-cell lung cancer.

Conclusions: Pralatrexate (120 mg/m2) plus docetaxel (35 mg/m2) plus vitamin supplementation is well tolerated with signs of efficacy against non–small-cell lung cancer that merit phase 2 testing.

Compared with methotrexate, the 10-deazaaminopterin class of antifolate drugs show superior intracellular transport via the one-carbon, reduced folate carrier (RFC-1) and increased accumulation within cells by enhanced polyglutamylation (1–3). As a result, 10-deazaaminopterins have greater cytotoxicity than methotrexate against a variety of human cancers in xenograft experiments.

The most potent 10-deazaaminopterin is pralatrexate (10-propargyl-10-deazaaminopterin; Fig. 1; refs. 4, 5). Pralatrexate is the most efficient substrate for RFC-1 and the most effective substrate for polyglutamylation by the enzyme folylpolyglutamate synthetase compared with aminopterin, methotrexate, and edatrexate (2). In laboratory studies, pralatrexate was superior to edatrexate with 3- to 4-fold greater growth inhibition against a series of human non–small-cell lung cancer (NSCLC) cell lines (LX-1 and A549) in mouse xenograft experiments (4).

A phase 1 trial of pralatrexate in patients with advanced cancer found stomatitis to be dose limiting, with a maximum tolerated dose (MTD) of 30 mg/m2 when delivered weekly and 170 mg/m2 when delivered every 2 weeks (6). Other observed toxicities included transaminase elevation, rash, pulmonary infiltrates, conjunctivitis, and epistaxis. No significant hematologic toxicities were noted. Pharmacokinetic analysis from the phase 1 trial revealed a mean area under the curve of 20.6 μmol h and a mean terminal half-life of 8 h at the 150 mg/m2 dose.

A phase 2 clinical trial of single-agent pralatrexate (135-150 mg/m2 every 2 weeks) in a total of 39 patients with previously treated advanced NSCLC showed a radiologic response rate of 10% (95% confidence interval, 3-25%) and median survival time of 13.5 months (7). Two (5%) patients experienced grade 4 stomatitis and 6 (15%) had grade 3. No clinically significant myelosuppression was observed.

The 10-deazaaminopterins show schedule-dependent synergy when combined with taxanes in mouse xenograft experiments. Pralatrexate has shown antitumor synergy with both paclitaxel and docetaxel in nude mice transplanted with the LX-1 tumor.8 Pralatrexate showed synergy with each taxane

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8 F.M. Sirotnak, unpublished data.
whether the agents were administered sequentially (pralatrexate first) or simultaneously.

This phase 1 clinical trial was initiated to determine the MTD and toxicity of pralatrexate when combined with paclitaxel or docetaxel. During the course of this trial, there was evolving evidence that vitamin B₁₂ and folic acid supplementation was a means to mitigate toxicity from antifolates. In clinical trials with the antifolate pemetrexed (Alimta, Eli Lilly, Indianapolis, IN), both alone and in combination with cisplatin, vitamin supplementation decreased toxicity without decreasing efficacy (8–10). A phase 3 trial treated patients with advanced mesothelioma with pemetrexed plus cisplatin, with and without vitamin B₁₂ and folic acid supplementation, and showed a statistically significant decrease in neutropenia with vitamins (8). There was a consistent trend to decreased rates of nonhematologic toxicity, including nausea, vomiting, diarrhea, and stomatitis, but these differences did not reach statistical significance (8). Inspired by this data, patients in the latter half of this phase 1 trial received vitamin B₁₂ and folic acid supplementation in an attempt to mitigate toxicity and allow pralatrexate dose escalation.

Materials and Methods

This was a phase 1, open-label, single-institution study (Memorial Sloan-Kettering Cancer Center, New York, NY). The primary objective of this study was to determine the MTD and toxicity of pralatrexate when combined with either paclitaxel or docetaxel. Eligible patients had pathologic evidence of an incurable solid tumor for which paclitaxel or docetaxel was an acceptable treatment; of ages ≥18 years; with Karnofsky performance status ≥70%; with adequate hematologic, liver, and kidney functions; and, if prior treatment for the cancer, completed prior chemotherapy or radiation therapy 3 or more weeks before entry into this study. The study excluded women who were pregnant, lactating, or of childbearing potential not using effective contraception, and any patient who had received prior treatment with antifolates. Patients who had received prior paclitaxel were excluded from the paclitaxel arm. Patients who had received prior docetaxel were excluded from the docetaxel arm. Patients with clinically significant pleural effusions or ascites, grade 3 or 4 edema, or prior pneumonectomy were excluded based on toxicity observed in such patients treated with 10-deazaaminopterin. Patients with baseline peripheral neuropathy (National Cancer Institute-Common Toxicity Criteria grade ≥1) were excluded. Patients with symptomatic or uncontrolled brain or leptomeningeal involvement, or other serious illness or medical condition, were excluded. All patients completed an institutionally approved process of informed consent.

Pretreatment evaluation included a complete medical history, physical examination, and laboratory studies including complete blood count, comprehensive panel, and serum pregnancy test for women of childbearing potential. All patients received a pretreatment chest radiograph and computed tomography scan of all relevant disease sites. The dosing schedule for this phase 1 trial was chosen based on the recommended doses of single-agent pralatrexate, paclitaxel, and docetaxel, as well as experience combining edatrexate with paclitaxel in prior clinical trials and pralatrexate with taxanes in mouse xenograft experiments (11, 12). Pralatrexate was delivered every 2 weeks as an i.v. push (days 1 and 15 of a 4-week cycle). The taxane was delivered either the day after or on the same day as pralatrexate, depending on the dose of pralatrexate or the taxane.

For this trial, pralatrexate was formulated at Memorial Sloan-Kettering Cancer Center (5). Bulk quantities of pralatrexate were manufactured by Ash-Stevens Pharmaceuticals (Detroit, MI) under a subcontract from the National Cancer Institute. Pralatrexate drug substance was supplied as a free acid in dry powder form. Weighed quantities of the pralatrexate drug substance were suspended in bacteriostatic sterile normal saline USP and brought into solution by adjusting the pH to 7 with 1 N NaOH. Each batch of pralatrexate was sterilized by means of filtration through a 0.20-μm Acrodisc filter, protected from light, and stored at 4°C for use within 28 days. Pralatrexate was administered as an i.v. push through the side arm of a freely running i.v. line containing normal saline. Paclitaxel (Taxol, Bristol-Myers Squibb, New York, NY) and docetaxel (Taxotere, Sanofi-Aventis, Bridgewater, NJ) were supplied commercially and administered according to label instructions.

Table 1. Dose and schedules explored

<table>
<thead>
<tr>
<th>Pralatrexate, mg/m²</th>
<th>Paclitaxel (P)/docetaxel (D), mg/m²</th>
<th>n</th>
<th>DLT (days 1-28)</th>
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</thead>
<tbody>
<tr>
<td>Without vitamin supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110, days 1, 15</td>
<td>P175, days 1, 15</td>
<td>3</td>
<td>Stomatitis</td>
</tr>
<tr>
<td>90, days 1, 15</td>
<td>P175, days 2, 16</td>
<td>3</td>
<td>Stomatitis, peripheral neuropathy</td>
</tr>
<tr>
<td>70, days 1, 15</td>
<td>D50, days 2, 16</td>
<td>3</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>60, days 1, 15</td>
<td>D35, days 2, 16</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>With vitamin B₁₂ and folic acid supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80, days 1, 15</td>
<td>D35, days 2, 16</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>100, days 1, 15</td>
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<td>120, days 1, 15</td>
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<td>D35, days 1, 15</td>
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</tr>
<tr>
<td>120, days 1, 15</td>
<td>D35, days 1, 15</td>
<td>11</td>
<td>Stomatitis, asthenia</td>
</tr>
<tr>
<td>140, days 1, 15</td>
<td>D35, days 1, 8, 15</td>
<td>5</td>
<td>Stomatitis</td>
</tr>
</tbody>
</table>
The dose cohorts defined by the doses and schedules for pralatrexate and the taxanes are outlined in Table 1. Patients in the paclitaxel arm received dexamethasone 20 mg orally the night before and the morning of paclitaxel treatment, and diphenhydramine 50 mg i.v. and ranitidine 50 mg i.v. before paclitaxel infusion. Patients in the docetaxel arm received dexamethasone 8 mg orally every 12 h for three doses beginning on the evening before docetaxel administration. All patients on the pralatrexate plus paclitaxel arm were treated without folic acid and vitamin B12 supplementation. After the first eight patients had been enrolled in the pralatrexate plus docetaxel arm, all additional patients were treated with vitamin supplementation (vitamin B12 1 mg i.m. every 8-10 weeks plus folic acid 1 mg oral daily dose for 7-14 days thereafter). A complete blood count was obtained before pralatrexate.

During treatment, medical history, physical examination, Karnofsky performance status, vital signs, weight, and toxicity/symptom evaluation (graded according to the National Cancer Institute-Common Toxicity Criteria version 2.0) were recorded weekly for the first cycle and then on treatment days thereafter. A complete blood count was obtained before each treatment. A comprehensive panel, including alkaline phosphatase, aspartate aminotransferase, total bilirubin, and serum creatinine, was obtained at the beginning of each cycle. A computed tomography scan or other imaging modality was done after serum creatinine, was obtained at the beginning of each cycle. A comprehensive panel, including alkaline phosphatase, aspartate aminotransferase, total bilirubin, and serum creatinine, was obtained at the beginning of each cycle.

Blood samples were drawn before therapy for RBC folate, methylmalonic acid, and homocysteine concentration measurements. For patients who received folic acid and vitamin B12 supplementation on study, these concentrations were checked again before the first dose of pralatrexate (i.e., after 7-14 days of initial vitamin supplements). All patients had serial homocysteine and methylmalonic acid concentrations measured before each cycle of chemotherapy. Follow-up RBC folate concentrations were not measured because they are not reliable after treatment with an antifolate.

Dose-limiting toxicity (DLT) was defined as any grade 3 non-hematologic toxicity using the National Cancer Institute criteria (excluding nausea and vomiting); any grade 4 hematologic toxicity or neutropenic fever; grade 3 hematologic toxicity requiring treatment delay beyond 2 weeks; or stomatitis requiring a dose reduction of pralatrexate or taxane. The MTD was defined as the dose at which two patients develop a DLT. Three patients were enrolled at each dose cohort. If no DLT was observed, three patients were enrolled at the next higher dose cohort. If one DLT was observed, the dose cohort was expanded to six patients. If two DLTs were observed, the MTD was reached. One cycle of therapy (two doses of pralatrexate over at least 4 weeks) was completed for all patients at any dose cohort without a DLT before patients were enrolled at the next cohort.

Up to nine additional patients were enrolled at one level below the MTD to collect additional toxicity data for the recommended phase 2 dose. If unacceptable toxicity was encountered, up to nine additional patients were enrolled at the next lower dose of pralatrexate or taxane. The MTD was defined as the dose at which two patients develop a DLT. Three patients were enrolled at each dose cohort. If no DLT was observed, three patients were enrolled at the next higher dose cohort. If one DLT was observed, the dose cohort was expanded to six patients. If two DLTs were observed, the MTD was reached. One cycle of therapy (two doses of pralatrexate over at least 4 weeks) was completed for all patients at any dose cohort without a DLT before patients were enrolled at the next cohort.

Pharmacokinetic studies were done on the final cohort of patients treated at the recommended phase 2 dose (n = 6). In this cohort, pralatrexate was administered by i.v. bolus infusion over 3 to 5 min, followed by docetaxel administered by i.v. infusion over 60 min. All blood was drawn into 6-mL heparinized tubes, frozen, and shipped to the Analytical Development Corporation Laboratory (Colorado Springs, CO) for pralatrexate and docetaxel quantitation. The sensitive, selective method for the determination of pralatrexate in human plasma involves extraction of pralatrexate from plasma using C18 solid-phase extraction cartridges followed by derivatization of the extract with...
acetyl chloride. The derivatized extracts are reconstituted in organic solvent and injected on a high-performance liquid chromatography column for quantitation by liquid chromatography-tandem mass spectrometry. Pralatrexate is quantitated by the internal standard method using quantitation standards (methotrexate) extracted from plasma and injected with each sample set. The limit of quantitation of pralatrexate is 0.5 ng/mL in human plasma samples. Docetaxel is measured in human plasma by protein precipitation with organic solvent, evaporation of the supernatant, reconstitution in mobile phase, injection on a reverse-phase high-performance liquid chromatography column for quantitation by liquid chromatography-tandem mass spectrometry followed by quantitation using external standards extracted from plasma, and injection with each sample set. The limit of quantitation of docetaxel is 5 ng/mL in human plasma samples.

Blood was drawn on cycle 1, day 1 at pre-pralatrexate, and 5, 10, 20, 30, 60 min and 2, 3, 4.5, 6, 24, 48 h following pralatrexate administration. On cycle 1, day 15, blood was drawn at pre-pralatrexate, and 5, 10, 20, 30, 60 min and 2, 3, 4.5, 6 h following pralatrexate.

Although radiologically measurable or evaluable disease was not required for entry into this study, serial computed tomography scans were obtained. The size of each measurable indicator lesion was recorded in centimeters as the product of the longest possible diameter and its longest associated perpendicular. Complete response was defined as the disappearance of all measurable and/or evaluable disease. Partial response was defined as a ≥25% decrease in the sum of the product of the diameters of the indicator lesions or the appearance of a new lesion. Progression of disease was defined as a ≥25% increase in the sum of the product of the diameters of the indicator lesions or the appearance of a new lesion. Stable disease was defined as any regression of the product of the diameters of the indicator lesions for a minimum of 4 weeks, with no radiological progression of disease.

Results

Patient characteristics. Patients were enrolled between December 1999 and April 2005. Forty-eight patients were treated. The median age was 61 years (range, 37-78 years). Forty-three patients had NSCLC, two patients had small-cell lung cancer, one patient had gastric carcinoma, one patient had thymic carcinoma, and one patient had carcinoma of unknown primary. There were 27 males and 21 females. Three (6%) patients had Karnofsky performance status of 70% and the rest had Karnofsky performance status of 80% to 90%. Eleven (23%) patients had a history of treated brain metastases and 7 (15%) had bone metastases. Fifteen (31%) patients had received no prior chemotherapy, 24 (50%) had one prior regimen, and 9 (19%) had two or more prior regimens.

MTD. The dose cohorts are summarized in Table 1. Due to toxicity, the dose of pralatrexate in combination with paclitaxel was decreased on two occasions. The first three patients treated at the first dose cohort developed dose-limiting stomatitis (grade 3), prompting the dose of pralatrexate to be lowered by 20% and the dosing schedule changed to give the paclitaxel on day 2 instead of day 1. Despite this adjustment, one additional patient developed dose-limiting stomatitis (grade 2) and a second patient developed dose-limiting peripheral neuropathy (grade 3). No additional patients were treated with pralatrexate plus paclitaxel.

Two of the six patients treated with pralatrexate plus paclitaxel experienced clinically significant neuropathy. Due to concern for peripheral neuropathy related to paclitaxel, attention was shifted to the combination of pralatrexate plus docetaxel, without B12 and folic acid supplementation. Two of the first three patients treated at the first cohort of this combination experienced dose-limiting diarrhea (grade 3), prompting dose reductions of both agents. By reducing the doses of pralatrexate and docetaxel, five patients were treated without DLT. In an attempt to mitigate toxicity and allow dose escalation, folic acid and vitamin B12 supplements were added.

With the addition of vitamin B12, and folic acid supplements and using a lower starting dose of docetaxel, dose escalation of the pralatrexate proceeded, followed by escalation of the docetaxel in turn. Alternating dose escalations showed that both drugs could be delivered safely on the same day. Further dose escalation suggested that patients could tolerate docetaxel at a dose of 35 mg/m² on days 1, 8, and 15, following which the dose of pralatrexate was increased to 140 mg/m² q2w plus docetaxel 35 mg/m² on days 1, 8, and 15 on a 28-day cycle. At this dose and schedule of pralatrexate and docetaxel, two of five patients did not tolerate the day 8 docetaxel during cycle 1 due to grade 1 stomatitis.

Additional patients were enrolled at the next lower dose cohort (pralatrexate 120 mg/m² q2w plus docetaxel 35 mg/m² on days 1, 8, and 15 in a 28-day cycle). Whereas the first three patients treated at this dose cohort did not experience a DLT, four of the next five patients treated at this dose and schedule experienced DLTs, including three patients with stomatitis (grades 2, 3, and 4) and one patient with grade 3 fatigue. Therefore, the protocol was amended to allow additional patients to be treated at the next lower dose, which omitted the day 8 docetaxel dose (pralatrexate 120 mg/m² q2w plus docetaxel 35 mg/m² on days 1 and 15 in a 28-day cycle). At this dose and schedule, six additional patients were treated without a DLT, making this the recommended phase 2 dose.

Toxicity. A summary of selected grade 2, 3, and 4 toxicities for each drug combination across all dose levels tested is presented in Table 2. Whereas neuropathy was a DLT in patients receiving pralatrexate with paclitaxel at 175 mg/m² every 2 weeks, it was not observed in patients receiving pralatrexate with docetaxel at 50 mg/m² every 2 weeks, or 35 mg/m² weekly. There was a lower rate of diarrhea in patients receiving pralatrexate and docetaxel plus vitamins, although these patients received a lower dose of docetaxel. Other side effects of note included grade 1 epistaxis (22 of 48, 46%), grade 1 to 2 tearing of the eyes (5 of 48, 10%), and grade 3 hypophosphatemia (5 of 48, 10%). These side effects occurred with equal frequency in paclitaxel- and docetaxel-treated patients.

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Methylmalonic acid and homocysteine status and toxicity. Vitamin B12 and folic acid supplementation for 7 to 14 days before chemotherapy consistently and significantly lowered baseline homocysteine concentrations from presupplementation median of 10.5 μmol/L to postsupplementation median of 7.2 μmol/L (P < 0.0001, paired t test). In addition, there were numerical trends toward lowering methylmalonic acid and increasing RBC folate concentrations with vitamins before chemotherapy (data not shown).

Patients were grouped according to the maximum grade of stomatitis experienced, and comparisons made of their methylmalonic acid, RBC folate, and homocysteine concentrations immediately before chemotherapy (regardless of dose level).
Data are presented in Table 3. A higher pretreatment homocysteine correlated with a higher grade of stomatitis by Kendall’s rank correlation \((n = 45, \tau = 0.203, P = 0.05)\). Of note, a previously reported phase 2 trial of single-agent pralatrexate in patients who did not receive vitamin supplementation found no significant correlation between baseline homocysteine and grade of stomatitis in a smaller sample size \((n = 24; \text{ref. 7})\).

**Pharmacokinetics.** The results of plasma pralatrexate and docetaxel concentrations from six patients who were treated at the recommended phase 2 dose are presented in Fig. 2. Plasma concentration data for both drugs were similar on day 1 and day 15, indicating consistency between dose days and no apparent difference in disposition of either pralatrexate or docetaxel between the first and second doses.

**Efficacy.** There were 40 patients with metastatic NSCLC treated with the combination of pralatrexate plus docetaxel on this study (with or without vitamin supplementation). Of these 40 patients, 14 were previously untreated, 26 were previously treated, 23 had received prior platinum (cisplatin or carboplatin), and 7 received two or more prior chemotherapy regimens. Six patients achieved radiologic partial response (15%; 95% confidence interval, 7-29%), 20 patients achieved stable disease (50%; 95% confidence interval, 35-65%), and 14 patients had progression of disease. For previously treated patients, the rate of partial response was 4 of 26 (15%). For previously untreated patients, the rate of partial response was 2 of 14 (14%). Three of the responses were seen at the three lowest dose levels.

The median progression-free survival for all 40 evaluable patients with metastatic NSCLC treated with the combination of pralatrexate plus docetaxel on this study (with or without vitamin supplementation) was 4 months (range, 1-56+ months). The median number of doses of pralatrexate administered with docetaxel was 6 (range, 1-38). Twelve (29%) patients received 16 or more doses of pralatrexate with docetaxel. Eleven of 40 patients (28%) had progression-free survival of >1 year (12, 12, 13, 13, 14, 15, 18, 18, 19, 30, and 56+ months). One patient remains disease-free at 56 months having undergone complete surgical resection of his stage IIIB NSCLC by pneumonectomy after a sustained (6-month) response to pralatrexate plus docetaxel. A Kaplan-Meier survival curve was generated for all 40 patients treated with pralatrexate plus docetaxel on this study (with or without vitamin supplementation), and the median overall survival was 18 months (95% confidence interval, 11-26 months; Fig. 3).

For the six patients treated with pralatrexate plus paclitaxel, one patient with NSCLC and one patient with thymic carcinoma, both with progressive cancer following initial chemotherapy, had clinically significant radiologic responses that did not meet criteria for partial response. One patient with stage IV NSCLC with progressive cancer following mitomycin plus vinblastine remained on pralatrexate plus paclitaxel for 9 months and showed a 12-month time to cancer progression.

**Discussion**

Due to the lack of myelosuppression and neuropathy seen in phase 1 and 2 trials of single-agent pralatrexate in patients with advanced solid tumors, and evidence of synergy when combining pralatrexate with taxanes in mouse xenograft

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<th>Homocysteine, μmol/L (45)</th>
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<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
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<tr>
<td>Median (n)</td>
<td>7.3 (18)</td>
<td>7.9 (7)</td>
<td>8.7 (15)</td>
<td>9.9 (5)</td>
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<th>RBC folate, ng/mL (35)</th>
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<th>Grade 2</th>
<th>Grade 3</th>
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<tr>
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<td>735 (17)</td>
<td>692 (5)</td>
<td>512 (10)</td>
<td>667 (3)</td>
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<td>141 (10)</td>
<td>129 (3)</td>
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<td>241 (3)</td>
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Fig. 2. Plasma concentration-time curves for docetaxel (A) and pralatrexate (B). Pralatrexate was administered by i.v. push over 3 to 5 min, followed by docetaxel administered by i.v. infusion over 60 min. Cycle 1, day 1: pre-pralatrexate, 5, 10, 20, 30, 60 min and 2, 3, 4, 5, 6, 24, 48 h post-pralatrexate push. Cycle 1, day 15: pre-pralatrexate, 5, 10, 20, 30, 60 min and 2, 3, 4, 5, 6 h post-pralatrexate.
experiments, there is interest in developing combinations of pralatrexate with taxanes for the treatment of patients with advanced solid tumors to exploit nonoverlapping toxicity and potential for increased clinical efficacy. This study showed that pralatrexate and docetaxel can be delivered safely to patients with previously treated advanced solid tumors and showed signs of clinical efficacy that merit testing in phase 2.

The initial dose cohorts for this phase 1 trial were chosen based on the recommended doses of single-agent pralatrexate, paclitaxel, and docetaxel. The pralatrexate plus paclitaxel arm of this study was abandoned due to dose-limiting stomatitis and neuropathy at the first two dose levels tested. The combination of pralatrexate plus docetaxel also proved to be intolerable at the first dose level tested due to diarrhea. Therefore, this clinical trial showed that a combination of pralatrexate and taxanes without vitamin supplementation requires dose reduction of both drugs to slightly below their individual MTDs. As each cohort experienced intolerable toxicity, the study was placed on prolonged hold, then amended and reopened with alternative dose levels, and subsequently the addition of vitamin supplementation. According to the trial design, additional patients were enrolled at the MTD of pralatrexate and docetaxel, with and without vitamin supplementation. The multiple, prolonged delays and amendments and additional patient accruals resulted in an unusually long total time of accrual (5 years) for a phase 1 study.

In this study, the addition of folic acid and vitamin B₁₂ supplementation allowed higher doses of pralatrexate to be delivered safely in combination with docetaxel. Given that vitamin supplementation was added coincident with dose escalation, it is not possible to compare, or comment on, the frequency of toxicities with and without vitamin supplementation.

Supplementation with folic acid and vitamin B₁₂ significantly lowered serum homocysteine concentrations. Although the use of variable doses of pralatrexate in this phase 1 trial limits the analysis, baseline homocysteine correlated with the grade of stomatitis experienced. This suggests that vitamin supplementation decreases risk of stomatitis from pralatrexate. Whereas vitamin supplementation may lower the risk of stomatitis from pralatrexate and improve drug delivery, its effect on efficacy is unknown. Vitamin supplementation clearly lowers the frequency of neutropenia in patients treated with pemetrexed and actually improves efficacy, possibly by improving drug delivery (8).

There was no clinically significant hematologic toxicity encountered in phase 2 testing of single-agent pralatrexate in patients with NSCLC (7). Similarly, neutropenia was not a DLT of the combination of pralatrexate plus taxanes in this study. In contrast, hematologic toxicity, especially neutropenia, is the most significant side effect for other antifolate plus taxane combinations (13–17).

Given the large number of patients (n = 40) with NSCLC treated with pralatrexate plus docetaxel on this phase 1 clinical trial, with or without vitamins, the efficacy data are worth noting. A substantial proportion of patients were treated who had received no prior chemotherapy (31%) than might normally be seen in a phase 1 trial. Chemotherapy-naïve patients with metastatic NSCLC are typically more fit, more likely to respond to chemotherapy, and have a longer survival than previously treated patients. Nevertheless, in this study, radiologic responses were observed in both chemotherapy-naïve and previously treated patients, and there were the same proportions of responders in the two groups (15%). Nearly one third of NSCLC patients treated with this combination showed progression-free survival of a year or more. The administration of eight or more cycles of this combination to nearly one third of patients treated is a testament of its tolerability and lack of cumulative side effects.

In conclusion, the combination of pralatrexate and docetaxel is safe and merits phase 2 testing in patients with NSCLC. Treatment should be preceded by folic acid and vitamin B₁₂ supplementation for at least 7 days, followed by pralatrexate 120 mg/m² plus docetaxel 35 mg/m² delivered on the same day every 2 weeks. A phase 1 trial of single-agent pralatrexate plus vitamins is currently under way to determine whether the addition of vitamins will allow a higher phase 2 starting dose of pralatrexate and, presumably, greater antitumor effects.

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