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ORIGINAL ARTICLE

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A phase I clinical pharmacologic study of pralatrexate in combination with probenecid in adults with advanced solid tumors

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Abstract *Purpose*: The antifolate pralatrexate (10-propargyl-10-deazaaminopterin, PDX) demonstrates greater in vitro and in vivo antitumor efficacy than methotrexate. Preclinical models indicated that the efficacy of pralatrexate may be enhanced by coadministration with probenecid. The aim of this phase I study was to determine the maximum-tolerated dose of pralatrexate when combined with probenecid given every 2 weeks in humans. *Methods*: The starting dose was pralatrexate 40 mg/m^2 intravenously and probenecid 70 mg/m^2 intravenously administered every 14 days, where one cycle of treatment was every 28 days. The pralatrexate dose was initially fixed while probenecid dose escalation was explored. The pralatrexate area under the curve (AUC), terminal-half life (t1/2), and maximum plasma concentration (Cmax) were determined in cycle 1. *Results*: Seventeen patients with advanced solid tumors were treated with a median of two prior chemotherapy regimens. Stomatitis was dose-limiting with pralatrexate 40 mg/m^2 and probenecid 233 mg/m^2. Mean pralatrexate AUC and half life (t1/2) increased with increasing doses of probenecid. No objective responses were seen. *Conclusion*: For patients with advanced solid tumors, the maximum-tolerated dose of this drug combination was pralatrexate 40 mg/m^2 and probenecid 140 mg/m^2. Vitamin B_{12} and folate supplementation may allow for further dose escalation of pralatrexate and probenecid. This is a suitable question for a future study.

Keywords Phase I · Pralatrexate · Probenecid · Solid tumors

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Introduction

The 10-deazaaminopterins comprise a class of folate analogues designed to possess greater anti-tumor effects than methotrexate. Pralatrexate (10-propargyl-10-deazaaminopterin, PDX) is structurally similar to methotrexate except for a propargyl group substitution at carbon 10 [1]. Efficacy studies using human tumor xenografts (MX-1 mammary carcinoma, LX-1 lung carcinoma, A549 squamous cell lung cancer) show that pralatrexate is significantly active against these tumors, but methotrexate is inactive against all three tumors in this model [2]. The improved activity of 10-deazaaminopterins may be due to the more effective internalization by the one carbon-reduced folate transporter and the subsequent accumulation in tumor cells through the formation of polyglutamated metabolites [3–7].

A phase I pralatrexate study enrolled 33 patients with advanced non-small cell lung cancer (NSCLC). Patients had received a median of two prior chemotherapy regimens. Vitamin supplementation was not administered in this protocol. Initially, PDX was administered weekly for 3 weeks in a 4-week cycle. Mucositis requiring dose reduction and/or delay in the first cycle occurred in four of six patients treated at the initial dose level (30 mg/m^2), making this the maximal tolerated dose for PDX given on this schedule. The treatment schedule was then modified to every 2 weeks. Twenty-seven patients were treated twice weekly. Mucositis was the dose-limiting toxicity (DLT), with grade 3 and 4 mucositis occurring in the first two patients treated at the 170 mg/m^2 dose level. Other toxicities were mild and reversible. No neutropenia was observed. The recommended phase II

dose from this study was determined to be 150 mg/m^2 every 14 days. Mucositis was the DLT, and the recommended phase II dose was 150 mg/m^2 every 14 days. Two patients with stage IV NSCLC had major objective responses. Stable disease was observed in five patients for 7–13 months [8].

In a phase II study of pralatrexate in 39 patients with NSCLC refractory to one prior chemotherapy regimen, the overall objective response rate was 10% (95% CI 3–25%). Initially, pralatrexate was administered at a dose of 150 mg/m^2 every 2 weeks at the recommended phase 2 dose as determined in the phase 1 study. However, 38% of patients required dose reductions at the 150 mg/m^2 dose level such that to decrease the frequency of stomatitis, the last 10 patients were treated at a dose of 135 mg/m^2 with improved tolerability [9].

In an effort to improve efficacy, pharmacologic modulation of antifolates with probenecid has been investigated. Probenecid increases the concentration of methotrexate in murine L1210 leukemia cells by inhibiting drug efflux [10]. Membrane ATPases of the multidrug resistance-associated protein (MRP) family mediate efflux of folate analogues from L1210 leukemia cells [11–14]. Over-expression of MRP-1, MRP-2, MRP-3, and MRP-4 in cancer cell lines (human embryonic kidney 293, human ovarian carcinoma 2008, and NIH 3T3 cells) has been associated with enhanced efflux and resistance to methotrexate and other folate analogues [15–17]. These observations support a model in which probenecid inhibits the activity of plasma membrane ATPases, resulting in net intracellular retention and greater cytotoxicity of folate analogues. Additionally, it has long been appreciated that the uricosuric drug probenecid decreases renal clearance of methotrexate by interfering with secretion in the proximal tubule [18, 19]. This yields increased serum concentrations of methotrexate.

In a xenograft model of JMN human mesothelioma, pralatrexate plus probenecid resulted in 5 (56%) complete tumor regressions in 9 animals, compared to 2 (17%) complete tumor regressions in 12 animals treated with pralatrexate alone [20]. Similarly, in xenograft models of other human solid neoplasms growing in mice (LX-1, prostate cancer, MX-1), pralatrexate plus probenecid consistently yielded superior efficacy compared to pralatrexate alone [21]. These preclinical results provide a rationale for the hypothesis that probenecid may improve the activity of pralatrexate in human solid tumors.

In this report, the results of a phase I trial of pralatrexate treatment in combination with intravenous administration of probenecid in patients with advanced solid tumors are presented. The primary objective was to establish the maximum tolerated doses (MTD) for intravenous probenecid and pralatrexate given in combination every 2 weeks. For all patients, pharmacokinetic (PK) studies were performed to determine the area under the curve (AUC), terminal half-life (t1/2), and maximal concentration (Cmax) of pralatrexate in the presence of probenecid.

Patients and methods

Patient eligibility

Patients ≥18 years of age with Karnofsky performance status of ≥70% and histologically documented advanced solid malignancies refractory to conventional therapy were eligible. Prior to enrollment, no chemotherapy or radiation therapy for 3 weeks was permitted. Patients were required to have WBC ≥4,000 cells/mm^3, platelet count ≥160,000 cells/mm^3, serum creatinine ≤1.2 mg/dl or creatinine clearance ≥60 ml/min, total bilirubin ≤1.0 mg/dl, AST and ALT < 2X upper limit of normal. Pretreatment labs included plasma folate, homocysteine, and RBC folate. Patients were not permitted to take folic acid or nephrotoxic agents which may alter the renal excretion of pralatrexate. Patients were excluded for: unstable angina, congestive heart failure, cardiac arrhythmia, significant pleural effusions or ascites, grade 3 or 4 edema, prior pneumonectomy, pregnancy, diagnosis of leukemia or lymphoma. Patients with treated or clinically stable brain metastasis were eligible. The study was approved by the Memorial Sloan-Kettering Cancer Center (MSKCC) Institutional Review Board (IRB). All patients provided written informed consent.

Pharmaceutical information

Probenecid was supplied as a 20 mg/ml solution in normal saline, prepared at MSKCC in accordance with the US pharmacopeia. Pralatrexate was prepared at MSKCC [1]. It was supplied as free acid in a dry powder. Pralatrexate was suspended in bacteriostatic sterile normal saline USP and brought into solution by adjusting the pH to 7.0 with 1 N NaOH. The solution was then sterilized by means of filtration through a 0.20-μm Acrodisc filter. Each batch of pralatrexate was checked for label strength greater than 97% using spectrophotometric criteria. The sterilized solution was protected from light and stored at 4°C for use within 60 days. Pralatrexate in solution was determined to be stable for > 60 days at 4°C.

Treatment plan

The recommended phase II dose for pralatrexate given every 2 weeks was determined to be 150 mg/m^2 [8]. Probenecid has been shown to decrease the renal clearance of methotrexate fourfold [18]. A starting dose for pralatrexate of 40 mg/m^2 was chosen. The initial treatment schedule was probenecid (70 mg/m^2) by intravenous push 10 min prior to pralatrexate (40 mg/m^2) by

intravenous bolus injection, administered every 2 weeks. The pralatrexate dose was to be fixed for the first 6 dose levels, with planned escalation of the dose of probenecid from 70 → 140 → 233 → 350 → 490 → 550 mg/m^2. Subsequently the PDX was planned to be dose-escalated from 40 → 50 → 60 mg/m^2 (dose levels 7 and 8). The maximum-tolerated dose was however exceeded at dose level 3 and doses administered are shown in Table 2.

Pretreatment evaluation included complete history and physical, complete blood count, electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, AST, ALT, PT, aPTT, urinalysis, chest X-ray, and EKG. History and physical as well as laboratory tests were performed weekly for 5 weeks, and biweekly thereafter. For patients experiencing grade 3 stomatitis, retreatment with a 50% dose reduction in pralatrexate after resolution was permitted at the discretion of the investigator. Imaging studies were obtained pretreatment and at least after every two cycles (8 weeks) of treatment. Criteria for radiologic response followed WHO guidelines [22].

Three to six patients were enrolled per dose level. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria scale, version 2.0. Dose-limiting toxicities (DLT) were defined as any grade 3 nonhematologic toxicity, any grade 4 hematologic toxicity or febrile neutropenia, or any grade 3 hematologic toxicity requiring treatment delay beyond 2 weeks. If zero of three patients experienced DLT at a dose level, three patients were entered at the next higher dose level. If one of three patients experienced DLT, three additional patients were entered at this dose level for a total of six. If none of these additional three patients experienced DLT, then the dose was escalated to the next higher dose level. If at least one of the additional patients experienced DLT, the MTD was exceeded and three to six more patients were treated at the next lowest dose. This lower dose at which patients tolerated the combination would then become the recommended phase II dose if no more than one of six patients experienced a DLT. No intrapatient dose escalation was permitted.

Pharmacokinetic (PK) analysis

Pharmacokinetic studies were performed to determine the pralatrexate AUC, t1/2, and Cmax. Heparinized blood samples were collected on the first two drug treatment dates (days 1 and 15 if no treatment delays) during cycle 1 only. After pralatrexate administration by IV bolus injection, blood samples for PK analysis were collected at the following time points: 5, 10, 20, 30, and 60 min and 2, 3, 4.5, 6, and 8 h. An additional 24-h time point was obtained in seven cases. HPLC assays using fluorescence detection for pralatrexate in plasma were performed according to the procedure described previously [23]. The $AUC_{0\rightarrow\infty}$, t1/2, and Cmax for pralatrexate were determined by noncompartmental analysis using WinNonLin version 4.1 software (Pharsight Corporation, Mountain View, CA, USA). Two patients missed an 8-h time point on one of the PK collection dates. For these patients, PK calculations were based on the remaining time points at which blood was collected.

Results

Patient characteristics

Between 8/27/2001 and 10/22/2002, 17 patients were enrolled in this single-institution study. The median age was 57 years (range 37–80 years). Pretreatment characteristics are summarized in Table 1. The majority (53%; 9/17) of patients had NSCLC. All had been treated previously with a median of two chemotherapy regimens (range 1–4). Homocysteine concentration was elevated in 15 (88%) patients. For all patients, plasma folate and RBC folate levels were at least normal. Eight (47%) patients had received prior radiation.

Exposure and adverse events at each dose level

The pralatrexate dose was fixed at 40 mg/m^2 and the probenecid dose was escalated at 70, 140, and 233 mg/m^2 in consecutive patient cohorts. All 17 patients enrolled had at least one dose of pralatrexate and probenecid, comprising the safety population. A median of two cycles (range 0.5–4 cycles) were completed for all patients (Table 2). Fifty-percent dose reduction of pralatrexate was required for one patient at dose level 2

Table 1 Baseline patient characteristics

Characteristics	Number of patients
Sex	
Men	8 (47%)
Women	9 (53%)
Karnofsky performance status	
80–90%	14 (82%)
70%	3 (18%)
Primary site	
Non-small cell lung cancer	9 (53%)
Colon adenocarcinoma	5 (30%)
Pancreatic adenocarcinoma	1 (6%)
Malignant mesothelioma of pleura	1 (6%)
Gastric neuroendocrine tumor	1 (6%)
Number of prior chemotherapy regimens	
One	4 (24%)
Two	5 (29%)
Three	4 (24%)
Four	4 (24%)
Prior radiotherapy	8 (47%)
Nutritional status	Median (interquartile range)
Plasma folate (nl range 2.8–13.5 ng/ml)	11.2 (8.4–14.3) ng/ml
RBC folate (nl > 220 μg/l)	547 (488–600) μg/l
Homocysteine (nl range 0–9 μM/l)	11.2 (9.3–12.3) μM/l

Table 2 Pralatrexate and probenecid exposure

Dose level	Pralatrexate (mg/m^2)	Probenecid (mg/m^2)	No. of patients treated	Median no. of cycles	No. of Patients with dose reductions
1	40	70	3	2	0
2	40	140	9	1.5	1
3	40	233	5	2	1

during cycle 1, and for one patient at dose level 3 during cycle 2. No other patients required dose reductions.

Four (24%) of 17 patients discontinued treatment due to treatment-related toxicity, which was stomatitis in all cases. Therapy was discontinued due to progression of disease in 12 (71%) of 17 patients. One patient elected to discontinue treatment after two cycles due to persistence of grade 2 fatigue which was present at the time of enrollment.

Table 3 summarizes adverse events (AEs) (CTC scale, version 2.0) at all dose levels that were assessed by the investigator as related to treatment with pralatrexate plus probenecid. At the first dose level, three patients were treated with pralatrexate 40 mg/m^2 and probenecid 70 mg/m^2. Grade 2 toxicities that occurred in one patient each were fatigue, anemia, peripheral edema, nausea, constipation, cough, and hepatic pain. Two patients experienced grade 1 rash. Other grade 1 toxicities occurred once, included fatigue, arthralgia, sensory neuropathy, and blurred vision. No DLTs occurred at this dose; therefore, dose escalation was permitted.

At the second dose level, three patients were treated with pralatrexate 40 mg/m^2 and probenecid 140 mg/m^2. One patient experienced grade 3 rash at the end of cycle 2, possibly related to the investigational treatment. Because the grade 3 rash did not occur during cycle 1, expansion of the patient cohort at the second dose level was not required. Grade 2 toxicities, occurring in one patient each, were: stomatitis, nausea, fatigue, and peripheral edema. Grade 1 nausea, vomiting, and diarrhea each occurred in two patients. Other grade 1 toxicities were observed in one patient each: stomatitis, peripheral edema, and myalgia.

Five patients were treated at the third dose level. Initially, three patients were enrolled at the third dose level (pralatrexate 40 mg/m^2 and probenecid 233 mg/m^2). The first patient enrolled at this dose experienced grade 2 stomatitis during cycle 1 and grade 3 stomatitis during cycle 2. The second patient treated at this dose level experienced grade 3 stomatitis during cycle 1; therefore, the dose level was expanded. The third patient tolerated treatment well, and the fourth patient experience grade 2 stomatitis. The fifth patient at this dose level, a 77-year-old patient who had received prior radiotherapy and four prior chemotherapy regimens, experienced grade 4 stomatitis, grade 3 leukopenia, and grade 3 thrombocytopenia after the first dose of pralatrexate and probenecid and was removed from the study.

Because two patients at the third dose level experienced dose-limiting stomatitis, the MTD was exceeded and dose level 2 (pralatrexate 40 mg/m^2 and probenecid 140 mg/m^2) was expanded initially by three additional patients.

Table 3 Treatment-related toxicities of probenecid plus pralatrexate (all doses) ($n = 17$ patients)

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Alopecia	1 (6%)	0	0	0
Bone marrow				
Leukopenia	0	1 (6%)	1 (6%)	0
Neutropenia	0	1 (6%)	0	0
Anemia	1 (6%)	3 (18%)	0	0
Thrombocytopenia	0	0	1 (6%)	0
Cough	2 (12%)	2 (12%)	0	0
Edema	1 (6%)	2 (12%)	0	0
Fatigue	5 (29%)	5 (29%)	0	0
Gastrointestinal				
Constipation	1 (6%)	1 (6%)	0	0
Diarrhea	2 (12%)	0	0	0
Nausea	5 (29%)	4 (24%)	0	0
Vomiting	5 (29%)	0	0	0
Myalgia	2 (12%)	0	0	0
Hepatic				
Bilirubin elevation	0	1 (6%)	0	0
ALT elevation	3 (18%)	0	0	0
AST elevation	2 (12%)	1 (6%)	0	0
Neuropathy	1 (6%)	0	0	0
Rash	6 (35%)	0	1 (6%)	0
Stomatitis	0	3 (18%)	3 (18%)	1 (6%)
Blurred vision	1 (6%)	0	0	0

One patient in this expansion group experienced grade 3 stomatitis, and the dose level was expanded to six patients. There were no further toxicities exceeding grade 2. The following grade 2 AEs each occurred once: stomatitis, nausea, cough, fatigue, and leukopenia.

Antitumor activity

Although response rate was not a primary endpoint of the study, 16 of the 17 patients treated were evaluated for antitumor activity. No objective tumor responses were noted for any patients. Four (25%) of 16 patients achieved stable disease lasting 1.9 (NSCLC), 2 (carcinoid), 3.7 (NSCLC), and 3.8 (NSCLC) months.

One patient demonstrated declining performance status that was likely due to progression of disease and suffered a fatal hip fracture before the date of first scheduled CT scan; this patient was therefore excluded from the evaluation for response.

Pharmacokinetic (PK) studies

Blood samples were obtained from all 17 patients on the first 2 treatment dates during cycle 1 and analyzed by HPLC for pralatrexate concentration. For each patient, the AUC, t1/2, and Cmax of pralatrexate are listed in Table 4. For four patients, PK analysis was performed only once (Table 4 legend).

There was significant variability of PK results for pralatrexate between patients at a given dose level. For example, at the second dose level AUC values ranged between 3.1 and 8.6 μg/ml h and half life ranged between 1.1 and 5.9 h (Table 4). With each dose escalation of probenecid, the mean t1/2, and AUC of pralatrexate increased. Cmax of pralatrexate showed minimal variation between the three dose levels (Table 5).

For 26 treatment administrations associated with grade 1–2 adverse events during cycle 1, mean pralatrexate AUC was 5.2 μg/ml h (SD 1.1). The three treatments resulting in grade 3–4 adverse events during cycle

Table 4 Pharmacokinetic (PK) studies during cycle 1 for each patient on study: AUC, terminal half-life (t1/2), and Cmax of pralatrexate (40 mg/m^2) co-administered with escalating doses of probenecid

Dose level	Pt. no.	Cycle 1, day 1			Cycle 1, day 15		
		AUC (μg/ml h)	t1/2 (h)	Cmax (μg/ml)	AUC (μg/ml h)	t1/2 (h)	Cmax (μg/ml)
First	1	5.2	1.5	7.7	5.1	3.0	3.1
	2	5.0	1.5	5.5	4.8	1.5	5.1
	3	4.3	1.9	3.3	4.8	1.4	4.8
Second	1[a]	4.9	5.9	4.7	6.4	3.9	4.6
	2	6.9	1.7	4.2	4.6	1.1	5.7
	3	5.0	3.0	3.6	5.6	1.5	5.1
	4	3.1	1.1	4.1	Not done[b]		
	5	5.8	1.9	6.0	7.2	1.9	5.0
	6	3.2	1.2	2.5	4.3	1.9	4.6
	7	8.6	1.2	3.1	Not done[b]		
	8	5.9	1.2	5.5	4.9	2.3	5.5
	9	6.3[c]	5.5	5.8	Dose reduction[d]		
Third	1	4.7	5.3	8.3	5.9	3.6	11.5
	2	4.6	2.8	5.4	6.0	1.5	12.6
	3	9.6[c]	1.3	6.7	Off study		
	4[e]	5.8	4.3	3.6	4.5	6.2	2.3
	5	8.1[f]	2.0	3.5	Off study		

[a]This patient experienced a viral URI after the first treatment which was unrelated to studying drugs. The second treatment with PK blood collection was delayed by 1 week until day 22

[b]Samples for PK were not drawn with these treatments

[c]Grade 3 stomatitis occurred

[d]Due to grade 3 stomatitis with the first dose, dose was reduced per protocol for the second treatment (pralatrexate 20 m/m^2 and probenecid 140 mg/m^2). AUC was 3.9 μg/ml h, t1/2 was 1.4 h, and Cmax was 1.4 μg/ml

[e]This patient experienced grade 3 mucositis during cycle 2. PK data were only collected for cycle 1 (the first and second drug administrations for each patient), so PK data are not available for the treatment that resulted in grade 3 toxicity for this patient

[f]Grade 4 stomatitis, grade 3 leukopenia, and grade 3 thrombocytopenia occurred

Table 5 Pralatrexate mean AUC, half life, and Cmax at the each dose level

Dose level	No. of patients	No. of PK studies	Mean AUC (μg/ml h) (SD)	Mean t1/2 (h) (SD)	Mean Cmax (μg/ml) (SD)
1	3	6	4.9 (0.3)	1.8 (0.6)	4.9 (1.7)
2	9	15	5.4 (1.4)	2.4 (1.6)	4.7 (1.0)
3	5	8	6.2 (1.8)	3.4 (1.8)	6.7 (3.8)
All	17	29	5.5 (1.4)	2.5 (1.6)	5.3 (2.3)

1 were associated with mean pralatrexate AUC of 8.0 μg/ml·h (SD 1.7).

Discussion

Compared to methotrexate, pralatrexate has been shown to have superior antitumor efficacy against tumor xenografts (MX-1, LX-1, A549 squamous cell lung cancer) [2]. In a previous phase II trial, pralatrexate monotherapy (135–150 mg/m^2 every 2 weeks) yielded a 10% objective response rate (95% CI 3–25%) in patients with NSCLC [9]. Preclinical studies suggest that anti-tumor efficacy of pralatrexate may be increased by probenecid [20, 21].

The current study was designed to determine a MTD of pralatrexate in combination with intravenous probenecid administered once every 2 weeks in patients with advanced solid tumors. At dose level 3 (pralatrexate 40 mg/m^2 and probenecid 233 mg/m^2), the MTD was exceeded with dose-limiting stomatitis occurring during the first cycle in 40% of patients. Additionally, at dose level 3, another patient experienced grade 3 stomatitis during cycle 2. Based on the results of this study, a recommended dose for future studies on this schedule is pralatrexate 40 mg/m^2 plus probenecid 140 mg/m^2 every 2 weeks.

The mean AUC of pralatrexate was slightly higher for patients who experienced grade 3–4 toxicities than for those patients who experienced grade 1–2 adverse events in the current study. However, several patients with relatively high AUC values only experienced grade 1–2 toxicities. Similar variability in pralatrexate AUC values in patients experiencing mild toxicities was seen in the phase I study of pralatrexate alone [8].

In the phase I study of pralatrexate alone, the mean AUC for pralatrexate 30 mg/m^2 given weekly was 2.5 μg/ml h (SD 0.6) for six PK experiments ([8] and L. Krug, unpublished data). In the current study, 17 patients received a comparable dose of pralatrexate (40 mg/m^2) in the presence of escalating doses of probenecid. For 29 PK analyses, the mean pralatrexate AUC was 5.5 μg/ml h (SD 1.4) (Table 5), suggesting that probenecid inhibited the renal excretion of pralatrexate. Impaired renal excretion of pralatrexate probably explains, at least in part, the dose-limiting stomatitis in this study with a relatively low dose of pralatrexate.

Although probenecid monotherapy does not cause stomatitis, mucosal toxicity in this study may reflect indirect effects of probenecid on mucosal epithelium. In the presence of probenecid, we observed significant stomatitis with lower pralatrexate AUC values than in the prior pralatrexate monotherapy study [8]. The recommended phase II regimen in the current study (pralatrexate 40 mg/m^2 plus probenecid 140 mg/m^2) yielded mean AUC of 5.4 μg/ml h (SD 1.4). In the pralatrexate monotherapy trial, the recommended phase II dose was 150 mg/m^2 and the mean AUC was 11.0 μg/ml h (SD 4.9) ([8] and L. Krug, unpublished data). Therefore, more stomatitis was observed in the current study than would have been predicted by the AUC value. This suggests a mechanism of enhanced mucosal toxicity with the pralatrexate plus probenecid combination, in which probenecid may inhibit ATP-dependent cellular efflux mechanisms, resulting in enhanced retention of pralatrexate in mucosal epithelial cells.

This comparative analysis of the toxicity and PK data from both phase I studies supports two mechanisms for enhanced stomatitis with the probenecid plus pralatrexate combination. First, probenecid increases the AUC of pralatrexate by inhibiting renal excretion. Second, probenecid may intensify the mucosal toxicities of pralatrexate by inhibiting drug efflux in mucosal epithelial cells, but the current study does not directly address this second possibility. The interpretation of the PK results is limited by small sample sizes and significant variability of PK results within a given dose level in both studies.

Plasma homocysteine concentrations were elevated in 15 (88%) of 17 patients, including all 4 patients who experienced grade 3-4 stomatitis. Elevated plasma homocysteine can indicate functional deficiency of folate or vitamin B12, which might not be apparent by measurement of vitamin concentrations [24]. Vitamin B12 and folate supplementation has been explored as a strategy to reduce the toxicities associated with folate analogues. In a study of the antifolate pemetrexed, vitamin B12 (1,000 μg IM every 9 weeks) and folate supplementation (350–1,000 μg orally daily) reduced the incidence of neutropenia, thrombocytopenia, diarrhea, mucositis, and drug-related death [25]. In a phase I study of pralatrexate plus docetaxel with vitamin B12 and folate supplementation, the vitamins lowered the homocysteine and methylmalonic acid concentrations, which indicates increasing vitamin B12 and folate pools (C. Azzoli, unpublished data). A recently initiated phase I trial at this institution will determine if vitamin supplementation will allow for a higher maximum-tolerated dose of pralatrexate, which may translate into greater anti-tumor efficacy.

In summary, the pralatrexate and probenecid combination was associated with significant stomatitis limiting the escalation beyond pralatrexate 40 mg/m^2 and probenecid 140 mg/m^2 given every 2 weeks. Given the clinical activity of pralatrexate in prior reports, further studies of single agent pralatrexate plus vitamin supplementation are ongoing. Similarly, vitamin supplementation may facilitate escalation of the dose of pralatrexate and probenecid in future studies.

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