

10-Propargyl-10-deazaaminopterin: An Antifolate with Activity in Patients with Previously Treated Non-Small Cell Lung Cancer¹

Lee M. Krug,² Christopher G. Azzoli, Mark G. Kris, Vincent A. Miller, Nushmia Z. Khokhar, William Tong, Michelle S. Ginsberg, Ennapadam Venkatraman, Leslie Tyson, Barbara Pizzo, Valerie Baez, Kenneth K. Ng, and F. M. Sirotnak

Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine [L. M. K., C. G. A., M. G. K., V. A. M., L. T., B. P., V. B., K. K. N.], Program of Molecular Pharmacology and Experimental Therapeutics [N. Z. K., W. T., F. M. S.], Departments of Radiology [M. S. G.] and Epidemiology and Biostatistics [E. V.], Memorial Sloan-Kettering Cancer Center, Weill Medical College of Cornell University New York, New York 10021

ABSTRACT

Purpose: 10-propargyl-10-deazaaminopterin (PDX) has superior antitumor efficacy in mouse xenograft models, likely attributable to increased uptake by the RFC-1 folate transporter and greater intracellular polyglutamylation. In a previous Phase I trial, stomatitis was the dose-limiting (and only clinically significant) toxicity of PDX. The recommended Phase II dose was 150 mg/m² i.v. every 2 weeks. Responses observed in patients with non-small cell lung cancer (NSCLC) in the Phase I trial prompted this Phase II trial.

Experimental Design: Patients had stage IIIB or IV NSCLC and either no previous chemotherapy or progression after initial response or stable disease to one previous chemotherapy regimen. Initially, PDX was administered at a dose of 150 mg/m² every 2 weeks. However, to decrease the frequency of stomatitis, the last 10 patients were treated at a dose of 135 mg/m². We planned to correlate PDX effects with folate and homocysteine levels and the expression of genes associated with folate transport and polyglutamylation.

Results: Thirty-nine patients were enrolled, 38 of whom were evaluable for response. Four patients had confirmed, major objective responses (10% based on intent to treat, 95% confidence interval 3–25) lasting 4, 9, 12, and 15 months. Twelve patients (31%) had stable disease. The me-

dian survival was 13.5 months. The predicted 1- and 2-year survival rates were 56 and 36%, respectively. Two patients (5%) suffered grade 4 stomatitis, and 6 (15%) had grade 3. No clinically significant myelosuppression occurred. No correlation between homocysteine or serum folate levels and severity of stomatitis was observed. Area under the curve (calculated using a limited sampling model) correlated with mucositis grade. A trend was noted between folate transporter expression and treatment effect.

Conclusions: The broad applicability of this new antifolate with limited toxicity and proven efficacy in NSCLC encourage further development of this compound. Several trials are now underway combining PDX with other chemotherapeutic agents and testing its efficacy in other cancers.

INTRODUCTION

Drugs that inhibit dihydrofolate reductase, such as methotrexate, were among the first chemotherapeutic agents discovered. Methotrexate remains one of the most widely applied chemotherapy drugs and is used to treat breast, bladder, and head and neck cancers; leukemias; and others. A class of rationally designed derivatives of methotrexate, the 10-deazaaminopterin, has demonstrated substantially greater antitumor effects than methotrexate against murine tumor models and human tumor xenografts (1–3), as well as excellent clinical activity in NSCLC³ (4, 5). The improved activity appears to be caused by more effective internalization by the one carbon, reduced folate transporter (RFC-1), and its subsequent accumulation in tumor cells through the formation of polyglutamylated metabolites (1, 6). The addition of a propargyl moiety at the carbon 10 position (PDX; Ref. 7) yielded the best agent with regard to uptake into cells by the RFC-1 transporter and retention in the cell by polyglutamylation (8). It subsequently demonstrated dramatic antitumor activity in mouse xenograft models of human breast and lung cancers.

Our Phase I trial reported previously of PDX established the excellent tolerance and potential efficacy of this agent (9). Thirty-three patients with NSCLC were enrolled. These patients had been treated with a median of two previous chemotherapy regimens, and 55% had previous radiation. PDX was administered i.v. push, starting at 30 mg/m². Stomatitis was the dose-limiting toxicity, and this occurred at the first dose level when PDX was given on a weekly schedule. However, on a biweekly schedule, the dose of PDX could be escalated to a maximum tolerated dose of 170 mg/m². Nearly all other toxicities were

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²To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-8420; Fax: (212) 794-4357; E-mail: krugl@mskcc.org.

³The abbreviations used are: NSCLC, non-small cell lung cancer; PDX, 10-propargyl-10-deazaaminopterin; FPGS, folylpolyglutamate synthetase; AUC, area under the curve; CI, confidence interval; LCSS, lung cancer symptom scale; C_T, threshold cycle; FPGH, folylpolyglutamate hydrolase.

grade 1 or 2 and readily reversible. No significant myelosuppression was noted. Two patients with NSCLC had objective tumor responses. On the basis of these results, this Phase II trial in patients with NSCLC was planned with PDX administered at a dose of 150 mg/m² every 2 weeks.

PATIENTS AND METHODS

Patient Eligibility. This trial enrolled adults with pathologically confirmed NSCLC, either stage IIIB (based on the presence of pleural or pericardial disease) or IV. In designing the trial, we wanted to allow patients the opportunity to receive standard first-line chemotherapy, because, based on extensive Phase III data, the majority of patients receive such treatment. However, we were aware of the usual low response rate of NSCLC in the second-line setting, especially for patients who progress outright on initial therapy. Therefore, we selected patients who had either: (a) no previous chemotherapy or (b) progression after initial response or stable disease with one previous chemotherapy regimen (except antifolates) documented radiologically after 2 months of therapy. This included patients who developed a recurrence after treatment with one preoperative or adjuvant chemotherapy regimen. If previous radiation therapy was necessary, it must have been completed ≥ 3 weeks previous to entry. Patients needed to have measurable or evaluable disease which had not been irradiated. Patients were required to have a Karnofsky performance status of $\geq 70\%$. Necessary hematological parameters were a WBC $\geq 4,000/\mu\text{l}$, hemoglobin ≥ 10 grams/dl, and a platelet count $\geq 160,000/\mu\text{l}$. Biochemical parameters were a total bilirubin ≤ 1 , aspartate aminotransferase $\leq 1.5 \times$ upper limits of normal, alkaline phosphatase $\leq 5 \times$ upper limits of normal, creatinine ≤ 1.5 mg/dl, or creatinine clearance ≥ 50 ml/min/1.7 m². Because of the potential for drug accumulation in fluid collections, patients with clinically significant pleural effusions or ascites, grade 3 or 4 edema, or previous pneumonectomy were excluded. Patients with brain metastases identified previously were eligible if they were neurologically stable. Patients with a concurrent active cancer or other unstable serious illness were excluded. Patients were required to discontinue folic acid supplements, including those in multivitamins, ≥ 7 days before starting therapy. Pregnant or lactating women, or fertile men or women not using effective contraception, were excluded. This trial was reviewed and approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center (Protocol 99-053). All patients signed informed consent.

Pharmaceutical Information. Initially, PDX was prepared at Memorial Sloan-Kettering as described previously (7). Subsequently, bulk quantities of PDX were manufactured by Ash-Stevens Pharmaceuticals under a subcontract from the National Cancer Institute. PDX was supplied as a free acid in a dry powder. The drug was suspended in bacteriostatic sterile normal saline USP and brought into solution by adjusting the pH to 7 with 1 N NaOH. The solution was then sterilized by means of filtration through a 0.20- μm Acrodisc filter. The sterilized solution was protected from light and stored at 4°C. Stability of PDX in solution is >1 year at 4°C. PDX was administered i.v. by bolus injection through the sidearm of a freely running i.v. line containing normal saline.

Treatment Plan. PDX was initially administered at 150 mg/m² i.v. push every 2 weeks. Two doses, or 4 weeks, were defined as one cycle. Because of the rate of stomatitis, a 10% dose reduction was implemented such that the first 29 patients were treated at 150 mg/m² and the last 10 patients were treated at 135 mg/m².

At baseline, all patients had a history and physical examination, a complete blood count, biochemical profile, and lactate dehydrogenase. Patients then had a physical exam weekly for the first 4 weeks and subsequently on days of treatment. A complete blood count and biochemical profile were repeated with each treatment. A computed tomography scan of the chest and any other imaging studies necessary to evaluate indicator lesions were obtained at baseline, after the first cycle, and then every two cycles thereafter.

The dose of PDX was adjusted if severe or persistent stomatitis occurred. Patients with grade 3 stomatitis at any time during the cycle were treated with a 50% dose reduction on resolution to grade 0. If stomatitis continued despite a dose reduction, a second 50% dose reduction was allowed at the investigator's discretion. For patients with any mucositis on the day of treatment, treatment was delayed a week until resolution. After an amendment, patients who had their dose reduced or delayed were instructed to begin p.o. folate supplements of 0.4–1 mg daily.

Biostatistics and Definitions. A Gehan two-stage design was used to calculate sample size (10). Initially, 19 patients were entered to determine whether the likelihood of the major response rate was $\geq 15\%$. Because responses were observed, accrual continued to 39 patients to better define the estimated major response rate with associated 95% CIs. Survival curves were generated using the methods of Kaplan and Meier (11).

For patients with measurable disease, response categories included complete response, partial response, no change, or progression. Responses were defined using standard criteria for bidimensional measurements. For patients with evaluable indicator lesions, response categories included complete response, improvement, no change, or progression. Responses were confirmed with a follow-up scan ≥ 4 weeks later.

Toxicities were graded using the National Cancer Institute Common Toxicity Criteria, version 2.0.

Pharmacodynamic Studies. In an attempt to determine whether drug metabolism impacted the likelihood of developing stomatitis, we conducted limited sampling pharmacodynamic studies. In the Phase I trial of PDX, a stepwise regression analysis was used to derive the following formula that would closely predict the true AUC using serum concentration values obtained at just two time points (9, 12):

$$\text{AUC} = -1136.066347 + 0.195951 \times C(t1) + 1.498976 \times C(t2),$$

where C(t1) and C(t2) = serum concentration of PDX obtained 5 and 20 min after injection, respectively.

In this Phase II trial, patients had heparinized blood samples drawn 5 and 20 min after the infusion of PDX in the first cycle (two treatments). The concentration of PDX at each time point was determined by high-performance liquid chromatography, the AUC was calculated using the above formula, and the result was correlated with the grade of stomatitis.

Determination of Folate Status. Homocysteine levels, which are frequently elevated in folate-deficient states (13),

have been correlated with mucositis grade in patients treated with pemetrexed (Alimta; Ref. 14). To determine whether folate deficiency correlated with stomatitis grade in patients treated with PDX, serum folate and homocysteine levels were drawn at baseline. Patients also had serial homocysteine levels drawn previous to starting each cycle. Follow-up folate levels were not measured, because the results are not reliable after treatment with an antifolate.

Quantitative PCR Analysis. We hypothesized that molecules which regulate internalization of PDX (*i.e.*, the RFC-1 folate transporter) and polyglutamylation (FPGS and FPGH) may correlate with drug activity. To evaluate this, we performed quantitative PCR analysis (using a Taqman automated PCR machine) on available pathologic samples.

Formalin-fixed, paraffin-embedded tissue samples were cut in 20- μ m-thick sections on a microtome with disposable blades. Two cut sections per sample were placed in autoclaved 1.5-ml Eppendorf tubes, deparaffinized in two changes of Citrisolv (Fisher Scientific) for 10 min, and washed twice with 100% ethanol. Total RNA was extracted using a modification of methodology described previously (15). Deparaffinized tissue was incubated for 16 h at 55°C in RNA digestion buffer containing 20 mM Tris/HCl (pH 7.5), 20 mM EDTA, 1% sodium dodecyl sulfate, and freshly added proteinase K. RNA purification was done by TRIzol liquid sample (Life Technologies, Inc.) and chloroform extraction, followed by precipitation with an equal amount of isopropanol in the presence of glycogen, at -20°C. The pellets were washed with 75% ethanol and resuspended in 20 μ l of RNase free water. DNase treatment was done using DNase I (Life Technologies, Inc.) following the manufacturer's protocol. First-strand cDNA was synthesized using Superscript II (Life Technologies, Inc.) primed with random hexamers. The relative quantitation of RFC-1, FPGS, and FPGH gene expression was carried out with the aid of an ABI Prism 7700 Sequence Detection System (Taqman; Applied Biosystems, Foster City, CA). Using the nuclease activity of Taq Polymerase, this method is based on the displacement and cleavage of a labeled fluorogenic probe specific to the target sequence flanked by PCR primers. This allows the quantitation of target genes, whereas PCR is in the log phase of amplification. A detailed description of this methodology has been provided previously (16–18). Specific cDNAs of interest (RFC-1, FPGS, and FPGH) and reference cDNA (β -Actin) were amplified separately with the Taqman using an oligonucleotide probe with a 5' fluorescent reporter dye (6FAM) and 3' quencher dye (TAMRA). Template cDNA was added to Universal Master Mix (Applied Biosystems) in a 25- μ l reaction with 700 nM primers and 200 nM probe/gene. The primer and probe sets were designed using Primer Express Software (Applied Biosystems), and the sequences are as follows: RFC-1, forward 5'-CCGCG-GCTCCTACCAGTT-3', reverse 5'-AAGACCAGGGCACA-GAGCTCTT-3', probe 6FAM-ATTCTGAACACCGTCGCTT-GGAAGACACT-3'; FPGS, forward 5'-GGCTGGAGGAGACC-AAGGAT-3', reverse 5'-TGAGTGTGTCAGGAAGCGGAAGT-3', probe 6FAM-CATGCCTTGCAATGGATCAGCCAA-TAMRA; FPGH, forward 5'-GCGAGCCTCGAGCTGTCTA-3', reverse 5'-ACTTATTACGGCATTTCATTA-3', probe 6FAM-CG-CCAAGAAGCCCATCATCGGAAT-TAMRA; β -actin, forward 5'-CTGGCACCCAGCACAATG-3', reverse 5'-GCCGATCCA-

Table 1 Patient characteristics (N = 39)

Stage IV	92%
Female	67%
Karnofsky performance status	
90%	18%
80%	67%
70%	15%
Lactate dehydrogenase above upper limits of normal	28%
Bone metastases	23%
Brain metastases	15%
Histology	
Adenocarcinoma	72%
Squamous	8%
Other	20%
Median age	57 (range 40–71)
Previous chemotherapy regimens	
Paclitaxel, carboplatin, ZD1839 ^a	18%
Paclitaxel, carboplatin	15%
Docetaxel, trastuzumab ^a	10%
Paclitaxel, trastuzumab ^a	10%
Mitomycin, vinblastine	8%
Docetaxel, carboplatin	5%
Gemcitabine, docetaxel, cisplatin ^{a,b}	5%
Paclitaxel, carboplatin ^b	5%
None	5%
Docetaxel	3%
Docetaxel, vinorelbine ^a	3%
Gemcitabine	3%
Gemcitabine, cisplatin	3%
Mitomycin, vinblastine, cisplatin ^b	3%
Retinoid (ALRT-1550) ^a	3%
Vinorelbine, cisplatin ^b	3%
Previous radiation	
Thoracic	18%
Brain	15%
Bone	8%
Mantle (for history of Hodgkin's disease)	3%

^a Administered in a clinical trial.

^b Administered as adjuvant or neoadjuvant therapy.

CACGGAGTACT-3', probe 6FAM-TCAAGATCATTGCTCCT-CCTGAGCGC-TAMRA. Relative quantitation was done using the comparative C_T method. The C_T indicates the fractional cycle number at which the amplified target reaches a fixed threshold. The amount of target gene normalized to an endogenous reference is given by $2^{-\Delta C_T}$ (2–4) where ΔC_T is C_T (target gene) - C_T (reference gene).

Lung Cancer Symptom Assessment. Patients' symptoms were evaluated at the beginning of every cycle (every 4 weeks) using the LCSS. The LCSS has been validated previously and includes an observer assessment and a linear analogue scale completed by the patient (19).

RESULTS

Patient Characteristics. Between August 1999 and June 2001, 39 patients were enrolled. The patient characteristics are detailed in Table 1. Three patients had not received previous chemotherapy (1 had received previously a retinoid). The other 36 patients had been treated with a variety of regimens. Six patients received their previous chemotherapy in the adjuvant or neoadjuvant setting.

Response and Survival. Thirty-eight patients completed at least one cycle of chemotherapy and were considered evalu-

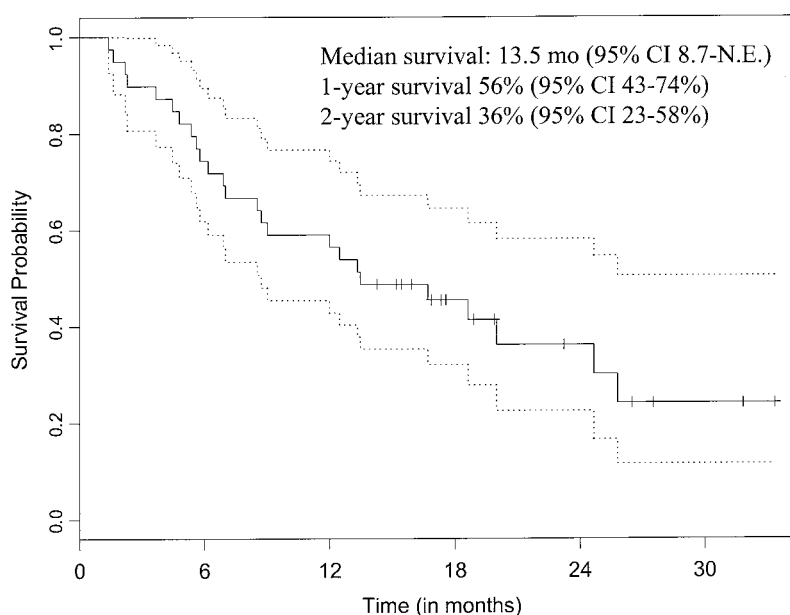


Fig. 1 Kaplan-Meier survival curve.

able for response. Four of the 39 patients had confirmed major objective responses (10% based on intent to treat, 95% CI 3–25). All 4 of these patients were treated with PDX at a dose of 150 mg/m². All had chemotherapy received previously; 2 with paclitaxel, carboplatin, and ZD1839; 1 with docetaxel and vinorelbine; and 1 with mitomycin and vinblastine. Three of these 4 patients had responded to their previous therapy, and 1 (treated with paclitaxel, carboplatin, and ZD1839) had stable disease. The duration of responses was 4, 9, 12, and 15 months. Twelve patients (31%) had stable disease. This includes 1 patient who achieved a partial response but progressed on a follow-up scan. We were unable to confirm stable disease with follow-up scans in 3 patients because of removal from study for toxicity (2 patients) and death (1 patient, see below). The median time to progression was 3 months. The median survival was 13.5 months (95% CI 8.7–not estimable). The predicted 1-year survival rate was 56% (95% CI 43–74%), and the 2-year survival rate was 36% (23–58%; Fig. 1).

Toxicity. As in the Phase I trial, the primary toxicity was stomatitis (Table 2). In all, 2 patients (5%) suffered grade 4 stomatitis, and 6 (15%) had grade 3. At a dose of 150 mg/m², 6 patients (21%) had grade 3 or 4 stomatitis, and 11 (38%) required a dose reduction (Table 3). At 135 mg/m², 2 patients (20%) had grade 3 or 4 stomatitis, and 1 (10%) required a dose reduction.

All other toxicities of PDX were mild. In fact, apart from 1 patient with a grade 3 elevation in alanine aminotransferase, only grade 1 or 2 toxicities occurred. No significant myelosuppression was observed in any patient. One patient died of sudden cardiac death the day before starting cycle 2, 2 weeks after treatment for a pulmonary embolism. An autopsy was not performed.

Folate and Homocysteine Levels. Baseline serum folate levels were obtained from 27 of the 39 patients and then grouped according to maximum grade of mucositis experienced

Table 2 Stomatitis rates by PDX dose

	PDX Dose	
	150 mg/m ² (29 patients)	135 mg/m ² (10 patients)
Stomatitis grade		
0	4 (14%)	5 (50%)
1	8 (28%)	0 (0%)
2	11 (38%)	3 (30%)
3	5 (17%)	1 (10%)
4	1 (3%)	1 (10%)
Dose reductions	11 (38%)	1 (10%)
Off study for stomatitis	7 (24%)	1 (10%)

after the first dose of PDX. The mean serum folate level of the patients who did not experience mucositis was 11.9 ng/ml (normal range 2.8–13.5 ng/ml). The mean folate level of patients with grade 1 mucositis was 9.3 ng/ml, with grade 2 mucositis 9.5 ng/ml, and with grade 3–4 mucositis 9.7 ng/ml. The correlation coefficient between folate level and stomatitis was not significant. (Kendall's rank correlation $\tau = -0.16$, $P = 0.24$).

Baseline homocysteine levels were obtained from 24 of the 39 patients (normal level < 9 μ M/liter). For this analysis, patients were again grouped according to maximum grade of mucositis after the first dose of PDX. Patients with no mucositis had a mean homocysteine level of 9.7 μ M/liter, grade 1–13.2 μ M/liter, grade 2–12.8 μ M/liter, and grade 3–4–9.6 μ M/liter. There were no trends observed to suggest that baseline homocysteine level predicted mucositis (Kendall's rank correlation $\tau = 0.14$, $P = 0.33$). In addition, patients had serial serum homocysteine levels drawn before each cycle of PDX. There were no trends observed to suggest that serial treatment with PDX affected serum homocysteine level over time.

Table 3 Selected toxicities (other than stomatitis)^a

	Grade 1	Grade 2	Grade 3	Grade 4
Nonhematological				
Fatigue	16 (41%)	7 (18%)	—	—
Nausea	17 (44%)	4 (10%)	—	—
Epistaxis	19 (49%)	—	—	—
Rash	15 (38%)	3 (8%)	—	—
Dyspnea	1 (3%)	8 (21%)	1 (3%)	—
Alopecia	6 (15%)	3 (8%)	N/A	N/A
AST ^b	3 (8%)	2 (5%)	—	—
ALT ^c	2 (5%)	1 (3%)	1 (3%)	—
Hematological				
Anemia	5 (13%)	—	—	—
Neutropenia	2 (5%)	—	—	—
Leukopenia	—	—	—	—
Thrombocytopenia	—	—	—	—

^a Tabulated by number of patients with their greatest toxicity in order of incidence. The percentage of patients is shown in parenthesis. All toxicities were graded using the National Cancer Institute Common Toxicity Criteria, version 2.0.

^b AST, aspartate aminotransferase.

^c ALT, alanine aminotransferase.

Pharmacodynamic Studies. AUC was calculated after the first dose of PDX for 27 of 39 patients and after the second dose for 25 of 36 patients using the limited sampling model described above (Table 4). Once again, patients were grouped according to a maximum grade of mucositis experienced after the first dose of PDX. A correlation was observed between predicted AUC and severity of stomatitis (Kendall's rank correlation $\tau = 0.25$, $P = 0.05$). This correlation was maintained ($P = 0.01$) when the results of this Phase II study were pooled with data from the previous Phase I trial.

Tumor Expression of the Folate Transporter and Sensitivity to PDX. Of the 39 patients enrolled, 13 had paraffin-embedded pathology specimen adequate to generate RNA. Each tissue block was determined to represent nearly all tumor by the inspecting pathologist before attempting RNA extraction. Adequate RNA was generated from 12 of the 13 paraffin-embedded specimens from which extraction was attempted. Five of these specimens belonged to patients who demonstrated immediate progression of disease within 1 month of PDX initiation. Seven specimens were from patients whose disease remained stable for ≥ 3 months (range 3–14 months). No specimens were available from patients who demonstrated a partial response to PDX.

Using human β -actin as an internal reference, real-time Taqman reverse transcription-PCR was performed to measure the relative expression of the following genes: (a) RFC-1 folate transporter (*RFC*); (b) *FPGH*; and (c) *FPGS*.

No clear trends were observed in the relative expression of *FPGH* and *FPGS* with regard to PDX response. However, the mean RFC level of the patients with progression of disease (2.28 \pm 0.91) tended to be lower than the patients whose disease remained stable on PDX for ≥ 3 months (4.5 \pm 2.22). This trend approached statistical significance despite the limited data set ($P = 0.063$ by *t* test). This suggests that tumors which express high levels of the RFC-1 folate transporter may be more susceptible to growth inhibition by PDX.

Lung Cancer Symptom Assessment. LCSS scores were analyzed for patients who received at least three cycles of

Table 4 Correlation of AUC (based on limited sampling model) with grade of mucositis

	Mucositis grade	<i>n</i>	Mean AUC ($\mu\text{mol} \times \text{h}$)	SE	Kendall's rank correlation
After first dose	0	12	9709	2620	$\tau = 0.25$ $P = 0.05$
	1	6	9297	2907	
	2	8	12222	7272	
After second dose	3–4	3	18568	2918	$\tau = 0.05$ $P = 0.64$
	0	17	9442	3807	
	1	6	9210	3529	
Phase I and II trials combined	2	2	8322	5790	$\tau = 0.26$ $P = 0.01$
	3–4	2	14213	5674	
	0	17	10095	4097	
	1	11	9677	3733	$\tau = 0.26$ $P = 0.01$
	2	10	12540	6522	
	3	4	13525	3882	
	4	2	20250	249	

treatment, which provided four questionnaire time points: baseline and three monthly follow-ups. Fifteen patients met this requirement, which included all 4 responding patients, 9 patients with stable disease, and 2 patients with progression. A significant improvement in shortness of breath and cough was noted for this subset of patients ($P = 0.044$ and 0.018 , respectively; Wilcoxon two-sided test). All other patient assessment and observer values were unchanged.

DISCUSSION

On the basis of strong preclinical data suggesting superior efficacy of PDX, a novel antifolate, and a previous Phase I trial demonstrating a favorable toxicity profile, we undertook this Phase II trial in advanced NSCLC. All but 3 of the 39 enrolled patients had received previous chemotherapy and had either stable disease or a response to that previous regimen. A portion (10%) of patients treated with PDX had confirmed durable responses, and another 31% had stable disease. These patients experienced an improvement in their symptoms of shortness of breath and cough. This response rate and symptomatic benefit are comparable with docetaxel, the Food and Drug Administration-approved agent for patients with platinum failure, which demonstrated response rates of 16–21% in Phase II trials and 6–11% in Phase III trials (20–23). The 13.5-month median survival with PDX is noteworthy, although patient selection needs to be taken into account, because outcomes are improved among patients who have experienced regressions with previous therapy. To decrease the frequency of stomatitis, the dose of PDX was lowered to 135 mg/m² every 2 weeks.

In an effort to determine which patients would benefit most from treatment with PDX, we studied RNA levels of targets implicated in the drug's mechanism of action, specifically the folate transporter (RFC-1) and the enzymes responsible for polyglutamylolation, *FPGS*, and *FPGH*. We found that patients with stable disease had a higher mean RFC level than patients with disease progression. This could correspond with PDX's increased affinity for the folate transporter in comparison with other antifolates. Unfortunately, adequate pathologic samples were not available for the patients who had a partial response. We plan to expand this data set in subsequent trials.

Recent data have brought to the forefront the issue of vitamin supplementation for patients treated with antifolates. In the past, two sequential Phase II trials conducted by the Cancer and Leukemia Group B with edatrexate with or without leucovorin rescue in mesothelioma raised concerns that vitamin supplementation would decrease efficacy (24). Patients treated with edatrexate plus leucovorin experienced less mucositis, myelosuppression, and rash. However, the response rate decreased from 25 to 16%, and the median survival decreased from 9.6 to 6.6 months with the addition of leucovorin. More recently, however, an improved toxicity profile with vitamin supplementation has been noted in trials with the pemetrexed (ALIMTA). In a multivariate analysis, it was determined that the rate of severe toxicities with this agent correlated with elevated homocysteine levels, a marker of folate deficiency, and methylmalonic acid, a marker of B₁₂ deficiency (14). Subsequently, all patients in trials with pemetrexed were supplemented with folic acid and vitamin B₁₂. Patients who received such vitamin supplementation had a marked decrease in the overall rate of grade 4 hematological or grade 3/4 nonhematological toxicity (25). In a Phase III trial of pemetrexed plus cisplatin *versus* cisplatin alone in patients with advanced mesothelioma, supplementation with folic acid and B₁₂ not only improved the toxicity profile but also was associated with improved response and survival (26). In our Phase II trial of PDX, stomatitis was essentially the only clinically significant toxicity. Twenty percent of patients developed grade 3 or 4 mouth sores.

We measured homocysteine and folate levels as well as drug levels to identify predictors of stomatitis. Homocysteine and serum folate levels did not correlate with severity of stomatitis, although AUC (calculated using a limited sampling model) did show a correlation. A larger sample size, or measurements of RBC folate, rather than serum folate levels, may have yielded more interesting results. We are conducting similar analyses with ongoing PDX trials, and the data will be pooled to better assess these factors. In addition, ongoing and future studies of PDX have incorporated folate and B₁₂ supplementation. Preclinical data suggest that even high levels of folic acid supplementation do not impact the efficacy of PDX.⁴ Once the means of predicting or minimizing stomatitis are identified in ongoing PDX trials, this will allow optimal dosing for greater antitumor effect.

PDX has shown remarkable activity in murine xenograft models of a number of tumor types, including non-small cell lung, breast, prostate, and cervical cancers; mesothelioma; and lymphoma (8, 27, 28). Furthermore, the lack of myelosuppression with PDX makes it an ideal agent to combine with other chemotherapeutic agents. Mouse xenograft data suggest synergy with paclitaxel and docetaxel⁴ and the platinum (27). A Phase I trial of PDX plus taxanes is under way, and a trial of PDX plus cisplatin is planned. The broad applicability of this new antifolate with limited toxicity and proven efficacy in NSCLC provide enthusiasm for further development of this compound.

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