Phase I and Pharmacokinetic Study of 10-Propargyl-10-deazaaminopterin, a New Antifolate

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ABSTRACT

The 10-deazaaminopterins are a new class of rationally designed antifolates demonstrating greater antitumor effects than methotrexate in murine tumor models and human tumor xenografts. Their design was aimed at improving membrane transport and polyglutamylation in tumor cells, resulting in increased intracellular accumulation and enhanced cytotoxicity. Compared with other 4-aminofolate analogues, 10-propargyl-10-deazaaminopterin (PDX) is the most efficient permeant for the RFC-1-mediated internalization and substrate for folypolyglutamate synthetase. PDX demonstrates greater in vitro and in vivo antitumor efficacy than methotrexate or edatrexate. We undertook a Phase I study with PDX to identify the potential toxicities and define an optimal dose and schedule. Thirty-three patients were enrolled, all of whom had non-small cell lung cancer (NSCLC) and were treated previously with a median of two prior chemotherapy regimens. 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 with a total of 102 four-week cycles (median, 2 cycles/patient). Mucositis was the dose-limiting toxicity, 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 is 150 mg/m^2 biweekly. At that dose level, the mean area under the curve was 20.6 μmolar h, and the mean terminal half-life was 8 h. Two patients with stage IV NSCLC had major objective responses, and five patients had stable disease for 7 (two patients), 9 (one patient), 10 (one patient), and 13 months (one patient). PDX is a new antifolate with manageable toxicity and evidence of antitumor activity in NSCLC. A Phase II trial in NSCLC and a Phase I trial with paclitaxel are under way. These studies will also quantitate the expression of genes controlling internalization (RFC-1) and polyglutamylation of PDX in tumor cells as correlates of response.

INTRODUCTION

The 10-deazaaminopterins are a new class of rationally designed antifolates demonstrating greater antitumor effects than methotrexate in murine tumor models and human tumor xenografts (1–3). Their design was aimed at improving membrane transport and polyglutamylation in tumor cells, resulting in increased intracellular accumulation and enhanced cytotoxicity. One of these analogues, edatrexate (10-ethyl-10-deazaaminopterin) has undergone extensive clinical testing and has been shown in Phase II trials to have significant activity in NSCLC, breast cancer, pleural mesothelioma, and malignant fibrous histiocytoma (4–10).

Further modification at the carbon 10 position with a propargyl group resulted in a compound with even greater in vitro and in vivo antitumor efficacy than edatrexate. PDX (Fig. 1) demonstrated cytotoxicity 10–20-fold greater than methotrexate and 3–4-fold greater than edatrexate in human tumor cell lines. Mouse studies using human tumor xenografts (MX-1 mammary carcinoma, LX-1 lung carcinoma, and A549 squamous cell lung cancer) showed 2–4-fold more complete regressions and cures with PDX compared with edatrexate. In the same model system, methotrexate only delayed tumor growth without sustained regression. PDX was also better tolerated than either methotrexate or edatrexate. Compared with aminopterin, methotrexate, and edatrexate, PDX is a more efficient permeant for RFC-1-mediated internalization and substrate for folypolyglutamate synthetase in tumors. This is felt to be the mechanism underlying its improved therapeutic activity (11).

The preclinical toxicity of PDX is similar to that of methotrexate and edatrexate. The most sensitive tissue is the gastrointestinal tract. Beagle dogs are most sensitive to toxicity at the 100 mg/m^2 dose level (5). PDX is the most efficacious antifolate (10×) for the treatment of cutaneous T-cell lymphoma (5, 6). Two patients with stage IV NSCLC had major objective responses, and five patients had stable disease for 7 (two patients), 9 (one patient), 10 (one patient), and 13 months (one patient). PDX is a new antifolate with manageable toxicity and evidence of antitumor activity in NSCLC. A Phase II trial in NSCLC and a Phase I trial with paclitaxel are under way. These studies will also quantitate the expression of genes controlling internalization (RFC-1) and polyglutamylation of PDX in tumor cells as correlates of response.

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3 The abbreviations used are: NSCLC, non-small cell lung cancer; AUC, area under the curve; PDX, 10-propargyl-10-deazaaminopterin; HPLC, high-performance liquid chromatography.

4 T. C. Chou and F. M. Sirotnak, unpublished results.
from PDX. A weekly \( \times 3 \) schedule was used based on previous experience with edatrexate and the clinical schedule of weekly methotrexate treatment in patients with solid tumors. On this schedule, dosages above 6 mg/kg in beagle dogs resulted in transient disturbances in liver function and mild to moderate myelosuppression. The LD\(_{50}\) in dogs was 8 mg/kg for PDX (120 mg/m\(^2\)) compared with 6 mg/kg for edatrexate (90 mg/m\(^2\)).

Based on this preclinical data, we undertook a Phase I study with PDX to identify the potential toxicities and define an optimal dose and schedule in humans.

**PATIENTS AND METHODS**

**Patient Eligibility.** Adults with pathologically confirmed, advanced solid tumors and a Karnofsky performance status \( \geq 70\% \) were eligible. Patients must not have received chemotherapy or radiation therapy for 3 weeks before entering the study. No prior treatment with antifolates was permitted. Patients were required to have adequate hematomatologic function (WBC \( \geq 4,000 \) mm\(^3\), platelets \( \geq 160,000 \) mm\(^3\)), renal function (serum creatinine \( \leq 1.2 \) mg/dl or creatinine clearance \( \geq 50 \) ml/min/1.7 m\(^2\) body surface area), and hepatic function (total bilirubin \( \leq 1.0 \) mg/dl, aspartate aminotransferase < 3 x the upper limits of normal). Patients with treated or clinically stable brain metastases were allowed. Patients with clinically significant effusions, ascites, or edema and patients with a prior pneumonectomy were excluded. Folic acid supplementation was not permitted. All patients gave written informed consent. This trial was reviewed and approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center.

**Pharmaceutical Information.** PDX was prepared at the Memorial Sloan-Kettering Cancer Center as described previously (12). It 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 7.0 with 1N NaOH. The solution was then sterilized by means of filtration through a 0.20-\( \mu \)m Acrodisc filter. Each batch of PDX was checked for purity greater than 97\% using spectrophotometric criteria. The sterilized solution was protected from light and checked for purity greater than 97\% using spectrophotometric criteria. PDX in solution is stable for 60 days at 4°C. PDX was administered i.v. by bolus injection through the side arm of a freely running i.v. line containing normal saline.

**Treatment Plan.** An initial schedule and dose were chosen based on previous clinical experience with methotrexate and edatrexate and the preclinical toxicity data for PDX in dogs. The initial schedule involved treatment weekly for 3 weeks in a 4-week cycle, and the starting dose was 30 mg/m\(^2\). Dose-limiting toxicity occurred at the starting dose on the weekly schedule. The schedule was then modified so that patients received treatment every 2 weeks in a 4-week cycle. With the biweekly schedule, dose escalation proceeded from 15 to 30 mg/m\(^2\) and then proceeded in roughly 10-mg/m\(^2\) increments until the maximal tolerated dose was reached.

One patient was enrolled per dose level, with expansion to three patients if any grade 2 toxicity or greater was observed in the first cycle of therapy. Dose-limiting toxicity was defined as any grade 3 nonhematological toxicity, any grade 4 hematological toxicity or neutropenic fever, or a grade 3 hematological toxicity requiring treatment delay beyond 2 weeks. If a dose-limiting toxicity occurred, that dose level was expanded to six patients. The maximal tolerated dose was defined as the dose level at which two of six patients developed dose-limiting toxicities. The dose level below the maximal tolerated dose was considered the recommended Phase II dose, and this level was expanded by six additional patients to further define potential toxicities. Toxicities were graded using National Cancer Institute Common Toxicity Criteria.

Doses of PDX were attenuated for mucositis. Grade 1 mucositis on the day of treatment resulted in a 50\% dose reduction. For patients with grade 2 mucositis, doses were held until resolution, and then patients were retreated at full dose. For patients with grade 3 or 4 mucositis, doses were held until resolution, and then patients were retreated with a 50\% dose reduction.

At baseline, all patients provided a history and underwent a physical examination, a complete blood count, biochemical profile, prothrombin time, activated partial thromboplastin time, lactate dehydrogenase, and urinalysis. Patients then underwent a physical exam weekly for the first 4 weeks and subsequently on days of treatment. A complete blood count, biochemical profile, prothrombin time, and activated partial thromboplastin time were repeated with each treatment. A chest X-ray and any other imaging studies necessary to evaluate indicator lesions (if present) were obtained at baseline and then monthly while the patient was on therapy.

**Pharmacokinetics.** Pharmacokinetic studies were performed on selected patients. Heparinized blood samples were obtained with the first two treatments and collected before the
Table 1 Patient characteristics

| N | 33 |
| NSCLC | 100% |
| % Female | 55% |
| Karnofsky performance status | |
| 90% | 24% |
| 80% | 48% |
| 70% | 27% |
| Median no. of prior chemotherapy regimens | 2 (range, 1–4) |
| Prior radiation | 55% |
| Median age (yrs) | 57 |

injection, at the end of the injection (time 0), and 5, 10, 20, 30, and 60 min and 2, 3, 4, 5, 6, 8, 24, 30, and 48 h after the injection. Urine was collected pretreatment (10 ml) and then from 0–4, 4–8, 8–24, and 24–48 h. Because of its fluorescence intensity, PDX can be detected in body fluids using a highly sensitive and specific HPLC assay (13). The HPLC assay uses an Econosphere C18 column and a mobile phase consisting of 15% acetonitrile (v/v) and 50 mM KH₂PO₄ (pH 7) at a flow rate of 1 ml/min. The AUC was calculated using the linear trapezoidal rule with extrapolation from the last time point to infinity using the slope of the linear regression line through the terminal elimination phase. The pharmacokinetic calculations were performed by noncompartmental analysis using the WinNonLin software package.

A limited sampling strategy was developed using the time versus concentration data from the first PDX injection as a training set and the data from the second PDX injection as a validation set (14). A stepwise regression analysis was applied to select a combination of time points at which the concentration levels could be used to predict the AUC.

RESULTS

Table 1 lists the characteristics of the 33 patients enrolled in this trial. All patients had NSCLC and had been treated previously with a median of two other chemotherapy regimens. More than half of the patients had received radiation therapy before entering this trial.

Initially, PDX was administered weekly for 3 weeks in a 4-week cycle starting at 30 mg/m². Six patients were treated on this schedule. Mucositis requiring a dose reduction and/or delay in the first cycle occurred in four patients at the initial dose level (30 mg/m²). This was the maximal tolerated dose for PDX given on this schedule.

The treatment plan was then modified to a biweekly schedule in 4-week cycles. Twenty-seven patients were treated in this fashion. A total of 102 four-week cycles of therapy were delivered (median, 2 cycles/patient). The dose levels and number of patients in each cycle are presented in Table 2.

Toxicities. Mucositis was the most common toxicity. It was also the dose-limiting toxicity, with grade 3 and 4 mucositis occurring in the first two patients treated at the 170 mg/m² dose level. The highest grade of mucositis seen in any patient at each dose level is summarized in Table 2.

Several other reversible toxicities were observed and are summarized in Table 3. Grade 2 transaminase elevation occurred in three patients. In each case, this occurred after several months of therapy and resolved after withholding a single treatment. All three patients subsequently resumed therapy without additional hepatotoxicity. One patient treated at the 170 mg/m² dose level developed a diffuse maculopapular, pruritic rash similar to that reported with methotrexate. One patient on the biweekly schedule (80 mg/m²) and one patient on the weekly schedule developed reticulonodular pulmonary infiltrates, but it was not possible to distinguish the drug effect from lung cancer progression or infection. Both patients were treated with steroids, and the patient on the biweekly schedule continued on therapy. One patient treated at the 130 mg/m² dose level who had significant eye irritation after treatment with docetaxel and vinorelbine developed reversible grade 3 conjunctivitis with PDX. Mild nausea was noted in four patients. Several patients reported blood-tinged nasal discharge, but no spontaneous epi-staxis occurred. No neutropenia was observed.

Pharmacokinetics. Serum and urine samples were obtained from three patients on the weekly schedule and from patients treated on the biweekly schedule at dose levels 120, 130, 150, and 170 mg/m². Samples were analyzed by HPLC; a sample chromatograph for a single patient is shown in Fig. 2. The small peak may be an uncharacterized metabolite of PDX because it often appears in the second time point. A summary of the pharmacokinetic parameters for each patient is presented in Table 4.

The calculated AUC for the initial dose for each patient is shown in Fig. 3. The mean AUC at each dose level sampled is as follows: (a) 30 mg/m², 5.0 μmol h (n = 3); (b) 120 mg/m², 8.2 μmol h (n = 1); (c) 130 mg/m², 29.8 μmol h (n = 3); (d) 150 mg/m², 20.6 μmol h (n = 9); and (e) 170 mg/m², 30.6 μmol h (n = 2). At 150 mg/m², the mean terminal half-life was 8 h, as measured after 17 infusions in 9 patients. The AUC did not change from the first to second dose on the biweekly schedule (P = 0.99, paired t test) or the weekly schedule. Additionally, the size of the early peak on the chromatogram, which likely represents a metabolite, remains small and without change between day 1 and day 15. This suggests that no induction or inhibition of metabolism occurred. Urinary excretion of the parent drug measured in two
patients was 6% and 20% at 24 h. The sample size was too small to establish a correlation between AUC and dose or between AUC and mucositis grade.

A limited sampling strategy was developed using a step-wise regression analysis to select a combination of time points at which the concentration levels could be used to predict AUC. The AUC can be best predicted ($R^2 = 0.93$) by obtaining sample concentrations after 5 [$C(t1)$] and 20 min [$C(t3)$] and applying the following formula: $AUC = -1136.066347 + 0.195951 \times C(t1) + 1.498976 \times C(t3)$.

**Responses.** Two patients with stage IV NSCLC had major objective responses. One patient at the 150 mg/m$^2$ dose level, who had been treated previously with vinorelbine plus docetaxel and then gemcitabine, had a 50% reduction in mediastinal lymphadenopathy. In one patient treated at the 170 mg/m$^2$ dose level, a biopsy-proven cervical lymph node metastasis disappeared after the first dose. One patient on the weekly schedule had stable disease for 7 months, and five patients on the biweekly schedule had stable disease for 7 months (90 and 130 mg/m$^2$), 9 months (80 mg/m$^2$), 12 months (150 mg/m$^2$), and 13 months (40 mg/m$^2$).

**DISCUSSION**

PDX is a 10-deazaaminopterin demonstrating markedly improved antitumor efficacy in human tumor xenografts compared with methotrexate and edatrexate. PDX has shown greater permeation and polyglutamylation in tumor cells, both of which are determinants of anticancer effects in model systems.

With weekly treatment for 3 weeks of a 4-week cycle, mucositis was dose limiting at the initial dose level of 30 mg/m$^2$. However, altering the schedule to every 2 weeks allowed much higher doses to be delivered (75 mg/m$^2$/week). Increased intracellular accumulation may explain, in part, why the weekly dosing used with other antifolates was more toxic with PDX. Mucositis was the dose-limiting toxicity, and the maximal tolerated dose was 170 mg/m$^2$. Other toxicities were mild and reversible. No neutropenia was observed in this heavily pre-treated group of patients. The pharmacokinetic parameters are similar to those reported for edatrexate (15). The recommended Phase II dose is 150 mg/m$^2$ every 2 weeks.

**Table 3** Selected toxicities for biweekly PDX

<table>
<thead>
<tr>
<th>Dose level (mg/m$^2$)</th>
<th>No. of patients entered</th>
<th>AST/ALT elevation</th>
<th>Rash</th>
<th>Pulmonary infiltrates</th>
<th>Conjunctivitis</th>
<th>Nausea</th>
<th>Epistaxis</th>
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*Numbers indicate the highest grade of toxicity seen for any patient at that dose level at any time on study. The National Cancer Institute Common Toxicity Criteria were used for grading. All toxicities listed occurred in one patient, except as noted in parentheses.

b AST, aspartate aminotransferase; ALT, alanine aminotransferase.

**Fig. 2** Sample chromatogram for a single patient.
Phase II trial of PDX as a first- or second-line therapy in patients with NSCLC has been initiated. Because levels of the RFC-1 transporter and the enzymes folylpolyglutamate synthetase and folylpolyglutamate hydrolase affect intracellular accumulation of PDX, reverse transcription-PCR will be performed on pathological samples to test the level of gene expression. In addition, a pharmacodynamic assessment will be conducted using the limited sampling strategy described above to determine whether AUC predicts toxicity (mucositis).

Schedule-dependent synergism between methotrexate or edatrexate and taxanes has been reported in cell culture models (16, 17), and these combinations have been evaluated in Phase I trials (18–20). One trial at the Memorial Sloan-Kettering Cancer Center treated 34 patients with edatrexate (days 1 and 15) and paclitaxel (days 2 and 16; Ref. 19). This therapy was well tolerated, with dose-limiting toxicity other than leukopenia apparently related to the taxane. Eight of 25 patients with NSCLC achieved partial responses. A second trial at Memorial Sloan-Kettering Cancer Center was carried out with edatrexate and paclitaxel given in the same sequence every 3 weeks to a total of 35 metastatic breast cancer patients (20). Therapy was well tolerated, with several dose-limiting but readily reversible nonhematological toxicities. A 48% major response rate (17% complete response rate) was obtained among 25 patients, some of whom had received prior adjuvant regimens containing methotrexate or doxorubicin. Evidence of marked potentiation and a lack of schedule-dependence in vivo was obtained for PDX with paclitaxel or docetaxel against the human LX-1 lung tumor in mice.5 Both of these combinations were curative in these experiments. The prior clinical results obtained with edatrexate and paclitaxel, the nonoverlapping toxicities, and the preclinical data described above support the development of a PDX and taxane combination. A Phase I study using an escalating dose of PDX administered together with a fixed dose of paclitaxel every 2 weeks has been initiated.

The uricosuric agent probenecid can enhance the efficacy of PDX. Net accumulation of classical antifolates and their polyglutamates is limited by their outward extrusion via a cMOAT/MRP-like ATPase (21). Probenecid will preferentially inhibit this enzyme. This was shown to result in an increase in net accumulation, greater cytotoxicity, and increased therapeutic efficacy of methotrexate against murine ascites tumors (22, 23). A marked improvement in the cytotoxicity and therapeutic efficacy of PDX in vivo was also obtained against human lung, breast, and prostate tumors by coadministration of probenecid (24). Based on these studies, a Phase I trial of this combination therapy is planned using simultaneous i.v. administration of PDX and probenecid.

In conclusion, the maximal tolerated dose of PDX is 30 mg/m² when given weekly for 3 weeks in a 4-week cycle or 170

Table 4 Pharmacokinetic results for patients treated with PDX

<table>
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<tr>
<th>Dose level (mg/m²)</th>
<th>First dose (mg)</th>
<th>AUC (µmol h)</th>
<th>t₁/₂ (h)</th>
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<sup>a</sup> Dose reduced due to toxicity.  
<sup>b</sup> Dose delayed due to toxicity.

**Table 4**

![Fig 3 AUC at selected dose levels for the first administration. Each data point represents one patient.](image)

and paclitaxel given in the same sequence every 3 weeks to a total of 35 metastatic breast cancer patients (20). Therapy was well tolerated, with several dose-limiting but readily reversible nonhematological toxicities. A 48% major response rate (17% complete response rate) was obtained among 25 patients, some of whom had received prior adjuvant regimens containing methotrexate or doxorubicin. Evidence of marked potentiation and a lack of schedule-dependence in vivo was obtained for PDX with paclitaxel or docetaxel against the human LX-1 lung tumor in mice.5 Both of these combinations were curative in these experiments. The prior clinical results obtained with edatrexate and paclitaxel, the nonoverlapping toxicities, and the preclinical data described above support the development of a PDX and taxane combination. A Phase I study using an escalating dose of PDX administered together with a fixed dose of paclitaxel every 2 weeks has been initiated.

The uricosuric agent probenecid can enhance the efficacy of PDX. Net accumulation of classical antifolates and their polyglutamates is limited by their outward extrusion via a cMOAT/MRP-like ATPase (21). Probenecid will preferentially inhibit this enzyme. This was shown to result in an increase in net accumulation, greater cytotoxicity, and increased therapeutic efficacy of methotrexate against murine ascites tumors (22, 23). A marked improvement in the cytotoxicity and therapeutic efficacy of PDX in vivo was also obtained against human lung, breast, and prostate tumors by coadministration of probenecid (24). Based on these studies, a Phase I trial of this combination therapy is planned using simultaneous i.v. administration of PDX and probenecid.

In conclusion, the maximal tolerated dose of PDX is 30 mg/m² when given weekly for 3 weeks in a 4-week cycle or 170

<sup>5</sup> F. M. Sirotnak, personal communication.
mg/m² when given biweekly, with mucositis as the primary toxicity. The recommended Phase II dose is 150 mg/m² every 2 weeks. Antitumor activity has been observed in NSCLC.

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Phase I and Pharmacokinetic Study of 10-Propargyl-10-deazaaminopterin, a New Antifolate

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