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ORIGINAL ARTICLE

A randomized phase II study of PX-12, an inhibitor of thioredoxin in patients with advanced cancer of the pancreas following progression after a gemcitabine-containing combination

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Abstract
Purpose This study evaluated PX-12, a novel small molecule inhibitor of the proto-oncogene (Trx-1), in patients with previously treated advanced pancreatic cancer (APC).
Methods PX-12 (54 or 128 mg/m^2) was administered by 3-hour IV infusion daily ×5 days every 21 days ($n = 17$). Patients were randomized to either 54 or 128 mg/m^2 and then stratified based on CA 19-9 level (≥1,000 vs. <1,000 U/ml) and SUV values on PET scans (≥7.0 vs. <7.0). The primary endpoint was based on a progression-free survival (PFS) at 4 months in ≥40% of patients, and required 40 patients in each arm. An amendment required elevated Trx-1 levels (>18 ng/ml) as an entry criteria after the first 17 patients were accrued.
Results Plasma Trx-1 levels were elevated in 3/28 (11%) patients screened for study. The grade of the expired metabolite odor was higher in the 128 mg/m^2 arm. Therapy was well tolerated, and Grade ≥3 adverse events were uncommon. The best response was stable disease in 2 patients. There was no consistent decrease in SUV, Trx-1 levels or CA 19-9 levels with therapy. No patients had a PFS of >4 months. Median PFS and survival were 0.9 months (95% CI 0.5–1.2) and 3.2 months (95% CI 2.4–4.2), respectively.
Conclusions Due to the lack of significant antitumor activity and unexpectedly low baseline Trx-1 levels, the study was terminated early. PX-12 does not appear to be active in unselected patients with previously treated APC.

Keywords PX-12 · Thioredoxin · Pancreatic cancer · Phase II · Second-line therapy

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Introduction

More than 200,000 deaths worldwide each year are attributable to pancreatic carcinoma [1]. The median survival for patients with metastatic or advanced pancreatic cancer (APC) is approximately 6 months [2]. Standard therapy for patients with APC consists of single agent gemcitabine or gemcitabine in combination with erlotinib, cisplatin or capecitabine [2–5]. Following first-line therapy with a gemcitabine-based regimen, a significant number of patients will maintain an adequate performance status and be able to tolerate a second-line therapy [6]. A recent phase III trial randomized patients to either 5-flurouracil (5FU), folinic acid or to the addition of weekly oxaliplatin to the same regimen of 5FU/folinic acid [7]. The interim results showed a statistically significant survival advantage for the oxaliplatin containing arm (26 vs. 13 weeks, $P = 0.014$). However, the outcome of patients who have progressed on a first-line

gemcitabine regimen is still poor with median survival of about 2–6 months [7, 8].

Thioredoxin-1 (Trx-1) is a low molecular weight (10–12 kDa) cellular redox protein found in the nucleus and cytoplasm [9]. Trx-1 is a proto-oncogene that stimulates tumor growth and inhibits both spontaneous and drug-induced apoptosis [8–10]. Plasma Trx-1 protein levels, presumably reflecting high tumor levels are significantly elevated (40–50%) in several human cancers types including gastric, colorectal, pancreatic, lung and breast cancer, and increased levels appear to correlate with shorter survival [11–14]. Elevated plasma Trx-1 levels as determined by an in-house ELISA assay have been defined in this study as being greater than threefold the median (5.4 ng/mL) of 89 normal volunteer samples [15]. Increased Trx-1 gene expression is also associated with increases in both hypoxia-induced HIF-1α levels and HIF-1 transactivation in cancer cells, resulting in increased VEGF production and enhanced tumor angiogenesis [16]. Trx-1 appears to have an important role in maintaining the transformed phenotype of some human cancers as well as their resistance to chemotherapeutic drugs and, thus, is a rational target for cancer drug development [9, 17].

PX-12 is a small molecular irreversible inhibitor of Trx-1 [18]. A Phase I study of PX-12 in patients with advanced solid tumors was previously reported [19]. Patients ($n = 38$), received treatment with PX-12, given intravenously for 1 or 3 h on a daily ×5 schedule repeated every 21 days. Therapy at doses from 9 to 226 mg/m^2 daily on this schedule was well tolerated. The metabolism of PX-12 was found to result in the release, in expired breath, of an irritant, 2-butanethiol, a low molecular weight thiol, the same compound that is added to natural gas to enable detection of gas leaks. The study demonstrated that cough and an odor related to the expiration of this metabolite was dose related. Dose-limiting toxicities observed were Grade 3 hypoxia and Grade 2 reversible bilateral pneumonitis at the 300 mg/m^2 dose level. Stable disease was seen in 6 patients. Circulating levels of Trx-1 were lowered with therapy at all dose levels, and a Trx-1 decrease of ≥25% was associated with increased survival [19].

We evaluated the activity of PX-12 in APC, a cancer that has been reported to have high levels of the target proto-oncogene Trx-1, and that also exhibits significant hypoxia with elevated HIF-1α levels [20, 21]. The two dose levels of PX-12 chosen for this Phase II study were 54 and 128 mg/m^2 administered IV over 3 hours daily for 5 days every 21 days. In Phase I, each of these dose levels produced mild, tolerable levels of odor and cough, and resulted in decreases in plasma Trx-1. The primary endpoint of this study was to evaluate progression-free survival (PFS) at 4 months. Additional endpoints included response rate, overall survival (OS), plasma Trx-1 levels, grade of metabolic type odor during the first hour of infusion on Day 1 of Cycle 1. We also evaluated changes in CA19-9 and PET scans [22], as novel intermediary biomarkers as a means of detecting early signs of activity with therapy. APC appears to be to glucose avid and thus easily visualized by PET scans [23]. In addition, recent studies suggest that a decrease in CA 19-9 level with therapy may be an early marker of response and improved overall survival [24, 25].

Patients and methods

Patient eligibility

Eligible patients had histologically or cytologically confirmed APC (stage IV disease only) and had progressed on gemcitabine or on a gemcitabine-containing combination regimen. Karnofsky Performance Status (KPS) was required to be ≥70%, and patients had received no more than two prior regimens for metastatic disease. All patients had discontinued previous anti-cancer therapy at least 3 weeks (or within 5 half lives of the drug) prior to entry (6 weeks for mitomycin C or nitrosureas), and all toxicities from prior treatment had resolved to Grade 1 or less; prior radiation therapy was discontinued at least 2 weeks prior to entry. A CA19-9 level >2 ×ULN, measurable disease by RECIST criteria and an 18F FDG-PET scan showing an standardized uptake value (SUV) of ≥5.0 in at least one lesion were required at study entry. Adequate bone marrow, liver and renal function was documented by ANC ≥1,500 cells/μL; platelets >100,000/μL; hemoglobin ≥9 g/dL, bilirubin ≤2.0 mg/dL; aspartate transaminase (AST) and alanine transaminase (ALT) ≤3.0× institutional upper limit of normal (ULN) OR <5 ×ULN if documented liver metastases, and creatinine ≤2.0 mg/dL. Patients with concurrent active infections, pulmonary disease (COPD, asthma) or evidence of interstitial pneumonitis or pulmonary fibrosis were excluded. Additionally, patients that were pregnant or breast feeding had known or suspected brain metastases were also excluded. The study was approved by institutional review boards at participating sites, and all patients provided written informed consent for participation in the trial prior to starting therapy. After 17 patients were accrued to the study, a protocol amendment was implemented that required all patients to have a plasma Trx-1 level of >18 ng/mL.

Treatment plan and dose modifications

Patients were stratified based on CA 19-9 level (≥1,000 vs. <1,000 U/ml) and SUV value (≥7.0 vs. <7.0) and then centrally randomized to receive PX-12, at either 54 mg/m^2 or 128 administered by 3-hour continuous intravenous

infusion (in 250 mL) daily for 5 days every 21 days (one cycle). Due to concerns of thrombo phlebitis in animal studies, PX-12 was administered via a central venous catheter, and patients were placed on low dose coumadin (1 mg/day) during therapy [18]. PX-12 was administered in a negative air pressure room with an in-room filtration system (i.e. carbon filter air purifier). Prophylactic antiemetics were not required for cycle 1. Treatment was discontinued for disease progression, unacceptable toxicity, more than 2 required dose reductions, patient non-compliance, or withdrawal of patient consent.

In patients experiencing Grade 3 or 4 toxicities determined to be related to study drug, the PX-12 dose was to be reduced by 25% for the next cycle of therapy. Dose reduction was not required in the case of any grade of alopecia or Grade 3 toxicity, such as nausea/vomiting or diarrhea that could be controlled with supportive care. In the absence of clinical benefit, patients requiring more than 2 dose reductions or a treatment interruption of greater than 2 weeks were withdrawn from the study. Patients that experienced any toxicity regardless of relationship were required to recover to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE-V3.0) Grade 1 or baseline severity prior to continuing study drug. If an interruption in treatment was required within a treatment cycle (during treatment administration days 1–5), missed doses were not made up and the patient resumed treatment on the next cycle once the toxicity had resolved. Delay in treatment was permitted for up to 2 weeks to recover from treatment-related toxicity.

Assessments

Baseline tumor evaluations were obtained within 30 days before the start of treatment and repeated every 2 cycles. Plasma Trx-1 protein levels were collected prior to study drug initiation as well as at the end of the infusion (within 15 min of the end of infusion) on Day 1 and Day 5 of each cycle, and on Day 8 and 15 of Cycles 1 and 2. Plasma Trx-1 levels were analyzed using an ELISA assay developed by ProlX Pharmaceuticals. A dedicated PET scan was performed at baseline (within 21 days of treatment start) and repeated at the end of Cycles 1 and 2; changes in SUV were assessed by the EORTC criteria [22]. Serum CA 19-9 levels were collected at baseline (within 30 days of treatment start) and prior to every cycle.

Safety evaluations, performed prior to the start of each cycle included serial physical exams, and hematology and chemistry laboratory studies as well as monitoring of adverse events. Assessments of expired air on Day 1 of Cycle 1 (30 min into the infusion, at the end of infusion and 2 h following the end of infusion) were used to determine the extent of odor generated by the 2-butanethiol metabolite of PX-12. The five point scale used to assess odor was as follows: no detectable odor**,** barely perceptible, easily perceptible, moderate and obnoxious.

Trial design and statistical methods

This was an open-label, randomized Phase II study of two dose levels of PX-12. Treatment allocation was performed using random permuted blocks with stratification by CA 19-9 level ($\geq$1,000 vs. <1,000) and SUV value ($\geq$7.0 vs. <7.0). Sample size was based on a clinically meaningful 4-month progression-free survival (PFS) rate of 40% or more, with a 4-month PFS rate of less than 20% not considered to be of interest. Based on an exact test for a single binomial proportion at the one-sided 0.10 significance level, a sample size of 40 patients in each arm was expected to provide a power of 92%. A planned interim analysis for assessment of futility was to be performed at the time when 23 total patients had been followed for 4 months (or until progression, if earlier). For the results of this study to be clinically meaningful, it was expected that $\geq$5 of the first 23 patients, irrespective of the dose level, would meet the PFS endpoint at the interim analysis.

Data from the phase IB study of PX-12 suggested that elevated levels of Trx-1 (>18 ng/ml) may correlate with efficacy [26]. Following enrollment of the first 17 patients (16 treated), of which three had elevated Trx-1 levels >18 ng/ml, the protocol inclusion criteria were amended to require that patients have a screening Trx-1 level of >18 ng/ml.

Results

Patient characteristics

A total of 17 patients were randomized between January 2007 and June 2008. One patient was not treated due to rapid disease progression prior to study drug administration. Patient characteristics are listed in Table 1. Fifteen patients had one regimen, and 2 had received 2 regimens prior to study entry. Median baseline CA19-9 values in the two arms were 5,344 and 5,773 U/mL, and median baseline SUV values were 13.4 and 14.4, respectively. After accrual of the first 17 patients, none of the next 11 patients had Trx-1 levels >18 ng/ml. As none of the initial treated 16 patients had a PFS of >4 months, the investigators closed the study to patient accrual in February 2009.

Treatment administration

The median duration of therapy was 2 cycles. The maximum number of cycles administered was 4 cycles. There were no dose reductions reported; dose delays or interruptions occurred in 3 patients due to flushing caused by an infusion reaction, tachycardia or persistent cough.

Table 1 Baseline patient characteristics

Characteristic	54 mg/m^2 No. (%)	128 mg/m^2 No. (%)
Age		
Median	69	59
Range	53–81	40–78
Sex		
Male	8 (80%)	6 (86%)
Female	2 (20%)	1 (14%)
Race		
White	10 (100%)	7 (100%)
KPS performance status		
100	4 (40%)	1 (14%)
90	2 (20%)	3 (43%)
80	4 (40%)	3 (43%)
Median	90	90
CA19-9		
>1,000 U/mL	7 (70%)	7 (100%)
<1,000 U/mL	3 (30%)	
SUV		
>7	7 (70%)	6 (86%)
<7	3 (30%)	1 (14%)
Trx-1*		
>18 ng/mL	2 (22%)	1 (14%)
<18 ng/mL	6 (67%)	4 (57%)
Hemolyzed sample	1 (11%)	2 (22%)

* Trx-1 levels were only collected for (treated) patients prior to first dose of study drug

Table 2 Selected toxicities (all cycles)

N = 16	Grade 1/2	Grade 3/4
Blood/bone marrow		
Hemoglobin	3 (19%)	0
Constitutional		
Diaphoresis	2 (13%)	0
Fatigue	7 (44%)	1 (6%)
Fever without neutropenia	1 (6%)	0
Infusion reaction	1 (6%)	0
Rigors/chills	2 (13%)	0
Gastrointestinal		
Anorexia	6 (38%)	0
Constipation	3 (19%)	0
Diarrhea	3 (19%)	0
Dry mouth	2 (13%)	0
Dysgeusia	1 (6%)	0
Nausea	7 (44%)	1 (6%)
Vomiting	1 (6%)	0
Metabolic/laboratory		
AP	2 (13%)	0
AST	1 (6%)	1(6%)
Neurology		
Cognitive disturbance	1 (6%)	0
Dizziness	2 (13%)	0
Tingling fingers/toes	2 (13%)	0
Ocular/visual		
Dry eye	2 (13%)	0
Pain		
Head/headache	1 (6%)	0
Joint	0	1 (6%)
Muscle	0	1 (6%)
Pulmonary/upper respiratory		
Cough	5 (31%)	0
Dyspnea	0	1 (6%)
Pulmonary emboli	0	1 (6%)

Scored by investigator as possibly, probably, or definitely related to therapy

AST serum aspartate aminotransferase, *AP* alkaline phosphatase

Safety

Overall PX-12 was well tolerated. Grade ≥3 events were uncommon (Table 2), and there were no significant difference in total adverse events between the two arms. Pertinent Grade 1 or 2 toxicities were increased in the 128 mg/m^2 arm compared to the 54 mg/m^2 arm (fatigue 71% vs. 30%, nausea 71% vs. 30%, anorexia 43% vs. 30%, cough 43% vs. 20%), respectively. Reports of moderate/obnoxious odor were also greater in the higher dose arm, 56% versus 28%, with maximum odor reported at 30 min into the infusion and at the end of the infusion. Of note, one patient treated at 128 mg/m^2 required interruption of the infusion on three occasions due to a cough considered related to the expired metabolite.

Efficacy

Median PFS for all patients was 0.9 months (95% CI 0.5–1.2 months), and 0.9 versus 0.6 months in the 54 and 128 mg/m^2 arms, respectively. No patients had PFS lasting >4 months. The median OS was 3.2 months (95% CI 2.4–4.2 months). There was no consistent decrease in CA 19-9 levels or SUV (Table 3). The best observed response was stable disease in two patients (12.5%, Table 3). Baseline and serial Trx-1 levels were available for 13 patients; in the 3 other patients, the baseline sample was hemolyzed and unsuitable for analysis. There was no significant decrease in Trx-1 levels during therapy in patients from either dose group.

Table 3 Summary of responses of patients treated

Subject ID	Dose (mg/m^2)	% change in SUV	Maximal % change in CA 19-9 (U/ml)	% change in Trx-1 from baseline (ng/ml)	Best overall tumor response
01-002	54	−27	+156.3	Hemolyzed	PD
01-004*	54	−28	+75.9	+105.5	SD
01-005*	54	−13	+448.7	ND	PD
01-006	54	+127	+198.7	+539.7	PD
01-007	54	+113	+431.7	+83.0	PD
01-010*	54	0	NA	NA	PD
01-011	54	+5	+254	+109.6	SD
03-004	54	+16	NA	NA	PD
03-005	54	−6	NA	NA	PD
01-012*	128	+25	+49.9	+91.6	PD
01-001	128	−6	−14.7	+136.7	PD
01-003	128	0	+2024.8	NA	PD
01-008*	128	NA	NA	NA	PD
03-001*	128	+20	NA	NA	PD
03-002	128	+47	+571.8	Hemolyzed	PD
03-003*	128	+39	+184.5	+169.2	PD

* Subjects 03-001, 03-004, 03-005, 01-003, 01-010 discontinued study at the end of C1

Subject 01-008 discontinued the study early on C1D8 due to disease progression, prior to C1 PET

Subject 01-012 discontinued the study at the end of C2 for disease progression and did not return for scan

CA19-9 maximal change FROM BASELINE, assessed at baseline and prior to every cycle

Trx-1 level plasma: Trx-1 levels measured from samples collected prior to study drug initiation on Day 1 and Day 5 of each cycle and at the end of the infusion, and on Day 8 and 15 of Cycles 1 and 2. Percent change in Trx-1 levels is calculated from C2 day 1 to baseline, for subject 01-011 percent change for C2, C3 and C4 are reported

SUV- PET scans performed at baseline and Day 21 of Cycles 1 and 2 (between Days 17 and 21)

ND not done, *NA* not applicable as patient discontinued study at the end of C1, *PD* progressive disease, *SD* stable disease

Discussion

Treatment with PX-12 was not effective in patients with gemcitabine pretreated APC. No responses were observed, and stable disease was observed in only 2/16 patients. Clinically meaningful PFS was not observed. Overall PX-12 was well tolerated. The most common side effects included mild to moderate cough, fatigue, nausea and anorexia. Although PX-12 was administered in a negative pressure room with in-room air filtration, a "garlic" like odor (secondary to 2-butanethiol expiration) was observed in the majority of patients during the PX-12 infusion, although less in the lower dose, and while generally tolerable for patients, was less well tolerated by some hospital staff**.** The need for negative pressure ventilation and filtration systems will limit the ability of most cancer centers to administer PX-12 as a 3-h infusion. Longer duration infusions may be better tolerated, and we have explored continuous administration of PX-12 over 24–72 h using an ambulatory pump device, as a means of limiting the impact of odor related to the expired metabolite on patients and staff [26].

Changes in CA19-9 and/or PET scans during therapy may correlate to outcomes. In a phase I/II study conducted by members of our group, patients with untreated APC were treated with gemcitabine and nab-paclitaxel [23]. A total of 62 patients were entered. This study demonstrated that a decrease in SUV as measured by the EORTC criteria [22] during therapy may be an early marker of response to therapy. Similarly changes in CA19-9 levels were seen, and a maximal decrease of CA19-9 by >40% correlated strongly with overall radiological response. Thus, both CA19-9 and PET-SUV changes with therapy can be useful early intermediary endpoints to assess effectiveness of new agents in APC. In addition, clinical trials using these intermediary endpoints would require smaller number of patients to establish efficacy of new agents compared to traditional study designs measuring response, PFS or OS.

Our observation that only a small percentage (11%) of pancreatic patients screened for our study had elevated plasma levels of Trx-1 was surprising, and seemingly inconsistent with the study reported by Nakamura et al. [12]. This percentage was also lower than that found using the in-house ELISA assay to assess a set of 20 pancreatic

cancer patients' plasma samples obtained from the NCI's Cooperative Human Tissue Network (CHTN) where 35% of the patients had plasma Trx-1 $\geq$18 ng/mL [15]. The level of plasma Trx-1 was found to be much lower (range 1.5–19.8 ng/mL; mean 6.6 $\pm$3.8 ng/mL) in normal volunteers using our assay compared to published studies using a commercially available Trx-1 assay (Redox Bioscience, Kyoto, Japan, range 13.2–36.8, median 18.0 ng/ml) [27]. The difference in antibody specificity between these assays or alternatively, differences in pre-analytical variables may also contribute to this observation. Trx-1 is made and released by platelets [28], and differences in centrifugation times, speed and other pre-analytical variables may influence the values measured. Extracellular Trx-1 (plasma) levels have been reported in a variety of diseases in addition to cancer including autoimmune disease, sepsis, coronary artery disease, sleep apnea and thus make interpretation of reported values difficult to interpret without corresponding clinical annotation documenting concomitant diseases in the patients from which the samples were obtained [29–32]. Strenuous exercise can also result in transient increases in plasma Trx-1[33]. Therefore, multiple factors may have contributed to our finding that plasma Trx-1 was lower than expected in patients otherwise eligible for our current study.

We believe that targeting Trx-1 remains a valid strategy. However, many methodological issues remain and a reproducible robust assay needs to be utilized and tumors that are likely to benefit from this strategy needs to be identified. Considering our observation that PX-12 showed no significant activity as monotherapy in this study population, future studies with thioredoxin-system inhibitors should perhaps be designed to investigate combination therapies with chemotherapies including platinum agents where Trx-1 overexpression is associated with drug-resistance [34, 35].

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