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A phase IB trial of 24-hour intravenous PX-12, a Thioredoxin-1 inhibitor, in patients with advanced gastrointestinal cancers

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Abstract

We investigated the safety, pharmacokinetics, and pharmacodynamics of PX-12, a thioredoxin-1 (Trx-1) inhibitor, administered as a 24-hour infusion every 7 or 14 days in patients with gastrointestinal malignancies. PX-12 is the first Trx-1 inhibitor to undergo clinical development. The first Phase 1 study of PX-12 demonstrated promising clinical activity, but the 1 and 3 hour-infusion schedules investigated were associated with a strong and irritating odor due to exhalation of one of its metabolites, 2-butanethiol. In an effort to achieve tolerability and achieve a drug exposure level necessary for biological activity, the current study was undertaken. While the maximally tolerated dose was estimated to be 300 mg/m² /24 hour once a week as the 2-butanethiol expirate was tolerable at that dose level, no evidence of clinical activity was observed. Pharmacokinetic studies of the parent compound PX-12 demonstrated rapid, irreversible binding to plasma components, resulting in low (ng/ml) peak plasma concentrations of non-bound PX-12 during infusion. DCE-MRI was performed pre-and post-infusion in three patients. There were no significant trends observed in changes in plasma Trx-1, vascular endothelial growth factor (VEGF), or beta fibroblast growth factor (FGF-2) pre- or post-treatment. However, there was a trend for a decrease in circulating Trx-1 during the first four PX-12 treatment cycles in patients that had a Trx-1 baseline level >18 ng/mL. Aggregate clinical trial results suggest that further clinical development of PX-12, as an intravenous infusion, is not feasible. However, the Trx-1 pathway remains a target of interest in patients with gastrointestinal malignancies.

Keywords

PX-12; Thioredoxin-1; GI Cancers; Redox

Introduction

Thioredoxin-1 (Trx-1) is a ubiquitously expressed 105 amino acid redox protein found in the cytoplasm and nucleus. Trx-1 undergoes reversible NADPH-dependent reduction by thioredoxin reductases^{1–4}. Trx-1 transfection of cancer cells stimulates tumor growth⁵ and inhibits both spontaneous and drug induced apoptosis⁶. Transfection with a redox inactive

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mutant Trx-1 fails to transform the cells, inhibits cell growth, potentiates apoptosis^{5,7} and blocks tumor formation by the cells in immunodeficient *scid* mice⁶. Trx-1 regulates several transcription factors including NF- κ B, glucocorticoid receptor, AP-1, and HIF-1 α ^{4;8-10}. Trx-1 over-expression increases VEGF production and stimulates neoangiogenesis, primarily via up-regulation of HIF-1 α ¹¹. Trx-1 is also secreted by cancer cells wherein it has growth promoting properties¹². Intracellular Trx-1 protein levels are significantly elevated in human gastrointestinal cancers including 50% of gastric cancer cases³, 55% of colorectal cancers¹³ and 41% of pancreatic cancer^{14;15}. Increased Trx-1 expression in colon cancer is an independent prognostic factor for decreased patient survival¹³. Elevation of plasma Trx-1 has also been reported in pancreatic and hepatocellular cancers^{15,16}.

PX-12 (1-methylpropyl 2-imidazolyl disulfide) is a novel small molecule inhibitor of Trx-1. The inhibitory action of PX-12 is primarily due to the irreversible thioalkylation of Trx-1 on its Cys73 residue in the non-catalytic domain¹⁷. PX-12 has also been shown to inhibit tubulin polymerization through cysteine oxidation¹⁸. PX-12 treatment results in anti-tumor activity in a variety of pre-clinical models, including the HT-29 human colon cancer cell line and is associated with a decrease in HIF-1 α and VEGF expression¹⁹. Consistent with this observation, pre-clinical investigations in HT-29 xenograft tumors demonstrated that PX-12 treatment caused a rapid 63% decrease in the average tumor microvascular permeability, measured by Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI), within 2 hours of drug administration. The decrease lasted 24 hours and returned to pretreatment values by 48 hours post-drug²⁰.

FGF-2 is a growth factor that can regulate a variety of activities including angiogenesis. FGF-2 can act synergistically with VEGF, and has been implicated in mediating resistance to anti-VEGF therapies [reviewed by²¹]. In a human microvascular endothelial cell (HMVEC) migration model, exogenous treatment with FGF-2 resulted in induction of thioredoxin interacting protein (TXNIP), a negative regulator of Trx-1. Further studies in this model demonstrate that FGF-2 treatment results in increased Trx-1 activity, and that repression of TXNIP with siRNA was sufficient to stimulate HMVEC migration²². Exogenous FGF-2 has recently been reported to induce the expression of Trx-1 in lung cancer cell lines²³. Together, these observations suggest that Trx-1 may exert some of its angiogenic activity via the FGF-2 pathway.

PX-12 has undergone early Phase 1 clinical testing in patients with various solid tumors²⁴, with the dose limiting toxicity being reversible chemical pneumonitis that occurred at a dose of 300 mg/m². Based on pharmacokinetic data and the observation that decreases in circulating Trx-1 levels were more pronounced after a 3 hour infusion compared to a 1 hour infusion, it was felt that prolongation of PX-12 infusion time may provide additional therapeutic benefit and improve tolerability. In a subsequent Phase 2 study of a 3-hour infusion every day for five days every 21 days was administered to patients with advanced cancer of the pancreas. This study was discontinued before an MTD was established for several reasons. These included an unexpectedly low level of circulating Trx-1, lack of significant antitumor activity, and toxicity, manifested as a cough and odor associated with the expired metabolite 2-butanethiol. A second Phase 1 trial of PX-12 investigated a 72-hour infusion every 21 days in 14 patients with advanced solid cancers. The longer infusion time

was associated with less odor and cough, but demonstrated limited clinical benefit. The best response was stable disease in a patient with rectal cancer [35].

Based on these results, we conducted a Phase IB trial of PX-12 in patients with advanced gastrointestinal tumor. The goal was to determine the maximally tolerated dose (MTD) of PX-12 delivered as a 24-hour infusion repeated every 7 or every 14 days. The secondary objectives were to assess pharmacokinetics, pharmacodynamics, and the efficacy of PX-12. The pharmacodynamic endpoints included dynamic-contrast-enhanced magnetic resonance imaging (DCE-MRI), pre- and post-dose drug administration in order to evaluate the effect of PX-12 on tumor perfusion and vascular permeability and plasma Trx-1, VEGF, and FGF-2 levels at baseline, and at the end of each treatment cycle.

Patients and Methods

Patient selection

Eligible patients were over 18 years of age with pathologically confirmed diagnosis of advanced or metastatic gastrointestinal (colorectal, gastroesophageal or pancreaticobiliary) cancer refractory to standard therapies. Other major criteria were: predicted life expectancy of at least 12 weeks, performance status of 0–2 Eastern Cooperative Oncology Group (ECOG), and no anticancer therapy or other investigational agent(s) for at least four weeks prior to study registration (six weeks for mitomycin C or nitrosureas). Patients had discontinued radiation therapy for at least four weeks prior to entry into the study and must have recovered (grade 1 or less) from all radiation-related toxicities. Adequate organ function was defined by WBC count $>2,500$ cells/ μ L, platelets $>100,000$ / μ L, hemoglobin >9 g/dL (may be transfused to this level), and an ANC >1500 cells/ μ L. Other laboratory requirements included creatinine <2.0 mg/dL, bilirubin <2.0 mg/dL, aspartate transaminase (AST/SGOT) and alanine transaminase (ALT) <3 times institutional upper limit of normal (ULN) or <5 times institutional ULN if the subject has documented liver metastases and international Normalized Ratio (INR) and activated partial thromboplastin time (PTT) within 1.5 times ULN unless subject was on prophylactic warfarin.

Patients were excluded from the study if they were pregnant or lactating, had any serious concomitant systemic disorders that in the opinion of the investigator would place the patient at excessive or unacceptable risk of toxicity or had surgery within 4 weeks of study entry. Other exclusion criteria were, a history of seizures, any untreated brain metastasis, unstable arrhythmias requiring control by medication, ischemic event experienced within the preceding six months, prior history of congestive heart failure requiring therapy, active infection requiring parenteral antibiotics at study entry, active (requiring continuous medical therapy) pulmonary disease (COPD, asthma) or evidence of interstitial pneumonitis or pulmonary fibrosis on baseline chest X-ray or chest CT scan, any active secondary malignancy diagnosed within the last 2 years, or inability to tolerate prophylactic (1 mg/day) warfarin.

Written informed consent was obtained from all patients before entering the study.

Study design

This was a Phase IB dose escalation study to establish the MTD of PX-12 administered every fourteen days. PX-12 was administered via a 24-hour infusion through a central venous catheter utilizing an infusion pump, on Day 1 of each 14-day cycle. Due to concern with local phlebitis observed in animal studies, patients also received a prophylactic dose of warfarin 1 mg/day. PX-12 dose started at 150 mg/m² and escalated to 200, 300, 400 and 450 mg/m² in cohorts of 3 patients per dose level. A standard “3+3” design was used. If one of three patients experienced dose limited toxicity (DLT), the cohort expanded to six patients. If there was no DLT, the dose was escalated to next cohort of three patients. If 2 DLT occurred at a dose level, then the previous dose was considered the MTD and dose escalation terminated. When the 14-day bi-weekly dosing cycle reached 450 mg/m² patient without evidence of drug accumulation or DLT's, the schedule was modified to evaluate cohorts on a weekly dosing schedule, starting at a lower dose level of 300 mg/m² of PX-12.

Drug administration

PX-12 was diluted with 5% dextrose solution, filtered, and administered using a portable ambulatory infusion device over 24 hours to allow outpatient treatment.

Safety assessments

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria CTCAE version 3.0. Dose-limiting toxicity (DLT) was defined as any of the following events related to PX-12 during the first treatment cycle (14 days): grade 4 hematologic toxicity or grade 3 or greater non-hematologic toxicity. The occurrence of a DLT or failure to recover to grade 1 within two weeks required patients to be removed from study. Patients who recovered to grade 1 or lower toxicity continued at the next lowest dose level or at 50% of the previous dose as long as their disease continued to be stable or responding.

In addition, a delay in the scheduled treatment for more than fourteen days due to ongoing treatment related toxicity was considered as a DLT. Patients receiving PX-12 in cycle 2 and beyond for bi-weekly and weekly dosing schedules with stable or responding disease who experienced DLT had their therapy held until their toxicity improved to grade 1 or less. Following this, therapy with PX-12 was continued at the next lower dose level. Any patient experiencing a second DLT at the lower dose was removed from study.

Pharmacokinetic analysis

Plasma was collected at pre-dose and end of the infusion for the first four cycles for pharmacokinetic analysis. For the bi-weekly schedule, plasma was also collected at 3 and 6 hours after the start of the first infusion. Plasma PX-12 concentrations were measured using liquid chromatography in tandem with mass spectrometry (LC-MS). Calibration curves were prepared in PBS for quantitative analysis because of the varying extents of interaction between PX-12 with human plasma obtained from different sources. Each calibration PBS standard or plasma sample was mixed with five volumes of acetonitrile. An aliquot of the supernatant was injected onto the LC-MS system. The chromatographic separation was achieved on a Hypercarb column (100 × 4.6 mm) with a gradient mobile phase consisting of 20 mM ammonium acetate and acetonitrile. PX-12 was detected by multiple reaction

monitoring with electrospray ionization in the positive mode. Linearity was established over the concentration range of 1 – 50 ng/ml. The reported plasma PX-12 levels represent the non-covalently bound PX-12 or PX-12 that has not undergone the thiol/disulfide exchange. Plasma concentrations of the primary metabolite 2-mercapto imidazole (2-MI) in the end of infusion samples were measured as described previously²⁴.

Plasma biomarker assessment

Normal donor blood samples were collected in heparinized tubes by the Red Cross or purchased from Bioreclamation Inc (City, State). The plasma was separated and stored at -70°C until analyzed. Patient plasma was isolated from whole blood collected in heparin coated tubes. Blood was centrifuged for 15 minutes at $1500 \times g$ at room temperature to sediment platelets and particulate matter. Plasma was aliquoted and frozen at -80°C until analyzed. Plasma samples for Trx-1, VEGF and FGF-2 protein level analysis were collected prior to study drug administration on day 1 and then 3 hours, 6 hours, and at the end of the infusion (24 hours) from the start of infusion in the first cycle. For three cycles thereafter plasma samples were taken prior to the infusion and at the end of the 24 hour infusion. The concentration of Trx-1 was measured as reported previously²⁵. The concentration of FGF-2-2, and VEGF was measured according to the protocol provided in R&D Systems (Minneapolis, MN) Human VEGF Quantikine ELISA Kit and Human FGF basic Quantikine HS ELISA Kit. All samples from each patient were analyzed together on one ELISA plate for each analyte (Trx-1, VEGF, FGF-2) in order to minimize day to day and plate to plate variation. Any sample with higher than 20% CV within triplicates was re-tested. Positive controls were run on each plate to ensure reproducibility between run days and reagent lots. Plasma samples were assessed for hemolysis to ensure analyte levels were not affected due to red cell lysis during sample processing.

Dynamic Contrast-Enhanced MRI

DCE-MRI was performed on a 1.5 T MRI scanner (GE) using an 8 channel phased-array coil (GE). DCE-MRI data were collected from subjects who repeated a “breathe-in, breathe-out and hold” pattern, with the imaging occurring during each held-expiration period. Prior to the dynamic portion of the scanning, four pre-contrast Three Dimensional Gradient Recalled Echo (3D-GRE) images were obtained at flip angles of 15° , 23° , 30° and 60° so that a pre-contrast T1 map could be calculated in each case. The dynamic series portion of the imaging comprised of 24–30 3D-GRE images collected during repeated “held exhalation” breath-holds. This afforded a temporal resolution of 16–18 seconds in the DCE-MRI series. Parameters for the 3D-GRE imaging were, 12 slices reconstructed to a matrix size of 256×256 , slice thickness=5 mm, TR=5.0 ms, TE=2.1 ms, and $\alpha=30^{\circ}$. After 1–2 pre-contrast images had been acquired as a baseline and to ensure adequate image quality, gadolinium contrast (Omniscan®, GE Healthcare) was injected at a dose of 0.1 mmol/kg at a rate of 4 mL/s using a power injector (Mallinckrodt Cincinnati, OH), and chased with a 20-ml saline flush also injected at 4 mL/s. Pixel-by-pixel pharmacokinetic analysis of DCE-MRI data was performed using a two-compartment model²⁶. Prior to pharmacokinetic analysis, sequential elastic registration was performed on the DCE-MRI images to reduce misregistration arising from inconsistent breathholding, as described previously²⁷. Three physiologically relevant parameters were calculated for each pixel, the volume transfer

constant (K^{trans}), volume fraction of the extravascular extracellular space (v_e), and plasma volume fraction (v_p), by fitting image data to the following equation:

$$C_t(t) = K^{trans} \int_0^t C_p(\tau) e^{-\frac{K^{trans}(t-\tau)}{v_e}} d\tau + v_p C_p(t) \quad (1)$$

where $C_p(t)$ is the arterial input function (AIF) calculated from a region-of-interest (ROI) placed over the descending aorta, and $C_t(t)$ is the volume-averaged concentration of the contrast agent in a pixel. $C_t(t)$ was calculated assuming a linear dependence of signal enhancement on the concentration of contrast agent, from the known in vitro longitudinal relaxivity of the contrast agent.

Efficacy assessment

Tumor assessments were performed within one week preceding PX-12 dosing and every 4th cycle (approximately every 8 weeks) until disease progression or study discontinuation.

Results

Patient characteristics

Eighteen patients were enrolled at Arizona Cancer Center, Tucson, AZ. All patients received at least one dose of PX-12. Patient characteristics are summarized in Table 1.

Treatment administration

The median time on study was 4 cycles or 8 weeks of therapy (Table 2). Five patients completed 4 cycles, four patients completed 2 cycles, four patients completed 1 cycle (all of them on a weekly schedule), three patients completed 3 cycles (one of which had weekly cycles), one patient completed 5 cycles and one completed 8 cycles. The reason for treatment discontinuation was progressive disease (PD) in 8 patients, adverse event in 2 patients, and death in 1 patient.

Toxicity

Treatment-emergent toxicities are summarized in Table 3. The only true dose –limiting toxicity (DLT), defined as occurring within first cycle of therapy was observed at a dose level of 400 mg/m². This involved grade 3 hyperbilirubinemia three days after the second dose in the first cycle, with resolution after twenty four hours. The same patient also had grade 3 fatigue and elevated serum alkaline phosphatase after the second dose of the first. Several patients experienced grade 3 or 4 toxicities beyond the first cycle of therapy. One of three patients at dose 150 mg/m² had a grade 3 fatigue after the fourth cycle. One of four patients at dose level 200 mg/m² developed a grade 3 pneumonia after the second cycle of study drug. Two of six patients at dose level 300 mg/m² (both of them received a weekly schedule) had grade 3 toxicity. One of these patients had a grade 3 cough, shortness of breath, hyponatremia, and hypoalbuminemia after the second cycle. The other patient at this dose level had grade 3 hyperbilirubinemia and elevated serum alkaline phosphatase after the third cycle (related to biliary tract obstruction from cancer). Two of three patients at the 450 mg/m² dose level developed grade 3 toxicities. One developed grade 3 fatigue, nausea, vomiting and small bowel obstruction 21 days after the second cycle of the study drug and

was then excluded from the study due to disease progression. The second patient at this dose level developed grade 3 small bowel obstruction, hypotension, and elevated INR following the third cycle.

Hematologic toxicity was uncommon, with only two of nineteen patients exhibiting grade 2 hematologic adverse events, one patient having anemia and the other patient having elevated prothrombin time. Another patient had grade 3 elevation of prothrombin time.

The most common non-hematologic toxicities were nausea, fever, vomiting, ascites, diarrhea, fatigue, garlic odor, sweating, weight loss, urinary tract infection, confusion, dysgeusia, constipation, abdominal distention, gastritis, anxiety, cold intolerance, flu like symptoms and cough. Most toxicities were grade 1 or 2.

Efficacy

There were no objective radiological responses based on the RECIST criteria. Two patients achieved apparent stable disease as their best response but only one stayed on study treatment longer than four months.

Pharmacokinetic Data

Figure 1 illustrates the cycle 1 individual concentration-time data from the 300 mg/m² bi-weekly cohort. PX-12 levels increased during infusion and reached a maximum concentration at the end of infusion. The collection times were limited to allow for calculation of pharmacokinetic parameters. Table 4 summarizes the plasma PX-12 and 2-MI concentrations at the end of the first infusion. Plasma concentrations of PX-12 were in the low ng/ml concentration range and seemed to increase as the dose increased. Plasma 2-MI concentrations were much higher than those of PX-12 and were in the low µg/ml concentration range. Analysis of pre- and post-cycle PX-12 concentrations from six subjects treated with up to 4 cycles indicated no apparent changes in the end-of-infusion PX-12 concentrations at different cycles.

Plasma Pharmacodynamic Data

Trx-1 plasma levels from frozen plasma of 89 healthy donors were measured via ELISA. The mean plasma Trx-1 level was 6.6 ± 3.8 ng/mL with a median value of 5.4 ng/mL and a range from 1.5 – 19.8 ng/mL (Figure 2). Increased plasma Trx-1 levels over the normal range were observed pretreatment in a sub-population of patients studied on this trial with concentrations ranging from 5.3 – 40.0 ng/mL (median = 17.4 ng/mL; mean = 19.8 ± 1.4 ng/mL). A cut off of >18 ng/mL (approximately 3-fold mean normal levels) was selected to stratify the data from this patient population to determine if starting Trx-1 could predict response to PX-12 treatment. In the six patients treated with bi-weekly PX-12 (regardless of treatment dose) that had Trx-1 levels of >18 ng/mL prior to treatment there was a trend towards decreased plasma Trx-1 following PX-12 treatment over the first 4 cycles. Due to the small number of patients in the study, statistical significance was only observed at the EOI time point in cycle 1 (paired Student T test p=0.03) (Figure 3A). Patients with Trx-1 levels under 18 ng/mL at the start of the study did not demonstrate a decrease in Trx-1 levels over the course of their treatment (Figure 3B). Two of the six patients (33%) whose plasma

Trx-1 levels were lowered following PX-12 treatment achieved apparent Stable Disease (SD) and stayed on study over 4 cycles (02-008 = 8 cycles, 02-009 = 5 cycles). No patients with pretreatment Trx-1 plasma levels under 18 ng/mL achieved SD on this study.

The concentrations of Trx-1, FGF-2, and VEGF at baseline (pre-dose) are provided in Table 5. The FGF-2 angiogenic factor was suppressed only in patients with PX-12-mediated decreases in Trx-1 levels. The two patients with apparent stable disease (subjects 02-008 and 02-009) had significantly lower plasma FGF-2 levels before cycle 3 ($p=0.03$) and cycle 4 ($p=0.05$) when compared to pre-treatment values by paired student T test analysis (Figure 4). VEGF levels, however, did not show any correlation with Trx-1 levels (data not shown) when stratified by Trx-1 pretreatment levels. Patients with stable disease did have minor decreases in plasma VEGF at the pre-dose cycle 3 (39% decrease) and cycle 4 (23%) time point compared to pretreatment values, but the decreases were not statistically significant.

Overall, decreases in plasma Trx-1, VEGF or FGF-2 were not predictive of clinical benefit (apparent stable disease) to PX-12 in this study. Two patients with apparent stable disease lasting beyond 4 cycles did have decreases in all three of these analytes over the first 4 cycles of treatment. Due to the very small number of patients with apparent stable disease in this study, it is not possible to conclude whether these changes in pharmacodynamic markers were mediated by PX-12 activity. Interestingly, the average plasma Trx-1 (19.8 ± 1.4 ng/mL) and VEGF levels (71.8 ± 4.6 pg/mL) were higher in this patient group as compared to the patients enrolled on the 72-hour infusion Phase 1 study of PX-12²⁸. This observation supports prior studies demonstrating high cellular expression of Trx-1 in GI tumors.

DCE-MRI

DCE-MRI imaging was performed on 3 subjects (Table 6). Figure 5 depicts example K^{trans} parameter maps calculated from a subject who received 400 mg/m² of the drug weekly by 24 hour infusion. In subjects with multiple tumors, the target lesion was chosen based on size (largest) and location within the liver (right lobe, less affected by cardiac pulsatility). In subject 02-004, dosed at 200 mg/m², no significant change in tumor K^{trans} was measured 24 hours following drug infusion. In subject 02-012, tumor K^{trans} appeared to decrease 24 hours following drug infusion at the 450 mg/m² dose level, rising back above baseline values by 7 days post-infusion. In subject 02-015, there was no significant change in tumor K^{trans} 24 hours following the first drug infusion at the 300 mg/m² dose level, but a decrease in tumor K^{trans} relative to baseline was observed following the 2nd of two biweekly infusions.

Discussion

PX-12 is the first targeted Trx-1 inhibitor to be investigated clinically. It has been evaluated in a total of four clinical trials. The current Phase 1B study in GI malignancies was designed for several reasons. The only minor response in the first Phase 1 trial of PX-12 was observed in a patient with appendiceal adenocarcinoma²⁴, and the best clinical response in the second Phase 1 study investigating the 72-hour infusion was SD observed in a patient with rectal cancer²⁸. Intracellular Trx-1 over-expression has been retrospectively correlated with advancing disease stage in colorectal cancers, and is associated with poorer survival¹³. In a

separate study, intracellular Trx-1 expression in resected liver metastases has also been shown to be an independent prognostic factor for poor prognosis in patients with colorectal cancers²⁸. Other gastrointestinal carcinomas have also been associated with Trx-1 levels. For example, intracellular Trx-1 protein has been reported to be over-expressed in gastric³ and pancreatic cancers²⁹.

In the current Phase 1B trial, a continuous 24 hour infusion schedule was chosen because of better tolerance observed with longer infusions, defined by less respiratory irritation, cough, and pungent sulfur odor from 2-buthanethiol, an exhaled metabolite of PX-12. This study was designed to optimize a dosing schedule and infusion rate that would circumvent this problem and still provide sufficient drug exposure for anti-tumor activity. In this trial we were able to escalate the dose of PX-12 beyond 300 mg/m² without encountering pneumonitis, which was a DLT with previous explored one and 3- hour infusion schedules.

Despite the ability to deliver higher doses and the improved tolerability of the exhaled metabolite 2-buthanethiol, we did not see any signs of objective anti-tumor activity. This lack of clinical activity could be due to failure to achieve optimal peak plasma concentrations of the parent PX-12 compound. In this study we measured the concentration of PX-12, as opposed to prior studies in which we were only able to detect 2-mercaptoimidazole²⁴, which is one of the main metabolites. Due to the highly reactive nature of PX-12, we observed low ng/mL plasma concentrations of PX-12 with the presence of µg/mL concentration of the inactive metabolite 2-MI. This observation suggests that prolonged infusion schedules resulted in inadequate intra-tumoral peak exposure to the active agent.

DCE-MRI was included as one of the pharmacodynamic endpoints in this study based on pre-clinical data indicating that PX-12 treatment results in decrease in tumor microvascular permeability, consistent with inhibition of VEGF expression²⁰. Only three patients were recruited to this optional portion of the study. Barriers to patient participation included patient anxiety associated with time in the scanner (approximately 30 minutes per scan) and difficulty in coordinating treatment administration with imaging time points. Interestingly, one patient with liver metastasis demonstrated a decrease in K^{trans} 24 hour post-treatment with 450 mg/m² PX-12, which was associated with a decrease in plasma VEGF levels from 19.3 pg/ml at baseline to 12.2 pg/ml at end of infusion.

Similar to prior studies with PX-12, there was a trend for patients starting with elevated plasma Trx-1 levels to have a reduction in Trx-1 plasma levels post-PX-12 treatment. Similarly, patients starting with the highest levels of FGF-2 had the greatest reductions in FGF-2 levels. Together, these observations suggest that PX-12 has some biological activity consistent with its reported mechanism of action in spite of the lack clinical activity observed in this study.

Given the lack of preliminary antitumor activity and an atypical toxicity profile (strong pungent odor, respiratory irritation and pneumonitis) it is difficult to propose further monotherapy studies with PX-12 in cancer patients. Nevertheless, because of mechanistic and translational studies, we believe that Trx-1 remains a target of interest for developing

anti-cancer therapies. Several other thioredoxin-system inhibitors are in pre-clinical development. These include PMX464, a thiol-reactive quinol, which inhibits Trx-1 function and has been shown to decrease proliferation and survival of HT-29 colon cancer cells, with enhanced activity in hypoxic conditions³⁰. Ethaselen, an organoselenium compound, inhibits thioredoxin reductase and has been shown to decrease cell growth of the RTO colorectal cancer cell line³¹. These and other Trx system inhibitors may prove to be more clinically viable, and should be investigated in the setting of GI malignancies.

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Figure 1.
Cycle 1 individual concentration-time data from the 300 mg/m² every 2 weeks cohort.

Figure 2.

Trx-1 plasma levels from frozen plasma of 89 healthy donors measured via ELISA. A cut off of 18 ng/mL (approximately 3-fold mean normal levels) was selected to stratify the pharmacodynamic data from this current PX-12 study.

Figure 3.

Plasma Trx-1 levels over 4 cycles of bi-weekly PX-12 treatment in patients with pretreatment plasma Trx-1 over 18 ng/mL. **3B.** Plasma Trx-1 levels over 4 cycles of bi-weekly or weekly PX-12 treatment in patients with pretreatment plasma Trx-1 under 18 ng/mL.

FGF-2 of patients with starting Trx-1 plasma Levels **OVER** 18 ng/mL

FGF-2 of patients with starting Trx-1 plasma Levels **UNDER** 18 ng/mL

Figure 4.

Plasma FGF-2 levels in patients with plasma Trx-1 values (Day 1) over or under 18 ng/mL. Only patients with evaluable starting FGF-2-2 levels are shown. In general, PX-12 treatment suppressed plasma FGF-2 in patients with high plasma Trx-1 yet had little effect on those patients with normal plasma Trx-1 levels.

Figure 5.

Anatomic reference MRI images and corresponding pixel-by-pixel maps of K^{trans} in subject 02-012. Target tumor (arrow) K^{trans} was low at baseline, decreasing at 24 h post-infusion, increasing above baseline by 7 days post-infusion. The color legend for K^{trans} runs from 0.0 min^{-1} (dark blue) through 0.6 min^{-1} (yellow). K^{trans} results for all study subjects are summarized in Table 5.

Table 1

Patient characteristics.

	N=18
Male	10
Female	8
Age Range	45–75
Median Age	65
Performance Status	
0	10
1	8
2	0
3	0
4	0
Tumor type	
Pancreatic cancer	4
Bile duct tumor	4
Colorectal cancer	8
Hepatocellular cancer	1
Neuroendocrine	1
Visceral metastases (lung and liver)	10

Table 2

PX -12 treatment administration.

PX-12 dose level mg/m ²	Number. of patients	Median number of cycles	Range of cycles
150	3	4	4
200	4	3	2--4
300	3	4	2--8
300*	3	2	1--3
400*	2	1	1--2
450	3	3	2--4

* Weekly PX-12 infusion schedule.

Table 3

List of treatment-emergent toxicities

PX-12 dose level (mg/m ²)	150	150	200	200	300	300	300	300*	300*	400	400	400*	400*	450	450*
Toxicity	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 3&4
Nausea			1										1		1
Vomiting			1										1		1
Ascites			1												
Diarrhea			1												
Sweating			1												
Fever			1												
Dyspnea			1											1	
Pneumonia				1								1			
Fatigue		1													
Pungent breath odor	1													2	
Weight loss														1	
Small bowel obstruct.															2
Urinary tract infection	1														
Confusion	1														
Dysgeusia	1														
Constipation				1											
Abdominal distention			1												
Anxiety	1														
Gastritis	1														
Cold intolerance	1														
Anemia														1	
Hyperbilirubinemia													1 ^a		
Hypoalbuminemia															
Elevated INR			1												1
Flu like symptom														1	
Cough														1	

PX-12 dose level (mg/m ²)	150	150	200	200	300	300	300*	300*	400	400*	400*	450	450*
Toxicity	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2	Grade 3&4	Grade 2
Elevated ALP							1				1		
Hyponatremia													
Hypotension							1						1

* Weekly PX-12 infusion schedule

^a Dose limiting toxicity (DLT)

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Table 4

Summary of PX-12 and 2-MI concentration at the end of the first infusion.

Dosing Schedule	PX-12 conc. (ng/mL) (mean $\pm$ SD)	N	2-MI conc. (μ g/mL) (mean $\pm$ SD)	N
150 mg/m ² bi-weekly	1.46 $\pm$ 0.04	2	1.79 $\pm$ 0.02	2
200 mg/m ² bi-weekly	1.58 $\pm$ 0.96	4	1.37 $\pm$ 0.13	4
300 mg/m ² bi-weekly	2.26 $\pm$ 0.12	3	2.24 $\pm$ 0.44	3
450 mg/m ² bi-weekly	4.58	1	4.10 $\pm$ 0.16	3
300 mg/m ² weekly	2.51 $\pm$ 1.19	3	1.36 $\pm$ 0.88	3
400 mg/m ² weekly	2.84	1	3.20	1

Table 5

Pretreatment levels of plasma proteins.

Treated Evaluable Patients	Trx-1 plasma Levels (N=17)	VEGF plasma Levels (N=17)	FGF-2 plasma Levels (N=15)
Range	5.3–40.0 ng/mL	19.3–236.6 pg/mL	<Lower Limit of Detection - 33.4 pg/mL
Median	17.4 ng/mL	60.3 pg/mL	14 pg/mL
Average	19.8 +/-1.4 ng/mL	71.8 +/- 4.6 pg/mL	17.4+/-1.7 pg/mL

Table 6

DCE-MRI 2-compartment pharmacokinetic model parameters for target tumors.

Subject (dose)	K^{trans} , baseline	K^{trans} , 24 h post 1 st infusion	K^{trans} , 7 days post 1 st infusion
Subject 02-004 (200 mg/m ²)	$1.2 \times 10^{-2} \text{ min}^{-1}$	$1.4 \times 10^{-2} \text{ min}^{-1}$	---
Subject 02-012 (450 mg/m ²)	$4.6 \times 10^{-2} \text{ min}^{-1}$	$3.9 \times 10^{-2} \text{ min}^{-1}$	$5.4 \times 10^{-2} \text{ min}^{-1}$
Subject 02-015 (300 mg/m ² bi-weekly)	$1.4 \times 10^{-1} \text{ min}^{-1}$	$1.6 \times 10^{-1} \text{ min}^{-1}$	$6.3 \times 10^{-2} \text{ min}^{-1}$ (48 h post 2 nd infusion)