Title:
A Multicenter Phase 1 Trial of PX-866, an Oral Irreversible Phosphatidylinositol 3-Kinase Inhibitor, in Patients with Advanced Solid Tumors

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ABSTRACT

Purpose: The objectives of the study were to evaluate the maximum tolerated dose (MTD), safety, pharmacodynamics, pharmacokinetics (PK), and antitumor activity of PX-866 in patients with incurable cancers.

Patients and Methods: This was a phase 1, open-label, dose-escalation study. Drug was administered orally once per day either on an intermittent (Arm 1; days 1-5 and 8-12 of a 28-day cycle) or continuous (Arm 2; days 1-28 of a 28-day cycle) schedule. Additional patients were treated at the Arm 2 MTD in a food effects sub-study.

Results: Eighty-four patients were treated in the Arm 1 (n=51), Arm 2 (n=20) and food effects (n = 13) cohorts. The most frequent study drug-related adverse events were gastrointestinal disorders (69.0%), with diarrhea being the most common (48.8%). The MTD was 12mg and 8mg for Arm 1 and 2, respectively. The dose-limiting toxicities (DLTs) consisted of grade 3 (g3) diarrhea (n=3) and g3 elevated AST (n=1). The PK profile was dose proportional, with no evidence of drug accumulation. PX-866-associated inhibition of platelet P-AKT SER473 was observed at the Arm 2 MTD. The best response per RECIST was stable disease in 22% of evaluable patients in Arm 1, 53% in Arm 2, and 11% in the food effects cohort. Eight patients were on study for 4 or more months.

Conclusions: This first-in-human study demonstrates that PX-866, an irreversible small molecule inhibitor of PI-3K, was well tolerated and was associated with prolonged stable disease, particularly when using a continuous dosing schedule.
TRANSLATIONAL RELEVANCE

The PI-3K/AKT/mTOR pathway is dys-regulated in a variety of solid tumors and is proposed to provide key growth and survival signals to tumor cells. Therefore, inhibitors of the PI-3K protein represent a promising class of therapeutic agents with several small molecule PI-3K inhibitors in clinical development. This article reports data from the phase 1, first-in-human study of PX-866, an oral, selective, irreversible inhibitor of PI-3K. Results from this trial demonstrate that PX-866 may be administered with a tolerable toxicity profile in patients with advanced solid tumors. Evidence of anti-tumor activity supports development as a single agent or in combination with other therapies.
INTRODUCTION

The phosphatidylinositol 3-kinase (PI-3K)/AKT signaling pathway is deregulated in many human cancers, leading to decreased expression of pro-apoptotic genes and increased expression of cell proliferation and cell survival genes, making it an attractive cancer target (1). PI-3K and AKT are oncogenes over-expressed or activated by mutation in many human cancers (2-6). \textit{PIK3CA}, the gene encoding PI-3K, is mutated in several tumor types including glioblastomas (27%) and gastric (25%), breast (18%), cervical (33%), and endometrial (39%) cancers, and is one of the most common activating mutations in head and neck squamous cell carcinoma (HNSCC) (6%-8%) (7-13). The PTEN (phosphatase and tensin homologue) tumor suppressor gene, which negatively regulates PI-3K signaling, may be lost via deletion (25% of melanoma, breast, and prostate cancers), mutation, or epigenetic suppression (14-18). Lastly, upstream growth factor receptors with increased activity in some cancers, such as epidermal growth factor receptor (EGFR), activate downstream PI-3K signaling (19).

There are eight mammalian PI-3K enzymes that are divided into three main classes based on sequence homology and substrate preference (20, 21). The Class IA enzymes, which are most commonly related to cancer biology, include the p110\(\alpha\), p110\(\beta\), and p110\(\delta\) catalytic subunits (the latter restricted to leukocytes). Mutations that activate p110\(\alpha\) result in greater signaling by PI-3K and oncogenicity (7). Mutations of the p85 regulatory subunit are also oncogenic (7, 22) and increase p110\(\alpha\) signaling (23).
PX-866 (acetic acid 4-diallylaminomethylene-6-hydroxy-1-α 12-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[a]phenanthren-11-yl ester) is a synthetic derivative of wortmannin, a natural furanosteroid metabolite product isolated from a strain of *Penicillium wortmannii*. The mechanism of action of PX-866 is consistent with irreversible inhibition of PI-3K as the 21 position of the agent interacts with the lysine-802 residue in the ATP catalytic site of PI-3K (24). PX-866 displays increased stability and activity, improved pharmacological profile, and reduced toxicity in mice compared with wortmannin (24). PX-866 is a potent, pan-isoform inhibitor of PI-3K with IC50s of 39 ± 21 nM, 88 ± 27 nM, 124 ± 26 nM, and 183 ± 25 against PI-3Kα, PI-3Kβ, PI-3Kδ, and PI-3Kγ, respectively. 17-OH PX-866 is an active metabolite with more potency against PI-3Kα (IC50 = 14 ± 6 nM) and PI-3Kβ (IC50 = 57 ± 7 nM) than the parent compound (25).

PX-866 blunts cell growth and decreases activation of PI-3K downstream targets *in vitro* and *in vivo*, including P-AKT (26) pS6 and p-mTOR (27). In preclinical studies, inhibition of P-AKT (S473) and P-S6 (S235/236) occur with IC50s of 60 and 74 nM, respectively, in A549 NSCLC cells *in vitro* (Oncothyreon, unpublished data). Inhibition of P-AKT (S473) was observed for up to 48 hours after PX-866 dosing in HT29 tumor models (25). PX-866 delayed tumor growth in OvCar-3 ovarian cancer (25), HT29 colon cancer (25), A-549 NSCLC (28), and U87 glioma (27) xenografts, with an association between anti-tumor activity and the presence *PIK3CA* activating mutations or PTEN loss.
The observation that PI-3K inhibition persists for several days following drug administration suggested that an intermittent dosing schedule might provide sufficient target inhibition with less toxicity than a daily dosing schedule. Therefore, this phase 1 study had two sequential arms: Arm 1 sought to identify the maximum tolerated dose (MTD) for an intermittent dosing schedule and Arm 2 identified the MTD for continuous daily dosing. The study objectives were to determine the MTD, toxicity profile, pharmacodynamics (PD), pharmacokinetics (PK), and antitumor activity of PX-866 in patients with advanced cancers. A food effects sub-study evaluated the impact of food on the PK profile of PX-866.
PATIENTS AND METHODS

Study Design

This was a phase 1, open-label, dose-escalation study with two arms conducted at the University of Texas MD Anderson Cancer Center and the University of Colorado Cancer Center after approval by the Institutional Review Boards of both centers.

In Arm 1, patients received drug orally once daily on days 1-5 and 8-12 of a 28-day cycle. Once the MTD in Arm 1 had been determined and confirmed with an expansion MTD Arm 1 cohort, the daily dosing cohort of Arm 2 started enrollment, with drug administered orally once daily on days 1-28 of a 28-day cycle. The starting dose for Arm 1 was 0.5mg once daily (1/10th the severely toxic dose identified in GLP toxicity studies). The starting dose for Arm 2 was two dose levels below the MTD for Arm 1. Dose escalation in both arms followed a 3+3 design, with expansion to six patients if one dose-limiting toxicity (DLT) was observed. Dose escalation was stopped when two or more DLTs occurred. Patients received repeated cycles in the absence of unacceptable toxicity or disease progression (PD).

A food effects cohort was enrolled once the MTD in Arm 2 was declared. The patients in the food effects sub-study were randomly assigned to one of two groups (group A or B). Each group consisted of approximately five patients. Each patient in the sub-study received PX-866 administered orally at the dose determined to be the MTD in the dose-escalation portion of Arm 2 (continuous daily dosing) of the protocol. For patients assigned to group A (first-dose-fasted treatment), PX-866 was administered...
orally on cycle 1 day -7 on an empty stomach. Following a 2-day washout period, PX-866 was administered orally on cycle 1 day -4 with food. For patients assigned to group B (first dose fed treatment), PX-866 was administered orally on cycle 1 day -7 with food. Following a 2-day washout period, PX-866 was administered orally on cycle 1 day -4 on an empty stomach. After a 3-day washout period, patients in both groups then began the expansion phase (Arm 2) of the protocol.

For the fasting treatment day, patients fasted overnight for approximately 10 hours. PX-866 was then administered with approximately 240 mL (8 ounces) of water. No food was permitted for at least 4 hours post-dose administration. Water was permitted as desired except for 1 hour before and after drug administration. For the fed treatment day, following an overnight fast of approximately 10 hours, patients began the recommended meal approximately 30 minutes prior to administration of PX-866. The recommended meal consisted of a standard high-fat breakfast, consisting of 2 fried eggs, 2 slices toasted white bread, 1 tablespoon butter, 1 tablespoon jam, 3 strips fried bacon, 4 ounces of hash brown potatoes, and 8 fluid ounces of whole milk (total caloric content of the breakfast was 951 kcals, with distribution of calories being 52% from fat, 33% from carbohydrates, and 15% from protein). Patients had to consume the entire meal in 30 minutes or less; however, PX-866 was administered 30 minutes after the start of the meal. If the patient could not complete the meal, an approximate percentage of the consumed meal was documented. PX-866 was administered with approximately 240 mL (8 ounces) of water. No food was permitted for at least 4 hours after
administration of PX-866. Water was permitted as desired except for 1 hour before and after drug administration.

**Definition of Dose-Limiting Toxicity and Maximum Tolerated Dose**

Using Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0 DLT was defined as: grade 3 or 4 (g 3-4) neutropenia accompanied by fever; g3-4 thrombocytopenia; g3-4 nausea, vomiting, or diarrhea that persisted despite optimal antiemetic or antidiarrheal therapy; any other g3-4 gastrointestinal toxicity; g3 elevation of transaminases for >7 days; any other g3-4 hepatic toxicity; g3-4 increase in serum glucose that persisted despite optimal therapy including insulin based therapy; or any other g3-4 toxicity, unless clearly related to an intercurrent illness or disease progression.

Patients who experienced a DLT could continue in the study, at the dose level below after recovery of the toxicity. Patients who required more than two weeks for recovery from a DLT were withdrawn. The highest dose level at which 0-1 of 6 patients experienced DLT was declared the MTD. In the first cycle of Arm 1, all patients who received 10 daily doses of PX-866 were evaluable for MTD determination. In Arm 2, all patients who received 21 daily doses of PX-866 were evaluable for MTD determination.

**Patients**

Inclusion criteria were: written signed informed consent; histologically-confirmed advanced solid tumor untreatable by standard therapy; age ≥18 years; Eastern}

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Cooperative Oncology Group (ECOG) performance status <2; life expectancy ≥12 weeks; discontinuation of anticancer therapy for ≥3 weeks (6 weeks for mitomycin C, nitrosureas, vaccines, or antibody therapy); recovery of previous therapy-related toxicities to baseline or ≤g1; adequate hematologic, hepatic, and renal function. Exclusion criteria were: active infection; diabetes or fasting blood glucose >160 mg/dL; significant concomitant disorders; surgery within four weeks; untreated or symptomatic brain metastasis; gastrointestinal conditions interfering with absorption. Patient safety was monitored by periodic physical exams, hematology and chemistry laboratory studies, and adverse events (AEs) assessment. Patients had radiographic tumor assessment at baseline and after every second cycle. Tumor response was evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.0.(29)

Pharmacokinetics

Levels of PX-866 and the metabolite 17-OH PX-866 were analyzed in samples collected during cycle 1 for both Arms 1 and 2. For Arm 1, PK samples were collected at baseline, and then on Cycle 1 Days 1, 5 and 12 at 20, 40 60 and 120 minutes post-dosing. For Arm 2, PK samples were collected at the same time points just on Cycle 1 Day 1. The PK profile was also evaluated in the food effects sub-study Samples for PK analyses were collected from patients on days -7 and -4 at baseline prior to PX-866 administration, and at 10 minutes, 20 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours after PX-866 administration.
Pharmacodynamic and Biomarker Testing

To noninvasively monitor PX-866 pharmacodynamics, assays were developed utilizing platelets to quantify PI-3K pathway inhibition using an enzyme linked immunosorbent assay (ELISA) to quantify total and phosphorylated AKT (P-AKT) protein in the fasted state (see supplementary methods). Optional, archival tumor tissue blocks were assessed for the presence of mutations in PIK3CA (G1624A, A1634G, A1633A, A3140G, A3140T) and KRAS (codons 12 and 13) using the shifted termination assay (TrimGen Corp, Sparks, Maryland) (30).

Statistics

Sample size was determined empirically, based upon a 3+3 escalation design. Descriptive statistics were used for analyses of safety, tumor response, PK, and pharmacodynamic measurements.
RESULTS

Patient Characteristics

Eighty-four patients were enrolled and treated with at least one dose of PX-866 while on the study across the intermittent (Arm 1, n=51), continuous (Arm 2, n=20), and food effects (n=13) cohorts (Table 1). The median age was 61 years (range: 29–83). Several tumor types were represented in the patient population, with the most common being colorectal cancer (n=21, 25.0%). Previous anticancer treatments included chemotherapy (90%), other therapy (33%), immune therapy (10%) and hormone therapy (10%). The majority (65%) of patients had received three or more anti-cancer therapies. Patient characteristics were comparable for the 3 arms of the study, although the overall percentage of patients with an ECOG performance status of 1 was higher for the food effects cohort (77%) than for Arm 1 (70%) or Arm 2 (60%).

Dose Escalation and MTD determination

PX-866 dose escalation started at 0.5mg in Arm 1 (intermittent), then explored 1, 2, 3, 4.5, 6, 8, 10, 12, and 16mg (Table 2). DLTs on the intermittent dosing schedule were g3 diarrhea (n=1) and grade 3 elevated AST (n=1) in two of five patients receiving 16 mg of PX-866. The MTD for PX-866 was determined to be 12mg for the intermittent schedule. Arm 1 was expanded to a total of 16 patients with no further DLTs.

Arm 2 started two dose levels below the MTD for Arm 1, which corresponded to 8mg. The DLT on the continuous dosing schedule was g3 diarrhea, which occurred in two of three patients receiving 10mg of PX-866. The MTD for PX-866 was determined
to be 8mg for the Arm 2 (continuous) dosing schedule. Arm 2 was expanded to a total of 17 patients with no further DLTs.

Safety

Patients who received at least one dose of PX-866 were evaluated for safety (n=84). The most frequent toxicities considered likely related to study drug were: gastrointestinal disorders (69%), with diarrhea being the most common (49%), followed by nausea (38%) and vomiting (25%) (Table 3). The majority (91%) of study drug-related toxicities were g1-2. Other study drug-related toxicities were g3 and were reported in patients treated at ≥8mg. In Arm 1, seven g3 study drug-related toxicities were reported in six of 51 patients (12%), all treated at 12mg or 16mg. These included fatigue (n=2), vomiting (n=1), diarrhea (n=1), hypertension (n=1), elevated aspartate aminotransferase (AST; n=1), and dehydration (n=1).

In Arm 2, five g3 study drug-related toxicities were reported in three of 20 patients (15%), all in the 10mg cohort and included nausea (n=1), vomiting (n=1), diarrhea (n=2), and elevated alanine aminotransferase (ALT)/AST (n=1). In the food effects cohort, 4 of 13 patients (31%), each treated at 8mg, experienced g3 toxicities, including diarrhea (n=2), anemia (n=1), and elevated liver transaminases (n=1). The incidence of ALT/AST elevations considered to be study drug-related was higher in patients on Arm 2 compared to Arm 1. Study drug-related hematologic toxicities were uncommon.
Outcome

Of the 84 patients treated, 28 discontinued prior to receiving a follow-up scan, including 20 patients (13 in Arm 1, three in Arm 2, and four in the food effects cohort) who came off study due to early PD; five patients in Arm 1 who withdrew due to AEs (considered study-drug related in only one patient who withdrew due to g1 nausea and diarrhea and g2 vomiting); and three patients (one in Arm 1, and two in Arm 2) who withdrew consent. Across all study arms, the median duration of treatment was 51 days (range: 1 to 552 days). The median duration of treatment was 51 days (range 1 to 229 days) for Arm 1 (range 1 to 229 days), 57 days for Arm 2 (range 3 to 552 days), and 46 days for the food effects arm (range 6 to 98 days).

Best response in the 56 evaluable subjects (defined as having a scan during or at the end of cycle 2) was stable disease (SD) in 7/32 patients (22%) in Arm 1, SD in 8/15 patients (53%) in Arm 2, and SD in 1/9 patients (11%) in the food effects cohort (Figure 1). Several patients experienced prolonged SD, including four patients in Arm 1 (melanoma, adenocystic carcinoma, non-small cell lung cancer, and chondrosarcoma) who received between 5 and 8 cycles; and four patients in Arm 2 (colorectal carcinoma (2) [CRC], metastatic pancreatic neuroendocrine tumor, and castration-resistant prostate cancer [CRPC]), who received between 6 and 20 cycles.

The three patients in Arm 2 who had the longest duration of SD had CRC, pancreatic neuroendocrine cancer and CRPC. The patient with CRC had PD on the regimen prior to enrollment and was on therapy for 6 cycles, experiencing the greatest...
degree of tumor shrinkage of any patient in the study. The patient with pancreatic neuroendocrine carcinoma had documented PD on study entry and experienced SD while receiving 10 cycles of PX-866 (8mg) before developing PD. The patient with CRPC had a history of progression after hormonal therapy and chemotherapy and entered the study with a normal PSA and bone metastases for which he received chronic pain medication. He initiated treatment at 10mg per day, and required two dose reductions to 8mg and 6mg due to a DLT (g3 diarrhea) first and recurrent g1 diarrhea later. The patient received 20 cycles before PD occurred, and discontinued all pain medications while on study.

Mutational status of $PIK3CA$ and KRAS was obtained from archival tumor specimens from 45 patients (Supplementary Tables 1 and 2). No differences in mean time on study based on mutational status were observed for Arm 1 patients, which may reflect conservative initial dosing and a lack of antitumor effects in the intermittent dosing arm. Mutational status and time on study for patients treated with continuous dosing are represented in Figure 2. While not statistically significant, an association with longer time on study was observed in patients with a $PIK3CA$ mutation ($PIK3CA$-mut) versus wild type ($PIK3CA$-WT). The mean time on study for $PIK3CA$-mut patients (n=4) was 204 days (range: 57-522) versus 115 days (range: 24-316) for $PIK3CA$-WT patients (n=8) (2-tailed t-Test; $P=0.28$). Two of the three patients with the longest duration of SD in Arm 2 had dual $PIK3CA$ point mutations, including the CRPC patient and the patient with CRC. The patient with pancreatic neuroendocrine tumor was $PIK3CA$-WT.
Pharmacokinetics

Plasma levels of PX-866 were undetected. Consequently, PK parameters were determined using an active metabolite of PX-866 (17OH-PX-866), which was identified in preclinical models (data on file, Oncothyreon, Inc.). PK parameters from the Arm 1, Arm 2, and the food effect cohorts are reported in Table 4. A mean plasma concentration-time curve for 17OH-PX-866 in a representative dose level is shown in Figure 3. The AUC for 17OH-PX-866 appears to be dose proportional from 4.5–16mg (R²=0.973) and Cmax is dose proportional across all dose levels (R²=0.78). No evidence of drug accumulation or drug reduction was seen with repeat dosing.

The effect of food intake on PX-866 PK parameters was evaluated in a food effects sub-study. Thirteen patients were enrolled, with evaluable data available for eight. In this small number of patients, Tmax was slightly delayed in the fed cohort and while AUC and Cmax were lower compared to the fasting state, although these differences were not significant (P=0.253 for AUC and P=0.063 for Cmax). Significant variability was observed due to two patients who exhibited high fasting Cmax values that were outside the normal distribution for the 8mg dose, and one patient who had drug levels below the limit of quantitation for all time points.

Pharmacodynamics

Pharmacodynamic assays evaluating quantitative changes in P-AKT and total AKT (T-AKT) in platelets was developed. Sampling was performed in 10 patients enrolled in the food effects sub-study who then went on to treatment with daily dosing.
using the Arm 2 schedule at the MTD of 8mg. Inhibition of P-AKT was observed within 4
hours in seven patients with P-AKT/T-AKT ratio decreases of 13%-94% (Supplementary
Figure 1), with four having P-AKT/T-AKT ratio decreases >80%. No correlation was
established between P-AKT/T-AKT ratios and AEs or antitumor activity.
DISCUSSION

This first-in-human study demonstrates that PX-866, an irreversible small molecule PI-3K inhibitor, is safe and well tolerated, with similar safety profiles when administered intermittently or continuously. The PX-866 MTD are 12mg and 8mg for the intermittent and continuous schedules, respectively.

The most common toxicity observed was diarrhea. This side effect was tolerable in most patients with the use of anti-diarrheal medications and, if needed, dose reduction of PX-866. Nausea, vomiting, and diarrhea are common side effects seen with other PI-3K inhibitors and were also tolerable with anti-emetics and anti-diarrheals. Interestingly, PX-866 was not associated with the significant hyperglycemia or skin toxicity reported with many other compounds targeting PI-3K (31, 32). This is not entirely unique as GDC-0941 in a phase Ib combination with chemotherapy with or without bevacizumab showed no hyperglycemia and only a mild rash (33). In addition, while dose-limiting toxicity associated with the alpha-specific inhibitor BYL719 included hyperglycemia, hyperglycemia was not reported at dose levels associated with disease stabilization (34). These results suggest that rash and hyperglycemia may not always be present with a PI-3K inhibitor, or may occur at levels of exposure greater than those needed for anti-tumor activity. Moreover, PX-866’s unique mechanism of action as an irreversible PI-3K inhibitor may make its toxicity profile different from reversible PI-3K inhibitors. Because PI-3K signaling has a well-established role in resistance to EGFR inhibitors (35), the lack of skin toxicity with PX-866 enables potential combinations of PI-3K and EGFR inhibitors (12).
The PK results indicate that the complex pharmacology of PX-866 is likely mediated by drug metabolites including, but not limited to, 17-OH PX-866. The half-life of PX-866 is short but daily dosing is supported by its irreversibility. On-target PI-3K inhibition was documented in patient platelet samples; however, there was no clear correlation between PI-3K pathway inhibition, drug PK, toxicity, or efficacy. This might be explained by inter-patient variation in PX-866 metabolism as well as with, the generation of other unidentified active metabolites. Further studies will examine the role of other metabolites in PI-3K pathway inhibition, efficacy, and drug tolerability. Results from the food effect sub-study suggest that food may decrease some of the variability observed in PX-866 PK. Although the Cmax and AUC appear to be lower in the fed group, these differences were not statistically different. In addition, the food effect PK data are limited by a small sample size and substantial inter-patient variability. Therefore, a food effect study in healthy volunteers is being performed (NCT01408316).

While no objective responses were observed, SD occurred in eight of 15 (53%) evaluable patients in Arm 2, with four (26.6%) of these patients having SD >4 months. The difference in SD rates between the intermittent (22%) and continuous (53%) dosing schedules is likely multifactorial, but the constant drug exposure seen with continuous dosing may induce higher PI-3K/AKT pathway signaling suppression. The two patients with the longest time on study (CRPC and pancreatic neuroendocrine carcinoma) both had progression prior to enrollment, and the patient with CRPC was able to discontinue pain medications during PX-866 treatment. A patient with CRC harboring dual PIK3CA mutations had tumor shrinkage with therapy. These outcomes support a direct
anticancer effect of PX-866 rather than variability in baseline tumors characteristics.
The stable disease seen in this study is consistent with other exclusive inhibitors of PI-
3K, where responses are rare (31, 32). For instance, the response rate with BKM120
was less than 3% in unselected phase 1 patients (32). Our study is limited by a
relatively high early discontinuation rate for clinical progression. This may have been
driven by several factors. First, the majority of early discontinuations occurred in the
intermittent dosing arm that was ultimately thought to be ineffective due to inadequate
drug exposure. Second, the food cohort contributed the second highest percentage of
early discontinuations. This group had the worst performance status of any cohort, and
the time on study for this group was calculated from the time continuous dosing began
on Cycle 1 Day 1 rather than the first day of dosing on day -7. Lastly, the enrollees in
this study represented a heavily pretreated population where over 65% had received
three or more previous lines of therapy.

An interesting finding was a possible association with longer time on PX-866 for
PIK3CA-mut vs. PIK3CA-wt patients, including two previously progressing patients with
PIK3CA-mut colorectal carcinoma and prostate carcinoma on study for 6 and 20
months, respectively. While this association could be explained by a small sample size
and an overall improved prognosis for patients with PI3KCA mutations, activation of the
PI-3K pathway is typically associated with worse prognosis in patients with ovarian or
prostate cancer (36, 37). Moreover, substantial pre-clinical data suggests that PI-3K
inhibition may be more effective in tumors harboring an activated PI-3K pathway. For
instance, PIK3CA mutation or PTEN loss were predictors for response to PX-866 in
human xenograft models of several tumors (38). Similarly increased anti-tumor activity has been seen in other pre-clinical PIK3CA-mut cancer models (39, 40). KRAS and p53 mutations may be indicators of resistance to PI-3K inhibition (38, 41). Consistent with this, a recent analysis of gynecologic malignancies who harbored PIK3CA mutations treated on P13K/AKT/mTOR inhibitors showed a higher response rate than patients without mutations in the MD Anderson Cancer Center phase I clinic (42).

The correlation between PI-3K/AKT pathway activation and outcome is limited as less than 50% of patients were tested for PIK3CA and KRAS mutations since the mutation analysis was not preplanned, and other potential biomarkers of PI-3K signaling (PTEN loss, PIK3CA amplification, or PI-3K over-expression) were not evaluated. Future studies will further investigate biomarkers predictive of benefit following PX-866 administration.

In conclusion, this first-in-human study of PX-866 established the MTD for two dosing schedules that were well tolerated, and 8mg of PX-866 daily is the recommended phase 2 dose. Tumor mutational analyses suggest an association with increased time on study in patients with PIK3CA-mut cancers, which will require prospective confirmation. PK and PD analyses demonstrate rapid absorption, and “on target” pathway inhibition. The agent’s favorable toxicity profile and antitumor activity support its further clinical development. PX-866 is currently in phase 2 trials for glioblastoma (NCT01259869) and CRPC (NCT01331083), and combination phase 1/2
studies with cetuximab (NCT01252628) or docetaxel (NCT01204099) for HNSCC/CRC and HNSCC/NSCLC, respectively.
Table 1: Baseline Demographics and Patient Characteristics

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<td>57 (68.7)</td>
</tr>
<tr>
<td>Number of Prior Anticancer Systemic Treatments for Metastatic Disease, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (5.9)</td>
<td>1 (5.0)</td>
<td>0 (0.0)</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>1</td>
<td>5 (2.0)</td>
<td>2 (10.0)</td>
<td>1 (7.7)</td>
<td>8 (9.5)</td>
</tr>
<tr>
<td>2</td>
<td>10 (19.6)</td>
<td>4 (20.0)</td>
<td>3 (23.1)</td>
<td>17 (20.2)</td>
</tr>
<tr>
<td>3</td>
<td>3 (5.9)</td>
<td>4 (20.0)</td>
<td>4 (30.8)</td>
<td>11 (13.1)</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>30 (58.8)</td>
<td>9 (45.0)</td>
<td>5 (38.4)</td>
<td>44 (52.4)</td>
</tr>
<tr>
<td>Tumor Type, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>9 (17.6)</td>
<td>7 (35.0)</td>
<td>4 (31.8)</td>
<td>20 (23.8)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>6 (11.8)</td>
<td>5 (25.0)</td>
<td>1 (7.7)</td>
<td>12 (12.3)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>8 (15.7)</td>
<td>1 (5.0)</td>
<td>0 (0.0)</td>
<td>9 (10.7)</td>
</tr>
<tr>
<td>Disease</td>
<td>Arm 1</td>
<td>Arm 2</td>
<td>Continuous</td>
<td>Intermittent</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td>4 (7.8)</td>
<td>0 (0.0)</td>
<td>2 (15.3)</td>
<td>6 (7.1)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>3 (5.9)</td>
<td>1 (5.0)</td>
<td>2 (15.3)</td>
<td>6 (7.1)</td>
</tr>
<tr>
<td>Breast</td>
<td>3 (5.9)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Other$^b$</td>
<td>16 (31.3)</td>
<td>4 (20.0)</td>
<td>4 (30.8)</td>
<td>24 (28.6)</td>
</tr>
</tbody>
</table>

$^a$ ECOG performance status was not available for one patient in Arms 1.

$^b$ Other includes: 2 each of leiomyosarcoma, skin (squamous cell), prostate, small cell lung, kidney, and anaplastic thyroid; and 1 each of adenocystic, hepatoma, gastrointestinal stromal, chondrosarcoma, endometrial, salivary gland, pancreatic islet cell, pancreatic neuroendocrine, pancreatic, esophageal, sarcoma, cholangiocarcinoma, urothelial, and gastric cancers

Abbreviations: Intermittent dosing (Arm 1); Continuous dosing (Arm 2); Eastern Cooperative Oncology Group Performance Status (ECOG PS)
Table 2: Dose Escalation and Dose-Limiting Toxicities

<table>
<thead>
<tr>
<th>Dose Cohort (mg)</th>
<th>No. of Patients in Dose Cohort (n)</th>
<th>No. of Patients with DLT&lt;sup&gt;a&lt;/sup&gt; (n)</th>
<th>DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>2</td>
<td>One patient with grade 3 diarrhea and one patient with grade 3 AST elevation and grade 2 diarrhea</td>
</tr>
<tr>
<td>Arm 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>2</td>
<td>Two patients with grade 3 diarrhea</td>
</tr>
</tbody>
</table>

<sup>a</sup>In patients during the first treatment cycle

<sup>b</sup>Includes MTD expansion cohort

Abbreviations: dose limiting toxicity (DLT); aspartate aminotransferase (AST); alanine aminotransferase (ALT); maximum tolerated dose (MTD); Intermittent (Arm 1); Continuous (Arm 2)
Table 3: Adverse Events Reported in the Safety Population Following Treatment with PX-866

<table>
<thead>
<tr>
<th>AE Type Reported</th>
<th>Arm 1&lt;sup&gt;a&lt;/sup&gt; N=51 n (%)</th>
<th>Arm 2&lt;sup&gt;b&lt;/sup&gt; N=20 n (%)</th>
<th>Food Effects N=13 n (%)</th>
<th>Total Population&lt;sup&gt;c&lt;/sup&gt; N=84 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AEs</td>
<td>50 (98.0)</td>
<td>19 (95.0)</td>
<td>13 (100.0)</td>
<td>82 (97.6)</td>
</tr>
<tr>
<td>Patients with Treatment-Related AEs</td>
<td>35 (68.6)</td>
<td>17 (85.0)</td>
<td>12 (92.3)</td>
<td>64 (76.2)</td>
</tr>
<tr>
<td>Treatment-Related AEs by Preferred Term in ≥ 5% of Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17 (33.3)</td>
<td>15 (75.0)</td>
<td>9 (69.2)</td>
<td>41 (48.8)</td>
</tr>
<tr>
<td>Nausea</td>
<td>18 (35.3)</td>
<td>8 (40.0)</td>
<td>6 (46.2)</td>
<td>32 (38.1)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10 (19.6)</td>
<td>7 (35.0)</td>
<td>4 (30.8)</td>
<td>21 (25.0)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 (11.8)</td>
<td>5 (25.0)</td>
<td>3 (23.1)</td>
<td>14 (16.7)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>2 (3.9)</td>
<td>4 (20.0)</td>
<td>2 (15.4)</td>
<td>8 (9.5)</td>
</tr>
<tr>
<td>AST increased</td>
<td>2 (3.9)</td>
<td>2 (10.0)</td>
<td>1 (7.7)</td>
<td>5 (6.0)</td>
</tr>
<tr>
<td>ALT increased</td>
<td>1 (2.0)</td>
<td>2 (10.0)</td>
<td>1 (7.7)</td>
<td>4 (4.8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Arm 1
<sup>b</sup> Arm 2
<sup>c</sup> Arm 1, Arm 2, and Food Effects

Abbreviations: adverse event (AE); alanine aminotransferase (ALT); aspartate aminotransferase (AST)
Table 4: Pharmacokinetics. In Arm 1, both inter-patient and intra-patient variability (CV%) was calculated for $C_{\text{max}}$, $T_{\text{max}}$, and AUC parameters for all patients on all dose levels for each of the three PK sampling days. Median composite variability was calculated for all three days. The composite CV% for both inter-patient and intra-patient variability for $C_{\text{max}}$ was 70% versus 47% and for AUC, 80% versus 45%, respectively. $T_{\text{max}}$ was similar between the two groups with a CV% of 40%.

<table>
<thead>
<tr>
<th>Study Arm</th>
<th>Dose Level (mg)</th>
<th>Pt (n)</th>
<th>$C_{\text{max}}$ (ng/ml ± SD)</th>
<th>$T_{\text{max}}$ (hr ± SD)</th>
<th>$V_{\text{z/F}}$ (L)</th>
<th>$\text{Cl/F}$ (L/hr)</th>
<th>$T_{1/2}$ (hr)</th>
<th>$AUC_{\text{inf}}$ (hr*ng/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>3</td>
<td>0.16±0.08</td>
<td>0.63±0.13</td>
<td></td>
<td></td>
<td></td>
<td>0.35±0.12</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>0.07±0.01</td>
<td>0.93±0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>3</td>
<td>0.96±0.04</td>
<td>0.82±0.28</td>
<td></td>
<td></td>
<td></td>
<td>1.82±0.31</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>1.36±0.07</td>
<td>1.11±0.19</td>
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<td></td>
<td>3.86±2.54</td>
</tr>
<tr>
<td>4.5</td>
<td>4</td>
<td>4</td>
<td>0.95±0.68</td>
<td>0.83±0.65</td>
<td></td>
<td></td>
<td></td>
<td>1.42±0.63</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>0.97±0.38</td>
<td>0.67±0.21</td>
<td></td>
<td></td>
<td></td>
<td>1.46±0.11</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4.02±0.75</td>
<td>1.07±0.21</td>
<td></td>
<td></td>
<td></td>
<td>8.77±4.32</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4</td>
<td>2.86±0.33</td>
<td>0.94±0.28</td>
<td></td>
<td></td>
<td></td>
<td>5.53±0.30</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td>16</td>
<td>2.84±0.57</td>
<td>0.88±0.22</td>
<td></td>
<td></td>
<td></td>
<td>6.82±0.42</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>5</td>
<td>2.44±1.14</td>
<td>0.95±0.19</td>
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<td></td>
<td></td>
<td>7.91±3.72</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>17</td>
<td>1.21±0.18</td>
<td>1.10±0.21</td>
<td>11180±2078</td>
<td>3346±730</td>
<td>3.88±0.99</td>
<td>4.88±1.03</td>
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<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>0.76±0.12</td>
<td>0.89±0.11</td>
<td>14209±4027</td>
<td>4574±1527</td>
<td>2.22±0.33</td>
<td>2.7±0.81</td>
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<tr>
<td>FE</td>
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</tr>
<tr>
<td>fast</td>
<td>8</td>
<td>6</td>
<td>2.39±1.28</td>
<td>0.94±0.22</td>
<td></td>
<td>6.47±3.15</td>
<td></td>
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<tr>
<td>fed</td>
<td>8</td>
<td>6</td>
<td>0.73±0.17</td>
<td>1.27±0.31</td>
<td></td>
<td>3.21±0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a PX-866 was administered while patients were fasting

*b PX-866 was administered with intake of food

Abbreviations: Intermittent (Arm 1); Continuous (Arm 2); patients (Pts); food effects (FE)
FIGURE LEGENDS

Figure 1. Best Responses by Time on Study (days), Mutational Status, and Tumor Type for Patients Receiving Continuous PX-866. Abbreviations: Colorectal carcinoma (CRC), squamous cell carcinoma of the head and neck (SCCHN), non-small cell lung cancer (NSCLC).

Figure 2. Duration of Clinical Benefit by Mutational Status.

Figure 3. Pharmacokinetics. Mean plasma concentration-time pharmacokinetic profile of 17-OH PX-866 from Arm 2 patients dosed with 10 mg (n=3) and 8 mg (n=17) PX-866. Data from the two arms were combined to increase statistical power. Samples were collected pre-dose, and at the following time points (±5 minutes) after the oral administration of PX-866: 10 minutes, 20 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours. Mean concentration and SD are presented as log 17-OH PX-866 ng/ml. Horizontal line represents the lower limit of quantification (LLOQ) for 17-OH PX-866.
REFERENCES


33. Besse B, Soria J, Gomez-Roca C, Ware A, Adjei A, Dy GK, et al. A phase Ib study to evaluate the PI3-kinase inhibitor GDC-0941 with paclitaxel (P) and carboplatin


Figure 1

Percent Change in Sum of Largest Diameters

- CRC
- Ovarian
- CRC
- Ovarian
- CRC
- Ovarian
- CRC
- Gastric
- SCCHN
- Sarcoma
- NSCLC
- Melanoma
- CRC
- CRC
- Ovarian
- Esophageal
- CRC
- CRC
- CRC
- Neuroendocrine
- CRC

Mutational Status:
A: PIK3CA-WT, KRAS-WT
B: PIK3CA-MUT, KRAS-WT
C: KRAS-MUT, PIK3CA-WT
D: PIK3CA-MUT, KRAS-MUT
E: Unknown

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Figure 2

Mean Duration on Study Following Continuous Treatment with PX-866 Compared with Mutational Status

<table>
<thead>
<tr>
<th>Mutational Status</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA-mut</td>
<td>4</td>
</tr>
<tr>
<td>KRAS-mut</td>
<td>5</td>
</tr>
<tr>
<td>PIK3CA/KRAS-mut</td>
<td>2</td>
</tr>
<tr>
<td>PIK3CA-WT</td>
<td>8</td>
</tr>
</tbody>
</table>
Clinical Cancer Research

A Multicenter Phase 1 Trial of PX-866, an Oral Irreversible Phosphatidylinositol 3-Kinase Inhibitor, in Patients with Advanced Solid Tumors

David S. Hong, Daniel W Bowles, Gerald S. Falchook, et al.

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