A multicenter phase 1 study of PX-866 in combination with docetaxel in patients with advanced solid tumours

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Background: This phase I, dose-finding study determined the safety, maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D), pharmacokinetics, and antitumour activity of PX-866, a phosphatidylinositol 3-kinase inhibitor, combined with docetaxel in patients with incurable solid tumours.

Methods: PX-866 was administered at escalating doses (4–8 mg daily) with docetaxel 75 mg m−2 intravenously every 21 days. Archived tumour tissue was assessed for potential predictive biomarkers.

Results: Forty-three patients were enrolled. Most adverse events (AEs) were grade 1 or 2. The most frequent study drug-related AE was diarrhoea (76.7%), with gastrointestinal disorders occurring in 79.1% (docetaxel-related) and 83.7% (PX-866-related). No dose-limiting toxicities were observed. The RP2D was 8 mg, the same as the single-agent MTD. Co-administration of PX-866 and docetaxel did not affect either drug’s PKs. Best responses in 35 evaluable patients were: 2 partial responses (6%), 22 stable disease (63%), and 11 disease progression (31%). Eleven patients remained on study for >180 days, including 8 who maintained disease control on single-agent PX-866. Overall median progression-free survival (PFS) was 73.5 days (range: 1–569). A non-significant association between longer PFS for PIK3CA-MUT/KRAS-WT vs PIK3CA-WT/KRAS-WT was observed.

Conclusion: Treatment with PX-866 and docetaxel was well tolerated, without evidence of overlapping/cumulative toxicity. Further investigation with this combination is justified.

The PI3K-serine-threonine kinase (AKT)/mammalian target of rapamycin (mTOR) signalling pathway is often altered in human cancers, leading to increased expression of cell proliferation and survival genes and decreased expression of pro-apoptotic signals (Nicholson and Anderson, 2002; Courtney et al., 2010). PI3K is an intracellular kinase consisted of the p110α, p110β, or p110δ

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catalytic subunits, and a p85 regulatory subunit; mutations to p110α and p85 can be oncogenic (Samuels and Ericson, 2006; Jaiswal et al., 2009). Activating mutations to PIK3CA, the gene encoding the p110α catalytic subunit of PI3K, are found in several tumour types, including glioblastoma (27%), breast (18%), colorectal (16% of non-hypermutated tumours), cervical (33%), endometrial (39%), squamous cell carcinoma of the head and neck (SCCHN; 6–8%), and non-small cell lung cancer (NSCLC; 2–6%) (Levine et al., 2005; Hayes et al., 2006; Samuels and Ericson, 2006; Miyake et al., 2008; Agrawal et al., 2011; Stransky et al., 2011; Cancer Genome Atlas Network, 2012). Increased PIK3CA copy numbers are seen in prostate cancer (28%), squamous histology NSCLC (33%), and SCCHN (45%) (Yamamoto et al., 2008; Agell et al., 2011; Morris et al., 2011). The phosphatase and tensin homolog (PTEN) tumour suppressor gene, which inhibits PI3K signalling, may be lost via deletion (25% of melanoma, breast, and prostate cancers), mutation, or epigenetic suppression (Peschke et al., 1998; Tokunaga et al., 2007; Cancer Genome Atlas Research Network, 2008; Carracedo and Pandolfi, 2008). Lastly, upstream growth factor receptors that activate PI3K signalling, such as epidermal growth factor receptor and insulin-like growth factor receptor, are often overexpressed (Bowles and Jimeno, 2011; Zhang et al., 2011).

PX-866 is a potent, pan-isozyme inhibitor of PI3K that is a synthetic derivative of wortmannin. PX-866 irreversibly inhibits PI3K by binding covalently to lysine-802 in the ATP catalytic site (Wipf et al., 2004). PX-866 and its active metabolite, 17-OH-PX-866, demonstrate potent inhibition of PI3K, with respective IC_{50}s of 39 ± 21 and 14 ± 6 nM against PI3Kα, and 88 ± 27 and 57 ± 7 nM against PI-3Kβ (Wipf et al., 2004). Single-agent PX-866 delays tumour growth in A549 NSCLC, OvCar-3 ovarian cancer, HT29 colon cancer, and U87 glioma xenografts, with an association between antitumour activity and the presence of PIK3CA-activating mutations or reduced PTEN expression (Ihle et al., 2005; Koul et al., 2010).

In the first-in-human, phase 1, single-agent study of PX-866, the recommended phase 2 dose (RP2D) was 8 mg daily (Hong et al., 2012). The most common adverse events (AEs) were diarrhoea, nausea, and vomiting. Best response per Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 (Eisenhauer et al, 2009) was stable disease (SD) in over 50% of evaluable patients at week 6. As with other investigational PI3K inhibitors, PIK3CA mutations were associated with longer duration of SD, but this was not statistically significant (Bendell et al., 2012; Hong et al., 2012). Given the drug’s favourable toxicity profile and the importance of PI3K/AKT/mTOR pathway signalling in numerous malignancies, PX-866 was deemed a good candidate for combination chemotherapy studies. Docetaxel is an effective cytotoxic chemotherapeutic agent for many tumours where PI3K signalling is important, including NSCLC, SCCHN, breast, and prostate cancer (Dreyfuss et al., 1996; Shepherd et al., 2000; Tannock et al., 2004; Harvey et al., 2006). The only significant overlapping toxicities between single-agent PX-866 and single-agent docetaxel are diarrhoea, nausea, and vomiting. Patient-derived SCCHN xenografts show enhanced tumour suppression when treated with PX-866 and docetaxel compared with either agent alone (Bowles et al., 2011). We conducted a phase 1 study of the combination of PX-866 and docetaxel to determine the maximum tolerated dose (MTD)/RP2D, toxicity profile, PK, antitumour activity, and predictive biomarkers of response for the combination in patients with advanced cancers.

**Patients and Methods**

**Patients.** Inclusion criteria included patients with: incurable, locally advanced, or metastatic cancer for which docetaxel administered at a dose of 75 mg m⁻² IV every 21 days is approved, considered standard of care, or is compendia listed; measurable disease per RECIST 1.1 or, for metastatic castrate resistant prostate cancer (mCRPC), evaluable for response or progression based on prostate-specific antigen (PSA) or bone scan; life expectancy > 3 months; adequate hepatic, haematological, and renal function; Eastern Cooperative Oncology Group performance status of ≤ 1; and completed previous treatment > 4 weeks. Exclusion criteria included: presence of any medical/social factors impacting patient safety; pregnancy or breastfeeding; previous treatment with docetaxel (except in CRPC) or a PI3K inhibitor; known human immunodeficiency virus; known or suspected clinically active brain metastases; grade ≥ 2 peripheral neuropathy; and/or history of hypersensitivity reactions to docetaxel or to other drugs formulated with polysorbate. The institutional review board of all participating centres granted approval and written informed consent was mandatory.

**Design.** This was an open-label, dose-escalation study of orally administered daily PX-866 in combination with docetaxel 75 mg m⁻² IV once every 21 days. In cycle 1 only, to allow for assessment of the effect of PX-866 on docetaxel PK, docetaxel was administered on day 1 followed by initiation of treatment with daily oral PX-866 on day 8. In all subsequent cycles, PX-866 was administered in a fasting state daily throughout the cycle. Prophylactic and therapeutic use of filgrastim or pegfilgrastim was permitted in any cycle. Patients with CRPC received dexamethasone 8 mg PO at 12, 3, and 1 h before docetaxel administration, and also received prednisone 5 mg twice daily (BID) throughout treatment; all other patients received dexamethasone 8 mg PO BID for 3 days starting the day before docetaxel. Prophylactic anti-emetics were allowed per institutional guidelines. At the investigator’s discretion, patients experiencing toxicity attributable to docetaxel were permitted to continue to receive single-agent PX-866 after receiving at least two cycles of combination treatment with PX-866 and docetaxel, and achieving SD or better.

**PX-866 dose escalation.** PX-866 was administered at 4, 6, or 8 mg daily to cohorts of 6–9 patients each. The 4-mg starting dose of PX-866 was chosen as representing 50% of the previously determined single-agent MTD of 8 mg. Each cohort initially enrolled up to three patients; once at least two patients completed therapy through day 21 without experiencing a DLT, the remaining patients in the cohort were allowed to enrol. Patients were assessed for response if they received at least 75% of the planned doses of PX-866 in cycles 1 and 2, unless the reason for not doing so was a DLT or other PX-866-related toxicity. If not more than one of the first six evaluable patients experienced a DLT, then the dose of PX-866 was escalated. A dose was considered not tolerated if the observed rate of DLT in 15 patients was 33%. After identification of the MTD/RP2D, 17 additional patients were treated in an expansion cohort. If ≥33% of the patients in the expansion cohort experienced a DLT, enrollment was to have been halted pending review by the study Safety Monitoring Committee.

Patients were evaluated for efficacy approximately every 6 weeks. Patients with SD or better received repeated cycles of treatment until PD, unacceptable toxicity, or withdrawal of consent. Efficacy assessments were performed following RECIST 1.1, except in mCRPC patients who could have been evaluated for PSA response per the Prostate Cancer Clinical Trials Working Group recommendations.

**Safety monitoring.** Safety assessments included: vital signs, laboratory assessments, and physical exams. AEs were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.02. Dose-limiting toxicities included: ≥ grade 3 events considered possibly, probably, or definitely
related to the combination study drug treatment, with the exception of nausea, vomiting, or diarrhoea without maximal anti-emetic or antidiarrhoeal therapy; >2-week delay in the start of cycle 2 or 3 as result of PX-866-related toxicity; grade 3 or 4 neutropenia with fever; platelets <25 000 per μl; absolute neutrophil count <500 per μl for >7 days; grade 3 transaminitis >7 days; grade 4 transaminitis; or >grade 3 increase in serum glucose despite optimal therapy. Patients who experienced a DLT were allowed to continue in the study at a reduced dose level.

**Pharmacokinetic and biomarker measurements.** Pharmacokinetics assessments included plasma measurements for: PX-866, metabolites of PX-866, and docetaxel. See Supplementary Data for additional details. Samples were collected to assess: docetaxel PK on cycle 1 day 1 and 2; PX-866 PK on cycle 1 days 8, 9, and 15 and cycle 2 day 15; and PK of both drugs on cycle 2 days 1 and 2. Optional archival tumour tissue blocks were evaluated for mutations in PIK3CA (G1624A, A1634G, A1633A, A3140G, and A3140T) and KRAS (codons 12 and 13) using the shifted termination assay (TrimGen Corporation, Sparks, MD, USA) (Hong et al, 2012), and expression of PTEN using immunohistochemistry (IHC) of archival biopsy samples was performed using the monoclonal antibody 6H2.1 (Al-Zaid et al, 2012). PTEN intensity in the tumour cells was scored relative to internal positive controls (0, completely absent; 1, markedly reduced; 2, mildly reduced; and 3, normal or increased). Only complete loss (score = 0) in >90% of the tumour cellularity assessed was considered significant.

**Statistics.** The planned enrollment for phase 1 was up to 36 evaluable patients, including up to 27 evaluable patients in three dose cohorts during dose escalation and approximately 9 evaluable patients in a safety expansion cohort at the MTD/RP2D. The sample size allowed for an approximately 33% early discontinuation rate due to non-PX-866-related events. With a sample size of 15 patients, if the true incidence of DLT was 10%, there would be a 79% probability of observing at least one DLT and a 45% probability of observing two or more DLTs. Progression-free survival (PFS) was measured from the time of consent to the date of progression or until death from any cause, or if unknown, the date that the patient was last known to be alive and progression-free.

### RESULTS

#### Demographics and baseline characteristics

**Patient demographics and baseline characteristics**. Patient demographics, baseline characteristics, and disposition are described in Table 1 and Figure 1. Forty-four patients were enrolled, with 43 patients receiving study treatment (N = 10 (4 mg), N = 10 (6 mg), and N = 23 (8 mg)). Of these, two patients in the 8 mg group did not receive PX-866, one because of early disease progression and another because of withdrawal of consent. Overall, the median number of prior treatments was 2 (range: 1–11). The most common tumour types were SCCCHN (N = 6 of out 43; 14%), NSCLC (N = 5 out of 43; 12%), ovarian cancer (N = 5 out of 43; 12%), and prostate cancer (N = 5 out of 43; 12%).

**Dose escalation and MTD/RP2D determination.** PX-866 dosing started at 4 mg and was escalated to 6 mg, and then 8 mg (single-agent MTD). No DLTs were seen in the dose-escalation 3 cohorts, and the 8-mg cohort was expanded to a total of 23 patients with no DLTs documented.

**Safety.** Patients receiving at least one dose of either drug were evaluated for safety (N = 43). The most frequent treatment-emergent AEs were diarrhoea (77%), fatigue (61%), nausea (58%), vomiting (51%), neutropenia (40%), and peripheral oedema (40%) (Table 2). The majority (85%) of toxicities were grade 1/2

| PX-866 dose cohort | 4 mg (N = 10) | 6 mg (N = 10) | 8 mg (N = 23) | Total (N = 43)(%) |
|-------------------|--------------|--------------|--------------|-----------------|
| Age, median (years) | 62 | 57 | 57 | 59 |
| ECOG PS* = 0 | 3 | 0 | 6 | 9 |
| ECOG PS* = 1 | 7 | 10 | 16 | 33 |
| Male | 4 | 4 | 12 | 20 (47) |
| Female | 6 | 6 | 11 | 23 (53) |
| White or Caucasian | 9 | 10 | 21 | 40 (93) |
| Black of African American | 1 | 0 | 1 | 2 (5) |
| Hispanic | 0 | 0 | 1 | 1 (2) |
| Number of prior treatments | 2.5 (range: 1–8) | 3 (range: 1–11) | 2 (range: 1–11) | 2 (range: 1–11) |
| Number of patients with tumour type | SCCCHN | 3 | 1 | 2 | 6 (14) |
| Ovarian | 2 | 1 | 2 | 5 (12) |
| Prostate | 0 | 1 | 4 | 5 (12) |
| NSCLC | 1 | 1* | 3 | 5 (12) |
| Other* | 4 | 6 | 12 | 22 (51) |

Abbreviations: ECOG = Eastern Cooperative Oncology Group; NSCLC = non-small cell lung cancer; PS = performance status; SCCCHN = squamous cell carcinoma of the head and neck.

*ECOG PS was not available for one patient.

*One patient was an ECOG PS of 1 at screening but was an ECOG PS of 2 at the time of the first dose.

*One patient was enrolled but did not receive study treatment.

*Small cell lung (N = 2); adenoid cystic (N = 2); bladder (N = 2); pancreatic adenocarcinoma (N = 2); endometrial (N = 2); neuroendocrine (N = 1); Merkel Cell (N = 1); gastrointestinal (N = 1); retinal (N = 1); nasopharyngeal (N = 1); anal (N = 1); and gastric (N = 1).

Table 2. Grade 3/4 AEs considered related to docetaxel and reported in 2 or more patients included the following: neutropenia (N = 19); diarrhoea (N = 4); diarrhoea (N = 3); vomiting (N = 3); nausea (N = 2); and mental status changes (N = 1). Two SAEs of diarrhoea were considered related to PX-866 and docetaxel. There were three deaths on study. Two patients died after radiographic evidence of progressive disease and discontinuation of study drugs, and one patient died of pneumonia considered unrelated to either study drug.

PX-866 dose reductions were required in 4 out of 43 (9%) of patients (all in the 8 mg cohort), due to vomiting and/or diarrhoea. Docetaxel dose reduction was required in 18 out of 43 (42%) of patients. Fourteen of these patients required dose reduction due to neutropenia. At the investigator’s discretion, docetaxel was discontinued in eight patients due to toxicity after achieving SD or better with combination therapy. These patients had received at least four (range: 4–11) cycles of combination treatment, and then continued to receive single-agent PX-866.
Patients allocated to sequential cohorts assigned to one PX-866 close cohort (n=44)

4 mg PX-866
Allocated to intervention (n=10)
Received allocated intervention (n=10)
Did not receive allocated intervention (n=0)

6 mg PX-866
Allocated to intervention (n=11)
Received allocated intervention (n=10)
Did not receive allocated intervention (n=1)

8 mg PX-866
Allocated to intervention (n=23)
Received allocated intervention (n=23)
Did not receive allocated intervention (n=0)

Active (n=0)
Inactive (n=10)
Discontinued due to:
Adverse event (n=0)
Progressive disease (n=7)
Patient decision (n=1)
Withdrawal of consent (n=2)
Physician decision (n=0)
Lost to follow-up (n=0)
Death (n=0)

Active (n=0)
Inactive (n=10)
Discontinued due to:
Adverse event (n=1)
Progressive disease (n=7)
Patient decision (n=0)
Withdrawal of consent (n=1)
Physician decision (n=1)
Lost to follow-up (n=0)
Death (n=0)

Active (n=0)
Inactive (n=23)
Discontinued due to:
Adverse event (n=1)
Progressive disease (n=16)
Patient decision (n=2)
Withdrawal of consent (n=2)
Physician decision (n=0)
Lost to follow-up (n=1)
Death (n=1)

Analysed for safety (n=10)
Analysed for antitumour response:
Evaluable (n=8)
Non-evaluable (n=2)

Analysed for safety (n=10)
Analysed for antitumour response:
Evaluable (n=9)
Non-evaluable (n=1)

Analysed for safety (n=23)
Analysed for antitumour response:
Evaluable (n=16)
Non-evaluable (n=7)

Figure 1. CONSORT diagram of the phase 1 portion of study PX-866-002. Enrollment and patient disposition in phase 1 of study PX-866-002. Forty-three patients were enrolled and treated with combination treatment (PX-866 and docetaxel), with 35 evaluable patients.

PFS and best response. Of the 43 patients treated, 12 discontinued treatment before progression for the following reasons, including 8 before a reassessment scan: AE (N=2), patient decision (N=3), withdrawal of consent (N=5), physician decision (N=1), or lost to follow-up (N=1). Of the 35 patients evaluable with at least one follow-up CT scan, best responses were 2 PR (6%), 22 SD (63%), and 11 PD (31%). The PRs were observed in patients with NSCLC and ovarian cancer. Ten patients (29%) had tumour shrinkage of ≥15%. Eleven patients had PFS of >180 days, including four patients with ovarian cancer, two patients with NSCLC, and one patient each with endometrial cancer, large cell neuroendocrine lung cancer, prostate cancer, nasopharyngeal carcinoma, and adenoid cystic carcinoma. Of the patients who discontinued docetaxel and continued on single-agent PX-866, disease control was maintained for up to 10 additional cycles, including a NSCLC patient with a PIK3CA mutation stable for 5 additional cycles on single-agent PX-866, and two patients with unknown mutational status who remained on single-agent PX-866 for 10 additional cycles.

Molecular correlation: PFS and best responses. We hypothesised that oncogenic PIK3CA mutation might be associated with improved response to PX-866, but that this effect could be overridden by mutant KRAS. Mutational status of PIK3CA and KRAS was obtained from archived tumour biopsies from 31 patients (Supplementary Table 1). Median PFS for PIK3CA-WT/KRAS-WT (N=20) was 49 days (range: 1–342) vs 175 days (range: 49–334) for PIK3CA-MUT/KRAS-WT (N=6) (two-tailed t-test; P = 0.23). Patients whose mutational status (N=12) was unknown had a median PFS of 77.5 days (range: 1–569). There were a limited number of patients with KRAS mutation only (PIK3CA-WT/KRAS-MUT) (N=3) and dual mutations (PIK3CA-MUT/KRAS-MUT) (N=2).
Pharmacokinetics. Full PK data are available in Table 3, Table 4, and the Supplementary Data (Supplementary Figures 1 and 2). The exposure of PX-866 in the presence of docetaxel was similar to historical levels, and docetaxel exposure was not affected by the presence of PX-866.

Table 2. Treatment-emergent adverse events by severity and preferred term occurring in ≥15% of phase 1 patients

| PX-866 dose | 4 mg (N = 10) | 6 mg (N = 10) | 8 mg (N = 23) | Total (N = 43) |
|-------------|---------------|---------------|---------------|---------------|
| Frequency by severity, N (%) | | | | |
| Grade 1 | 32 (56) | 36 (49) | 84 (50) | 152 (51) |
| Grade 2 | 10 (18) | 30 (41) | 62 (37) | 102 (34) |
| Grade 3 | 8 (14) | 5 (7) | 15 (9) | 28 (9) |
| Grade 4 | 7 (12) | 3 (4) | 8 (5) | 18 (6) |
| Grade 5 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Frequency by preferred term, N (%) | | | | |
| Diarrhoea | 7 (70) | 8 (80) | 18 (78) | 33 (77) |
| Fatigue | 6 (60) | 7 (70) | 13 (57) | 26 (61) |
| Nausea | 5 (50) | 4 (40) | 16 (70) | 25 (58) |
| Vomiting | 3 (30) | 5 (50) | 14 (61) | 22 (51) |
| Neutropenia | 6 (60) | 5 (50) | 6 (26) | 17 (40) |
| Oedema peripheral | 4 (40) | 6 (60) | 7 (30) | 17 (40) |
| Decreased appetite | 1 (10) | 4 (40) | 10 (44) | 15 (35) |
| Dehydration | 3 (30) | 1 (10) | 11 (48) | 15 (35) |
| Anaemia | 2 (20) | 2 (20) | 9 (39) | 13 (30) |
| Constipation | 2 (20) | 3 (30) | 8 (35) | 13 (30) |
| Alopecia | 2 (20) | 6 (60) | 4 (17) | 12 (28) |
| Asthenia | 1 (10) | 0 (0) | 6 (26) | 11 (26) |
| Pyrexia | 1 (10) | 4 (40) | 6 (26) | 11 (26) |
| Cough | 2 (20) | 5 (50) | 3 (13) | 10 (23) |
| Bone pain | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hypokalemia | 2 (20) | 1 (10) | 6 (26) | 9 (21) |
| Stomatitis | 1 (10) | 4 (40) | 0 (0) | 4 (9) |
| Dysgeusia | 2 (20) | 3 (30) | 3 (13) | 8 (19) |
| Arthralgia | 0 (0) | 2 (20) | 5 (22) | 7 (16) |
| Dizziness | 1 (10) | 2 (20) | 4 (17) | 7 (16) |
| Epistaxis | 1 (10) | 2 (20) | 4 (17) | 7 (16) |
| Oropharyngeal pain | 1 (10) | 1 (10) | 5 (22) | 7 (16) |
*Preferred terms were coded using Medical Dictionary for Regulatory Activities version 13.0.

Table 3. Summary of docetaxel pharmacokinetics

| Visit | Patient | T_max (h) | C_max (ng ml⁻¹) | AUClast (h*ng ml⁻¹) | AUCINF (h*ng ml⁻¹) | Cl (L h⁻¹) | Vz (L) | Half-life (h) | MRT_INF (h) |
|-------|---------|-----------|----------------|---------------------|-------------------|------------|------|-------------|-------------|
| C1D1  | N       | 43        | 43             | 43                  | 43                | 43         | 43   | 43          | 43          |
| Mean  | 0.19    | 799.12    | 1203.79        | 1530.81             | 143.66            | 2993.46    | 43   | 43          | 43          |
| SE    | 0.02    | 146.59    | 235.49         | 270.84              | 10.98             | 273.91     | 43   | 43          | 43          |
| CV%   | 56.1    | 120.3     | 128.3          | 116                 | 50.1              | 60         | 49.3 | 70.9        |
| C2D1  | N       | 35        | 35             | 35                  | 35                | 35         | 35   | 35          | 35          |
| Mean  | 0.19    | 538.86    | 850.85         | 1106.26             | 154.1             | 2691.45    | 35   | 35          | 35          |
| SE    | 0.02    | 60.66     | 88.15          | 127.4               | 15.23             | 235.19     | 35   | 35          | 35          |
| CV%   | 48.1    | 66.6      | 61.3           | 68.1                | 58.5              | 51.7       | 39   | 46.1        |

**Abbreviations:** AUC = area under the curve; Cl = clearance; CV = coefficient of variance; INF = infinity; D = day; MRT = mean resonance time; SE = standard error; Vz = volume of distribution.

**DISCUSSION**

This study demonstrates that PX-866, an oral, irreversible small-molecule PI3K inhibitor, can be safely combined with docetaxel 75 mg m⁻² every 21 days at the maximal-tolerated single-agent dose of 8 mg daily. The most common AEs of all grades included gastrointestinal toxicities (diarrhoea, nausea, and vomiting) and fatigue. Overall, PX-866-related gastrointestinal AEs were dose related. Although the overall incidence of grade 2 AEs increased with PX-866 dose escalation, there was no increase in the frequency of grade 3 or 4 AEs. The pattern of AEs was similar to those described with either single-agent PX-866 or docetaxel, though the incidence of neutropenia was somewhat higher than seen with some second-line docetaxel trials (Schuette et al, 2005). Although PX-866 has not been associated with neutropenia as a single agent, a drug–drug interaction that enhances docetaxel’s myelosuppressive effects cannot be ruled out. Unexpected increases in neutropenia have been seen in other docetaxel combination trials with agents thought not to be myelosuppressive (Jannie et al, 2013; Marshall et al, 2013). This may have been compounded by PX-866’s introduction at the neutrophil nadir on day 8 of cycle 1. PX-866 exposure in this study was similar to historical controls, and docetaxel exposure was not modified by PX-866 (Hong et al, 2012). PX-866 dose reductions were uncommon; docetaxel was reduced in less than half of patients. As combination therapies are most effective when all agents are given at their MTD, it is encouraging that PX-866 and docetaxel can be combined at the MTD of each single agent.

There were signs of anticancer activity in this phase 1 study. The partial response rate of 6% (2 out of 35) in evaluable patients, with a PR + SD rate of 69%, is consistent with what is expected in a heavily pretreated population and the second-line docetaxel responses in NSCLC (PR 13%, PR – SD 50%) (Roberts et al, 2004; Horstmann et al, 2005; Schuette et al, 2005). Overall, the median PFS for evaluable patients was 73.5 days (range: 1–569), including 31% (11 out of 35) of evaluable patients remaining on study in excess of 180 days, is encouraging and supports further investigation. It is noteworthy that several patients continued to experience SD on single-agent PX-866 after completing at least four cycles of concurrent docetaxel chemotherapy, including one patient with a PIK3CA mutation. One hypothesis is that PX-866 enhances the initial response to docetaxel, and then suppresses further growth through cell signalling inhibition without the cumulative toxicity of continued cytotoxic therapy. In prostate cancer xenografts, for instance, destruction of bulk tumour cells with docetaxel and cancer stem cell (CSC) suppression with a PI3K/mTOR inhibitor was more effective at suppressing growth.
and decreasing CSC populations than monotherapy with either agent alone (Dubrovská et al., 2010). In breast cancer in vivo and in vitro models, PI3K inhibition increased caspase-3-mediated apoptosis in cells in mitotic arrest from docetaxel therapy (Wallin et al., 2012). The ability to provide chronic tumour suppression with a PI3K inhibitor after initial induction of response would be of clinical value, because PX-866 may be given safely for over 1 year (Hong et al., 2012).

We did not identify a reliable predictive biomarker for response to therapy with PX-866 plus docetaxel. In this study, PIK3CA mutation, KRAS mutation, or PTEN level by IHC did not predict outcome. It has been difficult to correlate tumour mutations or protein expression profiles with responses to PI3K inhibitors. The presence of activating PIK3CA mutations did predict a higher response rate to PI3K/AKT/mTOR pathway inhibitors in a mixed phase 1 population, though this study included PI3K, AKT, mTOR, and combined inhibitors (Janku et al., 2011; Janku et al., 2012b). However, in the single-agent PX-866 phase 1 study there was a suggestion of longer time on study for patients with PIK3CA mutations that did not meet statistical significance (Hong et al., 2012). Similarly, in the phase 1 study of BKM120, an oral pan-PI3K inhibitor, no correlation was reported between antitumour activity and mutation status (PIK3CA or KRAS) or PTEN protein expression (Bendell et al., 2012). Moreover, alterations in PTEN level do not appear to predict responses to inhibitors of the PI3K/AKT/mTOR pathway, though this may reflect PTEN-deficient tumours’ dependence on p110β rather than p110α (Jia et al., 2008; Wee et al., 2008; Janku et al., 2012a). At this point, an accurate predictor of benefit to PI3K/AKT/mTOR inhibition remains elusive.

In conclusion, this phase 1 combination study of PX-866 and docetaxel established the RP2D as 8 mg of PX-866 when given with docetaxel at full dose. Tumour mutational analysis and protein expression of PTEN did not correlate with outcome. PK analysis revealed no drug–drug interaction between PX-866 and docetaxel. The combination’s favourable toxicity profile and antitumour activity support its further clinical development. A randomised phase 2 open-label study of docetaxel +/− PX-866 in second-line NSCLC and platinum-refractory SCCHN (NCT01204099) is ongoing.

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**CONFLICT OF INTEREST**

Alex A Vo, Scott Peterson, Luke Walker, and Diana Hausman are employees of Oncothyreon Inc. Antonio Jimeno has received laboratory research support from Oncothyreon Inc.; the University of Colorado holds intellectual property interests. The remaining authors declare no conflict of interest.

**REFERENCES**

Agell I, Hernandez S, Salido M, de Muga S, Juanpere N, Arumi-Uria M, Menendez S, Lorenzo M, Lorente JA, Serrano S, Lloreta J (2011) PI3K signaling pathway is activated by PIK3CA mRNA overexpression and
copy gain in prostate tumors, but PIK3CA, BRAF, KRAS and AKT1 mutations are infrequent events. Mod Pathol 24(3): 443–452.

Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang I, Wang J, Zhang N, El-Naggar AK, Jasser SA, Weinstein JN, Trevino L, Drummond JA, Muzny DM, Wu Y, Wood LD, Hruban RH, Westra WH, Koch WM, Califano JA, Gibbs RA, Sidransky D, Vogelstein B, Velculescu VE, Papadopoulos N, Wheeler DA, Kinzler KW, Myers JN (2011) Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 333(6046): 1154–1159.

Al-Zaide T, Dietelberg JS, Prieto VG, Lev D, Lutrha R, Davies MA, Diwan AH, Wang WL, Lazar AJ (2012) Trichilemmomas lose show of PI3T in Cowden syndrome but only rarely in sporadic tumors. J Cutan Pathol 39(5): 493–499.

Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanes DJ, De Buck SS, Ru QC, Peters M, Goldbrunner M, Baselga J (2012) Phase I dose, escalation-study of BKMK120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 30(3): 282–290.

Bowles DW, Keysar S, Anderson R, Sehrt D, Helber R, Song J, Hausman DF, Harvey V, Mouridsen H, Semiglazov V, Jakobsen E, Voznyi E, Robinson BA, Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, www.bjcancer.com | BRITISH JOURNAL OF CANCER (2012) Comprehensive characterization of human colon and rectal cancer. Nature 487(7407): 336–337.

Kurzrock R (2012a) PTEN assessment and PI3K/mTOR inhibitors: Importance of simultaneous assessment of MAPK pathway aberrations. J Clin Oncol 30(8): 777–782.

Kurzrock R, Timbersidor AM, Garrido-Laguna I, Westin SN, Moulder SL, Naing A, Bettegowda C, Chang K, Li RJ, Bowles DW, Keysar S, Anderson R, Sehrt D, Helber R, Song J, Hausman DF, Harvey V, Mouridsen H, Semiglazov V, Jakobsen E, Voznyi E, Robinson BA, Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, www.bjcancer.com | BRITISH JOURNAL OF CANCER (2012) Comprehensive characterization of human colon and rectal cancer. Nature 487(7407): 336–337.

Kurzrock R (2012b) PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol 30(8): 777–782.

Kauf D, Shen R, Kim YW, Kondo Y, Lu Y, Bankson J, Rones SM, Kirkpatrick DL, Powis G (2005) The phosphatidylinositol-3-kinase inhibitor PX-866 overcomes resistance to the epidermal growth factor receptor in human cancer xenografts. Mol Cancer Ther 4(9): 1349–1357.

Jaiswal BS, Janakiraman V, Klijavin NM, Chaudhuri S, Stern HM, Wang W, Han Z, Dhouk HA, Peters BA, Waring P, Dela Vega T, Kenski DM, Bowman KK, Lorenzo M, Li H, Wu J, Modrzen Z, Stinson J, Eby M, Yue P, Kaminker JS, de Sauvage FJ, Backer JM, Seshagiri S (2009) Spontaneous mutations in p8alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell 16(4): 463–474.

Janku F, Broadus R, Bakkar R, Hong DS, Stepanek VMT, Naing A, Falchook GS, Fu S, Wheler JI, Piha-Paul SA, Moulder SL, Luthra R, Tismberioud AM, Kurzrock R (2012a) PTEN assessment and PIK3K/mTOR inhibitors: Importance of simultaneous assessment of MAPK pathway aberrations. J Clin Oncol 30(8): abstract 10510.

Janku F, Timbersidor AM, Garrido-Laguna I, Westin SN, Moulder SL, Naing A, Bettegowda C, Chang K, Li RJ, Bowles DW, Keysar S, Anderson R, Sehrt D, Helber R, Song J, Hausman DF, Harvey V, Mouridsen H, Semiglazov V, Jakobsen E, Voznyi E, Robinson BA, Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, www.bjcancer.com | BRITISH JOURNAL OF CANCER (2012) Comprehensive characterization of human colon and rectal cancer. Nature 487(7407): 336–337.

Kurzrock R (2012b) PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol 30(8): 777–782.

Janns PA, Shaw AT, Pereira JR, Jeaninn G, Vansteenkiste J, Barrios C, Frank E, Gristedt L, Zaluzina V, Smith P, Smith I, Crino L (2013) Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 14(1): 38–47.

Jia S, Liu Z, Zhang S, Li P, Zhang L, Lee SH, Zhang J, Signoretto S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3k)-p110beta in cell growth, metabolism and tumorigenesis. Nature 454(7205): 776–779.

Kaul D, Shen R, Kim YW, Kondo Y, Lu Y, Bankson J, Ronen SM, Kirkpatrick DL, Powis G, Yung WK (2010) Cellular and in vivo activity of a novel PI3K inhibitor, PX-866, against human glioblastoma. Neuro Oncol 12(6): 559–569.

Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J (2012) Frequent mutation of the PIK3CA gene in ovarian and breast cancers. Clin Cancer Res 18(8): 2875–2878.

Marshall J, Shapiro GI, Uttenreither-Fischer M, Ould-Kaci M, Stopfer P, Gordon MS (2013) Phase I dose-escalation study of afatinib, an ErbB family blocker, plus docetaxel in patients with advanced cancer. Future Oncol 9(12): 271–281.

Miyake T, Yoshino K, Enomoto T, Takata T, Ugaki H, Kim A, Fujiiwara K, Miyatake T, Fujita M, Kimura T (2008) PIK3CA gene mutations and amplifications in uterine cancers, identified by methods that avoid confounding by PIK3CA pseudogene sequences. Cancer Lett 261(1): 120–126.

Morris LG, Taylor BS, Bivona TG, Gong Y, Deng S, Brennan CW, Kaufman A, Kastenhuber ER, Banerjee V, Singh B, Heguy A, Viale A, Mellinghoff IK, Huse J, Ganly I, Chen TA (2011) Genomic dissection of the epidermal growth factor receptor (EGFR)/PI3K pathway reveals frequent deletion of the EGFR phosphatase PTPRS in head and neck cancers. Proc Natl Acad Sci USA 108(7): 19024–19029.

Nicholson KM, Anderson NG (2002) The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal 14(5): 381–395.

Pesche S, Latil A, Muzeau F, Cussenot O, Fourrier G, Longy M, Eng C, Liderau R (1998) PTEN/MMAC1/TEP1 involvement in primary prostate cancers. Oncogene 16(22): 2879–2883.

Robertson JR, Gough BH, Quittieri L, Stallings SC, Halpern EF, Chabner BA, Gazelle GS, Finkelstein SN, Clark JW (2004) Trends in the risks and benefits to patients with cancer participating in phase 1 clinical trials. JAMA 292(17): 2130–2140.

Samuels Y, Ericson K (2006) Oncogenic PI3K and its role in cancer. Curr Opin Oncol 18(1): 77–82.

Schuette W, Nagel S, Blankenburg T, Lautenschlaeger C, Hans K, Schmidt EW, Dittrich I, Schweisfurth H, von Weikersthal LF, Raghavachar A, Reissig A, Serke M (2005) Phase III study of second-line chemotherapy for advanced non-small-cell lung cancer with weekly compared with 3-weekly docetaxel. J Clin Oncol 23(33): 8389–8395.

Shepherd FA, Dancey J, Bunn PA, Mattson K, Grailla R, O’Rourke M, Levitan N, Grossot L, Vincent M, Burkes R, Coughlin S, Kim Y, Berille J (2000) Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 18(10): 2095–2103.

Stansky N, Egloff AM, Tward AD, Kostic AD, Gibuliskis K, Swivchenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Sheller E, www.bjcancer.com | BRITISH JOURNAL OF CANCER (2012) Comprehensive characterization of human colon and rectal cancer. Nature 487(7407): 336–337.
Ramos AH, Stojanov P, Carter SL, Voel D, Cortes ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RB, Wang L, Rangel-Escareno C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zaigla J, Winckler W, Ardrie K, Gabriel SB, Meyerson M, Lander ES, Getz G, Golub TR, Garraway LA, Grandis JR (2011) The mutational landscape of head and neck squamous cell carcinoma. Science 333(6046): 1157–1160.

Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 351(15): 1502–1512.

Tokunaga E, Oki E, Kimura Y, Yamanaka T, Egashira A, Nishida K, Koga T, Morita M, Kakeji Y, Maehara Y (2007) Coexistence of the loss of heterozygosity at the PTEN locus and HER2 overexpression enhances the Akt activity thus leading to a negative progesterone receptor expression in breast carcinoma. Breast Cancer Res Treat 101(3): 249–257.

Wallin JJ, Guan J, Prior WW, Lee LB, Berry L, Belmont LD, Koeppen H, Belvin M, Friedman LS, Sampath D (2012) GDC-0941, a novel class I selective PI3K inhibitor, enhances the efficacy of docetaxel in human breast cancer models by increasing cell death in vitro and in vivo. Clin Cancer Res 18(14): 3901–3911.

Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao YM, Lengauer C (2008) PTEN-deficient cancers depend on PIK3CB. Proc Natl Acad Sci USA 105(35): 13057–13062.

Wipf P, Minion DJ, Halter RJ, Berggren ML, Ho CB, Chiang GG, Kirkpatrick L, Abraham R, Powis G (2004) Synthesis and biological evaluation of synthetic viridins derived from C(20)-heteroalkylation of the steroidal PI-3-kinase inhibitor wortmannin. Org Biomol Chem 2(13): 1911–1920.

Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, Soh J, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Date H, Lam WL, Minna JD, Gazdar AF (2008) PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 68(17): 6913–6921.

Zhang Y, Moerkens M, Ramaiahgari S, de Bont H, Price L, Meerman J, van de Water B (2011) Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. Breast Cancer Res 13(3): R52.

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