Published in final edited form as:

Clin Cancer Res. 2018 January 01; 24(1): 22–32. doi:10.1158/1078-0432.CCR-17-1807.

A multicenter phase I study evaluating dual PI3K and BRAF inhibition with PX-866 and vemurafenib in patients with advanced BRAF V600 mutant solid tumors

Clinton Yam^{1,2}, Xiaowei Xu¹, Michael A. Davies², Phyllis A. Gimotty³, Jennifer J.D. Morrisette⁴, Michael T. Tetzlaff², Khalida M. Wani², Shujing Liu¹, Wanleng Deng², Meghan Buckley³, Jianhua Zhao⁴, Ravi K. Amaravadi¹, Naomi B. Haas¹, Ragini R. Kudchadkar⁵, Anna C. Pavlick⁶, Jeffrey A. Sosman⁷, Hussein Tawbi^{2,8}, Luke Walker⁹, Lynn M. Schuchter¹, Giorgos C. Karakousis¹, and Tara C. Gangadhar¹

¹Abramson Cancer Center and the Division of Hematology & Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

²The University of Texas MD Anderson Cancer Center, Houston, TX

³Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

⁴Center for Personalized Diagnostics, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

⁵H. Lee Moffitt Cancer Center, Tampa, FL

⁶New York University Medical Center, New York, NY

⁷Vanderbilt-Ingram Cancer Center, Nashville, TN

⁸The University of Pittsburgh, Pittsburgh, PA

⁹Cascadian Therapeutics (formerly Oncothyreon) Inc., Seattle, WA

Abstract

Purpose—The objectives of the study were to evaluate the safety of daily oral PX-866 in combination with twice daily vemurafenib and to identify potential predictive biomarkers for this novel combination.

Experimental Design—We conducted a phase I, open-label, dose escalation study in patients with advanced BRAF V600 mutant solid tumors. PX-866 was administered on a continuous schedule in combination with vemurafenib. Patients underwent a baseline and on-treatment biopsy after 1-week of PX-866 monotherapy for biomarker assessment.

Results—24 patients were enrolled. The most common treatment-related adverse events were gastrointestinal side effects. One dose limiting toxicity (DLT) of grade 3 rash and one DLT of grade 3 pancreatitis were observed in cohort 2 (PX-866 6mg daily; vemurafenib 960mg twice

daily) and cohort 3 (PX-866 8mg daily; vemurafenib 960mg twice daily), respectively. Of 23 response evaluable patients, 7 had confirmed partial responses (PRs), 10 had stable disease and 6 had disease progression. Decreases in intra-tumoral pAKT expression were observed following treatment with PX-866. Patients who achieved PRs had higher rates of PTEN loss by immunohistochemistry (80% vs 58%) and pathogenic PTEN mutations and/or deletions (57% vs 25%). Two patients with durable PRs had an increase in intra-tumoral CD8 T-cell infiltration following treatment with PX-866.

Conclusion—PX-866 was well tolerated at its maximal tolerated single-agent dose when given in combination with a modified dose of vemurafenib (720mg twice daily). Response to treatment appeared to be associated with PTEN loss and treatment with PX-866 seemed to increase CD8 T-cell infiltration in some patients.

Keywords

PX-866; phase I study; BRAF V600 mutant cancers; PI3K inhibition; melanoma

INTRODUCTION

The phosphatidylinositol-3-kinase (PI3K) and AKT signaling pathway has been found to be aberrantly activated in multiple human cancers including melanoma, glioma, colon, non-small cell lung, breast, prostate and head and neck cancers (1–3). Deregulation of the PI3K/AKT pathway, which can cause resistance to BRAF inhibition, may be mediated by several mechanisms in melanoma, including loss of function of the phosphatase and tensin homolog (PTEN) tumor suppressor, upstream growth factor receptor activation or *PIK3CA* gene mutation and/or amplification (4–10). Dual inhibition of both PI3K and BRAF is a possible strategy to improve the efficacy of targeted therapy for BRAF V600 mutant melanoma. Identification of specific subsets of patients who will benefit from such combinations may help further individualize therapy and improve outcomes in melanoma.

PX-866 (Cascadian Therapeutics [formerly Oncothyreon], Inc.) is a synthetic wortmannin derivative and a potent, orally available, irreversible pan-isoform PI3K inhibitor (11), with half maximal inhibitory concentration (IC_{50}) values for the PI3K- α , PI3K- β , PI3K- γ and PI3K- δ isoforms being 39 nM, 88nM, 124 nM and 183 nM, respectively (PX-866 Investigator's Brochure V5.0, Cascadian Therapeutics, Inc.). The maximum tolerated dose (MTD) in a single agent phase I dose escalation study on a continuous schedule was 8mg daily with pharmacodynamic inhibition of pAKT observed at the MTD and a dose limiting toxicity (DLT) of diarrhea (12). Other common grade 1 and 2 gastrointestinal adverse events included nausea, vomiting and reversible aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevation. Subsequent studies further demonstrated the safety and tolerability of PX-866 in combination with other agents including docetaxel (13) and cetuximab (14) in patients with advanced solid tumors.

The combination of PX-866 and BRAF inhibition demonstrated synergistic inhibition of cell proliferation in melanoma cell lines (15,16). Furthermore, combined targeting of PI3K and mitogen-activated protein kinase (MAPK) pathways in PTEN null melanoma cell lines has been shown to result in enhanced cell killing (17). In addition, synergistic anti-tumor effects

have also been observed in pre-clinical xenograft mouse models of melanoma (15). These data suggest that the combination of PI3K inhibition and vemurafenib may improve outcomes in patients with melanoma harboring *PTEN* deletions or other changes that increase PI3K pathway activation. In addition, tumor profiling of baseline patient biopsies along with comparative analysis of pre-treatment and on-treatment biopsies may help to increase our knowledge of resistance mechanisms to BRAF inhibition and facilitate the interpretation of the clinical effects of PI3K inhibition in combination with BRAF inhibitor therapy. Based on this background and rationale, we conducted a multi-center phase I study of PX-866 and vemurafenib in patients with advanced BRAF V600 mutated cancers to determine the safety of daily oral PX-866 in combination with twice daily vemurafenib, and to assess biomarkers that may predict clinical benefit from this combination.

PATIENTS AND METHODS

Eligibility

Patients were eligible for the study if they were 18 years or older with pathologically confirmed advanced (unresectable stage IIIC or IV) BRAF V600 mutant (V600E or V600K) solid tumors, including melanoma. There were no restrictions placed on prior therapy received including the use of prior BRAF and MEK directed therapies. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ and marrow function. Patients with active untreated central nervous system (CNS) disease were excluded. Patients with previously treated CNS disease were required to be stable for 8 weeks prior to enrollment. Patients were also excluded if their corrected QT interval was greater than 480 milliseconds, had uncontrolled diabetes mellitus or if they were known to be human immunodeficiency virus positive. The trial was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects. The protocol was reviewed by the Institutional Review Boards of all participating centers and all patients provided written informed consent.

Study design and treatment

The primary objective of the trial was to determine the safety of daily oral PX-866 in combination with twice daily vemurafenib. Eligible patients were enrolled at participating centers from 7/5/2012 through 3/4/2014. We used a standard 3 plus 3 dose escalation study design to evaluate up to two dose levels of vemurafenib in order to identify the MTD of PX-866 when used in combination with vemurafenib. Treatment was administered on a 28-day cycle. During the initial 1-week lead-in period (cycle 1, days 1 through 7), patients received PX-866 alone. Starting on cycle 1, day 8 (C1D8), all patients received combination therapy according to their pre-specified dose cohorts (Table 1). For subsequent cycles, both PX-866 and vemurafenib were administered at the pre-specified doses from days 1 through 28. Patients who received at least one dose of either PX-866 or vemurafenib were included in the safety analysis patient set. Patients were considered evaluable for DLTs if they received at least 75% of the planned days of dosing with PX-866 and vemurafenib during Cycle 1. If the reason for not receiving 75% or more of the planned doses was a DLT, the patient was still considered DLT evaluable.

Evaluations before and during treatment consisted of a complete medical history, physical examinations, hematologic and metabolic laboratory profiles, cross sectional imaging and toxicity assessments. Patients remained on study until radiographic or clinical disease progression, unacceptable toxicity, or withdrawal of consent. Full supportive care was provided as indicated.

Dose escalation

The first dose level of PX-866 and vemurafenib to be evaluated was 6mg PO daily and 720mg PO twice daily, respectively. This dose level was chosen as representing 75% of the previously determined single-agent MTD for each drug. Each cohort initially enrolled up to 3 patients. Patients considered non-DLT evaluable were replaced. Patient enrollment and dose escalation proceeded according to a standard 3 plus 3 study design. To account for potential patient discontinuation from the study prior to being evaluable for DLT, enrollment of up to 2 additional patients per cohort was allowed.

Safety monitoring and dose modifications

Patients were evaluated for toxicity while on study. Severity was graded according to the National Cancer Institute's Common Terminology for Adverse Events (NCI CTCAE), version 4.03. A treatment-related adverse event (AE) was defined as an adverse event that first occurred or worsened in intensity after the administration of the study drug and considered related to PX-866 alone, vemurafenib alone or to both drugs. PX-866 was held for any grade 3 or greater adverse event considered to be related to PX-866 or the combination of PX-866 and vemurafenib until the toxicity resolved to grade 1 or less with the following exceptions: grade 3 neutropenia without fever, grade 3 skin cancer, and nausea, vomiting, or diarrhea in the absence of use of maximal antiemetic or antidiarrheal medications. PX-866 was discontinued if a delay of greater than two weeks was required for treatment-related toxicity. Likewise, vemurafenib was held for any grade 3 or greater adverse event considered to be related to vemurafenib or the combination until the toxicity resolved to grade 1 or less with the following exceptions: grade 3 neutropenia without fever; grade 3 skin cancer; or grade 3 nausea, vomiting, or diarrhea in the absence of use of maximal antiemetic or antidiarrheal medications.

Dose limiting toxicities

DLT was assessed during cycle 1 and defined as any grade 3 or greater AE that was possibly, probably or definitely related to PX-866 or the combination of PX-866 and vemurafenib, with the exception of nausea, vomiting or diarrhea without maximal antiemetic or antidiarrheal therapy; development of new non-melanoma skin cancer; asymptomatic increases in lipase or amylase; or grade 3 fatigue lasting for 7 days or less. Any patient who required a greater than 2-week delay in the start of cycle 2 due to unresolved toxicity related to PX-866 or the combination of PX-866 and vemurafenib was also considered to have a DLT. Toxicities that were unrelated to study treatment or related to vemurafenib alone were not considered DLTs. Patients who experienced a DLT were allowed to continue in the study at a lower dose according to the protocol specified dose modification following recovery of the toxicity to no more than grade 1 or the baseline level of severity.

Disease monitoring

Patients were evaluated for disease response or progression with CT/MRI scans every 8 weeks for the initial 24 weeks and every 12 weeks thereafter. Complete response (CR), partial response (PR) and progressive disease (PD) were defined and assessed according to Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). Patients who had PD by RECIST 1.1 criteria, but who were determined to have a clinical benefit from the treatment as determined by the clinical investigator, were allowed to continue treatment with the study combination after discussion and written approval from the medical monitor.

Tissue collection

All patients were asked to provide archived tumor biopsy specimens for assessment of potential biomarkers of PX-866 and vemurafenib response and resistance. Fresh tumor biopsies were also obtained at baseline (pre-treatment), following the 1-week lead-in treatment period with PX-866 alone (C1D8), after at least two weeks of combination therapy (when feasible) and at the time of progression (when feasible). All patient samples were collected and stored in appropriate specimen containers according to a protocol specified standard operating procedure.

Paired pre-treatment and C1D8 tumor specimens for each patient were analyzed by immunohistochemistry (IHC) for evidence of target inhibition and immune activation after initiation of PI3K inhibition. IHC of formalin-fixed paraffin-embedded (FFPE) sections of paired tumor specimens was performed on a Leica Bond-IIITM instrument using the Bond Polymer Refine Detection System (Leica Biosystems DS9800). Following heat induced epitope retrieval, commercially available antibodies against p-AKT Ser473 (Leica NCL-L-AKT-phos; 1:400), PTEN (DAKO M3627, clone 6H2.1; 1:1200), CD8 (DAKO M7103, clone C8/144B; 1:40) and PD-L1 (Cell Signaling Technology 15165BF, clone E1J2J; 1:2000) were incubated with the tumor specimens. Samples were then incubated with the appropriate polymer and endogenous peroxidase was blocked with hydrogen peroxide. The chromogenic reaction was carried out with 3,3'-diaminobenzidine chromogen solution and slides were counterstained with hematoxylin. The H-score (18) was used for semi-quantitative analysis of p-AKT Ser473 and membranous PD-L1 staining in this study. PTEN loss by IHC was defined as the complete absence of PTEN staining. The mean number of CD8 positive cells per high-power field was used to quantify the degree of CD8 T-cell infiltration. To ensure uniform evaluation, all slides were reviewed by a single pathologist (X.X.).

For identification of somatic tumor mutations, FFPE specimens were either macro-dissected from 5µM slides or used whole via 10µM curls. The samples were dewaxed with Qiagen Deparaffinization Solution and the tissue was lysed with Qiagen ATL Buffer. To further disrupt the tissue, an overnight incubation with Qiagen Proteinase K on a shaking incubating set to 56°C was needed. The following day, after crosslinking at 90°C, RNase A was added followed by Qiagen Protein Precipitation to remove RNA and proteins from the samples. The DNA was then precipitated using Isopropanyl and Glycogen and hydrated in Qiagen Hydration Solution. DNA quantification was performed using the Qubit Broad Range assay following manufacturer's protocols (Life Technologies, CA). We used a next generation

sequencing (NGS) panel which included hotspot regions of 20 genes that are frequently mutated in solid tumor malignancies (*AKT1*, *ALK*, *BRAF*, *CSF1R*, *EGFR*, *ERBB2*, *HRAS*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *NOTCH1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *RET* and *TP53*). Briefly, 1–10ng of DNA was amplified with 30 cycles of content-specific PCR primer pools. After cleanup with Ampure beads and washing twice with 200 μ L of 80% ethanol, samples were eluted in 40 μ L of low-EDTA-TE. If there was more than one primer pool, the resulting amplifications were combined at a given ratio and an aliquot was then PCR amplified with 8 cycles to add indices and sequencing adapters. After a second Ampure cleanup, libraries were quantified using Agilent High Sensitivity D1000 TapeScreens following the manufacturer's protocol (Agilent, CA). The designated threshold for quality control was a minimum 250 $\times$ read depth at any given position with 4000 $\times$ mean coverage across the entire panel. A validated calling threshold of 5% allele frequency was used and samples with mutations detected at low allele frequencies (<5%) were re-sequenced to exclude artifacts. Sequence reads were aligned to the human genome UCSC build hg19 (NCBI build 37.2) using a custom bioinformatics algorithm (19). Variants were then compared to an in-house developed knowledge base, which draws from publicly available sources such as PubMed, dbSNP database (20), COSMIC (21), 1000 Genomes (22), and the Exome Variant Server (<http://evs.gs.washington.edu>) to determine if variants were pathogenic or benign.

We also reported results on PTEN deletion in tumor samples by comparing the average depth of covered amplicons (PTEN exons 1, 3 and 5) in tumor specimens to normal controls. Tumor samples were considered positive, equivocal or negative for PTEN deletion if the ratio of the average depth of amplicons compared to normal controls was 0–0.5, 0.51–0.9, or >0.9, respectively.

To analyze the pharmacodynamic effects of PI3K inhibition by PX-866 on tumor protein expression, we performed reverse-phase protein array (RPPA) analysis on tumor specimens obtained pre-treatment and on C1D8. After reviewing H&E slides to identify samples with regions with $\geq$ 50% tumor cells, OCT-embedded tumors underwent H&E-guided macro-dissection, and protein lysates were isolated from tumor enriched regions as previously described (23,24). Protein concentrations were normalized and denatured proteins were submitted for RPPA analysis by the CCSG-supported University of Texas MD Anderson Functional Proteomics Core Facility. Expression levels of 302 proteins and phosphoproteins were measured (Supplementary Table S1). Log₂ expression values were corrected for differences in protein loading as previously described (23,24).

Statistical methods

As our study used a standard 3 plus 3 dose escalation study design, the escalation and stopping rules implied that the by-patient incidence rate of DLTs was to be approximately 25% at the MTD. The planned enrollment was up to 30 DLT evaluable patients for a maximum of 5 dose levels. The study protocol provided for enrollment of up to 20 additional patients to further evaluate pharmacokinetic or pharmacodynamic parameters in an optional MTD cohort. Objective response rate (ORR) was defined as the percentage of patients with a best response of either a confirmed CR or confirmed PR. Disease control rate

(DCR) was defined as the percentage of patients with a best response of either a confirmed CR, confirmed PR or SD. Exact confidence intervals for ORR and DCR were computed. For all patients, progression free survival (PFS) was defined as the time from the date of consent signing to the first documentation of objective progression (radiologically or symptomatically) or death due to any cause, whichever occurred first. For patients with a best response of CR or PR, duration of response (DOR) was defined as the time from the date of first documented CR or PR to the date of radiological or symptomatic progression of disease, or death from any cause.

For RPPA data, paired Student's t-tests were used to compare log₂-transformed protein expression levels obtained pre-treatment and on C1D8. The ratio of mean normalized linear expression value between C1D8 and pre-treatment samples was used to quantify differences in protein expression between the two time points. Unpaired Student's t-tests were used to compare pre-treatment log₂-transformed protein expression levels between patients that achieved a best response of CR, PR or SD (disease control) and patients whose best response was PD (did not achieve disease control). For purposes of quantifying the magnitude of differences in pre-treatment protein expression levels between patients who achieved disease control and those who did not, the ratio of expression for each protein was defined as the ratio of the mean normalized linear value in patients who achieved disease control to that in patients who did not achieve disease control. A two-sided p-value of less than 0.05 was considered statistically significant. All data were analyzed using STATA v14.0 (STATA, College Station, TX).

RESULTS

Patient characteristics

The baseline demographics and disease characteristics of all patients are summarized in Table 2. Details of prior BRAF/MEK inhibitor therapy in the 10 patients who previously received BRAF and/or MEK inhibitors are summarized in Supplementary Table S2. As the study allowed for enrollment of any patient with an advanced BRAF V600 mutant solid tumor, 23 patients with advanced melanoma and one patient with a confirmed BRAF V600E mutant gastrointestinal stromal tumor (GIST) were enrolled.

Safety and Toxicity

All patients were evaluated for safety (n=24). Table 3 summarizes the treatment-related AEs experienced by patients in the study. The most common all-grade treatment-related AEs were nausea (79%), diarrhea (71%), arthralgia (58%) and fatigue (54%). The most common grade 3–5 treatment-related AEs were lipase elevation (25%), alanine aminotransferase (ALT) elevation (13%), aspartate aminotransferase (AST) elevation (13%) and rash (8%). Serious treatment-related AEs included grade 2 uveitis in one patient in cohort 2, grade 4 hepatocellular injury in one patient in cohort 2 and grade 3 pancreatitis in one patient in cohort 3.

Dose limiting toxicities

A total of 15 patients were considered DLT evaluable. There were no DLTs in cohort 1. In cohort 2, there was one DLT of grade 3 rash that was considered by the treating investigator to be attributed to the study combination and therefore met the definition of DLT in this study. The patient required a dose reduction and the rash subsequently improved. The cohort was expanded with no additional DLTs in a total of 10 patients. One patient in cohort 2 had a reversible grade 4 AST/ALT elevation which was observed after the DLT evaluation period.

In cohort 3, there was one DLT of grade 3 pancreatitis. Based on the DLT event of pancreatitis, the study design would have required an expansion of cohort 3. However, cohort 3 was considered likely not to be tolerable based on the observed DLT event of pancreatitis as well as non-DLT toxicities of asymptomatic lipase elevation and reversible bilirubin elevation. Therefore, instead of expanding cohort 3, an alternate dosing cohort (cohort 4) of vemurafenib 720mg twice daily and PX-866 8mg once daily was opened according to the study protocol. There were no DLTs observed in cohort 4.

Dose interruptions and modifications

During the study, 33% (8/24) of patients across all dose cohorts required dose reductions of PX-866 and 50% (12/24) of patients required dose reductions of vemurafenib due to AEs. Rates of PX-866 dose reductions due to AEs in cohorts 1, 2, 3 and 4 were 25% (1/4), 30% (3/10), 60% (3/5) and 25% (1/4), respectively. Rates of vemurafenib dose reductions due to AEs in cohorts 1, 2, 3 and 4 were 25% (1/4), 70% (7/10), 80% (4/5) and 0% (0/5), respectively. The rates of PX-866 and vemurafenib dose interruptions across all cohorts due to AEs were 58% (14/24) and 71% (17/24), respectively. Rates of PX-866 dose interruptions due to AEs in cohorts 1, 2, 3 and 4 were 50% (2/4), 50%, (5/10), 100% (5/5) and 40% (2/5), respectively. Rates of vemurafenib dose interruptions due to AEs in cohorts 1, 2, 3 and 4 were 50% (2/4), 70% (7/10), 100% (5/5) and 60% (3/5), respectively. 33% (8/24) of patients experienced treatment-related AEs leading to study termination. The rate of study termination due to treatment-related AEs in cohorts 1, 2, 3 and 4 were 50% (2/4), 20% (2/10), 60% (3/5) and 25% (1/5), respectively.

Efficacy

Of the 24 patients in the study, 23 were evaluable for response (Supplementary Table S3, Fig. 2A). There were seven confirmed PRs and ten patients with SD as their best response, giving an objective response rate (ORR) of 29% (7/24) (95% confidence interval [CI]: 13–51%) and a disease control rate (DCR) of 71% (17/24) (95% CI: 49–87%). Patterns of progression of the fourteen patients who had disease progression while on study are summarized in Supplementary Table S4. Of the seven patients who had a partial response to therapy, 57% (4/7) experienced a grade 3 adverse event thought to be at least possibly related to PX-866. Similarly, among the sixteen patients whose best response on study was SD or PD, 56% (9/16) experienced a grade 3 adverse event thought to be at least possibly related to PX-866. Of the fourteen patients who had not received any prior BRAF or MEK inhibitor therapy, there were four confirmed PRs, eight patients with SD, one patient with PD and one non-evaluable patient, giving an ORR and DCR of 29% (95% CI: 8.4–58%) and 86% (95% CI: 57–98%), respectively. Of the ten patients who had received prior BRAF or

MEK inhibitor therapy, there were three confirmed PRs and two patients with SD, for an ORR and DCR of 30% (95% CI: 6.7–65%) and 50% (95% CI: 19–81%), respectively. The median PFS of all 24 patients was 6.1 months (95% CI: 2.9–11.5 months). The median PFS of patients who received and did not receive prior BRAF or MEK inhibitor therapy was 2.9 months (95% CI: 1.3 months-undefined) and 9.2 months (95% CI: 3.1–12.0 months), respectively. The median DOR among the seven patients who had objective responses was 8.3 months (95% CI: 5.7 months-undefined). The median DOR for patients who received and did not receive prior BRAF or MEK inhibitor therapy was 5.7 months (95% CI: 5.7 months-undefined) and 9.2 months (95% CI: 6.5 months-undefined), respectively.

Pharmacodynamic effect of PX-866 on tumor protein expression

23 patients successfully underwent the protocol specified paired tumor biopsies (pre-treatment and C1D8). Viable tumor for immunohistochemical (IHC) staining was present in both pre-treatment and C1D8 (on PI3K inhibition) samples for twelve of the 23 patients. Representative images of paired IHC stains for pAKT are shown (Fig. 1A). Of the twelve patients with adequate tumor specimens for paired analysis, eight patients had a decrease in pAKT expression by IHC (range: –63% to –10%, Fig 1B), two patients had no change in pAKT expression and two patients had an increase in pAKT expression (Fig 1B) after 1 week of PX-866 treatment.

Three patients had sufficient material from both pre-treatment and C1D8 biopsies for RPPA. Mean normalized linear and log₂ protein expression levels of paired samples are summarized in Supplementary Table S5. Expression of P-P70S6K_T389 was the protein most significantly downregulated by PX-866 treatment (Ratio=0.79, p=0.012). Two other markers of PI3K-AKT-mTOR pathway activation, P-PRAS40_T246 (Ratio=0.87, p=0.052) and P-GSK3α/β_S21/9 (Ratio=0.62, p=0.092), had decreased expression, suggesting inhibition of pathway signaling, although the differences did not reach statistical significance. Increased expression of P-IGF1R_Y1135/Y1136 (Ratio=1.13, p=0.040) was detected after treatment with PX-866, suggesting potential compensatory pro-survival signaling by IGF1R.

Baseline alterations in PI3K/AKT pathway as predictors of response

Eighteen of the 24 patients in our study had sufficient tumor for baseline PTEN IHC. Of these, five patients achieved an objective response (responders), twelve patients did not (non-responders) and the last patient was non-evaluable for response. PTEN loss by IHC was noted in 80% (4/5) and 58% (7/12) of responders and non-responders, respectively. All 24 patients had sufficient archived material for NGS. The rate of pathogenic PTEN mutations and/or deletions in responders and non-responders was 57% (4/7) and 25% (4/16), respectively (Table 4). When analysis was restricted to the ten patients who had received prior BRAF and/or MEK inhibitor therapy (Supplementary Table S2), PTEN loss by IHC was noted in 100% (2/2) and 80% (4/5) of responders and non-responders, respectively. The rate of pathogenic PTEN mutations and/or deletions in responders and non-responders was 33% (1/3) and 29% (2/7), respectively.

Six patients had sufficient material from the pre-treatment biopsy specimen for RPPA. Of these, three patients had PR or SD as their best response (disease control) and three patients had a best response of PD (failure of disease control). Significant differences in protein expression levels between patients who achieved disease control and those who did not are summarized in Supplementary Table S6. Notably, there were no significant differences identified in components of the PI3K-AKT pathway between the groups of patients.

Changes in the immune microenvironment following treatment with PX-866

Of the seven patients with confirmed PRs on this study, two had sufficient material from the pre-treatment and C1D8 biopsies for further IHC staining. Both patients demonstrated an increase in CD8 and PD-L1 staining following treatment with PX-866. Of note, the increase in CD8 T-cell infiltration and corresponding upregulation of PD-L1 expression following treatment with PX-866 was especially pronounced in one of the two patients (Fig. 2B). This patient had a PTEN deletion at baseline. Additionally, changes suggestive of successful PI3K pathway inhibition after treatment with PX-866 were also noted in the C1D8 biopsy specimen from this patient which, when compared to the pre-treatment biopsy, demonstrated a 29% and 84% decrease in the H-score (IHC) and normalized linear expression value (RPPA) of pAKT_S473, respectively. Interestingly, this patient also had the longest DOR (11 months) and PFS (13.2 months) observed on this study. Among the sixteen patients whose best response was SD or PD while on study, four had sufficient material from the pre-treatment and C1D8 biopsies for further IHC staining. Of these, 75% (3/4) demonstrated an increase in CD8 staining following treatment with PX-866. However, there was no corresponding increase in PD-L1 staining in these patients.

DISCUSSION

This study demonstrates that PX-866, an orally available, irreversible small molecule pan-isoform inhibitor of PI3K can be safely used at the MTD of 8mg daily in combination with a modified dose of vemurafenib in the treatment of patients with advanced BRAF V600 mutated cancers. To the best of our knowledge, this is the first completed clinical trial reporting the feasibility of combining PI3K and BRAF inhibition. Of note, the combination of vemurafenib and BKM120, another pan-isoform PI3K inhibitor, was found to be poorly tolerated in an earlier phase I study due to DLTs of myalgias, skin toxicity and febrile neutropenia (25). The most common all-grade treatment-related AEs observed in our study were gastrointestinal events (nausea, diarrhea and vomiting), arthralgia, fatigue and skin disorders (photosensitivity reactions and rash). The frequency of gastrointestinal events and fatigue was not unexpected, given similar patterns of toxicity observed in the PX-866 single agent phase I study (12). Arthralgias and skin disorders were observed and are known common side effects of vemurafenib (26). While only two DLTs were observed during the DLT evaluation period across all cohorts, several patients did require subsequent dose modifications in later treatment cycles due to late or cumulative toxicity, as is common with single agent vemurafenib as well. Of note, in the single agent phase I study of vemurafenib, the pharmacokinetic profiles of vemurafenib when dosed at 720mg twice daily and 960mg twice daily were comparable (26). Therefore, the combination of vemurafenib 720mg twice daily with PX-866 8mg daily was determined to be the MTD for this combination to allow

for a higher dose of PX-866 to be used in this combination. Consideration should be given to exploring intermittent dosing schedules in future studies to minimize toxicity and perhaps delay the onset of resistance (27).

Encouraging signs of anti-tumor activity were seen in this study. The ORR and DCR were 29% and 71%, respectively in the entire study cohort, which included ten patients with prior BRAF or MEK inhibitor therapy. Interestingly, although the DCR observed in patients with prior BRAF or MEK inhibitor therapy appeared lower than that observed in the entire cohort (50% vs 71%), the ORR was similar (30% vs 29%). It is important to note that the ORR of BRAF/MEK inhibitor naïve patients was 29% in this study which is lower than what has been reported for single agent vemurafenib in the front-line setting (28). However, 86% (12/14) of the BRAF/MEK inhibitor naïve patients on this study had received other prior systemic therapies, potentially explaining the lower response rates observed in this study. The ORR of patients who received prior BRAF/MEK inhibitor therapy was 30% in this study. Interestingly, a recent retrospective study reported that retreatment with BRAF-directed therapy after a median BRAF-free interval of 7.7 months was associated with an ORR of 43% (29). Although these response rates appear to be higher than what we report for patients who received prior BRAF/MEK inhibitor therapy, 60% (6/10) of such patients in our study were enrolled within 2 months of their most recent BRAF and/or MEK inhibitor therapy, potentially explaining the lower response rates. Interestingly, our results appear to be more consistent with an earlier prospective study of 25 patients reporting an ORR of 32% with the combination of dabrafenib and trametinib in patients who had progressed on prior BRAF inhibitor monotherapy and had been off therapy for at least 12 weeks (30). The median PFS for the entire study cohort was 6.1 months which is similar to the median PFS of 5.3 months reported in patients with previously untreated advanced BRAF V600 mutant melanoma who were treated with vemurafenib alone (28). For the seven patients who achieved a confirmed objective response (CR or PR), the median DOR was 8.3 months. Although our study was not powered to detect differences in PFS and DOR between patients who received and did not receive prior BRAF or MEK inhibitor therapy, patients who did not receive prior BRAF or MEK inhibitor therapy appeared to have improved PFS and more durable responses. However, favorable responses were not restricted to BRAF and MEK inhibitor naïve patients. Among the ten patients who had received prior BRAF or MEK inhibitor therapy, three confirmed PRs and two patients with SD were observed. One of these patients with a confirmed PR was the patient with the BRAF V600E mutant GIST. This patient had received seven prior lines of therapy, including prior BRAF and MEK inhibitor combination therapy with a best response of PR on any prior therapy. This patient had evidence of PTEN loss by IHC on the pre-treatment biopsy and had a confirmed PR lasting 8.3 months when treated with combination PI3K and BRAF inhibitor therapy on this study, suggesting that BRAF combination therapy can be further individualized to improve outcomes in patients with evidence of PI3K pathway activation. While BRAF and MEK inhibitor combination therapy is the standard of care for patients with BRAF V600 mutated melanoma based on increased survival compared to BRAF inhibition alone, the ability to delineate a subset of patients who could potentially benefit more from BRAF and PI3K inhibitor therapy than from BRAF and MEK inhibition would improve outcomes and should be assessed early on in the development of additional PI3K inhibitor combination therapy.

To the best of our knowledge, this is the first clinical study demonstrating pharmacodynamic changes in tumor tissue as a result of PI3K pathway inhibition in melanoma, with the majority of patients showing a decrease in tumor pAKT expression as assessed by IHC. In addition, although the exploratory proteomic analyses by RPPA were limited by the small number of samples that passed our quality-control (QC) standards, the analysis of paired pretreatment and on-treatment (C1D8) samples supports that PI3K-AKT activity was decreased, based on the detection of decreased expression of multiple phospho-proteins in the pathway (P70S6K, PRAS40, GSK3 α/β). Interestingly, increased levels of P-IGF-1R_Y1135/Y1136 were observed following treatment with PX-866, suggesting increased IGF-1R signaling in response to PI3K inhibition. Increased IGF-1R signaling has been implicated in the development of drug resistance to multiple targeted therapies (31).

Although limited by the number of exons assessed and the small patient cohort in the setting of a phase I study, our study also suggested that treatment response appeared to be associated with loss of PTEN expression by IHC and the presence of pathogenic PTEN mutations and/or deletions, identifying a subset of patients who could potentially derive greater benefit from simultaneous PI3K and BRAF inhibition. At present, it is unclear if the presence of pathogenic PTEN mutations and/or deletions or the lack of PTEN expression by IHC is the better surrogate marker of PI3K pathway activation and further studies are needed to validate their use in identifying patients who may benefit from PI3K pathway inhibition. In addition, the use of more comprehensive sequencing techniques like whole exome sequencing should be explored in future studies to provide a more definitive understanding of the mutational landscape of PTEN in melanoma and its role in determining response to targeted therapy.

It is also interesting to note that the patient with the most durable PR on this study was found to have a profound increase in CD8+ T-cell infiltration and upregulation of PD-L1 expression on tumor cells following treatment with PX-866. While the strength of inferences drawn from a single patient are limited, this observation appears to be consistent with previous data showing that activation of the PI3K pathway, particularly by loss of PTEN, is associated with suppression of the anti-tumor immune response (32–34). These studies also showed that inhibition of the PI3K pathway results in enhanced intra-tumoral CD8+ T cell infiltration as well as synergy with anti-PD1 therapy in animal models (32). Notably, this patient also had evidence of PTEN deletion at baseline and on-treatment biopsies demonstrated inhibition of the PI3K pathway inhibition with PX-866. We hypothesize that PI3K pathway inhibition with PX-866 in this patient resulted in an enhanced anti-tumor immune response as evidence by the increased CD8+ T cell infiltration, leading to compensatory upregulation of PD-L1 expression by the tumor. Taken together, these results suggest that PD-L1 upregulation may represent a mechanism of immune escape in response to enhanced T-cell mediated cytotoxicity following PI3K pathway inhibition, strengthening the rationale for evaluating the combination of anti-PD1/PD-L1 therapy and PI3K inhibitors in clinical trials.

This study provides the first clinical proof of the concept that the combination of PI3K and BRAF inhibition is feasible. While PX-866 is no longer in clinical development, several other PI3K inhibitors are in various phases of clinical testing. The signal of increased

efficacy in patients with PI3K pathway activation can be further evaluated in ongoing trials, with the goal of enriching for a patient population that may have increased benefit from individualized, tumor pathway-specific combination therapy.

In conclusion, this phase I study established the feasibility of the novel combination of BRAF and PI3K inhibition and demonstrated pharmacodynamic evidence of target inhibition at clinically tolerable doses in tumor tissue. Further, it appears that loss of PTEN may be a useful predictive marker of response to PI3K inhibition in combination with BRAF inhibitors in patients with BRAF V600 mutant melanoma. Together, these results support the need for continued biomarker assessment in early phase clinical studies to individualize combination therapy in patients with advanced BRAF V600 mutant cancer with the goal of improving clinical outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to thank the patients, caregivers and clinical and research personnel that made this work possible. This work was supported in part by grants from the Melanoma Research Alliance (Young Investigator Award to T.C.G.), the Young Friends of the Abramson Cancer Center (Young Investigator Award to T.C.G.), the Melanoma Research Foundation Breakthrough Consortium, the PENN/Wistar Melanoma SPORE (P50 CA174523), the Departments of Medicine at the University of Pennsylvania and the Corporal Michael J. Crescenz VA Medical Center (Frederick F. Samaha Award for Resident Scholarship to C.Y.), the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (M.A.D.) and Oncothyreon, Inc. The MDACC Functional Proteomics Core is supported by NCI CA16672.

Conflicts of interest: M.A.D. has served on the advisory boards for GlaxoSmithKline, Novartis, Roche/Genentech, Bristol-Myers Squibb, Sanofi-Aventis and Vaccines. M.A.D. has been the principal investigator of grants to MD Anderson Cancer Center from Oncothyreon, GlaxoSmithKline, Roche/Genentech, Merck, Myriad and Sanofi-Aventis.

References

1. Samuels Y, Ericson K. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol.* 2006; 18(1):77–82. [PubMed: 16357568]
2. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science.* 2011; 333(6046):1157–60. DOI: 10.1126/science.1208130 [PubMed: 21798893]
3. Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, et al. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res.* 2005; 11(8):2875–8. DOI: 10.1158/1078-0432.CCR-04-2142 [PubMed: 15837735]
4. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. *Oncogene.* 2008; 27(41):5527–41. DOI: 10.1038/onc.2008.247 [PubMed: 18794886]
5. Jiang CC, Lai F, Thorne RF, Yang F, Liu H, Hersey P, et al. MEK-independent survival of B-RAFV600E melanoma cells selected for resistance to apoptosis induced by the RAF inhibitor PLX4720. *Clin Cancer Res.* 2011; 17(4):721–30. DOI: 10.1158/1078-0432.CCR-10-2225 [PubMed: 21088259]
6. Zhang Y, Moerkens M, Ramaiahgari S, de Bont H, Price L, Meerman J, et al. Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. *Breast Cancer Res.* 2011; 13(3):R52.doi: 10.1186/bcr2883 [PubMed: 21595894]

7. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer*. 2009; 9(8):550–62. DOI: 10.1038/nrc2664 [PubMed: 19629070]
8. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. *Cancer Cell*. 2010; 18(6):683–95. DOI: 10.1016/j.ccr.2010.11.023 [PubMed: 21156289]
9. Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer*. 2011; 11(4):289–301. DOI: 10.1038/nrc3037 [PubMed: 21430697]
10. Shull AY, Latham-Schwark A, Ramasamy P, Leskoske K, Oroian D, Birtwistle MR, et al. Novel somatic mutations to PI3K pathway genes in metastatic melanoma. *PLoS One*. 2012; 7(8):e43369.doi: 10.1371/journal.pone.0043369 [PubMed: 22912864]
11. Ihle NT, Williams R, Chow S, Chew W, Berggren MI, Paine-Murrieta G, et al. Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. *Mol Cancer Ther*. 2004; 3(7):763–72. [PubMed: 15252137]
12. Hong DS, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL, et al. A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. *Clin Cancer Res*. 2012; 18(15):4173–82. DOI: 10.1158/1078-0432.CCR-12-0714 [PubMed: 22693357]
13. Bowles DW, Ma WW, Senzer N, Brahmer JR, Adjei AA, Davies M, et al. A multicenter phase 1 study of PX-866 in combination with docetaxel in patients with advanced solid tumours. *Br J Cancer*. 2013; 109(5):1085–92. DOI: 10.1038/bjc.2013.474 [PubMed: 23942080]
14. Bowles DW, Senzer N, Hausman D, Peterson S, Vo A, Walker L, et al. A multicenter phase 1 study of PX-866 and cetuximab in patients with metastatic colorectal carcinoma or recurrent/metastatic squamous cell carcinoma of the head and neck. *Invest New Drugs*. 2014; 32(6):1197–203. DOI: 10.1007/s10637-014-0124-3 [PubMed: 24916771]
15. Shannan B, Chen Q, Watters A, Perego M, Krepler C, Thombre R, et al. Enhancing the evaluation of PI3K inhibitors through 3D melanoma models. *Pigment Cell Melanoma Res*. 2016; doi: 10.1111/pcmr.12465
16. Deng W, Gopal YN, Scott A, Chen G, Woodman SE, Davies MA. Role and therapeutic potential of PI3K-mTOR signaling in de novo resistance to BRAF inhibition. *Pigment Cell Melanoma Res*. 2012; 25(2):248–58. DOI: 10.1111/j.1755-148X.2011.00950.x [PubMed: 22171948]
17. Maira SM, Stauffer F, Schnell C, Garcia-Echeverria C. PI3K inhibitors for cancer treatment: where do we stand? *Biochem Soc Trans*. 2009; 37(Pt 1):265–72. DOI: 10.1042/BST0370265 [PubMed: 19143644]
18. McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med*. 1985; 109(8):716–21. [PubMed: 3893381]
19. Daber R, Sukhadia S, Morrisette JJ. Understanding the limitations of next generation sequencing informatics, an approach to clinical pipeline validation using artificial data sets. *Cancer Genet*. 2013; 206(12):441–8. DOI: 10.1016/j.cancergen.2013.11.005 [PubMed: 24528889]
20. Smigielski EM, Sirotkin K, Ward M, Sherry ST. dbSNP: a database of single nucleotide polymorphisms. *Nucleic Acids Res*. 2000; 28(1):352–5. [PubMed: 10592272]
21. Forbes S, Clements J, Dawson E, Bamford S, Webb T, Dogan A, et al. Cosmic 2005. *Br J Cancer*. 2006; 94(2):318–22. DOI: 10.1038/sj.bjc.6602928 [PubMed: 16421597]
22. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, et al. Genomes Project C. A map of human genome variation from population-scale sequencing. *Nature*. 2010; 467(7319):1061–73. DOI: 10.1038/nature09534 [PubMed: 20981092]
23. Davies MA, Stemke-Hale K, Lin E, Tellez C, Deng W, Gopal YN, et al. Integrated Molecular and Clinical Analysis of AKT Activation in Metastatic Melanoma. *Clin Cancer Res*. 2009; 15(24):7538–46. DOI: 10.1158/1078-0432.CCR-09-1985 [PubMed: 19996208]
24. Gopal YN, Deng W, Woodman SE, Komurov K, Ram P, Smith PD, et al. Basal and treatment-induced activation of AKT mediates resistance to cell death by AZD6244 (ARRY-142886) in Braf-

- mutant human cutaneous melanoma cells. *Cancer Res.* 2010; 70(21):8736–47. DOI: 10.1158/0008-5472.CAN-10-0902 [PubMed: 20959481]
25. Algazi AP, Posch D, Ortiz-Urda S, Cockerill A, Munster PN, Daud A. A phase I trial of BKM120 combined with vemurafenib in BRAFV600^{E/K} mutant advanced melanoma. *Journal of Clinical Oncology.* 32(15 suppl):9101.
 26. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med.* 2010; 363(9):809–19. DOI: 10.1056/NEJMoa1002011 [PubMed: 20818844]
 27. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature.* 2013; 494(7436):251–5. DOI: 10.1038/nature11814 [PubMed: 23302800]
 28. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med.* 2011; 364(26):2507–16. DOI: 10.1056/NEJMoa1103782 [PubMed: 21639808]
 29. Valpione S, Carlino MS, Mangana J, Mooradian M, McArthur GA, Schadendorf DHA, et al. Re-challenge with BRAF-directed treatment: A multi-institutional retrospective study. *Journal of Clinical Oncology.* 2017; 35(Suppl):abstr 9512.
 30. Schreuer M, Jansen Y, Planken S, Chevolet I, Seremet T, Kruse V, et al. Combination of dabrafenib plus trametinib for BRAF and MEK inhibitor pretreated patients with advanced BRAFV600-mutant melanoma: an open-label, single arm, dual-centre, phase 2 clinical trial. *Lancet Oncol.* 2017; 18(4):464–72. DOI: 10.1016/S1470-2045(17)30171-7 [PubMed: 28268064]
 31. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell.* 2010; 141(1):69–80. DOI: 10.1016/j.cell.2010.02.027 [PubMed: 20371346]
 32. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discov.* 2016; 6(2):202–16. DOI: 10.1158/2159-8290.CD-15-0283 [PubMed: 26645196]
 33. Dong Y, Richards JA, Gupta R, Aung PP, Emley A, Kluger Y, et al. PTEN functions as a melanoma tumor suppressor by promoting host immune response. *Oncogene.* 2014; 33(38):4632–42. DOI: 10.1038/onc.2013.409 [PubMed: 24141770]
 34. Noh KH, Kang TH, Kim JH, Pai SI, Lin KY, Hung CF, et al. Activation of Akt as a mechanism for tumor immune evasion. *Mol Ther.* 2009; 17(3):439–47. DOI: 10.1038/mt.2008.255 [PubMed: 19107122]

STATEMENT OF TRANSLATIONAL RELEVANCE

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway has been implicated in a variety of solid tumors and has been proposed to be a mechanism of resistance to BRAF inhibition. Combining PI3K inhibitors with BRAF inhibitors therefore represents a rational therapeutic option in patients with BRAF V600 mutated cancers. This article reports data from a phase I study evaluating the novel combination of PX-866, an oral pan-isoform inhibitor of PI3K and vemurafenib, a BRAF inhibitor, demonstrating the safety and feasibility of this combinatorial treatment approach. Furthermore, this study is the first clinical study to demonstrate intra-tumoral changes in pAKT expression and the immune microenvironment in response to PI3K inhibition. In addition, our data also suggests that PTEN loss-of-function at baseline may be associated with improved responses, potentially identifying a subset of patients who would derive greater benefit from dual PI3K and BRAF inhibition.

FIGURE 1. Changes in pAKT expression following treatment with PX-866

Immunohistochemical stains showing reduced pAKT staining from three representative patients following 1 week of treatment with PX-866 alone (A) and a waterfall plot (n=12) showing the percentage change in pAKT expression by H score following 1 week of treatment with PX-866 alone (B).

FIGURE 2. Best percentage change from baseline in target lesion size during study and changes in CD8+ T cell infiltration and PD-L1 expression following treatment with PX-866

A waterfall plot (A) showing the best percentage change from baseline in the sum of target lesions for the 23 response evaluable patients and immunohistochemical stains (B) showing an increase in CD8+ T cell infiltration following 1 week of treatment with PX-866 (ii) as compared to baseline (i). There was also a corresponding increase in PD-L1 expression (iv) relative to baseline (iii).

TABLE 1**DRUG DOSING AND SCHEDULES**

	Cycle 1, days 1–7 (initial 1-week lead-in period)		Cycle 1, day 8 and onward	
	PX-866	Vemurafenib	PX-866	Vemurafenib
Cohort 1	6 mg daily	-	6 mg daily	720 mg BID
Cohort 2	6 mg daily	-	6 mg daily	960 mg BID
Cohort 3	8 mg daily	-	8 mg daily	960 mg BID
Cohort 4	8 mg daily	-	8 mg daily	720 mg BID

TABLE 2

BASELINE DEMOGRAPHICS AND DISEASE CHARACTERISTICS

Characteristic	Cohort 1 (720/6) N=4	Cohort 2 (960/6) N=10	Cohort 3 (960/8) N=5	Cohort 4 (720/8) N=5	Total N=24
Tumor type					
Melanoma 600E – no. (%)	4 (100)	8 (80)	5 (100)	5 (100)	22 (92)
Melanoma 600K – no. (%)	0	1 (10)	0	0	1 (4.2)
GIST (V600E) – no. (%)	0	1 (10)	0	0	1 (4.2)
Age (years)					
Median	64.5	48	52	63	53.5
Range	34–66	27–65	32–70	38–64	27–70
Sex					
Male – no. (%)	2 (50)	5 (50)	3 (60)	3 (60)	13 (54)
Female – no. (%)	2 (50)	5 (50)	2 (40)	2 (40)	11 (46)
ECOG PS (N=25)					
0 – no. (%)	3 (75)	4 (40)	4 (80)	1 (20)	12 (50)
1 – no. (%)	1 (25)	6 (60)	1 (20)	4 (80)	12 (50)
Number of previous systemic regimens	(range: 1–4)	(range: 0–7)	(range: 1–3)	(range: 1–6)	(range: 0–7)
0 – no. (%)	0	2 (20)	0	0	2 (8.3)
1 – no. (%)	3 (75)	1 (10)	1 (20)	2 (40)	7 (29)
2 – no. (%)	0	2 (20)	3 (60)	0	5 (21)
3 or more – no. (%)	1 (25)	5 (50)	1 (20)	3 (60)	10 (42)
Type of previous systemic therapy ¹					
Ipilimumab – no. (%)	0	3 (30)	2 (40)	3 (60)	8 (33)
Chemotherapy – no. (%)	0	3 (30)	1 (20)	2 (40)	6 (25)
BRAF/MEK inhibitor – no. (%)	2 (50)	4 (40)	1 (20)	3 (60)	10 (42)
Other immunotherapy – no. (%)	3 (75)	6 (60)	5 (100)	4 (80)	18 (75)
Stage					
IIIC – no. (%)	0	0	0	1 (20)	1 (4.2)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Characteristic	Cohort 1 (720/6) N=4	Cohort 2 (960/6) N=10	Cohort 3 (960/8) N=5	Cohort 4 (720/8) N=5	Total N=24
M1a – no. (%)	1 (25)	4 (40)	0	1 (20)	6 (25)
M1b – no. (%)	0	2 (20)	1 (20)	0	3 (13)
M1c – no. (%)	3 (75)	4 (40)	4 (80)	3 (60)	14 (58)
Lactate Dehydrogenase					
Elevated – no. (%)	2 (50)	4 (40)	3 (60)	2 (40)	11 (46)
Not elevated – no. (%)	2 (50)	6 (60)	2 (40)	3 (60)	13 (54)

1) Patients may appear in more than one type of systemic therapy if they had more than one line of systemic therapy

TABLE 3

TREATMENT-RELATED ADVERSE EVENTS BY PREFERRED TERM

	Cohort 1 (720/6) N=4	Cohort 2 (960/6) N=10	Cohort 3 (960/8) N=5	Cohort 4 (960/8) N=5	Total N=24
Frequency of all-grade treatment-related AEs by preferred term, n (%) ^{1, 2}					
Nausea	3 (75)	8 (80)	4 (80)	4 (80)	19 (79)
Diarrhea	3 (75)	7 (70)	3 (60)	4 (80)	17 (71)
Arthralgia	2 (50)	6 (60)	2 (40)	4 (80)	14 (58)
Fatigue	2 (50)	8 (80)	2 (40)	1 (20)	13 (54)
Photosensitivity Reaction	2 (50)	5 (50)	3 (60)	1 (20)	11 (46)
Vomiting	2 (50)	4 (40)	2 (40)	3 (60)	11 (46)
Rash	2 (50)	3 (30)	0	3 (60)	8 (33)
Decreased Appetite	1 (25)	4 (40)	2 (40)	0	7 (29)
Dry Skin	0	6 (60)	0	1 (20)	7 (29)
Dysgeusia	1 (25)	3 (30)	2 (40)	0	6 (25)
Myalgia	1 (25)	2 (20)	2 (40)	1 (20)	6 (25)
Alopecia	0	4 (40)	0	1 (20)	5 (21)
Pruritus	1 (25)	3 (30)	0	1 (20)	5 (21)
Pyrexia	0	3 (30)	1 (20)	1 (20)	5 (21)
Rash, Maculo-Papular	1 (25)	1 (10)	2 (40)	1 (20)	5 (21)
Alanine Aminotransferase Increased	1 (25)	0	2 (40)	1 (20)	4 (17)
Aspartate Aminotransferase Increased	1 (25)	0	2 (40)	1 (20)	4 (17)
Chills	1 (25)	2 (20)	0	1 (20)	4 (17)
Lipase Increased	0	0	3 (60)	1 (20)	4 (17)
Palmar-Plantar Erythrodysaesthesia Syndrome	1 (25)	2 (20)	0	1 (20)	4 (17)
Dry Eye	2 (50)	1 (10)	0	0	3 (13)
Edema, Peripheral	0	3 (30)	0	0	3 (13)
Pruritus Generalized	0	2 (20)	1 (20)	0	3 (13)
Rash, Generalized	0	3 (30)	0	0	3 (13)

	Cohort 1 (720/6) N=4	Cohort 2 (960/6) N=10	Cohort 3 (960/8) N=5	Cohort 4 (720/8) N=5	Total N=24
Frequency of grade 3–5 treatment-related AEs by preferred term, n (%). ^{1, 2}					
Lipase Increased	0	0	3 (60)	1 (20)	4 (25)
Alanine Aminotransferase Increased	1 (25)	0	2 (40)	0	3 (13)
Aspartate Aminotransferase Increased	1 (25)	0	2 (40)	0	3 (13)
Rash	1 (25)	1 (10)	0	1 (20)	3 (13)
Diarrhea	0	2 (20)	0	0	2 (8.3)
Rash, Erythematous	0	1 (10)	1 (20)	0	2 (8.3)
Rash, Generalized	0	2 (20)	0	0	2 (8.3)

Abbreviation, AE: adverse event

^{1/} Percentages in each cohort are calculated using the total number of patients as the denominator

^{2/} Only adverse events occurring in more than 10% of patients are shown

TABLE 4

IMPACT OF PTEN MUTATIONS, DELETIONS AND PROTEIN EXPRESSION ON CLINICAL RESPONSE.

	CR or PR (N=7)	SD or PD (N=16)	NE (N=1)	Total (N=24)
PTEN expression by IHC				
Total tested - N	5	12	1	18
Present – n (%)	1 (20)	5 (42)	1 (100)	7 (41)
Absent – n (%)	4 (80)	7 (58)	0	11 (17)
PTEN mutation and/or deletion				
Total tested - N	7	16	1	24
Wild Type – n (%)	3 (43)	12 (75)	1 (100)	16 (67)
Mutation and/or Deletion present – n (%)	4 (57)	4 (25)	0	8 (33)

Abbreviations: CR=Complete Response, PR=Partial Response, SD=Stable Disease, PD=Progression of Disease, NE=non-evaluable