Clinical Cancer Research

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

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

Conclusions: PX-866 was well tolerated at its maximum tolerated single-agent dose when given in combination with a modified dose of vemurafenib (720 mg 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. *Clin Cancer Res; 24(1); 22–32. ©2017 AACR.*

Introduction

The 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 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 nmol/L, 88 nmol/L, 124 nmol/L, and 183 nmol/L, 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 8 mg 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,

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Translational Relevance

The 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 intratumoral changes in pAKT expression and the immune microenvironment in response to PI3K inhibition. In addition, our data also suggest 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.

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 MAPK pathways in PTEN-null melanoma cell lines has been shown to result in enhanced cell killing (17). In addition, synergistic antitumor effects have also been observed in preclinical 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 pretreatment 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. On the basis of this background and rationale, we conducted a multicenter 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 + 3 dose-escalation study design to evaluate up to two dose levels of vemurafenib 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 prespecified dose cohorts (Table 1). For subsequent cycles, both PX-866 and vemurafenib were administered at the prespecified 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 6 mg orally daily and 720 mg orally 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 three patients. Patients considered non-DLT evaluable were replaced. Patient enrollment and dose escalation proceeded according to a standard 3 + 3 study design. To account for potential patient discontinuation from the study prior to being evaluable for DLTs, enrollment of up to two additional patients per cohort was allowed.

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

Abbreviation: BID, twice daily.

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 AE 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 AE 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 2 weeks was required for treatment-related toxicity. Likewise, vemurafenib was held for any grade 3 or greater AE 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.

DLTs

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 nonmelanoma 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), stable disease (SD), 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 (pretreatment), 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 pretreatment and C1D8 tumor specimens for each patient were analyzed by 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; 1:1,200), CD8 (DAKO M7103, clone C8/144B; 1:40), and PD-L1 (Cell Signaling Technology 15165BF, clone E1J2J; 1:2,000) 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 semiquantitative 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 $CD8^+$ 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. Xu).

For identification of somatic tumor mutations, FFPE specimens were either macrodissected from 5-μm slides or used whole via 10-μm 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). We used a next-generation sequencing (NGS) panel that 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 to 10 ng of DNA was amplified with 30 cycles of content-specific PCR primer pools. After cleanup with Ampure beads and washing twice with 200 μL of 80% ethanol, samples were eluted in 40 μL 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). The designated threshold for quality control was a minimum 250× read depth at any given position with 4,000× 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 resequenced 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 with 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 whether the 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 with 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 pretreatment and on C1D8. After reviewing hematoxylin and eosin (H&E) slides to identify samples with regions with ≥50% tumor cells, OCT-embedded tumors underwent H&E-guided macrodissection, and protein lysates were isolated from tumor enriched regions as described previously (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). Log_2 expression values were corrected for differences in protein loading as described previously (23, 24).

Statistical analysis

As our study used a standard 3 + 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 five 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 radiologic or symptomatic progression of disease, or death from any cause.

For RPPA data, paired Student *t* tests were used to compare log_2-transformed protein expression levels obtained pretreatment and on C1D8. The ratio of mean normalized linear expression value between C1D8 and pretreatment samples was used to quantify differences in protein expression between the two time points. Unpaired Student *t* tests were used to compare pretreatment log_2-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 pretreatment 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).

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 to 5 treatment-related AEs were lipase elevation (25%), ALT elevation (13%), 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.

DLTs

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 that was observed after the DLT evaluation period.

In cohort 3, there was one DLT of grade 3 pancreatitis. On the basis of 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 720 mg twice daily and PX-866 8 mg 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. Thirty-

Table 2. Baseline demographics and disease characteristics

Characteristics	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 V600E, *n* (%)	4 (100)	8 (80)	5 (100)	5 (100)	22 (92)
Melanoma V600K, *n* (%)	0	1 (10)	0	0	1 (4.2)
GIST (V600E), *n* (%)	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, *n* (%)	2 (50)	5 (50)	3 (60)	3 (60)	13 (54)
Female, *n* (%)	2 (50)	5 (50)	2 (40)	2 (40)	11 (46)
ECOG PS (*n* = 25)					
0, *n* (%)	3 (75)	4 (40)	4 (80)	1 (20)	12 (50)
1, *n* (%)	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, *n* (%)	0	2 (20)	0	0	2 (8.3)
1, *n* (%)	3 (75)	1 (10)	1 (20)	2 (40)	7 (29)
2, *n* (%)	0	2 (20)	3 (60)	0	5 (21)
3 or more, *n* (%)	1 (25)	5 (50)	1 (20)	3 (60)	10 (42)
Type of previous systemic therapy[a]					
Ipilimumab, *n* (%)	0	3 (30)	2 (40)	3 (60)	8 (33)
Chemotherapy, *n* (%)	0	3 (30)	1 (20)	2 (40)	6 (25)
BRAF/MEK inhibitor, *n* (%)	2 (50)	4 (40)	1 (20)	3 (60)	10 (42)
Other immunotherapy, *n* (%)	3 (75)	6 (60)	5 (100)	4 (80)	18 (75)
Stage					
IIIC, *n* (%)	0	0	0	1 (20)	1 (4.2)
M1a, *n* (%)	1 (25)	4 (40)	0	1 (20)	6 (25)
M1b, *n* (%)	0	2 (20)	1 (20)	0	3 (13)
M1c, *n* (%)	3 (75)	4 (40)	4 (80)	3 (60)	14 (58)
Lactate dehydrogenase					
Elevated, *n* (%)	2 (50)	4 (40)	3 (60)	2 (40)	11 (46)
Not elevated, *n* (%)	2 (50)	6 (60)	2 (40)	3 (60)	13 (54)

[a]Patients may appear in more than one type of systemic therapy if they had more than one line of systemic therapy.

three percent (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 10 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 14 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 $\geq$ grade 3 adverse event thought to be at least possibly related to PX-866. Similarly, among the 16 patients whose best response on study was SD or PD, 56% (9/16) experienced a $\geq$ grade 3 adverse event thought to be at least possibly related to PX-866. Of the 14 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 nonevaluable patient, giving an ORR and DCR of 29% (95% CI, 8.4%–58%) and 86% (95% CI, 57%–98%), respectively. Of the 10 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

Twenty-three patients successfully underwent the protocol-specified paired tumor biopsies (pretreatment and C1D8). Viable tumor for IHC staining was present in both pretreatment and C1D8 (on PI3K inhibition) samples for 12 of the 23 patients. Representative images of paired IHC stains for pAKT are shown (Fig. 1A). Of the 12 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 pretreatment and C1D8 biopsies for RPPA. Mean normalized linear and $\log_2$ 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

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 (720/8), $n = 5$	Total, $n = 24$
Frequency of all-grade treatment-related AEs by preferred term, *n* (%)[a,b]					
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)
Pruritis, generalized	0	2 (20)	1 (20)	0	3 (13)
Rash, generalized	0	3 (30)	0	0	3 (13)
Frequency of grade 3–5 treatment-related AEs by preferred term, *n* (%)[a,b]					
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: AEs, adverse events.

[a]Percentages in each cohort are calculated using the total number of patients as the denominator.

[b]Only adverse events occurring in more than 10% of patients are shown.

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-IGF-1R_Y1135/Y1136 (ratio = 1.13, $P = 0.040$) was detected after treatment with PX-866, suggesting potential compensatory prosurvival signaling by IGF-1R.

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), 12 patients did not (nonresponders), and the last patient was nonevaluable for response. PTEN loss by IHC was noted in 80% (4/5) and 58% (7/12) of responders and nonresponders, respectively. All 24 patients had sufficient archived material for NGS. The rate of pathogenic *PTEN* mutations and/or deletions in responders and nonresponders was 57% (4/7) and 25% (4/16), respectively (Table 4). When analysis was restricted to the 10 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 nonresponders, respectively. The rate of pathogenic *PTEN* mutations and/or deletions in responders and nonresponders was 33% (1/3) and 29% (2/7), respectively.

Six patients had sufficient material from the pretreatment 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 pretreatment 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. In addition, 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 with the pretreatment biopsy, demonstrated a 29% and 84% decrease in the H-score (IHC) and normalized linear expression value

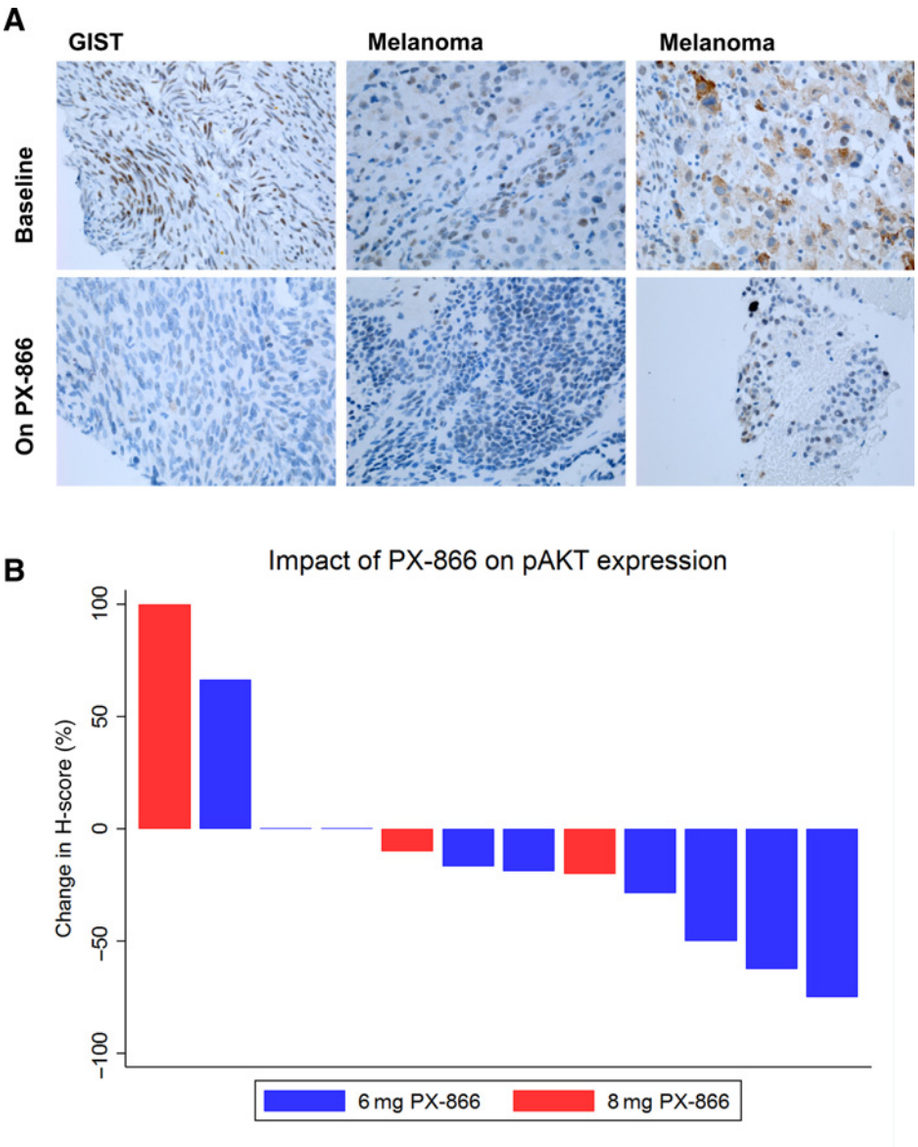

Figure 1.
Changes in pAKT expression following treatment with PX-866. IHC stains showing reduced pAKT staining from three representative patients following 1 week of treatment with PX-866 alone (**A**) and a waterfall plot ($n = 12$) showing the percentage change in pAKT expression by H-score following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

(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 16 patients whose best response was SD or PD while on study, four had sufficient material from the pretreatment 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 8 mg 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 720 mg twice daily and 960 mg twice daily were comparable (26). Therefore, the

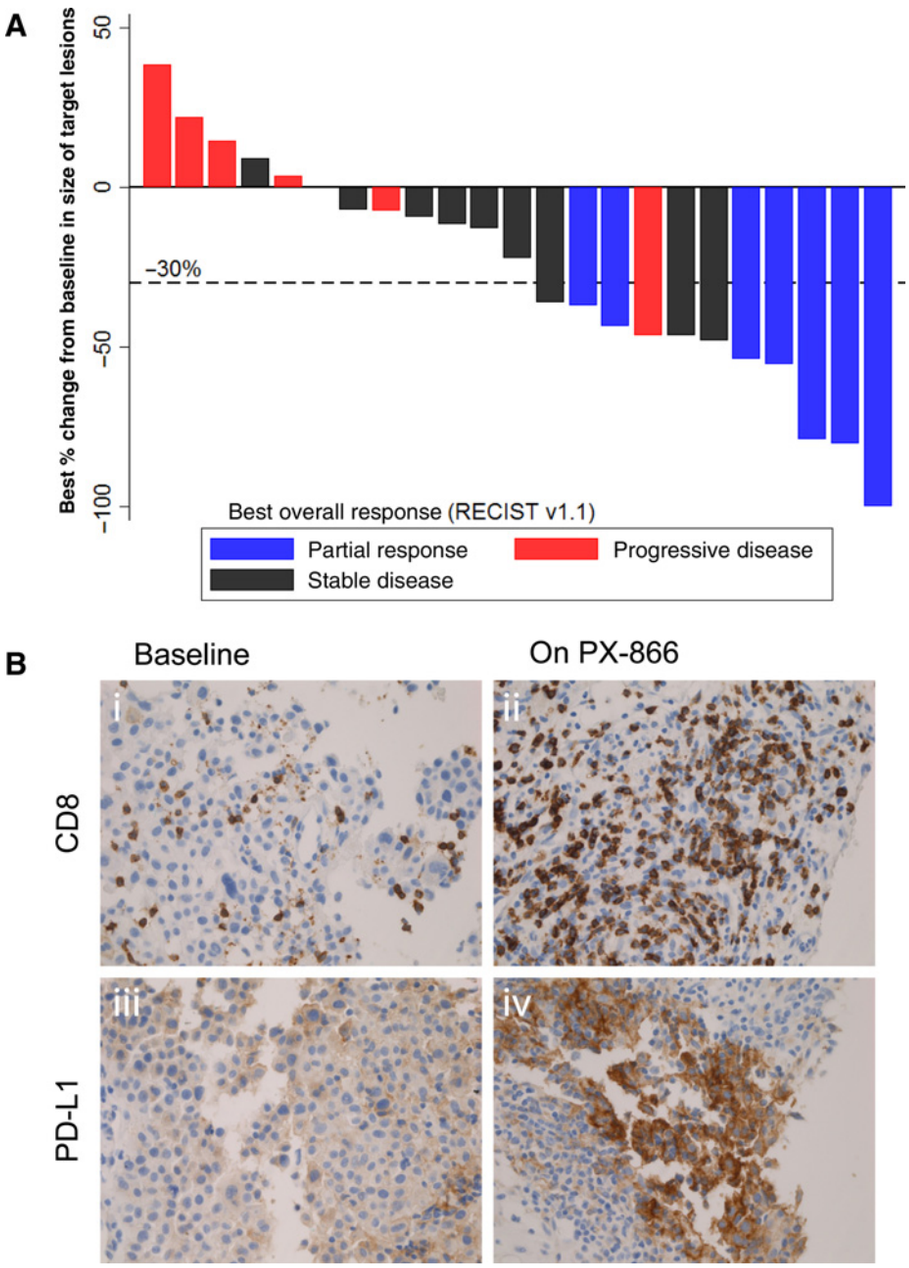

Figure 2.
Best percentage change from baseline in target lesion size during study and changes in CD8$^+$ T-cell infiltration and PD-L1 expression following treatment with PX-866. A waterfall plot (**A**) showing the best percentage change from baseline in the sum of target lesions for the 23 response evaluable patients and IHC stains (**B**) showing an increase in CD8$^+$ T-cell infiltration following 1 week of treatment with PX-866 (ii) as compared with baseline (i). There was also a corresponding increase in PD-L1 expression (iv) relative to baseline (iii).

combination of vemurafenib 720 mg twice daily with PX-866 8 mg 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 antitumor activity were seen in this study. The ORR and DCR were 29% and 71%, respectively, in the entire study cohort, which included 10 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 first-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.

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; NE, nonevaluable; PD, progression of disease; PR, partial response; SD, stable disease.

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 10 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 pretreatment 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 with BRAF inhibition alone, the ability the 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, GSK3α/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 whether 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 antitumor immune response (32–34). These studies also showed that inhibition of the PI3K pathway results in enhanced intratumoral $CD8^+$ 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 antitumor 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. Furthermore, 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.

Disclosure of Potential Conflicts of Interest

M.A. Davies is a consultant/advisory board member for Bristol-Myers Squibb, Novartis, Roche/Genentech, Sanofi Aventis, Vaccinex and reports receiving commercial research grants from AstraZeneca, GlaxoSmithKline, Merck, Oncothyreon, Roche/Genentech and Sanofi Aventis. H. Tawbi is a consultant/advisory board member for Bristol-Myers Squibb, Genentech/Roche and Novartis. No potential conflicts of interest were disclosed by the other authors.

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