Clinical Cancer Research

CDK 4/6 Inhibitor Palbociclib (PD0332991) in Rb$^+$ Advanced Breast Cancer: Phase II Activity, Safety, and Predictive Biomarker Assessment

Angela DeMichele[1,2,3], Amy S. Clark[1,3], Kay See Tan[2], Daniel F. Heitjan[2], Kristi Gramlich[1], Maryann Gallagher[1], Priti Lal[4], Michael Feldman[4], Paul Zhang[4], Christopher Colameco[1,2], David Lewis[1,2], Melissa Langer[1,2], Noah Goodman[1,2], Susan Domchek[1,3], Keerthi Gogineni[1,3], Mark Rosen[1,5], Kevin Fox[1,3], and Peter O'Dwyer[1,3]

Abstract

Purpose: The G_1–S checkpoint of the cell cycle is frequently dysregulated in breast cancer. Palbociclib (PD0332991) is an oral inhibitor of CDK4/6. Based upon preclinical/phase I activity, we performed a phase II, single-arm trial of palbociclib in advanced breast cancer.

Experimental Design: Eligible patients had histologically confirmed, metastatic breast cancer positive for retinoblastoma (Rb) protein and measureable disease. Palbociclib was given at 125 mg orally on days 1 to 21 of a 28-day cycle. Primary objectives were tumor response and tolerability. Secondary objectives included progression-free survival (PFS) and assessment of Rb expression/localization, KI-67, p16 loss, and *CCND1* amplification.

Results: Thirty-seven patients were enrolled; 84% hormone-receptor (HR)$^+$/Her2$^-$, 5% HR$^+$/Her2$^+$, and 11% HR$^-$/Her2$^-$, with a median of 2 prior cytotoxic regimens. Two patients had partial response (PR) and 5 had stable disease $\geq$ 6 months for a clinical benefit rate (CBR = PR + 6moSD) of 19% overall, 21% in HR$^+$, and 29% in HR$^+$/Her2$^-$ who had progressed through $\geq$2 prior lines of hormonal therapy. Median PFS overall was 3.7 months [95% confidence interval (CI), 1.9–5.1], but significantly longer for those with HR$^+$ versus HR$^-$ disease ($P = 0.03$) and those who had previously progressed through endocrine therapy for advanced disease ($P = 0.02$). Grade 3/4 toxicities included neutropenia (51%), anemia (5%), and thrombocytopenia (22%). Twenty-four percent had treatment interruption and 51% had dose reduction, all for cytopenias. No biomarker identified a sensitive tumor population.

Conclusions: Single-agent palbociclib is well tolerated and active in patients with endocrine-resistant, HR$^+$, Rb-positive breast cancer. Cytopenias were uncomplicated and easily managed with dose reduction. *Clin Cancer Res; 21(5); 995–1001. ©2014 AACR.*

Introduction

The G_1–S checkpoint is frequently dysregulated in breast cancer, leading to unchecked cellular proliferation. These include cyclin D1 overexpression or amplification, and loss of inhibitors p27 or p16 (1–3). Such tumors demonstrate increased proliferative indices, as measured by elevated levels of Ki-67 and increased phosphorylation of retinoblastoma (Rb) protein (4–7).

Palbociclib (PD0332991) is an oral inhibitor of the cyclin-dependent kinases (CDKs) 4 and 6 (8), preventing cell-cycle progression from G_1–S phase, thereby inhibiting cell proliferation and cellular DNA synthesis (9). Activity, resulting in blockade of Rb phosphorylation and G_1 arrest, was seen in Rb-positive tumor cell lines but not in Rb-negative MDA-MB-468 xenografts. In MDA-MB-435 xenografts, tumor growth suppression was shown to downregulate E2F-regulated genes (*CDC2*, *CCNE2*, *TK1*, *TOP2A*). MMTV-c-neu mice treated with palbociclib showed marked reduction in tumor volume and improved median survival compared with untreated controls (10), which was highly associated with presence of intact Rb and loss of p16.

In phase I, palbociclib was well tolerated and active, leading to a recommended phase II dose of 125 mg daily on a 3-week-on/1-week-off (3/1) schedule (11). Neutropenia was the dose-limiting toxicity; grade 3 neutropenia and anemia were seen in 12% and 7% of patients, respectively. The most common other toxicities were fatigue (34%), nausea (24%), constipation (17%), and vomiting (20%). While there were no partial responses (PR) in phase I, stable disease (SD) was seen in 35% of patients after 2 cycles, 27% of patients after 4 cycles and 16% of patients after 10 cycles. Notably, a heavily pretreated patient with ER$^+$/Her2$^-$ breast cancer achieved stable disease for 17 cycles.

On the basis of these findings, we conducted a phase II trial of palbociclib in patients with Rb-positive, advanced breast cancer. Primary endpoints were disease response and tolerability; secondary endpoints included progression-free survival (PFS) and

[1]Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania. [2]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania. [3]Hematology/Oncology Division, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. [4]Department of Pathology, University of Pennsylvania, Philadelphia, Pennsylvania. [5]Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Angela DeMichele, University of Pennsylvania School of Medicine, 3400 Civic Center Boulevard, Philadelphia, PA 19104. Phone: 215-614-1850; Fax: 215-615-3349; E-mail: Angela.demichele@uphs.upenn.edu

doi: 10.1158/1078-0432.CCR-14-2258

Translational Relevance

Cell-cycle alterations are common in cancer, and the G_1–S checkpoint is frequently disrupted in breast cancer, leading to unchecked cellular proliferation. Inhibitors of the cdk 4/6/cyclin D complex are currently under development. We performed a phase II trial of palbociclib, an oral, highly selective cdk 4/6 inhibitor, in patients with Rb-positive, advanced breast cancer with a median of 2 prior cytotoxic therapies. We demonstrated response or prolonged disease stability in patients with hormone receptor (HR) positive disease who had previously progressed on antiestrogen therapy. Neutropenia, though common, was uncomplicated and the drug was extremely well tolerated. These data suggest that palbociclib may have a role in the treatment of endocrine-resistant, HR^+, Rb-positive breast cancer. Despite its mechanism of action, however, Rb nuclear expression, Ki-67 proliferation index, p16 loss, and cyclin D amplification were not associated with response, underscoring the need for additional studies to better understand the biologic basis for the activity of palbociclib.

biomarker assessment to determine whether Rb localization, Ki-67 index, p16 loss, or CCND1 amplification were associated with response.

Patients and Methods

Study design

This was a single-institution, open-label, nonrandomized, single-arm trial (UPCC03909; NCT01037790) that included breast, testis, colon, and gastric cancers. The current report focuses exclusively on the breast cancer cohort. The trial was approved by the Institutional Review Board at the University of Pennsylvania and the Scientific Review Committee of the Abramson Cancer Center. P. O'Dwyer served as both the Principal Investigator and study sponsor for this investigator-initiated trial. Pfizer provided funding and palbociclib, but did not participate in data collection, analysis, or writing of the article.

Patients

Eligible patients had histologically confirmed metastatic breast cancer staining positive for Rb (>1+ staining intensity as defined below) in primary or metastatic tumor, at least one measureable lesion by RECIST (V1.0; ref. 12) and adequate organ and bone marrow function. There was no limit to the number of prior therapies allowed. Required washout was 21 days for prior cytotoxic chemotherapy and 28 days for prior investigational agents. Stable, previously treated brain metastases were allowed. Uncontrolled intercurrent illness, a baseline QTcB interval >470 ms, pregnancy, breastfeeding, or human immunodeficiency virus infection were exclusionary.

Treatment

Palbociclib was administered at 125 mg orally once daily, on days 1 to 21 of a 28-day cycle, continuing until disease progression or unacceptable toxicity. Toxicity was assessed using National Cancer Institute Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE V 3.0) in cycle 1 on days 1, 8, 15, and 21 and then on day 1 of subsequent cycles. In the event of grade 3/4 toxicities, palbociclib was withheld until resolution to <grade 2, and resumed at the next lower dose. Dose levels for dose reduction were 100, 75, and 50 mg; patients requiring reduction below 50 mg were discontinued. Supportive care was allowed at the investigator's discretion, but strong inducers or inhibitors of CYP3A4 were prohibited.

Assessments

Tumor assessments for response were conducted after every 2 cycles, using RECIST Version 1.0, by the RECIST core faculty of the Abramson Cancer Center blinded to patient identification and dose. Assessments were reduced to every 3 cycles for patients on treatment >18 months.

Biomarker analysis

Archival tumor tissue from either the breast primary tumor or a metastatic lesion was required, and stained for Rb, Ki-67, and p16 by immunohistochemistry. Rb was stained with a 1:50 dilution of antibody MS-107-P, clone 1F8 (Neomarkers/LabVision; ref. 13). Ki-67 was stained with a 1:100 dilution of antibody MIB-1 (DAKO), against the MK167 (FHA domain) interacting nucleolar phosphoprotein (14). p16 was stained with a 1:200 dilution of antibody MS218 (Neomarkers; ref. 15). *CCND1* amplification was assessed by FISH utilizing LSI Cyclin D1 SpectrumOrange/CEP11 SpectrumGreen (Vysis; ref. 16).

For each of the immunohistochemically stained markers, 2 independent pathologists determined subcellular location, staining intensity, and percentage of positively staining cells; discordance was resolved through adjudication. For Rb, categorical variables were created *a priori* by the method used by Shi and colleagues (13) based on a combination of percent tumor staining and intensity scores as follows: negative = 0 or 1+ intensity; equivocal = 2+ intensity or 3+ intensity in <30%; positive = 3+ staining in ≥30%, and separately assessed for nuclear and cytoplasmic compartments. p16 was categorized as 0%, 1–24%, 25–75% and >75% per previous reports (17, 18). Ki-67 staining was dichotomized at the 10% staining level (19). Amplification of *CCND1* by FISH was defined by an 11q13:CEP ratio ≥2.2 (16).

Statistical analysis

We defined tumor responses as "complete response' (CR), "partial response" (PR), stable disease (SD), and progressive disease (PD) per RECIST 1.0 criteria (12). Because of the cytostatic mechanism of action of palbociclib, SD was added to CR and PR in the definition of "clinical benefit" (CB) if SD lasted ≥6 months. A Simon two-stage design (20) was employed, with a clinical benefit rate of ≥15% (at least 1 response in 15 patients) in stage 1 required to move on to stage 2. On the basis of our observations in stage 1 of activity exclusively in HR^+ patients, we set our primary goal in stage 2 to estimate the response rate specifically in HR^+ disease, stratifying on this marker. A sample size of 30 response-evaluable patients with HR^+ disease was required to confirm a clinical benefit rate between 5% (p0) and 25% (p1; α and β both set at 0.10). We used Fisher exact test to assess association of categorical variables. PFS was defined as the time from the date of the initial palbociclib treatment to the date of death or date of progression (whichever occurred first), and PFS curves were planned *a priori* for the study population overall, by receptor subset (HR^+, $Her2^+$, and $ER^-/Her2^-$), and by prior treatments. Patients who discontinued due to toxicity were censored at the

Table 1. Study subjects

Characteristics	Total ($n = 37$)
Age (median; range)	59 (39–88)
HR status	
Any HR$^+$	33 (89%)
ER$^+$, PR$^-$	7 (19%)
PR$^+$, ER$^-$	4 (11%)
Both ER$^+$ and PR$^+$ (HR$^+$)	22 (60%)
Receptor group	
HR$^+$/Her2$^-$	31 (84%)
HR$^+$/Her2$^+$	2 (5%)
HR$^-$/Her2$^-$	4 (11%)
Prior hormonal therapy	
% Adjuvant	22 (59%)
% Advanced	31 (84%)
# Advanced lines (median, range)	2 (0–5)
0 or 1 line of therapy	13 (35%)
$\geq$2 lines of therapy	24 (65%)
Prior chemotherapy	
% Adjuvant	26 (70%)
% Advanced	34 (92%)
# Advanced lines (median, range)	2 (0–13)
0 or 1 line of therapy	9 (24%)
$\geq$2 lines of therapy	28 (76%)

date of study discontinuation. Log-rank tests were conducted to assess effects of receptor subsets and prior treatments on PFS. Cox proportional hazards models were used to assess the effects of biomarkers on PFS. We estimated the effects of predictors on the probability of response by logistic regression. All analyses were conducted in STATA 13 (StataCorp).

Results

Between April 2010 and March 2013, 128 patients with histologically confirmed breast cancer consented to screening for tumor Rb expression. Of these, 115 were Rb-positive, 5 were Rb-negative, and 8 had no tissue sample available. Thirty-seven patients who met other eligibility criteria subsequently provided informed consent and received treatment. Patient characteristics (Table 1) show a median age of 59 years (range 39–88). Eighty-four percent of tumors were HR$^+$/Her2$^-$, 5% HR$^+$/Her2$^+$, and 11% HR$^-$/Her2$^-$ (triple negative). This was also a heavily pretreated group, with a median of two prior chemotherapy regimens for metastatic disease, and up to 13 different regimens. Among those with HR$^+$ tumors, the majority had also received prior hormone therapy, with 70% having at least two prior regimens.

Efficacy

Among the first 15 patients with breast cancer enrolled on the protocol, 2 had PR, meeting our prespecified criteria for continued enrollment. We completed full enrollment within the HR$^+$ subset, but halted enrollment to the triple-negative cohort early because of observed rapid progression of enrolled patients and other treatment options available for this population.

Overall, CB was achieved in 7 of 37 patients, all in the 33 HR$^+$ patients, for a clinical benefit rate (CBR) of 19% in the overall trial cohort and 21% in those who were HR$^+$(Table 2). Among HR$^+$ patients, CB was seen exclusively in those patients who had already progressed through $\geq$2 prior lines of hormonal therapy (7/24, or 29%). With regard to prior chemotherapy, CBR was greater among patients with fewer than 2 prior regimens for metastatic disease compared with those who were more heavily treated (44% vs. 11% $P = 0.045$). Two patients in the HR$^+$ subset also had tumors with Her2 overexpression. One patient experienced a PR; one patient had SD lasting 5 months. Neither Her2$^+$ patient received concurrent Her2-directed therapy.

Figure 1 shows the waterfall plot for each patient's best tumor response by RECIST. Tumor shrinkage was greatest in a patient with HR$^+$/Her2$^+$ disease who was receiving no concurrent hormonal or Her2-directed therapy. To further illustrate the variability in duration of stable disease, the number of cycles received by both response assignment and receptor group is shown in Supplementary Fig. S1.

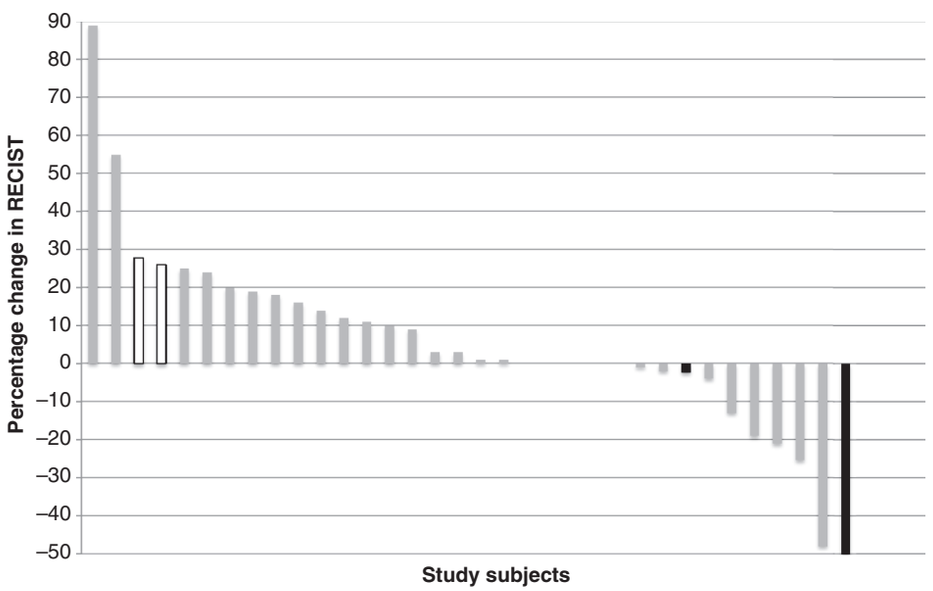

Figure 1.
Waterfall plot of the greatest percent change by RECIST and receptor group. Gray bars, subjects with HR$^+$/Her2$^-$ tumors; black bars, subjects with HR$^+$/Her2$^+$ tumors; white bars, subjects with HR$^-$/Her2$^-$ tumors.

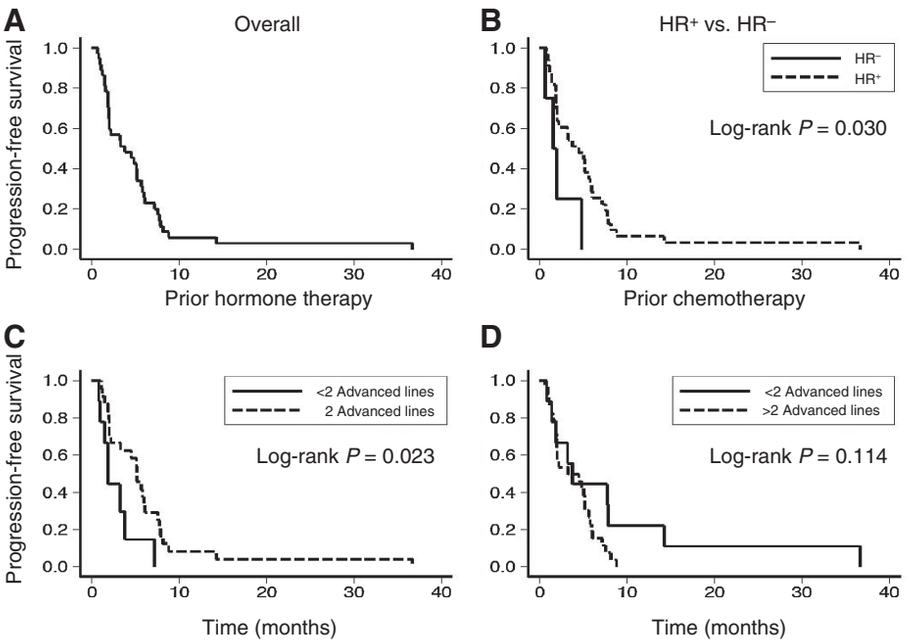

Figure 2.
Progression-free survival, overall and by HR status, and prior therapy. PFS is shown by the overall study cohort (A), subjects with HR^+ versus HR^- tumors (B), number of lines of prior hormonal therapy (HR^+ subjects only; C), and number of lines of prior chemotherapy (all subjects; D). All patients had progressed ($n = 36$) or discontinued study therapy for toxicity ($n = 1$) at the time of database lock and analysis.

Figure 2 shows the Kaplan–Meier curves for PFS. The median PFS for the group overall (Fig. 2A) was 3.7 months (95% CI 1.9–5.1). Median PFS was 3.8 months (1.9–5.8) for patients with $HR^+/Her2^-$ disease, 5.1 months (5.1–∞) for $HR^+/Her2^+$ disease and 1.5 months (0.62–∞) for $HR^-/Her2^-$ disease. Patients with HR^+ breast cancer (Fig. 2B) had significantly longer PFS compared with that of the HR^- group (4.5 months vs. 1.5 months, $P = 0.03$). Stratifying on degree of prior treatment (Fig. 2C and D), those with HR^+ tumors and ≥2 prior lines of hormone therapy had significantly longer PFS (5 months; 95% CI, 2–6); compared with those who received fewer than 2 prior lines of hormone therapy (2 months; 95% CI, 1–4; $P = 0.023$); there was no difference in median PFS in patients with <2 prior cytotoxic regimens compared with those with ≥2 (median 5 months; 95% CI, 2–6; $P = 0.114$).

Safety and tolerability

The incidence of adverse events by grade is shown in Table 3. One patient chose to discontinue study therapy after 2 cycles because of grade 2 fatigue. This patient had undergone three prior chemotherapy regimens for metastatic disease, but did not experience grade 3 or 4 neutropenia, anemia, or thrombocytopenia while on study therapy. No other patient was discontinued for toxicity and there were no treatment-related deaths.

Overall, there were 56 grade 3 events and 3 grade 4 adverse events, all due to myelosuppression. Nineteen patients (51%) experienced grade 3/4 neutropenia; 8 (22%) experienced grade 3/4 thrombocytopenia. Of those with grade 3/4 anemia (2 patients), 1 had grade 1 anemia at baseline. Grade 3/4 neutropenia was relatively isolated; only 6 of 19 (32%) had concurrent grade 3/4 anemia or thrombocytopenia. It was also largely uncomplicated, with only one episode of fever and sepsis in a neutropenic patient who was experiencing active disease progression simultaneously.

Myelosuppression, particularly neutropenia, led to dose interruptions and reductions (Table 4). Forty-six percent of all dose modifications were due to neutropenia, mostly grade 3. Among those with dose reductions, a significant fraction (19%) had more than one dose reduction for myelosuppression, with 7 patients being reduced to 50 or 75 mg daily. Of the 19 dose reductions, 14 (74%) occurred at the start of cycle 2, while only 3 occurred during cycle 1 (at the 2-week CBC), and just 1 occurred beyond cycle 2 (at cycle 5). These dose reductions did not appear to adversely affect response: among the 7 patients who experienced clinical benefit, only 2 remained at the 125 mg dose; 2 were reduced to 100 mg, 2 were reduced to 75 mg, and 1 was reduced to 50 mg, all with continued response.

Biomarker assessment

Representative sections for each of the biomarkers of interest, expression frequencies, and associations with odds of response and hazards of progression are shown in Supplementary Fig. S2 and Supplementary Table S1, respectively. None of the markers we evaluated were significantly associated with either clinical

Table 2. Response rates (overall and in the HR^+ group)

	Total ($n = 37$)	HR^+ ($n = 33$)
Complete response	0	0
PR	2 (5%)	2 (6%)
SD < 6 months	14 (38%)	13(39%)
SD ≥ 6 months	5 (14%)	5 (16%)
PD	16 (43%)	13 (39%)
CBR	7/37	7/33
(PR + ≥6 mo SD)	(19%)	(21%)
CBR by prior metastatic hormonal therapy		
0 or 1 prior lines hormone	N/A	0/9 (0%)
≥2 prior lines hormone		7/24 (29%)
Fisher exact test, *P*		0.081
CBR by prior metastatic chemotherapy		
0 or 1 prior lines chemotherapy	4/9 (44%)	4/9 (44%)
≥2 prior lines chemotherapy	3/28 (11%)	3/24 (13%)
Fisher exact test, *P*	0.045	0.068
Duration of response, months (median, range)	4 (2–5)	5 (2–6)

Table 3. Adverse events ($n = 37$ patients)

Adverse event	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)
Leukopenia	3 (8)	15 (41)	19 (51)	0 (0)
Neutropenia	2 (5)	13 (35)	19 (51)	1 (3)
Lymphopenia	9 (24)	4 (11)	10 (27)	1 (3)
Anemia	13 (35)	11 (30)	2 (5)	0 (0)
Thrombocytopenia	20 (54)	1 (3)	6 (16)	1 (3)
Sepsis	0 (0)	0 (0)	1 (3)	0 (0)
Fatigue	20 (54)	5 (14)	0 (0)	0 (0)
Arthralgia	3 (8)	0 (0)	0 (0)	0 (0)
Joint stiffness	1 (3)	0 (0)	0 (0)	0 (0)
Myalgia	4 (11)	1 (3)	0 (0)	0 (0)
Mucositis	5 (14)	2 (5)	0 (0)	0 (0)
Anorexia	10 (27)	0 (0)	0 (0)	0 (0)
Xerostomia	1 (3)	0 (0)	0 (0)	0 (0)
Nausea	7 (19)	2 (5)	0 (0)	0 (0)
Vomiting	2 (5)	0 (0)	0 (0)	0 (0)
Diarrhea	6 (16)	0 (0)	0 (0)	0 (0)
Constipation	3 (8)	0 (0)	0 (0)	0 (0)
Weight loss	0 (0)	1 (3)	0 (0)	0 (0)
Dysgeusia	3 (8)	0 (0)	0 (0)	0 (0)
Dry skin	2 (5)	0 (0)	0 (0)	0 (0)
Pruritus	3 (8)	0 (0)	0 (0)	0 (0)
Rash	1 (3)	0 (0)	0 (0)	0 (0)
Orthostasis	1 (3)	0 (0)	0 (0)	0 (0)

benefit or PFS, either at the *a priori* hypothesized cutoff points or in exploratory analyses of continuous data both in the overall and HR$^+$ groups (21).

Discussion

Palbociclib (PD0332991), an oral CDK4/6 inhibitor, demonstrates single-agent activity in this heavily pretreated population of patients with advanced breast cancer. The CBR was 21% for patients with HR$^+$ disease, but rose to 29% among those who had progressed through at least 2 prior lines of hormonal therapy, suggesting substantial activity in the setting of acquired endocrine resistance. These patients also had prolonged PFS (5 months) compared with those patients who had had only one line of prior endocrine therapy. Response duration in second line hormone therapy is typically 3 to 4 months, as evidenced by the 3.7-month PFS seen with both exemestane and fulvestrant in the phase III EFECT trial (22) and the 2.8 month PFS for the exemestane control arm in BOLERO-2 (23). Thus, the level of activity we saw in patients demonstrating prior endocrine resistance is encouraging, and will be further tested to some extent in the PALOMA-3 trial, combining palbociclib with fulvestrant in patients with endocrine-resistant breast cancer.

Table 4. Dose modifications (all evaluable patients, $n = 37$)

Event	Number (%)
Any dose modification (interruption, reduction, or discontinuation)	21 (57%)
Treatment interruption	9 (24%)
Dose reductions	19 (51%)
Discontinuation due to toxicity	1 (3%), fatigue
Reasons for dose modification	
Neutropenia	
Overall	17 (46%)
Grade 3	16 (43%)
Grade 4	1 (3%)
Thrombocytopenia	
Overall	3 (8%)
Grade 3	2 (5%)
Grade 4	1 (3%)
Final dose	
125 mg	18 (49%)
100 mg	12 (32%)
75 mg	6 (16%)
50 mg	1 (3%)

Palbociclib does appear to improve PFS in the first-line setting when combined with endocrine therapy compared with endocrine therapy alone, as seen in the recently completed randomized phase II PALOMA-1 trial (24). Because our study included only 1 first-line patient, it is impossible to draw conclusions about the single agent activity of palbociclib in first-line patients who are typically endocrine sensitive.

The finding of responses in HR$^+$/Her2$^+$ disease, as seen in the prolonged responder in this study, is supported by preclinical data suggesting sensitivity of Her2$^+$ cells to cell-cycle inhibition (25). The fact that this occurred in the absence of Her2-directed therapy suggests an alternative mechanism downstream of Her2 signaling. We enrolled only 4 patients with triple -negative disease, all of whom rapidly progressed on treatment. This, does not preclude the possibility that triple-negative disease could respond to palbociclib; combinations with chemotherapy and other targeted therapies are currently under investigation (26). Like the PALOMA-1 trial, our study did not identify any associations with hypothesized cell-cycle alterations, though we were unable to assess target suppression in the absence of on-treatment tumor biopsies.

Palbociclib was extremely well tolerated in this trial, and the absence of common systemic treatment-related symptoms such as alopecia, nausea, diarrhea, rash, or pain in our patients was notable, particularly in the setting of response. The emergence of uncomplicated grade 3/4 neutropenia suggests that the mechanism of myelosuppression with palbociclib may differ from that of traditional cytotoxic chemotherapy. The rarity of neutropenic fever/infection suggests that bone marrow progenitors, suppressed during treatment, may still be functional when faced with an infectious challenge, as preclinical studies have suggested (27). Sharpless and colleagues demonstrated that exposure of hematopoietic stem cells *in vitro* to PD0332991 reversibly decreased proliferation, but did not decrease total marrow cellularity, or alter apoptosis or viability of hematopoietic progenitor cells. These findings are corroborated by our clinical observation that short interruption followed by dose reduction was effective in restoring normal neutrophil counts. Thus, though the incidence of neutropenia is high, the relative paucity of fever and infection precludes this from being a major safety concern. It is not clear to what extent dose reductions altered response to therapy; however, response was seen at doses as low at 50 mg daily.

Recently, results were reported for two other cdk4/6 inhibitors currently in development. A phase I trial of LEE011 (Novartis) in advanced Rb-positive solid tumors and lymphoma (CLEE011 × 2101/NCT01237236) reported at ASCO, 2014 (28). At an MTD of 900 mg/day on a 3/1 schedule, 29% of patients had grade 3/4 neutropenia, 10% had grade 3/4 thrombocytopenia, and 3% had grade 3/4 anemia, fatigue, and/or diarrhea. Abemiclib (LY2835219, Lilly) was tested in a multitumor phase I/II trial, with breast cancer cohort results reported at AACR, 2014 (29). At the MTD of 200 mg twice daily on a continuous 28-day cycle, 19% of patients had grade 3 neutropenia and 10.6% had grade 3 thrombocytopenia. However, the most common toxicity was diarrhea, which occurred in 43%, 17%, and 6.4% of patients at

grades 1, 2, and 3, respectively. Nausea, fatigue, and vomiting additionally occurred in at least 15% of patients. There was one episode of febrile neutropenia. Among this heavily pretreated cohort (median 7 lines of prior therapy), 19% of patients experienced PR and 29.8% experienced $\geq$6 months SD. PR and 6-month SD rates increased to 25% and 36%, respectively, among women with HR^+ disease. The median PFS was 9.1 months with 18 patients still on study. The reasons for the increased single-agent response rate with this agent relative to LEE011 and palbociclib are not clear, but could be a result of a differing mechanism of action or a modulatory pharmacodynamic effect of continuous dosing. Continuous dosing has not been tested with palbociclib to date, precluding direct comparison of these agents.

Given the sample size constraints of this trial, we have interpreted our data with caution. We have reported only the prespecified analyses for HR^+ patients and comparison by prior therapy. We limited biomarker analyses to biologically hypothesized common cell-cycle changes at prespecified cutoff points and exploratory analysis of continuous variables, but because of the small samples, these findings in particular must be viewed as hypothesis-generating only, and warrant further study. Furthermore, other biomarkers may be important to predicting response to therapy. Recent data from the Engelman laboratory (30), for example, have suggested that PI3K alterations could sensitize tumors to the effects of cdk 4/6 inhibition and other alterations, such as Rb mutation or L0H could be important as well (31).

In summary, palbociclib is an active single agent in metastatic breast cancer, primarily in endocrine-resistant, HR^+ disease. These findings, as well as the promising findings in PALOMA-1 and its favorable side-effect profile have led to further development of palbociclib in breast cancer. Ongoing trials of this agent in HR^+ disease with endocrine therapy (PALOMA-2 and -3) and in combination with chemotherapy will further elucidate the role of palbociclib in the treatment of breast cancer.

Disclosure of Potential Conflicts of Interest

A. DeMichele is a consultant/advisory board member for Pfizer. K. Gogineni reports receiving a Medical Academic Partnership Research in Bioethics Grant from Pfizer. K. Fox is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. DeMichele, P. O'Dwyer
Development of methodology: A. DeMichele, A.S. Clark, M. Feldman, P. Zhang, P. O'Dwyer
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. DeMichele, A.S. Clark, M. Gallagher, P. Lal, M. Feldman, P. Zhang, C. Colameco, D. Lewis, M. Langer, N. Goodman, S. Domchek, K. Gogineni, K. Fox, P. O'Dwyer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. DeMichele, A.S. Clark, K.S. Tan, D.F. Heitjan, C. Colameco, P. O'Dwyer
Writing, review, and/or revision of the manuscript: A. DeMichele, A.S. Clark, K.S. Tan, D.F. Heitjan, P. Lal, M. Feldman, S. Domchek, M. Rosen, P. O'Dwyer
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. DeMichele, A.S. Clark, D.F. Heitjan, K. Gramlich, P. Zhang, D. Lewis, N. Goodman, M. Rosen
Study supervision: A. DeMichele, M. Feldman, N. Goodman, P. O'Dwyer

Grant Support

This study was supported by the Penn–Pfizer Alliance, Expedition Inspiration Fund for Breast Cancer Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 12, 2014; revised November 10, 2014; accepted November 30, 2014; published OnlineFirst December 11, 2014.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
2. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell 2002;2:103–12.
3. McIntosh GG, Anderson JJ, Milton I, Steward M, Parr AH, Thomas MD, et al. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene 1995;11:885–91.
4. Lange CA, Yee D. Killing the second messenger: targeting loss of cell cycle control in endocrine-resistant breast cancer. Endocr Relat Cancer 2011;18: C19–24.
5. Caldon CE, Daly RJ, Sutherland RL, Musgrove EA. Cell cycle control in breast cancer cells. J Cell Biochem 2006;97:261–74.
6. Buckley MF, Sweeney KJ, Hamilton JA, Sini RL, Manning DL, Nicholson RI, et al. Expression and amplification of cyclin genes in human breast cancer. Oncogene 1993;8:2127–33.
7. Dickson C, Fantl V, Gillett C, Brookes S, Bartek J, Smith R, et al. Amplification of chromosome band 11q13 and a role for cyclin D1 in human breast cancer. Cancer Lett 1995;90:43–50.
8. Pfizer. Palbociclib (PD0332991) Investigator's Brochure, December 2013.
9. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 2004; 3:1427–38.
10. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J Natl Cancer Inst 2012;104:476–87.
11. Flaherty KT, Lorusso PM, Demichele A, Abramson VG, Courtney R, Randolph SS, et al. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clin Cancer Res 2012;18: 568–76.
12. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
13. Shi SR, Liu C, Young L, Taylor C. Development of an optimal antigen retrieval protocol for immunohistochemistry of retinoblastoma protein (pRB) in formalin fixed, paraffin sections based on comparison of different methods. Biotech Histochem 2007;82:301–9.
14. Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, et al. An international Ki67 reproducibility study. J Natl Cancer Inst 2013;105:1897–906.
15. Milde-Langosch K, Bamberger AM, Rieck G, Kelp B, Löning T. Overexpression of the p16 cell cycle inhibitor in breast cancer is associated with a more malignant phenotype. Breast Cancer Res Treat 2001;67:61–70.
16. Roy PG, Pratt N, Purdie CA, Baker L, Ashfield A, Quinlan P, et al. High CCND1 amplification identifies a group of poor prognosis women with estrogen receptor positive breast cancer. Int J Cancer 2010;127: 355–60.
17. Zhang J, Lu A, Beech D, Jiang B, Lu Y. Suppression of breast cancer metastasis through the inhibition of VEGF-mediated tumor angiogenesis. Cancer Ther 2007;5:273–86.
18. Kerlikowske K, Molinaro AM, Gauthier ML, Berman HK, Waldman F, Bennington J, et al. Biomarker expression and risk of subsequent tumors after initial ductal carcinoma *in situ* diagnosis. J Natl Cancer Inst 2010; 102:627–37.

19. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011; 103:1656–64.
20. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989;10:1–10.
21. Clark AS, Lal P, Tan KS, Heitjan D, Lunceford N, Feldman M, et al., editors. Biomarkers to predict for response to PD0332991, an oral cdk 4/6 inhibitor: Examination in patients with metastatic breast cancer enrolled on UPCC 03909. San Antonio, TX: San Antonio Breast Cancer Symposium; 2013.
22. Chia S, Gradishar W, Mauriac L, Bines J, Amant F, Federico M, et al. Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT. J Clin Oncol 2008;26:1664–70.
23. Baselga J, Campone M, Piccart M, Burris HA III, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012;366:520–9.
24. Finn RD, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al., editors. Final results of a randomized Phase II study of PD 0332991, a cyclin-dependent kinase (CDK)-4/6 inhibitor, in combination with letrozole vs letrozole alone for first-line treatment of ER$^+$/HER2$^-$ advanced breast cancer (PALOMA-1; TRIO-18). San Diego, CA: American Association of Cancer Research; 2014.
25. Witkiewicz AK, Cox DW, Rivadeneira D, Ertel AE, Fortina P, Schwartz GF, et al. The retinoblastoma tumor suppressor pathway modulates the invasiveness of ErbB2-positive breast cancer. Oncogene 2013;33:3980–91.
26. Clark AS, O'Dwyer PJ, Heitjan D, Lal P, Feldman M, Gallagher MA, et al., editors. A phase I trial of palbociclib and paclitaxel in metastatic breast cancer. Chicago, IL: American Society of Clinical Oncology; 2014.
27. Johnson SM, Torrice CD, Bell JF, Monahan KB, Jiang Q, Wang Y, et al. Mitigation of hematologic radiation toxicity in mice through pharmacological quiescence induced by CDK4/6 inhibition. J Clin Invest 2010;120: 2528–36.
28. Infante JR, Shapiro GI, Witteveen PO, Gerecitano JF, Ribrag V, Chugh R, et al., editors. A phase I study of the single agent CDK4/6 inhibitor LEE011 in patients with advanced solid tumors and lymphomas. Chicago, IL: American Society of Clinical Oncology; 2014.
29. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, et al., editors. Clinical activity of LY2835219, a novel cell cycle inhibitor selective for CDK4 and CDK6, in patients with metastatic breast cancer. San Diego, CA: American Association of Cancer Research; 2014.
30. Vora SR, Juric D, Kim N, Mino-Kenudson M, Huynh T, Costa C, et al. CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell 2014;26:136–49.
31. Yu BD, Becker-Hapak M, Snyder EL, Vooijs M, Denicourt C, Dowdy SF. Distinct and nonoverlapping roles for pRB and cyclin D:cyclin-dependent kinases 4/6 activity in melanocyte survival. Proc Natl Acad Sci U S A 2003;100:14881–6.