4-14-2023

Phase II Study of Palbociclib (PD-0332991) in CCND1, 2, or 3 Amplification: Results from the NCI-MATCH ECOG-ACRIN Trial (EAY131) Subprotocol Z1B

Amy S Clark

Fangxin Hong

Richard S Finn

Angela M DeMichele

Edith P. Mitchell

See next page for additional authors

Authors

Amy S Clark, Fangxin Hong, Richard S Finn, Angela M DeMichele, Edith P. Mitchell, James Zwiebel, Fernanda I Arnaldez, Robert J Gray, Victoria Wang, Lisa M McShane, Larry V Rubinstein, David Patton, P Mickey Williams, Stanley R Hamilton, Mehmet S Copur, Samer S Kasbari, Ravneet Thind, Barbara A Conley, Carlos L Arteaga, Peter J O'Dwyer, Lyndsay N Harris, Alice P Chen, and Keith T Flaherty

Phase II Study of Palbociclib (PD-0332991) in *CCND1*, *2*, or *3* Amplification: Results from the NCI-MATCH ECOG-ACRIN Trial (EAY131) Subprotocol Z1B

Amy S. Clark[1], Fangxin Hong[2], Richard S. Finn[3], Angela M. DeMichele[1], Edith P. Mitchell[4], James Zwiebel[5], Fernanda I. Arnaldez[6], Robert J. Gray[2], Victoria Wang[2], Lisa M. McShane[7], Larry V. Rubinstein[7], David Patton[8], P. Mickey Williams[9], Stanley R. Hamilton[10], Mehmet S. Copur[11], Samer S. Kasbari[12], Ravneet Thind[13], Barbara A. Conley[14], Carlos L. Arteaga[15], Peter J. O'Dwyer[1], Lyndsay N. Harris[14], Alice P. Chen[16], and Keith T. Flaherty[17]

ABSTRACT

Purpose: Cyclin D/CDK4/6 is critical in controlling the G_1 to S checkpoint. *CCND*, the gene encoding cyclin D, is known to be amplified in a variety of solid tumors. Palbociclib is an oral CDK4/6 inhibitor, approved in advanced breast cancer in combination with endocrine therapy. We explored the efficacy of palbociclib in patients with nonbreast solid tumors containing an amplification in *CCND1*, *2*, or *3*.

Patients and Methods: Patients with tumors containing a *CCND1*, *2*, or *3* amplification and expression of the retinoblastoma protein were assigned to subprotocol Z1B and received palbociclib 125 mg once daily for 21 days of a 28-day cycle. Tumor response was assessed every two cycles.

Results: Forty patients were assigned to subprotocol Z1B; 4 patients had outside assays identifying the *CCND1*, *2*, or *3* amplification and were not confirmed centrally; 3 were ineligible and 2 were not treated (1 untreated patient was also ineligible), leaving 32 evaluable patients for this analysis. There were no partial responses; 12 patients (37.5%) had stable disease as best response. There were seven deaths on study, all during cycle 1 and attributable to disease progression. Median progression-free survival was 1.8 months. The most common toxicities were leukopenia (n = 21, 55%) and neutropenia (n = 19, 50%); neutropenia was the most common grade 3/4 event (n = 12, 32%).

Conclusions: Palbociclib was not effective at treating nonbreast solid tumors with a *CCND1*, *2*, or *3* amplification in this cohort. These data do not support further investigation of single-agent palbociclib in tumors with *CCND1*, *2*, or *3* amplification.

Introduction

One of the hallmarks of cancer is dysregulation of the cell cycle (1). Although the cell cycle is controlled by multiple pathways and proteins, the key regulator of the G_1 to S checkpoint is the retinoblastoma protein (Rb). Unphosphorylated active Rb inhibits the transition to S phase of the cell cycle by coupling to E2F transcription factors and blocking E2F-mediated gene transcription. Expression of cyclin D is highly regulated. Cyclin D complexes with and activates CDK 4 and 6 (CDK 4/6). This cyclin D/CDK4/6 complex phosphorylates and inactivates Rb, releasing E2Fs, and allowing progression into S phase. The key role of cyclin D/CDK4/6 is emphasized in that these complexes are also regulated by upstream mitogenic signaling pathways such as PI3K/AKT, Wnt, ER/PR, and MAPK (2–5). *CCND1, 2*, and *3* are the genes that encode the Cyclin D protein isoforms.

There are three CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) that are FDA-approved to treat estrogen receptor positive metastatic breast cancer in combination with endocrine therapy. Palbociclib received accelerated approval in combination with the aromatase inhibitor letrozole based upon a near doubling of progression-free survival (PFS) when compared with single-agent letrozole in the first-line metastatic setting, regardless of tumor *CCND 1, 2*, or *3* amplification (6, 7). Results were confirmed in a randomized double-blinded placebo-controlled phase III trial (8). Although the CDK4/6 inhibitors have been extensively studied in breast cancer, their efficacy shows some promise in other tumor types, though is less well explored. Palbociclib has been reported to stabilize Rb expressing growing teratoma in a group of 12 adults (9) as well as in a case report of a child with central nervous system growing teratoma syndrome who at the time of the report was receiving cycle 22 of therapy (10). Further, in a cohort of 17 heavily pretreated patients with mantle cell lymphoma, which due to the t(11:14) chromosomal translocation, express high

[1]University of Pennsylvania, Philadelphia, Pennsylvania. [2]Dana Farber Cancer Institute – ECOG-ACRIN Biostatistics Center, Boston, Massachusetts. [3]University of California, Los Angeles, Los Angeles, California. [4]Sidney Kimmel Cancer Center at Thomas Jefferson University Hospital, Philadelphia, Pennsylvania. [5]Investigational Drug Branch, Division of Cancer Treatment and Diagnosis, NCI, Bethesda, Maryland. [6]Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, NCI, Bethesda, Maryland. [7]Biometric Research Program, Division of Cancer Treatment and Diagnosis, NCI, Bethesda, Maryland. [8]Center for Biomedical Informatics and Information Technology, NCI, Bethesda, Maryland. [9]Frederick National Laboratory for Cancer Research, Frederick, Maryland. [10]City of Hope National Medical Center, Duarte, California. [11]Catholic Health Initiatives NCORP, Grand Island, Nebraska. [12]Southeast COR NCORP, Goldsboro, North Carolina. [13]University of Kentucky, Lexington, Kentucky. [14]Division of Cancer Treatment and Diagnosis, NCI, Bethesda, Maryland. [15]UT Southwestern Simmons Comprehensive Cancer Center, Dallas, Texas. [16]Early Clinical Trials Development Program, Division of Cancer Treatment and Diagnosis, NCI, Bethesda, Maryland. [17]Massachusetts General Hospital Cancer Center, Boston, Massachusetts.

Corresponding Author: Amy S. Clark, Division of Hematology-Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, 3400 Civic Center Boulevard, Philadelphia, Pennsylvania 19104. E-mail: Amy.clark@pennmedicine.upenn.edu

Clin Cancer Res 2023;29:1477–83

doi: 10.1158/1078-0432.CCR-22-2150

Translational Relevance

CCND1/2/3 amplifications are found in solid tumors and are assessable on commercially available genomic sequencing panels. *CCND* encodes the cyclin D protein, which complexes with CDK4/6 to allow progression from G_1 to S phase of the cell cycle. Thus, tumors that have amplification of *CCND1, 2*, or *3* may exhibit enhanced proliferation, and be particularly sensitive to palbociclib, a first-in-class oral CDK4/6 inhibitor approved to treat advanced breast cancer. We tested this hypothesis in a subprotocol of the NCI-MATCH trial. Our results do not support the use of palbociclib in nonbreast tumors containing a *CCND1, 2*, or *3* amplification.

levels of *CCND1* mRNA, treatment with palbociclib produced a modest objective response in 3 patients (11). Consistent across all of these trials, palbociclib has a favorable side-effect profile, with neutropenia being the most common toxicity. The Cancer Genome Atlas reported that *CCND1, 2*, or *3* amplification occur at variable rates across many tumor types. We hypothesized that palbociclib would be effective in nonbreast cancers that harbor amplification in the *CCND1, 2*, or *3* genes.

NCI-MATCH (EAY131, NCT02465060) is a national platform clinical trial designed to assess efficacy of targeted therapies in tumors with specific molecular alterations. The trial is run by the Eastern Cooperative Oncology Group (ECOG)-American College of Radiology Imaging Network (ACRIN) Cancer Research Group through the National Clinical Trials Network and the NCI Community Oncology Research Program. Here, we report the results of the NCI-MATCH Subprotocol Z1B, a phase II single arm study evaluating palbociclib in patients with nonbreast cancers containing a *CCND1, 2*, or *3* amplification and expression of Rb.

Patients and Methods

Clinical trial design

The Molecular Analysis for Therapy Choice (NCI-MATCH) trial, developed by ECOG-ACRIN Cancer Research Group (ECOG-ACRIN) and the NCI, aimed to find signals of efficacy for treatments targeted to actionable molecular alterations found in any tumor type. Each drug under investigation in the NCI-MATCH trial is vetted and must have at least preclinical evidence of target engagement (12). Each subprotocol was approved by the Central IRB for the NCI (the NCI Adult IRB). Patients undergo initial eligibility screening and metastatic tumor biopsy in Step 0, where targetable molecular alterations are identified. In Step 1, patients are assigned to subprotocols defined by the molecular alteration, which assessed the efficacy of a specific scientifically rational targeted therapy (or therapies). Patients undergo additional eligibility screening during Step 1 for each subprotocol. Subprotocol Z1B was designed to examine the clinical activity of palbociclib, a CDK4/6 inhibitor, in tumors with *CCND1, 2*, or *3* amplifications. By inhibiting CDK4/6, palbociclib was hypothesized to mitigate the increase in proliferation due to excess activated Cyclin D/CDK4/6, resulting from amplification of the *CCND1, 2*, or *3* genes. Because Cyclin D/CDK4/6 signaling is mediated through Rb and preclinical studies show lack of efficacy of CDK4/6i in Rb null tumor cells (13), tumor Rb expression was also required for eligibility on subprotocol Z1B. Written informed consent was obtained by all patients prior to any study activities; the study was conducted in accordance with the Declaration of Helsinki, Belmont Report, and U.S. Common Rule.

Patient selection

Adult patients with any nonbreast solid tumor, lymphoma or myeloma who progressed on standard treatment, or for whom no standard treatment was available, were eligible. Adequate hematopoietic, liver and kidney function, a performance status of ECOG ≤1 were required. Initially submission of fresh tissue was required, but an amendment on May 11, 2017, allowed patients to be enrolled using results from the designated lab network (instead of central testing). To be eligible for this subprotocol, tumors had to contain both an amplification in *CCND1, 2*, or *3* and Rb expression. *CCND* amplification was defined as seven or more copies of the gene; Rb expression was defined as 1+ or greater staining by IHC.

Tumor profiling

Actionable mutations were assessed using an NGS panel of 143 genes, including SNVs, indels, amplifications and selected fusions, and IHC assays for *PTEN*, *MLH1*, and *MSH2* (14, 15). If patients were identified as having a tumor with *CCND1, 2*, or *3* amplification, reflex testing for Rb expression by IHC was performed to confirm eligibility.

After completion of central testing of 5,954 patients' fresh tumor biopsies, trial accrual continued by identification of patients whose tumors were found to have eligible alterations by molecular profiling performed for clinical reasons at one of 25 CLIA accredited laboratories approved to screen for NCI-MATCH. Confirmatory central testing was required in order for these patients to be included in the primary analysis.

Assignment to treatment

Patients were assigned using a prospectively defined NCI designed informatics rules algorithm (MATCHBOX), as described previously (12).

Treatment

Patients assigned to subprotocol Z1B received palbociclib 125 mg by mouth once daily for 21 days followed by 7 days off, in 28-day cycles. A complete blood count was performed on day 1 of each cycle, as well as C1D15 and C2D15, or more frequently, as clinically required.

Evaluation of response

Response was evaluated every two cycles using criteria for solid tumors, lymphoma, glioblastoma multiforme, or multiple myeloma according to RECIST v1.1 (16–19).

Toxicity evaluation

Toxicity was evaluated using CTCAEv4. Dose modifications were according to the package insert for palbociclib.

Statistical considerations

The primary objective was to evaluate overall response rate (ORR) to palbociclib. A response rate of 5 of 31 patients (16%) or more was considered a signal of activity. This criterion allowed for 92% power to distinguish a 25% ORR from a null rate of 5%. The one-sided type I error rate was 1.8%. Secondary objectives were PFS at 6 months (PFS6), PFS, toxicity assessment, and evaluation of predictive biomarkers (comutations or other factors that potentially predict which patients will respond). The original accrual goal was 35 patients, to obtain 31 eligible patients. However, this subprotocol could accrue up to 70 patients (35 additional patients), after CTEP review of analysis from

the first 31 and would take into account disease histology. Accrual beyond the first 35 would only be allowed for cancer types with less than 10 patients enrolled.

Descriptive statistics were used to summarize patient characteristics, treatment, and study outcomes. A one-sided *P* value for the ORR was calculated using a one-sample binomial test against the null rate of 5%, and $P < 0.05$ for the first 31 eligible patients was deemed as statistically significant; if expansion to 70 was permitted, the ORR to be tested was one-sample binomial test against null rate of 5% with $P < 0.018$ All statistical analyses were performed with R, version 3.5.0 (R Foundation for Statistical Computing).

Data availability

The data underlying this article will be made available for request from the NCTN/NCORP Data Archive (https://nctn-data-archive.nci.nih.gov/) upon completing a Data Request Form for data from NCT04439201.

Results

From August 16, 2016, to December 5, 2017, 40 patients were identified as having *CCND1* (39 patients), *CCND2* (0 patients), or *CCND3* (1 patient) amplification in Step 0. Three patients did not meet eligibility criteria, and 1 patient died prior to starting study therapy. Of the remaining 36 patients, 4 had *CCND1* amplification by local testing only; because these were not centrally confirmed, they are excluded from the primary analysis. This left 32 evaluable patients who went on to Step 1 therapy with palbociclib. Patient characteristics are summarized in **Table 1**. The median age of the patients was 62, IQR 59–67 years. The majority (56%) of patients were male. See Supplementary Table S1 for a summary of the representativeness of our study population. Seventy percent of patients had received three or more prior lines of therapy (mostly chemotherapy) for their malignancies. A wide variety of malignancies was represented in this cohort (shown in **Table 2**). The majority of patients had adenocarcinoma ($n = 15$) or squamous ($n = 14$) histology. The four most commonly represented malignancies were squamous cell lung cancer ($n = 5$, 13.9%), adenocarcinoma of the prostate ($n = 4$, 11.1%), squamous cell carcinoma of the head and neck ($n = 3$, 8.3%), and adenocarcinoma of the colon ($n = 3$, 8.3%). The most common reason for discontinuation of study therapy was disease progression which occurred in 23 subjects (64%); one subject progressed during C1 and 14 progressed at the first disease assessment timepoint at the end of C2. There were seven deaths due to progression on study, all during cycle 1 of therapy.

There were no partial or complete responses. The best RECIST response observed was stable disease (SD) occurring in 12 patients (**Table 3**). Of the 32 patients in the final cohort, 8 patients were not evaluable for response due to death during cycle 1 ($n = 7$) or rapid

Table 1. Patient characteristics.

	Total
Enrolled	
Ineligible	3
Never started	2[a]
Treated	36
Unconfirmed CCND amplification status	4
Final cohort	32
(Total)	
Female *n* (%)	14 (44%)
Age (Y): median (range)	62 (38-78)
Race: White	28 (88%)
Black	1 (3%)
Hawaiian/Pacific Island	1 (3%)
Not reported	2 (6%)
Ethnicity: Hispanic	1 (3%)
ECOG PS 0	8 (25%)
N prior therapies: 1	3 (9%)
2	8 (25%)
3	10 (31%)
4	11 (34%)
Wt loss prev 6 mos:	
<5%	23 (72%)
5 to <10%	4 (12%)
10 to <20%	4 (11%)
≥20%	1 (3%)
Amplification[b]:	
CCND1	39 (97.5%)
CCND2	0 (0%)
CCND3	1 (2.5%)

Abbreviations: mos, months; prev, previous; PS, performance status; Tx, treatment; Wt, weight; Y, years.
[a]One subject also ineligible.
[b]Amplifications reported in all 40 patients assigned to subprotocol Z1B during Step 1, $N = 40$; other statistics are based on the 32 eligible and treated patients.

Table 2. Histologic tumor subtypes among analyzable patients in NCI MATCH subprotocol Z1B.

	Total ($n = 32$)
Adenocarcinoma (*n*)	14 (43.8%)
Prostate	3 (9.4%)
Colon[a]	3 (9.4%)
GEJ	2 (6.3%)
Stomach	2 (6.3%)
Endometrium (endometrioid)	1 (3.1%)
Lung	1 (3.1%)
Pancreas	1 (3.1%)
Rectum	1 (3.1%)
Squamous cell CA (*n*)	13 (40.1%)
Lung	5 (15.6%)
Oropharynx	3 (9.4%)
Glottis or larynx	2 (6.3%)
Esophagus	1 (3.1%)
Anus	1 (3.1%)
Vulva	1 (3.1%)
Transitional cell bladder (*n*)	2 (6.3%)
Serous CA, fallopian tube	1 (3.1%)
Adenoid cystic CA parotid	1 (3.1%)
Adenosquamous CA GEJ	1 (3.1%)
Neuroendocrine unknown primary	1 (3.1%)
Sarcomatoid CA	1 (3.1%)

Abbreviations: CA, cancer; GEJ, gastroesophageal junction.
[a]Two cases of mucinous colon CA.

Table 3. Best confirmed response.

	N
PR	0
SD	12
PD	12
NE	8
Total	32

Abbreviations: NE, not evaluable; PD, progressive disease; PR, partial response.

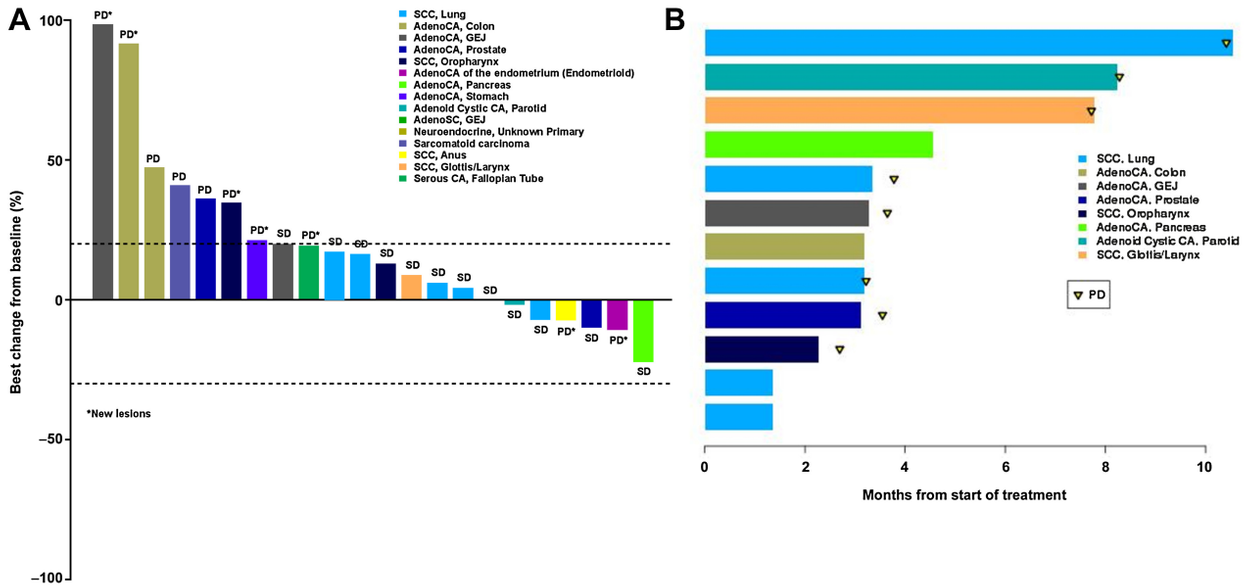

Figure 1.

Best response to palbociclib in patients with tumors containing a *CCND1*, *2*, or *3* amplification. **A,** Waterfall plot of best change from baseline for $n = 22$ patients with follow-up target lesion measurements. Color shows histology. For the remaining $n = 10$ patients: unevaluable ($n = 8$), PD due to new lesion ($n = 2$). **B,** Treatment duration for $n = 12$ patients who achieved SD. Abbreviations: PD, progressive disease; SCC, squamous cell carcinoma; AdenoCA, adenocarcinoma; GEJ, gastroesophageal junction; CA, carcinoma.

clinical disease progression during cycle 1 ($n = 1$), thus not having anatomic imaging with which to calculate a RECIST response. A waterfall plot depicting the percent change in tumor volume per RECIST v1.1 in each evaluable patient is shown in **Fig. 1A**. The largest reduction in tumor volume was 13% and was observed in a patient with adenocarcinoma of the pancreas. To further examine those patients with SD, **Fig. 1B** highlights the time on treatment with palbociclib. Most patients with SD experienced progression of disease by cycle 4 of therapy. Four patients remained on study for 4 cycles or longer: individual patients with squamous cell lung cancer, squamous cell carcinoma of the head neck larynx, adenoid cystic carcinoma of the parotid, and adenocarcinoma of the pancreas, were treated on study for 11, 8, 8, and 5 cycles, respectively. The range of prior lines of therapy in these four subjects was one to two, as compared with the eight subjects who came off trial in cycle 1 whose number of prior lines ranged from two to nine. The median PFS among patients treated with palbociclib was 1.8 months and the estimated 6-month PFS was 13% (90% CI, 5%–29%; **Fig. 2A**). The median overall survival was 7.7 months (**Fig. 2B**).

Adverse events (AE) assessed as possibly, probably, or definitely related to palbociclib are summarized in **Table 4**. The most commonly

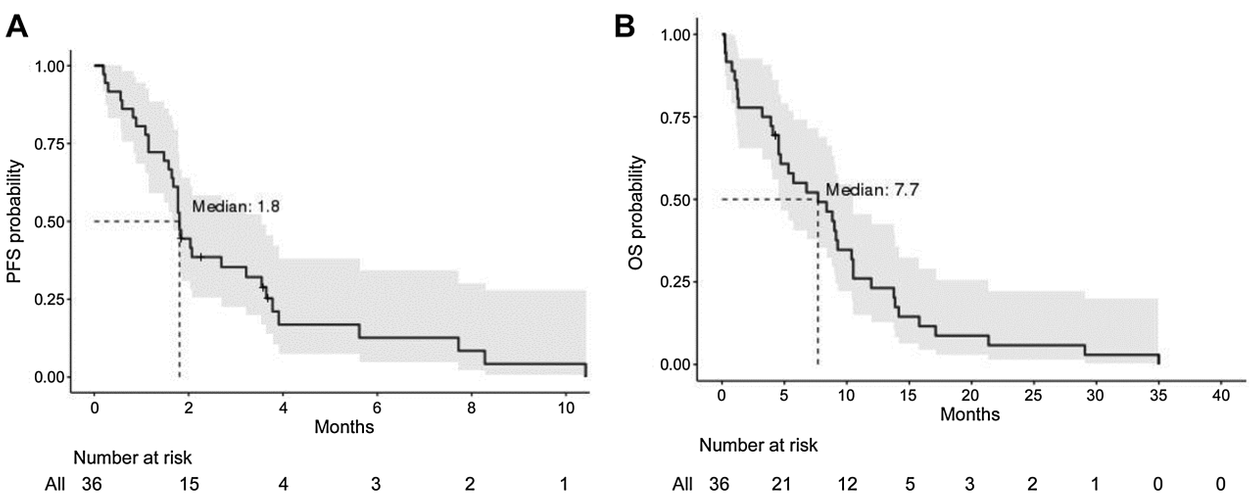

Figure 2.

A, PFS among patients with *CCND1, 2*, or *3* amplification receiving palbociclib on NCI-MATCH subprotocol Z1B. **B,** Overall survival among patients with *CCND1, 2*, or *3* amplification receiving palbociclib on NCI-MATCH subprotocol Z1B.

Table 4. Adverse events occurring in ≥ 10% of patients that possibly, probably, or definitely were related to palbociclib.

	Toxicity grade ($n = 38$)		
Toxicity type	**1, 2**	**3**	**4**
Alanine aminotransferase increased	3	1	—
Anemia	11	2	—
Anorexia	5	—	—
Aspartate aminotransferase increased	4	1	—
Constipation	4	—	—
Fatigue	8	2	—
Lymphocyte count decreased	8	1	—
Mucositis oral	5	—	—
Nausea	7	1	—
Neutrophil count decreased	7	11	1
Platelet count decreased	10	3	1
White blood cells decreased	14	7	—

reported treatment related AEs were due to myelosuppression: leukopenia ($n = 21$, 58.3%), neutropenia ($n = 19$, 52.8%), thrombocytopenia ($n = 14$, 38.9%), and anemia ($n = 13$, 36%). There were 19 grade 3/4 events, most of which were neutropenia (12/19, 63.2%). Other Grade 3/4 events that occurred in more than 1 patient were leukopenia (7, 36.8%), thrombocytopenia (4, 21%), anemia (2, 10.5%), and fatigue (2, 10.5%). There were only two grade 4 events (neutropenia and thrombocytopenia). Common nonhematologic toxicities included fatigue (10, 27.8%), nausea (8, 22.2%), elevation in aspartate aminotransferase (5, 13.9%), and elevation in alanine aminotransferase (4, 11.1%).

Molecular alterations co-occurring with *CCND1* or *CCND3* amplifications were common across the study population and were found in all but 2 patients enrolled on the Z1B subprotocol. The most commonly co-occurring alteration was *TP53* mutation that was found in 21 subjects. The second most common co-alterations were mutations in *KRAS* and *MYC*, which were found in four subjects each. Five subjects had only *CCND1* amplification on NGS analysis. There were no co-alterations that occurred more commonly among the 4 patients who had SD for 4 cycles or longer. Of note, *CCNE* amplification was NOT observed in any of these tumors. Finally, degree of *CCND* amplification was not associated with SD ($P = 0.5$ comparing mean degree of amplification among patients with SD compared with those with PD).

Discussion

Subprotocol Z1B of the NCI-MATCH trial examined the efficacy of palbociclib in patients with solid tumors (other than breast cancer) containing amplifications in *CCND1, 2*, or *3* with concomitant expression of the Rb protein. Of the 32 evaluable patients, 31 had a tumor with *CCND1* amplification and 1 had *CCND3* amplification. There were no objective responses observed in this NCI-MATCH subprotocol. Four patients had SD for at least six cycles, and no patient stayed on palbociclib longer than 10 cycles. The AE profile observed in this subprotocol was similar to those reported in the previously published trials of palbociclib in patients with breast cancer (7, 8, 20, 21): neutropenia was the most common AE and was also the most common grade 3/4 event, though febrile neutropenia was not observed.

Examination of genomic biomarkers assessed at the time of study enrollment failed to identify any additional genomic alterations that were unique to the tumors of the four individuals who derived potential clinical benefit. Although these results support the safety of palbociclib in the treatment of advanced solid tumors, they do not support its use in nonbreast cancers containing *CCND1 or 3* amplifications with Rb expression. These findings are consistent with recently published studies in metastatic breast cancer, which concluded that *CCND1* amplification did not predict response to palbociclib and letrozole. PALOMA1 (7), a phase I/II unblinded trial that randomized patients with breast cancer to receive letrozole alone or in combination with palbociclib in the first-line metastatic setting, was enriched for patients with tumors containing either *CCND1* amplification or loss of p16. In this study, the PFS of patients treated with palbociclib/letrozole was significantly better than that in patients treated with letrozole alone. However, *CCND1* amplification was not associated with superior clinical benefit from letrozole/palbociclib (6). *CCND1* amplification also failed to predict response to single-agent palbociclib in a smaller study in patients with metastatic breast cancer (21). There have been no predictive biomarkers discovered for palbociclib, although there are biomarkers that may predict lack of response: loss of Rb and high *CCNE* mRNA expression (22).

Our study is limited most significantly by the number of early events. Of the 40 patients who were enrolled into this subprotocol from Step 0, 1 patient died before reaching Step 1, 7 patients died during C1, 1 patient progressed during C1, and 14 additional patients progressed by C3. Thus, over half of patients either died or progressed within 2 months of identification of the *CCND* amplification. The eight early deaths were not due to prolonged time between Step 0 and Step 1 [median days 41 for the 8 patients who experienced early death compared with 42 for the remainder of the cohort ($P > 0.9$)]. This high rate of early events may be a result of the heavily pretreated nature of the study population and may reflect disease states too advanced to respond to a cytostatic drug. Although in the total study population, the median number of lines of therapy was 3 (1 to 10), the 8 patients who passed away had numerically more prior lines of therapy (median lines 3, range 2–10). Thus, rather than a biomarker of response to palbociclib, it may be a passenger mutation acquired over time during the course of multiple therapies and a biomarker of poor outcome. The idea of *CCND1* amplification being a biomarker of poor outcome is also supported by a study performed by Chen and colleagues that examined over 25,000 solid tumors. This study found that presence of *CCND*1 amplification correlated with worse overall survival and worse response to immune checkpoint inhibitors (23). Whether palbociclib might be effective as earlier-line metastatic treatment remains an open question.

Another limitation to the current analysis is the lack of pathway functional analysis. Further studies will be undertaken to more thoroughly examine the tumor genomes in patients enrolled onto this subprotocol to try to elucidate why some tumors progressed so quickly whereas others remained stable. It is plausible that there is an intricate interplay between *CCND* and its regulators, such as AMBRA1 (24–26), that may impact response to palbociclib.

In conclusion, this study is the first to examine the efficacy of palbociclib in *CCND1, 2*, or *3* amplified nonbreast solid tumors. Although our study confirms safety of the drug, our results do not support its use as a single agent in patients with solid tumors that contain amplification of the *CCND1* or *3* genes. Thus, future trials should concentrate on rational palbociclib drug combinations and use more in-depth functional assays of enzyme and pathway function to fully understand what molecular alterations are predictive biomarkers for this drug. Additional analyses are currently ongoing and may shed light on what drugs or drug combinations (with or without palbociclib) will be effective in patients with *CCND* amplified solid tumors.

Disclaimer

The Editor-in-Chief of *Clinical Cancer Research* is an author on this article. In keeping with AACR editorial policy, a senior member of the *Clinical Cancer Research* editorial team managed the consideration process for this submission and independently rendered the final decision concerning acceptability.

Authors' Disclosures

A.S. Clark reports grants from Novartis and Lilly outside the submitted work. R.S. Finn reports grants and personal fees from Pfizer during the conduct of the study as well as personal fees from AstraZeneca, Cstone, Exelixis, and Hengrui and grants and personal fees from Bayer, BMS, Eisai, Merck, Eli Lilly, and Adaptimmune outside the submitted work. A.M. DeMichele reports grants from Pfizer, Novartis, and Genentech outside the submitted work; in addition, A.M. DeMichele's spouse is on a Pfizer data and safety monitoring board for a GI drug (non-oncology). E.P. Mitchell receives institutional research funding from Genentech and Sanofi; has served as a consultant or advisor to BMS, Genentech, Merck, and Novartis; has received honoraria from Exelixis and Sanofi; serves on the speakers bureau of Ipsen; and has a leadership role in Corvus Pharmaceuticals. F.I. Arnaldez reports other support from AstraZeneca, PLC outside the submitted work. R.J. Gray reports grants from NCI during the conduct of the study. V. Wang reports grants from NIH/NCI during the conduct of the study. S.R. Hamilton reports other support from ECOG-ACRIN during the conduct of the study. C.L. Arteaga reports grants from Pfizer, Lilly, and Takeda and personal fees from Novartis, Lilly, Taiho Oncology, Daiichi Sankyo, AstraZeneca, Sanofi, Merck, OrigiMed, Immunomedics, Susan G. Komen Foundation, and Arvinas outside the submitted work; in addition, C.L. Arteaga has a patent for Provista with royalties paid. P.J. O'Dwyer reports grants from Pfizer during the conduct of the study. K.T. Flaherty reports personal fees from Clovis Oncology, Strata Oncology, Checkmate Pharmaceuticals, Kinnate Biopharma, Scorpion Therapeutics, PIC Therapeutics, Apricity, Oncoceutics, Fog Pharma, Tvardi, xCures, Monopteros, Vibliome, ALX Oncology, OMRx, Soley Therapeutics, Quanta Therapeutics, Lilly, Genentech, and Takeda; grants and personal fees from Novartis; and grants from Sanofi during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

A.S. Clark: Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **F. Hong:** Data curation, formal analysis, visualization, writing–original draft, writing–review and editing. **R.S. Finn:** Conceptualization, writing–review and editing. **A.M. DeMichele:** Conceptualization, supervision, investigation, writing–original draft, writing–review and editing. **E.P. Mitchell:** Supervision, investigation, methodology, writing–review and editing. **J. Zwiebel:** Supervision, writing–review and editing. **F.I. Arnaldez:** Supervision, writing–review and editing. **R.J. Gray:** Conceptualization, resources, supervision, methodology, writing–review and editing. **V. Wang:** Conceptualization, resources, supervision, methodology, writing–review and editing. **L.M. McShane:** Conceptualization, resources, supervision, methodology, writing–review and editing. **L.V. Rubinstein:** Conceptualization, supervision, methodology, writing–review and editing. **D. Patton:** Resources, supervision, methodology, writing–review and editing. **P.M. Williams:** Conceptualization, resources, supervision, writing–review and editing. **S.R. Hamilton:** Conceptualization, resources, supervision, methodology, writing–review and editing. **M.S. Copur:** Conceptualization, supervision, writing–review and editing. **S.S. Kasbari:** Conceptualization, supervision, writing–review and editing. **R. Thind:** Conceptualization, supervision, writing–review and editing. **B.A. Conley:** Conceptualization, supervision, methodology, writing–review and editing. **C.L. Arteaga:** Conceptualization, supervision, methodology, writing–review and editing. **P.J. O'Dwyer:** Conceptualization, supervision, investigation, writing–review and editing. **L.N. Harris:** Conceptualization, supervision, methodology, writing–review and editing. **A.P. Chen:** Conceptualization, supervision, methodology, writing–review and editing. **K.T. Flaherty:** Conceptualization, resources, supervision, investigation, writing–review and editing.

Acknowledgments

This study was coordinated by the ECOG-ACRIN Cancer Research Group (Peter J. O'Dwyer, MD, and Mitchell D. Schnall, MD, PhD, Group Co-Chairs) and supported by the NCI of the NIH under the following award numbers: U10CA180820, U10CA180794, UG1CA233341, UG1CA189809, U10CA180888, UG1CA189858, UG1CA233302, and UG1CA233180. Palbociclib was provided by Pfizer for this study. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. government.

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Note

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

Received July 18, 2022; revised October 7, 2022; accepted February 7, 2023; published first February 28, 2023.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
2. 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.
3. Caldon CE, Daly RJ, Sutherland RL, Musgrove EA. Cell cycle control in breast cancer cells. J Cell Biochem 2006;97:261–74.
4. 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.
5. 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.
6. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. 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). Cancer Res 2014;74 (19_suppl):CT101.
7. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncology 2015;16:25–35.
8. Finn RS, Martin M, Rugo HS, Jones S, Im S-A, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med 2016;375:1925–36.
9. Narayan V, Hwang W-T, Lal P, Rosen MA, Gallagher M, O'Dwyer PJ, et al. Cyclin-dependent kinase 4/6 inhibition for the treatment of unresectable mature teratoma: long-term follow-up of a Phase II study. Clin Genitourin Cancer 2016;14:504–10.
10. Schultz KAP, Petronio J, Bendel A, Patterson R, Vaughn DJ. PD0332991 (palbociclib) for treatment of pediatric intracranial growing teratoma syndrome. Pediatr Blood Cancer 2015;62:1072–4.
11. Lee C, Huang X, Di Liberto M, Martin P, Chen-Kiang S. Targeting CDK4/6 in mantle cell lymphoma. Ann Lymphoma 2020;4:1.
12. Flaherty KT, Gray R, Chen A, Li S, Patton D, Hamilton SR, et al. The molecular analysis for therapy choice (NCI-MATCH) trial: lessons for genomic trial design. J Natl Cancer Inst 2020;112:1021–9.
13. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol 2006;24:1770–83.
14. Lih C-J, Harrington RD, Sims DJ, Harper KN, Bouk CH, Datta V, et al. Analytical validation of the next-generation sequencing assay for a nationwide signal-finding clinical trial molecular analysis for therapy choice clinical trial. J Mol Diagn 2017;19:313–27.
15. Khoury JD, Wang W-L, Prieto VG, Medeiros LJ, Kalhor N, Hameed M, et al. Validation of immunohistochemical assays for integral biomarkers in the NCI-MATCH EAY131 clinical trial. Clin Cancer Res 2018;24:521–31.
16. Schwartz LH, Seymour L, Litière S, Ford R, Gwyther S, Mandrekar S, et al. RECIST 1.1-Standardisation and disease-specific adaptations: perspectives from the RECIST working group. Eur J Cancer 2016;62:138–45.

17. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 2014;2:3059.
18. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol 2010;28:1963–72.
19. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International myeloma working group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncology 2016;17:E328–46.
20. Turner NC, Ro J, André F, Loi S, Verma S, Iwata H, et al. Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med 2015;373:209–19.
21. DeMichele A, Clark AS, Tan KS, Heitjan DF, Gramlich K, Gallagher M, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015;21:995–1001.
22. Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, et al. Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. J Clin Oncol 2019;37:1169–78.
23. Chen Y, Huang Y, Gao X, Li Y, Lin J, Chen L, et al. CCND1 amplification contributes to immunosuppression and is associated with a poor prognosis to immune checkpoint inhibitors in solid tumors. Front Immunol 2020;11:1620.
24. Maiani E, Milletti G, Nazio F, Holdgaard SG, Bartkova J, Rizza S, et al. AMBRA1 regulates cyclin D to guard S-phase entry and genomic integrity. Nature 2021;592:799–803.
25. Chaikovsky AC, Li C, Jeng EE, Loebell S, Lee MC, Murray CW, et al. The AMBRA1 E3 ligase adaptor regulates the stability of cyclin D. Nature 2021;592:794–8.
26. Simoneschi D, Rona G, Zhou N, Jeong Y-T, Jiang S, Milletti G, et al. CRL4 (AMBRA1) is a master regulator of D-type cyclins. Nature 2021;592:789–93.