

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

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Abstract

Purpose: To identify the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the first-in-class, oral CDK4/6 inhibitor PD 0332991 administered once daily for 21 of 28 days (3/1 schedule) in patients with retinoblastoma protein (Rb)-positive advanced solid tumors and to describe pharmacokinetic–pharmacodynamic relationships relative to drug effects.

Experimental Design: This open-label phase I study (NCT00141297) enrolled patients who received PD 0332991 orally in six dose-escalation cohorts in a standard 3 + 3 design.

Results: Forty-one patients were enrolled. DLTs were observed in five patients (12%) overall; at the 75, 125, and 150 mg once daily dose levels. The MTD and recommended phase II dose of PD 0332991 was 125 mg once daily. Neutropenia was the only dose-limiting effect. After cycle 1, grade 3 neutropenia, anemia, and leukopenia occurred in five (12%), three (7%), and one (2%) patient(s), respectively. The most common non-hematologic adverse events included fatigue, nausea, and diarrhea. Thirty-seven patients were evaluable for tumor response; 10 (27%) had stable disease for ≥ 4 cycles of whom six derived prolonged benefit (≥ 10 cycles). PD 0332991 was slowly absorbed (median T_{max} , 5.5 hours), and slowly eliminated (mean half-life was 25.9 hours) with a large volume of distribution (mean, 2,793 L). The area under the concentration–time curve increased linearly with dose. Using an E_{max} model, neutropenia was shown to be proportional to exposure.

Conclusions: PD 0332991 warrants phase II testing at 125 mg once daily, at which dose neutropenia was the sole significant toxicity. *Clin Cancer Res*; 18(2); 568–76. ©2011 AACR.

Introduction

Phosphorylation of the retinoblastoma protein (Rb), mediated by cyclin-dependent kinase 4 (CDK4) or CDK6 (complexed with the activating subunit cyclin D), is required for entry of a cell into the cell cycle. Phosphorylation of Rb early in the G_1 phase initiates the changes in gene transcription that carry a cell through the G_1 –S tran-

sition and on to DNA replication (1). Thus, CDK4 and/or CDK6 are pivotal regulators of cell division.

Aberrations of the G_1 –S checkpoint are involved in the pathogenesis of many human cancers. Cyclin D is overexpressed and/or amplified in a wide variety of tumor types (2–4). Dysregulation of CDKs is among the most common aberration in human cancers (5): an activating mutation in CDK4 has been reported in some 40% of cases of melanoma (6), and CDK4/6 deregulation has been reported in liposarcoma, multiple myeloma, and mantle cell lymphoma (7–9).

The role of Rb as a tumor suppressor is well documented, and anticancer agents targeting the G_1 –S checkpoint are ineffective in tumors lacking functional Rb (10, 11). In tumors in which Rb function is intact and cellular proliferation is driven by upstream tumorigenic factors such as cyclin D1 overexpression, CDK4/6 is an attractive target for anticancer therapy. Although studies of CDK/cyclin function in mice have suggested that functional compensation is common among CDKs, it is apparent that tumor cells are sensitive to CDK inhibition, with outcome dependent on tumor cell type (12).

PD 0332991 is a novel, orally administered inhibitor of both CDK4 and CDK6 kinase activity. PD 0332991 is highly

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Translational Relevance

This study shows that an inhibitor of CDK4/6 may be administered with a tolerable toxicity profile and a pharmacokinetic–pharmacodynamic relationship that shows an expected cellular response (neutropenia). Evidence of antitumor activity supports further development in patients predicted to respond on the basis of the mechanism of action.

selective for CDK4 and CDK6, with IC_{50} values for CDK4/cyclinD1, CDK4/cyclinD3, and CDK6/cyclinD2 of 11, 9, and 15 nmol/L, respectively, and low activity against a panel of 36 additional protein kinases, including CDK2/cyclin E2, CDK2/cyclin A, and CDK1/cyclin B (11). PD 0332991, administered as a single agent has showed antiproliferative effects (selective G_1 arrest) on Rb-positive cells *in vitro* and tumor growth inhibition in several types of Rb-positive human breast and colon tumor xenografts (11), where PD 0332991 activity was associated with reduced Rb phosphorylation and decreased expression of the cell proliferation marker Ki-67. PD 0332991 showed no activity in Rb-negative tumor cell xenografts, consistent with CDK4/6 inhibition as the sole mode of action of PD 0332991 (11).

Results are reported from a first-in-human, phase I, dose-finding study of PD 0332991 conducted in patients with Rb-positive tumors (NCT00141297). Calculation of the initial phase I starting dose of PD 0332991 was based upon pivotal 3-week toxicology studies in rats and dogs that identified 300 mg/m² as the dose that produced severe toxicity in 10% of rats (STD10). One tenth of the rat STD10, 30 mg/m², did not result in severe, irreversible toxicity in the dog and is equivalent to 0.811 mg/kg in humans or approximately 50 mg in a 60-kg person. Toxicology data indicated that dogs were more sensitive than rats to testicular degeneration and bone marrow suppression produced by PD 0332991 exposure. Therefore, because of potential interspecies differences in sensitivity to PD 0332991, 25 mg was identified as an acceptable phase I starting dose in humans.

PD 0332991 has been evaluated in a phase I study to identify the recommended phase II dose (RP2D) on a treatment schedule comprising daily dosing for 14 days followed by 7 days off-treatment (2/1 schedule; ref. 13). The primary objective of this trial was to establish the safety profile of PD 0332991 and to identify the RP2D of a treatment schedule comprising daily dosing for 21 days followed by 7 days off-treatment (3/1 schedule).

Methods

Study design

This was a first-in-human, phase I, dose-finding, open-label, noncomparative study of PD 0332991 in Rb-positive solid tumors and non-Hodgkin lymphoma. The primary objective was to establish the safety profile of PD 0332991

by identifying dose-limiting toxicities (DLT), the maximum administered dose, the maximum tolerated dose (MTD), and the RP2D using a 4-week treatment cycle (3/1 schedule). Secondary objectives included characterization of single-dose and steady-state pharmacokinetics (PK) of oral PD 0332991 and evaluation of preliminary antitumor activity.

Written informed consent was obtained from all patients before any trial-specific activity was conducted. The final study protocol and amendments were reviewed and approved by the Institutional Review Board/Independent Ethics Committee at each participating site, in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki.

Study population

Eligible patients were men and women aged ≥ 18 years with Rb-positive solid tumors (except small cell lung cancer or retinoblastoma) or non-Hodgkin lymphoma, confirmed histologically or cytologically at original diagnosis, that were refractory to standard therapy or for whom no standard-of-care therapy was available. Additional key inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 , up to 3 prior cytotoxic chemotherapy regimens, the ability to swallow intact PD 0332991 capsules, and adequate organ function. Patients were ineligible if they met any of the following criteria: cytotoxic chemotherapy within 3 weeks prior to first treatment (8 weeks for mitomycin C or nitrosoureas); hormone therapy, radioimmunotherapy, immunotherapy, or other biologic therapy within 14 days prior to treatment; QTc interval > 470 msec; and untreated brain metastases (patients with brain metastases were eligible if they had completed treatment ≥ 10 days prior to the start of study medication, had discontinued corticosteroid treatment for these metastases for ≥ 5 days, and were neurologically stable). At screening, archival or fresh tumor tissue was assayed by a central laboratory (Oncotec Inc.) to evaluate Rb expression. Rb immunohistochemical staining intensity was assessed by a pathologist and deemed positive if a staining level of 1+ or greater above background was identified. The Rb expression assay was validated by Oncotec Inc.

Treatment

Cohorts of patients received escalating doses of PD 0332991 daily for 21 days, followed by 7 days off-treatment, repeated every 28 days. A standard 3 + 3 design was used, with provision for cohort expansion to 6 evaluable patients if a DLT was observed in the first cycle of treatment among the initial 3 patients. If 2 DLTs were observed during the first cycle in a cohort, dose-escalation was halted and dose finding continued at a lower level until the MTD (the highest dose level for which the incidence of first-cycle DLT was $< 33\%$) was identified. In the absence of DLTs, the dose was escalated in a modified Fibonacci scheme. To determine the RP2D, the MTD dose level was expanded to include a minimum of 12 patients.

Inpatient dose-escalations were permitted. After completing at least 2 treatment cycles at their assigned dose, patients could receive the next higher dose level, provided it was tolerable. Treatment cycles were repeated until occurrence of disease progression, unacceptable toxicity, or withdrawal of consent. Treatment was interrupted following a DLT, for serious adverse events (at the investigator's discretion), and for \geq grade 3 toxicity [including platelet counts $< 50,000/\mu\text{L}$, absolute neutrophil count (ANC) $< 1,000/\mu\text{L}$, and hemoglobin $< 8.0\text{ g/dL}$] in the first cycle; for subsequent cycles, ancillary supportive medications such as anti-diarrhea agents were to be used to maintain full dose.

A DLT was defined as one of the following adverse events occurring during cycle 1: grade 4 hematologic toxicity; grade 3 neutropenia with infection or fever $\geq 38.5^\circ\text{C}$; grade ≥ 3 non-hematologic treatment-related toxicity except those that had not been maximally treated (e.g., grade ≥ 3 nausea, vomiting, and diarrhea must have been fully treated) or that the patient considered tolerable, such as skin rash; confirmed grade 3 QTc prolongation (QTc > 500 msec) that persisted after correction of other possible causes such as electrolyte imbalance or hypoxia; or inability to receive the next dose of PD 0332991 within 1 week (± 1 day) of the last dose due to lack of hematologic recovery or prolonged non-hematologic toxicities of grade ≥ 3 . Patients resuming treatment following a DLT had their dose reduced to the next lower dose level previously tested on the same schedule or a 50% dose reduction if the interruption occurred at the starting dose.

Assessments

Safety (adverse events and laboratory parameters) and physical status (including ECOG performance status) were assessed at baseline, at regular intervals throughout the study, and within 1 week following treatment discontinuation. Adverse event severity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0 (14). Triplicate electrocardiograms (ECG) were taken at baseline as well as post-dose every 7 days during cycle 1 and on day 15 of cycles 2 to 6.

Tumor measurements derived from computed tomographic (CT) or MRI scans were obtained at baseline, after every 2 cycles during the study, and at the end of treatment/study withdrawal. Tumor responses were evaluated on the basis of Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 guidelines (15).

Pharmacokinetic assessments

Pharmacokinetic parameters of PD 0332991 were evaluated following a single dose (cycle 1, day 1) and following multiple dosing (cycle 1, day 8), with samples collected pre-dose and at 1, 2, 4, 7, and 10 hours post-dose in all patients. An additional sample was collected from patients in the expanded MTD cohort 24 hours post-dose on cycle 1, day 1. In the expanded MTD cohort, serial pharmacokinetic assessments were also obtained on cycle 1, day 21 and on cycle 2, day 1. At the end of cycle 1, 3 additional blood samples for

pharmacokinetic analysis were collected during the 7-day without-treatment interval (days 22, 23, and 24 of cycle 1), and trough pharmacokinetic samples were collected before dosing on days 8 and 15 of cycle 1. Urine pharmacokinetic assessments were conducted on day 1 of cycle 1, from urine samples collected from 0 to 12 hours in 13 patients in the expanded 125-mg RP2D cohort. Additional blood draws for pharmacokinetic assessment were obtained at the same time as ECG testing carried out at the projected time to first occurrence of the maximum observed plasma concentration C_{max} . Individual predicted area under the concentration–time curve (AUC) was estimated on the basis of dose, and individual predicted apparent clearance (CL/F) was obtained from population pharmacokinetic modeling. Additional pharmacokinetic parameters assessed were as follows: time to first occurrence of C_{max} (T_{max}), apparent volume of distribution during the terminal phase following oral administration (V_z/F), terminal elimination half-life ($t_{1/2}$), and drug accumulation ratio (R_{ac}).

Pharmacokinetic parameters were determined using non-compartmental analyses (WinNonLin version 4.1; Pharsight), and descriptive statistics for pharmacokinetic parameters were summarized by dosing group, day, and cycle.

Pharmacodynamics

An exploratory analysis was conducted to chart the course of changes in ANC and platelets over time during the first 2 cycles of treatment using data available from weekly safety laboratory tests. Relationships between PD 0332991 exposure and changes in (i) ANC and (ii) platelet counts were investigated further by fitting a simple model (without baseline) to determine the maximum effective concentration (E_{max}). Parameters to be determined were the E_{max} for ANC or platelets as a function of PD 0332991 exposure and the EC_{50} (the estimated plasma concentration resulting in a decrease from baseline of 50% in ANC or platelets). Data from all patients receiving PD 0332991 on the 3/1 schedule were included in the modeling analysis.

Results

Patient characteristics and disposition

Forty-one patients were enrolled in the study at 3 sites in the United States between September 2004 and June 2008 and received at least one dose of PD 0332991 on the 3/1 schedule. Patient baseline characteristics are presented in Table 1. Patients received PD 0332991 in 6 cohorts at doses ranging from 25 to 150 mg once daily.

Safety and tolerability

Five patients (12%) experienced DLTs (all neutropenia). Two patients in the 75-mg cohort experienced dose-limiting neutropenia (one grade 4 and one grade 3). Dose-escalation continued cautiously because the investigators considered these uncomplicated toxicities to be tolerable. DLTs were also documented in one patient receiving 125 mg once daily (grade 3 neutropenia) and two patients receiving 150 mg once daily (grade 3 neutropenia in one patient, grade 4

Table 1. Patient baseline characteristics

| | Patients (N = 41) |
|--|--------------------------|
| Median age (range), y | 54 (22–77) |
| Male/female (n) | 20/21 |
| ECOG performance status ^a , n | |
| 0 | 21 |
| 1 | 19 |
| 2 | 0 |
| Primary tumor type, n (%) | |
| Melanoma | 6 (15) |
| Breast | 5 (12) |
| Kidney | 4 (10) |
| Ovarian | 4 (10) |
| Liposarcoma | 4 (10) |
| Colon | 3 (7) |
| Other | 15 (37) |
| Prior therapy, n (%) | |
| Any | 41 (100) |
| Chemotherapy | 34 (83) |
| 1–2 regimens | 21 (51) |
| 3 regimens | 12 (29) |
| >3 regimens ^b | 1 (2) |
| Hormonal | 4 (10) |
| Immunotherapy/biologic | 7 (17) |
| Radiotherapy | 17 (42) |
| Surgery | 37 (90) |

^aECOG PS score not available for one patient.

^bOne patient who had received more than 3 prior cytotoxic chemotherapy regimens was enrolled into the study. Although the relevant inclusion criterion was such that patients were eligible with up to 3 prior cytotoxic chemotherapy regimens, this patient was enrolled with the agreement of the study sponsor and does not, therefore, represent a protocol deviation. The inclusion of this patient in the analysis populations did not affect the conduct or conclusions of the study.

neutropenia in the second patient). Of patients with dose-limiting neutropenia, additional treatment-related toxicities in cycle 1 included grade 2 anemia and grade 2 thrombo-

cytopenia in one patient receiving the 75-mg dose and grade 2 thrombocytopenia in one patient and grade 1 fatigue in a second patient receiving the 150 mg dose. The incidence and severity of neutropenia during cycle 1 is presented in Table 2. On the basis of these data, neutropenia was dose-limiting and 125 mg once daily was selected as the RP2D.

During cycle 1, 37 patients (90%) experienced an adverse event of any causality, with incidence decreasing to 30 patients (73%) after cycle 1. The most common cycle 1 non-hematologic adverse events were fatigue (14 patients, 34%), nausea (10 patients, 24%), vomiting (8 patients, 19.5%), and constipation (7 patients, 17%). After cycle 1, the most common non-hematologic adverse events included fatigue (10 patients, 24%), diarrhea (6 patients, 15%), nausea, dyspnea, and arthralgia (5 patients each, 12%). Grade 3 and 4 adverse events reported during and after cycle 1 are presented in Table 3. During cycle 1, a total of 16 patients (39%) experienced grade 3 adverse events; no patients reported grade 4 non-hematologic adverse events. The most common non-hematologic grade 3 adverse events in cycle 1 were fatigue, nausea, and abdominal pain (2 patients each, 5%; Table 3). After cycle 1, there were 10 patients (24%) with grade 3/4 adverse events (1 grade 4 adverse event of increased blood uric acid; Table 3). One grade 5 adverse event was also reported after cycle 1 in a patient who died of cardiac arrest, this death was not considered to be treatment-related.

A summary of patients whose hematologic laboratory abnormalities worsened relative to baseline during treatment is presented in Table 4. The majority of laboratory test abnormalities were grade 1 or 2; the most frequent grade 3 or 4 laboratory test abnormalities were lymphopenia, leukopenia, and neutropenia. Grade 3 or 4 thrombocytopenia was not reported. Hematologic abnormalities were adequately managed with standard supportive care, were not complicated, and resolved during the off-drug period with no cumulative events noted.

Analysis of QT interval changes corrected for heart rate (QTc) using both Bazett (QTcB) and Fridericia (QTcF) corrections was conducted (data not shown). Using Fridericia correction, 26 patients in the analysis had a maximum increase from baseline QTc of <30 msec, whereas no patients had a maximum on-treatment value \geq 500 msec. One patient on the 3/1 schedule had a maximum QTcF

Table 2. Incidence of treatment-emergent neutropenia during cycle 1

| Dose, mg QD | N | Neutropenia, CTC grade, n | | | | |
|-------------|----|---------------------------|---------|---------|---------|---------|
| | | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| 25 | 3 | 0 | 0 | 0 | 0 | 0 |
| 50 | 3 | 2 | 1 | 0 | 0 | 0 |
| 75 | 7 | 3 | 0 | 1 | 2 | 1 |
| 100 | 3 | 1 | 0 | 2 | 0 | 0 |
| 125 | 22 | 15 | 0 | 4 | 3 | 0 |
| 150 | 3 | 1 | 0 | 0 | 1 | 1 |

Table 3. Grade 3 and 4 AEs during and after cycle 1^a

| Adverse event, <i>n</i> | PD 0332991 schedule 3/1 (<i>n</i> = 41) | | | |
|-------------------------------|--|---------|---------------|---------|
| | Cycle 1 | | After cycle 1 | |
| | Grade 3 | Grade 4 | Grade 3 | Grade 4 |
| Hematologic | | | | |
| Neutropenia | 6 | 2 | 5 | 0 |
| Anemia | 0 | 1 | 3 | 0 |
| Leukopenia | 4 | 0 | 1 | 0 |
| Non-hematologic | | | | |
| Fatigue | 2 | 0 | 1 | 0 |
| Nausea | 2 | 0 | 0 | 0 |
| Abdominal pain | 2 | 0 | 0 | 0 |
| Dysphasia | 1 | 0 | 0 | 0 |
| Failure to thrive | 1 | 0 | 0 | 0 |
| Neck pain | 1 | 0 | 0 | 0 |
| Peripheral sensory neuropathy | 1 | 0 | 0 | 0 |
| Dyspnea | 1 | 0 | 1 | 0 |
| Hyponatremia | 0 | 0 | 1 | 0 |
| Hip arthroplasty | 0 | 0 | 1 | 0 |
| Gastrointestinal hemorrhage | 0 | 0 | 1 | 0 |
| Blood uric acid increased | 0 | 0 | 0 | 1 |
| Pleural effusion | 0 | 0 | 1 | 0 |

^aOne grade 5 event (cardiac arrest) occurred after cycle 1 and was not considered to be related to study drug.

increase from baseline of 67 msec that occurred only in cycle 1. Analysis of QT interval changes indicated that PD 0332991 has no clinically significant effect on cardiac repolarization.

Table 4. Hematologic toxicity by maximum toxicity grade among patients whose toxicity worsened relative to baseline, across all cohorts and all cycles

| Hematologic abnormalities, <i>n</i> (%) ^a | Schedule 3/1 (<i>n</i> = 41) | |
|--|-------------------------------|-----------|
| | Grade 1/2 | Grade 3/4 |
| Hemoglobin ^b | 22 (55) | 3 (8) |
| Lymphocytes ^c | 14 (36) | 14 (36) |
| Neutrophils ^c | 21 (54) | 8 (21) |
| Platelets ^b | 18 (45) | 0 |
| Leukocytes ^b | 27 (68) | 9 (23) |

^aLaboratory results by maximum toxicity grade among patients whose toxicity worsened relative to baseline.

^bForty of 41 patients.

^c39 of 41 patients in the schedule 3/1 group with baseline value and at least one post-baseline value patients assessable.

Antitumor activity

None of the 37 patients evaluable for response met RECIST guidelines for partial response. However, 13 patients (35%) maintained stable disease (SD) for at least 2 cycles. SD was observed in the following tumor types: liposarcoma (3 patients), testicular (2 patients), and kidney, ovarian, breast, appendiceal, peritoneal, melanoma, thymoma, and lung (1 patient each). SD lasted ≥ 4 cycles in 10 patients (27.0%) and ≥ 10 cycles in 6 patients (16.2%). Tumor types with SD lasting ≥ 10 cycles were testicular (1 patient receiving 125 mg once daily and 1 receiving 150 mg once daily), breast (1 patient receiving 50 mg once daily), and appendiceal, peritoneal, and thymoma (1 patient each at the 75, 100, and 150 mg once daily dose levels, respectively). Baseline expression of Rb varied among the 6 patients with prolonged disease stabilization. The patients with breast, appendiceal, and one of the patients with testicular cancer had high levels of Rb-positive cells (80%–100%), whereas the patients with thymoma, peritoneal cancer and the second patient with testicular cancer had low numbers of Rb-positive cells (10%) in their baseline biopsies. Two patients, one with appendiceal and one with testicular cancer, remain on therapy at the time of data cutoff, both with prolonged SD (39 and 23 cycles, respectively).

Pharmacokinetics

PD 0332991 plasma pharmacokinetics were determined in all 41 patients following a single dose on cycle 1, day 1

Table 5. PD 0332991 pharmacokinetic parameters on all doses, single-dose, and multiple dose^a

| PD 0332991 | | | | | | | | |
|-----------------------------|----|----------------------|--------------------------|-------------------------------|-------------------------------|----------------------|--------------------------|-------------------------------|
| Single-dose (day 1; N = 41) | | | | | Multiple dose (day 8; N = 31) | | | |
| Dose, mg | n | T _{max} , h | C _{max} , ng/mL | AUC ₀₋₁₀ , ng h/mL | n | T _{max} , h | C _{max} , ng/mL | AUC ₀₋₁₀ , ng h/mL |
| 25 | 3 | 4 (4-4) | 10 (63) | 58 (51) | 3 | 4 (2-7) | 16 (32) | 119 (32) |
| 50 | 3 | 4 (4-4) | 21 (3) | 134 (5) | 3 | 4 (2-7) | 36 (16) | 274 (15) |
| 75 | 7 | 4 (4-10) | 29 (24) | 199 (20) | 6 | 4 (4-9) | 59 (24) | 492 (27) ^b |
| 100 | 3 | 4 (4-4) | 47 (11) | 331 (17) | 3 | 7 (4-10) | 85 (11) | 592 (NA) ^c |
| 125 | 22 | 7 (2-24) | 52 (43) | 299 (44) | 13 | 4 (1-10) | 86 (34) | 724 (38) |
| 150 | 3 | 4 (4-4) | 91 (2) | 641 (5) | 3 | 7 (7-10) | 128 (42) | 1,084 (49) |

NOTE: Data are median (range) for T_{max}; arithmetic mean (% CV) for C_{max} and AUC₀₋₁₀.

^aSingle-dose (cycle 1, day 1) and multiple dose (cycle 1, day 8) parameters for all doses.

^bn = 5.

^cn = 2.

and in 31 patients following multiple dose administration on cycle 1, day 8 (Table 5). On day 1, all patients had detectable plasma concentrations of PD 0332991 at the first measured time point (1 hour) following oral administration (Supplementary Fig. S1A). Plasma pharmacokinetics parameters had low to moderate variability with a generally dose-dependent increase in exposure over the dose range of 25 to 150 mg, based on C_{max} and AUC from 0 to 10 hours (AUC₀₋₁₀). AUC was determined only up to 10 hours because of limited availability of data at later time points. Dose-normalized pharmacokinetics parameters for PD 0332991 after administration of 25 to 150 mg once daily showed an overall dose proportional increase in exposure with slightly higher exposures observed at the 150-mg dose level (Fig. 1A).

Following repeated daily dosing to steady-state for the RP2D cohort, pharmacokinetics parameters were estimated from day 21 data (Table 6; Supplementary Fig. S1>B and S1C). PD 0332991 was absorbed with a median T_{max} of about 5.5 hours and a mean PD 0332991 V_Z/F of 2,793 L, which is significantly greater than total body water (42 L), indicating substantial tissue binding. PD 0332991 was eliminated slowly, with a mean t_{1/2} of 26 hours and a mean CL/F of 80.6 L/h. The drug accumulated following repeated dosing with a median R_{ac} of 2.2, which is consistent with a half-life of about 26 hours. Renal excretion was a minor route of elimination with a mean of 1.8% of unchanged PD 0332991 found in urine (data not shown).

Pharmacodynamics

Analysis of changes from baseline of ANC and platelet levels showed a nadir occurring at the end of the dosing period in cycle 1 and cycle 2 for both cell types (Supplementary Fig. S2). In cycle 1, a rebound of both ANC and platelet levels was observed during the off-drug period that continued up to day 8 of the following dosing cycle. ANC did not return to baseline levels, whereas for platelets, the rebound exceeded baseline values. Changes from baseline

of ANC and platelet levels versus individual AUC for all 41 patients were assessed. For ANC, E_{max} was estimated to be 82.9% [SE, 7.8; 9.4% coefficient of variation (CV) with an

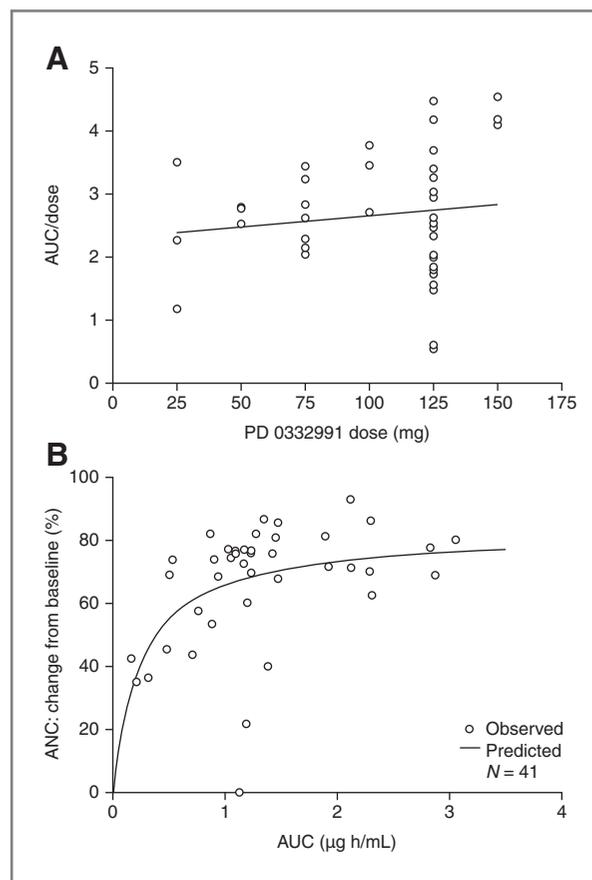


Figure 1. A, dose-normalized AUC following a single dose of PD 0332991 as a function of administered dose. B, changes from baseline in levels of ANC versus individual predicted AUC.

Table 6. Multiple-dose parameters on day 21 for the RP2D of 125 mg once daily

| Parameter | PD 0332991 125 mg (n = 4) |
|------------------------|------------------------------|
| T_{max} , h | 5.5 (2.0–9.8) |
| C_{max} , ng/mL | 97.4 (41) |
| C_{trough} , ng/mL | 47.0 (48) |
| AUC_{0-24} , ng h/mL | 1,733 (42) |
| $t_{1/2}$, h | 25.9 (29) |
| CL/F, L/h | 80.6 (34) |
| V_z/F , L | 2,793 (15) |
| R_{ac} | 2.2 (1.5–2.9) |

NOTE: Data are median (range) for T_{max} and R_{ac} , arithmetic mean (% CV) for AUC_{0-24} , CL/F, C_{max} , C_{trough} , and V_z/F . Abbreviations: CL/F, apparent oral clearance; V_z/F , apparent oral volume of distribution during the terminal elimination phase following oral administration.

estimated exposure of 0.253 $\mu\text{g hr/mL}$ (SE, 0.12; 49.1% CV) eliciting a 50% change from baseline (EC_{50}); Fig. 1B]. For platelets, the E_{max} was estimated to be 113.9% (SE, 33.3; 29.1% CV) and the EC_{50} was estimated to be 0.184 $\mu\text{g hr/mL}$ (SE, 0.97; 52.9% CV; data not shown).

Discussion

This study evaluated the selective CDK4/6 inhibitor PD 0332991 as an anticancer therapy in patients with advanced Rb-positive solid tumors or non-Hodgkin lymphoma and fulfilled its primary objective of establishing the safety profile of PD 0332991 and identifying a recommended 3/1 schedule dose for further investigation in phase II studies.

PD 0332991 had an acceptable safety profile in this population, and toxicities were both manageable and reversible. The DLT was dose-dependent neutropenia. This toxicity was predicted on the basis of preclinical data and is consistent with cell-cycle inhibition. Neutropenia was not cumulative in most patients; when compared with cycle 1, subsequent treatment cycles reported 1 additional patient with treatment-related neutropenia of any grade in the 50-mg once daily dose cohort (2 vs. 1) and 1 additional case in the 150-mg once daily cohort (3 vs. 2). In the 150-mg dose cohort, this included 1 additional patient with grade 3 treatment-related neutropenia compared with cycle 1 (2 vs. 1). The toxicity resolved quickly and so was not associated with complications. With regard to the dose-limiting neutropenia observed at 75 mg once daily, the toxicity appeared late in the cycle and reversed promptly. As the nature of the neutropenia, including its duration and recovery kinetics, could not have been predicted for this class of drug before the study, an exploratory approach was necessary during the study. Because the definitions of DLTs were written conservatively to protect from more prolonged

toxicity that could be associated with a drug with a long half-life, there was agreement from all investigators and Sponsor to proceed with careful monitoring. Subsequent data support that this was both safe and effective as a strategy and that the initial construct of the DLT definition was too conservative. The RP2D was determined to be 125 mg once daily.

The predominance of neutropenia as the major toxicity of PD 0332991, and especially the virtual absence of concomitant diarrhea, supports a very specific action of this drug on the cell cycle and a well-tolerated safety profile. Other candidate drugs that are described as cell-cycle inhibitors have multiple associated toxic effects, including many on organs with nondividing cells (16–21). For the first time, in PD 0332991, a compound may be available that permits adequate testing of cell-cycle inhibition as a therapeutic approach.

Although efficacy was not a primary endpoint of this study, tumor response data reflect a substantial degree of tumor control in a phase I trial and indicate that PD 0332991 has clinical activity in Rb-positive tumors. More than one quarter of patients (10 of 37; 27%) benefited from therapy with a best response of SD for ≥ 4 cycles, according to RECIST version 1.0 guidelines. Disease stabilization was noted in a range of tumor types, including liposarcoma, kidney, ovarian, breast, appendiceal, peritoneal, testicular, melanoma, and thymoma. Six patients (16%), including 2 patients with testicular cancer (22), achieved durable disease stabilization persisting for ≥ 10 cycles. This manifestation of patient benefit must inform the design of phase II and subsequent trials. A progression-free survival or similar endpoint is likely to be required to show activity, and the studies will need to be powered accordingly.

A number of CDK inhibitors, with varying modes of action that target the G_1 -S checkpoint of cell division are currently in clinical development. These include flavopiridol (CDK1, 2, 4, and 7 inhibitor; ref. 16), SCH 727965 (CDK1, 2, 5, and 9 inhibitor; ref. 17), SNS-032 (formerly BMS-387032; CDK2, 7, and 9 inhibitor; refs. 16–21, 23), AT7519M (CDK1, 2, 4, and 5 inhibitor; ref. 19), PHA 848125 (inhibitor of CDKs 1 and 2 in addition to neurotrophic tyrosine kinase receptor type 1, TRKA; ref. 18), and indisulam (E7070, which blocks cells in G_1 phase and reduces expression of cyclins A, B1, and CDK2; ref. 20). PD 0332991 is the only one of these agents that is orally bioavailable (although oral administration of SNS-032 may be feasible; ref. 21) and is a specific inhibitor of CDK4 and 6. Overall, the safety profile reported here for PD 0332991 is similar to that reported for the majority of other CDK inhibitors in development. Among them, myelosuppression is the predominant DLT, and the most commonly additional reported toxicities are nausea, vomiting, diarrhea, fatigue, along with myelosuppression, suggesting that these adverse events are not CDK subtype specific (16–21). However, there are some differences between the safety profile of PD 0332991 and that reported for CDK/cell-cycle inhibitors. Notably, the grade 3/4 vascular thrombotic events observed with flavopiridol in phase II were not seen

with PD 0332991, possibly due to the specificity of PD 0332991 for CDKs 4 and 6 (24). Neurologic adverse events were prominent in the safety profile reported for PHA 848125, with tremors and ataxia dose-limiting for this agent (18). Such events were not observed with PD 0332991, and it is possible that the neurologic adverse events seen with PHA 848125 arise from its inhibition of TRKA rather than CDK.

Pharmacokinetic assessments conducted in this study showed that PD 0332991 is slowly absorbed, with a median T_{max} of approximately 5.5 hours, penetrates extensively into the peripheral tissues, and is eliminated slowly with a half-life of 26 hours. Exposure generally increased with increasing dose and was moderately variable. The projected optimal dose based on preclinical pharmacokinetic/pharmacodynamic modeling was 400 mg once daily; however, the MTD in the clinic was 150 mg once daily for the 3/1 schedule. Pharmacokinetic/pharmacodynamic analysis of the data from 3 mouse human xenograft tumor models and projected human pharmacokinetics indicated that an estimated average steady-state PD 0332991 plasma concentration of approximately 1,000 ng/mL resulted in 80% to 90% inhibition of Rb phosphorylation and a 50% reduction in tumor growth for each of these human xenograft models. To date, there are no direct clinical data to assess this pharmacodynamic endpoint.

A pharmacokinetic/pharmacodynamic relationship could be established between changes in levels of ANC and platelets versus plasma exposure using a simple E_{max} model, with increasing exposure of PD 0332991 resulting in a saturable decrease from baseline for both ANC and platelets. Similarly, percentage decreases from baseline (to observed nadir) for both cell types were equivalent in each cycle. The extent of recovery of the cells during the 1-week off-drug period was dependent on the cell type. Multiple factors, both system-based (i.e., based on the cell type) and drug-based, could be implicated in the overall changes observed following treatment. System-based factors might include the mean transit time to maturation and baseline circulating levels of each cell type as well as feedback mechanisms involved in cell recovery as a response to drug-induced cell death. The drug-based effect may relate the plasma concentrations of PD 0332991 to the effect observed at the site of action (stem cells in the bone marrow). A semimechanistic physiologic model has been developed to describe the neutropenic effects of cytotoxic agents such as docetaxel, etoposide, paclitaxel, and irinotecan (25). This mechanistic population pharmacokinetic/pharmacodynamic modeling approach is currently being used to further explore the changes in levels of ANC and platelets observed following PD 0332991 treatment. It is possible that the use of ANC or platelet modeling may have use as surrogate pharmacodynamic markers of PD 0332991 activity.

In conclusion, PD 0332991 may be studied further at a dose of 125 mg once daily administered orally for the first 21 days of 28-day cycles; neutropenia is dose-limiting and predictable, based upon initial pharmacokinetic/pharmacodynamic modeling.

Preliminary evidence of antitumor activity warrants further clinical studies, and phase II trials of PD 0332991 as a single agent are planned/underway in patients with refractory solid tumors (NCT01037790) metastatic liposarcoma (NCT01209598), recurrent Rb-positive glioblastoma (NCT01227434), advanced hepatocellular carcinoma (NCT01356628), and Rb-wild-type non-small cell lung carcinoma with inactivated CDK N2a (NCT01291017). PD 0332991 is also currently being studied in phase II trials in combination with bortezomib and dexamethasone in patients with multiple myeloma (NCT00555906) and with letrozole in patients with hormone receptor-positive breast cancer (NCT00721409).

Disclosure of Potential Conflicts of Interest

P.M. LoRusso has received compensation (less than \$10,000) from Pfizer for attending an advisory board and has had travel expenses paid by Pfizer for attending an advisory board and her institution (Karnanos Cancer Institute) has received funding from Pfizer for the current study as well as for other research. The institution of A. DeMichele (University of Pennsylvania) has received major commercial research grants from Genentech and Wyeth. R. Courtney, S.S. Randolph, M.N. Shaik, and K.D. Wilner are employees of Pfizer and own stock/stock options in Pfizer. P.J. O'Dwyer has received a consulting fee (less than \$10,000) from Pfizer, for other research, and his institution (Abramson Cancer Center, University of Pennsylvania) has received funding from Pfizer for the current study and for other research. No potential conflicts of interest were disclosed by other authors.

Other Presentations

Work presented in the manuscript is original and has not been published elsewhere. Some of the data have been presented previously as listed below:

O'Dwyer PJ, LoRusso P, DeMichele A, Gupta V, Barbi A, Dials H, et al. A phase I dose-escalation trial of a daily oral CDK 4/6 inhibitor PD 0332991. *J Clin Oncol* 25:18S, 2007 (suppl; abstr 3550).

Vaughn DJ, Flaherty K, Lal P, Gallagher M, O'Dwyer PJ, Wilner K, et al. Treatment of growing teratoma syndrome (letter). *N Engl J Med* 2009;360:423-4.

Shaik MN, O'Dwyer P, LoRusso PM, Schwartz G, Wilner K, Courtney R. Population pharmacokinetic/pharmacodynamic evaluation of the effect of PD 0332991 on QTc in patients with advanced cancers. In: Proceedings of the 2010 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics; 2010 Mar 17-20; Atlanta, GA.

Tortorici MA, O'Dwyer P, LoRusso P, Schwartz G, Wilner K, Ruiz-Garcia A, et al. Population pharmacokinetics of PD 0332991 in patients with advanced cancers. In: Proceedings of the 2010 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics; 2010 Mar 17-20; Atlanta, GA.

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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

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