PHASE II TRIAL OF THE CYCLIN-DEPEDENT KINASE INHIBITOR PD 0332991 IN PATIENTS WITH CANCER

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SYNOPSIS	
TITLE	Phase II trial of the cyclin-dependent kinase inhibitor PD 0332991 in patients with cancer
PROTOCOL NUMBER	PD 0332991
CLINICAL PHASE	Phase II
RATIONALE	PD 0332991 is a novel, potent and selective inhibitor of cyclin-dependent kinases 4 & 6. PD 0332991 causes cell cycle arrest and induces apoptosis in cells containing detectable levels of the phosphorylated form of the retinoblastoma protein (Rb+). Dysregulation of the cell cycle is a hallmark of cancer, and frequently involves cdk4 and 6, or cyclin D, their principal cyclin partner. Tumor regression was observed in patients treated in Phase I trials of PD 0332991 at doses that were tolerable. The major toxicity was neutropenia, which was mild to moderate at the dose to be utilized in this Phase II trial. At these doses in a separate proof-of-concept study, evidence of inhibition of the cell cycle was demonstrated in imaging of tumor uptake of thymidine by FLT-PET. In this study, the activity of PD 0332991 will be sought in patients with a group of cancer types which for various reasons may be enriched for potential responders, and in whom detailed studies to elucidate PK/PD determinants of drug effect and correlative biological analyses will be performed.
OBJECTIVES	**The primary objectives of this study are:** • To determine the response rates following treatment with PD 0332991 in the following malignancies: 1) Metastatic breast cancer 2) Metastatic colorectal cancer that harbors the Kras or BRAF mutation 3) Advanced or metastatic esophageal and/or gastric cancer 4) Cisplatin-refractory, unresectable germ cell tumors 5) Any tumor type if tissue tests positive for CCND1 amplification, CDK4/6 mutation, CCND2 amplification OR any other functional alteration of the G1/S checkpoint. • To evaluate the safety and tolerability of PD 0332991, administered to subjects with refractory solid tumors **The secondary objectives of this study are:** • To assess the pharmacodynamic effects of PD 0332991 on tumor and non-tumor tissue • To investigate the relationship between selected biomarkers, PK and/or efficacy and safety outcomes.

	• To estimate the population pharmacokinetics for PD 0332991 and to correlate PK with efficacy outcomes. • • To evaluate the preliminary safety and efficacy of PD 0332991 in combination with trastusumab in patients with HER2-overexpressing tumors within the 5 cohorts under study • To perform a Phase II evaluation of PD0332991 in a population defined as potential responders on the basis of CCND1 gene amplification.

STUDY DESIGN	This is a phase II, non-randomized, open-label study, evaluating the response rates, time to progression, safety, tolerability, PK and pharmacodynamics of PD 0332991 in subjects with selected solid tumors. Patients will be treated with the drug at a dose of 125 mg QD on a 21-day schedule, with cycles repeating every 28 days. The study will consist of the following periods: Pre-Treatment Period: Subjects are consented and qualified for the study. Treatment Period: Subjects will be treated and monitored for safety (including laboratory assessments) and signs of toxicity. They will be evaluated every two to three cycles by conventional imaging modalities to determine response (measured using RECIST criteria). Imaging and biopsy of accessible disease pre-treatment and during the first cycle of therapy will be used to assess pharmacodynamic endpoints. In the absence of progressive disease (PD) and unacceptable PD 0332991-related toxicity, subjects may continue to receive drug treatment until progression. Post-Treatment Period: Additional follow-up will occur for significant adverse events related to study treatment that are not resolved by or occur after the time of the last visit.
NUMBER OF SUBJECTS	Approximately 100-205 subjects will be treated in 5 cohorts
TARGET POPULATION:	**INCLUSION CRITERIA:** **1. Disease Characteristics:** **All Subjects:** All subjects treated under this protocol will have histologically documented cancer of one of the following types: A. Metastatic breast cancer (7 triple negative, 23 ER+ after the first 15 patients are enrolled on the non-CCND1 cohort; in addition 10 HER2+ for combination trastuzumab and PD0332991 therapy) up to 55 total enrollment slots B. Metastatic colorectal cancer that harbors the Kras or BRAF mutation (15-30 enrollment slots) C. Advanced or metastatic esophageal and/or gastric cancer (15-30 enrollment slots) D. Cisplatin-refractory, unresectable germ cell tumors (15-30 enrollment slots) E. Any tumor type if tissue tests positive for *CCND1* amplification, *CDK4/6* mutation , *CCND2* amplification OR any other functional alteration at the G1/S checkpoint. (15-30 enrollment slots) **2. Biopsy Requirements:** **For Subjects with accessible disease amenable to biopsy:**

A biopsy will be obtained pre-treatment and during Cycle 1 (while patient is receiving drug) for molecular markers of the cell cycle, and its inhibition.

For all subjects:

1. Subjects will be ≥ 18 years old
2. The subject has disease that is assessable by tumor marker, physical, or radiologic means.
3. The subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
4. The subject has adequate organ function, defined as follows
   a. Bilirubin ≤ 1.5 x the upper limit of normal (ULN)
   b. Serum creatinine ≤ 1.5 x UNL or calculated creatinine clearance ≥ 60 mL/min, and
   c. **For subjects without liver metastases:** alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN
   d. **For subjects with liver metastases:** alanine aminotransferase (ALT) and aspartate aminotransferase ≤ 5 x ULN
5. All tumors must test positive for Rb expression with the exception of:
   a. ER positive metastatic breast tumors (data now shows all to be Rb positive.)
   b. Any tumor type if tissue tests positive for *CCND1* amplification, *CDK4/6* mutation , *CCND2* amplification OR any other functional alteration at the G1/S checkpoint.
6. The subject has adequate marrow function, defined as follows
   a. Absolute neutrophil count (ANC) ≥1500/mm^3
   b. Platelets ≥100,000/mm^3, and
   c. Hemoglobin > 9 g/dL
7. The subject is capable of understanding and complying with the protocol requirements and has signed the informed consent document.
8. Sexually active subjects (male and female) must use accepted methods of contraception during the course of the study and for 3 months after the last dose of protocol drug(s).
9. Female subjects of childbearing potential must have a negative pregnancy test at screening. Females of childbearing potential are defined as sexually mature women without prior hysterectomy or who have had any evidence of menses in the past 12 months.
10. However, women who have been amenorrheic for 12 or more months are still considered to be of childbearing potential if the amenorrhea is possibly due to prior chemotherapy, antiestrogens, or ovarian suppression.

EXCLUSION CRITERIA:

1. The subject has received cytotoxic chemotherapy (including investigational cytotoxic chemotherapy) within 3 weeks (or nitrosoureas or mitomycin C within 6 weeks) before the first dose of PD 0332991. Patients with HER2-overexpressing tumors may receive

	trastuzumab up to the date of starting therapy, and may continue to receive trastuzumab while receiving PD0332991. **2.** The subject has received any other type of investigational agent within 28 days before the first dose of study treatment. **3.** The subject has not recovered from clinically-meaningful toxicity due to prior therapy (i.e., back to baseline or Grade ≤ 1) with the exception of neurotoxicity and alopecia. **4.** The subject has untreated or uncontrolled brain metastases or evidence of leptomeningeal involvement of disease unless the subject has a teratoma in which case s/he may be eligible if all other eligibility criteria are met **5.** The subject has uncontrolled intercurrent illness including, but not limited to: a. ongoing or active infection b. diabetes mellitus c. hypertension d. symptomatic congestive heart failure, unstable angina pectoris, stroke or myocardial infarction within 3 months 6. The subject has a baseline corrected QT interval (QTc) > 470 ms. 7. The subject is pregnant or breastfeeding. 8. The subject is known to be positive for the human immunodeficiency virus (HIV). ***Note:*** baseline HIV screening is not required 9. The subject is unable or unwilling to abide by the study protocol or cooperate fully with the investigator or designee.
ESTIMATED LENGTH OF STUDY	Approximately 10 years for subject accrual and treatment.
ESTIMATED STUDY DATES	October 2009 (first patient, first visit) to September, 2019 (last patient, first visit)
INVESTIGATIONAL REGIMEN DOSE/ROUTE/ DURATION	Cycles will be 28 days in duration. For all subjects, PD 0332991 will be administered at a dose of 125mg daily as a single dose for 21 days out of 28, cycles defined as 28 days. Patients with HER2-overexpressing tumors have the option of receiving trastuzumab, at standard dose, scheduled every three weeks.
COMPARATOR DRUG	None
ASSESSMENTS:	
SAFETY	Safety will be assessed by evaluation of adverse events, vital signs, ECG, laboratory tests and concomitant medications. Adverse event seriousness, severity grade and relationship to study treatment will be assessed by the Investigator. Severity grade will be defined by the National Cancer

	Institute CTCAE v5.0. For patients receiving concurrent trastuzumab, cardiac evaluation with either echocardiogram or MUGA scan will be obtained prior to starting PD0332991 and every 12 weeks (+/- 1 week) while receiving PD0332991.
TUMOR ASSESSMENTS	Tumor response for subjects with measurable lesions will be assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Subjects with measurable lesions should be assessed using computerized tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and approximately every 9 weeks (± 4 days) from date of first dose of PD 0332991 until documented progression.
PHARMACOKINETICS	Blood samples will be obtained at limited time points, and matched with tumor biopsy, biomarker and hematology assessments.
PHARMACODYNAMICS	For all patients, blood specimens for analysis of PD0332991 will be collected: (1) On Cycle 1 Day 14 at pre-dose; (2) On Cycle 1 Day 15 at pre-dose, at 4 hours post-dose and between 7-10 hours post-dose, (2) matched with FLT-PET assessments except at baseline, (3) matched with tumor assessments except at baseline. A number of patients with accessible tumor will have biopsies, and have the tumor biopsy divided, with one aliquot flash frozen, and the other processed for FFPE. Samples to be analyzed for markers of cell cycle inhibition (such as Ki67, and others) and emerging markers of sensitivity to cell cycler inhibition.
STATISTICAL METHODS	The response rate of interest in this trial will be 15% for each of the indications tested. If we observe at least one response in the first fifteen subjects, we will enroll a further 15 subjects, for a total of 30. Observing zero responses in the first 15 subjects would exclude response rates as low as 15% with a 90% one-sided upper confidence bound. If at least three responses are observed in a total of 30 evaluable patients, we will conclude that the drug is active and merits further study; if the true response rate is 15% or greater, three or more responses will be observed with a probability of at least 85%. If the true response rate is only 3%, three or more responses will be observed with a probability of only 6%. In addition to responses of CRs and PRs, disease stabilization will be considered: if the proportion of patients with disease stabilization for 6 months exceeds 20%, we will conclude that the drug may warrant further investigation in that indication. Confidence intervals will be calculated for selected safety and exploratory variables. Adverse event terms will be standardized using the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by system organ class and preferred term. Laboratory parameters will be summarized using mean change in value and/or shift in category since baseline. Pharmacokinetic data from this study may be analyzed using mixed-effect

modeling approaches. The intent of this analysis will be to build upon a basic population pharmacokinetic model for PD 0332991 and to determine the inter-individual and residual variability in population PK parameters of interest. If data permit, PK/PD correlations to investigate any causal relationship between PD 0332991 exposure and efficacy outcomes will be explored.

SUV calculations will be used to assess PET responses. By convention a positive PET response will be taken as a decrease in summed SUV values of $\geq$ 25%. Relationships will be sought between PET responses and CT imaging response, as well as with PK indices in individual patients.

We will target a total accrual (for treatment) of 100-205 patients, depending on the availability of continued funding (the screening is projected to be the most resource-intensive part of this proposal, and our budget assumptions are based on a rate of 15% positivity – we will need to screen about 350 patients. A response rate of interest will be 20% (though clearly a high proportion of patients with stable disease could be considered interesting in some diseases). Taking that as a cut-off, we will treat patients with any one of these diseases until 15 have been accrued. If there have been no responses we will conclude with 95% confidence that the true response rate is less than 20% and no further patients will be screened. If one or more responses are observed, we will continue accrual in that disease type to a total of 30. For the population overall, a response rate of 15% or more, or prolonged (> 6 months) stable disease in 30% or more, would be convincing evidence of drug action in a targeted population, and would prompt additional studies.

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List of Abbreviations	
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AR	accumulation ratio
AST	aspartate aminotransferase
AUC	area under the plasma concentration time curve
$AUC_{0-\infty}$	area under the plasma concentration time curve from time of dosing to infinity
$AUC_{0-\tau}$	area under the plasma concentration time curve from time of dosing to τ, where τ is equal to dosing interval
BUN	blood urea nitrogen
CFR	code of federal regulations
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CL	plasma clearance after IV administration
CL/F	apparent plasma clearance after PO administration
CR	complete response
CRC	cohort review committee
CRO	contract research organization
CRF	case report form
CNS	central nervous system
CT	computerized tomography
CTCAE	common terminology criteria for adverse events
CYP	cytochrome p450
DLT	dose-limiting toxicity
DSMC	data safety monitoring committee
EC	ethics committee
ECG	electrocardiogram
ECOG	eastern cooperative oncology group
ED_{50}	dose required for 50% tumor inhibition
F	absolute bioavailability after PO administration
FDA	food and drug administration
FLT	fms-like tyrosine kinase
GCP	good clinical practice
HR	hazard ratio
ICH	international conference on harmonization
ICON 4	international collaborative ovarian neoplasm 4
INR	international normalized ratio

List of Abbreviations	
IRB	institutional review board
KDR	kinase insert domain receptor
MedDRA	medical dictionary for regulatory activities
MRI	magnetic resonance imaging
MAD	maximum administered dose
MTD	maximum tolerated dose
NOAEL	no observable adverse effect level
PD	Progressive disease
PFS	progression free survival
PK	pharmacokinetic
PR	partial response
PT/PTT	prothrombin time/partial thromboplastin time
RBC	red blood cell
RECIST	response evaluation criteria in solid tumors
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
$t_{1/2}$	terminal half-life
t_{max}	observed time to reach peak plasma concentration
UNL	upper normal limit
WBC	white blood cell

1.0 BACKGROUND AND RATIONALE

1.1 Cell Cycle Dysregulation and Cancer

One of the hallmarks of cancer is cell cycle dysregulation, either through overactivity of the proliferative machinery (as by activating mutations in cyclin/cdk components, gene amplification of regions of the genome that encode them, or overexpression of individual proteins that relate to cell cycle proteins) or through inactivation of the proteins that serve to block the cell cycle when appropriate (such as inactivating mutations in cell cycle inhibitors such as p53 or p16, or of components of the Rb complex), with the consequence that cells adopt a hyperproliferative program (1). A key regulator of the cell cycle, Rb activity is decreased or lost in a variable proportion of common epithelial malignancies (2). The Rb protein is a transcriptional repressor of the cell cycle, which in its unstimulated form binds the promoter region of E2F to shut down the transcription of genes required for DNA replication and mitosis. Phosphorylation of Rb (as initiated by cdk's 4 & 6 in complexes with cyclin D) displaces the protein from the E2F promoter, and permits the progression of the cell cycle through mitosis, when the action of phosphatases restores the hypophosphorylated form and again represses the E2F transcriptional program. The early association of Rb deficiency and retinoblastoma established the importance of the integrity of this pathway in protection from developing cancer, and led to a focus on the cell cycle as a target for cancer control (3-8). Work in recent years has elucidated the multiple interactions of Rb and other cell cycle regulators with cellular processes in addition to proliferation, including apoptosis, senescence, and genomic stability (9). It has become clear that inactivating lesions in many of these proteins, including Rb, may have consequences for cell survival that are cell type- and context-dependent. Therefore studies of agents designed to impact cell cycle regulatory pathways will be most informative if they assess drug effects at multiple levels, and establish in as detailed a manner possible the context in which the treatment is administered (ie, the molecular characteristics of the tumor).

1.2 Cell Cycle Abnormalities in specific cancers

Abnormalities of cell cycle regulation are characteristic of most solid tumors (2,9). Inactivation of p53 is believed to be a characteristic of some 50% of cancers, and the result is loss of the cdk inhibitor p21. In 40 – 60% of tumors p16 function is lost, and in a smaller proportion Rb function is compromised or lost. Interestingly, despite what is therefore a ubiquitous characteristic of the cancer cell, it is usually difficult to establish a clear relationship to prognosis for any of these abnormalities, and this perhaps serves to further emphasize the importance of context (accompanying molecular abnormalities) in determining the malignant behavior of these tumors. There are several examples of cancers in which dysregulation of specific components of the cell cycle can be shown to drive proliferation. With respect to the cyclins and cyclin-dependent kinases, overexpression of cyclin D1 is observed in several tumor types. This is most commonly achieved by gene amplification or transcriptional mechanisms, but in the case of mantle cell lymphoma, a translocation is responsible.

1.2.1 CDK inhibition in Breast Cancer

Overexpression of canonical cyclin D1a is common in breast carcinoma, with up to 50% of primary ductal breast carcinomas overexpressing cyclin D1a in various studies (10,11). Increased expression has been shown to be evident in all histological types of breast cancer and is present in both estrogen receptor positive and estrogen receptor negative malignancies (12,13). Once acquired, cyclin D1a overexpression is maintained through all stages of disease, including metastatic lesions (14). Despite the undisputed

presence of cyclin D1a in breast cancer, data thus far have produced conflicting results regarding the biological and clinical significance of cyclin D1a overexpression. Cyclin D1a overexpression alone has not been shown to induce malignant transformation in cell lines and mouse models, but nuclear cyclin D1 alleles have exhibited increased oncogenic potential. (15).

A polymorphism in the *CCND1* gene, G870A, results in an aberrantly spliced protein ("cyclin D1b") lacking the Thr-286 phosphorylation site necessary for nuclear export. Studies of murine fibroblasts have shown that although overexpression of canonical cyclin D1 ("cyclin D1a") alone is not sufficient to drive malignant transformation, expression of nuclear cyclin D1b is oncogenic. We recently reported the results of an analysis of cyclin D1a and D1b splice variant expression in a cohort of 118 patients with invasive breast cancer (Gupta, submitted). Cyclin D1b was expressed in 26% of tumors and cyclin D1a was overexpressed in 27%; co-expression occurred in 4%. Cyclin D1a and/or D1b expression were not significantly associated with ER/PR negativity, Her2 overexpression, young age, lymph node positivity, high tumor grade, or large tumor size. With a median follow-up of 44 months, the risk of recurrence was higher in those co-expressing D1a and D1b compared to either alone (RR=5.3, 95% CI 1.27 to 22.1, p = 0.02). The hazard ratio for those with co-expression compared with those without was 6.05 (p = 0.04). Thus, the presence of the splice variant in D1b in addition to overall cyclin D1 overexpression characterizes a breast cancer population at high risk of recurrence, who may be particularly suited to CDK 4/6 inhibition. The expression of cyclin D1b, and the presence of the mutation that prevents its nuclear export, will be assessed retrospectively in breast cancer samples.

1.2.1.1 CDK inhibition in HER2-Overexpressing Breast Cancer

Preclinical and unpublished work by Eric Knudson demonstrated efficacy of PD 0332991 in HER2 positive breast cancer cell lines as well as in mouse xenograft models (personal communication). Furthermore, in tumor models that have failed multiple HER2-directed therapies, PD 0332991 proved to be an effective anti-neoplastic (personal communications). This suggests that the Rb/CDK4/6 pathway may be instrumental in resistance to HER2 directed therapy. Studies in metastatic breast cancer have shown that dual HER2-directed targeted therapies have superior efficacy to trastuzumab alone, and that the addition of another targeted agent to trastuzumab in the setting of trastuzumab resistance can restore sensitivity to this therapy. However, there are currently no data on the comparative efficacy of PD0332991 alone vs. PD0332991 with concurrent trastuzumab. Toxicities of these agents are non-overlapping, suggesting an ability to combine these agents safely in patients with HER2-overexpressing disease.

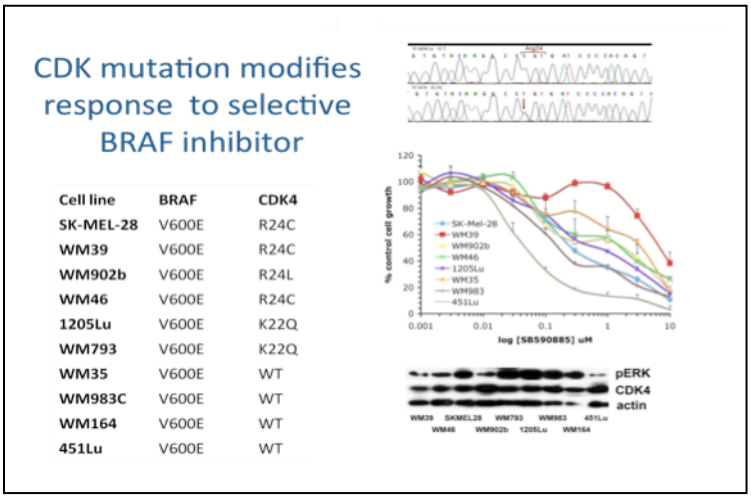
1.2.2 Colon Cancer

Abnormalities of the Rb family (pRB, pRB2/p130, p107) have been identified in a large proportion of colon cancers, and are implicated in tumor pathogenesis and progression (16,17). Some colon adenocarcinomas undergo allelic loss at the Rb locus (18,19), whereas almost half of colorectal carcinomas show nonrandom chromosomal 13 gains (20,21). The loss of Rb and CDK inhibitor p16(INK4a) correlates with the poor outcome in colon cancers (22,23). Approximately two thirds of intestinal tumors paradoxically have increased levels of Rb expression and protein production when compared with paired normal colonic tissue (24), and a mechanism through which Rb may play an antiapoptotic role through an interaction with BAG-1 has been proposed (25). In murine models, APC mutant mice engineered to have Rb deletion in the intestine demonstrate accelerated tumor formation especially in the large bowel (26). Thus substantial dysregulation of the Rb-cyclin axis exists in colon cancers. Specific abnormalities of cdk's and cyclins are also implicated in colon cancer progression. In MSI tumors harboring TGF-β receptor-II mutations, cdk4 expression is markedly increased and may even

provide a marker for drug effect (27). More recently, miRNA-mediated cdk6 dysregulation has been demonstrated in colon cancer models, but the incidence of this change in clinical samples has not yet been reported (28). Based on these findings, it is appropriate to accrue an unselected patient population in this colon cancer trial, with a view to a retrospective biological analysis of responders.

1.2.3 Melanoma

Mutations that underlie activation of the MAP kinase and PI3 kinase pathways and inactivate the p53/Rb pathway are the defining features of melanoma (29-31). The vast majority of melanomas harbor activating mutations in c-kit (3%), NRAS (15%), or BRAF (60%). The PI3 kinase pathway is activated through loss of PTEN (30%) or amplification of Akt3 (20%) in a large proportion of BRAF mutant melanomas. Overlying those alterations, p16 is very commonly mutated or deleted, allowing CDK4 to signal in an unopposed fashion. Less commonly cyclin D amplification has been described in melanoma. In cases where p16 is intact and cyclin D is not amplified, CDK4 harbors activating mutations allelic amplification driving overexpression. We have found that CDK4 mutations mediate resistance to BRAF inhibition, when they are present in conjunction with BRAF mutation in melanoma [Figure]. Therefore, CDK4 may be a superior target to BRAF in these instances. In our large panel of melanoma cell lines, approximately 8% harbor activating mutations in CDK4, most commonly the R24C and K22Q substitutions. In addition, we have identified a subset of melanomas which harbor high level amplification of CDK4 in conjunction with amplification of c-kit. Here, again, we believe that CDK4 inhibition is a rational approach. For the past several years, we have been prospectively evaluating patients' melanomas for the presence of BRAF and c-kit mutations for inclusion into clinical trials with relevant signal transduction inhibitors. This screening process can be easily modified to include CDK4 mutation or amplification as a prospective evaluation. In this way, we can identify patients with metastatic melanoma, who appear to be clinical trial candidates and match them with the most relevant targeted therapy in clinical trials. Enriching for patients with CDK4 mutations or amplification will enrich the population for patients with melanoma who may be most response to single-agent therapy. If this proof-of-concept is established, it would appropriate to further develop the agent for these patients while also exploring the role of the agent in combination with other signaling inhibitors in the broader melanoma population as CDK4 signaling appears to be aberrant in the vast majority of melanomas through



1.2.4 Germ cell Tumors

Deregulation of the retinoblastoma tumor suppressor (pRB) pathway in germ cell tumors (GCT) has been well documented (32). For example, embryonal carcinomas demonstrate little or no pRB expression. However, mature teratomas which are cisplatin- refractory strongly express pRB, suggesting an intact G1/S checkpoint. The extent of pRB expression in other cisplatin-refractory GCT has not been reported. Treatment of teratomas and other pRB-positive cisplatin-refractory GCT with CDK 4/6 inhibitor such as PD 0332991 is a rational approach.

We previously reported on the clinical course of 3 male patients aged 22 to 37 years with growing teratoma syndrome (GTS) treated on a phase I trial of oral daily PD 0332991 in refractory solid tumors (NCT00141297) (33). These patients initially presented with metastatic non-seminomatous GCT. They received cisplatin-based chemotherapy followed by two or more post-chemotherapy surgeries for GTS. All developed progressive disease not amenable to further surgery. These teratomas demonstrated strong nuclear expression of pRB in the epithelial component. The first patient received PD 0332991 150 mg daily for 21 days on an every 28 day cycle. The dose was decreased to 100 mg secondary to neutropenia. He demonstrated stable disease for 18 months. The second patient received treatment with PD 0332991 200 mg daily for 14 days on an every 21 day cycle. The dose was decreased to 150 mg because of headaches and vomiting. He also experienced neutropenia. He has demonstrated a partial response by Response Evaluation Criteria in Solid Tumors (RECIST) for 22 months and continues on treatment. The third patient was treated with PD 0332991 125 mg daily for 21 days on an every 28 day cycle. He has experienced neutropenia. He has demonstrated stable disease for 24 months and continues on treatment. This report is the largest published series of patients with GTS treated with a novel agent and, remarkably, all patients derived clinical benefit. We plan to expand our experience by treating additional patients with GTS and other pRB positive cisplatin-refractory GCT with PD 0332991 as part of this phase II study.

1.2.5 Esophageal and/or Gastric Cancer

The role of MAP kinase pathways in mediating multiple cell responses, including proliferation, is well-recognized (Wagner EF, Nebreda AR. Signal integration by the JNK and p38 pathways in cancer development. Nature Rev Cancer 9: 537-549, 2009). A recent publication has focused on signaling through the JNK pathway as a mediator of gastric cancer proliferation (Hayakawa Y, Hirata Y, Nakagawa H, et al. Apoptosis signal-regulating kinase 1 and cyclin D1 compose a positive feedback loop contributing toi tumor growth in gastric cancer. Proc Natl Acad Sci USA 108: 780-5, 2011). Hayakawa and colleagues found increased expression of ASK1 (a MAPKKK) in human gastric cancer specimens, and in 23/26 gastric cancer cell lines. Functional relevance oif this finding was suggested by the finding of activation of the pathway (elevated p-c-Jun), and cyclin D1, and confirmed through knockdown experiments in which ASK1 siRNA inhibited gastric cancer cell growth, while exogenous expression induced cell growth through a positive feedback loop involving cyclin D1. These findings raise the possibility that interrupting this growth-stimulatory loop could arrest the growth of gastric cancer in humans, a possibility to be tested in a pilot fashion in this trial.

1.3 PD 0332991 – an inhibitor of cyclin-dependent kinases 4 & 6

Because increased proliferation is a hallmark of most cancers, agents that specifically inhibit proliferation may have a role in the clinical management of cancer. One important target is the cell division cycle, which is controlled by the sequential activation of a family of related cyclin-dependent kinases (CDKs). CDK4 and CDK6 (CDK4/6) are closely related kinases that promote cell cycle progression from G_1 to S phase. This is a key regulatory step in the cell division cycle and is followed by commitment to cell division (G_2 followed by M phase)..

The only known natural substrate for CDK4/6 kinase activity is the retinoblastoma (Rb) protein. In a *minority* of tumors (eg, retinoblastoma, small-cell lung cancer), control of G_1/S progression is lost by Rb gene mutation. In most common tumors, however, other genetic and epigenetic changes lead to increases in CDK4/6 activity and uncontrolled tumor cell growth. Thus, tumor cell proliferation may be particularly sensitive to a selective inhibitor of CDK4/6.

The small molecule kinase inhibitor PD 0332991 is a potent inhibitor of both of these cdk's with in vitro

inhibitory constants in the nanomolar range (ref). The compound prevents cellular DNA synthesis by prohibiting movement from the G_1 phase of the cell cycle into the S phase, as demonstrated in laboratory models. PD 0332991 preclinical data indicate that it may be expected to have direct cytotoxic activity as well as the potential for growth arrest. Arrest in the G_1 phase is known to cause cell death in some tumor types, as demonstrated by this and other G_1 arresting agents (eg, Tamoxifen). Moreover, CDK inhibitors like PD 0332991 have the potential to enhance the effects of conventional therapies.

1.4 Preclinical Efficacy

Of the 16 phosphorylation sites on Rb, two are known to be specifically phosphorylated by CDK4/6;serine-780 and serine-795. The phosphorylation status of Rb at these specific sites in treated tumors provides an appropriate biomarker for target modulation that would give assurance that PD 0332991 is inhibiting its intended target. The IC_{50} values for reduction of Rb phosphorylation at serine-780 and serine-795 in MDA-MB-435 breast carcinoma were 0.066 μM and 0.063 μM, respectively. Similar effects on serine-780 and serine-795 phosphorylation were obtained in the Colo-205 colon carcinoma.
Time-course experiments indicate that the reduction of Rb phosphorylation begins to occur as quickly as four hours after exposure to PD 0332991 and reaches a maximum at 16 hours with continued exposure. The inhibition is completely reversible. After removal of the drug, phosphorylation on serine-780 and serine-795 begins to return in 2 hours and is complete within 16 hours. Active cellular proliferation returns concurrently with Rb phosphorylation. While this suggests that PD 0332991 will be most efficacious using continuous daily dosing, intermittent dosing using a variety of schedules has proven as efficacious as continuous daily dosing in animal xenograft models. Tumor regrowth is observed following discontinuation of therapy. This provides the basis for chronic intermittent dosing, which allows the integration of other agents as well as a period for recovery from anticipated toxicities, e.g. myelosuppression.

To determine whether tumors that regressed following discontinuation of PD 0332991 remained sensitive to further PD 0332991 treatment or whether they had acquired resistance, Colo-205 colon tumors that regressed post-PD 0332991 treatment were harvested and re-implanted into naïve mice. Following PD 0332991 treatment at a dose and schedule identical to the original experiment, the tumors responded with equal sensitivity to the drug and fully regressed, indicating that no resistance had developed during the initial treatment.

PD 0332991 exhibits significant antitumor efficacy, including tumor regressions, against multiple human tumor xenograft models in nude mice. PD 0332991 has produced striking tumor regressions in some models and tumor growth delay in others. In the MDA-MB-435 breast carcinoma model, the two dose levels that resulted in ≥50% tumor growth inhibition suppressed phosphorylation on serine-780 over the full 24-hour prior between doses. At two lower doses, which did not produce ≥50% tumor growth inhibition, phosphorylation returned over the 24-hour prior before the next dose. Similar results were observed with the Colo-205 colon carcinoma model.

In MDA-MB-435 breast carcinoma cells exposed for 24 hours to varying concentrations of PD 0332991, the percentage of cells in G_1 began to increase in the presence of as little as 0.04 μM PD 0332991 with a concomitant decrease in other phases of the cell cycle. Maximum effects were attained at 0.08 μM.
These data indicate that the three biological parameters that are expected for a CDK4/6 inhibitor, growth inhibition, Rb-dephosphorylation and G_1 arrest, all occur in drug-treated cells at comparable

concentrations of PD 0332991. This correlation suggests that these biochemical readouts are related and a result of the same mechanism of action (i.e., specific inhibition of CDK4/6).

1.5 Lack of Efficacy Against Rb-Negative Tumors

To ensure that PD 0332991 acts exclusively by inhibition of Rb phosphorylation, PD 0332991 was tested on Rb-negative tumor cells, the MDA-MB-468 human breast carcinoma and the H2009 human non-small cell lung carcinoma, both of which have deleted Rb. PD 0332991 had no antiproliferative activity on these cells at the highest concentration tested (3 μM), which is 1-2 orders of magnitude higher than the concentration necessary to inhibit Rb-positive tumor cells. Further evidence that the anti-tumor activity observed for Rb-positive tumors is due to inhibition of CDK4/6 was obtained by testing PD 0332991 in Rb-negative human tumor xenograft models, the MDA-MB-468 human breast carcinoma and the DU-145 human prostate tumor. Neither tumor responded to PD 0332991. These findings indicate that PD 0332991 is not expected to have an anti-tumor effect in tumors that do not express Rb, and that only patients with tumor types that express Rb should be included in a clinical trial of PD 0332991.

1.6 Preclinical Toxicity Data

PD 0332991 has been examined in various genetic toxicology and safety pharmacology (cardiovascular, neurofunctional, and pulmonary) studies. General toxicology (acute, oral-escalating dose, and 2-week dose range-finding) studies were conducted in rats and dogs. Pivotal toxicology studies were conducted in both species with PD 0332991 administered once by gavage daily for 3-weeks, followed by a 1-month reversal period.

The primary PD 0332991 toxicities in preclinical studies are to the bone marrow, lymphoid tissues, and testes. These toxicities occurred in both rats and dogs, and are consistent with cell cycle inhibition produced by the intended pharmacology of the drug. Pancytic bone marrow depletion resulted in decreases in various hematology parameters; however, the changes were reversible following cessation of dosing. Reversible myelosuppression is anticipated to occur in the clinic and to be dose-limiting. PD 0332991 demonstrated a potential for clastogenicity in the in vitro and in vivo micronucleus assays. Acute intravenous administration of PD 0332991 to dogs resulted in significant pulmonary effects, including apnea, which were reversible. Effects were transient, appeared related to peak plasma concentrations (≥2040 ng/mL), and consistent with centrally-mediated respiratory depression. No changes in pulmonary function occurred at plasma drug concentrations ≤414 ng/mL. Pulmonary changes were observed in rats administered PD 0332991 orally, which included rales, dyspnea, and atrophy of tracheal epithelium. The clinical relevance of these effects is unknown. Results of the Purkinje fiber and HERG in vitro assays, and cardiovascular study in dogs have indicated a potential for prolongation of the QT interval.

1.7 Phase I clinical trial

There are currently 4 ongoing trials. One study (A5481001) has been initiated to evaluate the safety of PD 0332991 in patients with advanced cancer. Enrollment for this study (Protocol A5481001) has been completed with 74 enrolled patients and 5 active patients. This study is evaluating two different dosing schedules of PD 0332991. The first dosing regimen to be evaluated was a 3/1 schedule (3 weeks on treatment/1 week off treatment) and started with an initial cohort receiving 25 mg of PD 0332991 administered orally once daily. The dose was escalated using a new cohort for each dose level to a dose

of 150 mg once daily. At this dose, 2/6 patients experienced DLTs related to myelosuppression. The dose was reduced to 125 mg once daily for the next cohort. This dose has been well tolerated and was considered the RP2D. As per protocol, a second dosing regimen, Schedule 2/1 (2 weeks on treatment/1 week off treatment) was also evaluated, beginning when there was 1 DLT in the Schedule 3/1. The initial dose level for Schedule 2/1 was 100 mg administered orally once daily which was escalated using a new cohort for each dose level to 225 mg once daily. At this dose level, 2/3 patients experienced myelosuppression-related DLTs and the dose was reduced to 150 mg once daily for the next cohort. This dose has been well tolerated and was considered the RP2D for this schedule. The most common adverse events other than those related to myelosuppression included fatigue, nausea, constipation, diarrhea, emesis and dyspnea; all of which had CTC Grade of 2 or less. For Schedule 3/1, 11/41 patients exhibited stable disease for ≥ 16 weeks including 7 patients exhibiting stable disease for ≥48 weeks in a variety of tumor types.

A second ongoing study (A5481002) is evaluating PET imaging modalities, target inhibition, clinical activity and safety of PD 0332991 in patients with previously treated mantle cell lymphoma. PD 0332991 is being administered on the Schedule 3/1 at an oral dose of 125 mg once daily**.** At this time, enrollment has been completed and 17 patients have entered the study but no efficacy data are available. So far, the safety profile has been similar to that observed in the dose escalation study.

A third study (A5481004) is a Phase 1/2 evaluation of PD 0332991 in combination with bortezomib and dexamethasone in patients with refractory multiple myeloma. Currently the Phase 1 portion of the study in ongoing and evaluating Schedule 3/1 with 6 patients dosed. Five of these patients have discontinued from the study due to progressive disease. The first cohort evaluated 100 gm PD 0332991, 1 mg/m^2 bortezomib and 20 mg dexamethasone. There were 2 DLT's due to Grade 3/4 thrombocytopenia in this cohort. Thus, this cohort was closed and the second cohort (75 mg PD 0332991, 1 mg/m^2 bortezomib and 20 mg dexamethasone) was opened.. Four patients have been dosed in this cohort with one patient having a DLT (Grade 3 thrombocytopenia). Enrollment into this cohort is currently ongoing.

A fourth study (A5481003) is a Phase 1/2 evaluation of PD 0332991 in combination with letrozole in the first-line treatment of ER+ and HER2- advanced breast cancer in postmenopausal women. The Phase 1 portion of the study is ongoing with 3 patients currently enrolled.

1.8 Pharmacokinetics

Pharmacokinetic data from the first in patient study (A5481001) indicated that PD 0332991 median time to peak concentration was approximately 4 hours after dosing with the exposure increasing in a generally dose proportional manner. The mean PD 0332991 volume of distribution was 3103 L, which is significantly greater than total body water (42 L), indicating that PD 0332991 extensively penetrates into peripheral tissues. PD 0332991 was eliminated slowly; the mean terminal half-life was 26.5 hours and the mean apparent oral clearance was 86.1 L/hr. PD 0332991 accumulated following repeated dosing with a median accumulation ratio of 2.4, which is consistent with a half-life of approximately 27 hours. Renal excretion was a minor route of elimination for PD 0332991 (1.7% of unchanged drug in urine). There was no clinically relevant effect of food on the PK of PD 0332991, indicating that PD 0332991 can be administered without regard to meals.

1.9 Pharmacodynamics

The phosphorylation status of Rb is the most direct way to measure CDK4/6 inhibition by PD 0332991. By blocking Rb phosphorylation via CDK4/6 inhibition, PD 0332991 activates Rb to suppress tumor

growth by repressing expression of genes required for proliferation. Changes in Rb phosphorylation will be monitored in clinical samples by immunohistochemistry (ICH) using antibodies specific for certain phospho-epitopes of Rb, such as phospho-Ser780 Rb, that are sensitive to CDK4/6 inhibition. To investigate biomarkers associated with efficacy in preclinical models, clinical samples will be assayed for Ki-67, a widely used marker of cell proliferation, and for the expression of certain proliferation-associated genes.

Genetic alterations in the Cyclin D1/p16/Rb/Cdk4/6 pathway that lead to increased Rb phosphorylation are among the most common in human cancer. Cyclin D1 complexes with and activates CDK4/6, facilitating movement through the cell cycle, and its expression is frequently unregulated due to gene amplification, chromosomal translocation, or oncogenic signaling. p16 is a tumor suppressor protein that normally inhibits CDK4/6 activity; its expression is frequently repressed in tumors, often by epigenetic mechanisms. The net effect is to increase CDK4/6 activity leading to Rb inactivation. Expression levels of Cyclin D1 and p16 will be assessed in clinical samples in this study using immunohistochemistry.

Genetic polymorphisms in K-*ras* and cyclin D1 are relatively common oncogenic alterations in human cancer. Preclinical data suggests a role for K-*ras* genetic polymorphisms in altering sensitivity to PD 0332991. The relation of Cyclin D1 polymorphism to PD 0332991 is under investigation, but is known to affect risk and outcome of several cancers. Genetic polymorphism in K- *ras*, cyclin D1, and Rb will be monitored in clinical tumor samples.

Amplification of CCND1 and PD0332991

Work at Pfizer has shown that breast cancer cell lines with amplified *CCND1*, the gene encoding cyclin D1, are especially sensitive to the drug. Further, broadening the focus of investigation, they have shown that sensitivity in these models may also depend on the presence of mutated or inactivated p16, a negative regulator of the cell cycle. And independent of these specific findings, gene expression profiling has identified a greater level of sensitivity for the luminal A breast cancer profile, suggesting that additional determinants of susceptibility can be identified. Shapiro in a review of this field distinguished key consequences of cell cycle inhibition: growth arrest and cessation of proliferation that could resume upon removal of the inhibitor, versus induction of cell death pathways and tumor shrinkage in certain models (34). While either effect could be construed as beneficial in cancer treatment, the latter is likely to be more demonstrably effective in advanced cancer, and is a reasonable focus for future trials. The data generated in this work will provide a basis for the interpretation of findings from the normal and tumor tissues of patients treated in these trials.

Data from studies at Pfizer have implicated *CCND1* amplification as a major association with sensitivity to PD0332991. Further, a recent study of Beroukhim and colleagues (35) analyzed 3,131 cancer specimens, belonging largely to 26 histological types, and identified 158 regions of focal copy number alteration that were altered at significant frequency across several cancer types. The gene that was the second-most frequently amplified was *CCND1*, and this particularly in esophageal and head and neck malignancies, liposarcomas and breast tumors. Together these findings prompt the addition of a study in a cohort particularly likely to respond on biological grounds.

1.10 Study Rationale

We have designed a Phase II trial enriched for potentially responding patients on either molecular or clinical grounds. We include patients with breast cancer, esophageal, gastric cancer and colorectal cancer, in whom the pathway can be activated by several mechanisms, and resistant germ cell tumors. The

inclusion of a resistant germ cell cancer population is based on our observations of significant activity in this population in the Phase I trial (33). Responses or significant proportion of patients with stable disease in these groups will prompt a more definitive confirmatory Phase II trial. In addition, these populations are likely to have tumors that take up 18F-fluorothymidine at baseline, which can then serve as a marker of cell cycle inhibition, and permit associations with clinical outcome, with PK, and allow the determination of pharmacodynamic variability. Positive PD findings could potentially guide the future development of this promising agent. In addition, given strong preclinical evidence to suggest the importance of the Rb pathway in HER2-overexpressing cancer cells as well as its potential role in secondary resistance mechanisms to HER2-directed therapy, subjects with HER2-overexpressing tumors may benefit from receiving trastuzumab in combination with PD 0332991. Thus additional patients with HER2-overexpressing tumors will be allowed to receive trastuzumab concurrently with PD0332991 in order to obtain preliminary safety data and efficacy of the combination.

2. STUDY OBJECTIVES

The primary objectives of this study are:

- To determine the response rates following treatment with PD 0332991 in the following malignancies:

  1 Metastatic breast cancer

  2 Metastatic colorectal cancer that harbors the Kras or BRAF mutation

  3.Advanced or metastatic esophageal and/or gastric cancer

  4 Cisplatin-refractory, unresectable germ cell tumors

  5. Any tumor type if tissue tests positive for *CCND1* amplification, *CDK4/6* mutation , *CCND2* amplification OR any other functional alteration at the G1/S checkpoint.

- To evaluate the safety and tolerability of PD 0332991, administered to subjects with refractory solid tumors

The secondary objectives of this study are:

- To assess the pharmacodynamic effects of PD 0332991 on tumor and non-tumor tissue
- To investigate the relationship between selected biomarkers, PK and/or efficacy and safety outcomes.
- To estimate the population pharmacokinetics for PD 0332991 and to correlate PK with efficacy outcomes.
- To evaluate the preliminary safety of PD 0332991 in combination with trastuzumab in patients with HER-2 overexpressing tumors within the 5 cohorts under study.
- To perform a Phase II evaluation of PD0332991 in a population defined as potential responders on the basis of *CCND1* gene amplification.

3.0 STUDY DESIGN

3.1 Overview of Study Design

This is a Phase II, open-label study evaluating the response rates, time to disease progression, safety, pharmacokinetics, and pharmacodynamics of PD 0332991 in subjects with selected solid tumors. Up to 30 patients in most cohorts will be accrued to the trial to determine the response rate. The cohorts are defined by disease type or *CCND1* amplification status. The ER-positive breast cohort will enroll up to 45 patients. In addition, 10 patients with HER2-positive cancer will receive PD 0332991 in combination with trastuzumab. An early stopping rule for lack of activity will be activated if there are no responses and no 6-month stable disease patients among the first 15 patients. The estimated length of the study is 1200 months for subject accrual and treatment.

A cycle of treatment is defined as 28 days. Subjects will take PD 0332991 daily for 21 days, with cycles repeating every 28 days.

4.0 ELIGIBILTY CRITERIA

4.1 INCLUSION CRITERIA:

1. Disease Characteristics:

All Subjects: All subjects treated under this protocol will have histologically documented cancer of one of the following types:

A. Metastatic breast cancer (7 triple negative, 23 ER+ after the first 15 patients are enrolled on the non-CCND1cohort; in addition 10 HER2+ for combination trastuzumab and PD0332991 therapy) up to 55 total enrollment slots

B. Metastatic colorectal cancer that harbors the Kras or BRAF mutation (15-30 enrollment slots)

C. Advanced or metastatic esophageal and/or gastric cancer (15-30 enrollment slots)

D. Cisplatin-refractory, unresectable germ cell tumors (15-30 enrollment slots)

E. Any tumor type if tissue tests positive for *CCND1* amplification, *CDK4/6* mutation, *CCND2* amplification OR any other functional alteration at the G1/S checkpoint. (15-30 enrollment slots)

2. Biopsy Requirements:

For Subjects with accessible disease amenable to biopsy: A biopsy will be obtained pre-treatment and in during cycle 1 (while patient is receiving drug) for molecular markers of the cell cycle, and its inhibition.

For all subjects:

3. Subjects will be $\geq$ 18 years old
4. The subject has disease that is assessable by tumor marker, physical, or radiologic means.
5. The subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

6. The subject has adequate organ function, defined as follows
   A. Bilirubin ≤ 1.5 x the upper limit of normal (ULN)
   B. Serum creatinine ≤ 1.5 x UNL or calculated creatinine clearance ≥ 60 mL/min, and
   C. For subjects without liver metastases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN
   D. For subjects with liver metastases: alanine aminotransferase (ALT) and aspartate aminotransferase ≤ 5 x ULN
7. All tumors must test positive for Rb expression except:
   A. ER positive metastatic breast tumors (data now shows all to be Rb positive.)
   B. Any tumor type if tissue tests positive for *CCND1* amplification, *CDK4/6* mutation, *CCND2* amplification OR any other functional alteration at the G1/S checkpoint.
8. The subject has adequate marrow function, defined as follows:
   A. Absolute neutrophil count (ANC) $\geq$1500/mm^3
   B. Platelets $\geq$100,000/mm^3, and
   C. Hemoglobin > 9 g/dL
9. The subject is capable of understanding and complying with the protocol requirements and has signed the informed consent document.
10. Sexually active subjects (male and female) must use accepted methods of contraception during the course of the study and for 3 months after the last dose of protocol drug(s).
11. Female subjects of childbearing potential must have a negative pregnancy test at screening. Females of childbearing potential are defined as sexually mature women without prior hysterectomy or who have had any evidence of menses in the past 12 months.
12. However, women who have been amenorrheic for 12 or more months are still considered to be of childbearing potential if the amenorrhea is possibly due to prior chemotherapy, antiestrogens, or ovarian suppression.

4.2 EXCLUSION CRITERIA

1. The subject has received cytotoxic chemotherapy (including investigational cytotoxic chemotherapy) within 3 weeks (or nitrosoureas or mitomycin C within 6 weeks) before the first dose of PD 0332991. . Patients with HER2-overexpressing tumors may receive trastuzumab up to the date of starting therapy, and may continue to receive trastuzumab while receiving PD0332991.
2. The subject has received any other type of investigational agent within 28 days before the first dose of study treatment.
3. The subject has not recovered from clinically-meaningful toxicity due to prior therapy (i.e., back to baseline or Grade ≤ 1), with the exception of neurotoxicity and alopecia.
4. The subject has untreated or uncontrolled brain metastases or evidence of leptomeningeal involvement of disease unless the subject has a teratoma in which case s/he may be eligible if all other eligibility criteria are met
5. The subject has uncontrolled intercurrent illness including, but not limited to:
   a. ongoing or active infection
   b. diabetes mellitus
   c. hypertension
   d. symptomatic congestive heart failure, unstable angina pectoris, stroke or myocardial infarction within 3 months

6. The subject has a baseline corrected QT interval (QTc) > 470 ms.

7. The subject is pregnant or breastfeeding.

8. The subject is known to be positive for the human immunodeficiency virus (HIV). ***Note:*** baseline HIV screening is not required

9. The subject is unable or unwilling to abide by the study protocol or cooperate fully with the investigator or designee.

4.3 Concomitant Medication and Treatment

Concomitant medications will be recorded on the CRF.

Subjects with HER-2 positive tumors will be allowed to receive concominant trastuzumab at the discretion of the treating physician. Trastuzumab will be given per standard of care protocol. If the subject is not currently receiving trastuzumab, a loading dose of 8mg/kg will be given intravenously on the first day of treatment and continued at 6mg/kg intravenously once every 3 weeks during the study period.

4.4 Tumor Biopsies

An archival tumor specimen (or a biopsy specifically to obtain tissue to establish eligibility) is required, and will be forwarded for testing as specified in section 6.1. A number of patients with accessible tumor will have biopsies, and have the tumor biopsy divided, with one aliquot flash frozen, and the other processed for FFPE. Samples will be analyzed for markers of cell cycle inhibition (such as Ki67, and others) and emerging markers of sensitivity to cell cycle inhibition. All patients with esophageal and/or gastric cancer will have tissue tested for phosphor-protein analysis, a putative marker of activated ASK1, JNK1 and 2, and c-Jun.

4.5 Anticancer Treatment, Including Radiotherapy

If a subject requires additional anticancer treatment, he or she must be withdrawn from the study (with the exception of trastuzumab for HER-2 positive tumors, palliative radiotherapy or other local therapy, which may be allowed during the study with the approval of the sponsor (Dr. O'Dwyer and the medical monitor). If anticancer treatment follows progression due to clinical deterioration determined by the investigator, the basis for this determination should be documented.

4.6 Other Medications

Subjects who develop nausea, vomiting, or diarrhea should be treated as clinically indicated. For subjects who have required treatment on study for nausea, vomiting, or diarrhea, prophylactic anti-emetic and anti-diarrheal medication may be used as per standard clinical practice before subsequent doses of study drugs, at the discretion of the investigator.

Pain medications administered as dictated by standard practice are acceptable while the subject is enrolled in the study. Colony-stimulating factors should not be administered prophylactically or therapeutically: myelosuppression should be managed by dose reduction as specified below..

No concurrent investigational agents will be permitted. No concurrent anticancer treatment will be permitted.

High dose (>60mg/day) or chronic (> 3months) steroid use is not allowed during the course of this study as it may interfere with metabolism of the study drug. Subjects who require anticoagulation (eg, warfarin, heparin compounds) while on study treatment must be monitored very closely; including frequent PT/INR and PTT testing.

5.0 STUDY TREATMENTS

5.1. Allocation to Treatment
This is a single-center study and a centralized registration system will be used. Patients will be enrolled into the Phase 2 study sequentially according to their disease type.

5.2. Trial Period
Patients will receive doses of PD0332991 on an outpatient basis. Clinic visits, hematology and other safety laboratory tests will be performed according to the Schedule of Activities. Additional assessments may be performed as necessary to evaluate specific adverse events until they resolve to baseline or CTC Grade ≤1. Additional clinic visits may be required at the discretion of the investigator.

5.3. Patient Dosing:
A cycle of therapy is 28 days duration. The starting dose of PD0332991 will be 125 mg. PD0332991 will be administered by mouth once daily for 21 days, followed by 7 days off drug. Repeated cycles of PD0332991 will be administered until patients develop progressive disease or unacceptable toxicity, whichever occurs first. Study treatment may also be discontinued for protocol non-compliance or administrative reasons detailed elsewhere in this protocol.

5.3.1. Patient Dose Modifications
PD0332991 should not be administered to patients with evidence of significant renal or hepatic impairment.
For Grade 3 and 4 toxicities, treatment with PD0332991 should be withheld until the toxicity resolves to Grade 1 or less, then reinstituted, if medically appropriate, per Sections 5.3.1.1 and Section 5.3.2.2. PD0332991 doses will not be made up at a later date. There has been extensive clinical trial experience with PD0332991 and has been approved for several indications, but clinical cirumstances vary considerably and cannot be prediected. So as to serve the patients' needs best (balancing toxicity and benefit) there may be occasions in which the treating physician takes an approach different to that outlined, as in clinical practice.

5.3.1.1. Dose Modification of PD 0332991 for Hematologic Toxicity
Decisions regarding dose reduction for myelosuppression will be made based on blood counts performed weekly during the initial two cycles of treatment, and every 28 days at the beginning of a cycle thereafter. For myelosuppression requiring drug dosing to be held as specified below, the drug will be held until counts recover to acceptable levels (see below). Doses of PD0332991 that are missed will not be made

up, and upon resumption of dosing, the cycle will stay on schedule. Counts will be performed twice weekly during a period of dose omission, and treatment may resume when the neutrophil count (or platelet count) has returned to an acceptable level as defined below. Doses will be modified according to the following table:

Within-Cycle Dose Modifications for Hematologic Toxicity

Neutrophils (per cubic mm)		Platelets (per cubic mm)	Action
≥ 1000	AND	> 50,000	Continue full dose
500-999	OR	25,000 to 49,900	Decrease PD0332991 dose to 100 mg after counts recover to Grade 2 or better
<500	OR	< 25,000	Decrease PD0332991 dose to 75 mg after counts recover to Grade 2 or better

This strategy is implemented with the recognition that exposure variability to this oral agent may be substantial, and that the area under the concentration-time curve is an excellent predictor of myelosuppression Hence what might look like a very low dose for one patient, may in fact reflect significant pharmacodynamic effect.

A new cycle of therapy will not commence until counts have recovered as outlined below:

Dose Modifications at the Start of a New Cycle Following Hematologic Toxicity

Neutrophils (per cubic mm) nadir previous cycle		Platelets (per cubic mm) nadir previous cycle	Action
≥ 1000	AND	> 50,000	Continue full dose
500-999	OR	25,000 to 49,900	Decrease PD0332991 dose to 100 mg daily
<500	OR	< 25,000	Decrease PD0332991 dose to 75 mg daily

Patients reduced to 100 mg whose nadirs are grade 2 or better in the subsequent cycle may be escalated to 125 mg, and will be monitored with weekly counts for at least two cycles to ensure its tolerability.

5.3.2.2. Dose Modifications of PD 0332991 for Non-Hematologic Toxicity

In general, for Grade 3 or 4 toxicities, attributable to PD0332991, treatment should be withheld until the toxicity resolves to ≤ Grade 2, then reinstituted, if medically appropriate, at a 50mg dose reduction. If treatment is withheld for longer than 3 weeks, discontinuation of study treatment should be considered.

Dose Limiting Non-hematologic Toxicity	Action
Any grade 3 or 4 non-hematologic toxicity	Hold till resolution to Grade 2 or less, then restart PD0332991 at a 50 mg dose reduction
Any Grade 2 toxicity, which in the opinion of the investigator, and after consultation with the Sponsor, is considered dose limiting	Hold till resolution to Grade 1 or better, then restart PD0332991 at a 25 mg dose reduction
Recurrent Grade 3 or 4 non-hematologic toxicity	Hold till resolution to Grade 2 or less, then restart PD0332991 at a 50 mg dose reduction
Inability for a patient to recover to < Grade 2 or baseline within 1 cycle, or miss more than 1 cycle of treatment	Consider discontinuation of study treatment

5.3.2.3 Dose Modification of trastuzumab and PD 0332991 for Cardiac Toxicity in patients receiving concurrent PD 0332991 and trastuzumab.

Per standard of care, patients receiving trastuzumab and PD 0332991 will be monitored every 3 months with an echocardiogram or MUGA.

a. **If the ejection fraction drops by >10 points, but remains normal, continue both therapies and repeat cardiac assessment in 4 weeks.**
b. **If the ejection fraction drops by 10-20 points but the ejection fraction is >40%, continue both therapies and repeat cardiac assessment in 2 to 4 weeks. If at the time of reassessment the ejection fraction has not improved, stop both drugs.**
c. **If the ejection fraction drops by >20 points, is <40%, hold both drugs and repeat cardiac assessment in 2 weeks. If the ejection fraction has improved to >45%, then restart both drugs. If at the time of reassessment the ejection fraction has not improved, stop both drugs.**

If a subject receiving trastuzumab and PD 0332991 has symptoms suggestive of heart failure, cardiac assessment should be obtained immediately.

a. **If the ejection fraction drops by <10 points, continue both drugs and repeat cardiac assessment in 2-4 weeks.**
b. **If the ejection fraction drops by >10 points but is >50%, continue both drugs and repeat cardiac assessment in 2-4 weeks.**
c. **If the ejection fraction drops by >30 points stop both drugs.**

5.4. Drug Supplies

5.4.1. PD0332991

5.4.1.1. Formulation and Packaging

PD 03322991 is formulated in 2-tone grey capsules containing 125mg, 100mg, or 75mg of study medication. The capsules can be distinguished by their size.

5.4.1.2. Preparation and Dispensing

Medication will be provided in non-patient specific bottles containing either 125mg, 100mg, or 75mg capsules. The patient number and the protocol number should be recorded on the bottle label in the spaces provided. Patients should be given a sufficient supply to last until their next study visit.

PD 0332991 is an agent that must be handled and administered with care. Patients should be instructed to keep their medication in the bottles provided and not transfer it to any other container. Due to possible unknown hazards associated with topical and environmental exposure to experimental agents, capsules must not be opened and/or emptied into any vehicle for oral ingestion; capsules must be swallowed intact.

5.4.1.3. Administration

Patients should be instructed to swallow PD 0332991 capsules whole and not to chew them prior to swallowing. No capsule should be ingested if it is broken, cracked, or otherwise not intact. Patients should be encouraged to take their dose at approximately the same time each day.

PD 0332991 dosing should be under fasting conditions, i.e. water only from 1 hour prior to until 2 hours following dosing.

5.4.1.4 Compliance

Study center personnel must ensure that outpatients clearly understand the directions for self-medication. Patients must be given a study medication diary to be completed each day that PD 0332991 is scheduled to be taken and be instructed how to complete it. Unused drug and/or empty bottles should be returned to the site at the next study visit.

5.4.1.5. Drug Storage and Drug Accountability

Patients and individuals dispensing study medication must not remove capsules from the sponsor's containers for dispensing or storage in any other containers.

The investigator, or an approved representative (eg, pharmacist), will ensure that all study drug is stored in a secured area, at controlled room temperature and in accordance with applicable regulatory requirements. Patients are to store study medication at room temperature.

5.5. Concomitant Medication(s)

Treatment intended as supportive care, such as analgesics, corticosteroids, megestrol acetate, erythropoietin, pamidronate, vitamins, and antidepressants may be used at the investigator's discretion. Prostate cancer patients may remain on luteinizing-hormone releasing hormone (LHRH) agonists while on study. Antiemetics may be used at the investigator's discretion for prevention and/or treatment of nausea and vomiting. Every effort should be made to ensure that nausea and vomiting are controlled, as these conditions may preclude a patient from taking or absorbing the oral doses of PD 0332991. Patients requiring palliative radiation therapy for bone pain from a pre-existing lesion after completing Cycle 1 may remain on study. Patients requiring radiation therapy in Cycle 1 are considered to have progressive disease and are to discontinue study treatment. Study medication must be interrupted while the patient receives a course of radiation therapy, as any potential interaction between PD 0332991 and radiation therapy is unknown. Such patients should be restarted on study medication after a post-radiation therapy recovery period of at least 1 week. Patients interrupting treatment to receive radiation therapy for ≥3 weeks, ie, ≥3 weeks elapsed from the planned start date of the next treatment cycle, should be considered for discontinuation of the study, unless there is evidence of clinical benefit.

There have been no clinical studies to evaluate the possibility for drug interactions between PD 0332991 and other medications. Because inhibition of CYP3A isoenzymes may increase PD 0332991 exposure leading to a potential increase in toxicities, the use of known strong potent CYP3A inhibitors (such as ketoconazole, miconazole, itraconazole, posaconazole, clarithromycin, erythromycin, tilithromycin, nefazodone, diltiazem, verapamil, indinavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, amprenavir, fosamprenavir, St. John's wort and grapefruit juice) are not recommended.

In addition, drugs that are known strong CYP3A inducers (such as phenobarbital, rifampin, phenytoin, carbamazepine, rifabutin, rifapentin, clevidipine, and St. John's Wort) are not recommended.

Concominant trastuzumab will be optional in subjects with HER2 positive tumors. There are no expected overlapping toxicities with trastuzumab and PD 0332991, however, safety assessments will be carried out closely on these subjects. Because of cardiac toxicity associated with trastuzumab, subjects receiving trastuzumab will be monitored with an echocardiogram or MUGA scan every 3 months per standard of care.

6. STUDY PROCEDURES

6.1. Screening

All patients being considered for the study and eligible for screening must sign an informed consent for the study prior to any study specific procedures. The required screening assessments and laboratory tests are summarized in the Schedule of Activities Tables. A historical or fresh biopsy sample will be obtained to evaluate the tumor for Rb expression. This screening test for Rb will be performed in the Department of Pathology. Note that Rb screening is not required for eligibility for subjects with ER positive breast cancer. Following completion of the pretreatment assessments and confirmation of eligibility, patients may be scheduled to begin study treatment.

6.2. Study Period

Patients will receive doses of PD0332991 on an outpatient basis. Clinic visits, hematology and other safety laboratory tests will be performed according to the Schedule of Activities. Additional assessments may be performed as necessary to evaluate specific adverse events until they resolve to baseline or CTC Grade ≙1. Additional clinic visits may be required at the discretion of the investigator.

6.3. Follow-Up Visit

The primary reason for a patient's discontinuation of the study medication will be clearly documented on the Case Report Form (CRF).

Adverse events that are serious, suspected to be related to study drug, or considered significant by the investigator or medical monitor must be followed after the time of therapy discontinuation until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and the medical monitor or his/her designated representative. Each serious adverse event (SAE) must be reported to Pfizer in accordance with the terms detailed in the Penn-Pfizer Alliance

Required tests and procedures for the follow up period are detailed in the Schedule of Activities. If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document patient outcome, if possible. The investigator should inquire about the reason for withdrawal, request the patient to return for a final visit and follow-up with the patient regarding any unresolved adverse events. If the patient withdraws from the trial and also

withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent.

6.4. Subject Withdrawal

Patients may withdraw from treatment at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor. Reasons for withdrawal include, but are not limited to the following:

- Progressive disease;
- Unacceptable toxicity;
- Global deterioration of health-related symptoms;
- Protocol non-compliance;
- Pregnancy;
- Patient request;
- Lost to follow-up;
- Study termination by Sponsor.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal, request the subject to return all unused investigational product(s), request the subject to return for a final visit,

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor, Dr. O'Dwyer, may retain and continue to use any data collected before such withdrawal of consent.

7. ASSESSMENTS

The baseline period is from 14 days prior to the patient's dose of PD0332991. The first treatment is Day 1 of Cycle 1. For laboratory tests, normal ranges will be provided from the site. Assessments required to evaluate the safety of PD0332991 are outlined in the following sections.

SCHEDULE OF EVENTS

Routine screening evaluations are to be performed within 2 weeks of starting treatment. Imaging may occur up to 4 weeks prior to treatment and testing for Rb expression may occur any time pre-treatment.

Test	Screening	Baseline	Week 1	Week 2	Week 3	Day 1 Subsequent Cycles	Week 6 or 7
Informed Consent	X						
PhysExam	X	X mini				X	
Weight		X				X	
Height	X						
EKG	X	X				X (cycle 2 only)	
Vital Signs/PS	X	X				X	
Rb testing	X						
CBC with diff	X	X	X	X	X	X	
OCOMP	X	X		X		X	
Urinalysis		X					
PK				X[1]			
PD		X					
Optional Biopsy		X			X		
AE assessment		X	X	X	X	X	
Con Meds	X	X	X	X	X	X	
Disease Assessment (scan, MRI)	X				X every other cycle		
Tumor Marker		X				X every other cycle	
FLT PET[2]	X				X		
FDG-PET[2]	X						X
ECHO/MUGA[3]		X					

1 Samples (1) On Cycle 1 Day 14 at pre-dose; (2) On Cycle 1 Day 15 at pre-dose, at 4 hours post-dose and between 7-10 hours post-dose, (2) matched with FLT-PET assessments (breast and colorectal patients only) except at baseline, (3) matched with tumor assessments except at baseline

2 FLT-PET will be performed in about 15 patients with colon cancer and breast cancer only. FDG PET will be performed in patients with colorectal cancer only. The follow-up scan will be performed in either week 6 or 7 for each study.

3 ECHO/MUGA will be obtained only for subjects with HER2-overexpressing tumors who will be receiving concominant trastuzumab. It will be repeated every 3 months to monitor cardiac function per standard of care.

7.1. Physical Examination
A complete physical examination will include the assessment of all body systems, the measurement of body weight, height, ECG, pulse, and assessment of ECOG performance status. All examinations must be performed by qualified health care professionals. Findings of all physical examinations should be recorded in the source documents, and any change from baseline considered by the investigator to be clinically significant should be recorded as an adverse event in the case report form.

7.2. Hematology and Coagulation Function
The following hematology tests will be performed at the intervals described in the Schedule of Activities: hemoglobin (Hgb), hematocrit (Hct), red blood cell count (RBC), white blood cell count with 5-part differential (WBC w/diff), and platelet count will be collected. Assessments will be performed within 72 hours prior to the designated timepoints on Day 1. Flexibility (1-3 days) will be allowed around the post-treatment blood tests for logistical reasons (eg, weekends and holidays); however, every effort should be made to obtain test specimens according to schedule.

7.3. Serum Chemistry
The following serum chemistry tests will be performed according to the Schedule of Activities: sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose (Glu), creatinine (Cr), blood urea nitrogen (BUN), total bilirubin (TBili), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), calcium (Ca), phosphorus (P), and alkaline phosphatase (AP). Assessments will be performed within 72 hours prior to the designated timepoints on day 1. Flexibility (1-3 days) will be allowed around the post-treatment blood tests for logistical reasons (eg, weekends and holidays); however, every effort should be made to obtain test specimens according to schedule.

7.4. Complete Urinalysis:
Complete microscopic urinalyses will be performed according to the Schedule of Activities on Day 1 of Cycle 1: color, appearance, specific gravity, pH, protein, glucose, occult blood, ketones, leukocyte esterase, nitrite, bilirubin, urobilinogen, and microscopic analysis of centrifuged sediment for casts, cells and crystals will be performed at baseline and as clinically indicated.

7.5. Cardiac Monitoring
All subjects with HER2-overexpressing tumors who will be receiving concominant trastuzumab will have a baseline assessment of ejection fraction with an echocardiogram or MUGA scan. This will be followed every 3 months per standard of care to assess for any changes in the ejection fraction while on trastuzumab therapy. Baseline ejection fraction must be at least 50% to receive trastuzumab. PD 0332991 and trastuzumab will be held according to section 5.3.2.3.

7.6. Tumor Assessments
All patients in this Phase 2 study will be considered evaluable for response. Baseline scans should be obtained within 28 days prior to enrollment. For the purpose of this study, patient's tumor assessments should be re-evaluated at the end of every second cycle (approximately every eight weeks). All patients with responding tumors (complete or partial response) must have the response confirmed no sooner than 4 weeks after the first documentation of response. Objective tumor response will be measured using the Response Evaluation Criteria in Solid Tumors (RECIST, Appendix 1). For details, see the Schedule of Activities, which summarizes information on the timing of study assessments. The determination of antitumor efficacy will be based on objective tumor assessments made according to the system of unidimensional evaluation. The same method and technique should be used to characterize each identified and reported lesion at baseline, during the study treatment period, and during follow-up. Imaging-based

evaluation rather than clinical examination is the required technique when either could be used to assess the antitumor effect of the treatment. Computed Tomography (CT) scan or Magnetic Resonance Imaging (MRI) is the preferred method for the tumor assessment. Whenever possible, clinical evaluation of superficial lesions should not be used as the sole form of measurement. X-ray, ultrasound and radionuclide scan should not be used to measure tumor lesions.

Tumor assessments can occur every 12 weeks for patients who have been receiving study drug for 18 months or more, at the discretion of the treating physician.

7.7 Pharmacokinetics of PD0332991

For all patients, blood specimens for analysis of PD0332991 will be collected: (1) On Cycle 1 Day 14 at pre-dose; (2) On Cycle 1 Day 15 at pre-dose, at 4 hours post-dose and between 7-10 hours post-dose, (2) matched with FLT-PET assessments (breast and colorectal patients only) except at baseline, (3) matched with tumor assessments except at baseline. The actual dosing time must be collected on Cycle 1 Days 13, 14 and 15 and on the day of the FLT-PET/tumor assessments. All actual PK collection times should be documented in the CRF.

Blood specimens (3 mL) to provide a minimum of 1.5 mL plasma will be collected into appropriately labeled tubes at times specified above. All efforts will be made to obtain the pharmacokinetic samples at the exact nominal time relative to dosing on Days 14 and 15, and if the patient has inadvertently taken that day's dose, no sample will be drawn, and the reason noted.

Following collection of 3 mL of blood into a K2EDTA tube, the tube should be inverted gently 15 times to completely mix whole blood with the anti-coagulant. The sample tubes should be kept on ice while awaiting processing and should be kept between 2 °C and 8 °C during all processing steps. Within 30 min of collecting the blood samples should be centrifuged at 3500 rpm (1000 x g) for 10 minutes at 4ºC. A clean (never used) pipette should be used to transfer the plasma layer (~1.0 mL) into a pre-labeled storage cryovial and the cryovial placed upright in a storage box and stored frozen at -20 °C.
.Samples will be analyzed using a validated analytical method in compliance with Pfizer's standard operating procedures.

7.8 Pharmacodynamics

Whole body PET/CT scans will be carried out on patients with colorectal cancer, and breast cancer if feasible, for an hour immediately following 10-15 mCi [18F]-fluoro-*L*-thymidine IV. These scans will enable estimation of the uptake of FLT into tumor. Surrogate values of relative uptake (SUV) will also be obtained. Before PET imaging, patients will be instructed to fast for at least 6 h, and to drink 1 L of water before imaging to stimulate ^{18}F-FLT excretion from the renal calyces and subsequent voiding.

For injection of the radiopharmaceuticals, a venous cannula will be inserted in each patient's forearm. All patients will be scanned in 8-10 positions from the skull base to the mid-thigh, with 5 min per bed position for emission scanning. PET images will be iteratively reconstructed (ordered-subset expectation maximization).The major marker to be assessed as an indicator of cell cycle inhibition will be FLT-PET. The surface expression of thymidine salvage transporters is a cell cycle dependent activity that should be markedly reduced in the presence of an effective cell cycle inhibitor.

18F-Fluoro-labelled thymidine (FLT), an analogue of thymidine and marker of proliferation, has been proposed as a novel PET radiotracer. In this study 18F-FLT will be used, under an Investigational New

Drug Application (IND #102,482), to assess cellular proliferation in a subset of patients with breast or colorectal cancer. Approximately 15 subjects will receive this investigational agent in this study.

Participants receiving 18F-FLT will be brought to the Division of Nuclear Medicine and Clinical Molecular Imaging for administration of the agent and subsequent PET/CT imaging of the whole body. A single dose of approximately 10 mCi to 15 mCi of 18F-FLT will be administered as a bolus intravenous injection. The injection will begin after adequate intravenous access is ensured (preferably in an antecubital vein). Subjects will be encouraged to void urine immediately prior to receiving the investigational agent and asked to remove metal objects that may be in the field of view. At completion of injection, the syringe and i.v. line will be flushed with saline solution. Subjects will be monitored for adverse events and a whole body imaging with PET/CT will begin approximately 60 minutes following administration of 18F-FLT.

18F-FLT PET/CT imaging

18F PET/CT imaging from the skull base to the mid-thigh will be performed approximately 60 minutes after 18F-FLT administration, per standard acquisition protocols in the Division of Nuclear Medicine and Clinical Molecular Imaging. Attenuation-corrected PET/CT images of the whole body will be processed and reviewed per Division guidelines by the Nuclear Medicine Authorized User associated with this research study.

7.9 Pathology Correlates

All patients entered in the study will be Rb positive. However we expect that certain tumor characteristics will predict for response among the diseases to be analyzed. For breast and colon cancers, the analyses of cdk 4/6 and of cyclin D will be retrospective, and will attempt to identify patients who either respond or have prolonged stable disease. Samples from patients with biopsiable disease will be evaluated to determine the degree of cell cycle inhibition achieved with PD0332991 treatment, its relationship to drug exposure, and to imaging markers, and will be analyzed for additional –omic studies as an approach to understanding markers of susceptibility to this therapy, in an exploratory fashion, and as funding plans evolve.

In addition, we will look at a cohort defined by the single determinant of *CCND1* amplification. Patients will qualify through quantitation of gene amplification by FISH, performed by Dr Paul Zhang in the Department of Pathology, University of Pennsylvania. The requirement for integral marker assay performance in a CLIA-approved environment makes this collaboration especially important. The criterion for positivity will be a 4:1 ratio of CCND1:CEP11 control in >15% cells. A large component of the study will involve screening, so to make this study practical from a budgetary as well as a patient perspective, we will enrich populations by selecting those for screening based on known populations with high incidence. The populations to be screened will include:

a. Esophageal cancer, especially squamous cell – cohorts to be analyzed separately.
b. Head and neck squamous cell cancer.
c. Breast, especially those with luminal B expression profile, ER positivity, and high Ki67 expression.
d. Liposarcoma
e. Any histology if already known to carry the amplification

In addition to CCND1, patients may enroll on this study if they express or amplify CDK4 or 6. As there are currently no CLIA-approved labs to performCDK 4/6 amplification, results from the University of Michigan's non-CLIA approved lab may be used until a CLIA-approved lab becomes available.

Documentation of how the test is performed, who performed the test, and the results will be obtained from the University of Michigan's lab and filed in the patient's medical record chart. As soon as a CLIA-approved lab becomes available at Penn or elsewhere, analysis for CDK 4/6 expression in a CLIA environment will be required. Patient's whose tissue has been tested for CDK4 mutational status at a CLIA certified lab may also be acceptable if adequate expression is reported.

7.10 Long-term Follow-up (Germ cell tumor participants only)

For participants enrolled to the germ cell tumor cohort, we will further clarify response and post-study treatment/progression through long term follow up via the participant's medical record and interaction with treating oncologist. Specifically, we will collect information regarding survival, date of last visit with oncology clinician, post-study treatments for cancer, outcome of post-study treatments for cancer, any cause hospitalizations, all with applicable associated dates.

The medical record will be reviewed at minimum every 6 months and data associated with the above recorded for the purposes of the study.

8 ADVERSE EVENT REPORTING

8.1 Adverse Events

The investigator must grade AEs according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) and provide an opinion regarding the causal relationship of the AE to study drug.

Patients will be carefully monitored for adverse events from the initial dose of study medication through the Follow-Up visit (Appendix C).

All observed or volunteered adverse events, regardless of treatment group or suspected causal relationship to study drug, will be recorded on the adverse event page(s) of the case report form.
Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of pre-existing illnesses should be recorded. Exacerbation of pre-existing illness, including the disease under study, is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the trial.

8.2 Serious Adverse Events

Reporting of Serious Adverse Events. Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening), Principal Investigator will report to FDA and Pfizer by facsimile any Serious Adverse Event ("SAE," as defined below) that occurs during the SAE reporting period (as defined below) in a Study subject assigned to receive the Pfizer Product or Compound (see Section 7, Pfizer Product or Compound). Principal Investigator will report such SAEs using a FDA MEDWATCH form and the *Reportable Adverse Event Fax Cover Sheet* provided by Pfizer. SAEs should be reported as soon as they are determined to meet the definition, even if complete information is not yet available.

a. SAE Definition. An SAE is any adverse event, without regard to causality, that is life-threatening or that results in any of the following outcomes: death; in-patient hospitalization or prolongation of existing hospitalization; persistent or significant disability or incapacity; or a congenital anomaly or birth defect. Any other medical

event that, in the medical judgment of the Principal Investigator, may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above is also considered an SAE. A planned medical or surgical procedure is not, in itself, an SAE. Also specifically excluded from this definition of SAE is any event judged by the Principal Investigator to represent progression of the malignancy under study, unless it results in death within the SAE Reporting Period.

b. SAE Reporting Period. The SAEs that are subject to this reporting provision are those that occur from after the first dose of the Pfizer Product or Compound through 28 days after discontinuation of the Pfizer Product or Compound.

c. Follow-Up Information. Institution will assist Pfizer in investigating any SAE and will provide any follow-up information reasonably requested by Pfizer.

d. Regulatory Reporting. Reporting an SAE to Pfizer does not relieve Institution of responsibility for reporting it to FDA, as required.

e. Pfizer-Provided Training. Pfizer will make available training material that provides information about the SAE reporting requirements for IIR studies. Principal Investigator will review this material and share it with any Study staff engaged in the reporting of SAEs.

Reporting Process to the IRB

Principal Investigators are required to submit reports of unanticipated problems posing risks to subjects or others using the "**Unanticipated Problems"** link in the HS-ERA system.

For reportable deaths, the initial submission to the IRB may be made by contacting the appropriate IRB Coordinator. IThe AE/Unanticipated Problem Form via HS-ERA is required as a follow up to the initial submission.

Reporting to DSMC

All SAEs for Penn subjects regardless of grade, attribution or expectedness must be submitted to the DSMC within 30 days. Unexpected deaths should be reported within 24 hours. Reports should be sent to the DSMC until the study is formally terminated with the IRB through Penn-CTMS.

IND SAFETY UPDATES/ALERTS
All IND Safety Updates/Alerts (sent by sponsors) with a grade 3 or higher, regardless of attribution or expectedness must be submitted to the DSMC within 30 days. Reports should be sent to the DSMC for 6 months from the date the last Penn subject was treated.

Please contact Cancer Center's Office for Compliance with any questions on reporting.

Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events, as noted above, as well as the construction and implementation of a site data and safety monitoring plan. Medical monitoring by an independent clinician Dr.Adam Cohen, Department of Medicine, Division of Hematology-Oncology will include a regular assessment of the number and type of serious adverse events on a periodic basis. Dr. Adam Cohen will be provided with copies of all SAEs and and AE tables every 3-6 months, depending on rate of accrual.

8.3 Abnormal Laboratory Test Results

Laboratory results will be assessed for adverse events as described in CTCAE v5.0 using normal ranges specified for the institution's laboratory.

The results of all laboratory tests required by the protocol will be recorded in the subject's case report form. All clinically important abnormal laboratory tests occurring during the study will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the investigator or until a diagnosis that explains them is made. The criteria for determining whether an abnormal laboratory test result should be reported as an adverse event are as follows:

1. Test result is associated with accompanying symptoms, and/or
2. Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
3. Test result leads to any of the outcomes included in the definition of a serious adverse event, and/or
4. Test result is considered to be an adverse event by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not meet Condition 2 above for reporting as an adverse event.

8.4 Abnormal Physical Examination Findings

Clinically significant changes, in the judgment of the investigator, in physical examination findings (abnormalities) will be recorded as adverse events.

8.5 Discontinuations

The reason for a subject discontinuing from the study will be recorded in the case report form. A discontinuation occurs when an enrolled subject ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation. Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response according to the definition of adverse event noted earlier. A discontinuation must be reported immediately to the Pfizer clinical monitor or his/her designated representative if it is due to a serious adverse event. The final evaluation required by the protocol will be performed at the time of study discontinuation. The investigator will record the reason for study discontinuation, provide or arrange for appropriate follow-up (if required) for such subjects, and document the course of the subject's condition.

8.6 Data Safety and regulatory issues

8.6.1 Data Monitoring Plan

Data will be monitored in accordance with the Cancer Center's Clinical Trials Scientific Review and Monitoring Committee (CTSRMC) Plan, approved by NCI during the Core Grant's most recent review. This plan requires that each investigator submit a study-specific plan for how s/he is reviewing data (see attached). In addition, this plan calls for an internal audit by the Cancer Center's Data Safety Committee (a subcommittee of the CTSRMC) of all data at least once per year for each study. Finally, toxicities, in particular are reviewed and discussed at each weekly meeting of the Developmental Therapeutics Program.

8.6.2 Data Plan

Case Report Forms (CRFs) developed by the Developmental Therapeutics Program will be modified to collect data points specific to meeting the data needs of a study. The CRFs will be reviewed and approved by the PI. These CRFs will then be converted to electronic CRFs that will be available only to select members of the Developmental Therapeutics Program. The CRFs include an audit trail to ensure data integrity. Source documents will be developed to ensure that each data point to be collected can be captured. In addition, all data enter via EPIC will be printed out and maintained in a shadow chart to ensure its ongoing access to the research staff and its auditors. A designated Research Coordinator with experience in data management will be responsible for ensuring that the data are generated, collected and transcribed appropriately.

On a quarterly basis, as specified by the Penn/Pfizer Alliance, study progress, but no specific patient data will be provided to Pfizer.

9 DATA ANALYSIS/STATISTICAL METHODS

All data for this trial will be summarized using tabulations and frequencies for dichotomous variables and means, medians, variances, and ranges for continuous variables. Summaries will be stratified by disease site, and characteristics of each population will be compared descriptively.

The response rate of interest in this trial will be 15% for each of the indications tested. If we observe at least one response in the first fifteen subjects, we will enroll a further 15 subjects, for a total of 30. Observing zero responses in the first 15 subjects would exclude response rates as low as 15% with a 90% one-sided upper confidence bound. If at least three responses are observed in a total of 30 evaluable patients, we will conclude that the drug is active and merits further study; if the true response rate is 15% or greater, three or more responses will be observed with a probability of at least 85%. If the true response rate is only 3%, three or more responses will be observed with a probability of only 6%. In addition to responses of CRs and PRs, disease stabilization will be considered: if the proportion of patients with disease stabilization for 6 months exceeds 20%, we will conclude that the drug may warrant further investigation in that indication. With n=30 subjects and a true response rate of 15%, the expected confidence interval width is ± 10.7% around the estimated proportion. Moreover we will have 90% power to detect any unforeseen adverse effect that occurs in at least 7% of cases.

A subject who completes at least 2 full cycles of therapy and undergoes the required imaging at 8 weeks is defined as evaluable for response. A subject who is not evaluable for response may be replaced.

We will compute confidence intervals for selected safety and exploratory variables. Adverse event terms will be standardized using the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by system organ class and preferred term. Laboratory parameters will be summarized using mean change in value and/or shift in category since baseline.

Subjects evaluable for response is defined as subjects who complete at least 2 full cycles of therapy and undergo the protocol required imaging at 8 weeks. By extension subject not evaluable for response may be replaced such that the accrual goal is reflective of evaluable subjects within the respective cohort.

Population Pharmacokinetic Analysis and PK/PD Modeling

Pharmacokinetic data from this study may be analyzed using mixed-effect modeling approaches. The intent of this analysis will be to build upon a basic population pharmacokinetic model for PD 0332991 and to determine the inter-individual and residual variability in population PK parameters of interest. If data permit, PK/PD correlations to investigate any causal relationship between PD 0332991 exposure and efficacy outcomes will be explored.

10 References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 100: 57-70, 2000

2. Sherr CJ, McCormick F. The Rb and p53 pathways in cancer. Cancer Cell 2: 103-112, 2003

3. Knudson AG, Jr. (1971) Mutation and cancer: Statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823.

4. Nakamura M, YonekawaY, Kleihues P, OhgakiH(2001) Promoter hypermethylation of the *RB1* gene in glioblastomas. *Lab Invest* 81:77–82.

5. Simpson DJ, Hibberts NA, McNicol AM, Clayton RN, Farrell WE (2000) Loss of pRb expression in pituitary adenomas is associated with methylation of the RB1 CpG island. *Cancer Res* 60:1211–1216.

6. Gonzalez-Gomez P, *et al.* (2003) CpG island methylation status and mutation analysis of the *RB1* gene essential promoter region and protein-binding pocket domain in nervous system tumours. *Br J Cancer* 88:109–114.

7. Edamoto Y, *et al.* (2003) Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. *Int J Cancer* 106:334–341.

8. Harbour JW, *et al.* (1988) Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science* 241:353–357.

9. Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer 8: 671-82, 2008

10. McIntosh, G. G., J. J. Anderson, et al. (1995). "Determination of the prognostic value of cyclin D1 overexpression in breast cancer." Oncogene **11**(5): 885-91.

11. Yu, Q., Y. Geng, et al. (2001). "Specific protection against breast cancers by cyclin D1 ablation." Nature **411**(6841): 1017-21.

12. Weinstat-Saslow, D., M. J. Merino, et al. (1995). "Overexpression of cyclin D mRNA distinguishes invasive and in situ breast carcinomas from non-malignant lesions." Nat Med **1**(12): 1257-60.

13. Wani, G., I. Noyes, et al. (1997). "Expression of molecular biomarkers in primary breast tumors implanted into a surrogate host: increased levels of cyclins correlate with tumor progression." Mol Med **3**(4): 273-83.

14. Bartkova, J., J. Lukas, et al. (1994). "Cyclin D1 protein expression and function in human breast cancer." Int J Cancer **57**(3): 353-61.

15. Lin, D. I., M. D. Lessie, et al. (2008). "Disruption of cyclin D1 nuclear export and proteolysis accelerates mammary carcinogenesis." Oncogene **27**(9): 1231-42.

16. Haigis K, Sage J, Glickman J, Shafer S, Jacks T (2006) The related retinoblastoma (pRb)

and p130 proteins cooperate to regulate homeostasis in the intestinal epithelium.

J Biol Chem 281:638–647.

17. Scambia G, Lovergine S, Masciullo V (2006) RB family members as predictive and

prognostic factors in human cancer. *Oncogene* 25:5302–5308.

18. Ali AA, *et al.* (1993) RB1 protein in normal and malignant human colorectal tissue and

colon cancer cell lines. *Faseb J* 7:931–937.

19. Vogelstein B, *et al.* (1989) Allelotype of colorectal carcinomas. *Science* 244:207–211.

20. Reichmann A, Martin P, Levin B (1981) Chromosomal banding patterns in human large

bowel cancer. *Int J Cancer* 28:431–440.

21. Muleris M, *et al.* (1987) Characteristic chromosomal imbalances in 18 near-diploid

colorectal tumors. *Cancer Genet Cytogenet* 29:289–301.

22. Palmqvist R, Stenling R, Oberg A, Landberg G (1998) Expression of cyclin D1 and

retinoblastoma protein in colorectal cancer. *Eur J Cancer* 34:1575–1581.

23. Cui X, *et al.* (2004) Aberrant expression of pRb and p16(INK4), alone or in combination, indicates poor outcome after resection in patients with colorectal carcinoma. *Hum Pathol* 35:1189–1195.

24. Yamamoto H, *et al.* (1999) Paradoxical increase in retinoblastoma protein in colorectal carcinomas may protect cells from apoptosis. *Clin Cancer Res* 5:1805–1815.

25. Clemo NK, Arhel NJ, Barnes JD, et al. The role for the retinoblastoma protein (Rb) in the nuclear localization of BAG-1: implications for colorectal tumour survival. Biochem Soc Trans 33: 676-8, 2005

26. Kucherlapati MH, Yang K, Fan K, et al. Loss of Rb in the gastrointestinal tract of Apc1638N mice promotes tumors of the cecum and proximal colon. Proc Natl Acad Sci USA 105: 15493-8, 2008

27. Grady WM, Willis JE, Trobridge P, et al. Proliferation and Cdk4 expression in microsatellite unstable colon cancers with *TGFBR2* mutations. Int J Cancer 118:600-8, 2005

28. Lujambio A, Ropero S, Ballestar E, et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 67: 1424-9, 2007

29. Smalley KS, Contractor R, Nguyen TK, et al. Identification of a novel subgroup of melanomas with KIT/cyclin-dependent kinase-4 overexpression. Cancer Res. 2008 Jul 15;68(14):5743-52.

30. Muthusamy V, Hobbs C, Nogueira C, et al. Amplification of CDK4 and MDM2 in malignant melanoma. Genes Chromosomes Cancer. 2006 May;45(5):447-54.

31. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005 Nov 17;353(20):2135-47.

32. Bartkova J, Lukas C, Sorenson CS, et al. Deregulation of the RB pathway in human testicular germ cell tumors. J Pathol 2003;200:149-56.

33. Vaughn DJ, Flaherty K, Lal P, et al. Treatment of growing teratoma syndrome. N Engl J Med 2009; 360:423-4.

34. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol 24:1770-83, 2006.

35.Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. Nature 463, 899-905, 2010