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A pharmacodynamically guided dose selection of PF-00337210 in a phase I study in patients with advanced solid tumors

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Abstract

Purpose—PF-00337210 is an oral, highly selective vascular endothelial growth factor receptor (VEGFR) inhibitor. We evaluated a composite of biomarkers in real time to identify the recommended phase 2 dose (RP2D) and preliminary anticancer activity of PF-00337210.

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Conflict of interest Glenn Liu and Patricia LoRusso have received research funding from Pfizer, Inc. Yanwei Zhang, Aurora Breazna, Michael Tortorici, David Shalinsky, and Alejandro Ricart have stock ownership in Pfizer, Inc. Aurora Breazna and David Shalinsky have remuneration from Pfizer, Inc. Alejandro Ricart has remuneration from Merck & Co., Inc. The other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Patients and methods—Patients (Pts) with advanced cancers were treated once (QD) or twice daily (BID) with escalating doses. Acute effects on tumor perfusion and vascularity were assessed using DCE-MRI, weekly BP readings, soluble VEGFR-2, and hemoglobin levels.

Results—Forty-six pts were treated with 0.67–9 mg QD and 4–6 mg BID of PF-00337210. Nineteen pts (41 %) previously received VEGF/VEGFR inhibitors. Two pts had dose-limiting toxicity (DLT) at 9 mg QD (troponin I increase and hypertension). The MTD at QD dose was 8 mg. Common drug-related adverse events were hypertension, fatigue, proteinuria, and nausea. Hypertension incidence and intensity corresponded with dose, but was well controlled with medication. Two confirmed partial responses and minor regressions (>10 to <30 % reduction in target lesions) were noted. Complete DCE-MRI was acquired in 21 pts (20 evaluable for vascular response). Ten pts were vascular responders, including 5/6 pts at BID doses. Greatest modulation of soluble VEGFR-2 was at 6 mg BID. The maximum change from baseline in diastolic BP was higher at BID doses. There were no significant differences for systolic BP and hemoglobin levels.

Conclusions—PF-00337210 has profound VEGFR inhibition effects at well-tolerated doses. Antitumor activity and VEGF inhibition effects were observed across BID doses. The RP2D was 6 mg BID.

Keywords

Pharmacodynamic analysis; Tyrosine kinase inhibitor; VEGF receptor; DCEMRI imaging

Introduction

Inhibition of vascular endothelial growth factor (VEGF) and its main binding site, VEGF receptor-2 (VEGFR-2), has been shown to suppress angiogenesis and tumor growth in preclinical models [1-4] and has been associated with clinical efficacy in patients with advanced cancer [5-8]. While several antiangiogenic agents have proven therapeutic efficacy, most phase I dose escalation studies of VEGF receptor (VEGFR) inhibitors report off-target toxicities (diarrhea, fatigue, palmar–plantar erythrodysesthesia, and liver enzyme elevations) that limit further dose escalation or the use of a continuous dosing schedule without drug holidays [9-17]. These adverse events are likely due to effects on cellular processes other than VEGFR signaling, as studies with VEGF ligands (e.g., bevacizumab, aflibercept) have not reported these types of dose-limiting toxicities [18-20]. There also has been a high attrition rate in phase 2/3 trials of VEGFR inhibitors in combination with chemotherapy, and more selective inhibitors with consistent pharmacokinetics (PK) and good tolerability profiles are needed [21-27]. A potential avenue to improve future clinical trials is the use of biomarkers in order to better predict and guide therapy with novel investigational agents rather than relying solely on dose-limiting toxicities. Treatment-induced hypertension has been proposed as a potential biomarker of clinical efficacy of antiangiogenic agents [7, 28]. A retrospective analysis of patients receiving sunitinib found a statistically significant association between treatment-induced hypertension and an improved clinical outcome [28]. However, there is no prospectively validated biomarker to consistently evaluate antiangiogenic agents. Additional studies to evaluate and validate biomarkers during therapy would be helpful to guide anticancer therapy.

PF-00337210 is a highly potent and selective ATP-competitive inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase that, based on IC_{50} , is >10-fold more selective for VEGFR-2 than other kinases (e.g., KIT and platelet-derived growth factor receptors [PDGFRs]) (see Supplementary Fig. 1) [3]. PF-00337210 was chosen from a series of compounds based on numerous favorable preclinical features: (1) availability of a robust salt/polymorph with improved oral bioavailability across species, doses, and formulations, with no food effect; (2) significantly lower inter-animal PK variability, with extensive distribution into body tissues; (3) reproducible preclinical pharmacodynamic (PD) effects, including statistically significant changes in blood pressure (BP) at no-observed-adverse-effect level (NOAEL); and 4) significant tumor regression in a model resistant to bevacizumab (Pfizer, data on file).

The key objectives of this first-in-human, phase I study were to evaluate the safety, PK, PD, and preliminary anticancer activity of PF-00337210. We employed multiple PD biomarkers (changes in hemoglobin levels, BP elevation, and modulation of the following soluble proteins: sVEGFR-2, sVEGFR-3, and sKIT) to evaluate PF-00337210 in real time and to determine the RP2D. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was also used to assess vascular permeability (the volume transfer constant, K^{trans}) of tumor masses or metastatic lesions, which was modeled through an innovative hierarchical mixed effect model to robustly evaluate the changes in K^{trans} on a per patient basis.

Patients and methods

Patient selection and eligibility criteria

Key inclusion criteria were age ≥ 18 years, histologically or cytologically confirmed advanced solid malignancy unresponsive to currently available therapies or for which no standard therapy existed, at least one measurable disease site as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.0); Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; adequate hematological, hepatic, and renal function; and life expectancy ≥ 12 weeks [29]. In the dose escalation phase, patients must have had three screening blood pressure readings ≥ 140/85 mmHg (taken over 3 days) without any antihypertensive medication. Patients who met the same blood pressure criterion on antihypertensive medications were accepted into the expansion cohort to confirm the RP2D.

Patients were excluded if they had: chemotherapy, radiotherapy, or other investigational agent within 4 weeks of study entry; a thromboembolic event or bleeding within 12 or 6 months of study entry, respectively; active brain metastases; concurrent use or anticipated need for treatment with cytochrome P450 3A4 inhibitors or inducers; clinically significant gastrointestinal abnormalities; or other serious medical conditions.

Efforts were made to identify patients with lesions eligible for DCE-MRI based on a selection criteria developed from a previous first-in-human experience; however, patient enrollment was not limited by the absolute requirement for DCE-MRI eligibility [30]. Prior anti-VEGF/anti-VEGFR therapy was allowed. All subjects gave written informed consent. The study was approved by local ethics committees and was conducted according to Good Clinical Practice regulations.

Study design and treatment

This was a phase I study with an accelerated dose escalation design. The starting dose chosen for the study was 0.67 mg. Patients were enrolled into one of nine PF-00337210 dose cohorts (0.67, 1, 2, 4, 6, 8, or 9 mg once daily [QD], and 4 or 6 mg twice daily [BID]) in continuous 28-day cycles. Cohorts of at least one evaluable patient were assigned to each dose level. Once a dose-limiting toxicity (DLT) was observed at any dose level, the design switched to a classical 3 + 3 design. Patients received therapy until disease progression, unacceptable toxicity, or investigator/patient decision to withdraw study consent.

DLTs were defined as any of the following drug-related toxicities occurring in cycle 1: grade 3 non-hematologic toxicity except blood pressure, grade 3 neutropenia with fever, grade 4 neutropenia > 7 days, grade 4 thrombocytopenia, hemoptysis of >2.5 mL of bright red blood/day, proteinuria of grade 2 over 24 h, or inability to resume PF-00337210 dosing at the current dose level within 14 days of stopping drug due to treatment-related toxicity. In the event of hypertension, DLT was defined as blood pressure ≥ 180/110 mmHg (three readings over 3 h), regardless of the use of antihypertensive medications, or >160/100 mmHg (three readings over 3 days) on maximum antihypertensive medication according to the judgment of the investigator. Intra-patient dose escalations were permitted for patients who completed the first two treatment cycles without drug-related toxicity of grade 2 and had a complete response, partial response, or stable disease.

Safety assessments

To be considered assessable for the safety of a given dose level, a patient must have received at least 75 % of the PF-00337210 doses or experienced treatment-related adverse events that prompted early treatment interruption or discontinuation. Patients who received <75 % of the dose were replaced. Safety evaluations included physical examination, assessment of ECOG performance status, vital signs, review of concomitant medications, toxicity assessment, and routine laboratories at baseline and weekly during treatment. An electrocardiogram (ECG) and troponin I/T samples were performed at baseline and predose on day 1 of each cycle. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 3.0).

Pharmacokinetic assessments

PF-00337210 concentrations in both plasma and urine were measured using validated analytical techniques. Blood samples for PK determinations were collected on Cycle 1, Day 1 (C1D1), Cycle 1, Day 15 (C1D15), and Cycle 2, Day 1 (C2D1) over 24 h from patients receiving QD dosing and over 8 h from patients receiving BID dosing. Urine was collected over 24 h on C1D1 and C2D1 for patients receiving QD dosing and over 12 h on C1D1 and C2D1 for patients receiving BID dosing. PF-00337210 concentrations in both plasma and urine were measured using validated liquid chromatography–tandem mass spectrometric methods. The calibration range was 0.200 to 200 ng/mL with the lower limit of quantification of 0.200 ng/mL.

Evaluation of food effect

Patients in selected cohorts participated in a pilot food effect study. On C2D1, patients ($n = 10$) were instructed to take their morning dose after an overnight fast. On C2D2, patients received their morning dose following a meal of 1000 kilocalories, with 50 % of the caloric content being fat. Standard PK parameters were analyzed at various time points and the comparison was made between the fasted and fed states.

Pharmacodynamic assessments

The acute effects of PF-00337210 on tumor vascular perfusion and permeability were assessed using DCE-MRI. Changes in vascular permeability were assessed using the vascular parameter change K^{trans} (volume transfer constant) by DCE-MRI. Each eligible patient underwent imaging at baseline (within 2 weeks before the first dose of PF-00337210) and at approximately C1D2 (2-h post-morning dose; the specific timing of the second scan was dependent on the PK profile observed in the first cohort of patients). A three-dimensional (3D) fast-spoiled gradient-recalled echo sequence scan was used to acquire T1-weighted images before, during, and after intravenous administration of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist®, Berlex Laboratories, Inc, Wayne, NJ) infused with a magnetic resonance-compatible injector at 3 mL/s and followed by a 20-mL saline flush at 3 mL/s. All data were acquired by using a GE 1.5-T EchoSpeed scanner (256 × 128 matrix; ten 5-mm slices acquired every 11.04 s for 5.53 min; 22- to 36-cm axial field of view). All pulse sequences used for the functional MRI data acquisitions were FDA-approved for clinical use. The functional DCE-MRI protocol had specific index lesion selection criteria, image acquisition, and analysis based on a previous first-inhuman VEGFR inhibitor study [30].

Soluble plasma proteins associated with angiogenesis (sVEGFR-2, sVEGFR-3, sKIT) were analyzed following collection of a whole blood sample on Day 1 of each treatment cycle (pre-dose) and at the end of study visit (day of last dose). Hematology assessments, including hemoglobin, hematocrit, red and white blood cells, and platelets, were conducted within 2 weeks prior to the first dose of treatment and weekly throughout the treatment.

Statistical methods

Correlation of PK (such as C_{max} and AUC) with primary AEs, tumor responses, DCE-MRI imaging assessments, and biomarkers of PF-00337210 was examined. For the biomarker analyses, the proportional odds ratio model was used to associate biomarker levels with PF-00337210 concentrations and with potential antitumor activity.

Hierarchical mixed effect model analysis for K^{trans}

K^{trans} was measured on a per lesion basis for each patient at baseline and follow-up. The analysis of K^{trans} was based on the natural logarithm of the ratio of K^{trans} at follow-up over baseline for each lesion of each patient (i.e., log ratio). The hierarchical mixed effect model, with baseline as a covariate and patient as random effect, was formulated to analyze the log ratio of K^{trans} . This model was able to accommodate an unbalanced number of lesions across patients, account for between- and within-subject variability, and leverage information across patients to provide a robust estimate of changes in K^{trans} on a per patient basis for

differentiating vascular responders and non-responders. Specifically, a patient was defined as a vascular responder if his/her percentage change from baseline (PCFB) in K^{trans} was statistically significantly lower than 0 % at false discovery rate (FDR, i.e., the proportion of false discoveries among total rejections) controlled within 0.2. The estimate of PCFB and its confidence interval (CI) were estimated by $(\exp(x) - 1) \times 100 \%$, where x was empirical best linear unbiased predictor (EBLUP) of log ratio or the bound of its CI on a per patient basis. The analysis results were presented by the CI plots (60 % CI) of the percent change from baseline in K^{trans} on a per patient basis, on which the vascular responders (FDR adjusted one-sided p value < 0.2) were highlighted. The analysis was conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Association analysis of BP and hemoglobin with dose

Systolic BP (sBP), diastolic BP (dBP), and hemoglobin were measured longitudinally for each patient. The analysis of covariance (ANCOVA) was applied to assess the association between dose level and the following variables: BP and hemoglobin with the corresponding baseline as covariate. BP was evaluated by the maximum change from baseline in both sBP and dBP across visits. Hemoglobin was evaluated by the maximum change from baseline across visits. Dose levels were classified into three categories: QD low dose (0.67–6 mg), QD at MTD and above (8 and 9 mg), and BID (4 and 6 mg). For sBP, dBP, or hemoglobin, all pair-wise comparisons between dose levels were performed with multiplicity adjusted by Tukey–Kramer test. No multiplicity adjustment was made across sBP and dBP. The analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Association analysis of hypertension severity with dose

The proportional odds ratio model was applied to assess the association between hypertension severity and dose. The odds ratio of experiencing a given hypertension severity was calculated for BID and QD doses, where hypertension severity was ordinal with three categories (mild, moderate, and severe), and dose levels were classified into two categories (QD [4, 6, 8, and 9 mg] and BID [4 and 6 mg]). The analysis was conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Correlation of pharmacodynamic and pharmacokinetic assessments

The relationship between PK parameters and PD endpoints was assessed. For all PK/PD analyses, PF-00337210 exposure was measured using the area under the concentration time curve from 0 to 24 h (AUC_{24}). PD endpoints were initially explored graphically; these included DCE-MRI (changes in K^{trans} and initial area under the curve [IAUC]), change in dBP, change in hemoglobin levels over time, and change sVEGFR-2, sVEGFR-3, and sKIT. Linear regression was conducted to correlate PK with the different PD endpoints. All analyses were conducted using SPLUS software (S-PLUS version 7.0, Insightful Corp.).

Assessment of antitumor activity

Patients were evaluated for response after every two cycles of therapy (approximately every 8 weeks), using RECIST 1.0. Any responses were confirmed after a minimum of 4 weeks.

Results

Patient characteristics and drug disposition

Forty-six patients, whose demographic characteristics are summarized in Table 1, received a total of 204 cycles of PF-00337210 at doses of 0.67–9 mg QD ($n = 30$), 4 mg BID ($n = 4$), or 6 mg BID ($n = 12$, Table 2). Forty-five patients (98 %) had received previous therapy, with an overall median of five prior therapies (range 0–10). Nineteen patients (41 %) had previously received VEGF/VEGFR inhibitors.

Safety and tolerability

The number of cycles of PF-00337210 received ranged from 1 to 9 for QD dosing and from 1 to 25 for BID dosing. The initial dose level was reduced in eight patients due to treatment-related adverse events (Table 2). Four patients discontinued therapy due to treatment-related adverse events and two patients due to non-treatment-related adverse events. At the time of data cutoff (January 28, 2011), two patients were receiving ongoing therapy (4-mg BID and 6-mg BID cohorts). Long-term follow-up noted that one patient in the 4-mg BID cohort received a total of 31 cycles (approximately 28 months), and one patient in the 6-mg BID cohort received 28 cycles (approximately 26 months) of therapy. At the RP2D, six patients received more than 6 cycles of therapy, and one of 12 patients discontinued therapy after 14 cycles (approximately 13 months) of therapy due to a treatment-related adverse event (recurrent, reversible bilirubin increase with liver enzymes within normal limits).

At doses from 0.67 to 6 mg QD, no DLTs were reported (Table 3). The 2-mg QD cohort was expanded due to the possibility of drug-related grade 2 proteinuria. Two patients in the 9-mg QD cohort experienced DLTs, and thus the MTD was exceeded. The first patient had atypical chest pain with a brief myocardial ischemia denoted by grade 3 troponin I levels, which were reversible within 24 h; the patient discontinued PF-00337210. The second patient had hypertension (systolic 180 mmHg over 3 h) at the anticipated time to $C_{\max}$ after the first dose and received 100 mg of oral labetalol after confirmation of DLT and remained on study at 6-mg QD with concomitant labetalol. His systolic blood pressure was slightly high, at 145 mmHg, after 24 h of the episode.

Patients were treated with PF-00337210 8 mg QD without DLT; however, the possibility of $C_{\max}$ -driven toxicity and the lack of consistent pharmacodynamic effects at 8-mg QD encouraged the exploration of BID dosing. One patient experienced a DLT at 6 mg BID (grade 3 palmar–plantar erythrodysesthesia). At the BID schedule, dose escalation was stopped as a result of PD effects rather than toxicity issues. Based on the safety profile and the significant pharmacodynamic findings, the 6-mg BID dose cohort was expanded to 12 patients and later determined to be the RP2D.

The most common toxicities of PF-00337210 were characteristic of other VEGFR inhibitors and included hypertension (50 %), and fatigue (37 %) (Table 3). The incidence and intensity of hypertension corresponded to dose level. With BID dosing, 81 % of patients had hypertension, but this event was only grade 3 in severity in 1 (25 %) patient at 4 mg BID and 2 (17 %) patients at 6 mg BID. Unlike other VEGFR inhibitors, there were few other common toxicities associated with PF-00337210, such as palmar–plantar erythrodysesthesia

(4.3 %) and gastrointestinal hemorrhage (2.2 %). No patients experienced grade 4 or 5 treatment-related toxicity. There was no significant myelotoxicity, stomatitis, or non-hematologic laboratory abnormalities. There were no reports of hypothyroidism, hemoptysis, gastrointestinal perforation, or clinically significant changes in ECG parameters.

Pharmacokinetics

Plasma concentrations of PF-00337210 exceeded the IC_{50} for VEGFR-2 inhibition (8 ng/mL) at doses 4 mg/day (Fig. 1a) and in almost all measurements from patients who received 6 mg BID (Fig. 1b). PF-00337210 administered in the fasted state was rapidly absorbed, with peak plasma concentrations occurring within 1 and 3 h after dosing (Supplementary Table 2). Plasma concentrations declined with a terminal plasma $t_{1/2}$ between 5 and 14 h after single doses. PF-00337210 plasma PK reached steady-state within 15 days, with no unexpected accumulation. The V_z/F ranged from 216 to 432 L and CL/F was between 20.8 and 35.7 L/h after the first initial dose. The PK of PF-00337210 was consistent across the dose levels; exposures (AUC_{24}) were generally linear (Fig. 1c), and coefficients of variability (% CV) ranged from 23 to 41 % at BID doses (on cycle 1 day 15, Supplementary Table 2).

The pilot food effect assessment indicated a similar rate and extent of absorption of PF-00337210 when administered in the fasted and fed state. Mean AUC_{24} was 546 ng.h/mL (CV, 38 %) in the fed state, which is similar to the value of 512 ng h/mL (31 %) obtained in the fasted state. Mean C_{max} was 31.3 ng/mL (CV, 46 %) in the fed state, which is similar to the value of 34.4 ng/mL (31 %) obtained in the fasted state. The median T_{max} ranged from 1 to 4 h in both the fasted and fed state.

Analysis of urine data obtained on days 1 and 29 (cycle 2 day 1) demonstrated that <1 % of the administered dose appeared as unchanged drug in the urine regardless of dose, which indicates that the drug is primarily eliminated through systemic metabolism.

Clinical activity

There was encouraging evidence of dose- and schedule-related control of tumor growth. Two patients had confirmed partial responses with BID dosing (Fig. 2): one patient with neuroendocrine tumor metastatic to the liver experienced a partial response in the 4-mg BID cohort; this response continues to be sustained at the reduced dose of 2 mg BID, and the patient received a total of 31 cycles. A partial response was also reported in a patient with melanoma metastatic to lung and mediastinum in the 6-mg BID cohort.

Seven patients receiving BID dosing had stable disease lasting 4 cycles of therapy, including five minor regressions (defined as a >10 to <30 % reduction in target lesions) in patients with neuroendocrine tumors ($n = 2$), endometrial cancer ($n = 1$), medullary renal cell carcinoma ($n = 1$), and colorectal cancer ($n = 1$). No responses were observed with the QD schedule, but a patient with colorectal cancer experienced a minor regression. Based on a proportional odds model for ordinal responses, the odds of experiencing a partial response or stable disease (versus progressive disease) were almost 14 times higher in patients who

received 4 or 6 mg BID than in patients who received 8 mg or 9 mg QD (odds ratio 13.998; 95 % CI 1.333–147.032, $n = 0.0279$) [31].

Meaningful tumor growth control (defined as a confirmed response plus stable disease for 4 cycles) was observed in nine evaluable patients (64 %) on BID dosing and six evaluable patients (27 %) on QD dosing. Overall, the BID dosing schedule produced a greater decrease in tumor size than QD dosing (Fig. 2). Furthermore, following BID dosing, minor regressions were observed in two of six patients previously treated with VEGF inhibitors.

DCE-MRI

Twenty-one patients had DCE-MRI scans, and 20 had adequate image-data quality for vascular response evaluation. A rapid decrease in tumor vascular parameters, as measured by mean K^{trans} and IAUC, could be seen on Day 2 after the PF-00337210 morning dose. As an example, Fig. 3 shows a patient with liver metastasis from a pancreatic neuroendocrine tumor (gastrinoma) who had a significant reduction in tumor vascular parameters and a confirmed partial response according to RECIST that continued after 25 cycles of therapy at cutoff date. An overall 31.8 % decrease in K^{trans} and an overall 22.6 % decrease in IAUC were demonstrated on Day 2 of therapy. Ten patients (50 %) were identified to be vascular responders based on the hierarchical mixed effect model analysis of K^{trans} (Supplementary Fig. 2). These included five of the six patients evaluated at BID doses, three patients at 9 mg QD, one patient on 8 mg QD, and one on 4 mg QD. Based on the hierarchical mixed effect model, 14 of 20 patients' 60 % CIs were below the reference line $y = 0$. However, four of these 14 patients had a one-sided p value (adjusted by FDR) of more than 0.2. Based on the criteria described in the "Patients and methods" section, only 10 (of 20) patients were therefore identified as vascular responders.

Soluble plasma proteins

PF-00337210 decreased sVEGFR-2 concentration to a much greater extent than sKIT, consistent with its proposed mechanism of action as a selective VEGFR-2 inhibitor. Supplementary Fig. 3 shows the effect of PF-00337210 on these soluble proteins in the 6-mg BID dosing cohort and the same endpoints for all patients combined. The greatest modulation of sVEGFR-2 was at 6 mg BID at C2D1: PF-00337210 treatment decreased sVEGFR-2, sVEGFR-3, and sKIT by 42, 38, and 12 %, respectively ($p < 0.01$ for sVEGFR-2 and sVEGFR-3 only). The selectivity of PF-00337210 for sVEGFR-2 and sVEGFR-3 was observed across all patients (Supplementary Fig. 4.)

Hemoglobin levels

A trend was seen toward increased levels of hemoglobin over time, suggesting an effect on erythropoiesis. Two patients developed erythrocytosis: one at 9 mg QD and the second at 6 mg BID. The first patient had a maximum level of hemoglobin of 19.5 g/dL during cycle 1. The second patient required a phlebotomy due to hemoglobin of 19.5 g/dL during cycle 8, and a subsequent dose reduction to 4 mg BID. No significant differences in hemoglobin levels were observed between QD and BID dose cohorts. Three patients received erythropoietic-stimulating agents during the study (one each at 2, 4, and 6 mg QD). One

further patient received one red cell transfusion after a grade 3 hemorrhage on Day 75 (outside the window analyzed).

Blood pressure

Supplementary Table 1 lists mean and median sitting sBP and dBP by dose cohort and treatment cycle. ANCOVA of the maximum change from baseline in dBP across visits demonstrated that dBP was significantly higher with BID dosing than with both high ($p = 0.0002$, two-sided) and low ($p = 0.0005$, two-sided) QD dosing. No significant differences were observed between QD low dose and QD high dose at significance level $\alpha = 0.05$, and no significant differences were found between QD and BID dosing for sBP. In general, sBP and dBP increased with dose level within the first two cycles of therapy and then stabilized, most probably due to the pharmacological treatment of hypertensive patients remaining in the study.

Hypertension severity

The proportional odds ratio model showed no significant association between hypertension severity and dose. Median AUC_{0-24} was numerically higher for patients who had dBP > 90 mmHg during study. All patients with target lesion regressions had hypertension; two patients with a confirmed partial response had mild and moderate hypertension. Of 14 patients with stable disease, mild, moderate, and severe hypertension was experienced by seven, four, and three patients, respectively.

Correlation between pharmacokinetic and pharmacodynamic endpoints

PF-00337210 exposure (AUC_{24} or C_{max}) was correlated with multiple PD endpoints, including DCE-MRI assessments (K^{trans} and IAUC), changes in dBP, and soluble proteins. Although the correlation of AUC versus K^{trans} demonstrated a trend toward increased exposure of PF-00337210, resulting in greater decreases in K^{trans} from baseline, this was not statistically significant ($R = 0.07$, $p = 0.4$) (data not shown). For the dBP versus PF-00337210 exposure analysis, patients were first categorized as having a dBP ≥ 90 mmHg (high dBP) or <90 mmHg (low dBP) at any time during the study. Patients who had a high dBP had a greater median (90 % CI) AUC_{24} (386 [135, 575] ng h/mL) than those who had a low dBP (223 ng h/mL [35, 384]). In addition, there was a significant trend between increasing exposure and increasing BP ($R = 0.4$, $p = 0.0009$) (see Supplementary Fig. 4). There is a trend between increasing exposure and decreasing sVEGFR-2 ($R^2 = 0.501$, $p = 6.76e-007$), sVEGFR-3 ($R^2 = 0.381$, $p = 0.00005$) and sKIT ($R^2 = 0.056$, $p = 0.090$), see Supplementary Fig. 5.

Discussion

The methods to recommend a dose after a phase I study have traditionally been based on convention, based on toxicity as opposed to an optimal biologic dose (OBD) [32, 33]. On the other hand, finding an OBD of a non-cytotoxic drug or determine dosing schedule is more challenging [33, 34]. The use of PD biomarkers in real-time during treatment with PF-00337210 is a unique strategy for determining the RP2D and the schedule of a novel VEGFR inhibitor because significant adverse events often occur outside the DLT window.

PF-00337210 was well tolerated up to a dose of 9 mg QD, when a DLT was observed. The possibility of $C_{\max}$ -driven toxicity and inconsistent pharmacodynamics at 9 mg QD led to the exploration of the BID schedule. PF-00337210 was well tolerated with BID dosing with only one DLT reported in 16 patients on this treatment schedule. The extent of exposure to PF-00337210 was significant as the median number of cycle ranges from 4.5 to 6.5 cycles at the BID doses (Table 2). This is relevant for targeted therapy usually administered in an oral chronic fashion, for which late or moderate adverse events deserve particular attention [32].

Hypertension was a common adverse event, as observed with other VEGF/VEGFR inhibitors [15, 20, 35, 36]. Although the incidence and intensity increased with dose in the present study, hypertension was well controlled with antihypertensive medications. Diastolic BP was significantly higher with BID dosing than QD dosing, and no significant association was noted between hypertension severity and dose. All patients with target lesion regressions (including confirmed responses) had hypertension during therapy. The incidence of gastrointestinal adverse events, commonly found in other VEGFR inhibitors [11, 12, 14, 16, 17], was relatively low with PF-00337210, denoting selectivity. The good tolerability profile of PF-00337210 is encouraging, given that other less-selective VEGFR inhibitors have been limited by poor long-term tolerability, particularly when combined with chemotherapy.

Patients treated on the BID dosing had more consistent changes in PD markers including reduction in tumor vascular parameters. Five (83 %) out of 6 patients in the BID group demonstrated significant changes in vascular effects on DCE-MRI, compared to 5 (36 %) out of 14 patients in the QD dosing group. Most of the responders in the QD schedule were at the toxic dose level (9 mg QD). Patients treated at the BID dosing also demonstrated decreases in tumor size or confirmed partial responses.

At 6 mg BID, PF-00337210 plasma concentrations were at VEGFR inhibitory levels in the majority of time points. One may speculate that BID dosing of PF-00337210 increases drug exposure with a lower $C_{\max}$, and that this may lead to a better therapeutic index than with multi-target tyrosine kinase inhibitors. The patient with DLT at 6 mg BID (grade 3 palmar-plantar erythrodysesthesia) had higher drug concentrations, and with concentrations outside the 95 % confidence interval was considered an outlier (Fig. 1b). The analysis of soluble proteins showed clinical selectivity for VEGFR-2 compared with other tyrosine kinases. Continued treatment with PF-00337210 was associated with rising hemoglobin levels over time, consistent with an effect on the VEGF signaling pathway and an effect on erythropoiesis, but hemoglobin levels seem to be a suboptimal measurement relative to other biomarkers.

For PK/PD analyses, PF-00337210 exposure was measured using AUC_{24} and $C_{\max}$. Although the correlation of AUC versus K^{trans} demonstrated a trend toward increased exposure of PF-00337210, resulting in greater decreases in K^{trans} from baseline, this was not statistically significant ($R = 0.07$, $p = 0.4$; data not shown). The lack of statistical significance for AUC versus K^{trans} might be due to a small sample of patients, different schedules of administration, heterogeneity in histology and previous exposure to anti-VEGF/VEGFR therapy. There were also notable associations between increasing exposure to

PF-00337210 and an increase in BP or decreasing sVEGFR-2 levels. These associations were significant despite a small sample size.

As demonstrated in this clinical trial, we encountered a DLT with QD dosing suggesting that some toxicity was related to $C_{\max}$ (e.g., hypertension), but did not see overt clinical activity with QD dosing. However, use of BID dosing resulted in an improvement in vascular effects, changes in biomarker activity, and a more consistent and positive clinical effect, and improved toxicity profile. As a comparison, sunitinib has a 40- to 60-h plasma half-life and is administered QD, but requires a treatment break to minimize drug accumulation [10]. Axitinib has a 4- to 6-h half-life and is administered BID [36]. It was anticipated based on initial PK determination that PF-00337210 could be administered QD given a plasma half-life of 10–14 h. However, the PD assessments during this study showed that the BID dosing resulted in improved changes in vascular parameters and eventually showed improved clinical activity.

We determined PF-00337210 6 mg BID to be the RP2D, based on significant PD findings and the safety profile, with some patients possibly requiring dose reductions over time. Analysis of PK and PD data in real time provided the opportunity of a dynamic approach with an efficient and safe patient accrual. These relevant biomarkers for on-target effects (including functional imaging of the tumors) helped to select the schedule of administration and to determine the OBD range.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Pharmacokinetic profile of PF-00337210. **a** Plasma concentration–time profiles for all PF-00337210 dose cohorts at steady-state. Target concentration: IC₅₀ for VEGFR-2 inhibition (8 ng/mL) represented by the *dashed black horizontal line*. **b** Plasma concentration–time profiles for PF-00337210 6 mg BID. Target concentration: IC₅₀ for VEGFR-2 inhibition (8 ng/mL) represented by the *dashed black horizontal line*. The patient with DLT at 6 mg BID (grade 3 palmar–plantar erythrodysesthesia) had higher drug concentrations outside the 95 % CI (outlier). **c** PF-00337210 dose linearity by area under the concentration–time curve from 0 to 24 h (AUC₂₄) after single dose

Fig. 2.

Best percentage change in target lesions for PF-00337210. **a** QD dosing, *VEGF* vascular endothelial growth factor. **b** BID dosing, * <1 % change ($n = 2$); no target entered ($n = 1$)

Fig. 3.
Representative dynamic contrast-enhanced magnetic resonance images from a patient with a liver metastasis from a pancreatic neuroendocrine tumor (gastrinoma) receiving PF-00337210 at 4 mg BID

Table 1

Patient baseline characteristics

	Total N = 46
Median age, years (range)	55 (19–86)
Male/female [<i>n</i> (%)]	25 (54)/21 (46)
ECOG performance status [<i>n</i> (%)]	
0	11 (24)
1	30 (65)
2	5 (11)
Primary tumor site [<i>n</i> (%)]	
Colorectal	8 (17)
Neuroendocrine	6 (13)
Renal cell carcinoma ^a	5 (11)
Sarcoma	4 (9)
Pancreatic	4 (9)
Other ^b	19 (41)
Prior systemic therapy	
Prior therapy [<i>n</i> (%)]	45 (96)
Median number of prior therapies (range)	5 (0–10)
Prior therapy with VEGF/VEGFR inhibitors [<i>n</i> (%)]	19 (41)

ECOG Eastern Cooperative Oncology Group, VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor

^aIncludes one patient with medullary carcinoma

^bIncludes non-small cell lung (*n* = 3), ovarian (*n* = 3), melanoma (*n* = 3), breast (*n* = 2), head and neck squamous cell (*n* = 2), bladder (*n* = 2), gastric (*n* = 1), endometrial (*n* = 1), esophageal (*n* = 1), small cell carcinoma with unknown primary site (*n* = 1)

Table 2

Dose escalation and summary of cycle 1 dose-limiting toxicities (DLTs)

PF-0037210 dose level	Median number of cycles (range)	Patients (n)		Patients with cycle 1 DLT	
		Treated	Evaluable		
0.67 mg QD	3	1	1	–	
1 mg QD	4	1 ^a	1	–	
2 mg QD	2 (2–4)	3	3	–	
4 mg QD	2 (2–3)	3	3	–	
6 mg QD	2 (1–5)	7	6	–	
9 mg QD	1.5 (1–9)	8 ^b	5 (MAD)	(1) Myocardial ischemia (with chest pain), denoted by grade 3 troponin levels (2) hypertension: systolic mmHg over 3 h	180
8 mg QD	2 (1–5)	7	6 (MTD)	–	
4 mg BID	6.5 (2–25)	4 ^c	4	–	
6 mg BID	4.5 (2–20)	12 ^d	RP2D	(1) Grade 3 palmar–plantar erythrodysesthesia	

BID twice a day, *MAD* maximum administered dose, *MTD* maximum tolerable dose, *QD* once a day, *R2PD* recommended phase 2 dose

^aDose was escalated to 2 mg QD

^bDose was reduced to 6 mg QD in three patients, one due to a treatment-related adverse event (DLT: hypertension) and two due to treating physician's decision after declaring 9 mg the MAD

^cDose was reduced to 3 mg BID in two patients (grade 3 palmar–plantar erythrodysesthesia and grade 3 transaminitis, dose was subsequently reduced to 2 mg BID due to another episode of grade 3 transaminitis)

^dDose was reduced to 4 mg BID in five patients (The DLT: grade 3 palmar–plantar erythrodysesthesia; others: erythrocytosis, transient grade 3 bilirubin increase, grade 3 hypertension, and persistent grade 2 fatigue)

Most frequently reported all-cycle, treatment-related adverse events	10 % of patients	adverse events of special interest
<p> Most frequently reported all-cycle, treatment-related adverse events </p>	<p> 10 % of patients </p>	<p> adverse events of special interest </p>

BID twice a day, *PPE* palmar-plantar erythrodysesthesia, *TEE* thromboembolic event (myocardial ischemia), *QD* once a day

^b Gastrointestinal hemorrhage, anal hemorrhage, skin neoplasm bleeding, tumor hemorrhage, urogenital hemorrhage. No patients experienced grade 4 or 5 treatment-related toxicity