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ORIGINAL REPORT

Phase I Dose-Escalation Trial of PT2385, a First-in-Class Hypoxia-Inducible Factor-2α Antagonist in Patients With Previously Treated Advanced Clear Cell Renal Cell Carcinoma

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A B S T R A C T

Purpose
The von Hippel-Lindau tumor suppressor is inactivated in the majority of clear cell renal cell carcinomas (ccRCCs), leading to inappropriate stabilization of hypoxia-inducible factor-2α (HIF-2α). PT2385 is a first-in-class HIF-2α antagonist. Objectives of this first-in-human study were to characterize the safety, pharmacokinetics, pharmacodynamics, and efficacy, and to identify the recommended phase II dose (RP2D) of PT2385.

Patients and Methods
Eligible patients had locally advanced or metastatic ccRCC that had progressed during one or more prior regimens that included a vascular endothelial growth factor inhibitor. PT2385 was administered orally at twice-per-day doses of 100 to 1,800 mg, according to a 3 + 3 dose-escalation design, followed by an expansion phase at the RP2D.

Results
The dose-escalation and expansion phases enrolled 26 and 25 patients, respectively. Patients were heavily pretreated, with a median of four (range, one to seven) prior therapies. No dose-limiting toxicity was observed at any dose. On the basis of safety, pharmacokinetic, and pharmacodynamic profiling, the RP2D was defined as 800 mg twice per day. PT2385 was well tolerated, with anemia (grade 1 to 2, 35%; grade 3, 10%), peripheral edema (grade 1 to 2, 37%; grade 3, 2%), and fatigue (grade 1 to 2, 37%; no grade 3 or 4) being the most common treatment-emergent adverse events. No patients discontinued treatment because of adverse events. Complete response, partial response, and stable disease as best response were achieved by 2%, 12%, and 52% of patients, respectively. At data cutoff, eight patients remained in the study, with 13 patients in the study for ≥ 1 year.

Conclusion
PT2385 has a favorable safety profile and is active in patients with heavily pretreated ccRCC, validating direct HIF-2α antagonism for the treatment of patients with ccRCC.

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INTRODUCTION

Although targeted therapies have considerably improved the prognosis for patients with clear cell renal cell carcinoma (ccRCC),[1] only 8%[2] to 11.7% of patients[3] with metastatic disease will survive 5 years. Thus, more effective therapies are needed.

Inactivation of the tumor suppressor gene von Hippel-Lindau (*VHL*) occurs in the majority of ccRCC patients.[4-8] The VHL protein (pVHL) is a component of an E3 ubiquitin ligase that mediates protein degradation by the proteasome. Among the proteins targeted by pVHL is the transcription factor hypoxia-inducible factor-2α (HIF-2α), an oncogenic driver in ccRCC.[4,8] Under normal oxygen tension, HIF-2α is hydroxylated on specific proline residues, a modification that is required for recognition by pVHL. As a result, HIF-2α is rapidly degraded in cells in normal oxygen conditions (Fig 1). In hypoxic conditions, the prolyl hydroxylases that perform the post-translational modification of HIF-2α are inactivated, and HIF-2α is stabilized. HIF-2α heterodimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT; also known as HIF-1β) to form an active transcription factor. This allows increased expression of hypoxia-inducible genes,
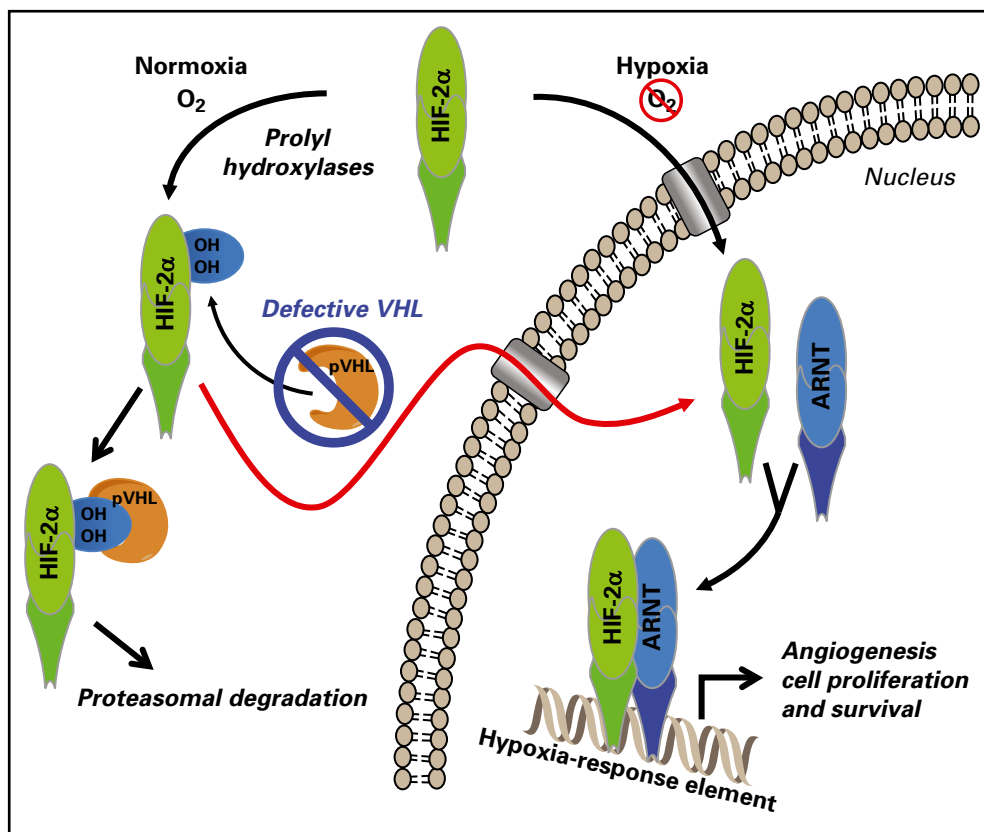
Fig 1. Hypoxia-inducible factor-2α (HIF-2α) inhibition and the von Hippel-Lindau (*VHL*)/HIF axis in renal cell carcinoma. Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; pVHL, VHL protein.

such as erythropoietin (*EPO*), which increase the production of red blood cells, resulting in increased oxygenation.[9] Loss of pVHL function, through mutation, hypermethylation, deletion, or other genomic alterations of *VHL*, results in constitutive stabilization of HIF-2α even in normoxic conditions. In ccRCC tumor cells with pVHL deficiency, HIF-2α upregulates the expression of genes that are important to tumor growth and metastasis, including those that encode cyclin D1 (*CCND1*), vascular endothelial growth factor A (*VEGFA*), transforming growth factor α, and C-X-C chemokine receptor 4.[8,10]

Currently approved therapies for ccRCC include inhibitors of vascular endothelial growth factor (VEGF) and VEGF receptor tyrosine kinases. These inhibitors provide therapeutic benefit because ccRCC is highly dependent on tumor angiogenesis through VEGF signaling. Because *VEGFA* is one of the target genes of HIF-2α, inhibition of HIF-2α transcriptional activity would be expected to inhibit tumor angiogenesis in ccRCC by inhibiting HIF-2α–induced upregulation of *VEGFA* expression. HIF-2α antagonism is also expected to impede tumor growth by inhibiting HIF-2α–induced upregulation of genes such as *CCND1* and *TGFA*, which promote tumor cell proliferation but are independent of the VEGF-signaling pathway. Therefore, HIF-2α antagonism in ccRCC may provide greater efficacy than is achieved with VEGF receptor kinase inhibitors.

Structural analyses have identified a ligand-binding pocket in the Per-ARNT-Sim (PAS)-B domain of HIF-2α,[11,12] an observation that was followed by the discovery of synthetic small molecules that occupy the pocket, including PT2385.[13] PT2385 disrupts HIF-2α/ARNT heterodimerization and inhibits HIF-2α target gene

expression. In a mouse xenograft model of ccRCC, PT2385 treatment resulted in decreased expression of HIF-2α target genes, decreased circulating human VEGF-A protein, and increased tumor cell apoptosis.[13] PT2385 treatment also resulted in regression of mouse tumor xenografts derived from the VHL-deficient human renal cell cancer (RCC) cell line 786-O, whereas treatment with the standard-of-care VEGFR tyrosine kinase inhibitor sunitinib resulted in tumor stasis. In addition, PT2385 inhibited growth of xenografts derived from a human ccRCC tumor refractory to sunitinib and the mammalian target of rapamycin inhibitor everolimus.[13] On the basis of these results, a first-in-human, phase I, dose-escalation trial of the first-in-class oral HIF-2α antagonist PT2385 was conducted in patients with advanced ccRCC previously treated with one or more VEGF inhibitors.

PATIENTS AND METHODS

Patients

This study enrolled patients at six centers between November 2014 and February 2016 and is still ongoing. The data cutoff date for this report is March 9, 2017. Patients had locally advanced or metastatic RCC with a clear cell component and had disease progression during treatment with one or more prior regimens that included a VEGF inhibitor. Eligible patients were $\geq$ 18 years of age, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and had adequate bone marrow (absolute neutrophil count $\geq 1.5 \times 10^9$/L; hemoglobin $\geq$ 9 g/dL; platelets $\geq 1 \times 10^5$/μL), hepatic (AST/ALT $\leq$ 2.5 × upper limit of normal; total bilirubin $\leq$ 2.0 mg/dL), and renal (serum creatinine < 2 × upper limit of normal or creatinine clearance $\geq$ 50 mL/min) function. Patients in the expansion cohort were required to have measurable disease per

Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1).[14] Patients with untreated brain metastases, uncontrolled or poorly controlled hypertension, a major cardiac event in the prior 6 months, and malabsorption as a result of prior gastrointestinal surgery or gastrointestinal disease, and those requiring warfarin anticoagulation were excluded.

All patients provided written informed consent. The study protocol was approved by institutional review boards at all participating institutions. The study (NCT02293980) was conducted in accordance with good clinical practice and the Declaration of Helsinki.

Study Design and Treatment

The primary objective of this phase I, open-label, multicenter, dose-escalation trial was to identify the maximum tolerated dose (MTD) and/or the recommended phase II dose (RP2D) of PT2385 in patients with advanced ccRCC. Secondary objectives were to evaluate the safety, pharmacokinetic (PK), and pharmacodynamic (PD) profiles, and to assess the antitumor activity of PT2385 in this patient population.

In a standard 3 + 3 design,[15] cohorts of three to six patients were treated with oral PT2385 twice per day. A starting dose of 100 mg twice a day was selected on the basis of results of preclinical toxicology studies (data not shown). Dose levels were increased by 100% until the first drug-related grade ≥ 2 toxicity was reported. Thereafter, dose increases were by $\leq 50\%$. Patients at each dose level had to be treated for a minimum of 3 weeks before enrollment occurred at the next dose level. Dose-limiting toxicity (DLT) was defined as febrile neutropenia; grade 4 neutropenia lasting for > 5 days; grade 4 thrombocytopenia (or grade 3 with bleeding); grade 4 anemia unexplained by underlying disease; grade 3 or 4 nausea, vomiting, or diarrhea persisting for > 72 hours despite optimal treatment; grade ≥ 3 increased transaminase levels; any other grade ≥ 3 non-hematologic toxicity; any other grade ≥ 3 laboratory toxicity not resolving within 72 hours and considered clinically significant by the investigator; or any PT2385-related toxicity resulting in discontinuation before 21 days of treatment. After determination of the MTD or RP2D, ≤ 25 patients were to be enrolled in the expansion portion.

Assessments

Blood samples for PK and PD assessment were drawn in patients under fasted conditions 1 hour before and 1, 1.5, 2, 4, 6, 8, 12, and 24 hours after PT2385 administration on day 1 of weeks 1 and 3, and before drug administration on day 1 of weeks 2, 4, 5, 6, 7, 10, 13, and 16, and every 12 weeks thereafter. The evening doses on day 1 of weeks 1 and 3 were withheld to allow for full 24-hour PK assessments. Area under the concentration-time curve (AUC), maximum concentration (C_{max}), time of maximum concentration (T_{max}), and terminal half-life ($T_{1/2}$) were determined. The plasma concentrations of PT2385 were determined using a validated liquid chromatography-tandem mass spectrometry method. Plasma Epo was measured with the Epo Access Immunoassay System (Beckman Coulter, Brea, CA).

Adverse events (AEs) were coded using Medical Dictionary for Regulatory Activities version 16 terminology. AE severity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 or later. Tumors were assessed by contrast or non-contrast computed tomography or magnetic resonance imaging and/or physical examination at baseline within 7 days before the week 7 visit and every 9 weeks thereafter. Tumor responses were evaluated using RECIST 1.1.[14] Patients were considered to have stable disease (SD) if they had SD at their first restaging scan.

Statistical Analyses

The sample size was based on the need to establish the MTD and/or RP2D (≤ 48 patients), and ≤ 25 additional patients were to be enrolled in the expansion cohort. All statistical analyses were performed using SAS version 9.3 or higher (SAS Institute, Cary, NC). PK analyses were performed using

Phoenix WinNonlin software (Certara, Princeton, NJ). Best response by RECIST and progression-free survival (PFS) time were investigator determined. Baseline patient and disease characteristics, efficacy, PK, and safety data were summarized. Cox proportional hazards regression models were used to identify factors potentially related to PFS (Appendix, online only).

RESULTS

Patient Characteristics

Twenty-six patients were enrolled in the dose-escalation portion, and 25 were enrolled in the expansion portion of the study. The majority of patients (71%) were male (Table 1). Most patients (69%) had an Eastern Cooperative Oncology Group performance status of 1. The median number of prior systemic therapies was four. All patients had been previously treated with VEGF-targeted therapy. The majority (61%) had also been treated with mammalian

Table 1. Patient Demographics and Baseline Treatment Characteristics

Characteristic	All Patients (N = 51)
Median age, years (range)	65 (29-80)
Sex	
Female	15 (29)
Male	36 (71)
ECOG PS	
0	16 (31)
1	35 (69)
Prior nephrectomy	42 (82)
Sites of metastases	
Lung	33 (65)
Node	22 (43)
Bone	19 (37)
Liver	12 (24)
Adrenal	11 (22)
Pancreas	8 (16)
Prior systemic therapies, median, No. (range)	4 (1-7)
Prior systemic therapies	
1	6 (12)
2	9 (18)
3	9 (18)
4	11 (22)
≥ 5	16 (31)
Prior anticancer therapies	
VEGF/VEGFR	51 (100)
mTOR	31 (61)
Cytokine	9 (18)
Immune checkpoint inhibitor*	11 (22)
Investigational/other	13 (25)
MSKCC score†	
Favorable risk (0 factors)	12 (24)
Intermediate risk (1-2 factors)	37 (72)
High risk (≥ 3 factors)	2 (4)

NOTE. Data are shown as No. (%) except where otherwise noted.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; LLN, lower limit of normal; MSKCC, Memorial Sloan Kettering Cancer Center; mTOR, mammalian target of rapamycin; ULN, upper limit of normal; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
*Immune checkpoint inhibitors included programmed cell death protein 1 and programmed death-ligand 1–targeted therapies.
†MSKCC Score is based on presence of five factors: interval from diagnosis to treatment < 1 year, Karnofsky performance status < 80, serum LDH > 1.5 × ULN, corrected serum calcium > ULN, and hemoglobin < LLN.

target of rapamycin inhibitors, and 39% had received prior immune checkpoint inhibitors (22%) and/or cytokines (18%).

Dose Escalation and MTD

No DLT was observed at the initial dose of 100 mg twice a day (n = 3) or at twice-a-day doses of 200 mg (n = 3), 400 mg (n = 4), 800 mg (n = 7), 1,200 mg (n = 6), or 1,800 mg (n = 3). Two days after the end of the 21-day DLT period, one patient in the 800-mg group experienced a grade 4 pulmonary embolism that was considered possibly related to PT2385 by the investigator in the context of disease progression. Following the consensus recommendation of the investigators, four more patients were treated at 800 mg twice a day and three at 1,200 mg twice a day to obtain additional safety, PK, and PD data. No patients experienced a DLT at any dose ≤ 1,800 mg twice a day; thus, an MTD was not identified.

PK and PD

Exposure to PT2385 was variable and increased up to 800 mg twice a day (Fig 2A). No additional increases were seen up to the highest dose level of 1,800 mg twice a day. At day 15, PT2385 (800 mg) was rapidly absorbed (median T_{max}, 2 hours) with a mean C_{max} of 3.1 μg/mL and a mean $T_{1/2}$ of 17 hours. A 2.5-fold accumulation in AUC was observed from day 1 to day 15. Similar values were observed when all available data at 800 mg twice a day

were combined (dose escalation plus expansion cohort; Fig 2A). Rapid reductions in plasma Epo were observed at all dosing levels (Fig 2B). Doses > 800 mg twice a day did not result in greater reductions in Epo. Because the MTD was not reached, the RP2D was based on the observed safety, PK, and PD data, and was determined to be 800 mg twice a day.

Safety

Treatment-emergent AEs occurring in > 10% of patients are listed in Table 2. The majority of AEs were grade 1 or 2 (Table 2). The most common all-grade AEs were anemia (45%), peripheral edema (39%), and fatigue (37%). Peripheral edema was not associated with thromboembolic events or cardiovascular toxicity. The most common grade ≥ 3 AEs were anemia (10%, all grade 3), hypoxia (10%, all grade 3), lymphopenia (8%; 4%, grade 3, 4%, grade 4), and hypophosphatemia (8%, all grade 3). Anemia was asymptomatic. Two patients each required a single prophylactic transfusion of packed red blood cells. Lymphopenia was not associated with infections. The only serious AE occurring in > 1 patient was hypoxia, which occurred in two patients treated at the 1,200-mg twice-a-day dose level.

There were five dose interruptions (dose range, 400 to 1,800 mg twice a day) and two dose reductions (one each at 800 mg twice a day and 1,200 mg twice a day) for AEs. No patients discontinued treatment because of AEs, and there were no deaths during the study. Reasons for
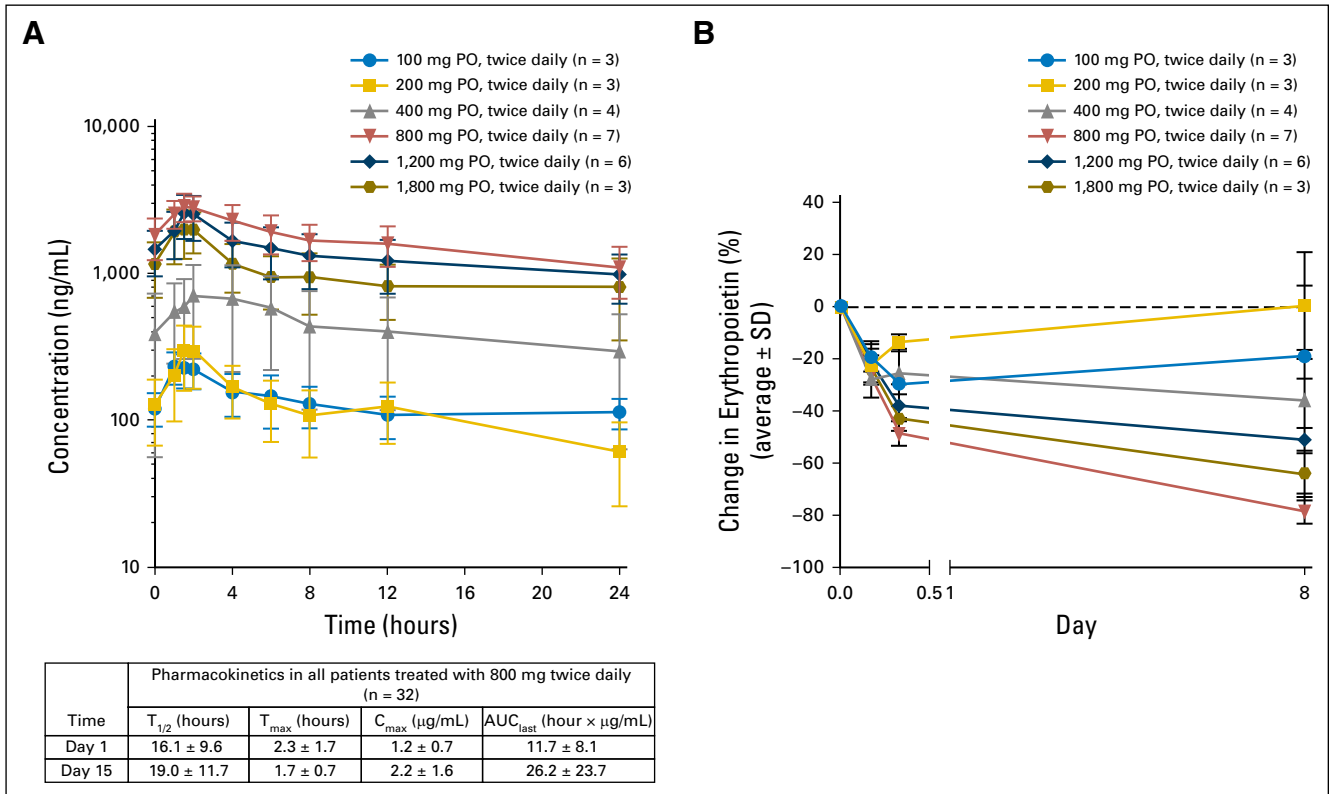

Time	Pharmacokinetics in all patients treated with 800 mg twice daily (n = 32)			
	$T_{1/2}$ (hours)	T_{max} (hours)	C_{max} (μg/mL)	AUC_{last} (hour × μg/mL)
Day 1	16.1 ± 9.6	2.3 ± 1.7	1.2 ± 0.7	11.7 ± 8.1
Day 15	19.0 ± 11.7	1.7 ± 0.7	2.2 ± 1.6	26.2 ± 23.7

Fig 2. Pharmacokinetics of PT2385. (A) Mean PT2385 plasma concentration, day 15, in all cohorts in the dose escalation phase. (Bottom) Pharmacokinetics in all patients (dose escalation and expansion cohorts; n = 32) treated with PT2385 at the recommended phase II dose (800 mg twice per day). (B) Pharmacodynamic response as assessed by decreases in the hypoxia-inducible factor-2α (HIF-2α) target erythropoietin. Plasminogen activator inhibitor-1, insulin-like growth factor-binding protein 3, and vascular endothelial growth factor A also were assessed as potential pharmacodynamic markers but no significant relationships with PT2385 exposure were observed. Abbreviations: AUC$_{last}$, area under the concentration-time curve from time 0 to the last quantifiable time point; C$_{max}$, maximum concentration; PO, orally; SD, standard deviation; T$_{1/2}$, terminal half-life; T$_{max}$, time of maximal concentration.

Table 2. Treatment-Emergent Adverse Events in > 10% of Patients

Adverse Event	All Patients (all doses; N = 51)			
	Grade 1/2	Grade 3	Grade 4*	All Grades
Any	29 (57)	17 (33)	4 (8)	50 (98)
Anemia	18 (35)	5 (10)	0 (0)	23 (45)
Peripheral edema	19 (37)	1 (2)	0 (0)	20 (39)
Fatigue	19 (37)	0 (0)	0 (0)	19 (37)
Nausea	17 (33)	0 (0)	0 (0)	17 (33)
Back pain	13 (25)	0 (0)	0 (0)	13 (25)
Lymphocyte count decreased	8 (16)	2 (4)	2 (4)	12 (24)
Weight increased	10 (20)	1 (2)	0 (0)	11 (22)
Arthralgia	9 (18)	1 (2)	0 (0)	10 (20)
Pain in extremity	9 (18)	1 (2)	0 (0)	10 (20)
Constipation	9 (18)	0 (0)	0 (0)	9 (18)
Cough	9 (18)	0 (0)	0 (0)	9 (18)
Dizziness	9 (18)	0 (0)	0 (0)	9 (18)
Hypoxia	4 (8)†	5 (10)	0 (0)	9 (18)
Insomnia	9 (18)	0 (0)	0 (0)	9 (18)
Blood creatinine increased	8 (16)	0 (0)	0 (0)	8 (16)
Hyperkalemia	8 (16)	0 (0)	0 (0)	8 (16)
Hypophosphatemia	4 (8)	4 (8)	0 (0)	8 (16)
Abdominal pain	7 (14)	0 (0)	0 (0)	7 (14)
Myalgia	7 (14)	0 (0)	0 (0)	7 (14)
Platelet count decreased	6 (12)	1 (2)	0 (0)	7 (14)
Vomiting	7 (14)	0 (0)	0 (0)	7 (14)
Decreased appetite	6 (12)	0 (0)	0 (0)	6 (12)
Diarrhea	6 (12)	0 (0)	0 (0)	6 (12)
Dyspnea	6 (12)	0 (0)	0 (0)	6 (12)
Hyperglycemia	4 (8)	2 (4)	0 (0)	6 (12)
Hyponatremia	4 (8)	2 (4)	0 (0)	6 (12)
Pyrexia	6 (12)	0 (0)	0 (0)	6 (12)
Urinary tract infection	5 (10)	1 (2)	0 (0)	6 (12)

NOTE. Data are shown as No. (%)
*Grade 4 adverse events were lymphocyte count decreased (n = 2); hypercalcemia; and pulmonary embolism.
†All grade 2; there is no grade 1 hypoxia per National Cancer Institute Common Terminology Criteria for Adverse Events.

treatment discontinuation (n = 43) were progressive disease (41 patients; 95%), patient decision (one patient; 2%), and palliative radiotherapy (one patient; 2%).

Efficacy

Fifty-one patients were treated, and 50 were evaluable for response. As best response, one patient (2%) had a complete response (CR), six (12%) had a partial response (PR), 26 (52%) had SD, and 17 (34%) had tumor progression. The disease control rate (CR plus PR plus SD) was 66%. Twenty-one patients (42%) had SD or better for ≥ 4 months. The CR occurred in a patient receiving the 200-mg twice-a-day dose (Fig 3). PRs occurred in one patient receiving the 1,200-mg twice-a-day dose, four patients receiving the 800-mg twice-a-day dose, and one patient receiving the 400-mg twice-a-day dose. Thirteen patients remained in the study for ≥ 1 year (Fig 4). At a median follow-up of 17.5 months, 25% of patients had PFS > 14 months.

An analysis of the relationship between PK and Memorial Sloan Kettering Cancer Center Score, a risk score that incorporates prognostic factors associated with survival in patients with previously treated metastatic RCC,[17] with PFS was performed (Appendix). The variable with the most significant effect on PFS was the trough PT2385 concentration at day 15, 12 hours postdose (P = .002). Notably, 16 of

26 patients (62%) with a PT2385 trough concentration ≥ 0.5 μg/mL had SD or better for ≥ 4 months, whereas only four of 22 patients (18%) with a PT2385 trough concentration of < 0.5 μg/mL had SD or better for ≥ 4 months (Appendix Fig A1, online only).

DISCUSSION

There is a growing body of evidence that HIF-2α is a major oncogenic driver in VHL-deficient ccRCC.[4,6-8] In contrast to many currently available therapies, which inhibit a single signaling pathway, direct inhibition of the transcriptional activity of HIF-2α has the potential to inhibit a number of pathways that contribute to tumor growth in ccRCC. However, to date, there have been no viable HIF-2α inhibitors for clinical use.

Targeting protein–protein interactions with small molecules for therapeutic applications has proven to be challenging.[18] Crystallographic studies identified a unique 290Å cavity in the PAS-B domain of HIF-2α that could accommodate a small molecule, which might disrupt heterodimerization with ARNT.[11,12] Iterative structure-based drug design was used to optimize candidate compounds, leading to the discovery of PT2385.[13] Occupancy of the HIF-2α PAS-B domain pocket results in a conformation change in HIF-2α and destabilization of its interaction with ARNT. As such, the mechanism of action of PT2385 involves an allosteric disruption of a protein–protein interaction between HIF-2α and ARNT. Recent data have shown that PT2385 is highly selective in its disruption of the HIF-2α/ARNT heterodimer and has no effect on the HIF-1α/ARNT heterodimer.[13] PT2385 has shown efficacy in ccRCC xenograft models, including one derived from a tumor refractory to sunitinib and everolimus.[13]

PT2385 is the first HIF-2α inhibitor to be tested in humans and to show activity in patients with ccRCC. PT2385 treatment resulted in rapid and pronounced decreases in Epo (known to be regulated by HIF-2α[9]) at all doses, demonstrating target engagement and biologic activity. PT2385 did not cause DLT at any dose, including those as high as 1,800 mg twice a day. Both PK and PD profiling indicated maximum compound exposure at 800 mg twice a day, establishing this as the RP2D.

A limitation of this study is the lack of a priori statistical calculation in selecting the size of the expansion cohort. However, in a heavily pretreated population with few treatment options, a response rate > 5% would be reasonable hypothesis-generating evidence that a compound warrants additional investigation. A sample of ≥ 25 patients provides a 95% lower confidence limit for overall response that excludes 5% if the observed response rate is ≥ 10%. Thus, the observed 14% response rate supports additional investigation of PT2385 efficacy in a larger patient population.

Drug exposure variability was observed in patients treated with PT2385, and greater exposure seemed to be correlated with longer PFS. The effect of administered dose and plasma trough levels of PT2385 on clinical activity should be confirmed in ongoing studies of PT2385. Preliminary data with a structurally related compound in clinical development do not show this variable drug exposure (data not shown).

In this study, PT2385 had a favorable safety profile, with most treatment-emergent AEs being grade 1 or 2, and no patients discontinuing treatment owing to AEs. Anemia, the most frequently reported treatment-emergent AE in this study, may be the result
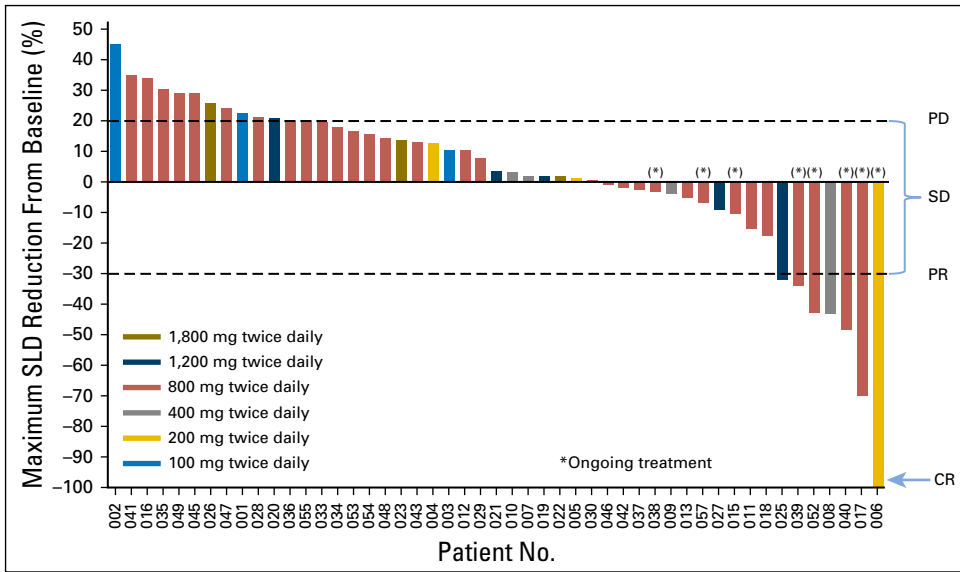
Fig 3. Maximum radiographic response (n = 49). Note that one patient discontinued the study because of clinical progression before the first scheduled radiologic assessment. Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; SLD, sum of the longest diameter.

of on-target effects of HIF-2α antagonism as reflected in reduced Epo, which functioned as a PD biomarker. Anemia was predominantly low-grade (grade 1 or 2, 35%; grade 3, 10%; no grade 4). During the course of the study, hypoxia observed by routine pulse oximetry monitoring emerged as a likely treatment-emergent AE (nine of 51 patients, 18%) and, along with anemia, was the most common grade ≥ 3 AE (n = 5 for each). All nine instances occurred in patients treated at doses ≥ 800 mg twice a day. Seven of

the nine events occurred during the first 5 weeks of therapy. PT2385 dosing was interrupted in two patients; one resumed the study drug within 1 week with no additional episodes of hypoxia, and one was taken out of the study for progressive disease. It should be noted that pulse oximetry was not a required measurement per the study protocol; thus, the incidence of hypoxia may be underreported. A possible mechanistic link to PT2385 is suggested by a recent study showing that HIF-2α plays a role in ventilatory
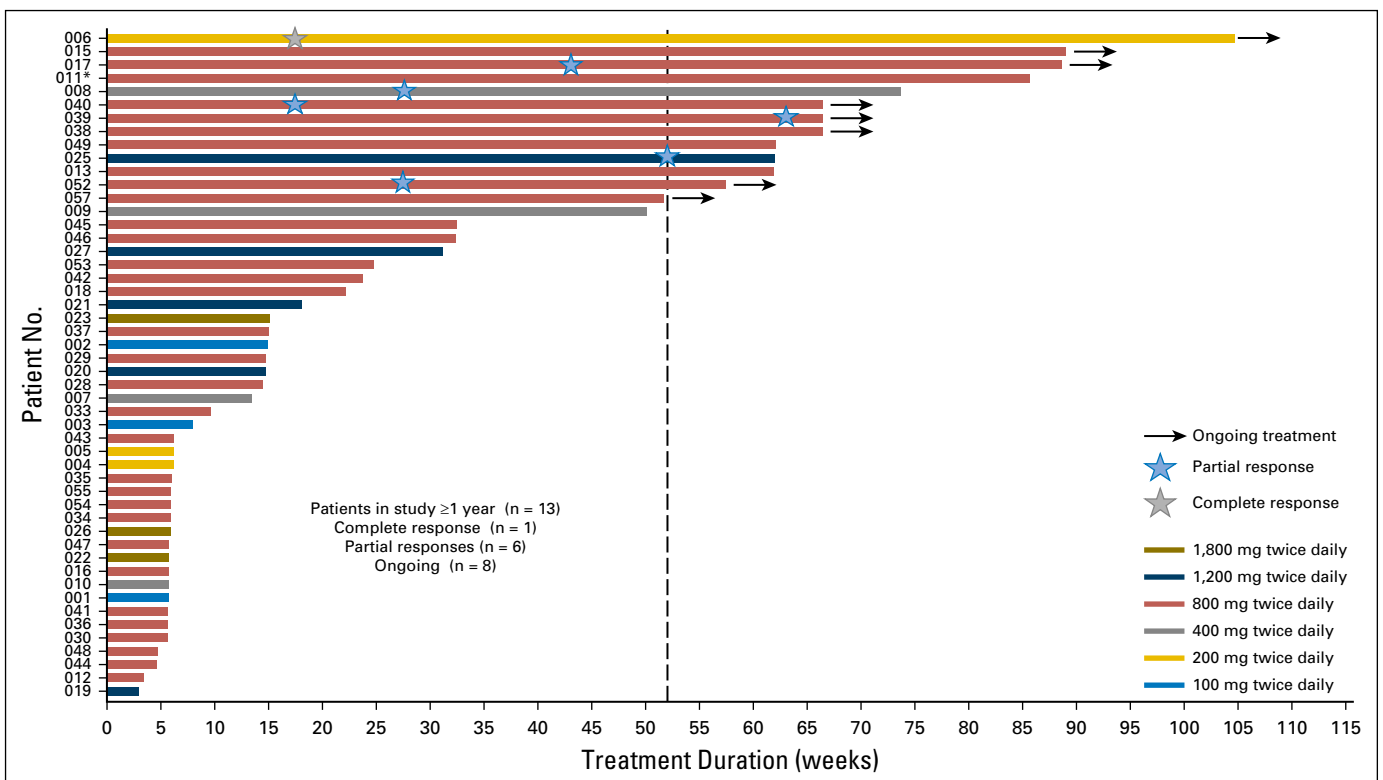

Fig 4. Treatment duration in all patients evaluable for response (n = 50). (*)The case of patient 11 was previously referenced.[16]

control and carotid body biology in rodents.[19] Alternatively, HIF-2α inhibition may lead to an impaired pulmonary vascular response to hypoxia.[20-22]

In this phase I dose-escalation study, PT2385 showed promising efficacy in a highly pretreated patient population (31% of patients received five or more prior therapies). Although many caveats necessarily apply to cross-trial comparisons, other phase I studies of targeted agents in heavily pretreated ccRCC populations, where drugs were dose-reduced or discontinued in a large number of patients, may be helpful in providing a context for these findings.[23,24] In this study, there were relatively few dose interruptions, two dose reductions, and no treatment discontinuations as a result of AEs. Interestingly, unlike VEGF signaling inhibitors,[25] PT2385 was not observed to cause hypertension or apparent cardiac toxicity. The favorable tolerability profile of PT2385 over time was evident in that 21 patients with SD or better remained in the study for ≥ 4 months, and 13 patients remained in the study for ≥ 1 year without discontinuation as a result of drug intolerability. Also, one patient previously treated with sunitinib and temsirolimus achieved a CR on single-agent PT2385, continuing to receive treatment for nearly 2 years at the time of data cutoff.

The favorable tolerability profile and activity of PT2385 monotherapy provide a rationale for exploring PT2385 in combination with other active agents for ccRCC. The second part of this study, therefore, investigates PT2385 plus the anti–programmed death ligand 1 monoclonal antibody nivolumab.[26,27] A third part assesses PT2385 plus the multitargeted tyrosine kinase inhibitor cabozantinib.[25,28] We anticipate these combination studies will build on the encouraging results from this first-in-human trial of the HIF-2α inhibitor PT2385.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Choueiri TK, Motzer RJ: Systemic therapy for metastatic renal-cell carcinoma. N Engl J Med 376:354-366, 2017

2. American Cancer Society: Kidney cancer (adult)—renal cell carcinoma. http://www.cancer.org/cancer/kidneycancer/detailedguide/

3. National Cancer Institute: Cancer stat facts: Kidney and renal pelvis cancer. http:// seer.cancer.gov/statfacts/html/kidrp.html

4. Kondo K, Kim WY, Lechpammer M, et al: Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. PLoS Biol 1:E83, 2003

5. Nickerson ML, Jaeger E, Shi Y, et al: Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. Clin Cancer Res 14:4726-4734, 2008

6. Cowey CL, Rathmell WK: VHL gene mutations in renal cell carcinoma: Role as a biomarker of disease outcome and drug efficacy. Curr Oncol Rep 11:94-101, 2009

7. Sato Y, Yoshizato T, Shiraishi Y, et al: Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet 45:860-867, 2013

8. Shen C, Kaelin WG, Jr.: The VHL/HIF axis in clear cell renal cancer. Semin Cancer Biol 23:18-25, 2013

9. Haase VH: Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev 27:41-53, 2013

10. Zhao J, Du F, Shen G, et al: The role of hypoxia-inducible factor-2 in digestive system cancers. Cell Death Dis 6:e1600, 2015

11. Scheuermann TH, Tomchick DR, Machius M, et al: Artificial ligand binding within the HIF2alpha PAS-B domain of the HIF2 transcription factor. Proc Natl Acad Sci USA 106:450-455, 2009

12. Rogers JL, Bayeh L, Scheuermann TH, et al: Development of inhibitors of the PAS-B domain of the HIF-2α transcription factor. J Med Chem 56:1739-1747, 2013

13. Wallace EM, Rizzi JP, Han G, et al: A small-molecule antagonist of HIF2α is efficacious in preclinical models of renal cell carcinoma. Cancer Res 76:5491-5500, 2016

14. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009

15. Le Tourneau C, Lee JJ, Siu LL: Dose escalation methods in phase I cancer clinical trials. J Natl Cancer Inst 101:708-720, 2009

16. Chen W, Hill H, Christie A, et al: Targeting renal cell carcinoma with a HIF-2 antagonist. Nature 539:112-117, 2016

17. Motzer RJ, Bacik J, Schwartz LH, et al: Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. J Clin Oncol 22:454-463, 2004

18. Koehler AN: A complex task? Direct modulation of transcription factors with small molecules. Curr Opin Chem Biol 14:331-340, 2010

19. Hodson EJ, Nicholls LG, Turner PJ, et al: Regulation of ventilatory sensitivity and carotid body proliferation in hypoxia by the PHD2/HIF-2 pathway. J Physiol 594:1179-1195, 2016

20. Smith TG, Brooks JT, Balanos GM, et al: Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med 3:e290, 2006

21. Petousi N, Croft QP, Cavalleri GL, et al: Tibetans living at sea level have a hyporesponsive hypoxia-inducible factor system and blunted physiological responses to hypoxia. J Appl Physiol (1985) 116:893-904, 2014

22. Frise MC, Robbins PA: The pulmonary vasculature–lessons from Tibetans and from rare diseases of oxygen sensing. Exp Physiol 100:1233-1241, 2015

23. Angevin E, Lopez-Martin JA, Lin CC, et al: Phase I study of dovitinib (TKI258), an oral FGFR, VEGFR, and PDGFR inhibitor, in advanced or metastatic renal cell carcinoma. Clin Cancer Res 19:1257-1268, 2013

24. Choueiri TK, Pal SK, McDermott DF, et al: A phase I study of cabozantinib (XL184) in patients with renal cell cancer. Ann Oncol 25:1603-1608, 2014

25. Moslehi JJ: Cardiovascular toxic effects of targeted cancer therapies. N Engl J Med 375:1457-1467, 2016

26. Motzer RJ, Escudier B, McDermott DF, et al: Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 373:1803-1813, 2015

27. Motzer RJ, Rini BI, McDermott DF, et al: Nivolumab for metastatic renal cell carcinoma: Results of a randomized phase II trial. J Clin Oncol 33:1430-1437, 2015.

28. Procopio G, Grassi P, Verzoni E, et al: Cabozantinib in the treatment of advanced renal cell carcinoma: design, development, and potential place in the therapy. Drug Des Devel Ther 10:2167-2172, 2016

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Appendix

Analyses of the Relationship Between Pharmacokinetics and Memorial Sloan Kettering Cancer Center Score with Progression-Free Survival

Univariable Cox regression models were used to evaluate the relationship between standard pharmacokinetic parameters and Memorial Sloan Kettering Cancer Center Score[17] with progression-free survival (PFS). The pharmacokinetic parameters investigated were maximum concentration (C_{max}), C_{max} normalized for dose, terminal half-life, area under the concentration-time curve extrapolated to infinity (AUC_{inf}), area under the concentration-time curve from time 0 to the last quantifiable time point (AUC_{last}), AUC_{inf} and AUC_{last} normalized for dose, median trough concentration, and time of maximum concentration at days 1 and 15, predose levels of PT2385 at days 8, 15, 22, 29, 36, 43, 64, 85, and 106, and 12 hr postdose levels at days 1 and 15 (Appendix Table A1, online only). The variables selected for inclusion in the multiple regression model (defined as $P < .10$, indicated in bold in Appendix Table A1) were C_{max} and normalized C_{max} at days 1 and 15, AUC_{last} and normalized AUC_{last} at day 15, predose and 12-hour postdose concentration at day 15, concentration at days 29 and 36, and Memorial Sloan Kettering Cancer Center Score. In the stepwise Cox model, the subset of variables that best predicted PFS ($P < .05$) was trough PT2385 concentration at day 15, 12 hours postdose, and predose PT2385 concentration at day 29. The variable with the most significant effect on PFS was the trough PT2385 concentration at day 15, 12 hours postdose ($P = .002$). The value of postdose concentration at day 15 that maximized the difference in PFS between patients with levels below the value versus at or above the value was determined to be 0.5 μg/mL. Specifically, 16 of 26 patients (62%) with a PT2385 trough concentration ≥ 0.5 μg/mL had SD or better for ≥ 4 months, whereas only four of 22 patients (18%) with a PT2385 trough concentration of < 0.5 μg/mL had SD or better for ≥ 4 months (Appendix Fig. A1)
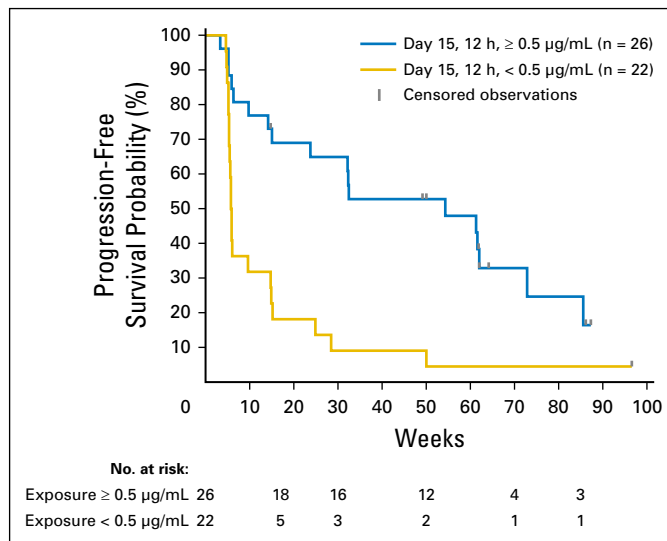

Fig. A1. Relationship between PT2385 exposure and progression-free survival in patients experiencing steady-state exposure trough concentrations < 0.5 μg/mL versus ≥ 0.5 μg/mL (all evaluable patients in dose escalation phase and expansion cohort; n = 48). Abbreviation: h, hours

Table A1. Univariable and Multivariable Analyses

Univariable Analyses		Multiple Regression Analyses With Stepwise Variable Selection		
Variable	χ^2 P	Step	Variable	χ^2 P
T_{max} day 1	.482	1	AUC_{last} day 15	.027
T_{max} day 15	.507		C_{max} day 1	.019
$T_{1/2}$ day 1	.732		C_{max} day 15	.026
$T_{1/2}$ day 15	.351		AUC_{last} normalized day 15	.017
AUC_{inf} day 1	.437		C_{max} normalized day 1	.028
AUC_{inf} day 15	.245		C_{max} normalized day 15	.013
AUC_{last} day 1	.283		Concentration day 15	.047
AUC_{last} day 15	**.074**		Concentration day 15 12-hour postdose	.012
C_{max} day 1	**.021**		Concentration day 29	.231
C_{max} day 15	**.048**		Concentration day 36	.142
AUC_{inf} normalized day 1	.495		MSKCC Score	.055
AUC_{inf} normalized day 15	.187			
AUC_{last} normalized day 1	.181	2*	AUC_{last} day 15	.019
AUC_{last} normalized day 15	**.039**		C_{max} day 1	.411
C_{max} normalized day 1	**.014**		C_{max} day 15	.519
C_{max} normalized day 15	**.017**		AUC_{last} normalized day 15	.589
Concentration day 1 12-hour postdose	.285		C_{max} normalized day 1	.162
Concentration day 8	.141		C_{max} normalized day 15	.372
Concentration day 15	**.091**		Concentration day 15	.024
Concentration day 15 12-hour postdose	**.038**		Concentration day 15 12-hour postdose	.005
Concentration day 22	.395		Concentration day 29	.180
Concentration day 29	**.095**		Concentration day 36	.105
Concentration day 36	**.068**			
Concentration day 43	.172	3†‡	AUC_{last} day 15	.224
Concentration day 64	.174		C_{max} day 1	.673
Concentration day 85	.744		C_{max} day 15	.571
Concentration day 106	.919		AUC_{last} normalized day 15	.878
Median trough concentration	.129		C_{max} normalized day 1	.346
MSKCC Score	**.083**		C_{max} normalized day 15	.685
			Concentration day 15	.095
			Concentration day 36	.800
			MSKCC score	.063

NOTE. Bolding indicates variables selected for inclusion in the multiple regression model.

Abbreviations: AUC_{inf}, area under the concentration-time curve extrapolated to infinity; AUC_{last}, area under the concentration-time curve from time 0 to the last quantifiable time point; C_{max}, maximum concentration; MSKCC, Memorial Sloan Kettering Cancer Center; T1/2, terminal half-life; T_{max}, time of maximum concentration.

*Concentration day 15 12-hour postdose was selected for the model. At step 2, the significance of the following variables, adjusting for this factor, was determined

†Concentration day 15 12-hour postdose and concentration day 29 were included in the model. At step 3, the significance of the following variables adjusting for these factors was determined

‡After step 3, no additional variables met the criterion ($P < .05$) to be included in the model. The final model included concentration day 15 12-hour postdose ($P = .002$) and concentration day 29 ($P = .007$)