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Phase I Safety and Pharmacokinetic Study of Bavituximab, a Chimeric Phosphatidylserine-Targeting Monoclonal Antibody, in Patients with Advanced Solid Tumors

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Abstract

Purpose: Bavituximab is a chimeric immunoglobulin G1 phosphatidylserine-targeting monoclonal antibody that triggers vascular disruption and enhances antitumor immune response. This phase I study assessed the safety and pharmacokinetics of bavituximab in patients with advanced solid tumors.

Experimental Design: Patients with refractory advanced solid tumors were enrolled into four sequential dose-escalation cohorts (0.1, 0.3, 1, or 3 mg/kg bavituximab weekly) with two dosing schedules. Patients in the 0.1 and 0.3 mg/kg cohorts received bavituximab on days 0, 28, 35, and 42. Patients in the 1 and 3 mg/kg cohorts were administered bavituximab on days 0, 7, 14, and 21. Safety, pharmacokinetics, and tumor response were assessed.

Results: Twenty-six patients were accrued. No maximum tolerated dose was reached. Six serious adverse events occurred in five patients, including one pulmonary embolism at 3 mg/kg, which was the only dose-limiting toxicity (DLT) in the study. Bavituximab half-life ranged from 37 to 47 hours, with no accumulation seen following administration of multiple doses. Activated partial thromboplastin time was modestly prolonged *in vitro* at the highest dose tested. As assessed on day 56, a total of 18 patients were evaluable for efficacy, of whom 10 had disease progression and none had an objective response.

Conclusions: Bavituximab was well tolerated at doses ranging up to 3 mg/kg weekly. Pharmacokinetic studies support a weekly dosing regimen. Additional phase I and II clinical trials are in progress to investigate bavituximab in combination with chemotherapy and other molecularly targeted agents. *Clin Cancer Res; 17(21); 6888–96. ©2011 AACR.*

Introduction

Bavituximab is a vascular targeting agent with a unique mechanism of action resulting in a high degree of specificity for tumor blood vessels. Specifically, bavituximab is an unconjugated, chimeric immunoglobulin G1 (IgG1) monoclonal antibody directed against anionic phospholipids expressed on tumor endothelium. In most tissues, the principal target of bavituximab, phosphatidylserine (PS), is restricted to the internal surface of the cell membrane (1). Various pathophysiologic processes disrupt this asymmetry, resulting in exposure of PS on the outer membrane leaflet, where it is available for binding by bavituximab (2). PS is exposed on tumor vascular endothelium within solid tumors, where hypoxia and other physiologic stresses induce PS exposure, rendering bavituximab vascular targeting tumor specific (3). In animal models, cytotoxic therapies, including chemotherapy and ionizing radiation, further induce PS exposure, thereby enhancing bavituximab binding (4, 5).

Bavituximab binds a complex of β_2 glycoprotein 1 (β_2GP1) and PS, triggering antitumor effects via a number of mechanisms. Principally, bavituximab acts as a vascular targeting agent. The bavituximab–β_2GP1–PS complex induces host effector processes such as antibody-dependent cellular cytotoxicity, resulting in vascular destruction (6). In addition, bavituximab has been shown to alter the immune milieu in the tumor microenvironment. Normally, PS exposure on apoptotic cells suppresses immune and inflammatory responses by binding to macrophage PS receptors, thereby signaling macrophage production of

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Translational Relevance

Vascular targeting agents have shown antitumor efficacy, but development and utility of many of these drugs are hampered by off-target effects. Bavituximab is a chimeric immunoglobulin G1 monoclonal antibody that seems relatively specific for tumor vasculature. Bavituximab is directed against the membrane phospholipid phosphatidylserine (PS). In normal cells, PS is restricted to the inner membrane leaflet, where it is not available for binding by bavituximab. However, in settings of physiologic stress and hypoxia, as occurs in tumor vasculature, PS is exposed on the outer membrane leaflet. Upon binding to exposed PS, bavituximab recruits host immune functions, resulting in tumor vessel obliteration. In this phase I study, bavituximab was well tolerated. There was one instance of pulmonary embolism and no significant bleeding events. On the basis of these findings, bavituximab is now under study in combination with other therapies for multiple cancer types.

anti-inflammatory cytokines such as TGFβ and interleukin (IL)-10 (7) and preventing dendritic cell maturation and antigen presentation (8). In preclinical models, PS-targeting antibodies countered both of these effects, resulting in production of proinflammatory cytokines such as TNFα and IL-1β, as well as induction of cytotoxic T-lymphocyte immunity (9).

In murine models of multiple tumor types, bavituximab inhibits tumor growth, prolongs survival, and enhances the effects of chemotherapy and radiation (4–6). This phase I clinical trial of bavituximab was carried out to evaluate its safety and pharmacokinetic profile in patients with relapsed and refractory malignancies. On the basis of preclinical modeling, dose escalation was halted at the anticipated biologically effective dose, rather than the maximum tolerated dose (MTD). Because activated platelets have exposed PS and pathologic endogenous antiphospholipid antibodies are associated with thrombosis, we monitored platelet function and coagulation parameters in addition to standard safety measures.

Patients and Methods

Patient selection

This study enrolled adults 18 years or older with evaluable, histologically, or cytologically confirmed refractory advanced solid malignancies. Key inclusion criteria included Eastern Cooperative Oncology Group performance status of 0–1; adequate hematologic, hepatic, and renal functions; prothombin time (PT)/Internationalized Normalized Ratio (INR) and activated partial thromboplastin time (aPTT) within institutional normal limits; and serum D-dimer 2 times or less than the upper limit of institutional normal. Major exclusion criteria included any history of thromboembolic events; clinically significant bleeding (defined as gross hematuria, hemoptysis, or gastrointestinal bleeding); history of bleeding diathesis; evidence or history of a hypercoagulable state (e.g., shortened aPTT); ongoing therapy with anticoagulants, nonsteroidal anti-inflammatory drugs, or antiplatelet drugs; any history of coronary artery disease or cerebrovascular accident; major surgery, chemotherapy, radiation, or investigational therapy within 4 weeks of study therapy initiation; and prior exposure to chimeric antibodies. Patients with clinically stable, previously treated brain metastases were eligible for the study.

Study design

Institutional Review Board approval was obtained at each participating clinical trial site before the start of the study. The study was conducted in accordance with the U.S. Food and Drug Administration Good Clinical Practice guidelines and the Health Insurance Portability and Accountability Act. Informed consent was obtained from each patient.

This was an open-label dose-escalation study. The dose levels selected for this clinical trial were 0.1, 0.3, 1, and 3 mg/kg, administered in a dose-escalating fashion. The starting dose was selected on the basis of safety data obtained in single- and repeat-dose rodent and primate toxicity studies, and efficacy data were obtained in a tumor-bearing mouse model. Dose escalation to the predicted biologically effective dose rather than the MTD was the goal of this study. This approach was selected because for monoclonal antibodies, the MTD may not correspond to optimal efficacy and, in some instances, may never be reached in monotherapy studies (10).

We determined the optimal biological dose of 3 mg/kg weekly, based on preclinical modeling and experience in other patient populations. In animal models, maximal efficacy of anti-PS antibody was achieved at doses of 0.5 mg/kg 3 times weekly, yielding a C_{max} of 5.5 μg/mL with a half-life of 48 hours and a simulated average blood concentration of 2 μg/mL over the course of treatment. Beyond that dose, PS binding by bavituximab was presumably saturated, based on observations that 1 to 2 μg/mL is the concentration at which binding of bavituximab to PS-expressing cells becomes saturated *in vitro* (11). Separately, *in vitro* transient elevations in aPTT, a potential safety signal, were noted in rats (the most sensitive species) at bavituximab doses of 20 mg/kg or more (C_{max} = 345 μg/mL). Therefore, capping the dose escalation in this study at 3 mg/kg (with simulated C_{max} in humans of 65.4 μg/mL, corresponding to an equivalent C_{max} of 11–111 μg/mL in rodents) provided a more than 5-fold safety margin while maintaining drug concentrations expected to be in excess of the therapeutic range based on animal models.

Additional guidance was derived from a phase I study in patients infected with hepatitis C virus (HCV; PS is also expressed on certain virally infected cells; refs. 12, 13). At doses up to and including 3 mg/kg, bavituximab did not cause detectable increases in D-dimer. However, patients treated at higher doses had transient increases in D-dimer levels up to twice pretreatment baseline. In addition, levels of β2GP1 (the plasma protein required for bavituximab

binding to PS) decreased by 40% in the 6 mg/kg dose cohort, potentially limiting PS binding by bavituximab.

There were 2 dosing schedules in this study. The trial was originally designed to provide detailed pharmacokinetic data on single- and multiple-dose bavituximab administration, similar to previous phase I antibody clinical trials (14). Accordingly, patients in the first 2 dose cohorts (0.1 and 0.3 mg/kg) received the first intravenous infusion of bavituximab on day 0, followed by a 28-day washout, and then received a single course of the assigned dose once weekly for 3 weeks (days 28, 35, and 42) and followed until day 70. The study was subsequently amended to eliminate the 4-week washout period after the first dose. Thus, patients in the 1 and 3 mg/kg dose cohorts were administered bavituximab on days 0, 7, 14, and 21 and then followed until day 56. Patients who achieved an objective radiographic response were eligible to receive continued therapy on an extension study.

Bavituximab, which was supplied as a sterile solution in 5 mL single-use borosilicate type I glass vials containing 20 $\pm$ 3 mg/mL bavituximab, acetate, and water for injection, was administered intravenously over approximately 90 minutes. Premedication was not administered.

Six patients were accrued to each dose level. Dose escalation was permitted if dose-limiting toxicity (DLT) occurred in 1 or none of 6 patients and serum bavituximab levels did not exceed a predetermined threshold (mean cohort $C_{max} \leq 65$ μg/mL). Intrapatient dose escalation was not allowed. The MTD was defined as the highest dose level at which a DLT was experienced in less than 33% of patients. DLTs were defined as any of the following adverse events observed through day 56 or 70 (depending upon cohort) considered causally related to bavituximab: grade 3 or greater hematologic or nonhematologic toxicity, grade 2 or greater prolongation of PT/INR [>1.5 $\times$ upper limit of normal (ULN) to 2 $\times$ ULN], grade 3 or greater prolongation of aPTT (>2 $\times$ ULN).

Assessments

Safety assessments included adverse events, concomitant medications, physical findings, complete blood cell count with differential, serum chemistries, coagulation parameters, and D-dimer. Platelet function (assessed by the PFA-100 assay) was evaluated on day 0 (predose), day 1, and day 21 (postdose). At one site, the platelet activation markers PAC-1 and P-selectin were evaluated serially on day 0 (predose), day 1, and day 21 (postdose) by flow cytometry. Blood samples for human antichimeric antibody analysis were collected on days 0 (predose), 28, 56, and 70. A complete coagulation evaluation was carried out 1 hour postdose on day 0 for all patients and as required for any patient exhibiting grade 2 or greater aPTT or PT/INR prolongation. This included the following assays: 1:1 mixing studies with immediate and delayed incubations, a specific clotting-based assay designed to test for the presence of a lupus anticoagulant (including a dilute Russell Viper Venom Time or hexagonal PTT), specific coagulation factor assays (II, V, VII, VIII, IX, X, and XI), fibrinogen, fibrin split products, thrombin time, and reptilase. Tumor response by Response Evaluation Criteria in Solid Tumors (RECIST; version 1.0) was assessed at prestudy and day 56 (or at time of study exit; ref. 15).

Pharmacokinetics

From patients in the 0.1 and 0.3 mg/kg dose cohorts, pharmacokinetic samples were collected prestudy and on days 0, 1, 2, 4 $\pm$ 1, 7, 10, 14, and every 7 days from days 21 to 70. From patients in the 1 and 3 mg/kg dose cohorts, pharmacokinetic samples were collected prestudy and on days 0, 1, 2, 4 $\pm$ 1, 7, 14, 21, 22, 23, 25 $\pm$ 1, and every 7 days from days 28 to 56.

Bavituximab blood levels were determined by a validated ELISA. Noncompartmental pharmacokinetic analyses were conducted on individual serum bavituximab concentration–time data with WinNonlin Professional (version 5.2; Pharsight Corporation).

Statistical analysis

Demographic and baseline disease characteristics for study patients were summarized by means, SDs, medians, and ranges for continuous variables and counts and proportions for categorical variables. Linear regression analysis was conducted for the following pharmacokinetic parameters versus the mg/kg bavituximab dose: C_{max}/dose (days 0 and 21); area under the plasma concentration-time curve from time zero to infinity (AUC_{inf})/dose (day 0); and area under the plasma concentration-time curve during a dosage interval [(tau)AUC_{tau}]/dose (day 21). Unless otherwise noted, all statistical testing was 2-sided and was carried out at the 0.05 significance level. All analyses and tabulations were carried out by SAS (version 8.2 or higher; SAS Institute) on a PC platform. There was no imputation of missing data.

Results

Study population, disposition, and drug exposure

A total of 26 patients were enrolled and received at least 1 infusion of bavituximab. Eight patients were enrolled in the 0.1 mg/kg dose cohort (2 patients were replaced because of inadequate multidose pharmacokinetic data), and 6 patients were enrolled in each of the 0.3, 1, and 3 mg/kg dose cohorts. Baseline patient characteristics are listed in Table 1. All 26 patients were evaluable for safety, and 18 patients were evaluable for tumor response. Overall, 6 patients (23%) discontinued study drug prior to study completion; 2 patients in 0.1 mg/kg dose cohort (both prior to day 28), 2 patients in 0.3 mg/kg dose cohort (1 prior to day 28; 1 prior to day 35), and 2 patients in 3 mg/kg dose cohort (1 prior to day 7; 1 prior to day 14). Fifteen patients discontinued study participation before day 56 for the following reasons: disease progression ($n = 12$), protocol violation (missed treatment, $n = 1$), initiation of other cancer therapy ($n = 1$), and DLT ($n = 1$).

Safety

All 26 patients experienced at least one adverse event, regardless of attribution (Table 2). Thirteen patients (50%)

Table 1. Baseline patient characteristics

Characteristics	Number (%) or median (range)
Total patients	26
Sex	
Male	7 (27)
Female	19 (73)
Age, y	57 (37–80)
Cancer type	
Breast	13 (50)
Colorectal	4 (15)
Pancreas	3 (12)
Other	6 (23)
Prior therapy	
Chemotherapy	26 (100)
Surgery	17 (65)
Radiation therapy	13 (50)
Biologic therapy	4 (15)

experienced at least one adverse event judged to be related to study treatment. The most frequent of these were fatigue (27%), nausea (15%), aPTT prolongation (12%), and pyrexia, rash, and dyspnea (each 8%). Most treatment-related adverse events were either grade 1 or 2. Adverse events seemed to occur with similar frequency across dose cohorts (Table 3). Infusion reaction was reported in 1 patient in the 0.1 mg/kg dose cohort who experienced grade 2 drug hypersensitivity (pruritus) during the second of 4 doses. Subsequent doses (without premedication) were administered without incident.

Ten patients (38%) experienced at least one grade 3 or 4 adverse event. One patient (4%) experienced a grade 3 or 4 adverse event considered possibly related to study drug. This event, a pulmonary embolism in the 3 mg/kg dose cohort, was the only DLT observed in the study. This occurred in a 42-year-old woman with stage IV breast cancer with multiple bone, lung, and mediastinal metastases. One day after the cycle 1 day 7 bavituximab infusion, she developed dyspnea. A computed tomographic angiogram showed subsegmental emboli in the right lower lobe basal artery, as well as tumor compression of central airways and vasculature. Elevations in PT and aPTT were noted. Treatment with unfractionated heparin was initiated, which was transitioned to low-molecular-weight heparin during the hospitalization. Study drug was permanently discontinued. The patient died 54 days after the last dose of bavituximab because of progressive compression of central airways and multiorgan system failure attributed to disease progression. Two patients died within 30 days of the last dose of bavituximab, both because of progression of the underlying malignancy.

One patient in the 3 mg/kg dose cohort developed grade 3 hypertension (i.e., requiring the addition of an antihypertensive agent), which was considered not causally related to bavituximab. This patient had a long-standing history (>10 years) of hypertension, which seemed to be poorly controlled at baseline. The recorded baseline blood pressure before study drug administration was 152/77 (specific blood pressure parameters were not included as eligibility criteria). Blood pressure remained elevated during the study. On day 35, 14 days after the fourth and final dose of bavituximab, the recorded blood pressure was 156/88. The investigator initiated treatment with 12.5 mg hydrochlorothiazide per os daily. At the end of study, blood pressure was 118/73, with antihypertensive therapy ongoing.

Coagulation parameters and platelet function

D-dimer monitoring was included in this study as a potential indicator of drug-related thrombotic risk. All 26 enrolled patients had serial D-dimer measurements over the course of the study. Consistent with the study population of patients with previously treated advanced cancer, only 10 patients (38%) had D-dimer levels within institutional normal limits at baseline. Twelve patients (46%) showed an increase in D-dimer level over the course

Table 2. Incidence of adverse events reported by 15% or more of study population

Preferred term	Bavituximab dose cohort				
	0.1 mg/kg ($n = 8$)	0.3 mg/kg ($n = 6$)	1 mg/kg ($n = 6$)	3 mg/kg ($n = 6$)	Total ($N = 26$)
Nausea	6 (75)	5 (83)	3 (50)	1 (17)	15 (58)
Fatigue	6 (75)	2 (33)	3 (50)	2 (33)	13 (50)
Headache	3 (38)	3 (50)	1 (17)	2 (33)	9 (35)
Constipation	1 (13)	2 (33)	2 (33)	1 (17)	6 (23)
Cough	1 (13)	1 (17)	2 (33)	1 (17)	5 (19)
Dizziness	1 (13)	1 (17)	2 (33)	1 (17)	5 (19)
Dyspnea	3 (38)	0 (0)	1 (17)	1 (17)	5 (19)
Chest pain	0 (0)	0 (0)	3 (50)	1 (17)	4 (15)

NOTE: The values in parenthesis are expressed in percentage.

Table 3. Overall summary of adverse events by dose cohort

Parameter	Bavituximab dose cohort				
	0.1 mg/kg ($n = 8$)	0.3 mg/kg ($n = 6$)	1 mg/kg ($n = 6$)	3 mg/kg ($n = 6$)	Total ($N = 26$)
Patients with any AE	8 (100)	6 (100)	6 (100)	6 (100)	26 (100)
Patients with study drug–related AEs	6 (75)	1 (17)	3 (50)	4 (67)	13 (50)
Patients with grade ≥3 AEs	3 (75)	3 (50)	1 (17)	3 (50)	10 (39)
Patients with study drug– related grade ≥3 AEs	0 (0)	0 (0)	0 (0)	1 (17)	1 (4)
Patients with SAEs	1 (13)	1 (17)	0 (0)	3 (50)	5 (19)
Patients with study drug–related SAEs	0 (0)	0 (0)	0 (0)	1 (17)	1 (4)

NOTE: The values in parenthesis are expressed in percentage.
Abbreviations: AE, adverse event; SAE, serious adverse event.

of the study. In general, these increased levels were correlated with disease progression, were not temporally associated with bavituximab administration, and did not predict thrombotic events. The one patient who did develop pulmonary embolism did not have an increasing D-dimer level at the time of the event. An antiphospholipid antibody effect (i.e., prolongation of aPTT associated with a positive clotting-based assay for a lupus anticoagulant) was detected in all 6 patients in the 3 mg/kg dose level but in none of the patients at lower dose levels. In all patients, the aPTT normalized by day 3 following the bavituximab dosing, consistent with the half-life of bavituximab.

Twenty-five patients (96%) had assessment of platelet function by PFA-100 analysis. One patient in the 3 mg/kg dose cohort had prolongation of a normal baseline PFA-100 after bavituximab administration. There were no clinical or laboratory adverse events associated with this finding. Platelet activation markers PAC-1 and P-selectin were unchanged in response to bavituximab therapy in the 2 patients evaluated.

Efficacy

There were no objective tumor responses according to RECIST on day 56 or study exit. Ten of 18 evaluable patients (55%) had progressive disease.

Pharmacokinetics

Bavituximab exhibited linear single-dose (day 0, for all cohorts) and multiple-dose (for the 1 and 3 mg/kg cohorts on day 21; there was insufficient data for the 0.1 and 0.3 mg/kg cohorts on day 42) pharmacokinetic characteristics (Table 4 and Fig. 1). Following time to peak concentration (T_{max}), mean serum bavituximab concentrations seemed to decline generally in an apparent monoexponential manner. Mean serum bavituximab concentrations on days 0 and 21 within dose groups were similar. Specifically, there were no significant differences in T_{max} ($P = 0.88$), area under the plasma concentration-time curve from time zero to time t (AUC_t; $P = 0.96$), AUC_{inf} (day 1) versus AUC_{tau} (day 21; $P = 0.37$), half-life [the time required to reduce the plasma concentration to

Table 4. Pharmacokinetic parameters of bavituximab following single-dose administration (day 0) and multiple-dose administration (day 21)[a]

Dose	Day 0					Day 21 (for 1 and 3 mg/kg)				
	No. of patients	Mean (CV %)				No. of patients	Mean (CV %)			
		C_{max} (μg/mL)	CL (mL/h/kg)	$t_{1/2}$ (h)	AUC_{inf} (d μg/mL)		C_{max} (μg/mL)	CL (mL/kg/d)	$t_{1/2}$ (h)	AUC_{inf} (d μg/mL)
0.1 mg/kg	8	2.11 (27.3)	1.1 (48.7)	43.9 (48.5)	113 (50.1)					
0.3 mg/kg	6	5.13 (42.4)	1.39 (34.3)	39.8 (34.1)	241 (39.8)					
1 mg/kg	6	16.6 (30.9)	1.14 (36.7)	40.3 (20.2)	966 (30)	6	18.7 (31.8)	1.12 (52.1)	46.8 (38.4)	1,053 (38)
3 mg/kg	6	56.4 (25.8)	1.34 (72.2)	37.2 (34.5)	3,017 (50.3)	4	59.6 (27.6)	1.51 (61.4)	46 (44.4)	2,672 (63.4)

Abbreviations: AUC_{inf}, area under the plasma concentration-time curve from time zero to infinity; CL, clearance; C_{max}, maximum concentration; CV, coefficient of variation; $t_{1/2}$, half-life.
[a]There were insufficient data for pharmacokinetic analysis of multiple-dose administration (day 42) in the 0.1 and 0.3 mg/kg cohorts.

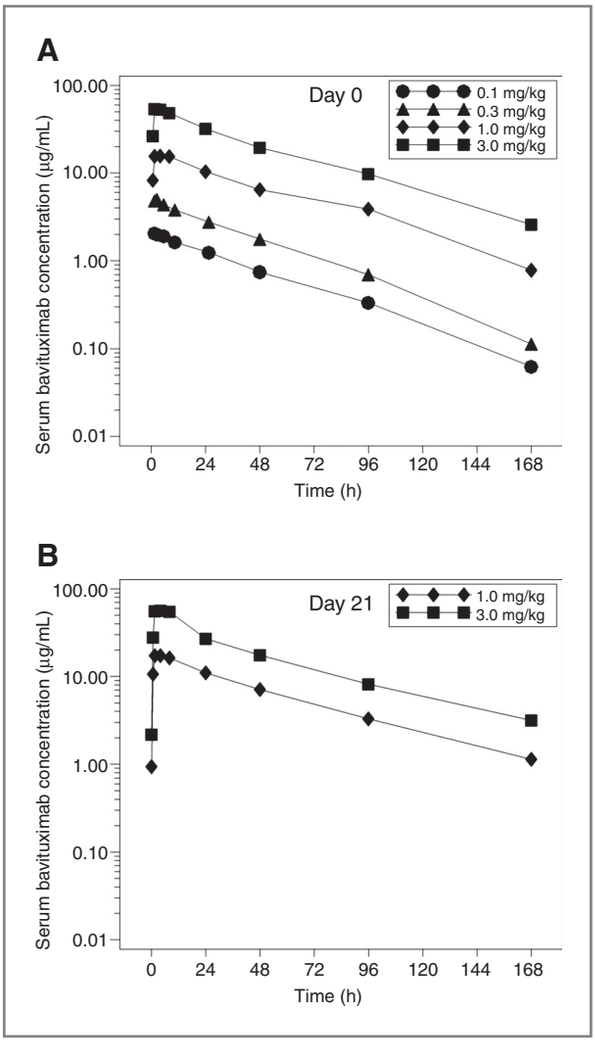

Figure 1. Mean serum bavituximab concentrations following (A) single-dose administration (day 0) of bavituximab 0.1 mg/kg (circle), 0.3 mg/kg (triangle), 1.0 mg/kg (diamond), and 3.0 mg/kg (square) and (B) multiple-dose administration (day 21) of bavituximab 1.0 mg/kg (diamond) and 3.0 mg/kg (square).

one half its initial value ($t_{1/2}$); $P = 0.22$], mean residence time [the average total time molecules of a given dose spend in the body (MRT); $P = 0.68$], apparent volume of distribution at steady state (V_z; $P = 0.07$), apparent volume of distribution during terminal phase (V_{ss}; $P = 0.16$), clearance [the volume of plasma in the vascular compartment cleared of drug per unit time by the processes of metabolism and excretion (CL); $P = 0.94$], or AUC_{inf}/dose (day 1) versus AUC_{tau}/dose (day 21; $P = 0.37$). Mean half-life estimates ranged 37 to 44 hours on day 0 and 46 to 47 hours on day 21. With the 1 mg/kg dose, bavituximab concentration remained above 2 μg/mL (the predicted therapeutic threshold based on preclinical modeling) for 6 days. With the 3 mg/kg dose, bavituximab concentration remained above 2 μg/mL for 7 days.

Discussion

Bavituximab is a chimeric IgG1 monoclonal antibody directed against the membrane phospholipid PS. In preclinical models, treatment with bavituximab resulted in highly specific targeting of tumor vasculature and apoptotic tumor cells and enhanced antitumor immune responses. This phase I, open-label, dose-escalation trial of bavituximab monotherapy, which enrolled 26 patients with refractory solid tumors, was carried out to determine the safety and tolerability profile and pharmacokinetic parameters of bavituximab. In doses ranging from 0.1 to 3 mg/kg, bavituximab was administered intravenously weekly (following an initial 28-day cycle in lower dose cohorts) for a total of 4 doses. The most common adverse events were fatigue (27%), nausea (15%), aPTT prolongation (12%), and pyrexia, rash, and dyspnea (each 8%). There were 6 serious adverse events reported by 5 patients, of which 1 event, pulmonary embolism (the one DLT of the study), was considered probably related to study drug. Although premedication was not administered, only one patient experienced a (grade 2) infusion reaction. Pharmacokinetic analysis showed that, at the highest dose tested, target exposure for efficacy (2 μg/mL) would be expected to be continuously exceeded by a weekly schedule of administration.

On the basis of its proposed mechanisms of action, bavituximab conveys a hypothetical risk of antiphospholipid antibody syndrome. This coagulation disorder, which arises from autoantibodies against cell membrane phospholipids associated with β_2GP1, is characterized by arterial and venous thromboses. The aPTT prolongation seen with antiphospholipid antibody syndrome represents an *in vitro* phenomenon, and there is no increase in bleeding propensity. Specifically, antiphospholipid antibodies bind to the phospholipid part of the PTT reagent. With insufficient reagent in the test tube, the specimen does not clot and the aPTT is falsely prolonged. Accordingly, this trial incorporated extensive laboratory monitoring for coagulation derangements. The presence of a concentration-dependent, transient antiphospholipid antibody effect was confirmed from blood drawn 1 hour (near C_{max}) after the first bavituximab dose in all patients treated with a 3mg/kg dose but in none of the patients treated with a dose below 3 mg/kg.

It is noteworthy that the single DLT in this trial, a pulmonary embolism, occurred 1 day after administration of 3 mg/kg bavituximab and was associated with aPTT prolongation. Although event timing suggests association with study drug, it must also be taken in context. Independent of therapy, a diagnosis of cancer conveys a 4- to 7-fold increased risk of venous thromboembolism (16–20). At baseline, this particular patient had multiple central lung and mediastinal metastases from breast cancer resulting in vascular compression, which may have contributed to her risk of thromboembolic events. In addition, of more than 80 patients with HCV infection—a population without an increased propensity

toward clotting—treated with bavituximab in early-phase trials to date, none has experienced venous thromboembolism (13, 21, 22).

Although hypertension is a known adverse effect of antiangiogenic drugs, the grade 3 hypertension event in this trial was considered not causally related to bavituximab. As described earlier, this patient had a long-standing history of poorly controlled hypertension, which did not clearly worsen during the course of bavituximab treatment. There were no other cases of hypertension in this trial. Furthermore, among the more than 80 HCV-infected patients treated with bavituximab to date, a single case of grade 1 hypertension (systolic blood pressure 120–139 or diastolic blood pressure 80–89) has been reported.

Reflecting concerns about potential procoagulant effects of bavituximab, D-dimer levels were mandated to be no more than twice the upper limit of institutional normal at screening. The prevalence of D-dimer elevations at baseline and throughout treatment (consistent with the well-recognized association between progressive malignancy, activation of coagulation, and fibrinolysis; refs. 23–28), along with a failure to predict thromboembolic events, render this assay noninformative in this population. Accordingly, the ongoing and future studies of bavituximab do not include D-dimer eligibility requirements or monitoring. Platelet function and activation were not affected by bavituximab treatment.

Comparison of bavituximab distribution and elimination characteristics in patients with refractory advanced cancer was similar to that observed in earlier clinical studies with bavituximab (in HCV-infected patients) and generally similar to other monoclonal antibodies (13, 21, 29, 30). After administration of single and multiple doses of bavituximab, pharmacokinetic analysis showed a half-life of approximately 2 days. This half-life is somewhat shorter than that of other chimeric (i.e., 65% human and 35% mouse) antibodies such as cetuximab (half-life 4 days; ref. 31) and may reflect the clearance of β_2GP1, the target protein to which bavituximab directly binds. In contrast to soluble targets such as VEGF, PS is static and saturable, so bavituximab levels may not necessarily need to be maintained over a prolonged period.

Patients were evaluated for potential antitumor activity of bavituximab. According to RECIST, there were no objective tumor responses. Of 18 evaluable patients, 8 patients (45%) did not have disease progression for at least two 28-day cycles. Given the design and small size of the study, no conclusions about dose–response can be drawn. The absence of objective responses to bavituximab monotherapy is somewhat expected, as early-phase clinical trials of other vascular directed therapies, including the antiangiogenic drug bevacizumab and the vascular disrupting agent combretastatin A4 phosphate (fosbretabulin), showed similar single-agent activity (14, 32). Preclinically, bavituximab was optimally effective when used in combination with cytotoxic chemotherapy or ionizing radiation, which increase PS expression on tumor cells and tumor-associated vasculature.

A principal limitation of this study is the lack of biomarker data, which has been a well-recognized feature of vascular directed therapies (33). Although *in vitro* aPTT prolongation provides some pharmacodynamic data, the association between this laboratory finding and therapeutic effect is not known. To provide more insight into the mechanism of bavituximab in humans, dynamic contrast enhanced MRI scans are being incorporated into selected ongoing and future bavituximab clinical trials. Similarly, this study did not include biomarkers of antitumor immunity, which represents another proposed mechanism of bavituximab therapeutic effect. Immune- and inflammation-associated cytokines were evaluated in a phase I trial of bavituximab for HCV patients (21). Induction of a proinflammatory reaction was evident several hours postdose, manifested by a transient increased serum TNFα/TGFβ ratio. However, because of the high background noise and nonspecific nature of these assays, cytokine profiles were not included in the current phase I study. We also recognize that limited drug exposure (4 weekly doses) and clinical follow-up (8–10 weeks) do not provide full insight into the clinical activity of bavituximab monotherapy. This conservative approach was taken because the principal focus of this study was to evaluate safety, tolerability, and pharmacokinetics. Similar to that of other vascular directed agents, it is expected that the clinical benefit of bavituximab is likely to lie in its combined use with other anticancer agents.

In conclusion, the vascular targeting agent bavituximab has shown anticancer properties in multiple preclinical models. This phase I trial has shown that this chimeric antibody can be administered safely, on a weekly basis, with low risk of infusion reactions. At the highest dose level studied, 3 mg/kg, target exposure for efficacy (2 μg/mL) was continuously exceeded, an antiphospholipid effect—manifested as *in vitro* prolongation of the aPTT—was apparent, and there was one instance of venous thromboembolism. Certain toxicities associated with anti-VEGF therapies, such as bleeding and proteinuria, were not observed (34, 35); nor were off-target effects of the tubulin-inhibiting vascular disrupting agents, such as cardiac toxicity and peripheral neuropathy, observed (32). Although a single case of grade 3 hypertension occurred, this episode seems not causally related to bavituximab. As a result of the pulmonary embolism that occurred at the 3 mg/kg dose level, close clinical monitoring for thromboembolic phenomena and laboratory monitoring for antiphospholipid effects are planned in future bavituximab trials. Given encouraging preclinical data and the safety profile documented from this phase I monotherapy study, phase I and II studies evaluating bavituximab in combination with other therapies are currently ongoing. On the basis of sustained achievement of predicted therapeutic concentrations in this phase I trial, both the 1 and 3 mg/kg doses are under study in these combination trials.

Disclosure of Potential Conflicts of Interest

D.E. Gerber, A.T. Stopeck, L.S. Rosen, P.E. Thorpe, and N.K. Ibrahim received research funding from Peregrine Pharmaceuticals. P.E. Thorpe is a paid consultant for and owns stock in Peregrine Pharmaceuticals. J. S. Shan is a paid employee of Peregrine Pharmaceuticals. L. Wong declared no potential conflicts of interest.

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