VOLUME 28 · NUMBER 5 · FEBRUARY 10 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase I Pharmacologic and Biologic Study of Ramucirumab (IMC-1121B), a Fully Human Immunoglobulin G_1 Monoclonal Antibody Targeting the Vascular Endothelial Growth Factor Receptor-2

Jennifer L. Spratlin, Roger B. Cohen, Matthew Eadens, Lia Gore, D. Ross Camidge, Sami Diab, Stephen Leong, Cindy O'Bryant, Laura Q.M. Chow, Natalie J. Serkova, Neal J. Meropol, Nancy L. Lewis, E. Gabriela Chiorean, Floyd Fox, Hagop Youssoufian, Eric K. Rowinsky, and S. Gail Eckhardt

From the University of Colorado at Denver, Aurora, CO; Fox Chase Cancer Center, Philadelphia, PA; Indiana University Simon Cancer Center, Indianapolis, IN; and ImClone Systems, New York, NY.

Submitted April 29, 2009; accepted October 6, 2009; published online ahead of print at www.jco.org on January 4, 2010.

Supported in part by ImClone Systems, a wholly owned subsidiary of Eli Lilly, and by Grant No. P30 CA006927 from the National Cancer Institute to Fox Chase Cancer Center. J.L.S., a senior fellow mentored by S.G.E., received funding from the National Cancer Institute of Canada through the Terry Fox Foundation and the Alberta Heritage Foundation for Medical Research.

Presented in part at the 20th Annual European Organisation for Research and Treatment of Cancer–National Cancer Institute–American Association for Cancer Research (EORTC-NCI-AACR) Symposium on Molecular Targets and Cancer Therapeutics, October 21-24, 2008, Geneva, Switzerland; 19th Annual EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, October 22-26, 2007, San Francisco, CA; 18th Annual EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, November 7-10, 2006, Prague, Czech Republic; 42nd Annual Meeting of the American Society of Clinical Oncology, June 2-6, 2006, Atlanta, GA.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Jennifer L. Spratlin, MD, FRCPC, Department of Medical Oncology, Cross Cancer Institute, 11560 University Ave, Edmonton, Alberta, Canada T5G 1Z2; e-mail: Jennifer.Spratlin@albertahealthservices.ca.

0732-183X/10/2805-780/$20.00

DOI: 10.1200/JCO.2009.23.7537

ABSTRACT

Purpose
To evaluate the safety, maximum-tolerated dose (MTD), pharmacokinetics (PKs), pharmacodynamics, and preliminary anticancer activity of ramucirumab (IMC-1121B), a fully human immunoglobulin G_1 monoclonal antibody targeting the vascular endothelial growth factor receptor (VEGFR)-2.

Patients and Methods
Patients with advanced solid malignancies were treated once weekly with escalating doses of ramucirumab. Blood was sampled for PK studies throughout treatment. The effects of ramucirumab on circulating vascular endothelial growth factor-A (VEGF-A), soluble VEGFR-1 and VEGFR-2, tumor perfusion, and vascularity using dynamic contrast-enhanced magnetic resonance imaging were assessed.

Results
Thirty-seven patients were treated with 2 to 16 mg/kg of ramucirumab. After one patient each developed dose-limiting hypertension and deep venous thrombosis at 16 mg/kg, the next lower dose (13 mg/kg) was considered the MTD. Nausea, vomiting, headache, fatigue, and proteinuria were also noted. Four (15%) of 27 patients with measurable disease had a partial response (PR), and 11 (30%) of 37 patients had either a PR or stable disease lasting at least 6 months. PKs were characterized by dose-dependent elimination and nonlinear exposure consistent with saturable clearance. Mean trough concentrations exceeded biologically relevant target levels throughout treatment at all dose levels. Serum VEGF-A increased 1.5 to 3.5 times above pretreatment values and remained in this range throughout treatment at all dose levels. Tumor perfusion and vascularity decreased in 69% of evaluable patients.

Conclusion
Objective antitumor activity and antiangiogenic effects were observed over a wide range of dose levels, suggesting that ramucirumab may have a favorable therapeutic index in treating malignancies amenable to VEGFR-2 inhibition.

J Clin Oncol 28:780-787. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Angiogenesis is regulated principally by interactions between vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs) and plays a key role in cancer growth and metastasis.[1-5] VEGF-A is the central regulator of tumor angiogenesis, endothelial proliferation, permeability, and survival.[1,6,7] VEGF-A binds with high affinity to two structurally similar tyrosine kinase receptors, VEGFR-1 and VEGFR-2, both expressed on tumor vasculature.[8,9] Blockade of the VEGF-A/VEGFR-2 interaction inhibits tumor angiogenesis and growth in preclinical studies and is a promising approach to anticancer treatment.[10-20]

Few anticancer therapeutics that directly and specifically inhibit VEGFR-2 have been evaluated.[21,22] Ramucirumab (IMC-1121B; ImClone Systems, New York, NY) is a fully human immunoglobulin G_1 monoclonal antibody (MAb) that binds with high affinity (approximately 50 pM) to the extracellular VEGF-binding domain of VEGFR-2. Both ramucirumab and its murine version, DC-101, were designed to bind to a VEGFR-2 epitope involved in

ligand binding and block VEGF ligands from binding this site and activating the receptor.[23,24] Inhibition of VEGF-stimulated VEGFR-2 activation by ramucirumab or DC-101 conferred significant antitumor activity in a range of malignancies in animal models as single agents and in combination with other therapeutics.[25-28] In nonclinical toxicology studies, ramucirumab was well tolerated, and a no observable effect level was not established.

The principal objectives of the present study were to establish the safety profile and maximum-tolerated dose (MTD) of ramucirumab administered weekly to patients with advanced solid malignancies; characterize the pharmacokinetics (PK), immunogenicity, and pharmacodynamic (PD) effects on serum VEGF-A, soluble (s) VEGFR-1, and sVEGFR-2; assess changes in tumor perfusion and vascularity evaluated by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI); and preliminarily evaluate antitumor activity.

PATIENTS AND METHODS

Patient Selection

Patients with advanced solid malignancies refractory to treatment or lacking standard therapeutic options were eligible. Other eligibility criteria included the following: age ≥ 18 years; adequate hematologic, hepatic, and renal function; and an Eastern Cooperative Oncology Group performance status ≤ 2. Key exclusion criteria were as follows: centrally located pulmonary lesions adjacent to or invading large blood vessels as assessed by the investigator; serious nonhealing active wound, ulcer, or bone fracture; deep venous thrombosis (DVT) within 6 months of entry; proteinuria ≥ 1+; prior left chest wall radiotherapy; anthracycline dose ≥ 300 mg/m^2 with abnormal left ventricular ejection fraction; prior treatment with VEGF or VEGFR inhibitors or any MAb (amended to within 6 weeks of entry); hypersensitivity reaction to a therapeutic protein; and use of thrombolytic agents, full-dose heparin or warfarin, aspirin, and nonsteroidal anti-inflammatory drugs. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the institutional review boards of the participating institutions. Written informed consent was obtained in accordance with federal and institutional guidelines.

Study Design

Ramucirumab was administered at escalating doses as a 1-hour intravenous infusion at a rate of ≤ 25 mg/min. Cycles consisted of four weekly ramucirumab infusions followed in the first cycle by a 2-week, treatment-free PK sampling period (eliminated in an amendment after preliminary PK assessment).

The initial ramucirumab dose level (2 mg/kg) was based on the results of PK and toxicology studies in nonhuman primates. The dose was increased sequentially by 100% (4 mg/kg), 50% (6 mg/kg), and 33% (8 mg/kg); thereafter, the dose escalation increment was fixed at 25% with no intrapatient dose escalation. Dose level assignment used a standard 3 + 3 design with at least three evaluable patients per cohort. If no dose-limiting toxicities (DLTs) occurred during cycle 1, three new patients were treated at the next higher level.

The MTD was defined as the highest dose level at which less than two patients experienced a DLT in cycle 1. DLT was defined as any grade 4 neutropenia lasting more than 7 days; grade ≥ 3 febrile neutropenia, thrombocytopenia, or anemia; or any grade ≥ 3 nonhematologic toxicity considered drug related by the investigator. Up to two dose reductions per patient were permitted. Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3).

Ramucirumab was supplied by ImClone Systems in 20-mL glass vials containing 100 mg of product formulated at a concentration of 5.0 mg/mL in phosphate-buffered saline. The total dose was calculated based on body weight.

Pretreatment and Follow-Up Studies

Clinical and laboratory evaluations (CBC, serum chemistries, and urinalysis) were performed before treatment, weekly in cycles 1 and 2, and every other week thereafter. An ECG was obtained before treatment. Disease assessments by modified Response Evaluation Criteria in Solid Tumors (RECIST) were performed before treatment, after the 2-week treatment-free period in cycle 1, and every other cycle thereafter. Patients continued treatment until disease progression or intolerable toxicity.

Serum PK Sampling and Assay

In preclinical xenograft studies, ramucirumab serum concentrations ≥ 20 μg/mL were associated with anticancer activity; accordingly, PK analyses were directed toward identifying doses for subsequent studies capable of producing trough concentrations more than 20 μg/mL.

To measure ramucirumab serum concentrations, heparinized blood samples were collected before, immediately after, and at 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours after the first and final infusion in C1; 264 and 336 hours after the final infusion in C1; before and 1 hour after the final infusion in each cycle; at study completion; and on the 45-day follow-up visit, whenever possible. A validated bridging enzyme-linked immunosorbent assay measured

Table 1. Patient Demographics and Clinical Characteristics

Demographic or Clinical Characteristic	No. of Patients (N = 37)
Age, years	
Median	56
Range	36-76
ECOG performance status	
0	11
1	26
Sex	
Male	23
Female	14
Previous therapy	
Chemotherapy	30
Radiotherapy	20
Hormonal	7
VEGF-targeting agents (antiligand, TKI)	5
Immunotherapy	2
Investigational agent	15
Other	12
Primary malignancy	
Colorectal	6
Head and neck	4
Hepatocellular	3
Pancreas	3
Carcinoid, esophagus, neuroendocrine, ovary, prostate, melanoma, stomach, uterine (leiomyosarcoma)	16*
Bladder, breast, thyroid (medullary), thyroid (papillary), biliary tract	5†
Tumor response assessment	
Measurable disease	27
Evaluable disease	10
Prostate	2
Neuroendocrine	2
Esophagus	2
Liver	1
Colorectal	1
Thyroid	1
Melanoma	1

Abbreviations: ECOG, Eastern Cooperative Oncology Group; VEGF, vascular endothelial growth factor; TKI, tyrosine kinase inhibitor.
*Two patients had each of the malignancies listed.
†One patient had each of the malignancies listed.

Table 2. Dose-Escalation Scheme

Cohort No.	Ramucirumab Dose Level (mg/kg)	No. of New Patients	No. of Doses		No. of Patients With DLT (cycle 1)
			Median	Range	
1	2	6	5	1-26	0
2	4	4	29.5	28-105	0
3	6	4	16.5	4-99	0
4	8	5	22	2-141	0
5	10	7	11	4-34	1*
6	13	5	16	5-69	0
7	16	6	4	1-81	2†

Abbreviation: DLT, dose-limiting toxicity.
*Grade 3 hypertension.
†One patient with grade 3 deep venous thrombosis and one patient with grade 3 hypertension in cycle 2 (after protocol-defined DLT window).

ramucirumab serum concentrations. The assay was linear for ramucirumab in the range of 1 ng/mL (lower limit of quantitation) to 200 ng/mL (upper limit of quantitation), with an inter- and intra-assay precision of coefficient of variation of ≤ 10%. Bioanalytical assay methods are provided in the Appendix (online only). Noncompartmental PK analyses were performed on samples obtained in cycle 1 using WinNonlin software version 3.3 (Pharsight, Mountain View, CA).

PD Studies

Circulating concentrations of VEGF-A, sVEGFR-1, and sVEGFR-2 were measured from blood samples obtained after the first ramucirumab infusion in cycle 1 and before and 1 hour after the last infusion in subsequent cycles. PD markers were measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Further details are provided in the Appendix.

Detection of Human Antihuman Antibodies to Ramucirumab

Blood sampling to detect human antihuman antibodies to ramucirumab was performed before the last infusion of each cycle, at the end of therapy, and at the 45-day follow-up visit. Analytic methods are provided in the Appendix.

DCE-MRI

DCE-MRI was performed in patients with appropriate liver lesions to minimize methodologic variability. Specific image acquisition protocols are provided in the Appendix.

Perceptive Informatics (Waltham, MA) performed postprocessing kinetic modeling using the compartmental Tofts model for calculating the following three quantitative end points: initial area under the signal-intensity curve for the first 60 seconds after gadolinium injection, uptake rate constant as a volume transfer constant between blood and extravascular extracellular space, and volume of extravascular extracellular space for interstitial leakage space.[29] Results of DCE-MRI were pooled with data obtained from another phase I study of ramucirumab administered once every 2 to 3 weeks[30]; studies had identical entry criteria.

RESULTS

Patient Demographics and Treatment

Thirty-seven patients, whose relevant demographic and disease characteristics are listed in Table 1, received 888 total infusions of ramucirumab, given weekly at seven dose levels ranging from 2 to 16 mg/kg. The median number of infusions administered was 11 (range, one to 141 infusions). The dose-escalation scheme, numbers of cycles,

Table 3. Noncompartmental Ramucirumab PK Parameters

Dose Level and Cycle 1 Infusion	No. of Patients	Half-Life (hours)		PK Parameters in Cycle 1*							
				Trough C_{min} (μg/mL)		C_{max} (μg/mL)		$AUC_{0-\infty}$ (h · μg/mL)		Cl (mL/h/kg)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2 mg/kg											
First infusion	6	68.4	10.7	6.83	1.60	43.7	6.71	3,914	534	0.519	0.075
Last infusion	3	104	22.0	104	21.8	21.3	7.37	78	27	0.222	0.076
4 mg/kg											
First infusion	6	92.5	34.5	18.8	10.7	80.3	11.7	9,143	4,192	0.508	0.220
Last infusion	4	200	51.1	55.5	24.6	155	77.7	25,839	9,174	0.179	0.092
6 mg/kg											
First infusion	4	86.8	22.4	41.7	1.53	183	25.3	19,099	2,331	0.318	0.0.041
Last infusion	4	136	89.6	118	26.3	364	124	45,850	15,040	0.140	0.045
8 mg/kg											
First infusion	5	123	34.9	93.4	21.0	325	62.2	43,824	10,341	0.190	0.038
Last infusion	5	318	140	176	57.9	497	168	132,789	47,746	0.067	0.025
10 mg/kg											
First infusion	7	110	30.4	166	138	406	152	40,333	9,134	0.264	0.085
Last infusion	7	205	61.3	313	157	616	100	156,840	49,016	0.069	0.022
13 mg/kg											
First infusion	5	90.1	25.2	103	44.0	429	134	45,710	25,058	0.399	0.275
Last infusion	5	300	136	328	73.8	883	225	236,290	89,906	0.061	0.019
16 mg/kg											
First infusion	6	120	34.7	177	42.1	558	132	67,871	13,361	0.245	0.059
Last infusion	5	283	87.0	280	98.1	934	508	190,592	68,357	0.096	0.046

Abbreviations: PK, pharmacokinetic; C_{min}, minimum concentration; C_{max}, maximum concentration; $AUC_{0-\infty}$, area under the concentration-time curve from zero extrapolated to infinity; Cl, clearance; SD, standard deviation.
*All PK parameters (first infusion) were calculated over 168 hours after the first infusion in cycle 1. All PK parameters (final infusion) were calculated over 336 hours after the final infusion in cycle 1 except C_{min}, which was calculated 168 hours after the final infusion of cycle 1.

and DLTs in cycle 1 by dose level are listed in Table 2. At the time of data analysis for this publication, one patient (16 mg/kg) was receiving treatment after 85 weeks.

Toxicity

Two patients experienced DLTs in cycle 1. The first DLT occurred in a patient who developed grade 3 hypertension after receiving the fourth dose of ramucirumab (10 mg/kg), resulting in expansion of this dose level without further DLTs. This event resolved rapidly with antihypertensive medication; however, the patient developed disease progression and was taken off study. The second DLT in cycle 1 was a DVT that occurred in a patient after the fourth 16 mg/kg dose of ramucirumab. Although this event resolved within 2 weeks during treatment suspension, the patient discontinued treatment for disease progression. Another patient treated with ramucirumab 16 mg/kg developed grade 3 hypertension after the first dose of cycle 2. Although this particular DLT was not considered preclusive for dose escalation because the event occurred after cycle 1, its temporal proximity to cycle 1, together with the other cycle 1 DLT, contributed to the determination of 13 mg/kg as the MTD of ramucirumab on a weekly schedule.

Twenty-two patients (60%) experienced grade 3 to 5 adverse events, irrespective of potential drug-related attribution. The most frequently reported serious events included hypertension (13.5%), abdominal pain (10.8%), and anorexia, vomiting, increased blood alkaline phosphatase, headache, proteinuria, dyspnea, and DVT (each in 5.4% of patients).

Hypertension seemed to be related to both dose and cumulative therapy at higher doses. Five patients experienced grade 3 hypertension at doses ranging from 8 to 16 mg/kg. Hypertension was generally noted in the peritreatment period and occasionally associated with other symptoms, particularly headache. Hypertension generally resolved after administration of common oral antihypertensive medications, enabling further ramucirumab treatment. However, a patient with a history of sunitinib-related hypertension developed grade 3 hypertension and headache after receiving two 8 mg/kg doses. Hypertension did not respond to antihypertensives, eventually resulting in study discontinuation and resolution of events.

Other DLTs included grade 3 proteinuria in cycle 7 (4 mg/kg) and grade 3 vomiting in cycle 2 (2 mg/kg). The most frequently reported ($\geq$ 25% of all patients) adverse events, regardless of relation to ramucirumab, included fatigue (51.4%), headache (51.4%), peripheral edema (35.1%), diarrhea (35.1%), nausea (32.4%), upper respiratory tract infection (32.4%), abdominal pain (29.7%), anorexia (29.7%), constipation (29.7%), epistaxis (29.7%), proteinuria (29.7%), arthralgia (27.0%), cough (27.0%), and dyspnea (27.0%).

PKs

Mean PK parameters derived from noncompartmental analyses of the ramucirumab concentration versus time data after the first and final doses in cycle 1 are listed in Table 3. The clearance rate of ramucirumab decreased disproportionately as dose increased from 2 to 16 mg/kg. This nonlinear effect, suggesting saturation of ramucirumab clearance mechanism(s), was less evident at doses exceeding 8 mg/kg. After the initial and final infusions in cycle 1, clearance rate decreased in a manner suggesting that the steady-state was being approached. Furthermore, maximum concentration and area under the concentration-time curve from zero extrapolated to infinity, after the first and last infusions of ramucirumab in cycle 1, and half-life values increased disproportionately as dose increased from 2 to 16 mg/kg. Ramucirumab half-life at steady-state ranged from approximately 200 to 300 hours at doses of 8 to 16 mg/kg. A large interpatient variability was also noted with respect to PK parameters.

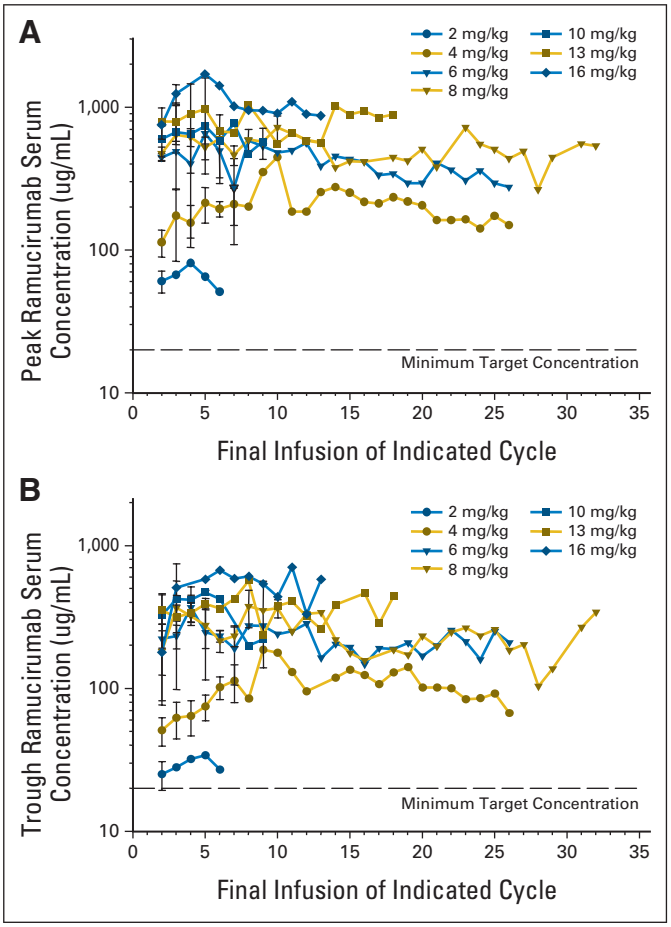

Fig 1. Scatterplots depicting mean values for (A) maximum concentration and (B) minimum concentration as a function of cycle number and at various ramucirumab dose levels (error bars indicate standard deviations).

Mean ramucirumab trough concentrations (C_{min}) were measured 7 days after treatment. In cycle 1, target C_{min} values ($\geq$ 20 μg/mL) were achieved at all doses, except for some patients after their first infusion at 2 to 4 mg/kg. Mean C_{min} and maximum concentration values as functions of ramucirumab dose and cumulative cycle number are shown in Figures 1A and 1B. C_{min} values measured in cycle 2 to cycle 32 averaged 29, 105, 222, 247, 353, 369, and 519 μg/mL after treatment with ramucirumab doses of 2, 4, 6, 8, 10, 13, and 16 mg/kg, respectively. There was negligible accumulation evident over at least 32 cycles of treatment (Figs 1A and 1B).

PDs

Serum biomarkers. The effects of ramucirumab on serum concentrations of VEGF-A, sVEGFR-2, and sVEGFR-1 after the first infusion in cycle 1 are shown in Figures 2A to 2F. Serum VEGF-A concentrations increased almost immediately after treatment, remaining elevated until the next treatment. At 7 days after treatment,

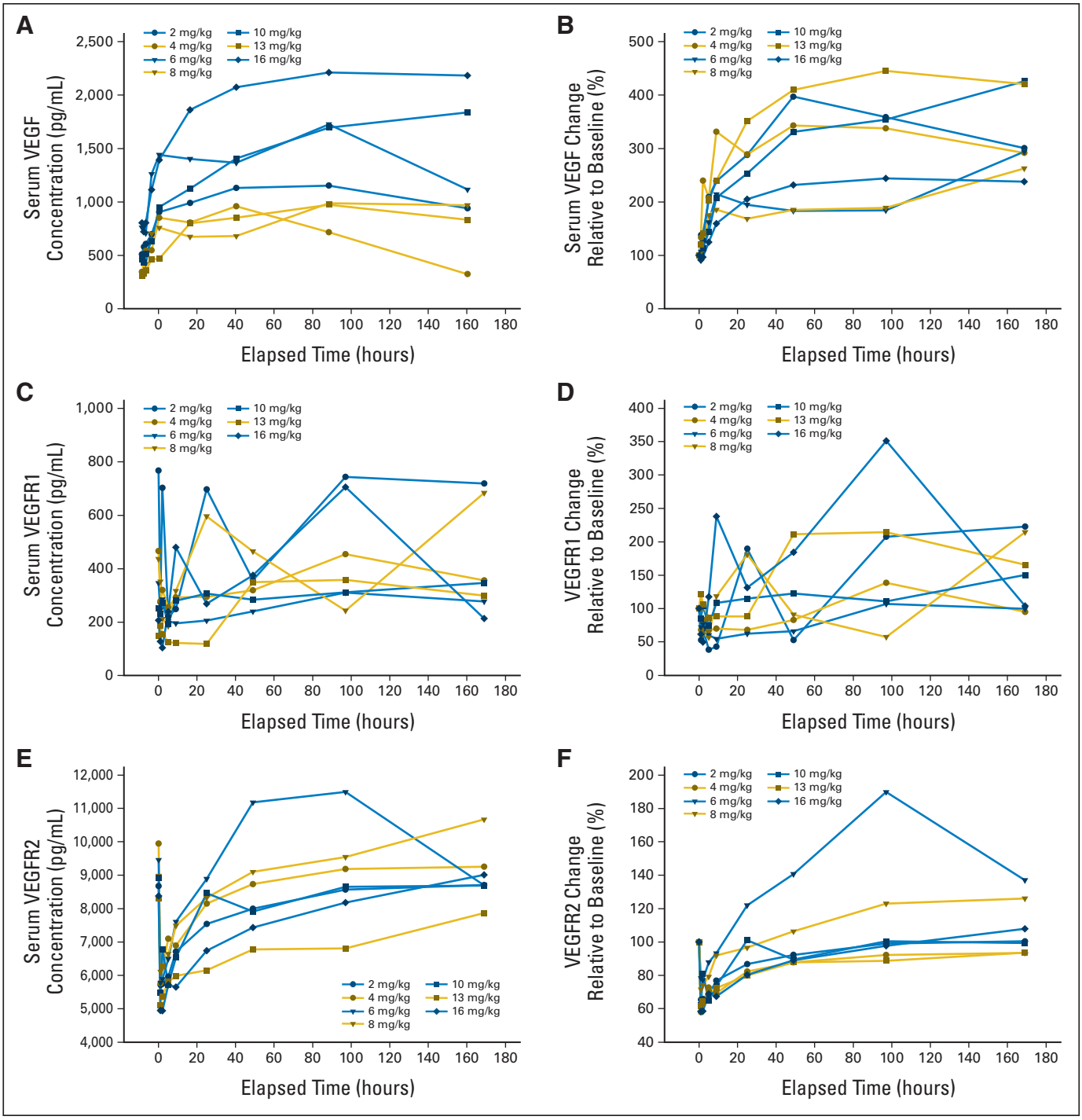

Fig 2. Scatterplots depicting (A, C, and E) raw data and (B, D, and F) mean percentage changes from pretreatment values in the following pharmacodynamic assessments over time after first infusion of ramucirumab: (A and B) serum vascular endothelial growth factor (VEGF)-A; (C and D) serum soluble VEGF receptor (sVEGFR)-1; and (E and F) serum sVEGFR-2.

VEGF-A concentrations were 1.5- to 3.5-fold higher than pretreatment concentrations. VEGF-A levels remained elevated through extended cycles (months for several patients) as long as ramucirumab was present (data not shown). Acute post-treatment elevations in VEGF-A concentrations were observed in all patients; the magnitude of this effect did not seem to be dose related. In contrast to VEGF-A concentrations, sVEGFR-1 and sVEGFR-2 concentrations generally decreased immediately after ramucirumab treatment and recovered to near-pretreatment levels. Similar to VEGF-A, the effects on sVEGFR-1 and sVEGFR-2 were not related to dose.

DCE-MRI. Post-treatment changes in tumor perfusion and vascularity were assessed using DCE-MRI parameters (initial area under the signal-intensity curve, volume of extravascular extracellular space, and uptake rate constant). Twenty-two patients had sequential

DCE-MRI performed. Kinetic modeling was completed for 13 patients (Appendix Table A1, online only).

Sequential DCE-MRI demonstrated decreased tumor perfusion and vascularity in nine (69%) of 13 patients, including five of seven patients in this study and four of six patients in the study with dosing every 2 to 3 weeks. A representative sequential DCE-MRI, demonstrating decreased perfusion and permeability in a liver metastasis after treatment with ramucirumab, is depicted in Appendix Figure A3 (online only). In patients for whom more than one tumor was assessed, decrements in tumor perfusion and vascularity consistently occurred in all target lesions.

Detection of Antibodies to Ramucirumab

Twenty-six patients with pre- and post-treatment samples were evaluated for development of antiramucirumab antibodies; none were detected.

Anticancer Activity

Twenty-seven patients had measurable disease, and 10 patients had evaluable disease. Table 4 lists the characteristics of patients who experienced confirmed partial response (PR) or stable disease (SD) lasting at least 12 weeks. Four patients, 11% of all patients and 15% of patients with measurable disease, had confirmed PRs, including two patients treated at 4 mg/kg (melanoma and gastric carcinoma, with PR lasting 31 and 103 weeks, respectively) and one patient each treated at 13 mg/kg (uterine leiomyosarcoma, with PR lasting 70 weeks) and 16 mg/kg (ovarian carcinoma, with PR lasting 86+ weeks). All patients received multiple prior therapies. Of 23 patients (62%) with SD as their best response, 11 patients with measurable disease had reductions in the sum of target lesions. Eleven patients (30%) experienced a confirmed PR or SD lasting ≥ 24 weeks across a range of doses. Other preliminary evidence of potential clinical benefit includes substantial reduction in tumor pain with reduced analgesic requirements (four patients) and significant reduction in refractory pleural effusions and frequency of palliative thoracenteses in a patient with papillary thyroid carcinoma.

Table 4. Patients With PR and SD (≥ 12 weeks)

Dose Level and Primary Malignancy	Best Response (≥ 12 weeks)	Duration of Response (weeks)
2 mg/kg		
Colorectal	SD	30
Breast	SD	15
Ovarian	SD	15
4 mg/kg		
Melanoma	PR	31
Gastric	PR	103
Colorectal	SD	31
6 mg/kg		
Head and neck	SD	105
Head and neck	SD	29
8 mg/kg		
Neuroendocrine (nasopharynx)	SD	151
Prostate	SD	41
Cholangiocarcinoma	SD	21
10 mg/kg		
Pancreatic	SD	37
Colorectal	SD	15*
Prostate	SD	14
Uterine leiomyosarcoma	SD	13
13 mg/kg		
Head and neck	SD	21
Uterine leiomyosarcoma	PR	70
Neuroendocrine	SD	23
16 mg/kg		
Ovarian	PR	86+*

Abbreviations: PR, partial response; SD, stable disease.
*Patient received prior anti–vascular endothelial growth factor therapy.

DISCUSSION

In contrast to other agents directed against the VEGFR-2/VEGF axis, ramucirumab binds a specific epitope on the extracellular domain of VEGFR-2, thereby blocking all VEGF ligands from binding to this therapeutically validated target.[23] Although a number of tyrosine kinas inhibitors are being used, their biochemical promiscuity and potential for off-target toxicities present potential limitations in cancer therapy. Because ramucirumab binds to VEGFR-2 specifically and with high affinity, it may offer a rational modulation advantage. Moreover, in contrast to bevacizumab, which binds to VEGF-A only, ramucirumab blocks all known VEGFs from binding to VEGFR-2. The combined effects of high specificity and more complete target inhibition could lead to a more complete blockade of angiogenesis.

In the present study, ramucirumab was well tolerated as a weekly 1-hour infusion. The principal toxicities encountered, including hypertension, vascular thrombotic events, and proteinuria, were similar qualitatively to those observed with other therapeutics targeting the VEGFR-2/VEGF axis.[31-41] Although some serious adverse effects occurred early at the 16 mg/kg dose, these events were uncommon and did not hinder ramucirumab administration for protracted periods. On the basis of the development of grade 3 hypertension associated with severe headache and a grade 3 DVT early into treatment at 16 mg/kg, the MTD on a weekly schedule was determined to be 13 mg/kg.

The ramucirumab PK profile was characterized by dose-dependent elimination and nonlinear exposure consistent with saturable clearance, similar to PK profiles exhibited by other antireceptor antibodies.[42-44] The large interindividual variability in PK parameters may be a result of the limited number of patients or unknown host effects. The ramucirumab PK profile served as a strong translational tool from preclinical experiments, supporting the notion that weekly doses are biologically relevant. The minimal target trough level (≥ 20 μg/mL), selected a priori based on PD and efficacy data in human tumor xenografts implanted in mice, was achieved in all treated patients (Fig 1B).

The primary elimination pathway for ramucirumab is likely receptor-mediated clearance; although target trough levels were reached at all doses, if drug clearance is rapid or not saturated, patients could have unblocked VEGFR-2 available to bind circulating VEGF and support tumor vascularity and growth. At doses more than 8 mg/kg, clearance is saturated, leading to a high probability that all VEGFR-2s are blocked by ramucirumab.

The affinity of ramucirumab for the VEGF-binding epitope on the extracellular domain of VEGFR-2 (dissociation constant = 50 pM) is much higher than the natural VEGF-A ligand.[6,26] Therefore, VEGF-A concentrations would be expected to increase after treatment as a result of displacement of the receptor-bound natural VEGF-A

ligand. Indeed, VEGF-A concentrations increased 1.5- to 3.5-fold across dose levels, particularly evident at doses ≥ 8 mg/kg; such elevations were sustained for protracted periods, likely because of the slow clearance of ramucirumab. The magnitude and duration of VEGF-A elevations after ramucirumab treatment may be useful PD indices to gauge adequacy of VEGFR-2 blockade. Some assay interference caused by the presence of ramucirumab was noted; however, the net effect decreased the sensitivity of the biomarker assay, suggesting that the results reflect a somewhat conservative presentation of the presence of these biomarkers. Early decrements in sVEGFR-1 and sVEGFR-2 receptors were also observed. These effects may be a result of ramucirumab binding to sVEGFR-2 and/or depletion of circulating VEGF-A binding sites as a result of the avid binding of both VEGFR-2 and sVEGFR-2 by ramucirumab. The magnitude of these effects, particularly the degree of elevated VEGF-A concentrations, did not seem to be dose related. These observations complement the results of PK studies in which ramucirumab target concentrations were exceeded at all dose levels, coupled with evidence of anticancer activity. The consistent and robust decrements in tumor perfusion and vascularity observed provide further preliminary support that the ramucirumab doses evaluated in this study, regardless of the small number of patients, are biologically relevant. However, it would not be prudent to relate these biomarker changes to anticancer activity because of the wide dosing range, the heterogeneous nature of patients with regard to tumor type and prior therapy, and the small patient population.

The apparent disease control rate, tabulated as the percentage of patients with PR or SD after ramucirumab monotherapy, was approximately 73%. Four patients had confirmed PRs with several types of refractory malignancies. Furthermore, 11 patients (30%) with an array of primary tumor types and dose ranges (2 to 16 mg/kg) experienced either PR or SD lasting ≥ 6 months.

These results provide proof that VEGFR-2 blockade with an MAb may be an effective anticancer strategy. Although ramucirumab is well tolerated at the MTD of 13 mg/kg, it may be reasonable to use ramucirumab doses as low as 8 mg/kg in disease-directed evaluations for several reasons. Ramucirumab PK clearance appears to be saturated at 8 mg/kg, suggesting receptor saturation because receptor-mediated clearance is likely the predominant clearance mechanism. Ramucirumab also has a tolerable safety profile, trough concentrations at least an order of magnitude higher than biologically relevant targets, favorable PD effects, preliminary evidence of decreased perfusion and vascularity on DCE-MRI, and anticancer activity. On the basis of these results, subsequent disease-directed development of ramucirumab is clearly warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Floyd Fox, ImClone Systems (C); Hagop Youssoufian, ImClone Systems (C); Eric K. Rowinsky, ImClone Systems (C) **Consultant or Advisory Role:** Roger B. Cohen, ImClone Systems (C) **Stock Ownership:** Hagop Youssoufian, ImClone Systems **Honoraria:** Roger B. Cohen, ImClone Systems; E. Gabriela Chiorean, Speaking honoraria, ImClone Systems **Research Funding:** Roger B. Cohen, ImClone Systems; D. Ross Camidge, ImClone Systems; E. Gabriela Chiorean, ImClone Systems; S. Gail Eckhardt, ImClone Systems **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Jennifer L. Spratlin, Roger B. Cohen, Neal J. Meropol, Floyd Fox, Hagop Youssoufian, Eric K. Rowinsky, S. Gail Eckhardt

Provision of study materials or patients: Jennifer L. Spratlin, Roger B. Cohen, Lia Gore, D. Ross Camidge, Sami Diab, Stephen Leong, Cindy O'Bryant, Laura Q.M. Chow, Neal J. Meropol, Nancy L. Lewis, E. Gabriela Chiorean, Hagop Youssoufian, S. Gail Eckhardt

Collection and assembly of data: Jennifer L. Spratlin, Roger B. Cohen, Matthew Eadens, Floyd Fox, Hagop Youssoufian

Data analysis and interpretation: Jennifer L. Spratlin, Roger B. Cohen, D. Ross Camidge, Natalie J. Serkova, Neal J. Meropol, Floyd Fox, Hagop Youssoufian, Eric K. Rowinsky, S. Gail Eckhardt

Manuscript writing: Jennifer L. Spratlin, Roger B. Cohen, Matthew Eadens, Lia Gore, D. Ross Camidge, Natalie J. Serkova, Neal J. Meropol, Floyd Fox, Hagop Youssoufian, Eric K. Rowinsky, S. Gail Eckhardt

Final approval of manuscript: Jennifer L. Spratlin, Roger B. Cohen, Matthew Eadens, Lia Gore, D. Ross Camidge, Sami Diab, Stephen Leong, Cindy O'Bryant, Laura Q.M. Chow, Natalie J. Serkova, Neal J. Meropol, Nancy L. Lewis, E. Gabriela Chiorean, Floyd Fox, Hagop Youssoufian, Eric K. Rowinsky, S. Gail Eckhardt

REFERENCES

1. Klagsbrun M, D'Amore PA: Vascular endothelial growth factor and its receptors. Cytokine Growth Factor Rev 7:259-270, 1996
2. Neufeld G, Cohen T, Gengrinovitch S, et al: Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 13:9-22, 1999
3. Ferrara N: Molecular and biological properties of vascular endothelial growth factor. J Mol Med 77:527-543, 1999
4. Aguayo A, Kantarjian H, Manshouri T, et al: Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 96:2240-2245, 2000
5. Carmeliet P, Jain RK: Angiogenesis in cancer and other diseases. Nature 407:249-257, 2000
6. Leung DW, Cachianes G, Kuang WJ, et al: Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246:1306-1309, 1989
7. Inoue M, Hager JH, Ferrara N, et al: VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic beta cell carcinogenesis. Cancer Cell 1:193-202, 2002
8. de Vries C, Escobedo JA, Ueno H, et al: The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255:989-991, 1992
9. Shibuya M, Yamaguchi S, Yamane A, et al: Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. Oncogene 5:519-524, 1990
10. Witte L, Hicklin DJ, Zhu Z, et al: Monoclonal antibodies targeting the VEGF receptor-2 (Flk1/KDR) as an anti-angiogenic therapeutic strategy. Cancer Metastasis Rev 17:155-161, 1998
11. Zhu Z, Witte L: Inhibition of tumor growth and metastasis by targeting tumor-associated angiogenesis with antagonists to the receptors of vascular endothelial growth factor. Invest New Drugs 17: 195-212, 1999
12. Zhu Z, Bohlen P, Witte L: Clinical development of angiogenesis inhibitors to vascular endothelial growth factor and its receptors as cancer therapeutics. Curr Cancer Drug Targets 2:135-156, 2002
13. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335-2342, 2004
14. Sandler A, Gray R, Perry MC, et al: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542-2550, 2006

15. Motzer RJ, Hutson TE, Tomczak P, et al: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115-124, 2007

16. Escudier B, Eisen T, Stadler WM, et al: Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 356:125-134, 2007

17. Giantonio BJ, Catalano PJ, Meropol NJ, et al: Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: Results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 25:1539-1544, 2007

18. Miller KD: E2100: A phase III trial of paclitaxel versus paclitaxel/bevacizumab for metastatic breast cancer. Clin Breast Cancer 3:421-422, 2003

19. Miller KD, Chap LI, Holmes FA, et al: Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 23:792-799, 2005

20. Adams VR, Leggas M: Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Ther 29:1338-1353, 2007

21. Posey JA, Ng TC, Yang B, et al: A phase I study of anti-kinase insert domain-containing receptor antibody, IMC-1C11, in patients with liver metastases from colorectal carcinoma. Clin Cancer Res 9:1323-1332, 2003

22. Jayson GC, Ton C, Parker GJ, et al: Phase I and DCE-MRI evaluation of CDP791, a di-Fab PEG conjugate that inhibits VEGFR2. J Clin Oncol 25: 143s, 2007 (suppl; abstr 3523)

23. Lu D, Jimenez X, Zhang H, et al: Selection of high affinity human neutralizing antibodies to VEGFR2 from a large antibody phage display library for antiangiogenesis therapy. Int J Cancer 97:393-399, 2002

24. Rockwell P, Neufeld G, Glassman A, et al: In vitro neutralization of vascular endothelial growth factor activation Flk-1 by a monoclonal antibody. Mol Cell Differ 3:91-109, 1995

25. Lu D, Shen J, Vil MD, et al: Tailoring in vitro selection for a picomolar affinity human antibody directed against vascular endothelial growth factor receptor 2 for enhanced neutralizing activity. J Biol Chem 278:43496-43507, 2003

26. Zhu Z, Hattori K, Zhang H, et al: Inhibition of human leukemia in an animal model with human antibodies directed against vascular endothelial growth factor receptor 2: Correlation between antibody affinity and biological activity. Leukemia 17: 604-611, 2003

27. Prewett M, Huber J, Li Y, et al: Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. Cancer Res 59:5209-5218, 1999

28. Bruns CJ, Liu W, Davis DW, et al: Vascular endothelial growth factor is an in vivo survival factor for tumor endothelium in a murine model of colorectal carcinoma liver metastases. Cancer 89:488-499, 2000

29. Tofts PS, Brix G, Buckley DL, et al: Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: Standardized quantities and symbols. J Magn Reson Imaging 10:223-232, 1999

30. Chiorean E, Sweeney C, Hurwitz H, et al: Phase I dose-escalation study of the anti-VEGFR-2 recombinant human IgG1 monoclonal antibody (MAb) IMC-1121B, administered every other week (q2w) or every 3 weeks (q3w) in patients (pts) with advanced cancers. 19th Annual EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, San Francisco, CA, October 22-26, 2007 (abstr B15)

31. Gordon MS, Margolin K, Talpaz M, et al: Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 19:843-850, 2001

32. Faivre S, Delbaldo C, Vera K, et al: Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 24:25-35, 2006

33. Britten CD, Kabbinavar F, Hecht JR, et al: A phase I and pharmacokinetic study of sunitinib administered daily for 2 weeks, followed by a 1-week off period. Cancer Chemother Pharmacol 61:515-524, 2007

34. Wilhelm SM, Carter C, Tang L, et al: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64:7099-7109, 2004

35. Clark JW, Eder JP, Ryan D, et al: Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. Clin Cancer Res 11:5472-5480, 2005

36. Awada A, Hendlisz A, Gil T, et al: Phase I safety and pharmacokinetics of BAY 43-9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. Br J Cancer 92:1855-1861, 2005

37. Moore M, Hirte HW, Siu L, et al: Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann Oncol 16:1688-1694, 2005

38. Drevs J, Siegert P, Medinger M, et al: Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. J Clin Oncol 25:3045-3054, 2007

39. Ryan CJ, Stadler WM, Roth B, et al: Phase I dose escalation and pharmacokinetic study of AZD2171, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinase, in patients with hormone refractory prostate cancer (HRPC). Invest New Drugs 25:445-451, 2007

40. Tamura T, Minami H, Yamada Y, et al: A phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. J Thorac Oncol 1:1002-1009, 2006

41. Holden SN, Eckhardt SG, Basser R, et al: Clinical evaluation of ZD6474, an orally active inhibitor of VEGF and EGF receptor signaling, in patients with solid, malignant tumors. Ann Oncol 16:1391-1397, 2005

42. Dirks NL, Nolting A, Kovar A, et al: Population pharmacokinetics of cetuximab in patients with squamous cell carcinoma of the head and neck. J Clin Pharmacol 48:267-278, 2008

43. Kuester K, Kloft C: Pharmacokinetics of Monoclonal Antibodies. Weinheim, Germany, Wiley-VCH, 2006, pp 45-91

44. Fracasso PM, Burris H 3rd, Arquette MA, et al: A phase 1 escalating single-dose and weekly fixed-dose study of cetuximab: Pharmacokinetic and pharmacodynamic rationale for dosing. Clin Cancer Res 13:986-993, 2007

