Clinical Cancer Research

First-in-Human Phase I Study of Single-agent Vanucizumab, A First-in-Class Bispecific Anti-Angiopoietin-2/Anti-VEGF-A Antibody, in Adult Patients with Advanced Solid Tumors

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Abstract

Purpose: Vanucizumab is an investigational antiangiogenic, first-in-class, bispecific mAb targeting VEGF-A and angiopoietin-2 (Ang-2). This first-in-human study evaluated the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of vanucizumab in adults with advanced solid tumors refractory to standard therapies.

Experimental Design: Patients received escalating biweekly (3–30 mg/kg) or weekly (10–30 mg/kg) intravenous doses guided by a Bayesian logistic regression model with overdose control.

Results: Forty-two patients were treated. One dose-limiting toxicity, a fatal pulmonary hemorrhage from a large centrally located mediastinal mass judged possibly related to vanucizumab, occurred with the 19 mg/kg biweekly dose. Arterial hypertension (59.5%), asthenia (42.9%), and headache (31%) were the most common toxicities. Seventeen (41%) patients experienced treatment-related grade $\geq$3 toxicities. Toxicity was generally higher with weekly than biweekly dosing. A MTD of vanucizumab was not reached in either schedule. Pharmacokinetics were dose-linear with an elimination half-life of 6–9 days. All patients had reduced plasma levels of free VEGF-A and Ang-2; most had reductions in K^{TRANS} (measured by dynamic contrast-enhanced MRI). Two patients (renal cell and colon cancer) treated with 30 mg/kg achieved confirmed partial responses. Ten patients were without disease progression for $\geq$6 months. A flat-fixed 2,000 mg biweekly dose (phamacokinetically equivalent to 30 mg/kg biweekly) was recommended for further investigation.

Conclusions: Biweekly vanucizumab had an acceptable safety and tolerability profile consistent with single-agent use of selective inhibitors of the VEGF-A and Ang/Tie2 pathway. Vanucizumab modulated its angiogenic targets, impacted tumor vascularity, and demonstrated encouraging antitumor activity in this heterogeneous population. *Clin Cancer Res; 24(7); 1536–45. ©2017 AACR.*

Introduction

Targeting angiogenesis is an attractive therapeutic approach in many cancers (1). Several inhibitors of the VEGF receptor (VEGFR) and family of ligands are approved, including the anti-VEGF-A mAb bevacizumab; the anti-VEGFR2 mAb ramucirumab; the dual VEGF-A/placenta growth factor (PlGF) inhibitor aflibercept; and the tyrosine kinase inhibitors sunitinib, sorafenib, pazopanib, regorafenib, and axitinib. Bevacizumab plus chemotherapy prolongs patient survival in many cancers compared with chemotherapy alone (2). However, patients who respond to VEGF/VEGFR inhibition invariably develop resistance due to activation of compensatory angiogenic signaling pathways (3).

Resistance to VEGF-targeted therapies may be partly mediated by Angiopoietin-1 and 2 (Ang-1 and Ang-2), the functional ligands of the Tie2 receptor tyrosine kinase found on endothelial cells (4, 5). Binding of Ang-1 to Tie2 stabilizes new blood vessels leading to maturation, whereas binding of Ang-2 to Tie2 triggers angiogenic sprouting and increased vessel plasticity (6). A shift in the Ang-1/Ang-2 ratio toward Ang-2 therefore represents a proangiogenic switch. Tumors expressing high levels of Ang-2 have a poor prognosis (7–10) and poor response to bevacizumab-based therapy (11, 12) and sunitinib (13). Several Ang-2 pathway inhibitors have been developed including the Ang-1/Ang-2–neutralizing peptibody trebananib (AMG 386); the fusion proteins CVX-060 and CVX-241(the latter binds both Ang-2 and VEGF); and the Tie2 receptor inhibitors ARRY614 and CEP-11981.

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Translational Relevance

Treatment of cancer by targeting VEGF-A with bevacizumab has been extensively validated in the clinic. However, not all tumors respond to VEGF-A blockade and escape mechanisms due to overlapping and compensatory pathways can limit the full potential of antiangiogenic agents. Angiopoietin-2 (Ang-2) and VEGF-A have complementary roles in regulating tumor angiogenesis and metastasis, suggesting that dual pathway inhibition is necessary to improve treatment outcomes. Vanucizumab is an investigational, first-in-class, bispecific IgG1-like mAb using CrossMab technology that simultaneously blocks VEGF-A and Ang-2 from interacting with their receptors. This first-in-human study demonstrated an acceptable safety and tolerability profile for vanucizumab in patients with advanced solid tumors. A MTD was not reached with either vanucizumab schedule (weekly/biweekly) tested at the highest dose (30 mg/kg). Vanucizumab also demonstrated consistent pharmacokinetic and pharmacodynamic effects, and showed encouraging signs of antitumor activity that support further evaluation of vanucizumab in clinical trials.

Dual Ang-2 and VEGF-A inhibition is attractive as both pathways show complementary and synergistic actions during tumor angiogenesis (14, 15). Tumors with elevated levels of both proteins have a worse prognosis than those expressing high levels of either protein alone (8, 16). Vanucizumab (RG7221/RO5520985) is a novel humanized IgG1-like bispecific mAb established using CrossMAb technology that binds both Ang-2 and VEGF-A with high affinity (17, 18). Vanucizumab inhibited angiogenesis, tumor growth, and metastasis formation *in vivo* more effectively than mono-specific anti-Ang-2 or anti-VEGF-A mAbs, and reduced microvessel density and promoted vessel maturation in a murine breast cancer model (17, 18). Normalization of vessel architecture appeared to improve chemotherapy drug delivery; combining vanucizumab with docetaxel resulted in complete and long-term tumor regression *in vivo* (18). Importantly, vanucizumab did not aggravate the known adverse effect of anti-VEGF-A treatment on healthy vessels.

Here we report results from part I of the first-in-human phase I study that investigated the MTD and/or recommended phase II dose (RP2D) of vanucizumab in patients with locally advanced or metastatic solid tumors.

Patients and Methods

The study was conducted according to the principles of the Declaration of Helsinki and was registered (clinicaltrials.gov NCT01688206). Institutional Review Board approval and written informed consents were obtained before study-related procedures commenced.

Eligibility criteria

Inclusion criteria included age $\geq$18 years; histologically/cytologically confirmed locally advanced or metastatic, nonresectable solid tumors that progressed despite standard therapy, or where no standard therapy existed; measurable disease by Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 criteria; Eastern Cooperative Oncology Group performance status $\leq$1; and adequate hematologic, coagulative, hepatic, renal, and cardiovascular function. Exclusion criteria included unstable primary central nervous system tumors or metastasis; squamous non–small cell lung cancer histology; hemoptysis grade (G) $\geq$2 within 4 weeks; bleeding diathesis or coagulopathy; major surgery within 4 weeks; anticancer therapy within 4–6 weeks (6 months for agents targeting VEGF/Ang-1/Ang-2/PlGF pathways); significant cardiovascular/cerebrovascular disease; or abdominal fistula/gastrointestinal perforation within 6 months.

Study design

This was part I (dose-escalation) of a four-part first-in-human, open-label, multicenter, phase I study. The primary objective was to determine the MTD and/or RP2D of vanucizumab. Secondary objectives were to characterize the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of single-agent vanucizumab.

Vanucizumab was administered by intravenous infusion without premedication on day (D) 1 of each cycle with two dosing schedules: weekly and biweekly. One cycle (C) corresponded to one vanucizumab dose. Vanucizumab was infused over 90 minutes for the first infusion. If the first and second infusion were tolerated without signs and symptoms of infusion-related reactions (CTCAE G$\geq$2), the second and all subsequent infusions were administered over 60 and 30 minutes, respectively.

The starting dose of 3 mg/kg for biweekly dosing was determined on the basis of nonclinical toxicology and efficacy studies, anticipated efficacious concentration based on dynamic pharmacokinetic/pharmacodynamic modeling, and subsequent prediction of the human pharmacokinetics of vanucizumab. This dose was anticipated to result in safety margins of 26-fold based on the AUC_{τ} to the exposure at the highest nonseverely toxic dose of 60 mg/kg and 52-fold based on C_{max}. This dose was close to the predicted minimum efficacious dose of 3.5 mg/kg. One patient was enrolled at the 3 mg/kg biweekly starting dose and at least three patients in subsequent cohorts. The first patient at each dose level was monitored for the first five days of C1. If no dose-limiting toxicity (DLT) occurred, the remaining patients in the cohort were treated simultaneously.

Dose escalation was guided by an Escalation With Overdose Control model with modified continual reassessment (Supplementary Methods; refs. 19, 20). This method seeks a dose level close to the MTD by using toxicity data (incidence of DLTs) from evaluable patients to estimate a dose–toxicity curve and minimizes the number of patients treated at possibly pharmacologically inactive doses. To our knowledge, this is the first reported phase I entry-into-human study to employ a continual reassessment method (CRM) design to assess the safety/tolerability at escalating dose levels of an investigational, large-molecule antiangiogenic drug.

Intra-patient dose escalation to a higher dose already considered safe was allowed after C6 (biweekly) and C12 (weekly).

DLTs, MTD, and RP2D

Toxicities were graded according to Common Terminology Criteria for Adverse Events version 4.03. A DLT was defined as an adverse event (AE) considered study drug–related occurring during the 28-day observation period (42 days in case of treatment delays) that met the criteria listed in Supplementary Table S1. The MTD was defined as the predicted dose at which the probability of DLT rate was 33%. The prediction was based on

two-parameter logistic dose–toxicity relationship model using a uniform prior for the toxicity at the starting dose and a truncated normal prior for the MTD.

The RP2D was selected on the basis of the integrated analysis of clinical safety/tolerability, antitumor activity, pharmacodynamic (including functional imaging), and pharmacokinetic data. Once a suitable dose had been established, pharmacokinetic modeling and simulation was conducted to identify a flat-fixed dose that had comparable steady-state systemic exposures, peak, and trough concentrations.

Pharmacokinetics

Blood for pharmacokinetic assessments was taken during C1, C2, and C4 at predose, 0.5 hours, end-of-infusion, and 2, 4, 6, 24, 96, and 168 (biweekly cohort only) hours post start of infusion. From C5, predose and end-of-infusion samples were taken every cycle (biweekly) or every second cycle (weekly). Additional samples were taken upon the occurrence of DLTs, dose reductions, disease response/progression, or study discontinuation. Plasma vanucizumab was measured using a validated ELISA (lower limit of quantification 1.00 ng/mL). Pharmacokinetic parameters were estimated using noncompartmental analysis (Supplementary Methods) with Phoenix WinNonlin (v6.2; Pharsight). Anti-drug antibodies (ADA) were measured using a bridging ELISA at baseline, every 4 weeks, and upon occurrence of a G$\geq$3 hypersensitivity reaction, to assess the potential immunogenicity of vanucizumab.

Pharmacodynamics

Circulating free and total (drug-bound and unbound) VEGF-A and Ang-2 levels were measured (see Supplementary Methods) in plasma obtained at baseline and D1, D2, D5 (biweekly), D8 (weekly), D15 (biweekly only), D18 (weekly only) D29, D57, and at disease progression/study discontinuation.

Multiparametric diffusion-weighted (DW) and dynamic contrast-enhanced (DCE) MRI scans were performed twice at baseline ($\geq$2 days apart) and on D5, D15, and D29 (biweekly dosing), or D8 and D18 (weekly dosing). Target lesions were selected on the basis of contrast uptake and lesion size ($\geq$2/$\geq$3 cm in areas subject to low/high respiratory motion, respectively). Parameters evaluated comprised K^{TRANS} derived from Extended Toft model, the blood gadolinium-normalized area under the gadolinium concentration–time curve (AUC_{BN}), and the apparent diffusion coefficient (ADC). Images were analyzed independently (VirtualScopics) blinded to the administered vanucizumab dose.

Response evaluation

Tumors were assessed using CT or MRI per RECIST criteria (version 1.1) at baseline and every 8 $\pm$ 1 weeks thereafter.

Statistical analysis

The planned sample size for this dose-escalation part of the study was 18 (minimum) to 60 (maximum) patients. Descriptive statistics were presented for all data. Logarithmically transformed, dose-normalized values of the area under the concentration–time curve from 0 hours to time t (AUC_{0-t}), minimum concentration (C_{min}), and maximum concentration (C_{max}) were analyzed separately by regimen using a linear mixed-effect model to evaluate accumulation and dose proportionality. Population pharmacokinetics and the relationship between pharmacokinetic, pharmadynamic biomarkers, and safety parameters were explored using nonlinear mixed-effect modeling (NONMEM version VI, ADVAN4, GloboMax LLC).

Results

Between October 2012 and November 2013, 42 patients (Table 1) were enrolled and treated at seven dose levels. Patients received a median of eight cycles of vanucizumab on both schedules [range 1–70 (biweekly) and 2–56 (weekly)]. The overall median treatment duration was 63 days. Twenty-two (52.4%) patients were treated on the biweekly schedule [3 mg/kg ($n = 1$), 6 mg/kg ($n = 3$), 12 mg/kg ($n = 4$), 19 mg/kg ($n = 8$), 30 mg/kg ($n = 6$)], and 20 (47.6%) patients on the weekly schedule [10 mg/kg ($n = 4$), 20 mg/kg ($n = 4$), 30 mg/kg ($n = 12$)]. At the data cutoff (December 02, 2016), 41 patients had discontinued the study due to disease progression ($n = 34$), consent withdrawal ($n = 1$), and toxicities ($n = 6$). The six toxicities leading to withdrawal were all assessed as study drug related by the investigator and comprised one death (described below); cerebral hemorrhage (G2), hypertension (G3), thrombotic microangiopathy (G3), and hypertensive encephalopathy (G4) all at 30 mg/kg weekly vanucizumab and vesicocutaneous fistula (G3) at 12 mg/kg biweekly vanucizumab.

DLT and MTD

Vanucizumab was reasonably well tolerated across dose levels and schedules. The MTD was not reached. One DLT (hemoptysis with fatal outcome) occurred in a female with colorectal cancer and a large, centrally located mediastinal mass. This patient

Table 1. Baseline demographics and disease characteristics of the treated patients ($N = 42$)

Characteristics	Value
Gender (male, female)	18, 24
Median age (range), years	63 (25–76)
Tumor stage at time of first diagnosis, *n* (%)	
III	11 (26.2)
IV	18 (42.9)
Other	13 (31.0)
Tumor stage at time of study entry, *n* (%)	
Metastatic	40 (95.2)
Locally advanced	2 (4.8)
Tumor type, *n* (%)	
Colon cancer	13 (31.0)
Breast cancer	3 (7.1)
Cervical cancer	3 (7.1)
Ovarian cancer	2 (4.8)
Gastric cancer	2 (4.8)
Pancreatic cancer	2 (4.8)
Rectum cancer	2 (4.8)
Uterine leiomyosarcoma	2 (4.8)
Cancer of unknown primary	2 (4.8)
Adenoid cystic carcinoma	2 (4.8)
Endometrial cancer	2 (4.8)
Other[a]	7 (16.7)
Median previous lines of therapy (range)	3 (1–10)
Antimetabolites, *n* (%)	33 (78.6)
Platinum compounds, *n* (%)	31 (73.8)
Topoisomerase inhibitors, *n* (%)	25 (59.5)
Cytotoxic agents, *n* (%)	19 (45.2)
Radiotherapy, *n* (%)	17 (40.5)
Bevacizumab	8 (19.0)

[a]One case each of bladder cancer, renal cell cancer, soft tissue (Ewing) sarcoma, thyroid cancer, non–small cell lung cancer, hypopharyngeal cancer, and ovarian/endometrial cancer.

Table 2. Adverse events regardless of relationship to vanucizumab occurring in ≥10% of the total population ($N = 42$)

	NCI-CTCAE		
Adverse event, *n* (%) MedDRA preferred term	**Grade 1/2**	**Grade 3/4[a,b]**	**Total, *n* (%)**
Hypertension	11 (26.2)	14 (33.3)	25 (59.5)
Asthenia	17 (40.5)	1 (2.4)	18 (42.9)
Headache	12 (28.6)	1 (2.4)	13 (31.0)
Diarrhea	10 (23.8)	1 (2.4)	11 (26.2)
Cough	10 (23.8)	—	10 (23.8)
Back pain	9 (21.4)	—	9 (21.4)
Constipation	9 (21.4)	—	9 (21.4)
Fatigue	8 (19.0)	—	8 (19.0)
Vomiting	8 (19.0)	—	8 (19.0)
Dysphonia	7 (16.7)		7 (16.7)
Anxiety	6 (14.3)	—	6 (14.3)
Arthralgia	6 (14.3)	—	6 (14.3)
Dyspnea	6 (14.3)	—	6 (14.3)
Musculoskeletal pain	6 (14.3)	—	6 (14.3)
Nausea	6 (14.3)	—	6 (14.3)
Toothache	6 (14.3)	—	6 (14.3)
Abdominal pain	4 (9.5)	1 (2.4)	5 (11.9)
Decreased appetite	5 (11.9)	—	5 (11.9)
Edema peripheral	5 (11.9)	—	5 (11.9)
Proteinuria	4 (9.5)	1 (2.4)	5 (11.9)

Abbreviations: MedDRA; Medical Dictionary for Regulatory Activities; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events.
[a]One patient had a G5 event (a DLT of pulmonary hemorrhage in a patient treated with 19 mg/kg biweekly).
[b]Other G3/4 AEs comprised thrombocytopenia in two (4.8%) patients and one case (2.4%) each of peripheral sensory neuropathy, ascites, dyspnea exertional, gamma−glutamyltransferase increased, stomatitis, appendicitis, blood alkaline phosphatase, blood bilirubin increased, hepatic failure, hypertensive encephalopathy, hyperuricaemia, hypokalaemia, infection, pleural effusion, pneumonia, subileus, thrombotic microangiopathy, urinary incontinence, and vesicocutaneous fistula. Multiple occurrences of the same adverse event in one individual counted only once with the highest grade.

received two doses of 19 mg/kg biweekly vanucizumab and died on D25.

Safety

The most common AEs were hypertension, asthenia, headache, and diarrhea (Table 2). Thirteen (31%) patients experienced 14 serious AEs (SAE); including seven SAEs considered to be study drug-related: cerebral hemorrhage, hypertension, hypertensive encephalopathy, and thrombotic microangiopathy (all 30 mg/kg weekly); vesicocutaneous fistula (12 mg/kg biweekly); hemoptysis (19 mg/kg biweekly); and blood creatinine increase (30 mg/kg biweekly).

Seventeen (40.5%) patients experienced G≥3 toxicities judged to be study drug-related. Table 3 summarizes severe AEs expected from the mode of action (MoA) of vanucizumab. Fourteen (33%) patients experienced G≥3 hypertension, particularly with weekly and higher vanucizumab doses. Eight of these patients had a history of preexisting arterial hypertension. Hypertension led to vanucizumab dose modification/interruption in three patients and vanucizumab discontinuation in one patient. Ten (24%) patients experienced hemorrhagic events. Eight events were non-serious and G1: epistaxis (three patients); hemoptysis (two patients); mouth hemorrhage, purpura, and melena (one patient each). The other two events were SAEs, study drug-related, and led to vanucizumab discontinuation: G5 hemoptysis (19 mg/kg biweekly; described above) and G2 cerebral hemorrhage (30 mg/kg weekly). Five patients (11.9%) experienced proteinuria: 1 G3 (12 mg/kg biweekly) and 4 G1/2. Edema occurred in 5 (11.9%) patients (all G1/2).

Five patients had G1 AEs suggestive of an infusion-related reaction: influenza-like illness (3 patients) and hot flush and pruritus (1 patient each). Infusions were not interrupted or stopped in any of these patients.

Pharmacokinetics

Vanucizumab pharmacokinetic profiles are shown in Fig. 1A and descriptive statistics for the parameters are provided in Supplementary Tables S2 and S3. C_{max} and $AUC_{0-\tau}$ increased dose proportionally for both schedules with low inter-individual variability (<43%). Across the doses and schedules investigated, the volume of distribution of vanucizumab ranged from 2.9 to 6.1 L, plasma clearance was similar (range 10.9–36.7 mL/h), and

Table 3. Adverse events of special interest grade ≥ 3 (regardless of relationship)

	Biweekly dosing						Weekly dosing			
Adverse event, *n* (%) MedDRA preferred term	**3 mg/kg (*n* = 1)**	**6 mg/kg (*n* = 3)**	**12 mg/kg (*n* = 4)**	**19 mg/kg (*n* = 8)**	**30 mg/kg (*n* = 6)**	**Total (*n* = 22)**	**10 mg/kg (*n* = 4)**	**20 mg/kg (*n* = 4)**	**30 mg/kg (*n* = 12)**	**Total (*n* = 20)**
Hypertension	—	—	—	—	4 (66.7)	4 (18.2)	1 (25.0)	1 (25.0)	8 (66.7)	10 (50.0)
Thrombocytopenia	—	—	—	1 (12.5)	—	1 (4.5)	—	—	1 (8.3)	1 (5.0)
Proteinuria	—	—	1 (25.0)	—	—	1 (4.5)	—	—	—	—
Hemoptysis	—	—	—	1 (12.5)[a]	—	1 (4.5)	—	—	—	—
Vesicocutaneous fistula	—	—	1 (25.0)	—	—	1 (4.5)	—	—	—	—
Hypertensive encephalopathy	—	—	—	—	—	—	—	—	1 (8.3)	1 (5.0)
Thrombotic microangiopathy	—	—	—	—	—	—	—	—	1 (8.3)	1 (5.0)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities. Multiple occurrences of the same AE in one individual are counted once.
[a]G5 adverse event.

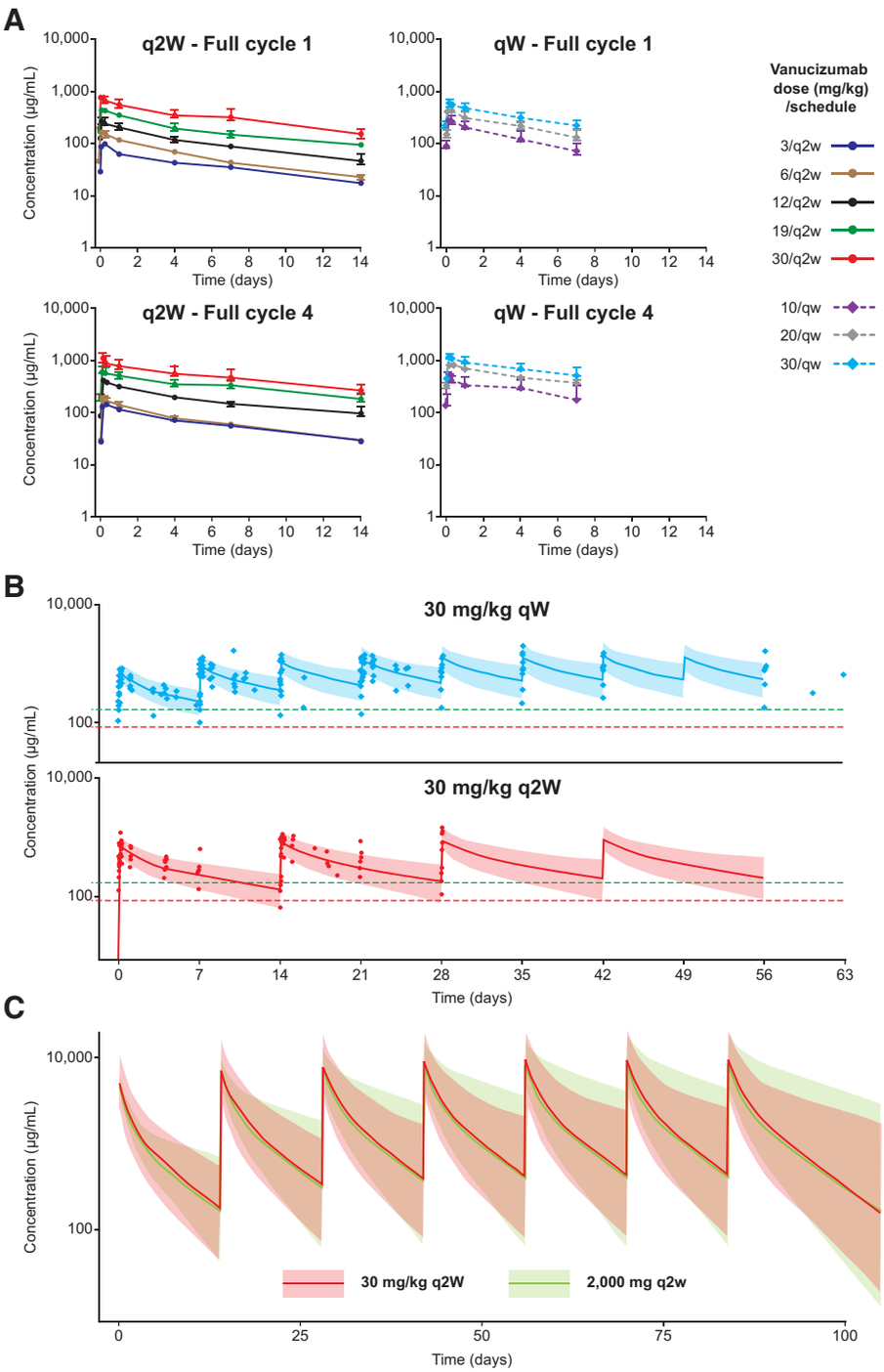

Figure 1.
Pharmacokinetics of vanucizumab. **A,** Plasma concentration–time course (mean ± SD) of vanucizumab on cycles 1 and 4 following a biweekly (q2w) or weekly (qw) schedule at doses of 3 mg/kg to 30 mg/kg. **B,** Observed and model predicted vanucizumab concentrations after multiple administrations of vanucizumab 30 mg/kg biweekly or weekly. Circles/diamonds indicate observed values, solid lines indicate median predicted values, and shaded regions are the 95% predicted intervals. Red and green dotted lines indicates the C_{trough} levels for bevacizumab 5 mg/kg biweekly and 10 mg/kg biweekly, respectively. **C,** Model predicted vanucizumab concentrations after multiple administrations of vanucizumab 30 mg/kg biweekly or 2,000 mg biweekly (solid lines indicate medians, and shaded regions are the 95% predicted intervals).

$t_{1/2}$ remained stable (range 6–9 days). Greater accumulation occurred with weekly dosing [accumulation ratios: 2.6-fold (C_{min}), 2.1-fold ($AUC_{0-\tau}$)] than with biweekly dosing [1.9-fold (C_{min}), 1.7-fold ($AUC_{0-\tau}$)].

Population pharmacokinetic modeling indicated that C_{trough} and C_{avg} vanucizumab concentrations comparable with those seen with 5 and 10 mg/kg bevacizumab (biweekly) would occur in 95% and 50%, respectively, of patients treated with 30 mg/kg biweekly vanucizumab by C2 (Fig. 1B). Simulations adjusted for an average 70 kg body weight predicted that a 2,000 mg biweekly flat dose would achieve similar vanucizumab exposure and peak/trough concentrations as 30 mg/kg biweekly dosing (Fig. 1C).

Two (5%) patients developed ADA following vanucizumab treatment (one after four cycles of 10 mg/kg weekly and one after 12 cycles of 30 mg/kg weekly); both without associated clinical symptoms or change in vanucizumab exposure. Accordingly, the ADA appeared to be non-neutralizing. Two patients had preexisting ADA.

Pharmacodynamics

The median baseline total plasma Ang-2 level was 2.5 ng/mL (range: 1.6–9.3 ng/mL) without apparent differences across tumor types. As expected, plasma levels of free VEGF-A and Ang-2 were depleted within 24 hours of administration, independent of dose and schedule (Fig. 2A and B). Conversely, total VEGF-A and Ang-2 levels increased upon first dose of vanucizumab.

MRI was conducted in 34 patients, including 29 and 25 patients, respectively, with two baseline DCE-MRI and DW-MRI scans. The median baseline coefficient of variability was 20% for K^{TRANS}, 9.0% for AUC_{BN}, and 11.2% for ADC. Twenty-two (64.7%) patients had moderate-to-significant sustained posttreatment reductions in DCE-MRI parameters reflecting changes in vascular permeability, leakiness, and perfusion. Patients were grouped as either weekly or biweekly $\leq$20 mg/kg and 30 mg/kg. At C2D1, percent decrease from baseline in median K^{TRANS} for $\leq$20 mg/kg biweekly was lower than $\leq$20 mg/kg weekly (–0.79 $\pm$9.68 vs. -24.06 ± 14.56), while for 30 mg/kg biweekly and 30 mg/kg weekly, the percent decrease from baseline in median K^{TRANS} was similar ($-31.37\% \pm 15.68$ vs. $-29.74\% \pm 6.53$; Fig. 2E). A trend toward sustained decreases in vessel permeability and leakiness was observed in weekly patients and biweekly patients treated with 30 mg/kg (Fig. 2E). Supplementary Figure S1 shows parametric K^{TRANS} map MRI images illustrating changes in vascular permeability for a colorectal cancer patient treated with 30 mg/kg biweekly. The median ADC for the 25 evaluable patients ranged from 769 to 4,805 μm/s. Seven (28%) patients had a >20% increase in posttreatment necrosis; six of whom were treated at 30 mg/kg (weekly or biweekly).

Clinical activity

Thirty-eight patients were evaluable for tumor response: Two had confirmed partial responses (PR), one had unconfirmed PR, 19 had stable disease, and 16 had progressive disease (Fig. 3). A 65-year-old male with metastatic colon cancer who switched from 30 mg/kg weekly to biweekly dosing after 6 months achieved a confirmed PR after one year of treatment. A 68-year-old male with metastatic renal cell cancer also exposed to 30 mg/kg biweekly had a confirmed PR upon 20 months of treatment and meanwhile remains on study for more than 3 years. A 48-year-old female with cervical cancer achieved a PR after six cycles of 30 mg/kg weekly, but discontinued the study due to brain hemorrhage before a confirmatory scan was available.

Eleven (55%) and 8 (44%) patients on the biweekly and weekly schedules, respectively, had reductions in the sum of target lesions at least once during the study. At the first 8-week tumor assessment, 13 (65%) and 9 (50%) evaluable patients on the biweekly and weekly schedules, respectively, had not progressed. Overall, 10 patients were without disease progression for at least 6 months.

Discussion

In this phase I study, vanucizumab, an investigational, first-in-class, bispecific Ang-2 and VEGF-A inhibitor, had an acceptable safety and tolerability profile in adult patients with advanced cancer consistent with that seen with bevacizumab and selective inhibitors of the Ang/Tie2 axis. Of note, the bispecific antibody generated using CrossMAb technology did not have a higher immunogenic potential compared with conventional antibody formats. One patient died due to pulmonary bleeding from a large centrally located mediastinal mass; this event was the only vanucizumab-related DLT. A MTD was not reached at the highest dose administered (30 mg/kg weekly /biweekly). Arterial hypertension was the most common G3/4 AE of vanucizumab, with an overall incidence of almost 60% (G$\geq$3: 33%) and which was higher than previously reported for other single-agent inhibitors of the VEGF-A or Ang/Tie2 axis. Hypertension was generally asymptomatic and easily controlled. High blood pressure appeared to be dose dependent and predominantly driven by the systemic vanucizumab-mediated VEGF-A inhibition. Across clinical studies, the frequency of G3/4 hypertension is 9%–32% with bevacizumab monotherapy (21–25) but $\leq$6% for single-agent Ang-1/2 antagonists (26–33). Moreover, hypertension rates with bevacizumab treatment did not increase by concurrent inhibition of the Ang/Tie-2 signaling pathway in patients with metastatic breast cancer [39%–40% (G$\geq$3: 18%–23%) compared with 38% (G$\geq$3: 19%) without concurrent Ang/Tie-2 inhibition; ref. 33)].

Ang-2 and VEGF may be involved in promoting the integrity of glomerular endothelia (34–36). Altered glomerular permeability with leakage of large proteins into the urine appears to be a direct result of VEGF-A and Ang-2 inhibition. Proteinuria is a common side effect of bevacizumab with an overall incidence (all grades) of 20% (37) and has been reported in 14% of patients treated with agents targeting the Ang/Tie2 pathway (28). Simultaneous inhibition of VEGF-A and Ang-2 with vanucizumab did not increase the rate or grade of proteinuria. Although the exact mechanism by which edema might occur is unclear, dual, nonselective Ang-1 and Ang-2 inhibition frequently induces edema formation in up to 30% of patients (27, 28). In preclinical models, Ang-1 acts as an antipermeability factor and protects adult vessels from leaking (38). We observed mild-to-moderate peripheral edema in 12% of our patients treated with the Ang-2–selective CrossMAb, thereby suggesting a mostly anti-Ang-1–mediated adverse effect.

Vanucizumab demonstrated a favorable pharmacokinetic profile. The elimination $t_{1/2}$ (6–9 days) was shorter than that of a typical IgG molecule. A low volume of distribution (2.9–6.1 L), consistent with other therapeutic mAbs (3–5 L; ref. 39), indicates that most of the drug was likely confined within the central compartment. Population pharmacokinetic simulations suggested that C_{trough} and C_{avg} levels after biweekly dosing of 30 mg/kg vanucizumab will result in serum exposures comparable with the clinically established bevacizumab dosages of 5–10 mg/kg biweekly. A flat dose of 2,000 mg vanucizumab biweekly was also predicted to achieve similar exposure as 30 mg/kg biweekly. Dosing every other week supports patient convenience and combination with other systemic anticancer therapies administered at this interval. Fixed dosing offers considerable advantages in ease of dose preparation, reduced potential of dose calculation errors, and lower costs (40).

Circulating VEGF-A and Ang-2 kinetics provided further evidence of the biologic effects of vanucizumab on angiogenic pathways. Consistent with the postulated MoA, drug-unbound (free) levels of both biomarkers decreased rapidly following treatment. Free circulating VEGF concentrations were also reduced upon treatment with bevacizumab in previous clinical trials (41–45). The paradoxical increases of total VEGF-A and total Ang-2 across all dose groups were likely due to the prolonged $t_{1/2}$ of vanucizumab-bound (inactive) targets and *de novo* synthesis of the ligands (43). The increase of total Ang-2 might also have been caused by the release of Ang-2 from Weibel–Palade–Bodies following vanucizumab treatment (46).

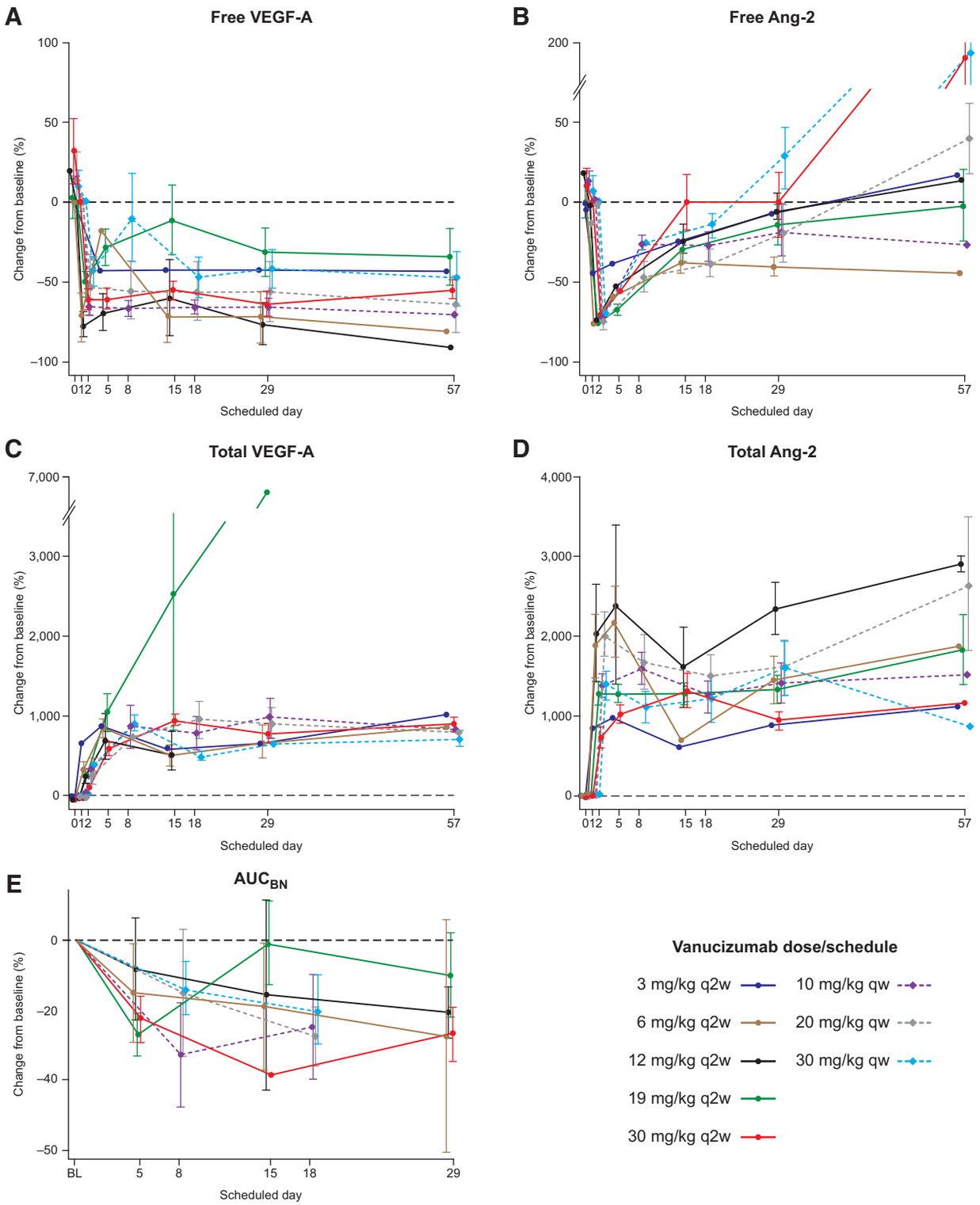

Figure 2.

Pharmacodynamics of vanucizumab. Scatterplots depicting mean changes from baseline (± SEM) plasma levels of free (**A** and **B**) and total (**C** and **D**) VEGF-A and Ang-2. There were overall no significant differences across dosing cohorts and administration schedules. The single patient within the 19 mg/kg group is considered an outlier. **E,** AUC_{BN} was measured by DCE-MRI.

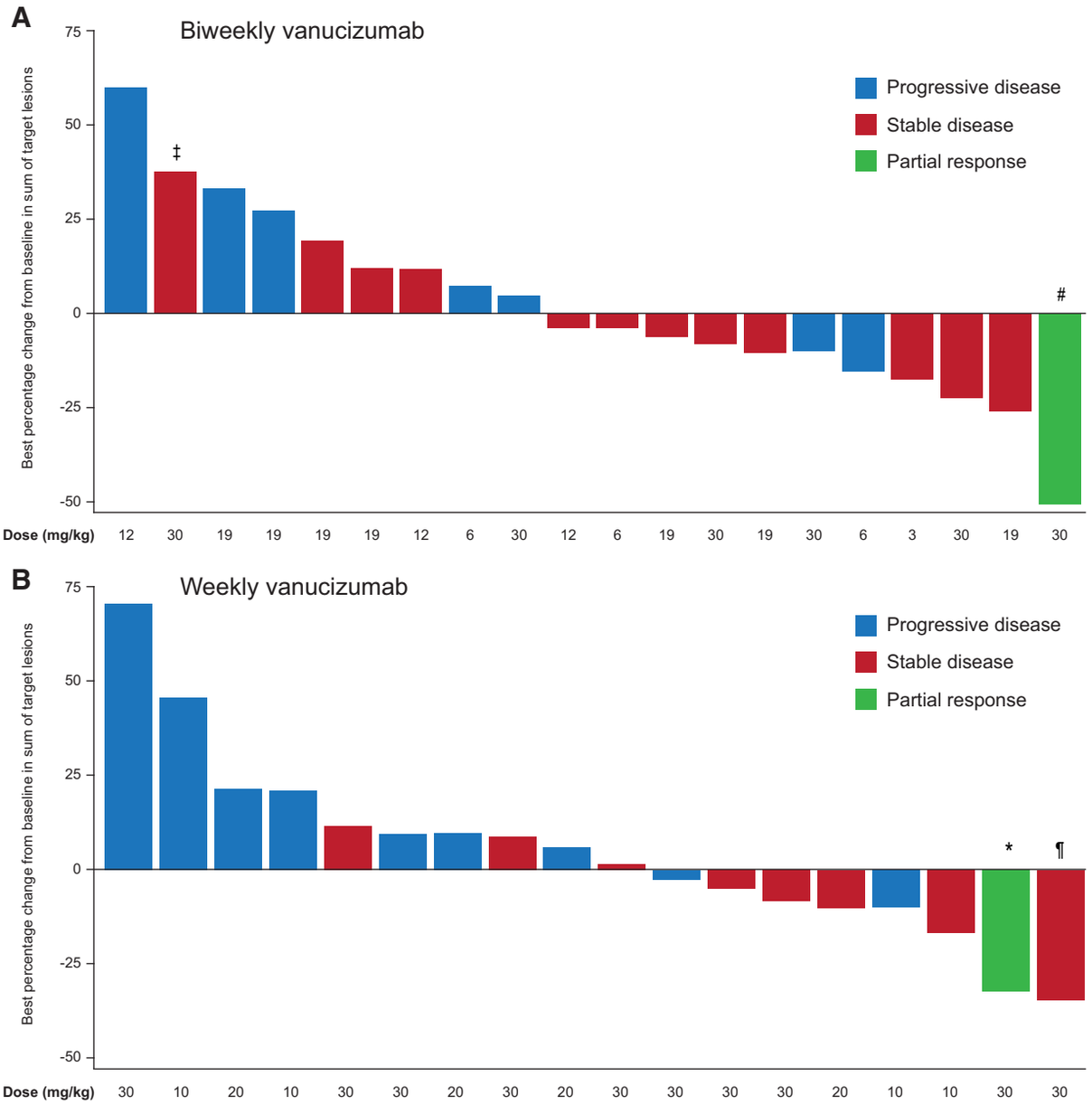

Figure 3.

Waterfall plot of best percentage change from baseline in the sum of the target lesions for patients treated biweekly (**A**) or weekly (**B**).The figure also shows the dose cohort. # Renal cell cancer patient with PR after approximately 20 months on treatment. *, Colon cancer patient who switched from weekly to biweekly dosing after 6 months and achieved a confirmed PR after approximately one year. ¶, Cervical cancer patient who achieved PR after six cycles and discontinued the study due to the AE of brain hemorrhage; therefore, no confirmatory scan was available. ‡, Patient had SD as the absolute increase from nadir was <5 mm.

DCE-MRI serves as a pharmacodynamic marker of biological activity for antiangiogenic cancer drugs by providing information about tumor microvessel structure and function, thereby helping to define the biologically active dose (47). DW-MRI provides complementary information about cellularity and necrosis within tumor tissue (48). Vanucizumab induced the anticipated effects on tumor perfusion and reduced vascular permeability in most patients and across a variety of tumor types. Notably, we observed a sustained decrease in tumor vessel permeability (measured by K^{TRANS}) and an increase in necrosis (measured as ADC) consistently with 30 mg/kg dosing in both schedules; the magnitude of reductions in K^{TRANS} was similar to those occurring with agents targeting the VEGF pathway (49, 50).

Vanucizumab also showed encouraging signs of antitumor activity in this heterogeneous population of patients with advanced solid tumors refractory to available therapy. Although overall three patients treated at the highest dose level achieved PR, there was no clear trend suggesting a relationship between the

dose and schedule of vanucizumab and the regression of tumors. Likewise, although limited by the small sample size and number of responders, there was no apparent relationship between baseline circulating Ang-2 levels and response.

In summary, the VEGF-A and angiopoietin signaling pathways represent attractive targets for cancer therapy. Vanucizumab simultaneously blocks VEGF-A and Ang-2 from interacting with their receptors and demonstrated an acceptable safety profile and evidence of antitumor effect at intravenous doses of up to 30 mg/kg biweekly. Clinical evaluations of vanucizumab combined with standard chemotherapy or novel cancer immunotherapies are in progress.

Disclosure of Potential Conflicts of Interest

J. Albanell reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Roche. T. Nayak has ownership interests (including patents) at F. Hoffman La Roche. O. Krieter has ownership interests (including patents) at Roche. No potential conflicts of interest were disclosed by the other authors.

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