

A Phase I First-in-Human Study of Nesvacumab (REGN910), a Fully Human Anti-Angiopoietin-2 (Ang2) Monoclonal Antibody, in Patients with Advanced Solid Tumors

Kyriakos P. Papadopoulos¹, Robin Kate Kelley², Anthony W. Tolcher¹, Albiruni R. Abdul Razak³, Katherine Van Loon², Amita Patnaik¹, Philippe L. Bedard³, Ariceli A. Alfaro², Muralidhar Beeram¹, Lieve Adriaens⁴, Carrie M. Brownstein⁴, Israel Lowy⁴, Ana Kostic⁴, Pamela A. Trail⁴, Bo Gao⁴, A. Thomas DiCioccio⁴, and Lillian L. Siu³

Abstract

Purpose: Nesvacumab (REGN910) is a fully human immunoglobulin G1 (IgG1) monoclonal antibody that specifically binds and inactivates the Tie2 receptor ligand Ang2 with high affinity, but shows no binding to Ang1. The main objectives of this trial were to determine the safety, tolerability, dose-limiting toxicities (DLT), and recommended phase II dose (RP2D) of nesvacumab.

Experimental Design: Nesvacumab was administered intravenously every two weeks with dose escalations from 1 to 20 mg/kg in patients with advanced solid tumors.

Results: A total of 47 patients were treated with nesvacumab. No patients in the dose escalation phase experienced DLTs, therefore a maximum tolerated dose (MTD) was not reached. The most common nesvacumab-related adverse events were fatigue (23.4%), peripheral edema (21.3%), decreased appetite,

and diarrhea (each 10.6%; all grade \leq 2). Nesvacumab was characterized by linear kinetics and had a terminal half-life of 6.35 to 9.66 days in a dose-independent manner. Best response by RECIST 1.1 in 43 evaluable patients included 1 partial response (adrenocortical carcinoma) of 24 weeks duration. Two patients with hepatocellular carcinoma had stable disease (SD) $>$ 16 weeks, with tumor regression and $>$ 50% decrease in α -fetoprotein. Analyses of putative angiogenesis biomarkers in serum and tumor biopsies were uninformative for treatment duration.

Conclusions: Nesvacumab safety profile was acceptable at all dose levels tested. Preliminary antitumor activity was observed in patients with treatment-refractory advanced solid tumors. On the basis of cumulative safety, antitumor activity, pharmacokinetic and pharmacodynamic data, the 20 mg/kg dose was determined to be the RP2D. *Clin Cancer Res*; 22(6); 1348–55. ©2015 AACR.

Introduction

Inhibition of tumor angiogenesis, particularly with VEGF/VEGF receptor antagonists, is a validated therapeutic approach for select oncologic indications (1–4). Angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), ligands for the vascular endothelial cell receptor tyrosine kinase Tie2 (5, 6) are proangiogenic factors selectively expressed during the angiogenesis process involved in tumor neovascularization (7, 8). Ang1 appears to play a role in

maturation and control of peripheral capillary permeability (9–11), while preclinical tumor models confirm that Ang2 is an important regulator of tumor angiogenesis and growth (12–16). Ang2 expression is upregulated in a range of human cancers (17–21), and high levels of circulating Ang2 are associated with a poor prognosis (22, 23).

Nesvacumab is a fully human immunoglobulin G1 (IgG1) VelocImmune monoclonal antibody that selectively binds Ang2 with high affinity (24 pmol), blocks Ang2 binding to the Tie2 receptor; but does not bind to Ang1. In human tumor cell line xenograft models, nesvacumab as a single agent demonstrated significant tumor growth inhibition in prostate adenocarcinoma (PC3), colorectal adenocarcinoma (Colo205), and epidermoid carcinoma (A431; ref. 13). Non-human toxicology studies in Sprague Dawley rats and cynomolgus monkeys showed no direct, definitive adverse toxic insult to any organ of either species (data on file, Regeneron). The no-observed-adverse-effect levels (NOAEL) in rat and monkey dosed every other week were 50 mg/kg by the intravenous route and 10 mg/kg by the subcutaneous route, the highest nesvacumab doses evaluated for each route of administration in these studies.

On the basis of these data, the selected starting dose of nesvacumab for this phase I study was conservatively chosen as 1 mg/kg administered by intravenous infusion every 2 weeks. This first-in-

¹South Texas Accelerated Research Therapeutics (START), San Antonio, Texas. ²Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, California. ³Princess Margaret Cancer Centre, Toronto, Ontario. ⁴Regeneron Pharmaceuticals, Inc., Tarrytown, New York.

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Corresponding Author: Kyriakos P. Papadopoulos, South Texas Accelerated Research Therapeutics, 4383 Medical Drive, San Antonio, TX 78229. Phone: 210-593-5250; Fax: 210-615-1121; E-mail: Kyri.Papadopoulos@start.stoh.com

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Translational Relevance

Angiopoietin-2 (Ang2) expression is a key regulator of tumor angiogenesis and growth. Ang2, which functions as a ligand for vascular endothelial cell receptor tyrosine kinase Tie2, is upregulated in various cancers and associated with poor prognosis. Preclinically, Ang-2 inhibition results in decreased tumor vascularity and significant inhibition of human xenograft tumor growth. Furthermore, Ang2 inhibition potentiates the antitumor effects of anti-VEGF agents. Nesvacumab (REGN910) is a novel IgG1 fully human anti-Ang2 monoclonal antibody. This article reports the first-in-human study of nesvacumab in patients with advanced tumors. The majority of analyzed tumors expressed Ang2, but none of several putative angiogenic biomarkers was informative for clinical efficacy. The manageable toxicity profile and preliminary evidence of antitumor activity support the further development of nesvacumab, particularly in combination with other targeted antiangiogenic and cytotoxic therapies.

human phase I study (NCT01271972) reports the safety, tolerability, pharmacokinetic, pharmacodynamic markers, preliminary antitumor efficacy, and recommended phase II dose for nesvacumab administered every 2 weeks in patients with advanced solid tumors.

Patients and Methods

This multicenter, phase I, nonrandomized, open-label study was conducted at two centers in the United States and one center in Canada. All patients provided written informed consent, and the study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and all applicable local regulatory requirements and laws.

Patient eligibility

Eligible patients were ≥ 18 years, with histologically proven advanced solid malignancies, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1 , and adequate organ function. Exclusion criteria included uncontrolled hypertension (systolic blood pressure >150 mm Hg, diastolic blood pressure >95 mmHg), brain metastases, presence of any atypical T1 contrast enhancing brain lesions in brain MRI (protocol amendment implemented following an atypical brain MRI finding in an index patient with neurologic symptoms), serious nonhealing wound or ulcer, active bleeding, significant cardiac event, and deep venous thrombosis or pulmonary embolism within 6 month prior to study enrollment. For patients with hepatocellular cancer (HCC), histologically proven disease was not required if classic tumor features were present on imaging consistent with accepted radiographic diagnostic criteria (24); HCC-specific inclusion/exclusion criteria were also applicable (Supplementary Data).

Study design and treatments

Nesvacumab was administered as a 30-minute intravenous infusion every 2 weeks at planned doses of 1, 3, 6, 12, or 20 mg/kg in 28-day cycles. During the dose escalation phase to

determine the MTD, enrollment was sequential and in a standard 3+3 design. Dose escalation was allowed if dose-limiting toxicity (DLT) occurred in 0/3 or $\leq 1/6$ patient in each cohort during cycle 1 (28 days). Patients not completing the first cycle for reasons other than DLT were considered nonevaluable and replaced. Expansion cohorts at candidate recommended phase II dose (RP2D) levels enrolled patients with advanced solid tumors (safety expansion cohorts) enriched for patients with HCC to further characterize safety and tolerability and assess preliminary antitumor activity. Enrichment for HCC in the expansion cohorts was a protocol amendment based on the observation of significant α -fetoprotein (AFP) decline and tumor regression in a patient with HCC treated in the dose escalation phase. Study treatment continued until disease progression, unacceptable toxicity (including DLT), or withdrawal of consent.

DLTs were defined during cycle 1, as any treatment-related grade 4 anorexia, nausea, vomiting, or diarrhea, or grade ≥ 3 nonhematologic toxicities except inadequately treated grade 3 anorexia, nausea, vomiting, or diarrhea. Hematologic toxicities defined as DLT were grade 3 or 4 neutropenia complicated by fever $\geq 101.3^{\circ}\text{F}$ (38.5°C) or infection, grade 4 neutropenia ≥ 7 days duration, grade 3 thrombocytopenia complicated by hemorrhage, or grade 4 thrombocytopenia.

Study assessments

Safety assessments were performed weekly during the first cycle and every two weeks throughout the study treatment period, followed by a posttreatment study visit 30 days (± 5 days) after the last dose. Assessments included vital sign measurements, physical examinations, clinical laboratory tests, urinalysis, brain MRIs (every 2 cycles, instituted following an index patient with neurologic symptoms, found to have an atypical brain finding), and collection of adverse event (AE) information. The severity of an AE was graded by the investigator using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0.

Tumor response radiologic assessments were performed at baseline and approximately every 8 weeks according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Serum tumor markers as appropriate for tumor type were used to assess the effect of nesvacumab therapy.

Pharmacokinetic analysis

Serum samples were collected on cycle 1 day 1 predose, 0 (end of infusion), 1, 2, 4, 8, 24, 48, and 168 hours postdose, day 15 (predose and end of infusion), and on day 22. In cycle 2, serum samples were collected on day 1 predose, 0 (end of infusion), 1, 2, 4, and 8 hours post dose. In cycles ≥ 3 , serum samples were collected on day 1 predose and end of infusion. Total nesvacumab concentration in serum was measured using a validated ELISA (Regeneron, on file). In the assay, the lower limit of quantification (LLOQ) of total nesvacumab was 0.078 mg/L. Noncompartmental pharmacokinetic parameters were calculated using Phoenix WinNonlin (version 6.3, Certara, L.P.).

Immunogenicity

Serum samples collected during the treatment period and at follow-up were evaluated for anti-nesvacumab antibody (ADA) using an electrochemiluminescence bridging immunoassay.

Biomarker studies

The pharmacodynamics effect of nesvacumab was explored in serum, and in mandated paired tumor biopsies obtained at baseline and after administration of 2 doses of nesvacumab on cycle 1 day 22 (± 2 days) in the solid tumor safety expansion cohorts. Biopsies were optional in HCC patients. Serum samples were collected at cycle 1 day 1 (predose), cycle 1 day 2, day 3, day 8, day 15 (predose), and day 22; and predose on days 1 and 15 for all subsequent cycles. Exploratory analysis of serum levels of Ang2 and other potential pharmacodynamic markers, including endothelial cell-specific molecule 1 (ESM-1), stromal cell-derived factor 1 (SDF-1), placental growth factor (PLGF), and soluble vascular cell adhesion molecule 1 (sVCAM-1) which may be affected by Ang2 inhibition, were performed by ELISA [R&D Systems, for total Ang2 (free and nesvacumab-bound) and SDF-1; Quest Diagnostics Clinical Trials Laboratory for PLGF and sVCAM-1; and Atila Biosystems, for ESM-1]. Archival tissue and paired tumor biopsies were processed for immunohistochemical analysis of Ang2, TIE-2 (Ang2 receptor), CD31, microvessel density (MVD), VEGFA, smooth muscle actin (SMA), Ki-67, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

Statistical analysis

Categorical and continuous data were summarized with frequencies and percentages or descriptive statistics, respectively. The safety population included all patients who received at least one dose of study drug; the efficacy population included all patients in the safety population with a baseline assessment and at least one postbaseline tumor assessment.

Results

Patient characteristics and disposition

A total of 47 patients were enrolled to the study between January 2011 and March 2013. Patient demographics and characteristics are listed in Table 1. The dose escalation phase initially tested the 4 planned 1 mg/kg to 12 mg/kg dose levels [1 mg/kg ($n = 4$), 3 mg/kg ($n = 4$), 6 mg/kg ($n = 3$), and 12 mg/kg ($n = 6$)]. One additional patient was enrolled concurrently in the 1 mg/kg cohort, and one patient withdrew consent and was replaced in the 3 mg/kg cohort. Following review of pharmacokinetic and safety data from the safety expansion cohort at 12 mg/kg ($n = 14$), an additional 20 mg/kg cohort ($n = 6$) was explored without defining an MTD, with a safety dose expansion cohort at 20 mg/kg ($n = 10$). Overall, 20 patients, including 6 with HCC were treated at 12 mg/kg; and 16 patients, including 8 with HCC, were treated at 20 mg/kg, respectively.

At the time of the data cutoff in September 2013, 46 patients (97.9%) had discontinued study treatment, including 38 patients (80.9%) because of progressive disease, 6 patients (12.8%) due to adverse events, and 1 patient each, due to decision of the investigator and withdrawal of consent. There was one death within 30 days of completing treatment, which was assessed as unrelated to nesvacumab.

Patients received a median of 4 doses (range 1–32) leading to a median duration of nesvacumab exposure of 56 days (range 14–448 days).

DLT and MTD

There were no DLTs observed in any of the 22 patients evaluable for DLT in the dose escalation phase. One patient in the 3 mg/kg

Table 1. Baseline demographics and primary cancer diagnosis

Characteristics	Total (N = 47)
Age (years)	
Mean (SD)	59.4 (14.21)
Median	59.0
Min: max	22:82
Gender, n (%)	
Male	23 (48.9)
Female	24 (51.1)
Race, n (%)	
American Indian or Alaska Native	1 (2.1)
Asian	7 (14.9)
Black or African American	3 (6.4)
White	36 (76.6)
ECOG performance status, n (%)	
0	14 (29.8)
1	33 (70.2)
Primary cancer diagnosis, n (%)	
Hepatocellular cancer	15 (31.9)
Other ^a	13 (27.7)
Breast cancer	5 (10.6)
Adrenocortical carcinoma	4 (8.5)
Colorectal cancer	3 (6.4)
Renal cell cancer	3 (6.4)
Non-small cell lung cancer	2 (4.3)
Pancreatic cancer	2 (4.3)

NOTE: ^achordoma, placental site trophoblastic tumor (PSTT), thyroid carcinoma, sarcoma, malignant fibrohistiocytoma, metastatic carcinoid, malignant neoplasm of floor of mouth, angiosarcoma, mucoepidermoid cancer, uterine carcinoma, uterine leiomyosarcoma, mesothelioma, lymphoepithelioma.

dose level withdrew consent prior to completing the first treatment cycle and was not evaluable for DLT. As no MTD was determined, the maximum administered dose of 20 mg/kg was designated the recommended phase II dose based on cumulative safety and pharmacokinetic data.

Safety

All 47 patients received at least one dose of nesvacumab and were evaluable for safety. All patients experienced at least one treatment-emergent adverse event (TEAE). The most common TEAEs of any grade were fatigue (53.2%; $n = 25$), diarrhea, nausea, and peripheral edema (each 25.5%; $n = 12$) (Supplementary Table S1). Thirty-six patients (76.6%) experienced at least one AE assessed as being nesvacumab-related (Table 2). The most common treatment-related AEs of any grade were fatigue (23.4%; $n = 11$), peripheral edema (21.3%; $n = 10$), decreased appetite, and diarrhea (each 10.6%; $n = 5$). The peripheral edema occurred in similar proportions of patients in the 12 mg/kg and 20 mg/kg cohorts and was mild to moderate (grade ≤ 2) in severity. Infusion-related reactions occurred in 3 patients (6.3%). Two patients, one each with grade 1 flushing and grade 3 back-pain, continued nesvacumab dosing without symptom recurrence; 1 patient (grade 2 bronchospasm) had dose interruption, and subsequently withdrew consent without resumption of dosing. Three patients, all in the 20 mg/kg dose group, each experienced at least possibly treatment-related AE assessed as grade ≥ 3 (transient infusion-related reaction, transient abdominal pain, and retinal detachment).

One patient receiving nesvacumab 6 mg/kg experienced a grade 1 cognitive disturbance during cycle 5, and dosing of nesvacumab was held as a precaution. Brain MRI showed atypical T2 white matter findings. At follow-up, both the symptoms and MRI

Table 2. Summary of treatment-related AEs occurring in ≥ 2 patients or grade ≥ 3 (CTCAE, version 4.0.)

Preferred term ^a	Nesvacumab dose											
	1 mg/kg (N = 4) ^b , n (%)		3 mg/kg (N = 4), n (%)		6 mg/kg (N = 3), n (%)		12 mg/kg (N = 20), n (%)		20 mg/kg (N = 16), n (%)		Total (N = 47), n (%)	
	All grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3
Number of patients with any TRAE	3 (75.0)	0	3 (75.0)	0	1 (33.3)	0	16 (80.0)	0	13 (81.3)	3 (18.8)	36 (76.6)	3 (6.4)
Fatigue	0	0	0	0	0	0	5 (25.0)	0	6 (37.5)	0	11 (23.4)	0
Edema peripheral	1 (25.0)	0	1 (25.0)	0	0	0	4 (20.0)	0	4 (25.0)	0	10 (21.3)	0
Decreased appetite	1 (25.0)	0	0	0	0	0	3 (15.0)	0	1 (6.3)	0	5 (10.6)	0
Diarrhea	0	0	1 (25.0)	0	1 (33.3)	0	3 (15.0)	0	0	0	5 (10.6)	0
Abdominal distension	0	0	1 (25.0)	0	0	0	3 (15.0)	0	0	0	4 (8.5)	0
Nausea	1 (25.0)	0	0	0	0	0	1 (5.0)	0	2 (12.5)	0	4 (8.5)	0
Central nervous system lesion	0	0	1 (25.0)	0	0	0	1 (5.0)	0	0	0	2 (4.3)	0
Cognitive disorder	0	0	0	0	1 (33.3)	0	0	0	1 (6.3)	0	2 (4.3)	0
Infusion-related reaction	0	0	1 (25.0)	0	0	0	0	0	1 (6.3)	1 (6.3)	2 (4.3)	1 (2.1)
Myalgia	0	0	1 (25.0)	0	0	0	0	0	1 (6.3)	0	2 (4.3)	0
Pleural effusion	0	0	0	0	0	0	2 (10.0)	0	0	0	2 (4.3)	0
Abdominal pain	0	0	0	0	0	0	0	0	1 (6.3)	1 (6.3)	1 (2.1)	1 (2.1)
Retinal detachment	0	0	0	0	0	0	0	0	1 (6.3)	1 (6.3)	1 (2.1)	1 (2.1)

NOTE: TRAE, treatment-related AE. A TRAE was defined as an AE that occurred in the period from the first nesvacumab dose to end of study visit or 30 days after last dose, whichever was later.

^aAll AEs were coded using the MedDRA, version 13.0.

^bAdditional patient recruited concurrently at two sites.

findings resolved; the patient had disease progression and was off treatment at the time of resolution. This AE prompted a protocol amendment implementing screening and on-study brain MRIs in all active and subsequently enrolled patients. Two additional on-study patients were found to have atypical T2 white matter findings on brain MRI, but without associated neurologic symptoms. These findings, respectively, improved or resolved following discontinuation of dosing. No baseline brain MRIs were available for comparison for these 2 patients nor the index patient. A total of 26 additional patients had both baseline screening and follow-up brain MRIs, none of whom developed any changes from baseline.

Five patients (10.6%) had at least one study-defined treatment-related SAE. These included the aforementioned 3 events of reversible central nervous system findings, with cognitive disturbance in 1 patient, and 1 event each of retinal detachment and vena cava thrombosis. The vena cava thrombosis occurred in the context of disease progression with retroperitoneal adenopathy and ureteric obstruction, but was considered possibly related to nesvacumab.

No dose reductions were required for nesvacumab-related AEs but 5 patients (10.6%) had nesvacumab dosing temporarily withheld due to a treatment-related AE, and 3 patients (6.4%) discontinued treatment permanently due to a treatment-related AE. One patient, an 82-year-old male with a diagnosis of HCC in the 12 mg/kg group died 14 days after the last dose of nesvacumab due to unrelated respiratory failure, following worsening end-stage liver disease and chronic obstructive lung disease.

Pharmacokinetics

Following the first intravenous administration of nesvacumab, the mean concentration–time profiles of total nesvacumab were characterized by an initial distribution phase followed by a single linear beta elimination phase (Fig. 1). A terminal target-mediated elimination phase was not observed within the scheduled every two weeks dosing interval. With every two-week interval retreat-

ment of nesvacumab, a slight increase in C_{max} (1.2- to 1.8-fold) was observed over the first 3 or 4 dosing intervals (6 to 8 weeks); however, there was no clear evidence of continued accumulation with longer term dosing, suggesting steady-state was mostly achieved (Fig. 1). At 20 mg/kg, following the first and subsequent doses, C_{min} of ≥ 122 mg/L was approximately twice the concentration associated with consistent antitumor activity in preclinical models (Fig. 1). Mean C_{max} and AUC_{last} value increases were dose-proportional (Table 3).

Consistent with the dose proportionality observed, total body clearance (CL) and volume of distribution at steady-state (V_{ss}) appeared to be dose-independent. The mean observed elimination $t_{1/2}$ ranged from 6.35 to 9.66 days in a dose-independent manner.

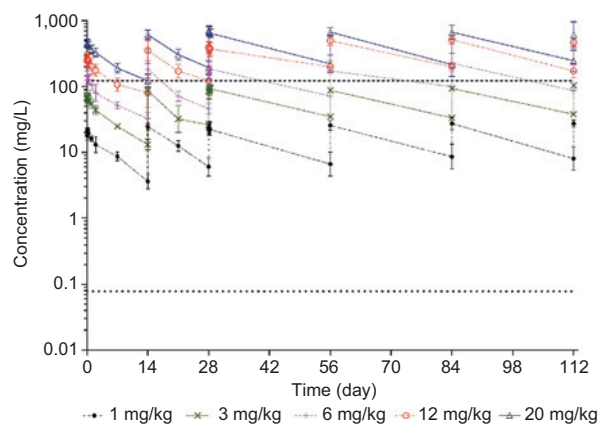


Figure 1. Mean (\pm SD) log-scaled concentrations of total nesvacumab versus time in patients receiving intravenous infusions of nesvacumab every 2 weeks. One standard deviation around the mean is presented. Horizontal dotted line = assay LLOQ (0.0870 mg/L). Horizontal dashed line = 122 mg/L.

Table 3. Summary pharmacokinetic parameters of total nesvacumab following intravenous infusion in patients (cycle 1 and cycle 2)

Parameter/units		Dose														
		1 mg/kg (n = 4)			3 mg/kg (n = 4)			6 mg/kg (n = 3)			12 mg/kg (n = 20)			20 mg/kg (n = 16)		
		Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Cycle 1/dose 1																
$t_{1/2}$	Day	6.35	6.05	1.52	7.32	8.00	1.75	9.66	11.2	2.98	9.46	8.71	3.22	8.94	8.26	2.36
t_{max}	Hour	3.04	3.08	1.68	3.54	2.58	3.82	1.86	2.48	1.18	19.6	1.58	74.9	2.70	1.68	2.56
CL	mL/day/kg	6.27	6.30	1.15	5.40	5.47	0.374	4.70	5.08	0.887	4.78	4.06	2.04	4.40	4.22	1.04
V_{ss}	mL/kg	53.4	53.0	11.5	53.6	57.3	12.0	62.1	57.8	19.6	58.8	56.6	14.1	52.9	50.8	10.2
AUC_{last}	day*mg/L	130	124	22.1	415	407	38.1	841	891	150	1785	1780	467	3087	3128	603
$AUC_{last}/dose$	day*kg/L	130	124	22.1	138	136	12.7	140	149	25.0	149	148	38.9	154	156	30.2
C_{max}	mg/L	22.1	22.3	2.53	74.8	75.7	12.2	152	153	16.0	285	264	65.6	487	488	66.0
$C_{max}/dose$	kg/L	22.1	22.3	2.53	24.9	25.2	4.06	25.3	25.5	2.67	23.7	22.0	5.47	24.4	24.4	3.30
C_{last}	mg/L	3.61	3.65	1.04	13.0	13.7	2.67	32.5	30.4	7.92	80.8	72.6	54.4	122	124	31.8
$C_{last}/dose$	kg/L	3.61	3.65	1.04	4.33	4.57	0.890	5.42	5.07	1.32	6.73	6.05	4.53	6.10	6.20	1.59
Cycle 1/dose 2																
C_{max}	mg/L	24.6	25.9	3.15	86.3	83.2	13.5	184	204	39.9	353	350	149	615	625	105
C_{last}	mg/L	6.00	6.31	2.28	26.1	3.55	27.1	45.8	47.4	8.81	123	136	38.9	194	186	52.2
Cycle 2 ^a																
C_{max}^a	mg/L	23.6	4.53	25.0	84.5	90.0	10.4	183	170	26.1	399	412	87.8	692	697	102
C_{last}^b	mg/L	6.58	7.39	3.44	34.7	34.7	— ^c	71.6	71.6	— ^c	199	179	48.5	221	193	79.4

Abbreviations: AUC, area under the curve; CL, clearance; C_{max} , maximum concentration; C_{last} , concentration at the end of the dosing interval; SD, standard deviation; $t_{1/2}$, half-life; t_{max} , time to maximum concentration; V_{ss} , volume of distribution at steady-state; $t_{1/2}$ was estimated over the every two weeks dosing interval.

^aMean concentration observed at the end of the first intravenous infusion of cycle 2.

^bMean concentration observed at the end of the second dosing interval of cycle 2.

^cn = 2.

Immunogenicity

One of the 47 patients, treated with nesvacumab at 3 mg/kg every two weeks, tested positive on the 30-day after last dose follow-up visit sample with an ADA titer of 30 (the minimum titer for the assay). This patient had no associated infusion-related reaction and no sudden and/or persistent drop in total nesvacumab concentration, indicating the ADA detected did not affect exposure in this individual.

Biomarker studies

Patients had a median baseline level of total serum Ang2 of 3.0 ng/mL (n = 47, range: 1.1–49.6 ng/mL). Patients with HCC had higher baseline levels of Ang2 (HCC median of 7.2 ng/mL versus non-HCC median of 2.9 ng/mL). Increase in total Ang2 levels was observed after the first dose of nesvacumab, and further accumulation to plateau was detected upon extended exposure (Fig. 2). When comparing the molar concentrations of total nesvacumab and total Ang2, and considering the ability of one nesvacumab molecule to bind two Ang2 molecules, Ang2 in serum was saturated by nesvacumab administered with the studied dose levels and dosing schedule (Supplementary Fig. S1). Serum levels of putative biomarkers of nesvacumab treatment (ESM-1, SDF-1,

PLGF-1, and sVCAM-1) identified in preclinical studies were also determined. No significant treatment-related changes in these biomarkers or correlations to treatment response or duration were observed (Supplementary Figs. S2–S4).

Archival tumor tissue (n = 38) and paired tumor tissue biopsy samples (n = 17) were available for analysis. In paired samples, expression of Ang2 was detected in most pretreatment biopsies [86.7% of evaluable baseline biopsies (n = 15) had Ang-2 positive vessel staining (H-score > 30)] and expression level in posttreatment biopsies was unaffected by nesvacumab treatment. Similar to Ang2, no nesvacumab-induced changes nor correlations to treatment response or duration were observed in tumor tissue for any of the other putative biomarkers (VEGF-A, TIE-2, SMA, MVD, TUNEL, Ki-67; Supplementary Figs. S5–S9).

Antitumor activity

Among the 47 patients enrolled, 43 patients were evaluable for tumor response. One patient with low-burden peritoneal adrenocortical carcinoma treated in the 1 mg/kg cohort achieved a confirmed PR by RECIST of 24 weeks duration, 23 patients (48.9%) achieved best response of stable disease, and 19 patients

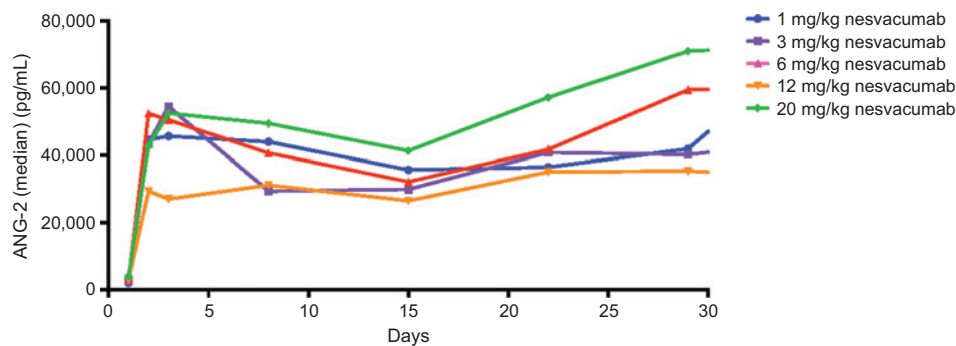
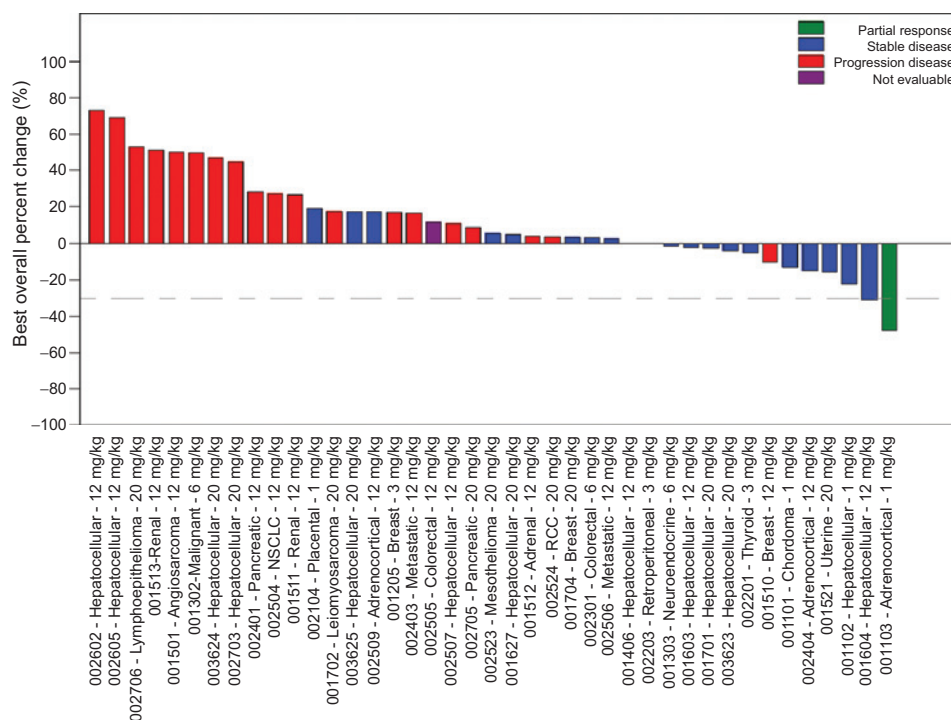


Figure 2. Total median Ang2 levels in serum versus time (first cycle) in patients receiving nesvacumab.

Figure 3.

Best overall percent change in target lesions from baseline. Best percentage change from baseline in the sum of the diameter of measurable target lesions for patients with pre- and postbaseline tumor assessments. Interrupted line indicates RECIST 1.1 threshold for response (-30% for partial response). "Not evaluable" patients had repeat CT performed <6 weeks from baseline.



(40.4%) had progressive disease as best response (Fig. 3). Five HCC patients enrolled across dose levels, 3 of whom had progressive disease in the 3 months preceding study entry, had SD > 16 weeks. Two HCC patients with SD, treated at 1 mg/kg and 20 mg/kg, demonstrated significant decline in AFP at best response; 20,506 ng/mL at baseline to 252 ng/mL and 42,983 ng/mL at baseline to 14,418 ng/mL, respectively.

Discussion

In this first-in-human study, nesvacumab safety profile was acceptable at doses up to 20 mg/kg every two weeks in patients with advanced solid tumors. No patients in the dose escalation phase experienced a protocol-defined DLT. Expansion cohorts at 12 and 20 mg/kg provided additional information on safety and preliminary antitumor activity in a population enriched for patients with HCC. The dose of 20 mg/kg was selected as the RP2D based on both clinical and preclinical data, including a C_{min} of 122 mg/L, which was approximately twice the concentration associated with consistent antitumor activity in preclinical models.

The most common treatment-related AEs with nesvacumab, all grade ≤ 2 , were fatigue, peripheral edema, diarrhea, and decreased appetite. With the caveat of limitations of cross-trial comparisons, these data are similar to findings reported in clinical trials of other inhibitors of Ang1 and Ang2, where fatigue and peripheral edema were also the most common clinical toxicities (25, 26). With the exception of fatigue, there were no obvious dose-related patterns in the occurrence or severity of treatment-related AEs. Nesvacumab-related peripheral edema ($n = 10$, 21.3%) was mild in severity in the majority of patients, and all events but one resolved without any action taken to the study drug. Factors that might increase the risk of nesvacumab-associated edema are unknown and require further study.

The toxicity profile of nesvacumab was remarkable for the lack of AEs typically associated with inhibitors of angiogenesis (27, 28). In particular, no treatment-related hypertension, hemorrhage, arterial thromboembolic events, or proteinuria were documented. Apart from the caveat of cross-trial comparisons, a mechanistic explanation of selective inhibition of Ang2 without Ang1 inhibition may account for the lack of proteinuria seen with nesvacumab (29–31), unlike the proteinuria grade ≥ 3 reported with the Ang1/Ang2 inhibitors trebananib and AMG780 (25, 26). Except for one patient with vena cava thrombosis, occurring in the context of progressive retroperitoneal disease, there were no other thrombotic events. Reversible CNS abnormalities seen on MRI in 3 patients were atypical in their clinical and radiologic appearance from those seen with other antiangiogenic agents (32, 33). The MRI findings suggest foci of restricted diffusion, distinct from the typical T2 weighted hyperintensity signal generally seen in the population at large over the age of 50 years (34, 35), and will require further elucidation. Infusion-related reactions were infrequent and easily managed, with patients receiving subsequent infusions without symptom recurrence. Anti-nesvacumab antibodies without clinical consequence were detected at a single timepoint in only 1 patient.

The pharmacokinetic profile of nesvacumab was dose-proportional, and serum concentration trough levels throughout the dosing interval at ≥ 12 mg/kg were above the level that correlated with consistent antitumor activity in preclinical models. Ang2 levels increased and plateaued with repeat dosing, likely reflecting saturated antibody–target (nesvacumab–Ang2) complex formation. However, additional studies of mRNA Ang2 levels are required to rule out a possible change in target production rates. Elevations of similar magnitude in Ang2 levels have been observed following therapy with other Ang1/Ang2 inhibitors (36). Analyses of Ang2 and other putative angiogenesis biomarkers in serum and tumor biopsies did not show association with

treatment response or duration, but were limited by small sample size and the heterogeneity of this population.

Evidence of antitumor effect included PR in 1 patient with adrenocortical carcinoma in the 1 mg/kg group and two HCC patients with tumor regression and >50% decrease in AFP.

In summary, the anti-Ang2 monoclonal antibody nesvacumab showed acceptable safety and evidence of objective treatment effects in 1 adrenocortical carcinoma patient and a few hepatocellular carcinoma patients when administered as monotherapy at intravenous doses up to 20 mg/kg every 2 weeks. Preclinical combination studies (13) and the distinct safety profile of nesvacumab suggest the feasibility and potential benefit of combination with anti-VEGF and other targeted or cytotoxic agents. Combination trials of nesvacumab with sorafenib and ziv-aflibercept/paclitaxel (37) have completed enrollment.

Disclosure of Potential Conflicts of Interest

A.W. Tolcher is a consultant/advisory board member for AbbVie, Akebia, AP Pharma, ArQule, Asana, Astex, Avid, Bayer, Bind, BioMed Valley Discoveries, Blend, Bristol-Meyers Squibb Japan, Celator, Clovis, Curis, Dicerna, Eisai, Eli Lilly, Endo, Genentech, Heron, Janssen, MedImmune, Mersana, Merus, Nanobiotix, Nektar, Neumedicines, Novartis, Pfizer, Pharmacyclics, Pierre Fabre, Sanofi-Aventis, Symphogen, Vaccinex, Vlaent, and Zyngenia. No potential conflicts of interest were disclosed by the other authors.

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Authors' Contributions

Conception and design: K.P. Papadopoulos, A.W. Tolcher, L. Adriaens, I. Lowy, P.A. Trail, A.T. DiCioccio, L.L. Siu

Development of methodology: L. Adriaens

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.P. Papadopoulos, R.K. Kelley, A.R. Abdul Razak, K.V. Loon, A. Patnaik, P.L. Bedard, A.A. Alfaro, A. Kostic, L.L. Siu

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.P. Papadopoulos, R.K. Kelley, A.R. Abdul Razak, A. Patnaik, P.L. Bedard, M. Beeram, I. Lowy, A. Kostic, P.A. Trail, B. Gao, A.T. DiCioccio, L.L. Siu

Writing, review, and/or revision of the manuscript: K.P. Papadopoulos, R.K. Kelley, A.W. Tolcher, A.R. Abdul Razak, K.V. Loon, A. Patnaik, P.L. Bedard, M. Beeram, L. Adriaens, C.M. Brownstein, I. Lowy, A. Kostic, P.A. Trail, A.T. DiCioccio, L.L. Siu

Study supervision: K.P. Papadopoulos, A.R. Abdul Razak, C.M. Brownstein, A. Kostic, L.L. Siu

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