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Clinical Cancer Research

A First-in-Human Phase I Study of MORAb-004, a Monoclonal Antibody to Endosialin in Patients with Advanced Solid Tumors

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Abstract

Purpose: Endosialin (TEM-1, CD248) is a protein expressed on the surface of activated mesenchymal cells, including certain subsets of tumors. Preclinical models suppressing endosialin function have shown antitumor activity. A humanized monoclonal antibody, MORAb-004, was engineered to target endosialin and is the first agent in clinical development for this mesenchymal cell target.

Experimental Design: This first-in-human, open-label, phase I study recruited patients with treatment-refractory solid tumors. MORAb-004 was administered intravenously once weekly in 4-week cycles. Objectives included determination of the safety of multiple infusions of MORAb-004, identification of the maximum tolerated dose (MTD), pharmacokinetic modeling, detection of any anti-human antibody response, and assessment of objective radiographic response to therapy.

Results: Thirty-six patients were treated at 10 dose levels of MORAb-004, ranging from 0.0625 to 16 mg/kg. Drug-related adverse events were primarily grade 1–2 infusion toxicities. Dose-limiting toxicity of grade 3 vomiting was observed at 16 mg/kg. Eighteen of 32 evaluable patients across all doses achieved disease stability, with minor radiographic responses observed in 4 patients (pancreatic neuroendocrine, hepatocellular, and sarcoma tumor types). Pharmacokinetics showed MORAb-004 accumulation beginning at 4 mg/kg and saturable elimination beginning at 0.25 mg/kg. Exposure increased in a greater-than-dose-proportional manner with terminal half-life increasing proportionally with dose. The MTD was identified as 12 mg/kg.

Conclusions: Preliminary antitumor activity was observed. Safety profile, pharmacokinetics, and early antitumor activity suggest that MORAb-004 is safe at doses up to 12 mg/kg and should be studied further for efficacy. *Clin Cancer Res; 21(6); 1281–8. ©2014 AACR.*

Introduction

MORAb-004 is a humanized $IgG_{1/\kappa}$ monoclonal antibody (mAb) directed against human endosialin [tumor endothelial marker-1] (TEM-1); CD248). First described as a component of the tumor endothelium (1, 2), endosialin is now understood to be expressed on the surface of cells of mesenchymal origin, including tumor-associated pericytes and activated fibroblasts, which are thought to play a key role in the development of tumor neovascular networks and stromal interaction (3). The interruption of endosialin function with antibody blockade or genetic knockouts negatively affects tumor growth and neovessel formation in numerous cancer types (2, 4–6). In some tumors, endosialin is expressed on the surface of the cancer cells, in addition to its expression on tumor-associated pericytes (5). This is true for tumors of mesenchymal origin as well as some epithelial tumors with mesenchymal features (7).

Endosialin is thought to enhance the stromal organization of human tumors. Reduced tumor growth and invasion have been observed in endosialin knockout mice (8), with fibronectin and collagen types I and IV, identified as specific ligands for endosialin, influencing the interaction among tumor cells, endothelia, and the stromal matrix (9). By blocking endosialin, fibronectin adhesion and cell migration were decreased (9). Endosialin has also been shown to play a role in the signaling pathways of human tumors, including platelet-derived growth factor-β (PDGF-β) and Notch receptor protein (10). Under normal conditions, pericytes that expressed high levels of endosialin were able to proliferate, respond to PDGF-BB stimulation by phosphorylation of the PDGF receptor and the MAPK ERK-1/2, and induce expression of c-Fos; however, with siRNA knockdown of endosialin expression, PDGF-BB–induced proliferation, ERK-1/2 phosphorylation, and c-Fos expression were significantly impaired (11).

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Note: Prior presentations: The results presented in this article were previously presented at the following meetings:

ASCO 2012 Annual Meeting: Diaz LA Jr and colleagues. A first-in-human phase I study of MORAb-004 (M4), a humanized monoclonal antibody recognizing endosialin (TEM-1), in patients with solid tumors.

ASCO 2011 Annual Meeting: Carvajal RD and colleagues. A first-in-human phase I study of MORAb-004 (MOR4), a humanized monoclonal antibody recognizing TEM-1 (endosialin), in patients with solid tumors.

Investigative sites: Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD; Memorial Sloan Kettering Cancer Center, New York, NY.

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Translational Relevance

This is a novel first-in-human clinical trial of an antibody to a new class of proteins on the surface of mesenchymal cells that includes the tumor vasculature and a subset of cancers. This study was conducted in patients with advanced solid tumor and shows some early signals of potential clinical benefit as a cancer therapy with relatively minimal toxicity even at high doses.

This study presents the results of the first-in-human phase I dose escalation trial of MORAb-004, a novel antitumor agent that targets cells of mesenchymal origin.

Materials and Methods

Study population

This study was conducted at two centers in the United States between March 27, 2009, and September 21, 2011 (Clinical Trials.gov identifier: NCT00847054). Each participant provided written informed consent before initiating study procedures. All enrolled patients were greater than 18 years old and were required to have treatment-refractory solid tumors and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) v.1.0 (12) or evaluable by clinical signs/symptoms (e.g., ascites, pleural effusion, or lesions of less than 2 cm) within 4 weeks before study entry. Patients were required to have a Karnofsky performance status of at least 70% (13) and adequate hematologic and coagulation parameters (absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, hemoglobin ≥ 10 g/dL). Patients who had received chemotherapy, biologic therapy, radiotherapy, or immunotherapy must have had a washout period of at least 3 weeks before enrollment. Patients with known CNS tumor involvement, other active malignancy, clinically significant cardiac disease, active serious systemic disease or infection, evidence of immune or allergic reaction, or documented human antihuman antibodies (HAHA) after prior monoclonal antibody therapy were excluded.

Study design and treatment

This first-in-human, open-label, phase I study recruited patients with extracranial solid tumors who had failed standard chemotherapy. All documents pertaining to study design, informed consent, and patient information received Institutional Review Board approval in accordance with the Declaration of Helsinki before the study began.

Patients were treated at escalating dose levels using a standard 3+3 design. Objectives included determination of the safety of multiple infusions of MORAb-004, identification of the maximum tolerated dose (MTD), pharmacokinetic (PK) modeling, detection of any anti-human antibody response, and assessment of objective radiographic response to therapy. Doses were escalated in cohorts of 3 to 6 patients, based on the incidence of dose-limiting toxicity (DLT) during the first 4-week cycle of therapy.

MORAb-004 was administered i.v. once weekly in 4-week cycles. Patients were allowed to receive additional cycles of therapy at the original dose until disease progression or prohibitive toxicity occurred. Dose levels from 0.5 to 16 mg/kg per week were originally planned. No intrapatient dose escalation was allowed. Each higher dose level of MORAb-004 was administered only after the safety of the previous lower dose had been established.

Premedications were recommended but not required. Diphenhydramine 50 mg by mouth (p.o.) and cimetidine 300 mg p.o. could be administered 1 hour before treatment in response to a grade 1 drug hypersensitivity reaction. Diphenhydramine 50 mg i.v., ranitidine 50 mg i.v., and dexamethasone 20 mg i.v. could be administered at least 0.5 hour before treatment in response to a grade 2 reaction. Patients who experienced grade 3 or 4 hypersensitivity reactions were discontinued from study drug.

Dose-limiting toxicity

A DLT was defined as any grade 3, 4, or 5 toxicity [National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE) definition; ref. 14], except alopecia, that was definitely, probably, or possibly related to MORAb-004 administration. Infusion-related toxicities that could be treated or controlled to grade 2 or less by maximal medical management were not considered DLTs. Anaphylactic or anaphylactoid reactions of grade 3 or greater were not considered DLTs for the purpose of dose escalation and cohort expansion because these events are not related to dose. With regard to grade 3 or higher liver function test abnormalities, when supportive evidence (radiographic) indicated that these were due to tumor progression, these events were not considered DLTs.

Safety and efficacy evaluations

All safety evaluations (physical examination, 12-lead ECG, hematology and chemistry laboratory panels, coagulation times, and urinalysis) were performed at screening, day 1 of every treatment cycle, and at the final visit. An ECG was repeated on day 22 of each cycle, and laboratory assessments were repeated on day 15 of each cycle. Coagulation times and adverse events were assessed at every visit; adverse events were collected for 30 days after the last dose.

Efficacy evaluations were performed at screening, every 8 weeks during treatment, and at the final visit, if applicable. Tumor response was measured using CT or MRI.

Pharmacokinetic and correlative studies

Serum samples for PK assessment were taken at every visit during treatment and at the final visit. Patients were monitored for the presence of HAHA at screening, day 15 of cycle 1, days 1 and 15 of each additional cycle, and at the final visit. Both PK and HAHA were assessed during follow-up at 2, 4, 6, and 12 weeks after the final dose. Samples were analyzed using an endosialin antigen–based electrochemiluminescent immunoassay to capture and quantify the serum concentration of free/partial-loaded MORAb-004; the lower limit of quantitation for the method was 7 ng/mL in undiluted serum. Detection of MORAb-004–specific antibodies in patients' serum samples was accomplished using an immunoassay bridging format based on the formation of MORAb-004 HAHA complexes detected in a quasiquantitative manner via electrochemiluminescence. The sensitivity of this method using a surrogate anti–MORAb-004 IgG antibody as a positive control standard in human serum was 19 ng/mL in undiluted serum.

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Statistical analysis

The sample size for this study was not based on statistical considerations but was determined by the study design. If a DLT was observed in a cohort, 3 additional patients were accrued to the same dose level. No statistical comparisons that would have required a minimum sample size for the study were planned.

Safety analyses were performed on all patients who had received at least one dose of MORAb-004. Safety data, presented by dose group, were summarized using descriptive statistics [i.e., mean and standard deviation for continuous variables, *n* (%) for categorical variables].

Progression-free survival (PFS) was assessed on all enrolled patients. Patients who were alive with no documented radiologic disease progression had their PFS censored at the date of their last evaluable tumor assessment. Best overall response by RECIST v.1.0 (12) was assessed for all patients who had at least one post-baseline evaluation.

Results

Patient characteristics

Thirty-six adults with refractory, extracranial solid tumors were enrolled (Table 1) and received at least one dose of MORAb-004. Of these, 11 patients had received at least 3 prior chemotherapy regimens (median, 1; range, 0–5).

The first patient dosed in this study (0.5 mg/kg), a 69-year-old woman with advanced uterine cancer, developed significant vaginal bleeding following 17 days of exposure to MORAb-004 and an expanding chest wall hematoma after placement of a central venous catheter with a port following 21 days on study medication. The patient also experienced isolated activated partial thromboplastin time (aPTT) elevations (77.2 seconds 1 day after dose and 71.0 seconds 2 days later) with no other significant coagulation abnormalities; aPTT normalized by 6 days after dose. No connection could be made between the aPTT elevations, the hematoma event, and administration of MORAb-004. However, it was deemed appropriate to assess any bleeding risk by treating additional patients at lower doses; hence, the dosing schedule was altered, and the next two patients received 0.0625 mg/kg (one-eighth of the original planned starting dose). A dose-doubling schedule followed, with two patients enrolled in each of the next two cohorts (0.125 mg/kg and 0.25 mg/kg). Because these patients showed no evidence of significant bleeding or coagulation abnormalities after 4 weeks, the original dose schedule was resumed with continued careful monitoring of bleeding and laboratory coagulation abnormalities (Table 2).

Table 1. Patient demographics and baseline disease characteristics

	All patients ($N = 36$)
Median age, y (range)	56.5 (23, 74)
Gender, *n* (%)	
Male	21 (58.3)
Female	15 (41.7)
Race, *n* (%)	
Caucasian	32 (88.9)
African American	2 (5.6)
Asian	1 (2.8)
Other	1 (2.8)
Karnofsky performance status, *n* (%)	
100%	3 (8.3)
90%	21 (58.3)
80%	12 (33.3)
Cancer diagnosis, *n* (%)	
Colorectal	11 (30.6)
Sarcoma	8 (16.7)
Non–small cell lung	2 (5.8)
Mesothelioma	1 (2.8)
Pancreatic neuroendocrine	1 (2.8)
Hepatocellular	1 (2.8)
Ovarian	1 (2.8)
Pancreatic	1 (2.8)
Other	12 (33.3)

Table 2. All grade 3 and/or serious TEAEs

Dose cohort	Dose (mg/kg)	*N*	Grade 3 and/or serious events
1	0.0625	2	Abdominal pain—SAE, grade 3 Weight decreased—grade 3 Back pain—SAE, grade 3
2	0.0125	2	Hypokalemia—grade 3 Drug hypersensitivity—SAE, grade 3 Angioedema—grade 3
3	0.25	2	None
4	0.5	4	Vaginal hemorrhage—grade 3 Hematoma[a]—SAE, grade 3 Hydronephrosis—SAE, grade 3 Urinary retention—SAE, grade 3 Acute respiratory distress syndrome—SAE, grade 3
5	1	4	None
6	2	3	None
7	4	3	Pericardial effusion—SAE, grade 3 Bacteremia—SAE, grade 3 Urinary tract infection—SAE, grade 3
8	8	3	Abdominal pain—SAE, grade 2 Leukopenia—grade 3 Pyrexia[a]—SAE, grade 1 Infusion-related reaction[a]—SAE, grade 1
9	12	8	Diarrhea—SAE, grade 3 Hyperglycemia—grade 3 Hyponatremia—SAE, grade 3 Noncardiac chest pain—grade 3 Alanine aminotransferase increased—grade 3 Hyperbilirubinemia—SAE, grade 2 Pyrexia—Two SAEs, grade 2 Sinus tachycardia—SAE, grade 1 Contrast medium allergy—SAE, grade 2 Blood creatinine increased—SAE, grade 2 Confusional state—SAE, grade 2
10	16	5	Vomiting[a]—One SAE, grade 3, DLT; one SAE, grade 2 Gastroesophageal reflux—grade 3 Nausea[a]—One SAE, grade 3; one SAE, grade 2 Fatigue[a]—grade 3 Depressed level of consciousness[a]—SAE, grade 3 Somnolence[a]—SAE, grade 3 Pain in extremity—grade 3 Pyrexia[a]—SAE, grade 2 Infusion-related reaction[a]—SAE, grade 2 Diplopia[a]—SAE, grade 2 Speech disorder[a]—SAE, grade 2

[a]Considered related to MORAb-004.

Maximum tolerated dose

One patient developed a DLT of grade 3 vomiting on the 16-mg/kg dose; however, as an additional grade 3 event of vomiting (not considered a DLT per protocol) and other serious adverse events (SAE) of nausea, diplopia, depressed level of

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Table 3. MORAb-004–related TEAEs that occurred in more than one patient by grade

MedDRA preferred term[a,c]	0.0625 (mg/kg) (*n* = 2)	0.125 (mg/kg) (*n* = 2)	0.25 (mg/kg) (*n* = 2)	0.5 (mg/kg) (*n* = 4)	1 (mg/kg) (*n* = 4)	2 (mg/kg) (*n* = 3)	4 (mg/kg) (*n* = 3)	8 (mg/kg) (*n* = 3)	12 (mg/kg) (*n* = 8)	16 (mg/kg) (*n* = 5)	Total (*N* = 36)
Grade 1, *n* (%) patients[b]											
Fatigue	1 (50.0)	0	0	1 (25.0)	1 (25.0)	1 (33.3)	0	2 (66.7)	2 (25.5)	1 (20.0)	9 (25.0)
Headache	0	0	0	0	0	0	0	3 (100.0)	4 (50.0)	1 (20.0)	8 (22.2)
Pyrexia	0	0	0	0	1 (25.0)	1 (33.3)	1 (33.3)	2 (66.7)	1 (12.5)	1 (20.0)	7 (19.4)
Chills	0	0	0	1 (25.0)	1 (25.0)	2 (66.7)	1 (33.3)	0	1 (12.5)	1 (20.0)	7 (19.4)
Nausea	0	0	0	0	0	0	0	1 (33.3)	1 (12.5)	0	2 (5.6)
Myalgia	0	0	0	0	0	0	1 (33.3)	0	0	1 (20.0)	4 (11.1)
Vomiting	0	0	0	0	0	0	0	1 (33.3)	0	0	1 (2.8)
aPTT prolonged	0	0	0	1 (25.0)	0	0	0	0	1 (12.5)	1 (20.0)	3 (8.3)
Cough	0	0	0	0	2 (50.0)	0	0	1 (33.3)	0	0	3 (8.3)
Malaise	0	0	0	0	0	0	0	2 (66.7)	0	0	2 (5.6)
Pruritis	1 (50.0)	0	0	0	0	0	0	0	0	1 (20.0)	3 (5.6)
Rash	0	0	0	0	1 (25.0)	0	0	0	0	2 (40.0)	3 (8.3)
Anemia	0	0	0	0	0	0	0	1 (33.3)	0	1 (20.0)	2 (5.6)
Diarrhea	0	0	0	1 (25.0)	0	0	0	0	0	1 (20.0)	2 (5.6)
Flushing	1 (50.0)	0	0	1 (25.0)	0	0	0	0	0	0	2 (5.6)
Infusion-related reaction	0	0	0	0	0	0	0	1 (33.3)	0	0	1 (2.8)
Grade 2, *n* (%) patients											
Fatigue	0	0	0	1 (25.0)	1 (25.0)	1 (33.3)	0	2 (66.7)	2 (25.0)	0	7 (19.4)
Headache	0	0	0	0	0	1 (33.3)	1 (33.3)	0	0	3 (60.0)	5 (13.9)
Pyrexia	0	0	0	0	0	0	0	0	0	1 (20.0)	1 (2.8)
Nausea	0	0	0	0	0	0	0	0	0	2 (40.0)	2 (5.6)
Vomiting	0	0	0	0	0	0	0	0	0	1 (20.0)	1 (2.8)
Malaise	0	0	0	0	0	0	0	0	0	1 (20.0)	1 (2.8)
Pruritis	0	0	0	0	1 (25.0)	0	0	0	0	0	1 (2.8)
Infusion-related reaction	0	0	0	0	0	0	0	0	0	0	1 (2.8)
Proteinuria	0	1 (50.0)	0	0	0	0	0	0	1 (12.5)	0	2 (5.6)
Grade 3, *n* (%) patients											
Fatigue	0	0	0	1 (25.0)	0	0	0	0	0	0	1 (2.8)
Nausea	0	0	0	0	0	0	0	0	0	1 (20.0)	1 (2.8)
Vomiting	0	0	0	0	0	0	0	0	0	2 (40.0)	2 (5.6)
Pruritis	0	0	0	0	0	0	0	0	0	0	1 (2.8)

Abbreviations: aPTT, activated partial thromboplastin time; MedDRA, Medical Dictionary for Regulatory Activities v.11.1.

[a]For each preferred term, a patient with 2 or more events in that category is counted only once.

[b]No grade 4 or 5 events were observed.

[c]Percentages are based on the total number of patients in each dose group.

consciousness, somnolence, and speech disorder were observed at this dose level, the decision was made to open an additional cohort of 6 patients at 12 mg/kg rather than continue accrual to the 16-mg/kg dose. Two patients in the 12-mg/kg cohort did not complete cycle 1 due to disease progression; these were replaced with 2 additional patients for a total of 8 patients at 12 mg/kg. No DLT was observed at this dose level. Despite the fact that protocol definition of MTD was not achieved (i.e., 2 DLTs at 16 mg/kg), the *de facto* MTD was defined as 12 mg/kg based on the safety findings noted above.

Toxicity

Treatment-emergent adverse events (TEAE) included those that occurred from the first day of MORAb-004 administration until 30 days after the last dose or events that were present before the first day of study drug administration and worsened in severity during the study. The most common TEAEs were fatigue (47.2%), headache (36.1%), pyrexia (22.2%), chills, (19.4%) and nausea (13.9%).

Thirty-three SAEs were reported, of which only five SAEs occurred in more than one patient: pyrexia in 3 (8%) patients and abdominal pain, nausea, vomiting, and infusion-related reaction in 2 (6%) patients each. Thirteen SAEs in 5 (14%) patients were considered related to MORAb-004. Table 2 presents all grade 3 TEAEs, SAEs, and DLTs by dose cohort. No grade 4 or 5 TEAEs occurred. One patient died 30 days after the last infusion of MORAb-004 due to disease progression; this was not considered to be related to MORAb-004.

Table 3 presents all MORAb-004–related TEAEs occurring in more than one patient by severity. The majority of treatment-related SAEs occurred at dose levels of 12 mg/kg (3 of the 8 patients treated at that dose level) and 16 mg/kg (3 of 5 patients).

Antitumor activity

Eighteen of 32 evaluable patients (56%) demonstrated a best overall response of stable disease by RECIST (Table 4). Three patients were not evaluable as they had no post-baseline CT/MRI due to discontinuation before first scheduled assessment (two due to AEs and one due to clinical determination of disease progression). One patient was not evaluable by CT/MRI as he only had one kidney and could not tolerate contrast medium; he was assessed by positron emission tomography. Median PFS based on 22 events in 36 patients was 8.4 weeks (95% confidence interval, 7.1–16.4 weeks).

Of the patients who achieved stable disease, 4 had minor decreases in tumor size based on the sum of the longest diameters; however, the magnitude of tumor reduction was less than 30% (1 patient with a 10% decrease in pancreatic neuroendocrine

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Table 4. Summary of antitumor activity of MORAb-004

Patient ID	Dose group (mg/kg/wk)	Tumor type ($N = 36$)	Cycles (n)	PFS (days)	Best overall response	Best % change from baseline in sum of longest diameters
101-002	0.0625	Colorectal	2	53	SD	1
101-004	0.0625	Pancreatic neuroendocrine	12	330	SD	−10
101-003	0.125	Colorectal	5	127	SD	8
101-005	0.125	Colorectal	1	24	PD	39
101-006	0.25	Colorectal	4	106	SD	0
101-010	0.25	Colorectal	4	106	SD	16
101-001	0.5	Sarcoma, uterus	1	24[a]	SD	6
101-011	0.5	Mesothelioma	6	148[a]	SD	2
102-001	0.5	Colorectal adenocarcinoma	2	50	PD	48
102-004	0.5	Carcinoid small bowel	4	106[a]	SD	2
101-012	1	Pancreatic adenocarcinoma	2	36	PD	20
101-014	1	Colorectal adenocarcinoma	1	29[a]	SD	3
102-008	1	Adenoid cystic	1	1[a]	NE	[b]
102-010	1	Hepatocellular carcinoma	15	400[a]	SD	−21
102-012	2	Renal cell	1	32	PD	29
102-013	2	Adenocarcinoma, unknown primary	1	1[a]	NE	[b]
102-014	2	Myxoid chordrosarcoma	4	106[a]	SD	10
101-015	4	Colorectal adenocarcinoma	2	56	PD	13
101-016	4	Pleural mesothelioma	2	1[a]	NE	[b]
102-015	4	Hemangiopericytoma	8	222	SD	−13
101-017	8	Sarcoma	1	37	PD	[c]
101-018	8	Chondrosarcoma	4	115	SD	8
102-016	8	Osteosarcoma, scalp	2	59	PD	16
101-022	12	Small round cell, inguinal area	1	13	PD	27
101-023	12	Colorectal adenocarcinoma	6	1[a]	NE	[b]
101-024	12	Colorectal adenocarcinoma	2	50	PD	1
101-025	12	Colorectal adenocarcinoma	4	106	SD	10
102-019	12	Adenocarcinoma, ampullary	1	19	PD	44
102-020	12	Unknown primary	2	32	PD	[c]
102-021	12	Non–small cell lung	3	53[a]	SD	12
102-024	12	Non–small cell lung	2	50	PD	23
101-019	16	Metastatic transitional cell, bladder	2	50	PD	[c]
101-020	16	Pancreatic adenocarcinoma	1	24	PD	31
101-021	16	Undifferentiated pleomorphic sarcoma	2	50[a]	SD	14
102-017	16	Ovarian adenocarcinoma	3	51[a]	SD	−1
102-018	16	Rhabdomyosarcoma	8	218[a]	SD	1

Abbreviations: NE, not evaluable; PD, progressive disease; SD, stable disease.
[a]Censored at last progression-free observation.
[b]No post-baseline imaging evaluation or target lesions not evaluable.
[c]Response was defined as PD because of the appearance of new lesions.

tumor and PFS of 330 days, 1 patient with a 21% decrease in hepatocellular tumor and PFS of 400 days, 1 with a 13% decrease in sarcoma and PFS of 222 days, and 1 with a 1% decrease in ovarian tumor and PFS of 51 days). Figure 1A presents the best percentage change in tumor size by individual patient. Figure 1B shows the percentage of change for each patient over the course of the study.

Endosialin expression

Diagnostic biopsy samples from 28 enrolled patients were collected and stained with an anti-endosialin antibody. Analysis of archival tumor in 7 representative cases revealed endosialin stromal expression and $CD34^+$ vessel staining in all cases, with 1+ to 3+ endosialin tumor cell staining observed in 4 of the 7 cases (Fig. 2). Three of these 4 cases showed a tumor shrinkage of 10% to 21% and stable disease as the best overall response (see Table 4 for anti-tumor activity in subjects 101-004, 102-010, and 102-015). The fourth case (subject 101-001) had a 6% increase in tumor size despite 3+ endosialin tumor staining but did achieve stable disease as the best overall response.

Immunogenicity

To better understand the potential immunogenicity of MORAb-004, patients were evaluated for drug hypersensitivity adverse events (DHAE), defined as drug hypersensitivity reactions per NCI CTCAE v.3.0 and occurring with associated positive HAHA. Six events in 6 (17%) patients were deemed DHAEs, the most common being pyrexia (11%). Two of these patients demonstrated preexisting HAHA at screening; one developed grade 1 myalgia, edema, and rash with multiple samples positive for HAHA after dose, and the other had grade 2 fever and grade 2 infusion–related reaction. Four patients had DHAEs associated with treatment-emergent positive HAHA: 3 patients with grade 1 pyrexia and 1 patient each with grade 1 flushing, chills, infusion-related reaction, and rash.

Pharmacokinetics

Systemic exposure to MORAb-004, as assessed by both maximum serum concentration (C_{max}) and area under the serum concentration–time curve up to the last quantifiable concentration time point (AUC_{0-t}), increased in an approximate dose-proportional manner for C_{max} and greater than dose proportional

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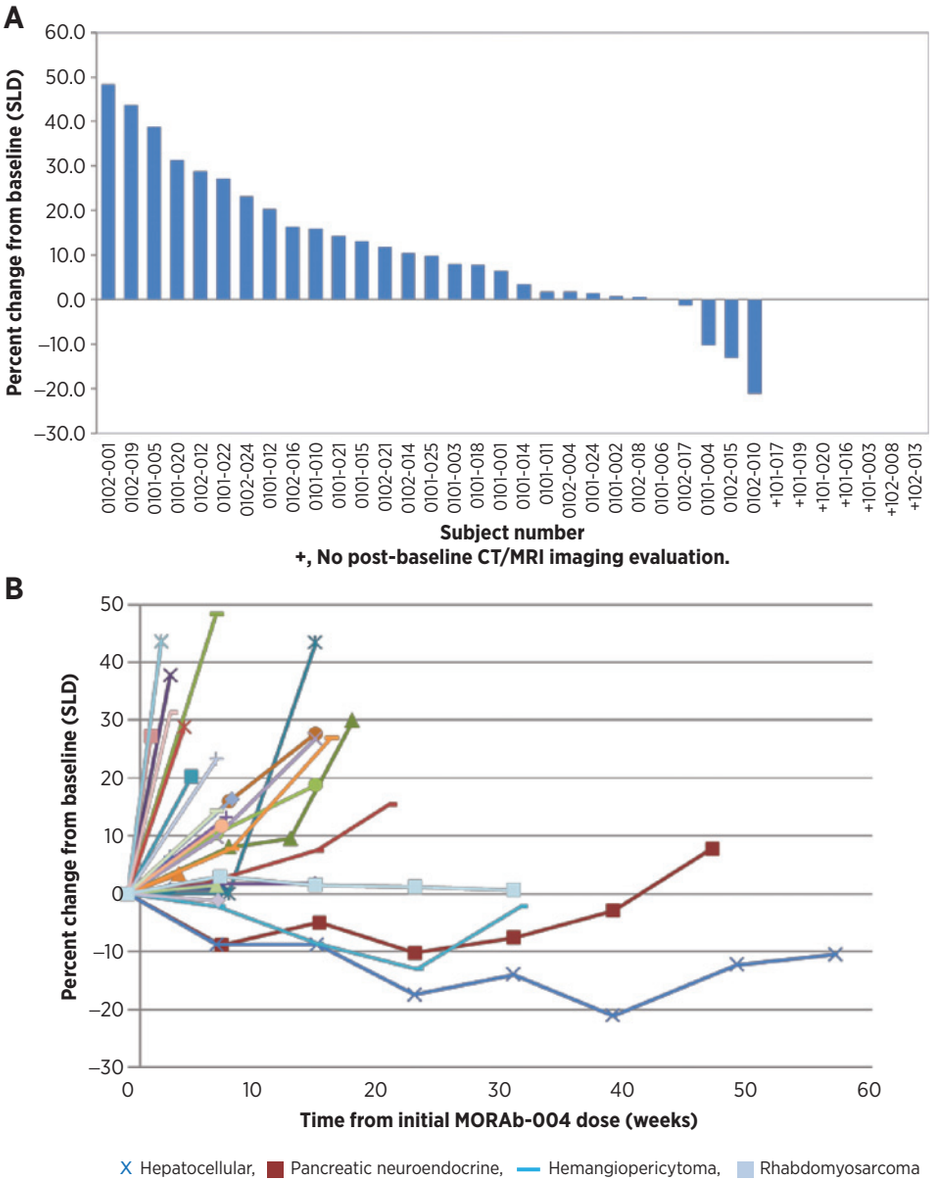

Figure 1.
A, percentage change from baseline in the sum of the longest diameters for each patient's target lesion(s) best overall radiographic response. B, minor tumor responses were noted in sarcoma (hemangiopericytoma), hepatocellular carcinoma, and pancreatic neuroendocrine tumor. One patient with bladder cancer had a response in existing lesions, but developed progressive disease due to the appearance of new lesions. The responses noted in hemangiopericytoma, hepatocellular carcinoma, and pancreatic neuroendocrine tumor were of 6 months, 9 months, and 11 months duration, respectively.

for AUC_{0-t} across the range of 0.0625 to 16 mg/kg, following both single and multiple weekly infusions (Table 5). A review of the individual PK profiles indicated that saturable clearance may play a role in the PK of MORAb-004, even following the first infusion of a relatively low dose. Following multiple weekly infusions, the half-life estimates ranged from approximately 20 to 200 hours, indicating a slow clearance of MORAb-004, especially at the higher doses. No accumulation was evident with the limited data available on day 22 when compared with day 1 in doses up to 2 mg/kg, whereas there was evidence of accumulation at dose levels of 4 mg/kg and above.

Discussion

Here, we report the first clinical trial results for MORAb-004, a humanized monoclonal antibody targeting endosialin. Prolonged stable disease of at least 106 days was observed in 12 patients having tumor subtypes generally thought of as epithelial in origin, including colorectal carcinoma (Table 4). This represents only modest activity; however, it does support further investigation into such tumors, using the higher doses (8–12 mg/kg).

Endosialin is known to be expressed in the stromal compartment of virtually every human tumor (1, 2, 4–7). In addition to the stromal compartment expression, endosialin is expressed by tumor cells in a subset of tumors, primarily of mesenchymal origin. Direct tumor cell expression has been observed in subtypes of soft-tissue sarcoma, melanoma, and neuroblastoma (15, 16). In addition, tumor cell expression has been noted in subsets of epithelial malignancies, including colorectal cancer (17). This expression in the tumor cell compartment, in addition to stromal cell expression, may factor into the responses observed

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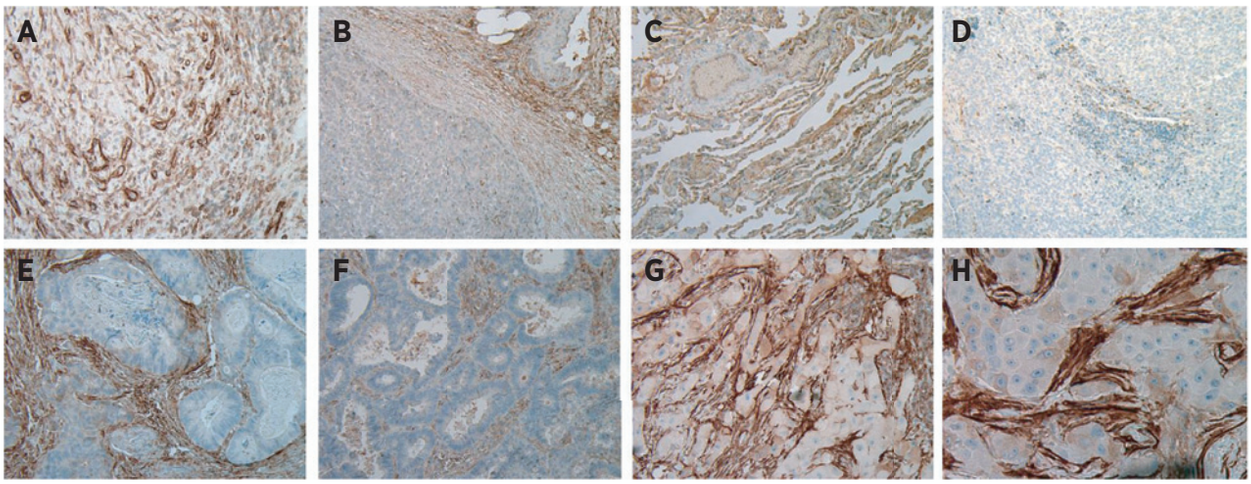

Figure 2.
Diagnostic biopsy samples from patients enrolled on the trial were collected an stained with an anti-endosialin monoclonal antibody (clone 1-55). A, uterine sarcoma (patient ID 101-001), 3+ tumor and stroma staining; B, pancreas adenocarcinoma (patient ID 101-004), 3+ stromal staining and 2+ tumor cell staining; C and D, hemangiopericytoma (patient ID 102-015) 3+ stromal staining, 0–1+ tumor cell staining; E, Colon carcinoma (patient ID 101-023), 2–3+ stromal staining, 0+ tumor cell staining; F, colon carcinoma (patient ID 101-025), 2–3+ stromal staining, 0+ tumor cell staining; G and H, hepatocellular carcinoma (patient ID 102-010), 1–2+ tumor cell staining and 3+ stromal staining.

with single-agent MORAb-004 in this phase I trial. However, this could not be confirmed due to the small number of subjects assessed.

Although the mechanism of action of MORAb-004 is not completely understood, preclinical models suggest that endosialin is removed from the cell surface upon MORAb-004–mediated internalization, while MORAb-004 has no antibody-dependent cellular cytotoxicity activity (D.J. O'Shannessy; unpublished data). Endosialin has been shown to be involved in signaling pathways thought to be critical for mesenchymal cell types (e.g., PDGF-β receptor and Notch protein receptor; ref. 10), as well as to participate in extracellular matrix protein interactions (9). Therefore, it is possible that MORAb-004 may be affecting cellular signaling as well as protein–protein interactions that serve to communicate signals in the tumor microenvironment between tumor and stromal cells. Additional work is under way to further elucidate the exact mechanism of action of MORAb-004.

Based on the safety and tolerability data from this phase I dose-escalation study, an MTD of 12 mg/kg was defined for MORAb-004. Preliminary evidence of antitumor activity was observed, with prolonged disease stabilization in patients with advanced solid tumors. Taken together, the safety profile, PK, and potential antitumor activity suggest that MORAb-004 has efficacy at doses up to and including 12 mg/kg. Phase II studies in melanoma, metastatic colorectal cancer, and soft tissue sarcoma studies have been initiated based on the results of this study.

Table 5. Mean PK parameters for MORAb-004-001 in humans in cycle 1 days 1 and 22

Dose (mg/kg)	Cycle day	Number	C_{max} μg/mL (SD) (*n*)	AUC_{0-t} μg·h/mL (SD) (*n*)	$t_{1/2}$ h (SD) (*n*)
0.0625 mg/kg	C01D01	2	0.495 (0.177)	10.8 (1.88)	23.09 (–)
0.0625 mg/kg	C01D22	2	0.500 (0.141)	11.6 (0.403)	44.97 (–)
0.125 mg/kg	C01D01	2	0.960 (0.523)	24.9 (14.2)	30.7 (10.7)
0.125 mg/kg	C01D22	2	0.840 (0.721)	20.7 (19.5)	25.2 (0.177)
0.25 mg/kg	C01D01	2	4.50 (1.51)	148 (74.7)	24.74 (1.74)
0.25 mg/kg	C01D22	2	3.91 (0.898)	141 (70.9)	21.0 (10.2)
0.5 mg/kg	C01D01	4	11.9 (4.90)	351 (144)	26.19 (2.42)
0.5 mg/kg	C01D22	3	11.0 (2.15)	442 (152)	29.46 (3.68)
1 mg/kg	C01D01	4	23.0 (4.06)	1289 (306)	37.79 (8.47)
1 mg/kg	C01D22	3	24.3 (2.78)	1390 (349)	52.25 (17.2)
2 mg/kg	C01D01	3	46.8 (5.87)	2702 (481)	52.35 (18.7)
2 mg/kg	C01D22	3	50.1 (7.30)	3739 (1595)	83.81 (25.3)
4 mg/kg	C01D01	3	108 (4.58)	7971 (597)	87.12 (12.21)
4 mg/kg	C01D22	3	160 (8.49)	14205 (1783)	103.93 (3.84)
8 mg/kg	C01D01	3	200 (22.3)	15636 (975)	106.18 (10.6)
8 mg/kg	C01D22	3	280 (58.4)	22908 (723)	163.55 (54.7)
12 mg/kg	C01D01	8	317 (68.1)	24951 (3968)	119.08 (24.7)
12 mg/kg	C01D22	6	587 (91.2)	64542 (15061)	197.17 (41.2)
16 mg/kg	C01D01	5	447 (145)	31244 (12700)	120.24 (13.1)
16 mg/kg	C01D22	3	685 (169)	83697 (27846)	178.40 (22.4)

Abbreviations: C01D01, cycle 1 day 1; C01D22, cycle 1 day 22; SD, standard deviation.

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Disclosure of Potential Conflicts of Interest

C.M. Coughlin has ownership interests (including patents) in Morphotek. No potential conflicts of interest were disclosed by the other authors.

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