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A Phase I Dose Escalation and Expansion Study of the Anticancer Stem Cell Agent Demcizumab (Anti-DLL4) in Patients with Previously Treated Solid Tumors

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Abstract

Purpose: This phase I trial evaluated the safety, pharmacokinetics, and pharmacodynamics of demcizumab (OMP-21M18), a humanized IgG_2 mAb targeting the Notch ligand DLL4 in adult patients with advanced malignancies.

Experimental Design: Standard 3+3 design, with demcizumab 0.5, 1, 2.5, or 5 mg/kg weekly or 2.5, 5, or 10 mg/kg every other week, with an expansion cohort at 10 mg/kg every other week. Dose-limiting toxicities (DLT) were assessed during the first 28 days.

Results: Fifty-five patients received demcizumab (15 weekly, 18 every other week, 21 expansion cohort, 1 loading dose). No more than one DLT was seen at any dose level. The MTD was not reached for either schedule. Treatment-related adverse events occurring in >10% of patients were hypertension or blood pressure increased (47%), fatigue (31%), anemia (22%), headache (20%), nausea (13%), hypoalbuminemia (11%), dizziness (11%), and dyspnea (11%). One patient dosed at 2.5 mg/kg developed reversible right-sided heart failure after 63 days on treatment and 4 dosed at 10 mg/kg developed congestive heart failure after ≥98 days on treatment. Five patients were hospitalized with bleeding episodes (2 episodes of tumor-associated bleeding). Sixteen of 25 (64%) evaluable patients at 10 mg/kg had evidence of stabilization of disease or response.

Conclusion: Demcizumab was generally well tolerated at doses ≤5 mg weekly with disease stabilization and decreases in tumor size demonstrating antitumor activity. Hypertension was the most common adverse event that was clearly related to treatment. Prolonged administration was associated with an increased risk of congestive heart failure. *Clin Cancer Res; 20(24); 6295–303. ©2014 AACR.*

Introduction

The Notch signaling pathway is an intracellular signaling system that plays a critical role in cellular survival. Composed of four receptors (Notch 1–4) and 5 ligands [Jagged 1, 2 and Delta-like ligands (DLL) 1, 3, and 4], the diversity of receptors and ligands allows an array of tissue-specific effects (1). These include the specification of cell fate, differentiation, proliferation, survival, and cellular self-renewal. Notch plays an important role in embryonic development, tissue repair, hematopoiesis, and the maintenance of endothelial and gut epithelial stem cell homeostasis (2). Given this key role, alterations in the Notch signaling pathway result in abnormal cellular function, including the development of malignancies. Notch mutations stimulate proliferation, restrict differentiation, promote cellular survival, and are associated with oncogenesis in several malignancies (3, 4). Notch plays a critical role in the maintenance of cells within tumors with stem cell properties; specifically, the ability to self-renew, differentiate into multiple cell types, and relative chemo- and radioresistance (5). Although DLL4 binds all four Notch receptors, the interaction with Notch 1 is preferred (6). DLL4 is commonly expressed in solid tumors and associated with chemoresistance in pancreatic cancer models, whereas Notch 1 expression has been associated with a poor prognosis in lung cancers (7–9). DLL4 is expressed at sites of angiogenesis and is the

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The Notch pathway plays a critical role in cellular differentiation and survival, and inhibition of signaling via this pathway decreases the ability to serially propagate tumors, pointing to its importance in the maintenance of cancer stem cells. Demcizumab is a humanized antibody directed against delta-like ligand 4 (DLL4), a key ligand in the pathway. This clinical study evaluated the safety, tolerability, pharmacokinetics, pharmacodynamic effects in blood and hair follicles, and preliminary antitumor activity of demcizumab in patients with advanced solid tumors. Pharmacodynamic changes indicative of downregulation of stem cell–related genes and upregulation of vascular/endothelial genes were observed starting at 2.5 mg/kg every other week. The majority of patients treated at 10 mg/kg every other week had disease stabilization. Prolonged exposure (>12 weeks) resulted in the emergence of delayed cardiac toxicity thought to be due to dysangiogenesis mediated by inhibition of signaling through DLL4 receptors on endothelial cells.

specific ligand of the pathway in endothelial cells (10). DLL4 interacts with VEGF through a negative feedback loop in which Notch signaling limits the response to VEGF (11). VEGF acts upstream of Notch and induces expression of DLL4 in cultured human endothelial cells. In multiple models, inhibition of Notch signaling results in endothelial cell hyperproliferation (12). In xenograft models, DLL4 blockade results in both ineffective angiogenesis and inhibition of tumor growth (12). Demcizumab (OMP-21M18), a humanized IgG_2 mAb, targets DLL4, blocking the interactions with Notch receptors. In minimally passaged human xenograft models of breast, colon, ovarian, non–small cell lung and pancreatic cancer both alone and in combination with chemotherapy, demcizumab markedly reduced tumor growth, regrowth, and the number of cells expressing markers associated with cancer stem cells (13–15). Limiting dilution assays confirmed that the number of tumor-initiating cells (cancer stem cells) in tumors treated with the combination of chemotherapy and demcizumab were reduced significantly over tumors treated with chemotherapy alone (13). We conducted a multi-center, open-label, dose escalation phase I trial of this first-in-class anti-DLL4 antibody, demcizumab. The objectives were to determine the MTD, safety, immunogenicity, pharmacokinetics, biomarkers, and efficacy in patients with advanced malignancies.

Patients and Methods

Patients

Eligible patients were $\geq$21 years old, with histologically confirmed metastatic or unresectable malignancy with $\geq$1 lesion measuring 2 $\times$ 2 cm and for which there was no remaining curative therapy and no therapy with a survival benefit, an Eastern Cooperative Oncology Group performance status $\leq$1, a life expectancy of >3 months, normal hematologic and clotting parameters, total bilirubin $\leq$1.5 $\times$ institutional upper limit of normal (IULN), aspartate aminotransferase (AST; SGOT) and alanine aminotransferase (ALT; SGPT) $\leq$2.5 $\times$ IULN, and creatinine <1.5 $\times$ IULN or creatinine clearance >60 mL/min/1.73 m^2. Patients were excluded who had received their last therapy <4 weeks before enrollment, had a history of an allergic reaction to a monoclonal antibody, known HIV infection, had bleeding disorder or coagulopathy, were receiving anticoagulants, had clinically significant gastrointestinal disease, had uncontrolled hypertension, or were receiving >2 antihypertensives. Pregnant or nursing women were also excluded and women of childbearing potential were required to have a prior hysterectomy or a negative serum pregnancy test and be using contraception. Men were also required to use contraception.

During the study, the protocol was amended to exclude patients with New York Heart Association Classification II, III, or IV heart disease, evidence of cardiac ischemia, acute myocardial infarction or heart failure within 6 months, receiving any medications for cardiac ischemia, B-type natriuretic peptide (BNP) value of >200 pg/mL, or a total cumulative doxorubicin dose of $\geq$400 mg/m^2 (after 16 patients had been enrolled). A second amendment excluded patients with brain metastases, uncontrolled seizure disorder, or active neurologic disease, squamous cell carcinoma of the lung, unresected luminal tumors of the gastrointestinal tract or significant intercurrent illness (after 21 patients had been enrolled). The original and amended study protocol and informed consent were reviewed and approved by the Institutional Review Boards of the participating institutions, and informed consent was obtained from patients (clinicaltrials.gov registration number: NCT00744562).

Study design

The objectives were to determine the MTD and assess the safety, immunogenicity, pharmacokinetics (PK), biomarkers, and efficacy in patients with advanced malignancies. The study was designed as a standard 3+3 dose escalation trial. Dose-limiting toxicity (DLT) was defined as any Grade 3 or 4 adverse event (except hypertension) that occurred during the first 28 days, as assessed by the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0, unless it could be clearly attributed to another cause. Hypertension was only a DLT if the blood pressure was $\geq$150/90 mmHg after receiving $\leq$2 antihypertensives for 14 days within the first 28 days or if $\geq$3 antihypertensive medications were required. The MTD was defined as the highest dose at which 0 to 1 of 6 patients experienced a DLT. All patients in a cohort were required to complete their day 28 assessment before dose escalation. Doses were initially 0.5, 1, 2.5, and 5 mg/kg weekly (QW) for 9 doses and then once every other week. The protocol was subsequently amended to enroll cohorts of 6 patients treated with

2.5, 5, and 10 mg/kg every other week (Q2W) and an expansion cohort of up to 32 patients at the highest tolerated dose. On the basis of initial pharmacokinetic analysis, an additional loading dose schedule of 10 mg/kg weekly × 3 followed by every other week dosing designed to rapidly bring drug levels to a steady state was added.

Study drug administration

Patients received demcizumab at the assigned dose through a 0.22-μm filter over 30 minutes.

Study assessments

Safety was monitored with weekly physical examination, vital signs, clinical laboratory testing (complete blood counts and comprehensive metabolic panel), and assessment of performance status. Electrocardiogram, anti-demcizumab antibody (ADA), and urinalysis were performed every 28 days. CT or MRI was performed at baseline and every 8 weeks. Response was assessed by RECIST 1.0 (16). While the study was ongoing, cardiotoxicity was identified as a potential toxicity in a cynomolgus monkey study, and the protocol was amended to include monitoring with BNP (every 28 days) and echocardiogram (baseline and every 8 weeks).

Pharmacokinetic specimens were obtained at baseline, end of infusion, 0.5, 1, 3, 6, 24, 72, and 168 hours after infusion on study days 0 and 56 on the weekly schedule and at baseline, end of infusion, 0.5, 1, 3, 6, 24, 72, and 168 hours after infusion on study days 0 and 42 of the every other week schedule. Pre- and postinfusion samples were drawn on all other infusion days, and a sample was obtained on any visit not associated with an infusion and at treatment termination. Plasma was harvested with sodium heparin as anticoagulant, and analyzed for OMP-21M18 concentration in a fully validated antigen-binding ELISA.

Biomarkers

Specimens for pharmacodynamic (PD) biomarkers were obtained at baseline, days 28 (hair follicles, whole blood, and plasma), 56, 140, and treatment termination (whole blood and plasma only). Whole blood was collected in PAXgene tubes (BD Biosciences), and blood for plasma biomarkers was collected in K_3EDTA vacutainer tubes (BD Biosciences). Hair follicles were preserved in RNAlater (Qiagen) until extraction of RNA (PicoPure RNA Isolation Kit from LifeTechnologies). In addition, control plasma and whole blood samples were sourced and collected (Conversant Bio) using the same methods described above from 8 patients with cancer who were not treated with demcizumab. Affymetrix human gene chip *U133 Plus 2.0* arrays were used for profiling the gene expression levels in the whole blood and in the hair follicle samples (Almac CLIA–certified laboratory). To obtain the expression levels of each probe set, the raw CEL files in each dataset were processed for background adjustment and signal intensity normalization with GC Robust Multi-array Average (GCRMA) algorithm in the open-source bioconductor software (www.bioconductor.org). For the plasma biomarker assays, Rules Based Medicine Human MAP v1.6 protein panel was performed (RBM CLIA–certified laboratory), and ELISA analysis was used for placental growth factor (PIGF) (R&D Systems).

Statistical considerations

This was a phase I dose escalation study with a standard 3+3 dose escalation. Therefore, the sample size was not statistically determined. If 1 of 3 subjects experienced a DLT, that dose level was expanded to 6 subjects. If 2 or more of the 6 subjects experienced a DLT, no further subjects were dosed at that level and 3 additional subjects were added to the preceding dose cohort unless 6 subjects had already been treated at that dose level. Subjects were assessed for DLTs from the time of the first dose through day 28. Dose escalation, if appropriate, occurred after all subjects in a cohort had completed their day 28 DLT assessment.

The general analytical approach for all endpoints was descriptive in nature. No statistical hypotheses were tested. For continuous variables, descriptive statistics were used. For categorical variables, descriptive statistics include counts and percentages per category. Time-to-event variables were estimated by the Kaplan–Meier method.

For the microarray gene expression analysis, genes were considered significant at a 95% confidence interval (CI) and a gene expression change of greater than 1.5-fold. Paired-sample bootstrapping was used to generate the CI (SAS, R). The 95% CI (bias-corrected adjusted, BCa) was calculated according to standard methods, applying a nonparametric bootstrap procedure (17, 18). Each patient was compared with their own pretreatment sample in a paired-sample analysis. Only those genes with an absolute fold change of greater than 1.5 and within a 95% CI were considered significant. The limits of the CIs cannot cross zero for statistical significance. Thus, for the upregulated genes, the lower confidence limit (lb) had to be greater than +1.1; for the downregulated genes, the upper confidence limit (ub) had to be less than −1.1. Fewer than 2% of the probesets on the microarray chips were significantly changed by demcizumab treatment. The untreated control subjects without demcizumab treatment were analyzed using the same methods. The plasma biomarkers were analyzed using paired-sample Student *t* test to assess significance comparing each patient post-treatment with its own pre-treatment sample. A P value <0.05 was considered significant for plasma proteins. The plasma samples from untreated control subjects without demcizumab treatment were analyzed using the same methods.

Results

Patients and treatment

A total of 60 patients were enrolled from September 1, 2008, to March 7, 2011, and 55 patients received demcizumab. Baseline characteristics of the enrolled patients are summarized in Table 1. Five patients were not dosed due to decline in hemoglobin (3), disease progression (1), and

Table 1. Patient characteristics

Enrolled/treated	60/55
Mean age (years)	59.2
Gender	
Male, *n* (%)	33 (55)
Female, *n* (%)	27 (45)
Ethnicity	
Hispanic or Latino	4 (6.7)
Non-Hispanic or Latino	56 (93.3)
Race, *n* (%)	
White	55 (91.7)
Asian	2 (3.3)
Black or African	1 (1.7)
Missing	2 (3.6)
Prior therapy	
Surgery, *n* (%)	39 (65)
Chemotherapy, *n* (%)	60 (100)
Immunotherapy, *n* (%)	0 (0)
Radiotherapy, *n* (%)	31 (51.7)
Tumor types	
Colorectal	10
Lung	9 (7 non–small cell, 2 small cell)
Pancreatic	8
Salivary gland	6
Breast	5
Sarcoma	5 (1 each histiocytoma, leiomyosarcoma, synovial, clear cell, pleomorphic high grade)
Kidney	3
Melanoma	3 (2 cutaneous, 1 ocular)
Head and neck	2
Other	9[a]

[a]One each: gastric, prostate (neuroendocrine), bladder, esophageal, ovarian granulosa cell, penile, testicular, mesothelioma, and unknown primary.

elevated BNP (1). A total of 15 patients were treated on the weekly schedule and 18 on the every other week schedule. Subsequently, 21 patients were treated at 10 mg/kg once every other week in the expansion cohort. A single patient was treated in the loading dose schedule. The 55 treated patients had received a median of 4 (range, 1–12) prior therapies.

Safety and toxicity

Demcizumab was generally well tolerated. The median number of infusions per patient was 6 (range: 1–12). Three patients had grade 3 to 5 adverse events that met the definition of a DLT. These DLTs were grade 3 anemia and dyspnea (2.5 mg/kg weekly), grade 3 colitis with bleeding (2.5 mg/kg every other week), and grade 4 hypertension with grade 5 brain metastases with bleeding leading to death (10 mg/kg every other week). These cohorts were subsequently expanded to 6 patients without another DLT. The MTD was not reached on either schedule. Four additional patients had grade 3 to 5 events occurring during the first 28 days which were clearly attributed to their underlying disease and therefore not DLTs. Three of these were secondary to tumor-associated pain and disease progression (grade 3 neuralgia, grade 3 flank, abdominal, and pleuritic pain, and grade 3 pleuritic pain). The fourth patient had grade 3 atelectasis and grade 4 pulmonary embolism and pulmonary infarction.

Treatment emergent adverse events from any cause occurring in >15% of patients are summarized in Supplementary Table S1. The majority of these events were grades 1 to 2, independent of the dose and unrelated to study drug. Treatment emergent adverse events related to study drug and occurring in >5% of patients are summarized in Table 2. The most common of these were hypertension or blood pressure increased (47%), fatigue (31%), anemia (22%), headache (20%), nausea (13%), hypoalbuminemia (11%), dizziness (11%), and dyspnea (11%). The grade 3 to 5 events related to study drug occurring in >5% of the patients were hypertension and blood pressure increased (36%), congestive heart failure (7%), and dyspnea (7%).

Hypertension was the most common treatment-related adverse event. Twenty-eight of 55 (51%) patients enrolled had a prior history of hypertension. Thirty (55%) patients had an adverse event of hypertension or blood pressure increased, with 26 considered related to study drug. The majority were grade 3 events defined as requiring a modification of the patient's antihypertensive regimen. In 14 patients, this was new-onset hypertension, whereas 16 had an exacerbation of preexisting hypertension. In most cases, hypertension was easily controlled by administration of or a change in antihypertensives.

A total of 44 serious adverse events were reported in 22 patients. Two patients died while receiving study drug, one each from an unsuspected brain metastases from a sarcoma and one from bleeding from an unresected primary rectal tumor. An additional 4 patients died from adverse events occurring within 30 days of study termination which were attributed to disease progression (2 cases), one with aspiration pneumonia, and one with chronic obstructive pulmonary disease. Four patients were hospitalized for a bleeding episode, one each with tumor-associated bleeding from an abdominal mass eroding into the stomach and duodenum, a Mallory Weiss tear, bleeding secondary to reflux gastritis from a biliary stent, and menorrhagia. These episodes were considered unrelated to study therapy based on the timing of drug administration, but the tumor-associated gastrointestinal bleeding prompted a protocol amendment to exclude patients having luminal gastrointestinal tumors.

Four of 10 patients who received 10 mg/kg once every other week for a minimum of 98 days developed symptomatic congestive heart failure. Symptoms were reported between study days 107 and 112. An additional patient who received 2.5 mg/kg once every week developed right

Table 2. Treatment emergent adverse events related to study drug in >5% of patients

	Weekly dose escalation cohort				Every other week dose escalation cohort			Expansion cohort	Loading	Total
Dose = mg/kg (*n*)	0.5 (3)	1.0 (3)	2.5 (6)	5.0 (3)	2.5 (6)	5.0 (6)	10 (6)	10 (21)	10 (1)	(55)
Hypertension	1 (33)	1 (33)	1 (17)		1 (17)	2 (33)	2 (33)	10 (48)	1 (100)	19 (35)
Fatigue	1 (33)	1 (33)	1 (17)			1 (17)	4 (67)	9 (43)		17 (31)
Anemia						2 (33)		9 (43)	1 (100)	12 (22)
Diarrhea			2 (33)		2 (33)	2 (33)	1 (17)	4 (19)		12 (22)
Headache					3 (50)	2 (33)	3 (50)	3 (14)		11 (20)
Nausea	1 (33)	1 (33)	1 (16)		1 (16)		2 (33)	1 (5)		7 (13)
BP increased			1 (17)		4 (67)			1 (5)		6 (11)
Hypoalbuminemia								6 (29)		6 (11)
Dyspnea					1 (17)		1 (17)	4 (19)		6 (11)
Hyponatremia							1 (17)	4(19)		5 (9)
Dizziness						1 (17)		4 (19)		5 (9)
Anorexia					1 (17)	1 (17)		3 (14)		5 (9)
LV[1] dysfunction								4 (19)	1 (100)	5 (9)
Dyspnea		1 (33)						4 (19)		5 (9)
Edema peripheral		1 (33)	1 (17)					2 (10)		4 (7)
ALT increased	1 (33)							3 (14)		4 (7)
AST increased	1 (33)							3 (14)		4 (7)
Creat[2] increased								4 (19)		4 (7)
CHF								4 (19)		4 (7)
Chills	1 (33)							3 (14)		4 (7)

NOTE: 1. LV, left ventricular. 2. Creat, Creatinine.

ventricular failure on study day 63. Four of the 5 patients with heart failure developed dyspnea and edema and the fifth patient only had dyspnea. In all 5 patients, treatment was discontinued and the symptoms responded to medical management. BNP levels were elevated from normal at baseline to >200 pg/mL in all 5 patients. In the patient with right heart failure, the estimated left ventricular ejection fraction (LVEF) was preserved (60% at baseline vs. 65% on day 35), whereas the other 4 patients had significant decreases in estimated LVEF. Right ventricular function was also noted to be impaired in two of these patients with pulmonary hypertension in one. An additional asymptomatic patient treated at the 10 mg/kg once every other week dose level developed moderate pulmonary hypertension without a rise in BNP.

Pharmacokinetics

Noncompartmental analysis (NCA) was initially conducted on data from each individual subject ($n = 55$) receiving at least one dose of demcizumab with adequate samples for analysis. Drug accumulation was observed in all dose cohorts. The accumulation ratios in the QW dosing cohorts ranged from 2.12 to 2.83 and from 1.50 to 1.65 in the Q2W dosing cohorts. NCA results are summarized in Supplementary Table S2. Because of the long half-life, short dosing intervals, and the large degree of extrapolation required in the NCA, estimation of PK parameters such as clearance and half-life was deemed unreliable with NCA. Compartmental analysis was subsequently conducted on the composite of the group mean data from dose groups 1, 2.5, and 5 mg/kg QW and 2.5, 5, and 10 mg/kg Q2W. A linear two compartment model was adopted to estimate the pharmacokinetic characteristics of the composite data. Parameter estimation was conducted using PK/PD modeling software ADAPT V with model fits in Fig. 1 and results summarized in Supplementary Table S3 for the every other week dosing. Demcizumab pharmacokinetics were linear at

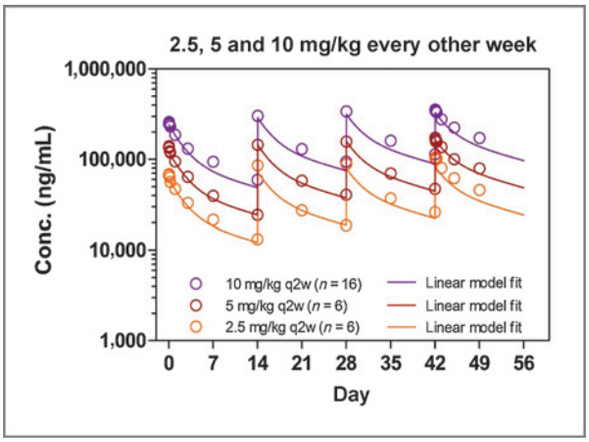

Figure 1. Group mean concentration–time profiles and the linear 2-compartment model fit. Symbols are the observed concentration–time data, by cohort, averaged over nominal time; solid lines are the model predictions by fitting to all the data.

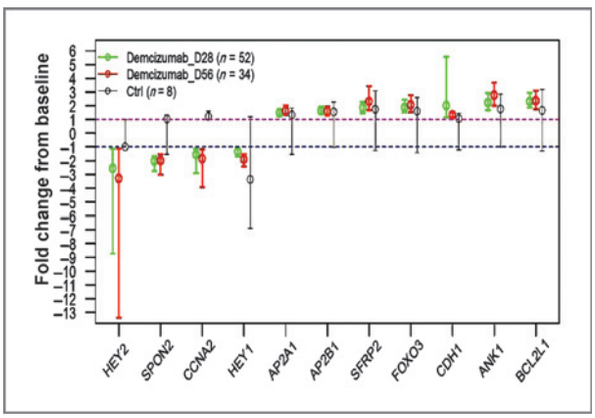

Figure 2. Effect of demcizumab on gene expression in whole blood. Demcizumab significantly affected gene expression changes of Notch pathway–related markers in blood cells consistent with suppression of the Notch pathway. RNAs were isolated from whole blood, and gene expression profiles were assayed. The fold change represents the gene expression ratio comparing posttreatment with pretreatment (day 0) samples. Posttreatment time points were day 28 (green bars, $n = 52$) and day 56 (red bars, $n = 34$). Genes were significant at a 95% CI and a gene expression change of greater than 1.5-fold. Gray bars indicate fold change in control whole blood samples that were analyzed from 8 sourced cancer patients which were not treated with demcizumab. As depicted, the blood biomarkers were not significantly regulated in control samples as shown.

doses of 1.0 mg/kg once weekly and 2.5 mg/kg once every 2 weeks and above, corresponding to concentrations above approximately 10 μg/mL. Within the linear range, clearance was estimated to be 4.17 mL/day/kg, and half-life was estimated to be 15.9 days. Model fits for the weekly dosing cohorts are in Supplementary Fig. S1. Clearance was slightly faster in the 3 patients in the 0.5 mg/kg weekly cohort, suggesting target-mediated clearance at this dose level which is a typical phenomenon for antibody therapeutics.

Immunogenicity

Six of 55 (11%) patients were confirmed to be positive for ADAs in a confirmatory immunodepletion assay. ADA formation was generally late emerging, occurring after treatment termination in 5 of the 6 patients. Formation of ADA did not appear to impact drug exposure, safety, or biologic activity.

Biomarkers

Whole blood samples ($n = 52$ patients), plasma samples ($n = 50$ patients), and hair follicles ($n = 28$ patients) were analyzed. In blood cells, demcizumab downregulated Notch and Wnt pathway genes (Fig. 2). In contrast, genes involved in the negative regulation of the Notch and the Wnt pathways were upregulated by treatment. These changes are consistent with suppression of the Notch pathway. In addition, angiogenesis and vasculature genes were upregulated in blood cells. Similarly, in hair follicles, stem cell–related genes were downregulated, whereas vascular/endothelial genes were upregulated by demcizumab (Supplementary Fig. S2). Pharmacodynamic changes were observed starting at 2.5 mg/kg every other week (Fig. 3). Interestingly, the differentiation marker KRT19 was also upregulated by treatment with demcizumab. Plasma vascular and endothelial markers were observed to be elevated by demcizumab (Supplementary Fig. S3).

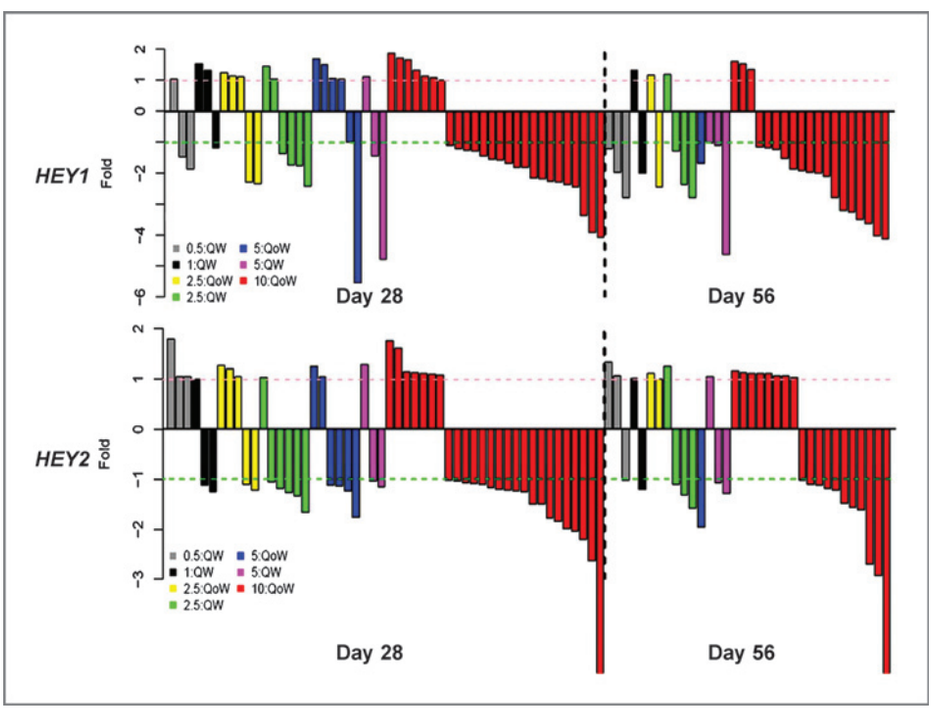

Figure 3. Dose effect of demcizumab on HEY1 and HEY2 genes. Demcizumab downregulated Notch target genes HEY1 and HEY2 in blood cells. The fold change represents the gene expression ratio comparing posttreatment with pretreatment (day 0) samples. Posttreatment time points were day 28 (left) and day 56 (right). Colors indicate the different dosing groups: gray = 0.5 mg/kg QW; black = 1 mg/kg; yellow = 2.5 mg/kg QoW; green = 2.5 mg/kg QW; blue = 5.0 mg/kg QoW; pink = 5 mg/kg QW; red = 10 mg/kg QoW.

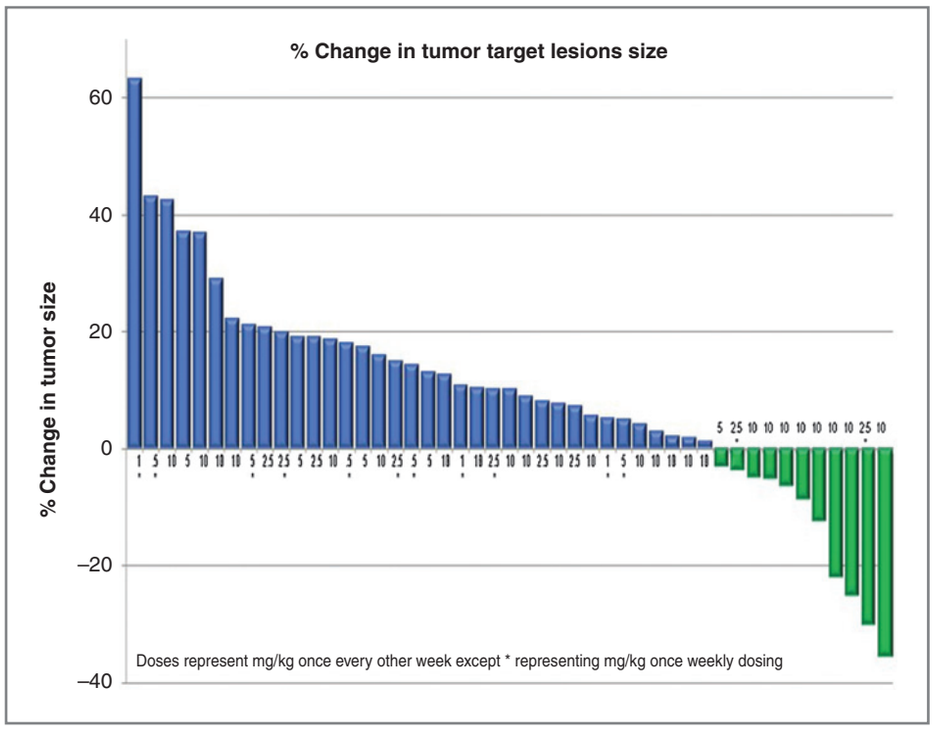

Figure 4. Waterfall plot of individual patient best percent change in target lesions size.

Efficacy

Forty-eight patients were evaluable for response by RECIST criteria, with one unconfirmed partial response in a patient with pancreatic cancer treated at 10 mg/kg once every other week. Twenty-one patients had stable disease as their best response with reductions in target lesion size in patients with non–small cell lung cancer, renal cell carcinoma, colorectal cancer, and additional patients with pancreatic cancer. The overall disease control rate (PR+stable) was 40% (22/55). Figure 4 is a waterfall plot of the percent change in target lesion size by patient. Stabilization of disease allowed subjects to remain on treatment for prolonged periods (Supplementary Fig. S4). The majority of evaluable patients (16/25; 64%) treated at 10 mg/kg once every other week had stable disease (Supplementary Table S4). A patient with ovarian granulosa cell carcinoma who received 10 mg/kg once every other week remained on therapy for 518 days.

Discussion

Demcizumab is a first-in-class anti-DLL4 antibody, which interrupts Notch pathway activation. The MTD was not reached on either the weekly or every other week schedule on this study. Patients treated at 10 mg/kg had higher rates of stable disease which was prolonged in a number of patients including one patient with ovarian cancer treated for 17 months. Four of the 10 patients (40%) treated for at least 98 days at the highest dose level developed congestive heart failure between days 107 and 112. With the emergence of this toxicity, the study was halted after the enrollment of the first patient in the loading dose phase. The recommended phase II dose is 5 mg/kg every other week or less based on the safety data from cohorts treated at this dose.

Therapy was generally well tolerated, with fatigue and hypertension the most common adverse events. Hypertension developed at all dose levels, but was readily manageable with oral antihypertensives. Tumor-associated bleeding was seen in two patients with gastrointestinal tract tumors. Hypertension, bleeding, and the development of cardiac dysfunction are consistent with the toxicities associated with antiangiogenic agents, and in the case of demcizumab, likely result from dysfunctional angiogenesis which has been associated with inhibition of DLL4. Similar rates of cardiac dysfunction were reported with another anti-DLL4 antibody, suggesting that this is likely a class effect (19). The patients who developed cardiac failure responded to medical treatment and had at least partial reversal of their heart failure (although 1 patient had a persistently elevated right ventricular systolic pressure and dyspnea) over the 2 to 3 months that they were followed after discontinuation of demcizumab. Echocardiogram findings indicate that the cardiac dysfunction may involve both ventricles and in some cases is associated with pulmonary hypertension. BNP levels appear to be an indicator of cardiotoxicity with significant increases occurring before the development of heart failure. Given the long half-life of the drug, longer follow-up is necessary to fully evaluate whether the heart failure will completely reverse following dug discontinuation.

Reduction in tumor size was seen in patients with a variety of solid tumors and there was one unconfirmed

partial response in a patient with pancreatic cancer. Preclinical models suggest that the mechanism of the antitumor effects of demcizumab may be due to a combination of the inhibition of the stem cell population and dysangiogenic effects (12). The clinical observations from this study are consistent with similar effects in humans. Prolonged periods of disease stabilization might occur with an agent that inhibits cancer stem cells as the balance between cell death and renewal is altered. Biomarker data from hair follicles, plasma, and whole blood confirm that demcizumab alters Notch pathway gene expression and enhances expression of genes associated with angiogenesis. The pharmacokinetic parameters of demcizumab are typical for those associated with an IgG2 antibody.

In summary, this phase I trial of demcizumab demonstrated a tolerable short-term safety profile with hypertension and fatigue as common adverse events. Pharmacokinetics were typical of a humanized antibody, and pharmacodynamic assays show alterations in gene expression consistent with interruption of the Notch signaling pathway and the induction of dysangiogenesis. Disease stabilization and, in some cases, decreases in tumor size suggest antitumor activity and resulted in prolonged drug administration. This prolonged exposure allowed the emergence of delayed cardiac toxicity. Tumor-associated bleeding consistent with dysfunctional angiogenesis also occurred. It is not clear whether these toxicities are due to cumulative dose or duration of exposure; thus, we recommend a dose of 5 m/kg or less for further evaluation. Further evaluation of demcizumab will require the optimization of dosing and strategies to mitigate toxicities. These strategies will likely include the exclusion of patients with significant risk of cardiac toxicity and gastrointestinal (GI) luminal tumors, modified dosing regimens, use of BNP and echocardiographic monitoring, and the use of cardioprotective agents such as acetylcholinesterase (ACE) inhibitors or carvedilol. The indications of antitumor activity and target modulation warrant further investigation of this agent, and phase Ib studies in combination with chemotherapy are ongoing.

Disclosure of Potential Conflicts of Interest

D.C. Smith reports receiving commercial research grants from MedImmune and OncoMed. R.J. Stagg, L. Xu, and J. Dupont are employees of and hold ownership interest (including patents) in OncoMed Pharmaceuticals Inc. B. Sikic reports receiving a commercial research grant from OncoMed Pharmaceuticals Inc. No potential conflicts of interest were disclosed by the other authors.

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