A Phase I, First-in-Human Study of AMG 780, an Angiopoietin-1 and -2 Inhibitor, in Patients with Advanced Solid Tumors

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Abstract

Purpose: To assess the toxicity, pharmacokinetics, tumor vascular response, tumor response, and pharmacodynamics of AMG 780, a mAb designed to inhibit the interaction between angiopoietin-1 and -2 and the Tie2 receptor.

Experimental Design: This was a phase I dose-escalation study of patients with advanced solid tumors refractory to standard treatment without previous antiangiogenic treatment. AMG 780 was administered by intravenous infusion every 2 weeks in doses from 0.1 to 30 mg/kg. The primary endpoints were incidences of dose-limiting toxicity (DLT) and adverse events (AE), and pharmacokinetics. Secondary endpoints included tumor response, changes in tumor volume and vascularity, and anti-AMG 780 antibody formation.

Results: Forty-five patients were enrolled across nine dose cohorts. Three patients had DLTs (0.6, 10, and 30 mg/kg), none of which prevented dose escalation. At 30 mg/kg, no MTD was reached. Pharmacokinetics of AMG 780 were dose proportional; median terminal elimination half-life was 8 to 13 days. No anti-AMG 780 antibodies were detected. At week 5, 6 of 16 evaluable patients had a >20% decrease in volume transfer constant (Ktrans), suggesting reduced capillary blood flow/permeability. The most frequent AEs were hypoalbuminemia (33%), peripheral edema (29%), decreased appetite (27%), and fatigue (27%). Among 35 evaluable patients, none had an objective response; 8 achieved stable disease.

Conclusions: AMG 780 could be administered at doses up to 30 mg/kg every 2 weeks in patients with advanced solid tumors. AMG 780 treatment resulted in tumor vascular effects in some patients. AEs were in line with toxicity associated with antiangiopoietin treatment. Clin Cancer Res; 22(18); 4574–84. ©2016 AACR.

Introduction

Angiogenesis, the process of new blood vessel formation, is required for tumor growth and metastatic spread and is regulated by a number of different pathways (1). VEGF pathway family members play a critical role in angiogenesis by stimulating proliferation, migration, and survival of endothelial cells (2). The angiopoietin axis is a distinct angiogenic pathway that activates complementary angiogenic processes through binding of the ligands angiopoietin-1 and -2 (Ang1 and Ang2) to the tyrosine kinase receptor Tie2 (1). Ang1 is primarily involved in the maturation and stabilization of blood vessels, whereas Ang2 contributes to vascular remodeling and new vessel sprouting (3–5). Many tumor cells overexpress Ang1, and transcription of Ang2 is significantly upregulated in tumor-associated endothelium (3). Studies have also identified increased levels of Ang2 in the tumor vasculature across various types of tumors; these elevations have been associated with disease progression and poor prognosis (6–9).

Inhibition of angiogenesis has been shown to improve outcomes in clinical studies. A number of VEGF pathway inhibitors have been shown to be effective in the treatment of a variety of solid tumor types (10–15). Inhibition of the angiopoietin pathway has been shown to result in tumor growth inhibition in preclinical models, with simultaneous inhibition of Ang1 and Ang2 resulting in greater tumor growth suppression compared with targeting either ligand in isolation (16, 17). In early-phase clinical studies, treatment with trebananib (AMG 386), a peptide that inhibits the interaction between Ang1 and Ang2 and the Tie2 receptor (18), showed antitumor activity, reducing tumor size and inducing objective responses in some patients (19–23).

In a randomized, double-blind, phase III clinical study, trebananib plus weekly paclitaxel prolonged progression-free survival of patients with recurrent ovarian cancer compared with placebo plus paclitaxel [7.2 months vs. 5.4 months; HR, 0.66; 95% confidence interval (CI), 0.57–0.77; P < 0.0001; ref. 24]. Adverse events (AE) occurring with greater frequency in the trebananib group included edema, ascites, pleural effusion, and blurred vision (24).

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

Prior presentation: The results were presented in part as a poster at the American Society of Clinical Oncology 50th Annual Meeting, Chicago, IL, May 30 to June 3, 2014.

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doi: 10.1158/1078-0432.CCR-15-2145

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AMG 780 in Patients with Advanced Solid Tumors

Translational Relevance

Inhibition of angiogenesis can slow tumor growth and improve outcomes for patients with metastatic disease. The angiopoietin axis plays an important role in angiogenesis and is distinct from currently approved angiogenesis inhibitors which target the VEGF pathway. Several investigational drugs that target the angiopoietin axis are currently in clinical trials. AMG 780 is a mAb that binds to angiopoietin-1 and -2 (Ang1 and Ang2), preventing their binding to the Tie2 receptor to suppress angiogenesis. In this phase 1 first-in-human study in patients with advanced cancer, AMG 780 was administered once every 2 weeks at doses up to 30 mg/kg, with median terminal elimination half-life ranging from 8 to 13 days and evidence of reduced capillary blood flow. No MTD was reached. Dosing once every 2 weeks supports both patient convenience and combination with other anticancer regimens.

AMG 780 is an investigational, intravenously administered fully human monoclonal immunoglobulin G2 antibody that, like trebananib, binds to Ang1 and Ang2, thereby preventing their interaction with the Tie2 receptor. In mice bearing Colo205 human colorectal cancer xenografts, twice weekly administration of AMG 780 significantly inhibited tumor growth (66%), viable tumor fraction (81%), and tumor endothelial cell proliferation (84%) compared with a control antibody (25). The inhibitory activities of AMG 780 [IC50 (nmol/L): Ang1, 4.5; Ang2, 0.06] were similar to those of trebananib [IC50 (nmol/L): Ang1, 3.5; Ang2, 0.03; ref. 25]. Preclinical studies indicate that AMG 780 and trebananib have similar inhibitory activities and toxicity profiles and that AMG 780, a mAb, will have a longer half-life than trebananib, a peptibody, potentially providing longer target inhibition. This report presents results from a first-in-human phase I dose-escalation study that assessed the toxicity, pharmacokinetics, tumor vascular response, tumor response, and pharmacodynamics of AMG 780 in patients with advanced solid tumors (Clinical Trial registration ID: NCT01375552).

Materials and Methods

Patients

Eligible patients were ≥18 years of age and had pathologically confirmed advanced solid tumors refractory to standard treatment, or for which standard treatment was not available or had been refused; measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (26); and Eastern Cooperative Oncology Group performance status ≤2. Patients were excluded if they had untreated or symptomatic primary central nervous system tumors or metastases; head and neck cancer; history of hematopoietic stem cell transplant; bleeding diathesis or adrenal hemorrhage; stroke, arterial/venous thrombosis, pulmonary embolism, myocardial infarction, or unstable/uncontrolled cardiac disease; pulmonary hemorrhage or gross hemoptysis within 6 months; history of peripheral vascular disease; prior treatment with another agent targeting the angiopoietin-Tie2 pathway; concomitant therapeutic dose anticoagulation therapy (prophylactic therapy was allowed); or inadequate hematologic, hepatic, or renal function. Patients with unexplained clinical signs and symptoms suspicious for adrenal insufficiency (including but not limited to hyperkalemia, hyponatremia, and hypotension) had a cosyntropin test performed prior to enrollment and were ineligible if their serum cortisol was ≤18 μg/dL 30 to 60 minutes after stimulation. Patients provided written informed consent, and study procedures were approved by an institutional review board at each site.

Study design and treatment

Patients received AMG 780 by intravenous infusion once every 2 weeks beginning on the first day of treatment in week 1. At week 9, patients did not receive AMG 780; radiologic assessment was done and, if disease progression had not occurred, biweekly treatment recommenced at week 10. Treatment with AMG 780 was discontinued for disease progression per RECIST version 1.1 (26), clinical progression, or intolerance. To determine the MTD, AMG 780 was administered to patients enrolled sequentially into ascending dose cohorts 1 to 9 (0.1, 0.3, 0.6, 1.2, 2.5, 5, 10, 20, and 30 mg/kg, respectively). The starting dose was estimated to provide a twofold exposure margin over the concentration required for 50% tumor growth inhibition in a mouse Colo205 xenograft model (26). Initially, 3 patients were enrolled in cohort 1. If none of the patients had a dose-limiting toxicity (DLT) during the first 28 days of treatment, then 3 patients were enrolled in the next dose cohort. If one of the initial 3 patients had a DLT, the cohort was expanded to 6 patients. If no additional DLTs occurred in those 6 patients, then 3 patients were enrolled in cohort 2. If 2 or more of the initial 3 patients in cohort 1 experienced a DLT, no additional patients were enrolled. Patients who dropped out during the first 28 days of treatment for reasons other than DLT could be replaced. Dose escalation continued until all planned cohorts were enrolled or an MTD (defined as the highest dose level with a DLT patient incidence rate of <33% in a minimum of 6 patients treated at that dose level) was determined. If the criteria for MTD were not met for any of the cohorts, no MTD was reached. DLT was defined as any treatment-related grade ≥3 AE occurring in the first 28 days of treatment. To be considered a DLT, grade 3 infusion reactions had to last >2 hours, grade 3 fatigue had to last >7 days, and grade 3 proteinuria had to last >2 weeks.

Endpoints and analysis

The primary endpoints were toxicity, as indicated by patient incidence of DLTs and AEs, and pharmacokinetic parameters of AMG 780. Secondary endpoints were tumor response per RECIST version 1.1 (26), changes in tumor volume, anti-AMG 780 antibody formation, and change in tumor vascularity from baseline to week 5. An exploratory endpoint was pharmacodynamic changes in the levels of angiogenesis-related biomarkers across dose and time. All patients who received more than one dose of AMG 780 and had available radiologic imaging at baseline and at least once postbaseline were included in tumor response analysis.

Adverse events

All AEs occurring from study day 1 until 4 weeks after the last dose of AMG 780 (end of study) were recorded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 by the investigators. In preclinical studies, histologic changes were noted in the liver, pancreas, and adrenal cortex of rats receiving AMG 780 (Amgen Inc., data on file). Therefore, in addition to routine safety
laboratory assessment, which included alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, for evaluation of liver function, we also evaluated pancreatic function by monitoring amylase and lipase, and adrenal function by measuring both baseline and cosyntropin-stimulated cortisol concentrations. Cosyntropin testing was conducted with measurement of baseline and stimulated cortisol levels (serum cortisol before and 30 to 60 minutes after an intravenous injection of 0.25 mg of cosyntropin) at screening, predose at week 5, at weeks 9 and 18, and every 8 weeks thereafter. A Jonckheere-Terpstra test (27) was used to evaluate associations between AMG 780 dose and amylase/lipase levels.

**Pharmacokinetics**

Serum samples for evaluation of AMG 780 pharmacokinetics were collected immediately before and after infusion of AMG 780 at weeks 1, 3, 5, 7, 10, every 2 weeks beginning at week 12, and every 8 weeks after week 18. Additional samples were collected at week 1 at 0.5, 8, 24, 72, 168, and 240 hours postdose, at week 5 at 48 and 168 hours postdose, and at week 7 at 8, 24, 72, 168, and 336 hours postdose. Pharmacokinetic analyses were conducted using noncompartmental methods with Phoenix WinNonlin version 6.3 software on Citrix (Pharsight).

**Immunogenicity**

Antidrug antibodies were detected using a modified electrochemiluminescent bridging immunoassay as previously described for trebananib (28). Serum samples were collected for evaluation of anti-AMG 780 antibodies at week 1 (predose); weeks 5, 9, and 10; every 8 weeks thereafter; and at the end-of-study visit. Samples were first analyzed for anti-AMG 780 antibodies using a validated immunoassay. Positive samples were subsequently tested with a validated receptor-binding assay to determine whether these antibodies neutralized the activity of AMG 780.

**Tumor vascular response**

To evaluate changes in tumor perfusion and vessel permeability, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was performed at baseline and postdose at week 5 using either ProHance or MultiHance as contrast agents (both from Bracco Imaging). DCE-MRI was analyzed by an independent core laboratory that evaluated percentage change from baseline in the volume transfer constant ($K_{trans}$) and blood-normalized area under the enhancement curve (IAUC; ref. 29).

**Tumor response**

Contrast-enhanced computed tomography or MRI was performed at baseline, weeks 9 and 18, and every 8 weeks thereafter. The same imaging modality used for baseline studies was employed for subsequent scans. Tumor response was evaluated by investigators per RECIST version 1.1 (26). Radiologic imaging was also submitted to an independent imaging core laboratory for assessment of tumor volume.

**Pharmacodynamics**

Serum samples for evaluation of circulating angiogenic cytokines were collected at day 1 (predose), and days 4, 11, 15 (predose), 29 (predose), 31, 43 (predose), 46, 57, every 8 weeks thereafter, and at the end-of-study visit. Circulating angiogenic cytokines assessed included soluble VEGF receptor 2 (sVEGFR2), placental growth factor (PLGF), VEGF, Soluble Kit, soluble VEGF receptor 1 (sVEGFR1), soluble vascular cell adhesion molecule-1 (sVCAM-1), Ang1, Ang2, and soluble Tie2. A detailed description of these biomarker assays was previously reported (30).

**Results**

**Patients**

Between August 2010 and December 2013, 45 patients were enrolled across each of the nine dose cohorts (0.1–30 mg/kg; Supplementary Fig. S1). All patients received at least one dose of AMG 780 except for one patient in the 10 mg/kg dose cohort who withdrew consent before receiving AMG 780 and was not included in the analyses. The median (range) number of doses of AMG 780 administered was 4 (1–20). At the time of analysis, all patients had discontinued AMG 780 because of disease progression ($n = 35$), AEs ($n = 5$), death ($n = 1$), withdrawal of consent ($n = 2$), and other reasons ($n = 2$). Patient characteristics are described in Table 1. The median (range) age was 63 (45–76) years and 57% of patients were female. Patients who received AMG 780 most frequently had cancer of the large intestine (27%), non–small cell lung cancer (NSCLC) (21%), ovarian cancer (11%), or mesothelioma (9%).

**Dose escalation and toxicity**

Three patients had DLTs. One patient in the 0.6 mg/kg dose cohort had treatment-related grade 3 thrombocytopenia beginning
on day 18 that resulted in discontinuation of AMG 780. One patient in the 10 mg/kg dose cohort had treatment-related grade 2 proteinuria beginning on day 2 that worsened to a DLT of grade 3 proteinuria on day 15, and resulted in discontinuation of AMG 780. Finally, one patient in the 30 mg/kg dose cohort with NSCLC who entered the study with a mild pericardial effusion was hospitalized for dyspnea on day 4 (post one dose of AMG 780) and experienced worsening of the pericardial effusion to a grade 3 serious AE. In addition, the patient had an obstructing lesion in the left upper lung lobe and a possible pneumothorax. The pericardial effusion continued to worsen over the next several days and was treated with placement of a pericardial window and drainage. The investigator reported there was a reasonable possibility the worsening effusion was related to AMG 780. The patient discontinued AMG 780 because of disease progression. None of these DLTs prevented dose escalation and no other DLTs occurred in any of the other cohorts; therefore, an MTD was not reached in this study.

All patients who received AMG 780 had at least one treatment-emergent AE. The most frequently occurring treatment-emergent AEs were hypoalbuminemia, peripheral edema, decreased appetite, and fatigue (Table 2). Twenty-seven patients (61%) had grade ≥3 treatment-emergent AEs; there did not appear to be an association between incidence of grade ≥3 events and dose. Two patients had grade 4 AEs (esophageal ulcer and sepsis); both occurred in the 10 mg/kg cohort and neither was considered related to administration of AMG 780 by the investigators. The patient with an esophageal ulcer had a history of hepatobiliary disease; therefore, neither met the criteria for Hy's law. There was no trend for increase or decrease in amylase or lipase levels with increasing dose of AMG 780 per Jonckheere-Terpstra test performed at each patient visit. One patient in the 5 mg/kg cohort had an elevated postbaseline serum amylase level >150 IU/L that resolved by the end-of-study visit. One patient in the 30 mg/kg cohort had serum lipase >800 IU/L that resulted in discontinuation of AMG 780 but was not considered related to treatment by investigators. No significant decreases in serum cortisol levels, either before or after cosyntropin stimulation, were observed at any of the doses or time points tested.

Pharmacokinetics

AMG 780 exhibited linear pharmacokinetics after single-dose administrations over the dose range of 0.1 to 30 mg/kg (Fig. 1). Exposure [as measured by mean maximal plasma concentration (Cmax) and area under the concentration–time curve during the dosing interval (tau; AUCtau), with tau equal to 336 hours] increased in a dose-proportional manner up to a dose of 30 mg/kg (Table 3). Median time to Cmax (i.e., tmax) was equal to 1 hour across all doses. The mean volume of distribution at steady state (Vss) at all dose levels was 31.4 mL/kg. For doses from 0.6 to 30 mg/kg, the estimated mean serum clearance after intravenous infusion was 0.16 mL/h/kg. Steady state was reached after three doses of AMG 780 (Fig. 1), with a mean accumulation ratio of 1.6. At doses of 2.5 mg/kg and above, the minimum terminal elimination half-life (t1/2,z) was between 8 and 13 days, and minimum plasma concentration (Cmin) exceeded the 50% (1.2 μg/mL) and 90% (10.4 μg/mL) inhibitory concentrations obtained in a mouse Colo205 xenograft model (Amgen Inc., data on file).

Tumor vascular response

Baseline DCE-MRI was conducted in 41 consenting patients (3 patients did not have DCE-MRI per their decision). Among these 41 patients, 22 had baseline and at least one postbaseline scan after AMG 780 was administered whereas the remainder lacked a postbaseline scan because they were off-study at week 5 (n = 14), their lesions were too small at baseline (n = 3), or they lacked baseline scans (n = 2). Sixteen of these 22 patients were considered evaluable in that they had interpretable data from baseline as well as from both the predose and the postdose week 5 scans. In 2 of these 16 patients, two evaluable lesions were present. Data were considered not interpretable (evaluable) in 6 patients because either week 5 predose and/or postdose scans were not
Table 2. Patient incidence of treatment-emergent AEs irrespective of relationship to treatment

<table>
<thead>
<tr>
<th>AMG 780 dose cohort</th>
<th>All patients (N = 44)</th>
<th>AMG 780 0.1 mg/kg (n = 3)</th>
<th>AMG 780 0.3 mg/kg (n = 3)</th>
<th>AMG 780 0.6 mg/kg (n = 6)</th>
<th>AMG 780 1.2 mg/kg (n = 6)</th>
<th>AMG 780 2.5 mg/kg (n = 9)</th>
<th>AMG 780 5 mg/kg (n = 3)</th>
<th>AMG 780 10 mg/kg (n = 9)</th>
<th>AMG 780 20 mg/kg (n = 3)</th>
<th>AMG 780 30 mg/kg (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AE, n (%)</td>
<td>44 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>6 (100)</td>
<td>3 (100)</td>
<td>9 (100)</td>
<td>3 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>22 (50)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>3 (50)</td>
<td>1 (33)</td>
<td>3 (50)</td>
<td>2 (67)</td>
<td>4 (44)</td>
<td>2 (67)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Grade 4*</td>
<td>2 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (22)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grade 5</td>
<td>3 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
<td>1 (13)</td>
</tr>
</tbody>
</table>

AEs occurring in >10% of patients, n (%)

Hypoalbuminemia
- Grade 3: 15 (34) 1 (33) 1 (33) 3 (50) 2 (67) 3 (50) 1 (33) 3 (33) 0 1 (13)
- Grade 5: 3 (7) 0 0 0 0 1 (17) 0 1 (11) 0 1 (13)

Peripheral edema
- Grade 3: 13 (30) 1 (33) 1 (33) 3 (50) 1 (33) 2 (33) 1 (33) 3 (33) 0 1 (13)

Decreased appetite
- Grade 3: 12 (27) 1 (33) 0 0 2 (67) 2 (33) 1 (33) 4 (44) 1 (33) 1 (13)

Fatigue
- Grade 3: 12 (27) 1 (33) 1 (33) 3 (50) 0 1 (17) 1 (33) 4 (44) 0 1 (13)

Hypotension
- Grade 3: 9 (20) 1 (33) 0 0 2 (33) 1 (33) 0 2 (22) 0 1 (13)

Lymphopenia
- Grade 3: 9 (21) 2 (67) 1 (33) 1 (17) 1 (33) 1 (17) 0 2 (22) 0 1 (13)

Nausea
- Grade 3: 1 (2) 0 0 0 0 1 (17) 0 2 (22) 0 1 (13)

Cough
- Grade 3: 8 (18) 2 (67) 0 0 0 0 0 2 (22) 0 1 (13)

Dyspnea
- Grade 3: 8 (18) 1 (33) 0 0 1 (33) 1 (33) 0 2 (22) 0 1 (13)

Headache
- Grade 3: 1 (2) 0 0 0 0 1 (17) 0 0 0 0

Abdominal pain
- Grade 3: 7 (16) 0 1 (33) 0 2 (67) 1 (17) 0 4 (44) 0 1 (13)

Constipation
- Grade 3: 7 (16) 0 1 (33) 0 1 (33) 1 (33) 0 2 (22) 0 1 (13)

Anemia
- Grade 3: 6 (14) 1 (33) 0 0 0 0 1 (17) 1 (33) 0 1 (13)

Blood alkaline phosphatase increased
- Grade 3: 6 (14) 0 0 1 (17) 0 2 (33) 1 (33) 0 1 (11) 0 1 (13)

Dizziness
- Grade 3: 6 (14) 0 0 1 (17) 1 (33) 0 2 (22) 0 1 (13)

Hypokalemia
- Grade 3: 6 (14) 1 (33) 0 0 1 (33) 1 (33) 0 2 (22) 0 1 (13)

Hypoglycemia
- Grade 3: 6 (14) 0 0 0 0 0 0 0 0 0

Myalgia
- Grade 3: 5 (11) 0 0 0 0 1 (17) 0 1 (17) 0 2 (22) 0 1 (13)


Clinical Cancer Research

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Published OnlineFirst April 13, 2016; DOI: 10.1158/1078-0432.CCR-15-2145

*Two patients had grade 4 AEs (esophageal ulcer and sepsis).

*There were three fatal AEs (suicide, malignant lung neoplasm, and dyspnea). None were considered to be related to treatment.
evaluable \((n = 4)\) or baseline scans were inadequate \((n = 2)\). At week 5, 6 patients had a >20% decrease in \(K_{\text{trans}}\) (a measure of capillary blood flow), and 5 patients had IAUC decreased by >20% relative to baseline (Fig. 2). Four patients had a >20% decrease in both \(K_{\text{trans}}\) and IAUC. There were no effects on tumor vascularity among the other patients.

**Tumor response**

Thirty-five patients were evaluable for tumor response. No patients achieved a complete or partial response. Eight patients had a best response of stable disease (one each receiving AMG 780 0.1, 0.3, 0.6, 2.5, 20, and 30 mg/kg, and 2 receiving AMG 780 10 mg/kg). Stable disease lasted >6 months for the patient in the 0.6 mg/kg cohort (a patient with NSCLC); however, there was no reduction in tumor volume. The remaining 27 patients had progressive disease. Tumor size decreased relative to baseline in 4 patients (all had stable disease as best response). Best response reductions in tumor size were 3.8% in a patient with the large intestine (2.5 mg/kg cohort; evaluated as having stable disease that lasted 33 weeks), 4.5% in a patient with ovarian cancer (2.5 mg/kg cohort; progressive disease at initial scan), 7.4% in a patient with NSCLC (0.6 mg/kg cohort; stable disease lasting 3 weeks), and 25% in a patient with endometrial cancer (30 mg/kg cohort; stable disease lasting 8 weeks). Across all patients, tumor size changes relative to baseline ranged from −25% to 135%.

**Anti-AMG 780 antibodies**

For anti-AMG 780 antibody analyses, serum samples were available for all 44 patients at baseline and for 33 patients postbaseline. No patients developed binding or neutralizing anti-AMG 780 antibodies during the study. One patient had preexisting neutralizing, anti-AMG 780 binding antibodies at baseline, but did not test positive for anti-AMG 780 antibodies at any other point during the study. The presence of these neutralizing antibodies did not affect AMG 780 concentrations.

**Pharmacodynamics (biomarkers)**

Dose-proportional changes in biomarkers were observed for sVCAM-1 and PLGF during AMG 780 treatment (Fig. 3A and B). Maximum increases occurred at treatment day 4 for PLGF and treatment day 11 for sVCAM-1. There was also a small decrease

**Table 3.** AMG 780 pharmacokinetic parameters

| Dose (mg/kg) | \(C_{\text{max}}\) (µg/mL) | AUC\(_{\text{tau}}\) (mg ⋅ h/mL) | \(t_{1/2,\omega}\) (hours) | Week 1 | Mean | CV (%) | Mean | CV (%) | Mean | CV (%) | Mean | CV (%) | Mean | CV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | 3 | 2.46 | 22.2 | 2.70 | 50.4 | 55.9 | 58.0 | 2 | 2.38 | 20.2 | 260 | 77.0 | 0.547 | 77.0 | 36.8 | 24.9 | 0.183 | 141.4 |
| 0.3 | 3 | 8.11 | 33.0 | 6.94 | 51.9 | 55.5 | 48.5 | 2 | 6.85 | 26.2 | 557 | 55.7 | 0.638 | 55.7 | 29.2 | 9.2 | 0.0828 | – |
| 0.6 | 6 | 16.0 | 15.1 | 1,860 | 35.5 | 79.7 | 47.1 | 4 | 19.8 | 10.3 | 2,570 | 33.3 | 0.255 | 35.6 | 32.2 | 12.0 | 2.45 | 73.7 |
| 1.2 | 3 | 40.8 | 37.1 | 6,310 | 42.6 | 147 | 16.3 | 3 | 55.6 | 27.5 | 7,730 | 20.6 | 0.159 | 18.2 | 26.3 | 42.8 | 11.4 | 3.7 |
| 2.5 | 4 | 55.9 | 26.7 | 8,600 | 29.5 | 198 | 31.0 | 3 | 98.0 | 18.4 | 15,700 | 17.7 | 0.161 | 18.3 | 31.6 | 17.5 | 28.1 | 13.5 |
| 5 | 3 | 121 | 15.9 | 19,500 | 6.3 | 240 | 62.8 | 1 | 202 | – | 36,300 | – | 0.135 | – | 32.8 | – | 76.5 | – |
| 10 | 9 | 265 | 29.5 | 35,900 | 41.3 | 179 | 23.7 | 2 | 394 | 6.1 | 56,600 | 32.0 | 0.186 | 32.3 | 34.3 | 37.0 | 75.0 | – |
| 20 | 3 | 431 | 14.6 | 75,800 | 16.1 | 300 | 25.2 | 2 | 811 | 13.6 | 165,000 | 28.6 | 0.126 | 29.3 | 30.2 | 27.1 | 387 | 21.9 |
| 30 | 8 | 724 | 15.7 | 113,000 | 19.9 | 289 | 53.4 | 4 | 1,360 | 20.6 | 243,000 | 29.9 | 0.132 | 32.1 | 29.0 | 23.1 | 625 | 19.4 |

**Abbreviations:** CL, serum clearance after intravenous infusion; CV, coefficient of variation.
in sVEGFR2 over the first 57 days of treatment with AMG 780; this decrease was not dose proportional (Fig. 3C). None of the measured circulating angiogenic factors were associated with baseline tumor measurements or changes in tumor size during treatment. All evaluable patients (n = 38) expressed detectable levels of Ang1 and Ang2. Mean (SD) serum baseline Ang1 and Ang2 concentrations were 27.8 (10.3) ng/mL and 3.50 (2.24) ng/mL, respectively.

Discussion

In this first-in-human, phase I dose-escalation study, we found that AMG 780, a mAb that blocks the interaction between Ang1 and Ang2 and the Tie2 receptor, could be administered at doses up to 30 mg/kg, had pharmacologic characteristics that allowed for dosing once every 2 weeks, and had biologic effects consistent with an antiangiogenic mechanism of action. An MTD was not reached at the highest dose administered (30 mg/kg every 2 weeks). Three patients had DLTs (thrombocytopenia, proteinuria, pleural effusion), none of which prevented dose escalation. There were two grade 4 events (esophageal ulcer and sepsis) and three on-study deaths (two disease progressions and one suicide), none of which were considered related to AMG 780. All other AEs were of grade 0-3, and there did not appear to be an association between dose and AE severity.

Patterns of toxicity occurring during the study were consistent with those anticipated for an antiangiopoietin agent. Peripheral edema, which has been reported during treatment with...
Figure 3.
Pharmacodynamic effects of AMG 780 on the circulating angiogenic factors sVCAM-1 (A), PLGF (B), and sVEGFR2 (C). Arrows indicate AMG 780 administration. EOS, end of study; PLGF, placental growth factor; sVCAM-1, soluble vascular cell adhesion molecule-1; sVEGFR2, soluble vascular endothelial growth factor receptor 2.
trebananib (19–24, 31, 33), occurred in 30% of patients, although all events were grade 1 or 2. The mechanism by which peripheral edema might occur during AMG 780 and trebananib treatment is uncertain. There did not appear to be an association between administration of AMG 780 and hepatic, pancreatic, or adrenal cortex functioning. Although 2 patients had significant hepatic laboratory abnormalities, the presence of grade 3 elevations in alkaline phosphatase is suggestive of underlying hepatobiliary tract disease rather than drug-induced liver injury. Events typically associated with anti-VEGF therapy (e.g., bleeding events, hyperension, thromboembolic events, delayed wound healing) occurred either infrequently or not at all. Because AMG 780 inhibits the angiopoietin pathway, its mechanism of action and AE profile are unique and different from those of angiogenesis inhibitors targeting the VEGF pathway. This is consistent with what has been observed in studies evaluating other agents targeting the angiopoietin pathway (24).

AMG 780 had approximately dose-linear pharmacokinetics, with steady-state reached after three every 2 weeks doses and no evidence of accumulation. At doses of 2.5 mg/kg and above, median t1/2,α was between 8 and 13 days for this mAb compared with 3.1 to 6.3 days for the peptide trebananib (19). This difference in half-life is due to molecular weight and size differences between the two molecules (antibody vs. peptide). Although an MTD was not reached (as frequently occurs in phase I studies evaluating biologic agents; ref. 34), AMG 780 concentrations remained above the IC90 for tumor growth inhibition in xenograft models throughout a 2-week cycle. Thus, every 2 weeks administration of AMG 780 is feasible and provides serum concentrations at levels required for antitumor activity. Dosing once every 2 weeks supports both patient convenience and combination with chemotherapy regimens administered at this interval.

Dynamic contrast-enhanced magnetic resonance imaging has been used in clinical development of antiangiogenic drugs to evaluate their effects on tumor vasculature (29, 35). The reductions in Ktrans and IAIC reported in this study suggest that AMG 780 induced the anticipated effects on tumor perfusion and vessel permeability (as measured by Ktrans and IAIC), with effects observed across a variety of tumor types. Notably, the magnitude of reductions in Ktrans and IAIC was similar to those occurring with trebananib (19) and smaller than what has been measured with agents targeting the VEGF pathway (29, 36–38).

Further evidence of the biologic effects of AMG 780 on angiogenic pathways was provided by evaluation of circulating angiogenic factors. During AMG 780 treatment, serum concentrations of PLGF and sVCAM-1 were increased, whereas serum sVEGFR2 decreased. PLGF is a ligand for VEGFR1 (39), VCAM-1 is expressed by endothelial cells in response to VEGF (40), and sVEGFR2 is a VEGF receptor variant that appears to have antilymphangiogenic activity (41). The observed changes in these molecules are indicative not only of effects of AMG 780 on angiogenesis but also of interactions between the angiopoietin axis and VEGF pathway. Although we did not see an association between change from baseline in PLGF, WCA-M, and sVEGFR2 and outcomes, it must be noted that the small magnitude of changes in tumor size, which is not unexpected for a heavily pretreated patient population with advanced disease, may have made it difficult to identify such associations if they exist. It is also possible that identification of a biomarker for AMG 780 activity will require evaluation of alternative biomarkers. For example, it has been suggested that tumor expression of a panel of proteins (AngI, EGF, and Emmprin) may have predictive value for tumor growth inhibition by the anti-Ang2 mAb CVX-060 (42).

Evaluation of tumor expression of other components of the angiopoietin pathway (including Tie2) might also be appropriate. However, such analyses may be challenging given the need to localize expression within tumor versus endothelial versus stromal cells and the heterogeneity of expression of Tie2 (43).

Tumor response was a secondary endpoint in this phase I dose-escalation study. Although no patients met the criteria for complete or partial response, 4 patients had reductions in tumor size during AMG 780 treatment and one patient, who had been treated with surgery, radiation, and seven prior anticancer regimens (including one course of bevacizumab), had stable disease lasting 7 months. The lack of complete or partial responses must be evaluated in the context of the enrolled population of patients with advanced solid tumors refractory to available therapy.

The angiopoietin pathway represents an attractive target in the treatment of cancer. Both Ang1 and Ang2 play key roles in neovascularization, with Ang1 regulating maturation of new blood vessels and Ang2 regulating vascular remodeling and sprouting of new vessels (3–5). In addition to the extensive data on the role of the angiopoietin pathway in angiogenesis, several studies have demonstrated increased expression of Tie2 in cancer cells and in cancer-associated stromal cells. This, coupled with the expression of Ang1 and/or Ang2 by cancer cells and studies of cell lines and in vivo tumor models, suggests that Tie2 activation may contribute to cancer cell growth via additional autocrine mechanisms that promote a stem cell–like phenotype with enhanced adhesion, invasion, and metastatic potential (44–46).

Results from the phase III TRINOVA-1 study of trebananib plus paclitaxel versus placebo plus paclitaxel in women with recurrent ovarian cancer have validated the angiopoietin pathway as a target in the treatment of cancer (24). In addition to trebananib and AMG 780 (which target the interaction between Ang1 and Ang2 and Tie2), agents using a diverse range of alternative strategies to inhibit the angiopoietin pathway have been evaluated in the treatment of cancer (47). Agents investigated in clinical studies include CVX-060, a fusion of a peptide and a carrier antibody scaffold that blocks the interaction between Ang2 and Tie2 (42); CEP-11981, a small-molecule inhibitor of VEGFR1, VEGFR2, and Tie2 (48); MED3617 (49) and REGN910 (50), mAbs that bind Ang2 to prevent its interaction with the Tie2 receptor; and small-molecule inhibitors such as regorafenib (51, 52), cobazotinib (53), MCIG265 (54), and foretinib (55) that block the activity of multiple protein kinases, including Tie2 and VEGF receptors. All of these agents focus on inhibition of Ang2 or Tie2, either alone or in combination with sometimes multiple other targets, but none aim at inhibiting both Ang1 and Ang2, alone. The potential role of targeting angiopoetins and other angiogenic factors outside of the well-studied VEGF pathway in the treatment of cancer continues to be an area of scientific interest (56, 57). Although treatment with anti-VEGF or anti-VEGF receptor agents has been shown to result in objective responses in some patients (particularly when combined with chemotherapy), not all patients respond and tumor shrinkage has not always translated into improvements in overall survival in phase III studies (57).

As a result, combinations employing agents targeting both the VEGF and angiopoietin pathways may be worthy of investigation. In conclusion, AMG 780 could be...
administered at doses up to 30 mg/kg once every 2 weeks with toxicity as anticipated for an angiopoietin agonist. No MTD was reached. AMG 780 had dose-proportional pharmacokinetics with serum concentrations remaining above levels anticipated for an antitumor effect (based on preclinical studies) throughout a 2-week treatment cycle.

Disclosure of Potential Conflicts of Interest

E. Rasmussen, Y.C. Hwang, M.B. Bass, G. Friberg, and C.A. Pickett have ownership interest (including patents) in Amgen. No potential conflicts of interest were disclosed by the other authors.

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References


Acknowledgments

The authors thank Don Zhong, PhD (Amgen Inc.), for performing anti-AMG 780 antibody measurements and data analysis; and Terrance J. Williams, PhD (formerly of Amgen Inc.), and Ali Hassan, PhD (Complete Healthcare Communications, Inc.), whose work was funded by Amgen Inc., for assistance in the preparation of this manuscript.

Grant Support

This study was supported by Amgen Inc.


A Phase I, First-in-Human Study of AMG 780, an Angiopoietin-1 and -2 Inhibitor, in Patients with Advanced Solid Tumors

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