

## A First-in-Human Study of Conatumumab in Adult Patients with Advanced Solid Tumors

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### Abstract

**Purpose:** To determine the safety, tolerability, pharmacokinetics, and maximum tolerated dose (MTD) of conatumumab, an investigational, fully human monoclonal agonist antibody against human death receptor 5, in patients with advanced solid tumors.

**Experimental Design:** In the dose-escalation phase, patients received escalating intravenous doses of conatumumab (0.3, 1, 3, 10, or 20 mg/kg, 3–9 per cohort) every 2 weeks. In the dose-expansion phase, 10 patients with colorectal cancer (CRC) and 7 with non–small cell lung cancer (NSCLC) received 20 mg/kg of conatumumab every 2 weeks.

**Results:** Thirty-seven patients received 1 or more doses of conatumumab. Conatumumab seemed to be well tolerated; there were no dose-limiting toxicities. Of adverse events possibly related to treatment, only 3 patients (8%) had a grade 3 event (fatigue and/or elevated lipase), and no anti-conatumumab antibodies were detected. An MTD was not reached. Conatumumab exhibited dose linear kinetics from 3 to 20 mg/kg, with a mean terminal half-life of 13 to 19 days. One patient with NSCLC (0.3 mg/kg) had a confirmed partial response (PR) at week 32 (38% reduction in tumor size), with further reduction (48%) by week 96; this patient remains on conatumumab after 4.2 years with a sustained PR. Fourteen patients had a best response of stable disease, 2 for 32 weeks or more. One patient with CRC (0.3 mg/kg) and stable disease for 24 weeks had a 24% reduction in tumor size by RECIST (Response Evaluation Criteria in Solid Tumors) and a 35% reduction in the sum of standardized uptake values of all lesions measured by [<sup>18</sup>F]fluorodeoxyglucose positron emission tomographic scan. Changes in tumor levels of activated caspase-3 did not appear to be associated with tumor response.

**Conclusions:** Conatumumab can be administered safely up to the target dose of 20 mg/kg every 2 weeks. *Clin Cancer Res*; 16(23); 5883–91. ©2010 AACR.

Apoptosis is triggered via 2 signaling pathways: the intrinsic apoptosis pathway and the extrinsic apoptosis pathway. The intrinsic pathway is activated by mitochondrial signals triggered by cell stress, whereas the extrinsic pathway is activated by the binding of apoptosis-inducing ligand/tumor necrosis factor (TNF) receptor–related, apoptosis-inducing ligand (Apo2L/TRAIL) to receptors belonging to the TNF receptor superfamily (1–4). Apo2L/TRAIL

binds to 5 receptors: death receptors 4 (DR4) and 5 (DR5), which transduce apoptotic signals, and decoy receptors DcR1, DcR2, and osteoprotegerin, which do not induce apoptosis. Binding of Apo2L/TRAIL to DR4 and DR5 induces formation of a death-inducing signaling complex, leading to the activation of effector caspases and cell death (1–4).

Apoptosis is dysregulated in cancer, making it an important target for the development of anticancer treatments (5, 6). Many conventional anticancer therapies act by triggering the intrinsic apoptosis pathway; however, these agents are cytotoxic and rely on an intact *p53* tumor suppressor gene, a key regulator of the intrinsic pathway, which is inactivated in many human tumors, resulting in resistance to treatment (2–4, 7). The resistance of tumor cells to standard cytotoxic agents may be circumvented by death receptor agonists, which bind to DR4 and/or DR5 and activate the extrinsic and intrinsic apoptosis pathways independently of *p53*. *In vitro* studies have shown that tumor cells are sensitive to death receptor agonists, whereas normal cells are resistant (8). The mechanism for enhanced tumor sensitivity to these agents is not clearly understood but may be associated with alterations in

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### Translational Relevance

Conatumumab is an investigational, fully human monoclonal agonist antibody directed against human death receptor DR5, which is expressed in many tumor types. In preclinical studies, conatumumab inhibited tumor growth in a number of *in vitro* and *in vivo* models of human cancer, both as monotherapy and in combination with chemotherapeutic agents. In this first-in-human study, conatumumab seemed to be well tolerated and showed preliminary evidence of antitumor activity in adult patients with advanced solid tumors. Further clinical trials evaluating conatumumab in combination with chemotherapy and targeted agents are ongoing.

intracellular factors and/or differences in levels of death receptors (9–12).

A number of death receptor agonists are currently in clinical development, including the monoclonal antibodies DR4 agonist mapatumumab and DR5 agonists conatumumab (AMG 655), CS-100, and lexatumumab, all of which mimic the effects of Apo2L/TRAIL, and the proapoptotic ligand recombinant human Apo2L/TRAIL, which is directed against DR4 and DR5 (13–23). Conatumumab is an investigational, fully human monoclonal agonistic antibody (IgG1) directed against human DR5, which is expressed in many tumor types (11, 24–27). In preclinical studies, conatumumab increased caspase activation, decreased cell survival, and inhibited tumor growth in a number of *in vitro* and *in vivo* models of human cancer, both as monotherapy and in combination with chemotherapeutic agents (28).

This first-in-human study was designed to determine the safety, tolerability, pharmacokinetics, and maximum tolerated dose (MTD) of conatumumab administered every 2 weeks in adult patients with advanced tumors.

### Methods

#### Key eligibility criteria

Inclusion criteria included signed institutional review board–approved informed consent; age of 18 years or older; diagnosis of an advanced solid tumor or Hodgkin or non-Hodgkin lymphoma refractory to standard treatment or for which no curative therapy was available; Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1; willingness to provide paraffin-embedded tumor samples (in the dose-expansion phase, patients were required to have tumors accessible for biopsy and to consent to provide tumor biopsy samples); no primary central nervous system tumor; no hematologic malignancy (except Hodgkin or non-Hodgkin lymphoma); adequate cardiac, hematologic, hepatic, and renal functions; and no concurrent or prior (within 30 days of study day 1) anticoagulation therapy (low-dose warfarin for prophylaxis against thrombosis was allowed).

#### Study design

This was a first-in-human, open-label clinical trial with dose-escalation and dose-expansion phases. Conatumumab was administered as a 1-hour intravenous infusion every 2 weeks. The starting dose was 0.3 mg/kg every 2 weeks, which provided an exposure margin of more than 400-fold on the basis of no observed adverse effect level of 300 mg/kg from the nonhuman primate toxicology study. The 0.3-mg/kg dose was predicted to reach the concentration level that was observed at the dose required to achieve 50% maximal tumor growth inhibition (ED<sub>50</sub>) in preclinical models. The 20-mg/kg maximum planned dose was predicted to maintain serum trough concentrations of conatumumab at the ED<sub>90</sub> observed in preclinical models.

The every-2-week dose interval for multiple dose administration was based on the projected pharmacokinetics in humans.

In the dose-escalation phase, patients were sequentially enrolled into 1 of the 5 preplanned dose cohorts of 0.3, 1, 3, 10, or 20 mg/kg of conatumumab administered every 2 weeks (3–9 patients per dose cohort utilizing a 3 + 3 + 3 design). Enrollment in the next dose level occurred if none of the first 3 patients at the previous dose level experienced a dose-limiting toxic effect [DLT; any conatumumab-related grade  $\geq$ 3 hematologic or nonhematologic toxic effect according to the Common Terminology Criteria for Adverse Events (CTCAE), except alopecia, in the first 28 days of treatment]. If 1 of 3 or 2 of 6 patients experienced a DLT, 3 additional patients would be enrolled at that dose level. If 1 of 6 or 2 of 9 patients experienced a DLT, dose escalation to the next dose level would occur. Dose escalation continued until the MTD (the highest dose level with an observed incidence of a DLT in  $<$ 33% of treated patients) or the maximum planned dose was reached. Adverse events were recorded for all patients who received 1 or more doses of conatumumab and were graded according to CTCAE, Version 3.0.

Patients received conatumumab on days 1, 15, and 29 followed by a 28-day treatment-free period; no conatumumab was administered on day 43 to allow for the assessment of terminal pharmacokinetic parameters. If no DLT was observed, conatumumab was resumed every 2 weeks on day 57 in patients with an objective tumor response (complete or partial response [CR or PR]) or stable disease. No inpatient dose adjustment was allowed.

The dose-expansion phase was designed to detect post-treatment changes in caspase-3 activity in patient tumor samples as well as to provide additional safety and efficacy data. The planned sample size was 20 patients in 2 cohorts: 10 with colorectal cancer (CRC) and 10 with non-small cell lung cancer (NSCLC) and who were to receive the target dose of 20 mg/kg of conatumumab every 2 weeks (including day 43). The number of patients enrolled in each cohort was based on extrapolation of preclinical caspase data, which indicated that 10 patients per cohort were required to provide 90% power to detect at least an 88% change in activated caspase-3 from post- versus pretreatment biopsies with a 10% level of significance.

**Table 1. Patient demographics and baseline characteristics<sup>a</sup>**

|                                       | All patients (N = 37) |
|---------------------------------------|-----------------------|
| Sex                                   |                       |
| Female                                | 13 (35)               |
| Male                                  | 24 (65)               |
| Race                                  |                       |
| White or Caucasian                    | 30 (81)               |
| Black or African American             | 3 (8)                 |
| Hispanic or Latino                    | 1 (3)                 |
| Asian                                 | 2 (5)                 |
| Japanese                              | 1 (3)                 |
| Age, median (range), y                | 56 (29–79)            |
| ECOG performance score                |                       |
| 0                                     | 11 (30)               |
| 1                                     | 26 (70)               |
| Disease stage                         |                       |
| III                                   | 5 (14)                |
| IV                                    | 32 (86)               |
| Primary tumor type                    |                       |
| Colorectal                            | 23 (62)               |
| Non–small cell lung cancer            | 7 (19)                |
| Bone sarcoma                          | 1 (3)                 |
| Breast                                | 1 (3)                 |
| Mesothelioma                          | 1 (3)                 |
| Oral                                  | 1 (3)                 |
| Pancreatic endocrine tumor            | 1 (3)                 |
| Stomach                               | 1 (3)                 |
| Thymoma                               | 1 (3)                 |
| Prior radiotherapy                    | 19 (51)               |
| Number of prior chemotherapy regimens |                       |
| 2                                     | 4 (11)                |
| ≥3                                    | 33 (89)               |

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

<sup>a</sup>Values are number (percentage) unless indicated otherwise.

Patients received conatumumab every 2 weeks until disease progression, intolerable adverse event, or consent withdrawal. After the last dose of conatumumab, patients were monitored for 4 or more weeks.

### Study endpoints

Endpoints included incidence of a DLT, a severe adverse event, clinically significant changes in laboratory test results, MTD (if reached), pharmacokinetic parameters, expression of activated caspase-3 in pretreatment and post-treatment biopsy samples (in the expansion phase only), tumor response by Response Evaluation Criteria in Solid Tumors (RECIST) or [18F]fluorodeoxyglucose positron emission tomographic scan (<sup>18</sup>FDG-PET), and formation of anticonatumumab antibodies.

### Treatment procedures

Screening procedures included complete medical history, vital signs, physical examination, urinalysis, electrocardiogram, safety laboratory tests (hematologic and coagulation tests, comprehensive chemistry panel, creatine kinase, amylase, and lipase), and tumor biopsy (optional for dose escalation, required for dose expansion) for assessment of caspase-3 activation.

Assessments before each dose of conatumumab included physical examination, safety laboratory tests, and urinalysis. Safety laboratory tests and urinalysis were also performed on days 2 and 8.

Tumors were evaluated with computed tomographic and/or magnetic resonance imaging (MRI) scans (within 28 days before day 1 and every 8 weeks thereafter) and <sup>18</sup>FDG-PET (baseline and day 36 ± 5 days, except for patients with prostate or bladder cancer). Tumor response was assessed by RECIST, and <sup>18</sup>FDG-PET tumor-response criteria were defined on the basis of the recommendations of the European Organization for Research and Treatment of Cancer (29), with a metabolic PR defined as a greater than 25% reduction in maximum standardized uptake value (SUV<sub>max</sub>).

### Evaluation of anticonatumumab antibodies

Serum for assessment of anticonatumumab antibodies was collected predose at weeks 1, 5, and 9, and every 8 weeks thereafter, and samples were tested using a validated Meso Scale Discovery electrochemiluminescence bridging immunoassay (30) as described in the Supplementary Methods section.

### Pharmacokinetics

Serum samples for pharmacokinetic parameters were collected on day 1 [predose, 0.5 hour, 1 hour (end of infusion), and 6 hours]; days 2, 3, 5, 8, and 11; day 15 (predose and end of infusion); day 29 [predose, 0.5 or 1 hour (end of infusion), and 6 hours]; days 30, 31, 33, 36, 39, 43 (predose and end of infusion), and 50; day 57 (predose and end of infusion); every 8 weeks thereafter; and 4 or 8 weeks after the end of treatment. The end-of-infusion samples were collected within 5 minutes before the end of the infusion.

Serum pharmacokinetic parameters were estimated using noncompartmental methods with WinNonlin Enterprise software (Version 5.1.1; Pharsight Corp.).

### Measurement of tumor caspase-3 activity

Patients in the dose-expansion phase underwent imaging-guided, fine-needle tumor aspiration within 4 weeks prior to study day 1 and then 24 to 48 hours after the first dose of conatumumab [the estimated postdose sampling time was based on maximal induction of activated caspase-3 observed in preclinical models (ref. 31)]. Activated caspase-3 was measured directly by immunocytochemical detection. Pathway activation was defined as a 100% or greater increase in caspase-3 activity in post- versus pretreatment tumor biopsies as quantified by laser scanning cytometry (LSC).

**Table 2. Patient disposition**

|   | Conatumumab dose cohorts |              |               |             |             |                 |                   | All patients  |
|---|--------------------------|--------------|---------------|-------------|-------------|-----------------|-------------------|---------------|
|   | Dose escalation          |              |               |             |             | Dose expansion  |                   |               |
|   | 0.3 mg/kg                | 1 mg/kg      | 3 mg/kg       | 10 mg/kg    | 20 mg/kg    | 20 mg/kg<br>CRC | 20 mg/kg<br>NSCLC |               |
| Patients enrolled, <i>n</i>                                 | 3                        | 3            | 3             | 4           | 9           | 10              | 5                 | 37            |
| Patients who received conatumumab, <i>n</i>                 | 3                        | 3            | 3             | 4           | 9           | 10              | 5                 | 37            |
| Patients who discontinued study, <i>n</i>                   | 2                        | 3            | 2             | 4           | 9           | 10              | 5                 | 35 (95%)      |
| Reasons   |                          |              |               |             |             |                 |                   |               |
| Disease progression   | 2                        | 1            | 1             | 3           | 3           | 5               | 4                 | 19 (51%)      |
| Lost to follow-up   | 0                        | 0            | 0             | 0           | 2           | 2               | 0                 | 4 (11%)       |
| Consent withdrawn   | 0                        | 0            | 0             | 1           | 2           | 0               | 0                 | 3 (8%)        |
| Death   | 0                        | 1            | 0             | 0           | 0           | 1               | 0                 | 2 (5%)        |
| Adverse event   | 0                        | 1            | 0             | 0           | 0           | 0               | 0                 | 1 (3%)        |
| Other   | 0                        | 0            | 1             | 0           | 2           | 2               | 1                 | 6 (16%)       |
| Patients still receiving conatumumab, <sup>a</sup> <i>n</i> | 1                        | 0            | 1             | 0           | 0           | 0               | 0                 | 2             |
| Number of doses per patient <sup>a</sup>                    |                          |              |               |             |             |                 |                   |               |
| Median  | 10                       | 3            | 23            | 3           | 3           | 4               | 4                 | 3             |
| Range   | 3 to 54+                 | 3 to 5       | 5 to 43+      | 2 to 5      | 3 to 11     | 1 to 4          | 3 to 12           | 1 to 54+      |
| Time on treatment, <sup>a</sup> wk                          |                          |              |               |             |             |                 |                   |               |
| Median  | 24.1                     | 13.0         | 50.4          | 11.3        | 9.9         | 8.1             | 15.0              | 11.1          |
| Range   | 8.1 to 109.9+            | 13.0 to 16.6 | 13.3 to 96.0+ | 7.7 to 14.0 | 5.4 to 30.1 | 2.0 to 13.9     | 8.1 to 23.3       | 2.0 to 109.9+ |
| Dose-limiting toxic effects, <sup>b</sup> <i>n</i>          | 0                        | 0            | 0             | 0           | 0           | NE              | NE                | 0             |

Abbreviation: NE, not estimable.

<sup>a</sup>As of February 4, 2008. One patient with thymoma and a best response of stable disease received his or her last dose on April 4, 2008, and ended the study 2 weeks later. The patient received a total of 46 doses and was on treatment for approximately 2 years. One patient with NSCLC and a PR is currently (as of the date of this report) receiving conatumumab, has received at least 106 doses, and is on study after more than 4 years.

<sup>b</sup>Evaluated during the dose-escalation phase.

### Immunocytochemical detection of cleaved caspase-3

Immediately after acquisition, the tumor samples were fixed in 4% paraformaldehyde, gently resuspended by pipetting, and washed twice with PBS solution. Cells were processed and slides were generated as described in the Supplementary Methods section. The slides were mounted and the cells were analyzed by LSC.

### Laser scanning cytometry

Cytometric measurements were performed using a laser scanning cytometer (iCyte; Compucyte Corp.) as described in the Supplementary Methods section.

## Results

### Patient characteristics and disposition

Thirty-seven patients [22 dose escalation, 15 dose expansion (10 CRC and 5 NSCLC)], whose characteristics are

shown in Table 1, were enrolled between December 2005 and August 2007 and received 1 or more doses of conatumumab (Table 2). Most patients (51%) discontinued the study because of disease progression. The median number of doses of conatumumab received per patient was highest at 0.3 and 3 mg/kg. The 2 most responsive patients were enrolled in these dose cohorts: 1 patient (0.3 mg/kg) was still receiving conatumumab as of March 2010 and had received at least 106 doses of conatumumab (3 missed doses) for at least 218 weeks (4.2 years); a second patient (3 mg/kg) who completed the study in April 2008 had received approximately 46 doses (6 missed doses) for 105 weeks (>2 years). No DLTs were observed at any dose, and an MTD was not reached.

### Safety and tolerability

Conatumumab was generally well tolerated up to the highest planned dose of 20 mg/kg every 2 weeks. All patients

**Table 3. Adverse events by dose<sup>a</sup>**

|  | All patients |              | 0.3 mg/kg |              | 1 mg/kg   |              | 3 mg/kg   |              | 10 mg/kg  |              | 20 mg/kg <sup>b</sup> |              |
|--|--------------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------------------|--------------|
|  | Any grade    | Grade<br>≥ 3 | Any grade | Grade<br>≥ 3 | Any grade | Grade<br>≥ 3 | Any grade | Grade<br>≥ 3 | Any grade | Grade<br>≥ 3 | Any grade             | Grade<br>≥ 3 |
| Number of patients reporting at least 1 adverse event, n (%) | 37 (100)     | 14 (38)      | 3 (100)   | 1 (33)       | 3 (100)   | 2 (67)       | 3 (100)   | 3 (100)      | 4 (100)   | 1 (25)       | 24 (100)              | 7 (29)       |
| Pyrexia  | 17 (46)      | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 2 (67)    | 0            | 2 (50)    | 0            | 11 (46)               | 0            |
| Fatigue  | 14 (38)      | 4 (11)       | 3 (100)   | 1 (33)       | 1 (33)    | 0            | 2 (67)    | 1 (33)       | 1 (25)    | 0            | 7 (29)                | 2 (8)        |
| Chills   | 9 (24)       | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 0         | 0            | 6 (25)                | 0            |
| Nausea   | 9 (24)       | 0            | 0         | 0            | 0         | 0            | 2 (67)    | 0            | 2 (50)    | 0            | 5 (21)                | 0            |
| Anemia   | 8 (22)       | 0            | 0         | 0            | 2 (67)    | 0            | 1 (33)    | 0            | 2 (50)    | 0            | 3 (13)                | 0            |
| Anorexia   | 8 (22)       | 0            | 0         | 0            | 0         | 0            | 1 (33)    | 0            | 0         | 0            | 7 (29)                | 0            |
| Cough  | 8 (22)       | 0            | 0         | 0            | 0         | 0            | 0         | 0            | 2 (50)    | 0            | 6 (25)                | 0            |
| Dyspnea  | 8 (22)       | 2 (5)        | 0         | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 0         | 0            | 6 (25)                | 2 (8)        |
| Vomiting   | 8 (22)       | 0            | 0         | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 0         | 0            | 6 (25)                | 0            |
| Constipation   | 7 (19)       | 0            | 1 (33)    | 0            | 0         | 0            | 1 (33)    | 0            | 0         | 0            | 5 (21)                | 0            |
| Diarrhea   | 7 (19)       | 0            | 2 (67)    | 0            | 0         | 0            | 1 (33)    | 0            | 0         | 0            | 4 (17)                | 0            |
| Chest pain   | 6 (16)       | 0            | 1 (33)    | 0            | 0         | 0            | 2 (67)    | 0            | 0         | 0            | 3 (13)                | 0            |
| Anxiety  | 5 (14)       | 0            | 1 (33)    | 0            | 1 (33)    | 0            | 0         | 0            | 0         | 0            | 3 (13)                | 0            |
| Hyperhidrosis  | 5 (14)       | 0            | 0         | 0            | 1 (33)    | 0            | 2 (67)    | 0            | 1 (25)    | 0            | 1 (4)                 | 0            |
| Hypomagnesemia   | 5 (14)       | 0            | 0         | 0            | 0         | 0            | 1 (33)    | 0            | 1 (25)    | 0            | 3 (13)                | 0            |
| Peripheral edema   | 5 (14)       | 0            | 0         | 0            | 0         | 0            | 1 (33)    | 0            | 1 (25)    | 0            | 3 (13)                | 0            |
| Upper respiratory tract infection                            | 5 (14)       | 0            | 0         | 0            | 0         | 0            | 1 (33)    | 0            | 0         | 0            | 4 (17)                | 0            |

NOTE: Grade 4 adverse events were hyperbilirubinemia, acute respiratory failure, and pulmonary infarction (1 patient each); deaths (grade 5 events) were due to hepatic failure and progression of NSCLC (1 patient each).

<sup>a</sup>In 5 or more patients.

<sup>b</sup>From the dose-escalation and dose-expansion cohorts combined.

completed the DLT observation window, that is, they all received at least 2 doses of conatumumab. Adverse events (regardless of investigator attribution) were generally mild to moderate, the most common being pyrexia, fatigue, and chills (Table 3). Fourteen patients (38%) had an adverse event of worst grade 3 or higher; 3 had a grade 4 event (1 hyperbilirubinemia, 1 acute respiratory failure, and 1 pulmonary infarction). The patient with hyperbilirubinemia died of hepatic failure. This patient had a diagnosis of stage IV, moderately differentiated, metastatic CRC with multiple metastases within the liver and bulky periportal lymphadenopathy, as well as metastases in the lungs. Within 11 days of starting on study, the patient's liver function test results continued to increase above baseline elevations, and the patient's white blood cell count increased. Scans revealed progressive pulmonary and hepatic metastases (with increased biliary dilatation and portal vein compression). The investigator attributed the elevated hepatic values to disease progression, and the patient elected to forgo further aggressive interventions. A second patient died because of progression of NSCLC.

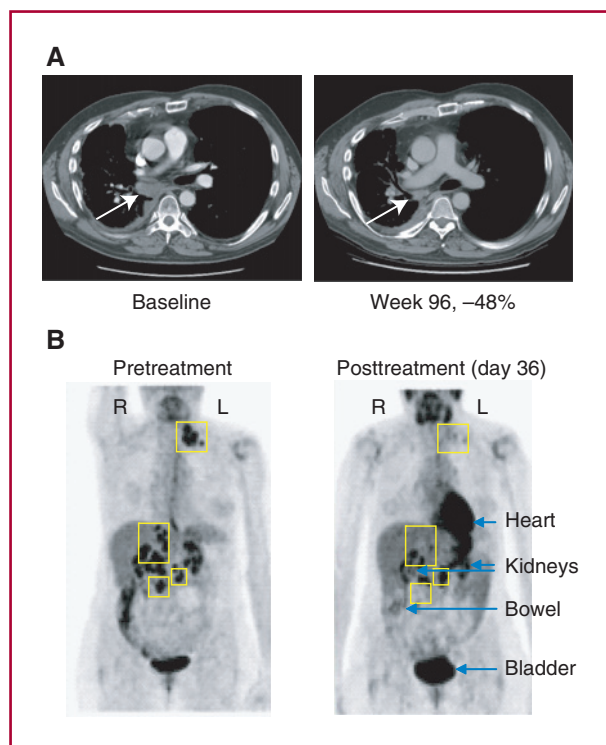
Adverse events deemed at least possibly related to conatumumab treatment were reported in 21 patients (57%), of whom 3 (8%) had a grade 3 adverse event: 1 with grade 3

fatigue and grade 3 elevated lipase, 1 with grade 3 elevated lipase, and 1 with grade 3 fatigue (Supplementary Table S1). The elevations were not associated with aminotransferase elevations or with clinical symptoms consistent with pancreatitis. There were no grade 4 or grade 5 treatment-related adverse events.

Ten patients had adverse events that were considered serious, only 1 of which, pyrexia, was attributed as possibly related to conatumumab treatment. No deaths were attributed to conatumumab treatment, and no anticonatumumab antibodies were detected in any patient.

#### Antitumor activity

One of the seven patients with NSCLC, a 51-year-old man with poorly differentiated adenocarcinoma (0.3-mg/kg dose level), had a confirmed PR at week 32 (38% reduction in tumor size), with a further reduction in tumor size by week 96 (48% reduction; Fig. 1A) that was maintained at week 104 (43% reduction). He had previously received chest radiotherapy along with carboplatin, docetaxel, and bevacizumab, to which the tumor initially responded, but the disease progressed after 5 months of treatment. He remains on conatumumab after 4.2 years with a sustained PR.



**Fig. 1.** A, PR in a patient with NSCLC (computed tomographic scan). B, metabolic response in a patient with colorectal cancer ( $^{18}\text{F}$ -FDG-PET scan).

Fourteen additional patients had stable disease (range, 5–89 weeks), 2 of whom had stable disease for more than 32 weeks [1 with mesothelioma (3 mg/kg) whose disease progressed at week 47, and 1 with thymoma (3 mg/kg) who received conatumumab for 105 weeks]. Of the 23 patients with CRC, 1 with signet ring adenocarcinoma (0.3 mg/kg),

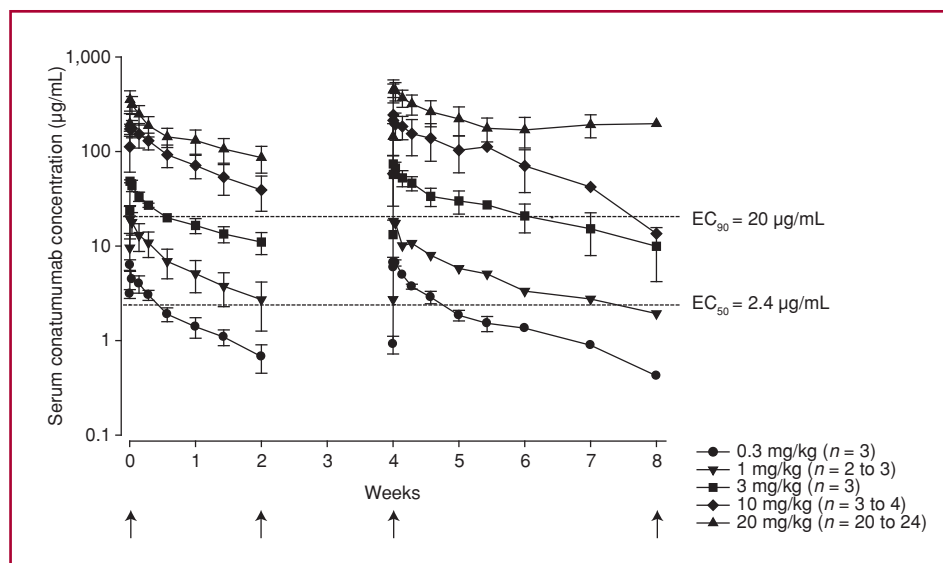
who previously received bevacizumab plus FOLFOX and then capecitabine plus cetuximab, had stable disease for 24 weeks with a 24% reduction in tumor size by RECIST and a metabolic PR with a 35% reduction in  $\text{SUV}_{\text{max}}$  at the day 36  $^{18}\text{F}$ -FDG-PET assessment (Fig. 1B). A second patient with CRC (adenocarcinoma, 20 mg/kg) had a 48% reduction in  $\text{SUV}_{\text{max}}$  at the same time point but was removed from the study 1 week later after the discovery of a previously undiagnosed bone metastasis by MRI (best response by RECIST was progressive disease); the investigator recommended alternative treatment. In addition to the 2 patients with a metabolic response, a further 6 of 34 patients with evaluable  $^{18}\text{F}$ -FDG-PET scans had an  $\text{SUV}_{\text{max}}$  reduction of greater than 10% at day 36 with a mean reduction of  $-18\%$ . However, these reductions did not fulfill the criteria defining a metabolic response.

### Pharmacokinetics

Conatumumab serum concentration data from 37 patients were used for the analysis. Conatumumab exhibited dose-linear kinetics in the dose range from 3 to 20 mg/kg (Fig. 2), with an estimated mean clearance of 0.26 to 0.29 mL/h/kg and mean terminal half-life ( $t_{1/2,z}$ ) of 13 to 19 days (Supplementary Table S2). Mean clearances were similar among the 3-, 10-, and 20-mg/kg dose groups but apparently were higher among the lower dose groups of 0.3 and 1 mg/kg, resulting in lower than expected exposures and shorter  $t_{1/2,z}$  (Supplementary Table S2). The time to reach maximum concentration ( $C_{\text{max}}$ ) was generally 1 to 6 hours after the start of infusion. The accumulation ratios ranged from 1.21 to 1.70 when comparing  $\text{AUC}_{0-336\text{ h}}$  values on days 1 and 29.

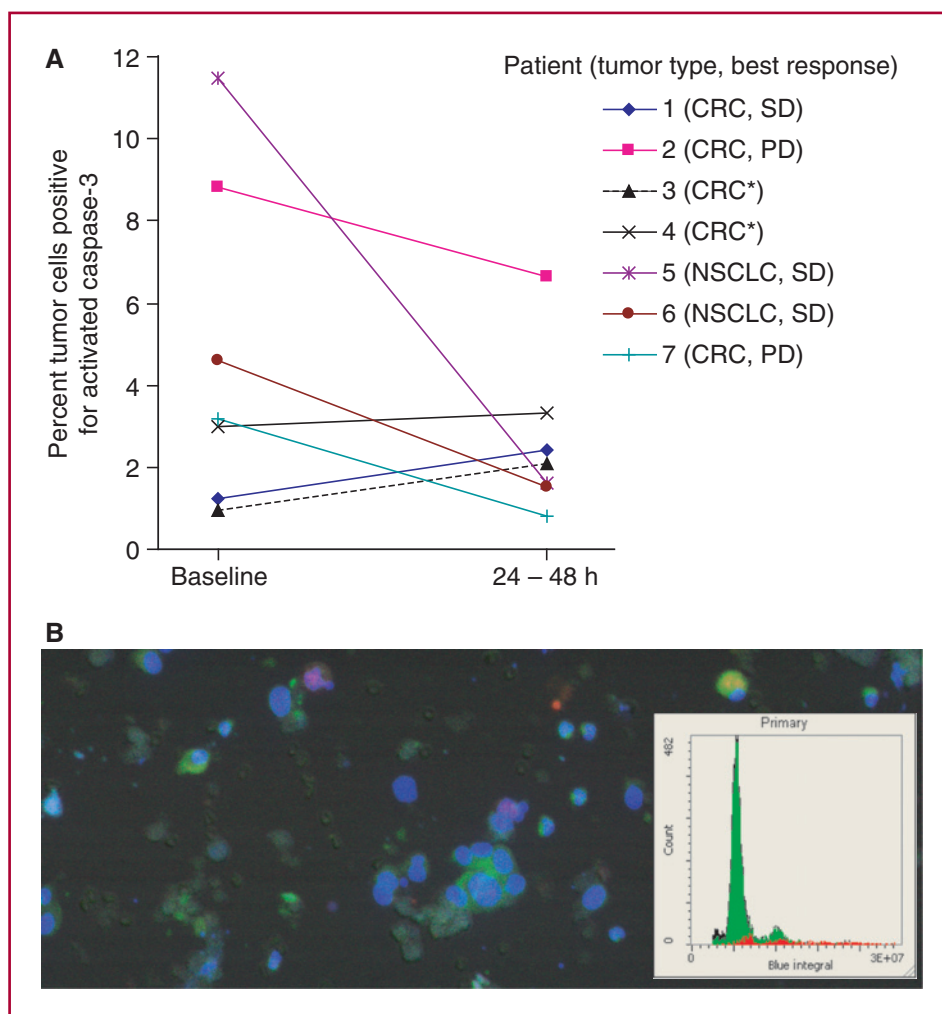
### Tumor caspase-3 activity

Preclinical data showed that conatumumab rapidly increased serum levels of activated caspase-3 in xenograft models of CRC, with peak levels detected approximately 12 to 24 hours after treatment (28). We investigated



**Fig. 2.** Serum concentration-time profiles of conatumumab on days 1 and 29 following intravenous administration every 2 weeks. Data points represent means  $\pm$  standard deviations. Arrows represent dosing with conatumumab.  $\text{EC}_{50}$ , conatumumab concentration required to achieve 50% maximal reduction in tumor volume;  $\text{EC}_{90}$ , conatumumab concentration required to achieve 90% maximal reduction in tumor volume.

**Fig. 3.** A, changes in levels of activated caspase-3 in tumor samples. \*No postbaseline tumor assessment. PD, progressive disease; SD, stable disease. B, activated caspase-3 in NSCLC. The representative field shows tumor cells with activated caspase-3. Images were captured on an iCyte laser scanning cytometer, using a 20 $\times$  objective. Activated caspase-3 staining, red; EpCAM staining, green; DNA staining (Hoechst stain), blue. Inset is a DNA content histogram of tumor cells (green) with overlaid activated caspase-3-positive cells (red) before treatment with conatumumab.



whether levels of activated caspase-3 similarly increased in the tumors of patients with CRC or NSCLC after dosing with conatumumab (preliminary antitumor activity was observed in CRC and NSCLC earlier in the study). Among the 7 patients who had sufficient tumor samples ( $\geq 1,000$  cells) matched from pretreatment and posttreatment (vs. the 15 total patients who enrolled in the expansion portion of the study), 2 had a posttreatment increase in activated caspase-3 (1.2%–2.4% and 0.9%–2.1%), 2 had an approximately stable level (8.8%–6.7% and 3.0%–3.3%), and 3 had a decreased level (3.2%–0.8%, 11.5%–1.6%, and 4.6%–1.5%). There was no clear association between change in level in activated caspase-3 and tumor response (Fig. 3A). Figure 3B shows quantitation of epithelial cells, activated caspase-3, and DNA content in fine-needle aspirate from a patient with NSCLC (prior to conatumumab exposure) as measured by LSC. The aspirate contained EpCAM-positive tumor cells (at various stages of the cell cycle, as determined by DNA content), a small percentage of which contained activated caspase-3 (2%, as expected).

## Discussion

Targeting the extrinsic apoptosis pathway may circumvent tumor-cell resistance to conventional chemotherapies and may offer the opportunity to do so with few added toxic effects for patients. In this first-in-human trial in 37 adult patients with advanced solid tumors, the DR5 agonist conatumumab seemed to be well tolerated up to the maximum planned dose of 20 mg/kg administered every 2 weeks. Adverse events (regardless of attribution) were generally mild to moderate, including only 2 grade 4 events (1 of which was eventually fatal) and 1 death due to disease progression. Adverse events likely due to treatment with conatumumab occurred in 57% of patients; only 3 patients reported a related grade 3 adverse event (fatigue and/or increased lipase), and there were no related grade 4 events and no related deaths. There were no DLTs at any dose; thus, an MTD was not reached.

The  $t_{1/2,z}$  of 8 to 19 days supports intravenous administration of conatumumab either every 2 weeks or every 3 weeks in patients with cancer. Following 3 doses of

conatumumab at 3 mg/kg every 2 weeks,  $C_{max}$  was approximately 67  $\mu\text{g/mL}$  and  $C_{min}$  was approximately 20  $\mu\text{g/mL}$ . The  $C_{min}$  value at 3 mg/kg approximated the mean steady-state minimal concentration value required to achieve 90% of maximal reduction in tumor volume ( $EC_{90}$ ) in a non-clinical xenograft model (unpublished data). Modeling of the pharmacokinetic data suggests that a dose regimen of 10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks will provide a mean minimum trough concentration of 61 or 50  $\mu\text{g/mL}$  and that more than 85% of patients will have trough levels above the  $EC_{90}$  (20  $\mu\text{g/mL}$ ). Although the 2 most responsive patients were treated at the 0.3- or 3-mg/kg dose level, based on the pharmacokinetic modeling data, 10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks will be the target doses in phase II studies.

Antitumor activity of conatumumab was suggested with the observation of a PR in a patient with NSCLC, metabolic PRs in 2 patients with CRC, and stable disease for more than 32 weeks in 2 patients (1 with mesothelioma and 1 with thymoma). Interestingly, the response in NSCLC (lasting over 4 years) on this study was observed in the lowest dose cohort (0.3 mg/kg); the generalizability of this observation is unknown, and subsequent studies to evaluate the potential for dose optimization are ongoing.

Because CRC tumor xenografts treated with conatumumab showed increases in activated caspase-3, we investigated whether activated caspase-3 could be used as a pharmacodynamic marker for conatumumab-associated activation of the extrinsic apoptotic pathway in patients with CRC or NSCLC (in whom the most robust anticancer signals were observed in the escalation phase). Had this endeavor provided meaningful data, our intent would be to test lower doses of conatumumab to evaluate effects on the pharmacodynamic marker.

To our knowledge, this is among the first published descriptions of the use of LSC to detect tumor markers in solid tumor samples from a clinical trial with a pro-apoptotic agent. LSC allows direct, *in situ* measurement of caspase-3 activity in tumor samples on a per cell basis by using DNA content information (32). In this study, we were unable to show that the level of activated caspase-3 is associated with clinical outcome (no patients in the dose-expansion phase showed tumor shrinkage). These results may have been limited by any or all of the following:

1) amount and/or quality of the aspirate (blood contamination, low cellular content, fixation problems, and/or excessive debris); 2) timing of tumor collection (apoptotic cells are cleared rapidly); and 3) biological mechanisms such as potential overexpression of negative regulators of apoptosis such as c-FLIP (33), which inhibits signaling downstream of the death-induced signaling complex, and overexpression of inhibitor of apoptosis proteins (34), which inhibit caspase activation. Alternatively, increased expression of Bcl-2 family members or decreased DR5 expression may have contributed to this result. Understanding these biological factors and their potential relevance for patient selection will be important considerations for future trials. Furthermore, studies to better predict variability in the quality of fine-needle aspiration biopsies (such as the value of real-time cytologic assessment) may help to improve future biomarker assessment.

In summary, conatumumab appeared to be well tolerated and showed preliminary evidence of antitumor activity in adult patients with advanced solid tumors. On the basis of all of the available clinical and preclinical evidence, multiple phase II clinical trials evaluating conatumumab in combination with chemotherapy and targeted agents are currently ongoing in a number of indications (35).

### Disclosure of Potential Conflicts of Interest

Employment and ownership interest of Amgen Inc. (C.-P.H., L.G., G.J., Y.C.H., S.W., J.S.H., G.F.), consultant for Amgen Inc. (R.S.H.), honoraria and research funding from Amgen Inc. (R.K.), research funding from Amgen Inc. (R.S.H., D.S.H.), nothing to disclose (M.V.), research funding from Amgen Inc. (P.M.L.).

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