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ORIGINAL ARTICLE

Phase 1 study of conatumumab, a pro-apoptotic death receptor 5 agonist antibody, in Japanese patients with advanced solid tumors

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Abstract
Purpose Conatumumab is a fully human monoclonal agonist antibody against human death receptor 5 (DR5). The primary objectives of this phase 1 study were to assess the safety, tolerability, and pharmacokinetics (PK) of conatumumab in Japanese patients with advanced solid tumors.
Methods This is an open-label ascending dose study with a starting dose level of 3 mg/kg. Subsequent doses of 10 and 20 mg/kg were planned. Six patients were enrolled into 1 of 3 dose cohorts (3, 10, or 20 mg/kg) of conatumumab administered intravenously once every 2 weeks as a single agent. No conatumumab was administered on day 43 to allow the assessment of terminal PK parameters. The primary endpoints were the incidence of dose-limiting toxicities (DLTs) and assessment of PK parameters of conatumumab.
Results Eighteen patients received at least 1 dose of conatumumab. There were no DLTs observed as defined in the protocol. No patients had an adverse event leading to conatumumab discontinuation. Conatumumab demonstrated dose-linear kinetics. A best response of stable disease was reported in nine patients. Monocytes were found to express DR5 and showed a high degree of conatumumab receptor occupancy after treatment at all dose levels.
Conclusions Conatumumab administered up to 20 mg/kg once every 2 weeks was well tolerated in Japanese patients with advanced solid tumors. Adverse events and PK in these patients were similar to those in the first in human (FIH) study.

Keywords Conatumumab · Advanced solid tumors · Pharmacokinetics · Phase 1 study

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Introduction

Apoptosis is an evolutionarily conserved process for removing unwanted cells from the body. Dysregulation of this process contributes to the development of many diseases including cancer. Apoptosis can be triggered by intracellular events, such as DNA damage, or by extracellular signals, such as tumor necrosis factor (TNF) and TNF-related molecules. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor superfamily and can induce apoptosis by binding to 2 cell-surface receptors called death receptor 4 (DR4) and death receptor 5 (DR5), respectively [1, 2]. TRAIL binding to DR4 and DR5 initiates an intracellular caspase cascade-inducing apoptosis in many transformed cell lines, but not in most normal cells [3].

Conatumumab, a fully human monoclonal agonist antibody (immunoglobulin class G1) against human DR5, is being developed as an anticancer therapy. Conatumumab mimics endogenous TRAIL by binding DR5 and activating caspases, thereby inducing apoptosis in sensitive cells. DR5 agonists may be an effective anticancer therapy in

humans [4]. Conatumumab induced a dose-dependent inhibition of cancer xenografts. This activity significantly enhanced in combination with chemotherapeutic agents. Conatumumab has been shown to have additive or synergistic activity in several cancer cell lines and in xenograft models [5–9]. Conatumumab is designed to induce selective apoptosis in cancer cells and may enhance the activity of standard cancer therapy, molecularly target agents, or both.

The first in human (FIH) study of conatumumab conducted in the United States was a phase 1, open-label multiple-dose, dose-escalation study evaluating the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics of conatumumab administered as monotherapy with advanced solid tumors that were refractory to standard treatment [10]. The doses evaluated in the FIH study were 0.3, 1, 3, 10, and 20 mg/kg. Conatumumab was infused over 30–60 min every 2 weeks to characterize and evaluate the safety, tolerability, and PK after multiple administrations until the disease progression or intolerability of the investigational product. The results of the study showed no apparent relationship between dose level and frequency or severity of adverse events. No dose-limiting toxicities (DLTs) were observed. In addition, no infusion reactions were reported, and no antibodies against conatumumab were detected in the FIH study. Therefore, conatumumab has been considered well tolerated up to 20 mg/kg administered intravenously (IV) in this study. Furthermore, when conatumumab was infused 3 mg/kg administered IV once every 2 weeks, C_{max} was approximately 65 μg/mL, and C_{min} was approximately 20 μg/mL. As this C_{min} value at 3 mg/kg approximated the ED_{90} in a nonclinical xenograft model, the dose of 3 mg/kg is considered to be a potentially active clinical dose. The mean half-life ($t_{1/2,\ z}$) value ranged from 13 to 19 days at the dose of 3–20 mg/kg.

This study is intended to evaluate the safety, tolerability, and PK of conatumumab in three dose schedules in patients with advanced tumors in Japan. Therefore, starting dose was decided at 3 mg/kg, and the preliminary human PK profile supports IV administration once every 2 weeks.

Patients and methods

Patients

Eligible patients had histologically or cytologically confirmed advanced solid tumors that were refractory to standard therapy or for which no curative therapy; age of 20–74 years; life expectancy ≥3 months; Eastern Cooperative Oncology Group performance status ≤1; previous chemotherapy ≥4 weeks before enrollment (6 weeks for prior mitomycin or a nitrosourea); absolute neutrophil count ≥1,500/μL; platelets ≥100,000/μL; hemoglobin ≥9 g/dL; prothrombin time or activated partial thromboplastin time ≤1.5 times the upper limit of normal (ULN); estimate of glomerular filtration rate ≥60 mL/min by Cockcroft and Gault equation; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN (if liver metastases ≤5 × ULN); total bilirubin, amylase, and lipase ≤1.5 × ULN; urinary protein excretion ≤100 mg per day or 0, 1+ using dipstick analysis; absence of pregnancy; and no coexisting severe medical conditions. This protocol was approved by the each institution's institutional review board. Patients gave written informed consent according to institutional guidelines before enrollment.

Study design and drug administration

Conatumumab was to be administered IV over 30 min every 2 weeks. This study is intended to evaluate the safety, tolerability, and PK of conatumumab in three dose schedules in patients with advanced tumors in Japan. Therefore, starting dose was decided at 3 mg/kg. The preliminary human PK profile supports IV administration once every 2 weeks. When conatumumab at a dose of 3 mg/kg was administered as an IV infusion every 2 weeks, observed trough serum concentration (C_{min}) was approximately 20 μg/mL. As this C_{min} value at 3 mg/kg approximated the mean trough concentration required for 90% reduction in a nonclinical xenograft model. Planned dose levels of 3, 10, and 20 mg/kg were examined in cohorts of six patients in the dose-escalation phase. A DLT was defined as a treatment-related, grade 3 or higher hematologic or nonhematologic toxicity (according to the Common Terminology Criteria for Adverse Events [CTCAE] version 3.0), except AST and ALT, and infusion reaction, occurred during the first 28 days after the initial administration. For AST and ALT, the DLT criterion was defined as >300 IU/L.

Patients received conatumumab on days 1, 15, and 29. No conatumumab was administered on day 43 to allow assessment of terminal PK parameters. Conatumumab administration was to resume every 2 weeks starting on day 57, so long as subjects did not experience medication intolerance or disease progression, or did not withdraw consent. Patients were evaluated for tumor response by computed tomography (CT) or magnetic resonance imaging every 8 weeks.

Conatumumab was presented as a sterile, clear, colorless protein solution. Conatumumab was diluted in 0.9% normal saline in a final volume of 100 mL and administered by continuous IV infusion by controlled infusion pump over 60 min for the first dose. Infusion over 30 min in the subsequent dose was allowed, if the first dose was well tolerated.

Dose escalation

Six patients in each cohort were assigned sequentially. A patient was to receive conatumumab in this study until disease progression, inability to tolerate the investigational product, or withdrawal of consent. Cohort dose escalation was to occur if none or 1 of the initial 6 patients experienced a DLT during the first 28 days of the study treatment. If 33% or more of the patients enrolled in a cohort experienced DLTs, then the sponsor, in consultation with the principal investigator, was to decide whether the next cohort can be initiated.

Dose modification

No intrapatient dose modification will be allowed during dose escalation. Patients who achieved complete response, partial response, or stable disease were allowed to continue at the same dose until disease progression or drug intolerance.

Pretreatment and follow-up studies

Pretreatment evaluation included a complete history; physical examination, and routine laboratory studies, including a complete blood count (CBC), white blood count, chemistry (electrolytes, albumin, glucose, blood urea nitrogen, creatinine, uric acid, total bilirubin, ALT, AST, alkaline phosphatase, amylase, lipase, and creatine kinase), urinalysis, electrocardiogram, relevant radiologic studies, and tumor markers. During the study, radiologic studies for disease status were repeated every 8 weeks, and tumor response was assessed by modified Response Evaluation Criteria in Solid Tumors (RECIST). CBC and chemistry were obtained on days 1, 2, 8, 15, 29, 43, and 57, and every 2 weeks thereafter.

Serologic evaluation for human anti-human antibodies

Serum samples for assessment of anti-conatumumab antibodies were collected at predose, on days 15, 29, and 57, and every 8 weeks thereafter. Anti-conatumumab binding antibodies in serum were detected using a validated immunoassay.

Assays for anti-conatumumab antibodies

Serum samples were treated with acetic acid to dissociate antibody complexes and were incubated with a mixture of biotinylated conatumumab, ruthenylated conatumumab, and Tris buffer (pH 9.5) for 15–24 h. The mixture was added to an MSD 6000 streptavidin microtiter plate (blocked with 1X phosphate-buffered saline and 1% bovine serum albumin), the plate was washed, and captured complexes that consisted of anti-conatumumab antibodies bound to both labeled conatumumab were detected by electrochemiluminescence (ECL) in the presence of tripropylamine containing buffer. ECL was measured by an MSD 6000 plate reader (Meso Scale Discovery, Gaithersburg, MD). The limit of detection for this assay was 10 ng/mL of anti-conatumumab antibodies.

Pharmacokinetic assessment

Serum samples for PK assessment were collected at predose, 0.5 (day 1 only), 1, 6, 24, 48, 96, 168, 240, and 336 h postdose, on days 1 and 29. In addition, serum samples were collected at 504 and 672 h postdose on day 29. Serum concentrations of conatumumab were determined using a validated immunoassay. PK parameters for each patient were estimated using noncompartmental methods with WinNonlin Professional (version 5.2.1; Pharsight, Mountain View, CA).

Assay for serum conatumumab

Serum samples were loaded into a 96-well microplate after precoated with a murine anti-conatumumab monoclonal antibody (Amgen Inc., Thousand Oaks, CA, USA). The plate was washed, and a biotinylated rabbit anti-conatumumab polyclonal antibody (Amgen Inc.) and NeutrAvidin conjugated horseradish peroxidase (HRP: Pierce Biotechnology Inc., IL) was added. After another washing step, tetramethylbenzidine (TMB) substrate solution (Bio-Fx, MD) was added and color development was stopped by 2 N sulfuric acid. Intensity of the color (optical density, OD) was measured at 450–650 nm using a microplate reader. The conversion of OD units to concentrations was achieved through the computer software (Watson version 7.0.0.01 data reduction package). The limit of quantification for this assay was 29.9 ng/mL of conatumumab.

Pharmacodynamic assessments

Pharmacodynamic assessments were done by measuring occupancy of the DR5 on peripheral monocytes before and after conatumumab treatment. Whole blood samples were collected on days 1 (predose), 2 (predose), and 15 (predose).

Measurement of DR5 occupancy on monocytes

A flow cytometry assay was used to measure DR5 occupancy on circulating monocytes. It was performed on whole blood specimens using the FACSCalibur (Becton–Dickinson, San Jose, CA, USA) at Mitsubishi Chemical Medience Cooperation (Tokyo, Japan).

Peripheral blood monocytes were gated based on side scatter, CD45 and $CD4^{dim}$ expression. DR5 staining was then evaluated using two monoclonal antibody clones M413 (Amgen Inc.) a fully human monoclonal antibody that is not blocked by conatumumab and DJR2-4 (eBioscience, San Diego, CA)—a mouse IgG1 antibody that is blocked by conatumumab. Median background fluorescence was assessed for each sample as well.

To calculate receptor occupancy (RO), the following formula was used.

$$\mathrm{RO} = 1 - \frac{(DJR2 - 4_{\mathrm{day}n} - \mathrm{background}_{\mathrm{day}n})/(M413_{\mathrm{day}n} - \mathrm{background}_{\mathrm{day}n})}{\mathrm{day}n(DJR2 - 4_{\mathrm{baseline}} - \mathrm{background}_{\mathrm{baseline}})/(M413_{\mathrm{baseline}} - \mathrm{background}_{\mathrm{baseline}})}$$

Similarly, to evaluate normalized changes in DR5 staining for the two monoclonal antibodies (M413 and DJR2-4) individually, the following formulas were used.

$$M413_{\mathrm{day}n} = \frac{(M413_{\mathrm{day}n} - \mathrm{background}_{\mathrm{day}n})}{(M413_{\mathrm{baseline}} - \mathrm{background}_{\mathrm{baseline}})}$$

$$DJR2 - 4_{\mathrm{day}n} = \frac{(DJR2 - 4_{\mathrm{day}n} - \mathrm{background}_{\mathrm{day}n})}{(DJR2 - 4_{\mathrm{baseline}} - \mathrm{background}_{\mathrm{baseline}})}$$

Results

General

Patient demographics are provided for the 18 patients who enrolled in this study in Table 1. All 18 patients received at least three doses of conatumumab. The median treatment days were 64.0 days for 3 mg/kg cohort, 28.5 days for 10 mg/kg cohort, and 99.5 days for 20 mg/kg cohort. At the time of data cutoff, two patients (1 with rectal carcinoid in 3 mg/kg and 1 with leiomyosarcoma in 20 mg/kg) were on treatment with stable disease in week 67 and week 40, respectively.

For the 18 patients, there were no DLTs within the first 28 days of study treatment. No subject had treatment related, serious adverse events. In addition, no infusion reactions have been reported. Anti-conatumumab antibodies were not detected in any of the patients tested.

Averse events

All 18 patients experienced at least one conatumumab-related adverse event during the treatment or the safety follow-up period. The most common conatumumab-related adverse events (i.e., those reported in ≥20% of all patients) were pyrexia (8 [44%] patients), fatigue (7 [39%] patients), nausea (6 [33%] patients), and headache (5 [28%] patients) (Table 2 Hlk252870014). All of the treatment-related events were grade 1 or grade 2 in severity. No apparent dose-related trend was found in the incidence or severity of treatment-related adverse events across the cohorts. One serious adverse event of pain was reported for patient at 10 mg/kg. This serious adverse event was not considered by the investigator to be related to conatumumab. No deaths occurred during the treatment or safety follow-up period. No patients had an adverse event leading to conatumumab discontinuation or withdrawal from the study.

No adverse events of infusion-related reactions were reported in this study.

In order to further explore adverse events that might be attributable to infused conatumumab, an additional ad hoc analysis using broader search terms derived from the CTCAE criteria for allergic reactions and cytokine release syndrome for events that occurred on the same day or the day after dosing was performed. In this analysis, 14 (78%) patients had symptoms that occurred on the same day or the day after dosing. Multiple infusion-related events associated with a single infusion and occurring on the same date were counted as a single infusion-related episode. A total of 31 separate infusions-related episodes were associated with the onset of symptoms in 14 patients. The infusion-related events that occurred in two or more patients were pyrexia (8 [44%] patients); nausea (4 [22%] patients); vomiting (3 [17%] patients); chills, and headache (2 [11%] patients each). Seven patients had multiple infusion-related episodes, and seven patients had a single episode. The infusion-related events occurred with the first infusion in 9 (50%) patients. Among those 9 patients, 1 had recurrent infusion-related episodes (nausea and vomiting). There were no grade 3 or higher infusion-related reactions. Pyrexia occurred in 8 patients (3 patients in cohort 1, 2 patients in cohort 2, and 3 patients in cohort 3). All eight patients experienced pyrexia on day 1. However, pyrexia resolved on the day or the next day of the onset in seven patients, except one patient. In this patient, pyrexia was resolved on

Table 1 Baseline demographics and other patient characteristics

	3 mg/kg Q2W Cohort 1 ($n = 6$)	10 mg/kg Q2W Cohort 2 ($n = 6$)	20 mg/kg Q2W Cohort 3 ($n = 6$)	All patients ($n = 18$)
Age (years)				
Median	57.5	50.5	65.5	58.0
Range	53–67	30–65	52–70	30–70
Sex (n, %)				
Men	4 (67)	3 (50)	2 (33)	9 (50)
Women	2 (33)	3 (50)	4 (67)	9 (50)
ECOG performance status (n, %)				
0	4 (67)	4 (67)	4 (67)	12 (67)
1	2 (33)	2 (33)	2 (33)	6 (33)
Tumor type (n, %)				
Colon or rectum	2 (33)	2 (33)	1 (17)	5 (28)
NSCLC	2 (33)	0 (0)	2 (33)	4 (22)
Stomach	0 (0)	1 (17)	1 (17)	2 (11)
Soft tissue sarcoma*	0 (0)	0 (0)	2 (33)	2 (11)
Pancreatic	1 (17)	0 (0)	0 (0)	1 (6)
Other†	1 (17)	3 (50)	0 (0)	4 (22)

Q2W, once every 2 weeks; *ECOG* Eastern Cooperative Oncology Group

* Includes two leiomyosarcomas, † Includes one adenoid cystic carcinoma, one rectal carcinoid, one invasive thymoma, and one epithelial type malignant pleural mesothelioma

Table 2 Incidence of conatumumab-related adverse events during the first course and all courses

	3 mg/kg Q2W Cohort 1 ($n = 6$)	10 mg/kg Q2W Cohort 2 ($n = 6$)	20 mg/kg Q2W Cohort 3 ($n = 6$)	All patients ($n = 18$)
No of patients reporting at least one event	6 (100)	6 (100)	6 (100)	18 (100)
Pyrexia	3 (50)	2 (33)	3 (50)	8 (44)
Fatigue	2 (33)	3 (50)	2 (33)	7 (39)
Nausea	2 (33)	2 (33)	2 (33)	6 (33)
Headache	2 (33)	3 (50)	0 (0)	5 (28)
Anorexia	2 (33)	1 (17)	0 (0)	3 (17)
Hypoalbuminaemia	2 (33)	1 (17)	0 (0)	3 (17)
Lymphocyte count decreased	2 (33)	1 (17)	0 (0)	3 (17)
Rash	3 (50)	0 (0)	0 (0)	3 (17)
Vomiting	2 (33)	0 (0)	1 (17)	3 (17)
Chills	1 (17)	1 (17)	0 (0)	2 (11)
Lipase increased	1 (17)	1 (17)	0 (0)	2 (11)

Reported-related adverse events were worst grade and occurred with ≥2 patients. All conatumumab-related adverse events were grade 1 or 2

day 3. No patients except two patients experienced pyrexia at second dose or after.

Serologic evaluation for human anti-human antibodies

A total of 18 subjects were tested for the presence or development of anti-conatumumab antibodies. All samples were negative for the presence or development of anti-conatumumab antibodies.

Pharmacokinetic analysis

The serum concentration–time profiles and the PK parameters of conatumumab are shown in Fig. 1 and Table 3, respectively. After IV administrations of conatumumab at 3, 10, and 20 mg/kg once every 2 weeks, the mean $t_{1/2,z}$ value ranged from 10.4 to 11.9 days on day 1 and from 16.8 to 18.8 days on day 29. The accumulation ratio was 1.80, 1.67, and 1.75 for dose cohorts of 3, 10, and 20 mg/kg, respectively. The values of systemic serum clearance (CL) remained similar following three doses of conatumumab in each dose cohort. The mean CL values at 20 mg/kg were slightly higher than those at 3 or 10 mg/kg, resulting in slightly less than dose proportional exposures at 20 mg/kg. The mean values of volume of distribution at steady state on day 29 were slightly higher (<two fold) than those on day 1 in each dose cohort.

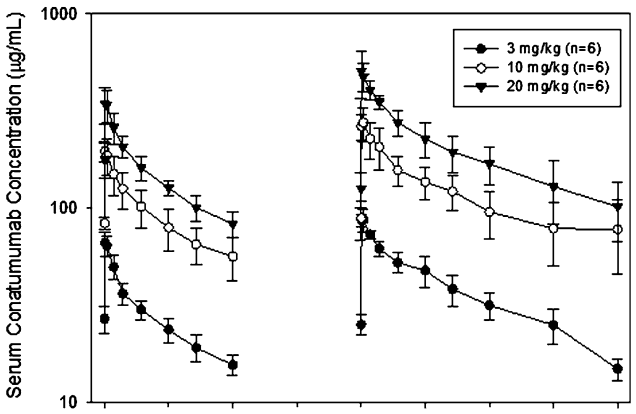

Fig. 1 Mean (±SD) serum concentration–time profiles on day 1 and day 29 after intravenous administrations of 3, 10, or 20 mg conatumumab once every 2 weeks

Pharmacodynamic analysis

Occupancy of DR5 was measured on circulating monocytes by flow cytometry using two monoclonal antibody clones M413 (not blocked by conatumumab) and DJR2-4 (blocked by conatumumab). Overall levels of DR5 expression (as determined by M413) were relatively unchanged on monocytes after treatment while diminished staining with DJR2-4 was observed. Predose and postdose ratios of bound versus total DR5 staining were then used to express receptor occupancy. Levels of DR5 occupancy were approximately 80% and similar in 3, 10, and 20 mg/kg treatment cohorts indicating that even at the lowest dose occupancy reached a maximal level as determined by the detection limit of the assay (Table 4).

Antitumor activity

All 18 patients enrolled in the study had measurable disease at baseline and evaluable postbaseline measurements. Across all 18 patients, 9 had a best tumor response of stable disease based on investigator's evaluation including 3 patients (2 patients with nonsmall cell lung cancer [NSCLC] and 1 patient with rectal carcinoid) in cohort 1, 2 patients (1 patient with adenoid cystic carcinoma and 1 patient with invasive thymoma) in cohort 2 and 4 patients (soft tissue sarcoma, stomach cancer, rectal cancer, NSCLC) in cohort 3 (Table 5).

The maximum percentage reductions in tumor burden, as assessed by RECIST version 1.0 and measured using the sum of the longest tumor diameters. Stable disease defined as failure to achieve a partial response but with no evidence of progression for first CT scan after treatment. Of 18 patients, 5 showed some reduction in tumor burden. One patient with NSCLC showed the maximum tumor reduction of 14.3%.

Discussion

Conatumumab specifically binds the extracellular domain of human DR5 and not DR4 or decoy receptors such as TRAIL R3, TRAIL R4, and osteoprotegerin (OPG) [11, 12]

Table 3 Summary of PK parameters following intravenous administration of conatumumab once every 2 weeks on day 1 and day 29

Dose (mg/kg)	Day	*N*	t_{max} (h)	C_{max} (µg/mL)	$AUC_{0–336}$ (h µg/mL)	$t_{1/2,\ z}$ (day)	CL (mL/h)	Vss (mL)	$AUC_{0–336}$ AR
3	1	6	1.0 (1.0–6.0)	67.7 (7.92)	9,037 (1,101)	11.2 (2.8)	10.84 (2.52)	4,004 (1,173)	1.80 (0.171)
	29	6	1.0 (1.0–6.0)	88.8 (10.4)	16,186 (1,903)	16.8 (3.5)	10.03 (2.19)	5,176 (718)	
10	1	6	1.0 (1.0–6.0)	201 (25.3)	30,083 (6,390)	11.9 (3.8)	10.90 (2.42)	4,228 (692)	1.67 (0.220)
	29	6	3.5 (1.0–6.0)	282 (46.6)	49,717 (9,980)	18.8 (6.2)	11.40 (1.92)	6,837 (2,914)	
20	1	6	1.0 (1.0–6.0)	345 (70.8)	48,517 (5,684)	10.4 (3.4)	15.55 (3.85)	5,180 (1,010)	1.75 (0.247)
	29	6	1.0 (1.0–24)	513 (136)	84,748 (13,136)	17.3 (4.1)	14.27 (2.84)	7,839 (2,554)	

All parameters are reported as mean (SD) values, except for t_{max}, which is reported as a median (range) value

$AUC_{0–336}$ and V_{ss} summary statistics are presented to 0 decimal places. CL and $t_{1/2,\ z}$ summary statistics are presented to 2 and 1 decimal place, respectively. C_{max} summary statistic is presented to 3 significant figures. t_{max} summary statistic is presented to 2 significant figures

$AUC_{0–336}$ AR: $AUC_{0–336}$ accumulation ratio was calculated by dividing the individual $AUC_{0–336}$ value on day 29 by the corresponding individual $AUC_{0–336}$ value on day 1. $AUC_{0–336}$ AR values are presented to 3 significant figures

t_{max}: The time the maximal serum concentration was observed

C_{max} = The maximal observed serum concentration after dosing

$AUC_{0–336}$ = The area under the serum concentration–time curve from time zero to 336 h postdose

$t_{1/2,\ z}$ = Estimated terminal phase half-life

CL = Apparent clearance ($AUC_{0–336}$ was used to estimate CL on day 29)

V_{ss} = Volume of distribution at steady state

Table 4 Summary statistics for DR5 occupancy on circulating monocytes

	3 mg/kg Q2W Cohort 1 ($n = 6$)				10 mg/kg Q2W Cohort 2 ($n = 6$)				20 mg/kg Q2W Cohort 3 ($n = 6$)			
Day	1	2	15	29	1	2	15	29	1	2	15	29
Monocytes (10^9/L)												
Mean	0.314	0.282	0.321	0.301	0.461	0.382	0.328	0.364	0.309	0.174	0.226	0.268
SD	0.1452	0.1142	0.2086	0.1791	0.2701	0.2440	0.0980	0.1455	0.0392	0.0776	0.0718	0.0900
Occupancy (%)												
Mean	0.0	86.4	81.3	–	0.0	86.5	84.5	–	0.0	86.3	81.8	–
SD	–	1.5	3.1	–	–	5.8	4.6	–	–	4.7	4.2	–

Table 5 Tumor response

	3 mg/kg Q2W Cohort 1 ($n = 6$)	10 mg/kg Q2W Cohort 2 ($n = 6$)	20 mg/kg Q2W Cohort 3 ($n = 6$)	All patients ($n = 18$)
Best overall response, *n* (%)				
CR or PR	0 (0)	0 (0)	0 (0)	0 (0)
SD	3 (50)	2 (33)	4 (67)	9 (50)
PD	3 (50)	4 (67)	2 (33)	9 (50)
Disease control rate, *n* (%)				
CR + PR + SD	3 (50.0)	2 (33.3)	4 (66.7)	9 (50.0)
95% CI	11.8–88.2	4.3–77.7	22.3–95.7	26.0–74.0
Maximum percent tumor reduction, %				
Mean	4.1	24.3	4.7	11.0
SD	15.7	29.9	12.0	21.7
Range	−14.3 to 33.2	−5.7 to 79.1	−9.1 to 26.1	−14.3 to 79.1

and mimics the effect of endogenous TRAIL, triggering death in the sensitive cells. Conatumumab induces apoptosis in various human tumor cell lines in vitro. DR4 and DR5 agonists have been demonstrated to induce apoptosis through Fas-associated death signal and caspase activation in a variety of cancer cell lines [13]. The in vivo antitumor activity of conatumumab was found to be significantly enhanced in combination with the chemotherapeutic agents [14]. It was reported that this drug may potentially act synergistically with existing cancer therapies, including targeted agents [11]. These findings suggest that conatumumab could be useful for cancer therapy in combination with a range of existing antitumor therapies on a wide variety of tumor types.

Conatumumab was well tolerated at doses of 3, 10, and 20 mg/kg IV administered once every 2 weeks in patients with advanced tumors in Japan. No dose-related trends were found in the incidence or severity of treatment-emergent adverse events. No adverse events leading to investigational products discontinuation were reported. No treatment-related serious or grade 3 adverse events were reported. All 18 patients experienced at least 1 adverse event that was considered by the investigator to be at least possibly related to conatumumab administration. The most frequently reported of these included pyrexia (44%), fatigue (39%), nausea (33%), and headache (28%). Pyrexia occurring within 24 h of infusion of a monoclonal antibody and resolving within 48 h is a well-described infusion reaction attributed to cytokine release [15].

Hyperamylasemia or hepatic toxicity with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin was reported in other investigational products targeting TRAIL receptors [16–19]. Hypomagnesemia and elevated serum lipase were reported in conatumumab FIH study. In this study, three elevations of lipase (2 grade 1 events and 1 grade 3 event) and 1 elevation of amylase (grade 2 event) were reported; however, there have been no other investigator defined the cases of clinical pancreatitis. No notable hepatic toxicity and hypomagnesemia occurred. The results of this study were consistent with the results of FIH study. In FIH study, no DLTs were observed. The common reported adverse events were pyrexia (46%); fatigue (38%); chills and nausea (24% each); anemia, vomiting, anorexia, dyspnea, and cough (22% each).

Pharmacokinetic analyses from this study confirmed that IV infusion of conatumumab at 3, 10, and 20 mg/kg once every 2 weeks was appropriate to maintain drug

concentrations above target thresholds from preclinical studies. The mean $t_{1/2,z}$ values on day 29 for the dose cohorts ranged from 16.8 to 18.8 days and roughly close to the serum half-life (23 days) of immunoglobulin gamma (IgG) in human [20] and the result in the US study. The accumulation ratio for each dose cohort was below two, indicating no remarkable accumulation after repeat IV administration of conatumumab at the dose levels. Slightly higher clearance of conatumumab was indicated at 20 mg/kg, compared with that at 3 or 10 mg/kg.

In addition, analyses of DR5 levels were conducted via a receptor occupancy assay in peripheral blood. Monocytes were found to express DR5 [21–24]. The receptor occupancy on circulating monocytes showed that >80% of DR5 on the monocytes is bound by conatumumab at the lowest dose for up to 2 weeks after dosing. These results suggest that 3 mg/kg of conatumumab may be sufficient for occupancy of DR5, at least for cells in circulation. Despite conatumumab bound to DR5 on the monocytes, a decrease in the percentage of circulating monocytes was not generally observed with conatumumab treatment. Although DR5 mRNA is expressed in most cell types [25]; most normal cells are resistant to TRAIL or TRAIL receptor agonistic antibodies [3, 13, 26, 27]. Additional conditions or stimulations may be essential for induction of apoptosis in normal cells. TRAIL-deficient mice display increased susceptibility to tumor metastasis, autoimmune disease progression, and possible defects in thymocyte negative selection [28, 29]. TRAIL receptor-deficient mice are not lethal and develop normally but show enhanced cytokine production in macrophages and dendritic cells to ex vivo or in vivo challenges [30], suggesting that the TRAIL receptors may regulate innate immune responses. Conatumumab is an agonist of the DR5 and is anticipated to have effects opposite to those noted in TRAIL-deficient mice. It should be noted that DR5 occupancy was measured on circulating cells, but the results may not always reflect that on tumors. It may be difficult to assess DR5 occupancy on the tumor unless biopsy would be performed following conatumumab administration. Therefore, surrogate markers for it should be developed.

The levels of several intracellular molecules that could be released into serum or activated upon apoptosis induction were examined as pharmacodynamic markers of conatumumab. These included both direct and indirect markers of TRAIL-induced apoptosis. Administration of conatumumab resulted in an increase in serum caspase-3 activity in colorectal cancer tumor xenograft model. In the conatumumab FIH study, the preliminary analyses demonstrated a statistically significant difference among time points with respect to percent change in caspase 3/7 from baseline [10]. In addition, dose-dependent increases in serum cleaved caspase 3/7 and circulating genomic DNA were also noted in ~50% of patients with colorectal, NSCLC, and sarcoma treated with Apo2L/TRAIL in the phase I study [31]. We examined some possible serum or plasma markers such as caspases, cytokeratins, and genomic DNAs. Most of these markers are almost stable from baseline to day 50; no apparent trend was observed (data not shown). Since it is difficult to find biomarker candidates in such a study with small patient numbers, further investigation should be taken over in the next clinical studies.

In this study, tumor response to conatumumab treatment was measured by CT scan using modified RECIST version 1.0. Of 18 patients, 5 showed some reduction in tumor burden. The patient with NSCLC showed the maximum tumor reduction in 14.3%. Of 18 patients, 9 had a best tumor response of stable disease based on investigator's evaluation. In fact, 2 patients remained on study as of the data cutoff date (February 22, 2009) in week 67 and week 40 of their treatment. Follow-up information on these two patients indicated that, as of January 04, 2010, one remained on study for 85 weeks (over 1.5 years) and had received approximately 40 doses of conatumumab. The second patient discontinued the study on May 13, 2009, due to progressive disease. This patient had been on study for 80 weeks (over 1.5 years) and had received approximately 37 doses. These results provide the evidence of long-term clinical benefit in these two patients. The best response was confirmed in one patient with adenocarcinoma NSCLC who had failed first-line treatment (paclitaxel and carboplatin). This patient had received low dose of conatumumab (3 mg/kg). In previous FIH study, two objective tumor responses were seen each one patient with NSCLC and colorectal cancer at lower dose (0.3 mg/kg every 2 weeks).

In summary, conatumumab was shown to be well tolerated with an acceptable safety profile. Despite doses as high as 20 mg/kg, a maximum tolerated dose was not reached, and no DLTs were observed. The safety and PK profiles of conatumumab in this study were similar to those in the FIH study. Furthermore, at least two patients demonstrated evidence of clinical activity as demonstrated by a RECIST response or disease stabilization of substantial duration. These results warrant further investigation of conatumumab, in combination with other therapies, as a potential therapy for advanced cancers.

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