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**Original**

# Thirteen-week Intravenous Toxicity Study of a Novel Humanized Anti-Human Death Receptor 5 Monoclonal Antibody, CS-1008, in Cynomolgus Monkeys

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**Abstract:** CS-1008, a humanized monoclonal antibody that is agonistic to human death receptor 5, was intravenously administered to cynomolgus monkeys twice a week for 13 weeks at 3 different dose levels (5, 15 and 42 mg/kg) in order to evaluate its potential toxicity. A control group received phosphate buffered saline containing 0.01% polysorbate 80. Each of the 4 groups consisted of 3 male and 3 female cynomolgus monkeys. No animal in any group died during the dosing period. No toxic changes in clinical signs, food consumption, body weight, electrocardiography, ophthalmology, urinalysis, hematology, blood chemistry, gross pathology, organ weights or histopathology were noted in any group during the dosing period. In the toxicokinetic analysis, the values for the maximum concentration of CS-1008 in plasma and the area under the curve generally increased with increasing dose. No clear differences in the toxicokinetic parameters or profiles were observed between the sexes. Development of anti-CS-1008 antibodies was not detected in any sample. The no-observed adverse-effect level (NOAEL) of CS-1008 in cynomolgus monkeys under the conditions of this study was concluded to be 42 mg/kg in both sexes, when administered intravenously twice a week for 13 weeks. This study supports the development of CS-1008 as a therapeutic biopharmaceutical. (J Toxicol Pathol 2010; **23**: 11–17)

**Key words:** death receptor 5, humanized monoclonal antibody, CS-1008, cynomolgus monkey

## Introduction

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily of cytokines, induces apoptosis in cancer cells *in vitro* and has potent anti-tumor activity against tumor xenografts of various cancers[1]. Although TRAIL is not thought to show cytotoxic effects on normal human hepatocytes, several published studies suggest potential liver toxicity[2–4]. Human death receptor 5 (DR5, or TRAIL-R2), which is 1 of the 5 receptors for TRAIL, can be detected in human cancers, including pancreatic, gastric, colon, breast and non-small cell lung cancer, with low or no expression in normal tissues[5–8]. An agonistic monoclonal antibody (mAb) against DR5 would be expected to be a therapeutic cancer antibody, as it preferentially induces apoptosis of tumor cells while having little or no effect on normal cells. TRA-8, a murine anti-DR5 mAb, shows *in vitro* cytotoxicity in various human tumor cell lines and *in vivo* anti-tumor efficacy in murine xenograft models of human cancer[9]. CS-1008 is a humanized mAb composed of the complementarity determining regions of TRA-8 and the variable region framework and constant regions of human immunoglobulin IgG1. In previous pharmacologic evaluations, CS-1008 induced cell death in various DR5-expressing human tumor cell lines, without inducing cell death of human primary hepatocytes[10].

The objective of this study was to investigate the potential toxicity of CS-1008 following 13 weeks of intravenous dosing in a relevant animal species.

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## Materials and Methods

### *Animals*

In order to determine a relevant animal species for toxicologic assessment, the following investigations were conducted in accordance with the 1997 International

Conference on Harmonization "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" guidelines. Analysis of the cDNA sequence of cynomolgus-monkey DR5, measurement of the binding affinity of CS-1008 to human and cynomolgus-monkey DR5, cross-reactivity survey of CS-1008 immunohistochemical staining in human and cynomolgus-monkey tissues and confirmation of the functional pathway for DR5-mediated apoptosis in a monkey-cell line were all performed. The cDNA sequences of monkey DR5 were similar to those of human DR5. Based on comparative binding kinetics of CS-1008 to human and cynomolgus monkey DR5, CS-1008 can be considered to have similar affinities for human DR5 and monkey DR5. Cross-reactivity testing demonstrated immunohistochemical-staining similarities between normal human and cynomolgus monkey tissues. In addition, the pharmacological activity observed in a cynomolgus monkey-derived cell line that expresses DR5 suggests that the mechanism for CS-1008-mediated cell-death induction is functioning. The results indicated that the cynomolgus monkey is a relevant animal species for toxicology assessment for this study.

In this study, 12 male and 12 female purpose-bred cynomolgus monkeys (*Macaca fascicularis*), aged 3–5 years and weighing 2.4–3.9 kg at the initiation of dosing, were purchased from Guangdong Scientific Instruments & Materials Import / Export Corporation (Guangzhou, China) and used. The monkeys were randomly assigned to 1 of 4 groups in order to achieve approximately equal mean body weights among the groups. The animals were housed individually in stainless steel cages residing in a room maintained at 24.4–27.5°C with 45–67% relative humidity and a 12-hour light and dark cycle. Harlan Teklad Global Certified 25% Protein Primate Diet (Harlan Sprague Dawley Inc.) was provided once daily to each animal in the afternoon. Water was available *ad libitum* from an automatic supply. This study was approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd.

### *CS-1008 and control*

CS-1008 in a concentration of 10.60 mg/mL in phosphate buffered saline (PBS) containing 0.01% polysorbate 80 was prepared in-house and stored at or below –70°C until use. As the control, PBS containing 0.01% polysorbate 80 was also prepared and stored in the same manner. CS-1008 was returned to room temperature prior to use. The control was thawed under running water and returned to room temperature prior to use.

### *Treatment*

Twice a week for 13 weeks, 1 of 3 dose levels of CS-1008 (5, 15 or 42 mg/kg; 0.47, 1.42 or 4 mL/kg) or the control (PBS containing 0.01% polysorbate 80, 4 mL/kg) was administered in accordance with the dosing group to which the animal had been assigned. Each group consisted of 3 male and 3 female cynomolgus monkeys. The CS-1008 or control was intravenously injected into the cephalic vein of the forearm at an injection rate of 5 mL/min using a disposable syringe and either a needle or an indwelling needle. The highest dose level was set by taking into account the concentration of CS-1008 and the maximum feasible dose volume. The lower dose levels were set in a geometric ratio of approximately 3.

### *Clinical evaluations*

Clinical signs of all the animals were observed 3 times daily on the dosing days, once daily on non-dosing days and once on the day of gross pathology. Food consumption of all animals was recorded daily and was ascertained from the amount of food supplied to each animal and the amount remaining. For each week, the mean value consumed per day was taken as the daily food consumption. All animals were weighed using an electronic balance (HP-40K, A&D Company, Limited) twice during the acclimation period, once weekly after initiation of dosing and once on the day of gross pathology. Electrocardiograms were recorded without anesthesia using an electrocardiograph system for animals (Cardisuny α6000AX-D, Fukuda M-E Kogyo Co., Ltd.) by the standard lead method before initiation of dosing (time corresponding to approximately 1 hour after administration) and approximately 1 hour after administration at Week 13 of dosing. Heart rate, PR interval, QRS duration, QT interval and QTc from the wave of lead II were measured. Ophthalmologic examinations were performed under ketamine hydrochloride anesthesia (Fuji, approximately 10 mg/kg, Fuji Chemical Industry Co., Ltd.) by intramuscular injection before initiation of dosing and after administration at Week 13 of dosing. The optic media and ocular fundus were observed after instillation of a mydriatic drug (Mydrin®-P, Santen Pharmaceutical Co., Ltd.). The anterior portion of the eye and the optic media were examined visually using a penlight and slit lamp (SL-14, Kowa Co., Ltd.). The ocular fundus was examined using an indirect ophthalmoscope (Genesis, Kowa Co., Ltd.).

### *Clinical laboratory tests*

Blood was drawn from the femoral vein before initiation of dosing and once at Weeks 4, 8 and 13 of dosing with a syringe containing 3.8% weight/volume (w/v) sodium citrate solution as an anticoagulant. Plasma was obtained by centrifugation, and coagulation parameters were measured using an automatic blood coagulation-measuring apparatus (CA-5000, Sysmex Corporation). For measurement of other hematologic parameters, whole blood was drawn with a syringe and treated with an anticoagulant (EDTA-2K). Blood smears were prepared for measurement of differential leukocytes. The hematologic parameters were measured using a hematology system (ADVIA120, Bayer Diagnostics Manufacturing Ltd.). In addition, blood was drawn from the femoral vein and left at room temperature for 20–60 minutes. Serum was obtained by centrifugation, and blood-chemistry parameters were measured with an automatic analyzer (JCA-BM8, JEOL Co., Ltd.). Urinalysis was performed before

initiation of dosing and after administration at Week 13 of dosing. Urine samples at 2 hours and 16 hours were collected in a metabolic cage. Color, pH, glucose, ketone bodies, bilirubin, occult blood, urobilinogen, protein and sediments in fresh urine were evaluated using an automatic urine analyzer (Clinitek 200+, Miles Labs., Inc.) or an automated urine quantitative analyzer (UM-3410, Arkray Factory, Inc.). Urine volume, specific gravity, N-Acetyl-beta-D-glucosaminidase activity, and osmolarity in 16-hour preserved urine were also evaluated using a measuring cylinder, a urinary refractometer (URICON-JE, Atago Co., Ltd.), a spectrophotometer (U-3200, Hitachi, Ltd.) or a fully Automated Osmotic Pressure Meter OM-6050 (Osmostation™, Arkray Factory, Inc.).

*Pathology*

At the end of the dosing period, all animals were weighed and euthanized by exsanguination under anesthesia by an intravenous injection of sodium pentobarbital solution (Tokyo Kasei Kogyo Co., Ltd.) into the tail vein. External appearance, internal organs and tissues were observed macroscopically. Organs were weighed using an electronic balance (HR-200 and HF-3000, A&D Company, Limited). Relative organ weights were calculated from the body weight on the day of gross pathology. In the case of bilateral organs weighed separately, the total bilateral weight was calculated. The organs and tissues were fixed in 10% neutral buffered formalin. The eyeballs and optic nerves were fixed in a solution of formaldehyde and glutaraldehyde, and the testes were fixed in Bouin's solution. The organs and tissues were embedded in paraffin and sectioned. The paraffin sections were hematoxylin-eosin-stained and examined microscopically.

*Toxicokinetics*

Blood was drawn from the femoral vein with a syringe containing sodium heparin before administration of CS-1008 or the control and 1, 7, 24 and 72 hours after administration on Day 1; 72 hours after administration at Weeks 4 and 8; and before administration and 1, 7, 24 and 96 hours after administration at Week 13. The samples were immediately centrifuged to obtain plasma. The plasma obtained was stored in a freezer. The plasma concentrations of CS-1008 were measured using an enzyme-linked immunosorbent assay (ELISA), and the toxicokinetic parameters were calculated.

*Anti-CS-1008 antibody titer measurement in plasma*

Blood was drawn from the femoral vein with a syringe containing sodium heparin on Day 1 of dosing, 72 hours after administration at Weeks 4 and 8 of dosing and 96 hours after administration on the final dosing day (the day of gross pathology). The samples were immediately centrifuged to obtain plasma. The plasma obtained was frozen. The plasma concentrations of anti-CS-1008 antibody were determined using an ELISA method.

*Statistical analysis*

Data on food consumption, body weight, electrocardiography, urinalysis, hematology, blood chemistry and organ weights were first analyzed for homogeneity of variance by Bartlett's test. If the variance was homogeneous, Dunnett's test was applied to compare the means of the CS-1008 groups with that of the control group. If the variance was heterogeneous by Bartlett's test, a non-parametric Dunnett's test was applied to compare the mean ranks of the CS-1008 groups with that of the control group. Data from clinical signs, ophthalmology, urinalysis (except for quantitative data), gross pathology and histopathology were not analyzed statistically.

## Results

No animals died, and no abnormalities were observed in any group during the dosing period in terms of clinical signs, food consumption or body weight (Fig. 1). In electrocardiography, no abnormal waveforms were noted in any group, and no CS-1008-related changes were noted in the heart rate, PR interval, QRS duration, QT interval or QTc. No CS-1008-related abnormalities were noted in any group at Week 13 of dosing in the ophthalmologic examination. In addition, no test article-related changes were noted in any group during the dosing period in the urinalysis or the hematologic examination (Table 1).

Blood-chemical examinations revealed no CS-1008-related changes in any group throughout the dosing period (Table 2). When compared with the control group, a high bilirubin level was noted in male monkeys in the 42 mg/kg group at Week 13 of dosing. However, this change was judged unrelated to CS-1008 administration as there were no differences from the pre-dosing values. No changes were seen in any group for the serum liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase. Low blood urea nitrogen was noted in males in the 5 mg/kg group at Week 13 of dosing; however, this change was judged unrelated to the test article since the individual values were within the range of the background data.

No CS-1008-related changes were noted in any group at the end of the dosing period in the organ weight measurements (Table 3). When compared with the control group, low kidney weights were noted in females in the 5 and 15 mg/kg groups; however, these changes were judged toxicologically insignificant since they were not dose-related, and the individual values were within the range of the background data. In regard to gross pathology, no CS-1008-related changes were noted in any organ in any group at the end of the dosing period. Histopathology revealed glomerulosclerosis with thickening of the Bowman's capsule, regeneration of tubules and interstitial fibrosis in the kidneys in 1 male from the 42 mg/kg group; however, these changes were judged toxicologically insignificant as they were unilateral and focal. No CS-1008-related changes including apoptotic bodies were found in the liver.

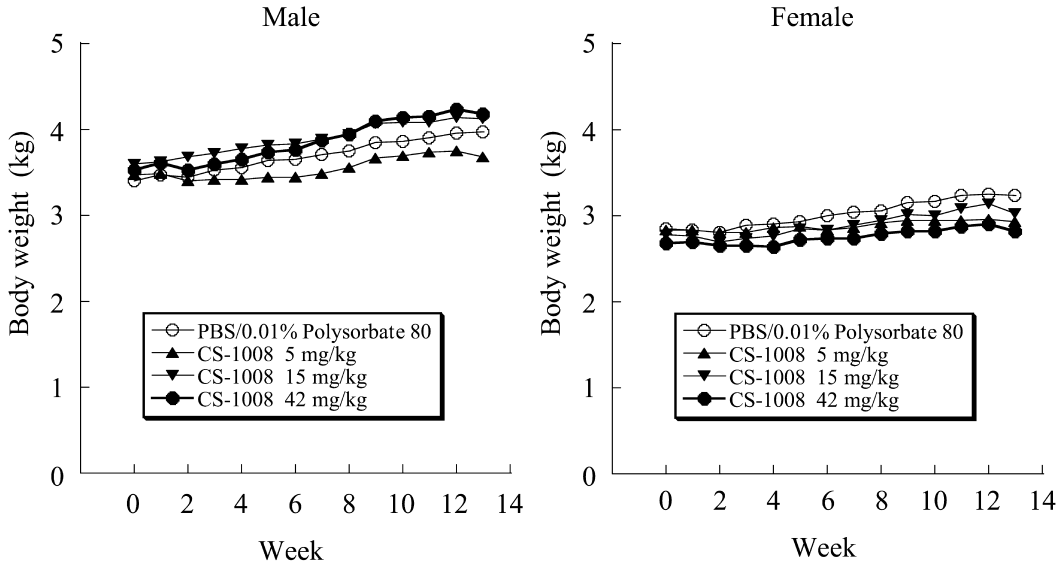


**Fig. 1.** Body weights of cynomolgus monkeys (males, left panel; females, right panel) treated for 13 weeks with 1 of 3 doses of CS-1008 (5, 15 or 42 mg/kg) or phosphate buffered saline (PBS) containing 0.01% polysorbate 80.

**Table 1.** Primary Hematology Parameters in Cynomolgus Monkeys Treated with CS-1008 or Phosphate Buffered Saline Containing 0.01% Polysorbate 80 (Control)

| Parameter | Sex | Time of analysis | Control | CS-1008 dose (mg/kg) | | |
|---|---|---|---|---|---|---|
| | | | | 5 | 15 | 42 |
| Red blood cell count ($\times 10^6$/mm$^3$) | Male | Before dosing | 5.62 ± 0.48 | 6.08 ± 0.16 | 5.15 ± 0.38 | 5.89 ± 0.36 |
| | | Week 4 | 5.24 ± 0.38 | 5.92 ± 0.11 | 5.03 ± 0.43 | 5.50 ± 0.43 |
| | | Week 8 | 5.27 ± 0.29 | 5.96 ± 0.25 | 5.14 ± 0.44 | 5.45 ± 0.42 |
| | | Week 13 | 5.30 ± 0.20 | 6.02 ± 0.13 | 5.31 ± 0.54 | 5.66 ± 0.38 |
| | Female | Before dosing | 5.43 ± 0.28 | 5.16 ± 0.05 | 5.12 ± 0.50 | 5.82 ± 0.36 |
| | | Week 4 | 5.43 ± 0.18 | 5.02 ± 0.09 | 5.03 ± 0.32 | 5.41 ± 0.24 |
| | | Week 8 | 5.28 ± 0.15 | 4.79[a)] ± 0.04 | 5.11 ± 0.11 | 5.31 ± 0.16 |
| | | Week 13 | 5.51 ± 0.21 | 5.06 ± 0.17 | 5.49 ± 0.02 | 5.56 ± 0.39 |
| White blood cell count ($\times 10^3$/mm$^3$) | Male | Before dosing | 14.98 ± 5.66 | 14.58 ± 6.69 | 8.93 ± 2.05 | 17.68 ± 7.26 |
| | | Week 4 | 13.73 ± 4.12 | 12.81 ± 6.12 | 8.39 ± 1.87 | 12.02 ± 6.50 |
| | | Week 8 | 15.13 ± 3.99 | 14.82 ± 5.54 | 10.56 ± 2.69 | 11.63 ± 5.55 |
| | | Week 13 | 14.91 ± 2.42 | 14.80 ± 7.05 | 11.83 ± 2.82 | 10.88 ± 1.44 |
| | Female | Before dosing | 11.04 ± 1.61 | 13.44 ± 6.37 | 8.98 ± 1.82 | 12.40 ± 2.36 |
| | | Week 4 | 11.88 ± 1.68 | 12.11 ± 6.50 | 8.36 ± 1.90 | 11.75 ± 4.35 |
| | | Week 8 | 13.29 ± 0.63 | 12.42 ± 6.24 | 8.39 ± 1.83 | 11.76 ± 3.97 |
| | | Week 13 | 11.55 ± 0.91 | 11.84 ± 5.41 | 10.89 ± 1.77 | 12.46 ± 4.48 |
| Lymphocyte count ($\times 10^3$/mm$^3$) | Male | Before dosing | 11.72 ± 5.34 | 11.12 ± 6.43 | 5.19 ± 1.07 | 12.06 ± 6.31 |
| | | Week 4 | 10.73 ± 3.68 | 10.14 ± 5.67 | 5.15 ± 1.16 | 6.38 ± 2.19 |
| | | Week 8 | 11.72 ± 3.16 | 11.83 ± 5.87 | 6.12 ± 1.14 | 7.46 ± 2.05 |
| | | Week 13 | 11.16 ± 2.94 | 11.85 ± 6.39 | 7.39 ± 1.30 | 7.89 ± 0.72 |
| | Female | Before dosing | 7.71 ± 1.86 | 8.57 ± 4.02 | 5.64 ± 0.90 | 7.32 ± 2.57 |
| | | Week 4 | 8.01 ± 1.04 | 8.11 ± 4.26 | 5.29 ± 0.42 | 6.57 ± 3.28 |
| | | Week 8 | 9.92 ± 1.09 | 8.31 ± 4.59 | 5.50 ± 1.41 | 7.42 ± 3.14 |
| | | Week 13 | 8.97 ± 1.24 | 8.10 ± 3.74 | 6.92 ± 1.31 | 7.86 ± 3.63 |
| Neutrophil count ($\times 10^3$/mm$^3$) | Male | Before dosing | 2.44 ± 1.25 | 2.79 ± 0.68 | 3.16 ± 1.54 | 4.77 ± 3.14 |
| | | Week 4 | 2.26 ± 0.82 | 2.08 ± 0.29 | 2.65 ± 1.84 | 5.10 ± 4.30 |
| | | Week 8 | 2.74 ± 1.05 | 3.02 ± 0.69 | 3.65 ± 2.37 | 3.59 ± 3.45 |
| | | Week 13 | 2.97 ± 1.53 | 2.26 ± 0.62 | 3.80 ± 2.19 | 2.41 ± 0.76 |
| | Female | Before dosing | 2.75 ± 1.53 | 4.04 ± 2.48 | 2.86 ± 0.81 | 4.43 ± 0.42 |
| | | Week 4 | 3.18 ± 1.23 | 3.30 ± 1.95 | 2.64 ± 1.49 | 4.65 ± 1.31 |
| | | Week 8 | 2.48 ± 1.77 | 3.41 ± 1.45 | 2.48 ± 0.67 | 3.78 ± 0.63 |
| | | Week 13 | 2.01 ± 0.54 | 3.07 ± 1.48 | 3.46 ± 0.91 | 4.02 ± 0.63 |

All values given as means ± standard deviation.

[a)]: Significantly different from the control group at $P<0.01$ compared with control.

**Table 2.** Primary Blood-Chemistry Parameters in Cynomolgus Monkeys Treated with CS-1008 or Phosphate Buffered Saline Containing 0.01% Polysorbate 80 (Control)

| Parameter | Sex | Time of analysis | Control | CS-1008 dose (mg/kg) | | |
|---|---|---|---|---|---|---|
| | | | | 5 | 15 | 42 |
| AST | Male | Before dosing | 26 ± 8 | 26 ± 7 | 30 ± 8 | 25 ± 8 |
| (IU/L) | | Week 4 | 23 ± 5 | 27 ± 5 | 31 ± 13 | 33 ± 22 |
| | | Week 8 | 28 ± 11 | 24 ± 1 | 30 ± 9 | 23 ± 4 |
| | | Week 13 | 23 ± 3 | 22 ± 3 | 27 ± 6 | 23 ± 7 |
| | Female | Before dosing | 21 ± 1 | 25 ± 6 | 33 ± 8 | 29 ± 6 |
| | | Week 4 | 21 ± 2 | 25 ± 8 | 39 ± 13 | 28 ± 14 |
| | | Week 8 | 28 ± 7 | 27 ± 4 | 32 ± 12 | 32 ± 18 |
| | | Week 13 | 22 ± 5 | 24 ± 7 | 24 ± 7 | 25 ± 11 |
| ALT | Male | Before dosing | 26 ± 11 | 32 ± 20 | 36 ± 13 | 27 ± 10 |
| (IU/L) | | Week 4 | 28 ± 14 | 21 ± 6 | 41 ± 21 | 24 ± 6 |
| | | Week 8 | 32 ± 15 | 23 ± 5 | 36 ± 14 | 26 ± 8 |
| | | Week 13 | 28 ± 12 | 20 ± 7 | 36 ± 14 | 23 ± 6 |
| | Female | Before dosing | 35 ± 4 | 40 ± 27 | 49 ± 22 | 81 ± 48 |
| | | Week 4 | 37 ± 4 | 22 ± 3 | 72 ± 52 | 67 ± 45 |
| | | Week 8 | 48 ± 15 | 47 ± 18 | 64 ± 50 | 69 ± 48 |
| | | Week 13 | 36 ± 8 | 19 ± 3 | 35 ± 13 | 40 ± 32 |
| ALP | Male | Before dosing | 1387 ± 207 | 1302 ± 237 | 1210 ± 469 | 1476 ± 284 |
| (IU/L) | | Week 4 | 1326 ± 295 | 1291 ± 315 | 1302 ± 212 | 1535 ± 294 |
| | | Week 8 | 1307 ± 295 | 1275 ± 291 | 1304 ± 192 | 1420 ± 169 |
| | | Week 13 | 1300 ± 327 | 1272 ± 202 | 1363 ± 163 | 1435 ± 135 |
| | Female | Before dosing | 772 ± 308 | 593 ± 161 | 410 ± 140 | 632 ± 311 |
| | | Week 4 | 833 ± 210 | 588 ± 109 | 452 ± 205 | 601 ± 334 |
| | | Week 8 | 774 ± 229 | 508 ± 85 | 468 ± 196 | 563 ± 290 |
| | | Week 13 | 772 ± 110 | 580 ± 156 | 457 ± 179 | 546 ± 234 |
| LDH | Male | Before dosing | 556 ± 252 | 549 ± 158.9 | 510 ± 43 | 522 ± 29 |
| (IU/L) | | Week 4 | 721 ± 221 | 693 ± 232.6 | 596 ± 200 | 872 ± 623 |
| | | Week 8 | 966 ± 723 | 514 ± 70.6 | 611 ± 110 | 590 ± 145 |
| | | Week 13 | 730 ± 57 | 545 ± 119.1 | 549 ± 134 | 727 ± 276 |
| | Female | Before dosing | 379 ± 88 | 420 ± 132.9 | 524 ± 207 | 390 ± 29 |
| | | Week 4 | 425 ± 72 | 624 ± 316.7 | 626 ± 49 | 410 ± 102 |
| | | Week 8 | 699 ± 223 | 676 ± 285.1 | 486 ± 84 | 403 ± 56 |
| | | Week 13 | 541 ± 230 | 748 ± 546.2 | 404 ± 99 | 440 ± 110 |
| Total bilirubin | Male | Before dosing | 0.20 ± 0.03 | 0.19 ± 0.04 | 0.20 ± 0.03 | 0.28 ± 0.02 |
| (mg/dL) | | Week 4 | 0.20 ± 0.05 | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.30 ± 0.13 |
| | | Week 8 | 0.21 ± 0.05 | 0.20 ± 0.02 | 0.19 ± 0.02 | 0.27 ± 0.01 |
| | | Week 13 | 0.18 ± 0.04 | 0.18 ± 0.01 | 0.21 ± 0.02 | 0.26[a)] ± 0.04 |
| | Female | Before dosing | 0.16 ± 0.01 | 0.19 ± 0.08 | 0.22 ± 0.09 | 0.18 ± 0.05 |
| | | Week 4 | 0.17 ± 0.05 | 0.20 ± 0.08 | 0.27 ± 0.12 | 0.19 ± 0.05 |
| | | Week 8 | 0.18 ± 0.06 | 0.22 ± 0.04 | 0.23 ± 0.06 | 0.21 ± 0.03 |
| | | Week 13 | 0.16 ± 0.02 | 0.22 ± 0.05 | 0.19 ± 0.03 | 0.18 ± 0.03 |
| BUN | Male | Before dosing | 23.1 ± 2.8 | 19.9 ± 5.4 | 23.8 ± 6.7 | 21.8 ± 4.5 |
| (mg/dL) | | Week 4 | 26.3 ± 7.3 | 19.5 ± 3.7 | 23.7 ± 5.1 | 21.5 ± 1.7 |
| | | Week 8 | 28.2 ± 4.6 | 20.7 ± 4.2 | 24.8 ± 5.0 | 22.2 ± 0.5 |
| | | Week 13 | 29.8 ± 4.6 | 20.5[a)] ± 3.3 | 23.7 ± 4.3 | 23.2 ± 0.9 |
| | Female | Before dosing | 17.5 ± 3.5 | 21.9 ± 8.5 | 19.0 ± 5.9 | 18.1 ± 4.0 |
| | | Week 4 | 20.7 ± 4.3 | 21.6 ± 3.4 | 22.0 ± 4.1 | 19.7 ± 4.5 |
| | | Week 8 | 20.9 ± 5.3 | 21.1 ± 2.6 | 23.4 ± 2.2 | 22.0 ± 8.0 |
| | | Week 13 | 20.9 ± 2.7 | 19.8 ± 5.0 | 23.7 ± 1.0 | 20.6 ± 6.1 |
| Creatinine | Male | Before dosing | 0.67 ± 0.12 | 0.69 ± 0.02 | 0.61 ± 0.02 | 0.53 ± 0.02 |
| (mg/dL) | | Week 4 | 0.60 ± 0.81 | 0.67 ± 0.04 | 0.64 ± 0.07 | 0.60 ± 0.06 |
| | | Week 8 | 0.62 ± 0.09 | 0.68 ± 0.02 | 0.61 ± 0.03 | 0.60 ± 0.06 |
| | | Week 13 | 0.64 ± 0.09 | 0.70 ± 0.04 | 0.68 ± 0.05 | 0.65 ± 0.03 |
| | Female | Before dosing | 0.51 ± 0.06 | 0.62 ± 0.07 | 0.53 ± 0.05 | 0.58 ± 0.03 |
| | | Week 4 | 0.50 ± 0.08 | 0.59 ± 0.12 | 0.53 ± 0.03 | 0.59 ± 0.05 |
| | | Week 8 | 0.48 ± 0.03 | 0.60 ± 0.10 | 0.54 ± 0.10 | 0.56 ± 0.05 |
| | | Week 13 | 0.50 ± 0.08 | 0.61 ± 0.14 | 0.54 ± 0.10 | 0.59 ± 0.07 |

All values given as means ± standard deviation. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AST: aspartate aminotransferase. BUN: blood urea nitrogen. LDH: lactate dehydrogenase. [a)]: Significantly different from the control group at $P<0.05$.

**Table 3.** Primary Organ Weights at the End of the Dosing Period in Cynomolgus Monkeys Treated with CS-1008 or Phosphate Buffered Saline Containing 0.01% Polysorbate 80 (Control)

| Organ weight (g) | Sex | Control | CS-1008 dose (mg/kg) | | |
|---|---|---|---|---|---|
| | | | 5 | 15 | 42 |
| Liver | Male | 74.6 ± 12.8 | 59.5 ± 8.5 | 70.2 ± 9.4 | 76.5 ± 2.5 |
| | Female | 65.8 ± 12.7 | 53.0 ± 6.0 | 54.2 ± 4.7 | 55.8 ± 7.0 |
| Kidney[a)] | Male | 14.5 ± 2.0 | 13.7 ± 1.3 | 17.1 ± 2.3 | 16.9 ± 1.5 |
| | Female | 13.4 ± 1.9 | 10.8[b)] ± 0.6 | 10.4[b)] ± 0.4 | 12.0 ± 0.6 |
| Spleen | Male | 6.2 ± 2.4 | 3.6 ± 1.0 | 4.0 ± 0.9 | 7.0 ± 1.6 |
| | Female | 5.4 ± 2.0 | 2.8 ± 0.5 | 2.7 ± 0.2 | 4.0 ± 0.7 |
| Thymus | Male | 5.4 ± 1.7 | 3.7 ± 1.7 | 3.9 ± 1.9 | 2.9 ± 0.3 |
| | Female | 3.0 ± 1.6 | 2.1 ± 0.5 | 2.6 ± 1.0 | 2.2 ± 1.1 |

All values given as means ± standard deviation. [a)]: The sum of the right and left kidneys. [b)]: Significantly different from the control group at $P<0.05$.

**Table 4.** Toxicokinetic Parameters in Cynomolgus Monkeys Treated with CS-1008

| Time of analysis | Parameter | Sex | CS-1008 dose (mg/kg) | | |
|---|---|---|---|---|---|
| | | | 5 | 15 | 42 |
| Day 1 | $C_{max}$ ($\mu$g/mL) | Male | 135.0 ± 15.4 | 333.2 ± 7.2 | 1124.0 ± 172.0 |
| | | Female | 124.1 ± 22.1 | 430.3 ± 31.9 | 1067.3 ± 56.1 |
| | $AUC_{0-3d}$ ($\mu$g•day/mL) | Male | 245 ± 13.0 | 728 ± 27.2 | 2300 ± 281 |
| | | Female | 262 ± 34.4 | 929 ± 106 | 2280 ± 167 |
| Week 13 | $C_{max}$ ($\mu$g/mL) | Male | 731.7 ± 132.0 | 1763.0 ± 142.0 | 4754.7 ± 383.0 |
| | | Female | 783.0 ± 59.7 | 2174.0 ± 230.6 | 5523.7 ± 1207.9 |
| | $AUC_{0-3d}$ ($\mu$g•day/mL) | Male | 2630 ± 311 | 5560 ± 505 | 15500 ± 1560 |
| | | Female | 2260 ± 295 | 7210 ± 936 | 17600 ± 3430 |

All values given as means ± standard deviation. $C_{max}$: maximum concentration of CS-1008 in plasma. $AUC_{0-3d}$: area under the curve (of the plot of the concentration of CS-1008 in plasma vs. time) for 3 days.

**Table 5.** Trough Plasma Concentrations of CS-1008 ($\mu$g/mL) in Cynomolgus Monkeys Treated with CS-1008

| Sex | Time of analysis | CS-1008 dose (mg/kg) | | |
|---|---|---|---|---|
| | | 5 | 15 | 42 |
| Male | Week 1 (72 h after 1st dosing) | 64.30 ± 3.58 | 204.0 ± 5.6 | 540.3 ± 40.1 |
| | Week 4 (72 h after 7th dosing) | 410.3 ± 32.7 | 1002.3 ± 43.0 | 2508.7 ± 200.8 |
| | Week 8 (72 h after 15th dosing) | 494.0 ± 10.5 | 1349.7 ± 181.1 | 2990.0 ± 202.9 |
| | Week 13 (72 h after 25th dosing) | 639.0 ± 90.4 | 1487.0 ± 200.7 | 3810.0 ± 397.5 |
| | Week 13 (96 h after 26th dosing) | 660.7 ± 66.8 | 1342.0 ± 185.7 | 3727.7 ± 392.2 |
| Female | Week 1 (72 h after 1st dosing) | 67.03 ± 9.61 | 238.7 ± 27.6 | 632.0 ± 50.9 |
| | Week 4 (72 h after 7th dosing) | 297.7 ± 166.9 | 1262.3 ± 120.8 | 2972.7 ± 273.4 |
| | Week 8 (72 h after 15th dosing) | 374.3 ± 111.0 | 1597.0 ± 202.2 | 3640.3 ± 572.9 |
| | Week 13 (72 h after 25th dosing) | 540.7 ± 53.3 | 1680.7 ± 338.5 | 4539.3 ± 1132.9 |
| | Week 13 (96 h after 26th dosing) | 503.7 ± 72.2 | 1725.3 ± 286.7 | 4205.0 ± 931.4 |

All values given as means ± standard deviation.

In the toxicokinetic analysis, the values for the maximum concentration of CS-1008 in plasma ($C_{max}$) and the area under the curve (AUC) generally increased with dose (Table 4). The trough plasma concentrations markedly increased at Week 4 compared with Day 1 at all doses; however, the ratio increase after Week 4 was much lower than that seen at Week 4 (Table 5). The profiles of the trough plasma concentrations suggest that the toxicokinetic parameters had almost reached a steady state by Week 4. There were no clear differences between the sexes in terms of the toxicokinetic parameters or profiles. The anti-CS-1008 antibody concentration obtained for each plasma sample was below the lower limit of quantification (0.2 $\mu$g/mL).

## Discussion

TRAIL-induced apoptosis has been seen in many tumor cells but not in normal cells *in vivo*[11]. The TRAIL receptors are attractive targets for cancer treatment. Agonistic antibodies against TRAIL-R1 (DR4) and TRAIL-R2 (DR5) are currently in preclinical or clinical studies[12]. Current evidence suggests that some of these antibodies might potentially be toxic to the liver. An agonistic anti-mouse DR5 mAb treatment has been reported to induce cholangitis and cholestatic liver injury in C57BL/6 mice[13]. In a phase 1 clinical trial of mapatumumab (HGS-ETR1, a fully human mAb against TRAIL-R1), two patients receiving 10 mg/kg every 14 days had elevated results of liver function tests[14]. Grade 3 elevation of ALT and grade 2 elevation of AST were noted in a patient given a single dose of Apomab (a fully human mAb against TRAIL-R2) at a dose of 10 mg/kg in a phase 1 study[15]. In two patients treated with lexatumumab (HGS-ETR2, a fully human mAb against TRAIL-R2) at 20 mg/kg, grade 4 elevations of AST and ALT and grade 3 hyperbilirubinemia were noted in a phase 1 study[16]. Meanwhile, TRA-8, a murine anti-DR5 mAb, and CS-1008, a humanized mAb composed of the complementarity determining regions of TRA-8, do not appear to induce cell death in human primary hepatocytes[9, 10]. In the present study, it was confirmed that CS-1008 did not induce toxic changes in any tissues or organs, including the liver, in cynomolgus monkeys. Based on the results of the present study, the no-observed adverse-effect level (NOAEL) of CS-1008 in cynomolgus monkeys of both sexes was concluded to be 42 mg/kg when administered intravenously twice a week for 13 weeks. These safety profiles support the development of CS-1008 as a therapeutic biopharmaceutical.

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