of sunitinib treatment. Given that patients discontinued sunitinib treatment due to insufficient clinical response, the subsequent worsening in health status was more likely due to disease progression than to drug toxicity in this single-arm study of limited sample size.

Based on the dose-corrected C_{trough} data, the pharmacokinetics of sunitinib and its metabolite in advanced/metastatic gastric cancer patients were consistent with previous experience with sunitinib at 50 mg/day on Schedule 4/2 in patients with advanced solid tumors [40]. No unexpected accumulation of sunitinib or its metabolite was observed throughout the study. Assessment of soluble protein levels versus measures of clinical outcome only showed associations between clinical benefit and high sKIT ratio to baseline at cycle 1 day 28 (P=0.0081), and between clinical benefit and low VEGF-C ratio at cycle 2 day 1 (P=0.0326). However, there were a limited number of patients with clinical benefit to include in these analyses. In general, the patterns of pharmacodynamic changes in soluble protein levels observed were similar to those seen in previously reported sunitinib studies [41].

In exploring the potential of sunitinib in gastric cancer, several hypotheses can be proposed that may have had an impact on the limited efficacy observed in this trial. Firstly, in the absence of known predictive biomarkers, it was not possible in this trial to select a subset of gastric cancer patients who might be more likely to respond to sunitinib, which is in contrast to the ability to preselect HER2-positive patients likely to be sensitive to trastuzumab in the ToGA trial [37]. Further understanding of who may benefit from treatment could help to refine the target population for future studies. It is also notable that ORR assessed using RECIST was selected as the primary endpoint of this study. However, observations with targeted agents in other tumor types, for example imatinib in GIST [42] and sunitinib in RCC [43], suggest that one-dimensional RECIST measurements can miss important information about changes in tumor density and metabolic response. This raises the question as to whether ORR is the most suitable endpoint for assessing sunitinib in gastric cancer.

Dose selection and schedule could also be explored further. On the intermittent schedule used in this and other studies of sunitinib, pharmacodynamic modulation of several plasma proteins associated with angiogenesis was reversible during the off-treatment period [38, 43–45]. This raises the question of whether continuous administration of sunitinib might be of benefit, to ensure continuous suppression of angiogenesis. Ultimately, these hypotheses would all require testing in a trial setting.

In summary, the preliminary activity and manageable toxicity observed with sunitinib in this study suggest that although single-agent sunitinib has insufficient clinical value as second-line treatment for advanced gastric cancer, its role in combination with chemotherapy is worthy of further study. The results of ongoing phase I trials in the first-line treatment setting will provide more insight into the use of multiple-RTK inhibitors such as sunitinib in the treatment of advanced gastric cancer.

Acknowledgments Thanks to the global network of investigators who participated in this trial:

CHINA: Rongcheng Luo, Shukui Qin, Lin Shen, Yan Sun. HONG KONG: Wai-Tong Ng, Winnie Yeo. ITALY: Stefano Cascinu, Alberto Sobrero. JAPAN: Narikazu Boku, Toshihiko Doi, Kuniaki Shirao. PORTUGAL: José Evaristo Sanches. REPUBLIC OF KOREA: Yung-Jue Bang, Hyun-Cheol Chung, Won Ki Kang, Yoon-Koo Kang. TAIWAN: Jen-Shi Chen, Kun-Huei Yeh.

Editorial assistance was provided by Jenni Macdougall of ACUMED® (Tytherington, UK) and was funded by Pfizer Inc. The authors also acknowledge data analysis from Charles Harmon (an employee of Atrium Inc., and a paid consultant to Pfizer Inc.) and Zhixiao Wang (Outcomes Research, Pfizer Oncology, New York, NY, USA), and input and review of the manuscript from Richard Chao, Darrel Cohen, and Kristen Letrent (Pfizer Oncology, La Jolla, CA, USA, and New York, NY, USA).

This trial was sponsored by Pfizer Inc.

Conflict of interest Y-J Bang has received commercial research support, honoraria from speakers bureau, and compensation for consultation from Pfizer.

Y-K Kang has received honoraria from speakers bureau from Pfizer.

HC Chung has received research funding from Pfizer for the clinical trial.

T Doi has received compensation for consultation for Pfizer's Sutent GIST program.

JM Tursi, MJ Lechuga, DR Lu, and A Ruiz-Garcia are employees of Pfizer.

JM Tursi, MJ Lechuga, and DR Lu own stock in Pfizer.

A Ruiz-Garcia owns restricted stocks in Pfizer.

A Sobrero participated as chairman to advisory boards for Pfizer in 2009 and 2008.

WK Kang, N Boku, JS Chen, Y Sun, L Shen, SK Qin, and WT Ng have nothing to disclose.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108. doi:10.3322/canjclin.55.2.74
2. Inoue M, Tsugane S (2005) Epidemiology of gastric cancer in Japan. Postgrad Med J 81:419–424. doi:10.1136/pgmj.2004.029330
3. Jung KW, Yim SH, Kong HJ, Hwang SY, Won YJ, Lee JK, Shin HR (2007) Cancer survival in Korea 1993–2002: a population-based study. J Korean Med Sci 22 Suppl:S5–S10.:S5–S10. doi:10.3346/jkms.2007.22.S.S5

4. Scartozzi M, Galizia E, Verdecchia L, Berardi R, Antognoli S, Chiorrini S, Cascinu S (2007) Chemotherapy for advanced gastric cancer: across the years for a standard of care. Expert Opin Pharmacother 8:797–808. doi:10.1517/14656566.8.6.797
5. Wilson D, Hiller L, Geh JI (2005) Review of second-line chemotherapy for advanced gastric adenocarcinoma. Clin Oncol (R Coll Radiol) 17:81–90. doi:10.1016/j.clon.2004.10.006
6. Wohrer SS, Raderer M, Hejna M (2004) Palliative chemotherapy for advanced gastric cancer. Ann Oncol 15:1585–1595. doi:10.1093/annonc/mdh422
7. Glimelius B, Ekstrom K, Hoffman K, Graf W, Sjoden PO, Haglund U, Svensson C, Enander LK, Linne T, Sellstrom H, Heuman R (1997) Randomized comparison between chemotherapy plus best supportive care with best supportive care in advanced gastric cancer. Ann Oncol 8:163–168
8. Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE (2006) Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. J Clin Oncol 24:2903–2909. doi:10.1200/JCO.2005.05.0245
9. Ohtsu A (2008) Chemotherapy for metastatic gastric cancer: past, present, and future. J Gastroenterol 43:256–264. doi:10.1007/s00535-008-2177-6
10. Drescher D, Moehler M, Gockel I, Frerichs K, Muller A, Dunschede F, Borschitz T, Biesterfeld S, Holtmann M, Wehler T, Teufel A, Herzer K, Fischer T, Berger MR, Junginger T, Galle PR, Schimanski CC (2007) Coexpression of receptor-tyrosine-kinases in gastric adenocarcinoma—a rationale for a molecular targeting strategy? World J Gastroenterol 13:3605–3609
11. Zhang H, Wu J, Meng L, Shou CC (2002) Expression of vascular endothelial growth factor and its receptors KDR and Flt-1 in gastric cancer cells. World J Gastroenterol 8:994–998
12. Becker JC, Muller-Tidow C, Serve H, Domschke W, Pohle T (2006) Role of receptor tyrosine kinases in gastric cancer: new targets for a selective therapy. World J Gastroenterol 12:3297–3305
13. Katano M, Nakamura M, Fujimoto K, Miyazaki K, Morisaki T (1998) Prognostic value of platelet-derived growth factor-A (PDGF-A) in gastric carcinoma. Ann Surg 227:365–371
14. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M (1996) Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer 77:858–863
15. Takahashi Y, Cleary KR, Mai M, Kitadai Y, Bucana CD, Ellis LM (1996) Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. Clin Cancer Res 2:1679–1684
16. Doi T, Koizumi W, Siena S, Cascinu S, Ohtsu A, Michael M, Takiuchi H, Swaisland H, Gallagher N, Van Cutsem E (2003) Efficacy, tolerability and pharmacokinetics of gefitinib (ZD1839) in pretreated patients with metastatic gastric cancer. Proc Am Soc Clin Oncol 22:Abstract 1036.
17. Rojo F, Tabernero J, Albanell J, Van CE, Ohtsu A, Doi T, Koizumi W, Shirao K, Takiuchi H, Cajal S, Baselga J (2006) Pharmacodynamic studies of gefitinib in tumor biopsy specimens from patients with advanced gastric carcinoma. J Clin Oncol 24:4309–4316. doi:10.1200/JCO.2005.04.2424
18. Shah MA, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, O'Reilly E, Tse A, Trocola R, Schwartz L, Capanu M, Schwartz GK, Kelsen DP (2006) Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. J Clin Oncol 24:5201–5206. doi:10.1200/JCO.2006.08.0887
19. Pinto C, Di FF, Siena S, Cascinu S, Rojas Llimpe FL, Ceccarelli C, Mutri V, Giannetta L, Giaquinta S, Funaioli C, Berardi R, Longobardi C, Piana E, Martoni AA (2007) Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study). Ann Oncol 18:510–517. doi:10.1093/annonc/mdl459
20. Han SW, Oh DY, Im SA, Park SR, Lee KW, Song HS, Lee NS, Lee KH, Choi IS, Lee MH, Kim MA, Kim WH, Bang YJ, Kim TY (2009) Phase II study and biomarker analysis of cetuximab combined with modified FOLFOX6 in advanced gastric cancer. Br J Cancer 100:298–304. doi:10.1038/sj.bjc.6604861
21. Dragovich T, McCoy S, Fenoglio-Preiser CM, Wang J, Benedetti JK, Baker AF, Hackett CB, Urba SG, Zaner KS, Blanke CD, Abbruzzese JL (2006) Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas: SWOG 0127. J Clin Oncol 24:4922–4927. doi:10.1200/JCO.2006.07.1316
22. Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM (2003) SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. Mol Cancer Ther 2:471–478
23. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray LJ, Carver J, Chan E, Moss KG, Haznedar JO, Sukbuntherng J, Blake RA, Sun L, Tang C, Miller T, Shirazian S, McMahon G, Cherrington JM (2003) In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 9:327–337
24. O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW, Wong LM, Hong W, Lee LB, Town A, Smolich BD, Manning WC, Murray LJ, Heinrich MC, Cherrington JM (2003) SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. Blood 101:3597–3605. doi:10.1182/blood-2002-07-2307
25. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG (2006) Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368:1329–1338. doi:10.1016/S0140-6736(06)69446-4
26. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA (2007) Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115–124
27. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van GM, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216. doi:10.1093/jnci/92.3.205
28. Bello CL, Sherman L, Zhou J, Verkh L, Smeraglia J, Mount J, Klamerus KJ (2006) Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multi-targeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects. Anticancer Drugs 17:353–358
29. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, de Haes JC (1993) The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 85:365–376. doi:10.1093/jnci/85.5.365
30. Blazeby JM, Conroy T, Bottomley A, Vickery C, Arraras J, Sezer O, Moore J, Koller M, Turhal NS, Stuart R, Van CE, D'haese S, Coens C (2004) Clinical and psychometric validation of a questionnaire module, the EORTC QLQ-STO 22, to assess quality

of life in patients with gastric cancer. Eur J Cancer 40:2260–2268. doi:10.1016/j.ejca.2007.07.005
31. Giuliani F, Gebbia V, De Vita F, Maiello E, Di Bisceglie M, Catalano G, Gebbia N, Colucci G (2003) Docetaxel as salvage therapy in advanced gastric cancer: a phase II study of the Gruppo Oncologico Italia Meridionale (G.O.I.M.). Anticancer Res 23:4219–4222.
32. Jo JC, Lee JL, Ryu MH, Sym SJ, Lee SS, Chang HM, Kim TW, Lee JS, Kang YK (2007) Docetaxel monotherapy as a second-line treatment after failure of fluoropyrimidine and platinum in advanced gastric cancer: experience of 154 patients with prognostic factor analysis. Jpn J Clin Oncol 37:936–941. doi:10.1093/jjco/hym123
33. Hironaka S, Zenda S, Boku N, Fukutomi A, Yoshino T, Onozawa Y (2006) Weekly paclitaxel as second-line chemotherapy for advanced or recurrent gastric cancer. Gastric Cancer 9:14–18. doi:10.1007/s10120-005-0351-6
34. Kodera Y, Ito S, Mochizuki Y, Fujitake S, Koshikawa K, Kanyama Y, Matsui T, Kojima H, Takase T, Ohashi N, Fujiwara M, Sakamoto J, Akimasa N (2007) A phase II study of weekly paclitaxel as second-line chemotherapy for advanced gastric Cancer (CCOG0302 study). Anticancer Res 27:2667–2671
35. Chun JH, Kim HK, Lee JS, Choi JY, Lee HG, Yoon SM, Choi IJ, Ryu KW, Kim YW, Bae JM (2004) Weekly irinotecan in patients with metastatic gastric cancer failing cisplatin-based chemotherapy. Jpn J Clin Oncol 34:8–13. doi:10.1093/jjco/hyh006
36. Hartmann JT, Quietzsch D, Daikeler T, Kollmannsberger C, Mayer F, Kanz L, Bokemeyer C (1999) Mitomycin C continuous infusion as salvage chemotherapy in pretreated patients with advanced gastric cancer. Anticancer Drugs 10:729–733
37. Van Cutsem E, Kang Y, Chung H, Shen L, Sawaki A, Lordick F, Hill J, Lehle M, Feyereislova A, Bang Y (2009) Efficacy results from the ToGA trial: A phase III study of trastuzumab added to standard chemotherapy (CT) in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC). J Clin Oncol 27:abstr LBA4509.
38. Burstein HJ, Elias AD, Rugo HS, Cobleigh MA, Wolff AC, Eisenberg PD, Lehman M, Adams BJ, Bello CL, DePrimo SE, Baum CM, Miller KD (2008) Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 26:1810–1816. doi:10.1200/JCO.2007.14.5375
39. Socinski MA, Novello S, Brahmer JR, Rosell R, Sanchez JM, Belani CP, Govindan R, Atkins JN, Gillenwater HH, Pallares C, Tye L, Selaru P, Chao RC, Scagliotti GV (2008) Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. J Clin Oncol 26:650–656. doi:10.1200/JCO.2007.13.9303
40. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E (2006) Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 24:25–35. doi:10.1200/JCO.2005.02.2194
41. DePrimo SE, Bello C (2007) Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib. Ann Oncol 18 Suppl 10:x11–9.:x11–x19. doi:10.1093/annonc/mdm409
42. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Benjamin RS (2007) Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol 25:1753–1759. doi:10.1200/JCO.2006.07.3049
43. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, Theuer CP, George DJ, Rini BI (2006) Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol 24:16–24. doi:10.1200/JCO.2005.02.2574
44. Norden-Zfoni A, Desai J, Manola J, Beaudry P, Force J, Maki R, Folkman J, Bello C, Baum C, DePrimo SE, Shalinsky DR, Demetri GD, Heymach JV (2007) Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. Clin Cancer Res 13:2643–2650. doi:10.1158/1078-0432.CCR-06-0919
45. Yoon SY, Kim SY, Cho YH, Chung HW, So Y, Lee HM (2009) Hepatic metastases of gastric adenocarcinoma showing metabolic remission on FDG-PET despite an increase in size on CT. Cancer Res Treat 41:100–103. doi:10.4143/crt.2009.41.2.100

Cancer Chemother Pharmacol (2011) 68:733–741
DOI 10.1007/s00280-010-1544-1

ORIGINAL ARTICLE

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

T. Doi · H. Murakami · A. Ohtsu · N. Fuse · T. Yoshino · N. Yamamoto · N. Boku · Y. Onozawa · C-P. Hsu · K. S. Gorski · G. Friberg · T. Kawaguchi · T. Sasaki

Received: 1 October 2010 / Accepted: 26 November 2010 / Published online: 16 December 2010

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

T. Doi (✉) · A. Ohtsu · N. Fuse · T. Yoshino
Gastrointestinal/Oncology Division,
National Cancer Center Hospital East, 6-5-1,
Kashiwanoha, Kashiwa, Chiba 277-8577, Japan
e-mail: todoi@east.ncc.go.jp

H. Murakami · N. Yamamoto · N. Boku · Y. Onozawa
Shizuoka Cancer Center, Nagaizumi, Japan

C-P.Hsu · K. S. Gorski · G. Friberg
Amgen Inc., Thousand Oaks, CA, USA

T. Kawaguchi · T. Sasaki
Takeda Bio Development Center Ltd., Tokyo, Japan

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.

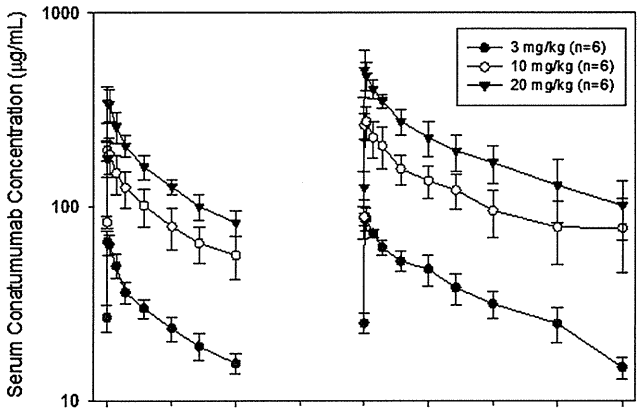

Fig. 1 Mean (±SD) serum concentration–time profiles on day 1 and day 29 after intravenous administrations of 3, 10, or 20 mg conatumumab once every 2 weeks

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.

Acknowledgments We would like to thank Francesca Civoli and Steven J. Swanson for clinical immunology analysis and John Thomas for providing training at Mitsubishi Chemical Medience Cooperation (Tokyo, Japan) to evaluate receptor occupancy by flow cytometry. Takeda Bio Development Center Ltd., Tokyo, Japan.

References

1. Pitti RM, Marsters SA, Rupper S et al (1996) Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 271:12687–12690
2. Wiley SR, Schooley K, Smolak PJ et al (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3:673–682
3. Kelly S, Ashkenazi A (2004) Targeting death receptors in cancer with Apo2L/TRAIL. Curr Opin Pharmacol 4:333–339
4. Fesik SW (2005) Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Can 5:876–885
5. DeRosier LC, Vickers SM, Zinn KR et al (2007) TRA-8 anti-DR5 monoclonal antibody and gemcitabine induce apoptosis and inhibit radiologically validated orthotropic pancreatic tumor growth. Mol Cancer Ther 6(12 pt1):3198–3207
6. DeRosier LC, Huang ZQ, Sellers JC et al (2006) Treatment with gemcitabine and TRA-8 anti-death receptor-5 mAb reduces pancreatic adenocarcinoma cell viability in vitro and growth in vivo. J Gastrointest Surg 10:1291–1300
7. Ibrahim SM, Ringel J, Schmidt C et al (2001) Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. Pancreas 23:72–79
8. Xu ZW, Kleeff J, Friess H et al (2003) Synergistic cytotoxic effect of TRAIL and gemcitabine in pancreatic cancer cells. Anticancer Res 23:251–258
9. Hylander BL, Pitoniak R, Penetrante RB et al (2005) The anti-tumor effect of Apo2L/TRAIL on patient pancreatic adenocarcinomas grown as xenografts in SCID mice. J Transl Med 3:22
10. LoRusso P, Hong D, Heath E et al (2007) First-in-human study of AMG 655, a pro-apoptotic TRAIL receptor-2 agonist, in adult patients with advanced solid tumors. ASCO Meeting Abstracts 25:3534
11. Ashkenazi A (2002) Targeting death and decoy receptors of the tumor-necrosis factor superfamily. Nat Rev Cancer 2(6):420–430
12. Johnstone RW, Frew AJ, Smyth MJ et al (2008) The TRAIL apoptotic pathway in cancer onset, progression and therapy. Nat Rev Cancer 8:782–798
13. Ashkenazi A, Pai RC, Fong S et al (1999) Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 104:155–162
14. Kaplan-Lefko P, Bush T, Belmontes B et al (2008) AMG 655, a fully human agonistic antibody against death receptor 5, enhances the anti-tumor activity of gemcitabine in MiaPaCa2/T2, a pancreatic cancer model. AACR Meeting Abstracts 49:3999
15. Calogiuri G, Ventura MT, Mason L et al (2008) Hypersensitivity to last generation chimeric, umanized and human recombinant monoclonal antibodies for therapeutic use. Curr Pharmaceutical Design 14:2883–2891
16. Camidge D, Herbst RS, Gordon M et al (2007) A phase I safety and pharmacokinetic study of Apomab, a human DR5 agonist antibody, in patients with advanced cancer. ASCO Meeting Abstracts 25:3582
17. Tolcher AW, Mita M, Meropol NJ et al (2007) Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. J Clin Oncol 25:1390–1395
18. Plummer R, Attard G, Pacey S et al (2007) Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. Clin Cancer Res 13:6187–6194
19. Patnaik A, Wakelee H, Mita M et al (2006) HGS-ETR2—A fully human monoclonal antibody to TRAIL-R2: results of a phase I trial in patients with advanced solid tumors. ASCO Meeting Abstracts 24:3012
20. Lobo ED, Hansen RJ, Balthasar JP (2004) Antibody pharmacokinetics and pharmacodynamics. J Pharmaceut Sci 93:2645–2668
21. Griffith TS, Wiley SR, Kubin MZ et al (1999) Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. J Exp Med 189:1343–1353
22. Washburn B, Weigand MA, Grosse-Wilds A et al (2003) TNF related apoptosis-inducing ligand mediates tumoricidal activity of human monocytes stimulated by Newcastle disease virus. J Immunol 170:1814–1821
23. Hayakawa Y, Screpanti V, Yagita H et al (2004) NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy. J Immunol 172:123–129
24. Wilson NS, Dixit V, Ashkenzi A et al (2009) Death receptor signal transducers: nodes of coordination in immune signaling networks. Nat Immunology 10(4):348–355
25. Sheridan JP, Marsters SA, Pitti RM et al (1997) Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 277:818–821
26. Walczak H, Miller RE, Ariail K et al (1999) Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 5(2):157–163
27. Pollack IF, Eriff M, Ashkenazi A (2001) Direct stimulation of apoptotic signaling by soluble Apo2L/tumor necrosis factor-related apoptosis-inducing ligand leads to selective killing of glioma cells. Clin Cancer Res 7:1362–1369
28. Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA et al (2003) Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL−/− mice. Nat Immunol 4:255–260
29. Cretney E, Takeda K, Yagita H et al (2002) Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. J Immunol 168:1356–1361
30. Diehl GE, Yue HH, Hsieh K et al (2004) TRAIL-R as a negative regulator of innate immune cell responses. Immunity 21:877–889
31. Pan Y, Xu R, Peach M, et al (2007) Application of pharmacodynamic assays in a phase Ia trial of Apo2L/TRAIL in patients with advanced tumors. ASCO Meeting Abstracts, 3535

Gastric Cancer (2011) 14:226–233
DOI 10.1007/s10120-011-0030-8

ORIGINAL ARTICLE

A phase II study of biweekly mitomycin C and irinotecan combination therapy in patients with fluoropyrimidine-resistant advanced gastric cancer: a report from the Gastrointestinal Oncology Group of the Japan Clinical Oncology Group (JCOG0109-DI Trial)

Tetsuya Hamaguchi · Kuniaki Shirao · Atsushi Ohtsu · Ichinosuke Hyodo · Yasuaki Arai · Hiroya Takiuchi · Hirofumi Fujii · Motoki Yoshida · Hiroshi Saito · Tadamichi Denda · Wasaburo Koizumi · Hiroaki Iwase · Narikazu Boku · Gastrointestinal Oncology Study Group of Japan Clinical Oncology Group

Received: 26 October 2010 / Accepted: 24 January 2011 / Published online: 19 April 2011

Abstract
Background Preclinical studies have shown that mitomycin C (MMC) acts synergistically with irinotecan (CPT-11). In this phase II study, we evaluated the efficacy and toxicity of MMC/CPT-11 therapy as second-line chemotherapy for patients with fluoropyrimidine-resistant advanced gastric cancer.
Methods Eligible patients had evidence of tumor progression despite prior treatment with fluoropyrimidine-based regimens or had relapsed within 6 months after completion of therapy with adjuvant fluoropyrimidines. Treatment consisted of MMC (5 mg/m^2) and CPT-11 (150 mg/m^2) administered i.v. every 2 weeks. The primary endpoint was the response rate (RR). Our hypothesis was that this combination therapy was efficacious when the lower boundary of the 95% confidence interval (CI) of the RR exceeded 20% of the threshold RR.

T. Hamaguchi (✉) · K. Shirao
Division of Gastrointestinal Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: thamaguc@ncc.go.jp

K. Shirao
Department of Medical Oncology, Faculty of Medicine, Oita University, Oita, Japan

A. Ohtsu
National Cancer Center Hospital East, Research Center for Innovative Oncology, Chiba, Japan

I. Hyodo
Department of Internal Medicine, National Hospital Organization Shikoku Cancer Center, Ehime, Japan

Y. Arai
Department of Diagnostic Radiology, Aichi Cancer Center Hospital, Aichi, Japan

H. Takiuchi · M. Yoshida
Cancer Chemotherapy Center, Osaka Medical College Hospital, Osaka, Japan

H. Fujii
Department of Medical Oncology, Tochigi Cancer Center, Tochigi, Japan

M. Yoshida
Department of Gastroenterology, Kumamoto Regional Medical Center, Kumamoto, Japan

H. Saito
Department of Gastroenterology, Yamagata Prefectural Central Hospital, Yamagata, Japan

T. Denda
Division of Hematology/Oncology, Chiba Cancer Center Hospital, Chiba, Japan

W. Koizumi
Department of Internal Medicine, Kitasato University East Hospital, Kanagawa, Japan

H. Iwase
Department of Gastroenterology, National Hospital Organization Nagoya Medical Center, Aichi, Japan

N. Boku
Division of Gastrointestinal Oncology, Shizuoka Cancer Center, Shizuoka, Japan

Results Between April 2002 and July 2003, 45 eligible patients were registered and analyzed. Among the 45 patients, 40 (89%) had previously received chemotherapy for metastasis and 24 (53%) had a performance status (PS) of 0. Thirteen partial responses were obtained among the 45 patients, resulting in an overall RR of 29% (95% CI, 16–42%). The median time to progression was 4.1 months, and the median survival time was 10 months, with a 1-year survival rate of 36%. Grade 4 neutropenia was observed in 29% of the patients, whereas febrile neutropenia occurred in 9%. The incidence rates of grade 3 nausea and diarrhea were 13 and 2%, respectively.

Conclusions Although this study did not achieve the per-protocol definition of activity, the progression-free survival and overall survival appeared to be promising, with acceptable tolerability. Thus, MMC/CPT-11 therapy as second-line chemotherapy for fluoropyrimidine-resistant advanced gastric cancer presents a potential treatment option in patients with a good PS.

Keywords Gastric cancer · Mitomycin-C · Irinotecan · Fluoropyrimidine-resistant · Second-line chemotherapy

Introduction

Gastric cancer is the most common malignancy in Asian countries, with approximately 50,000 deaths in Japan annually [1]. The treatment of choice for this malignancy is primary tumor resection. In patients with curatively resected stage I–III gastric cancer, the 5-year survival proportion is >50%; however, this proportion remains at <10% in stage IV or recurrent disease. Randomized trials have demonstrated that fluorouracil-based regimens improve survival proportions in patients with advanced gastric cancer (AGC) compared with best supportive care (BSC) alone as first-line chemotherapy [2–4]. Moreover, combination chemotherapy results in superior outcomes compared with monotherapy. In Japan, the efficacy and toxicity of the combination of an oral fluoropyrimidine (S-1) and platinum was previously evaluated in the phase III SPIRITS (S-1 plus cisplatin vs. S-1 alone for first-line treatment of AGC) trial. S-1 plus cisplatin resulted in superior overall survival (OS) compared with S-1 alone [hazard ratio (HR), 0.77; 95% confidence interval (CI), 0.61–0.98%; $P = 0.04$], with an impressive median OS of 13.0 months [5]. The Japan Clinical Oncology Group (JCOG) 9912 trial (5-fluorouracil [FU] alone vs. S-1 alone vs. irinotecan [CPT-11] plus cisplatin [CDDP] combination for the first-line treatment of AGC) was also conducted in Japan. S-1 showed significant noninferiority for progression-free survival (PFS) and OS compared with 5-FU alone; however, CPT-11 plus CDDP showed no significant superior effects on PFS and OS compared with 5-FU alone [6]. In Japan, S-1 plus CDDP combination therapy is considered the standard first-line treatment for AGC.

Thuss-Patience et al. [7] reported at the 2009 Annual Meeting of the American Society of Clinical Oncology (ASCO) that CPT-11 monotherapy significantly prolonged OS compared with BSC as second-line chemotherapy. Although that report was the first randomized phase III study investigating second-line chemotherapy for AGC, no objective responses were observed. Thus, a consensus regarding the standard regimen for second-line chemotherapy has not yet been obtained.

Many AGC patients who failed to respond to first-line chemotherapy showed symptoms of pain, weight loss, or nausea due to their progressive disease. Thus, the induction of a tumor response is as important as delaying tumor progression for as long as possible. Patients who received combination chemotherapy showed higher response rates than those who received single-agent chemotherapy alone. Therefore, combination chemotherapy is preferable to single-agent chemotherapy for palliation. Moreover, combination chemotherapy may prolong OS compared with single-agent chemotherapy alone.

CPT-11 is a potent topoisomerase I inhibitor and is effective against AGC. In a phase II trial, the response rate (RR) to CPT-11 alone was 16% in previously treated AGC patients [8]. The administration of a CDDP and CPT-11 combination in AGC patients resulted in a higher RR and longer time to progression (TTP) [9–11]. As mentioned above, CDDP/CPT-11 did not significantly prolong OS over 5-FU, but induced a significantly higher RR than 5-FU in the JCOG9912 trial [6]. A 5-FU, leucovorin (LV), and CPT-11 combination produced a higher RR and longer TTP than CDDP/CPT-11 in AGC patients [12]. In another randomized phase III trial, 5-FU/LV/CPT-11 showed a trend to have superiority in TTP over CDDP/5-FU (5.0 vs. 4.2 months, respectively; HR, 1.23; 95% CI, 0.97–1.57%; $P = 0.088$), and a better safety profile [13]. These results support the finding that CPT-11 is active against AGC.

Mitomycin C (MMC) is also effective against AGC. Preclinical studies have shown that a MMC and CPT-11 combination synergistically inhibits tumor growth in vitro [14]. This is due to the possible induction of topoisomerase I gene expression by MMC, thereby increasing tumor cell sensitivity to CPT-11. A phase I/II study of this combination recommended an MMC dose of 5 mg/m^2 and a CPT-11 dose of 150 mg/m^2 administered biweekly [15]. The dose-limiting toxicities of this combination regimen when administered at 10 mg/m^2 for MMC and 150 mg/m^2 for CPT-11 were grade 4 neutropenia with or without febrile neutropenia and grade 3 diarrhea. The overall RR was 50% (15/30 patients), and 5 of 14 patients (36%) with prior chemotherapy showed a partial response (PR). We

previously showed that MMC and CPT-11 combination chemotherapy was effective and well tolerated in patients with fluoropyrimidine-resistant metastatic colorectal cancer; the RR, median TTP, and median survival time (MST) were 34% (95% CI, 20–49%), 4.2 months, and 11.9 months [16], respectively.

These results led us to conduct the present phase II clinical trial to investigate the efficacy and toxicity of MMC/CPT-11 therapy in patients with AGC resistant to a fluoropyrimidine-containing regimen in the JCOG0109-DI study.

Patients and methods

Eligibility

A patient was considered eligible if there was evidence of a refractory response to one prior chemotherapy containing fluoropyrimidine, which was any of the following types of history of chemotherapy:

1. In the case of unresectable gastric cancer, disease progression detected while receiving front-line chemotherapy containing fluoropyrimidine, or confirmed immediately after the discontinuation for any reason other than disease progression.
2. In the case of recurrent gastric cancer, recurrence detected within 24 weeks from the last dose of postoperative adjuvant chemotherapy containing fluoropyrimidine, and further chemotherapy was not administered after recurrence.
3. In the case of recurrent gastric cancer detected 25 weeks after the last dose of postoperative adjuvant chemotherapy, disease progression detected while receiving front-line chemotherapy containing fluoropyrimidine after recurrence, or confirmed immediately after the discontinuation for any reason other than progression.
4. In the case of recurrent gastric cancer treated with neoadjuvant chemotherapy, the effect of neoadjuvant chemotherapy containing fluoropyrimidine was stable disease, progressive disease, or not evaluated, and recurrence was identified after curative resection. Chemotherapy was not performed following recurrence.
5. In the case of recurrent gastric cancer treated with neoadjuvant chemotherapy, the chemotherapy effect was a complete response or PR, and progression was detected during one chemotherapy containing fluoropyrimidine after recurrence, or confirmed immediately after discontinuation for any reason other than progression.

Disease progression and the nonefficacy of neoadjuvant chemotherapy were believed to represent clinical failure by treating physicians. Elevation of the level of a tumor marker, such as carcinoembryonic antigen (CEA), was not accepted as adequate evidence for treatment failure. Documentation of evidence of a refractory response by computed tomography (CT) and magnetic resonance imaging was required.

For the other eligibility criteria, patients must be between 20 and 75 years of age, and have an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2, adequate baseline bone marrow function [white blood cell (WBC) and platelet counts $\geq$4,000 and 100,000/mm^3, respectively], adequate hepatic function (serum bilirubin level $\leq$1.5 mg/dl and both serum aspartate aminotransferase and alanine aminotransferase levels $\leq$100 U/l), adequate renal function (serum creatinine level $\leq$1.5 mg/dl), adequate respiratory function (arterial partial pressure of oxygen $\geq$70 mmHg), and have received no blood transfusion within 14 days before enrollment. All patients were required to have $\geq$1 measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST).

Patients were excluded if they had symptomatic brain metastasis, symptomatic ascites and/or pleural effusion, previous history of MMC or CPT-11 chemotherapy, pre-existing diarrhea of >4 times/day, suspicion of existing active bleeding which needed blood transfusion at 14 days prior to registration in this study, or a high risk of a poor outcome due to concomitant nonmalignant disease (i.e., cardiac, pulmonary, renal, or hepatic disease; poorly controlled diabetes; or uncontrolled infection), or severe psychiatric disease. Pregnant or lactating women were excluded.

The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review board of each participating hospital. All patients gave their written informed consent.

Treatment plan

The treatment schedule consisted of one MMC dose (5 mg/m^2, bolus injection), then CPT-11 (150 mg/m^2, 90-min i.v. infusion) repeated every 2 weeks, as described previously [16]. All patients were treated on an outpatient basis and were recommended to receive both a 5-hydroxytryptamine-3-receptor antagonist and dexamethasone to prevent emesis. Subsequent treatment cycles were withheld until the WBC and platelet counts were $\geq$3,000 and 100,000/mm^3, respectively; diarrhea was $\leq$grade 1; and there were no infection symptoms such as pyrexia ($\geq$38°C). When the treatment course was delayed within 8 days from the planned schedule, the same dosage levels as those used previously were administered. When the treatment course was delayed beyond 8 days and within 21 days from the planned schedule, one lower dose level (CPT-11 level -1,

125 mg/m^2; level -2, 100 mg/m^2) than the previous level was administered, while the MMC dose was maintained at 5 mg/m^2. The treatment course was discontinued if it could not be started within 21 days from the planned schedule. When grade 4 leukopenia or thrombocytopenia occurred in a previous treatment course causing a delay within 8 days, the same dosage levels as those used previously were administered. When grade 2 diarrhea or higher was observed in a preceding course, dosages 1 level lower than the previous dosages were administered.

Treatment was repeated until disease progression or when severe toxicity was observed. The total MMC dose was limited to 50 mg/m^2, to prevent cumulative toxicity (e.g., interstitial pneumonia and hemolytic uremic syndrome), and thereafter CPT-11 alone was administered. This indicates that the maximum number of total treatment cycles of MMC/CPT-11 therapy is 10 cycles.

Evaluation of response and toxicity

During protocol treatment, the patient's signs and symptoms, as well as laboratory data (i.e., WBC with differential counts, liver function tests, urea nitrogen, creatinine, electrolytes, and urinalysis) were examined biweekly. Adverse events were evaluated using the National Cancer Institute-Common Toxicity Criteria version 2.0. Tumor response was assessed by CT every 4 weeks. The response of measurable and evaluable disease sites was assessed by each investigator in accordance with RECIST, and then reviewed by central review at the group meeting.

Statistical analysis

For this study, the primary endpoint was the RR and the secondary endpoints were OS and toxicity. Here, we used the standard design (attained design) of the Southwest Oncology Group [17]. Based on reports of RRs of 22% with paclitaxel alone [18] and 16% with CPT-11 alone [8] in the second-line setting and an RR of 36% in phase I/II studies of MMC/CPT-11 therapy [15], the RR in this study was expected to be within 30–40% for a future phase III trial. Here, the required sample size was calculated to be 45 patients, with the following parameters: $\alpha = 0.05$, $\beta = 0.10$, threshold response rate $(p_0) = 20\%$, and expected response rate $(p_a) = 40\%$. Interim analysis was performed when the number of enrolled subjects reached 25. The significance level for the interim analysis was set as $P < 0.02$. Furthermore, when the number of patients who reached RR was <5 at the interim analysis, the study was prematurely discontinued because it would have been difficult to exceed the expected RR despite further patient accumulation, or because it would not be worth advancing this regimen to an ensuing clinical study. When the study was not completed after the interim analysis, the number of patients was increased to 45 in order to allow the null hypothesis (threshold RR) to be tested. When α was <0.05, or when the lower boundary of the 95% CI of the RR exceeded 20% of the threshold RR, this therapy was considered to be efficacious as chemotherapy for gastric cancer patients who had received pretreatment. That is, when ≥16 of 45 patients had a PR, this study was judged to be positive. Here, patient enrollment was not temporarily discontinued.

OS was defined as the time from the registration date to death as a result of any cause. PFS was defined as the time from the registration date to the first documentation of objective tumor progression. Time-to-event and OS data were summarized using the Kaplan–Meier method.

Results

Patient population and study treatment

Between April 2002 and July 2003, 45 patients (33 men, 12 women) from 12 hospitals were enrolled and analyzed. Table 1 shows the demographic data, baseline disease, and regimens of prior chemotherapy. The median age was 64 years (range 36–75), and all patients had a good PS of 0 or 1. Eighteen patients (40%) had diffuse-type gastric cancer. As for prior chemotherapy, 40 (89%) had previously received chemotherapy for metastasis, whereas 5 had received adjuvant chemotherapy. In the first-line chemotherapy, 33 patients (73%) had received 5-FU or S-1 alone.

In all 45 patients, MMC/CPT-11 therapy was administered 281 times, and the median number of doses was 6 (range 1–10). Of the 45 patients, 10 (22%) completed the planned 10 chemotherapy cycles. In the remaining 35 patients, the reasons for treatment discontinuation were disease progression in 25, toxicity in 6, patient's refusal in 3, and death in 1. Regarding CPT-11 administration, 11 patients (24%) required -1 level dose reduction and 8 (18%) required -2 level reduction because of leukopenia and thrombocytopenia.

Efficacy

Of the 45 patients, 13 showed a PR (RR: 28.9%; 95% CI, 15.6–42.1%) (Table 2). The median PFS was 4.1 months (Fig. 1). The median OS time was 10.1 months (95% CI, 7.3–12.6 months), and the 1-year survival rate was 38% (Fig. 2).

Because the lower boundary of the 95% CI of the RR (15.6%) did not exceed the threshold RR (20%), the

Table 1 Patient characteristics ($n = 45$)

Age (years)	
Median	64
Range	36–75
Gender	
Male	33
Female	12
ECOG performance status	
0	24
1	21
2	0
Borrmann macroscopic type of primary cancer	
0	1
1	1
2	17
3	18
4	5
Unknown	3
Histological type	
Intestinal	25
Diffuse	18
Unclassified	2
Prior chemotherapy	
5-FU alone	18
S-1 alone	15
S-1 + CDDP	6
MTX + 5-FU	2
Others	4

ECOG Eastern Cooperative Oncology Group, *5-FU* 5-fluorouracil, *CDDP* cisplatin, *MTX* methotrexate

Table 2 Evaluation of response ($n = 45$)

Tumor response	Patients	
	n	% (95% CI)
Complete response	0	0
Partial response	13	28.9 (15.6–42.1)
Stable disease	17	37.7 (23.6–51.9)
Progressive disease	14	31.1 (17.6–44.6)
Not evaluated	1	4.4 (0–6.5)
Survival	Months (95% CI)	
PFS	4.1 M (2.5–5.7)	
OS	10.1 M (7.3–12.6)	

CI confidence interval, *PFS* progression-free survival, *OS* overall survival

MMC/CPT-11 combination as second-line chemotherapy could not be definitively concluded as efficacious for further investigation.

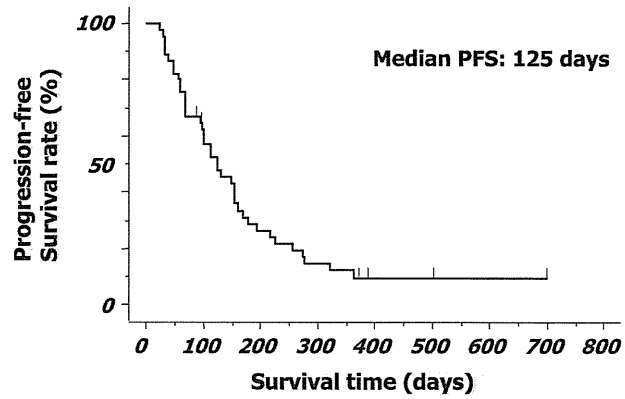

Fig. 1 Kaplan–Meier estimates of progression-free survival (*PFS*) rates

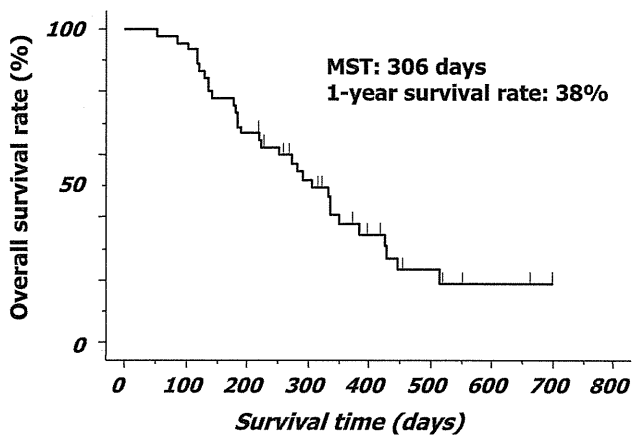

Fig. 2 Kaplan–Meier estimates of overall survival. *MST* Median survival time

Toxicity

The toxicities of the MMC/CPT-11 therapy are summarized in Table 3, with myelosuppression and gastrointestinal toxicity as major toxicities. Grade 3 and 4 neutropenia occurred in 24 and 29% of the patients, respectively, whereas grade 3 and 4 thrombocytopenia developed in only 7%. As for the nonhematological toxicities, the incidence rate of grade 3 diarrhea was 2%, and nausea and vomiting were mild. Early death due to interstitial pneumonitis within 30 days from the last chemotherapy occurred in 1 patient, which was considered by the JCOG Data and Safety Monitoring Committee to have been possibly related to the treatment.

Discussion

In second-line chemotherapy for AGC, the potential benefits remain unclear because of the few prospective studies that have been conducted thus far. These trials demonstrated that

Table 3 Grade 2–4 adverse events according to NCI-CTC ver. 2.0 ($n = 45$)

	Grade 2	Grade 3	Grade 4	Grade 3–4 (%)
Hematological WBC	24	8	5	29
Neutrophils	10	11	13	53
Hb	25	3	3	13
Platelets	1	2	1	7
Febrile neutropenia	0	4	0	9
Non-hematological Anorexia	13	11	0	24
Nausea	11	6	0	13
Diarrhea	4	1	0	2
Infection with grade 3/4 neutropenia	0	2	0	4
Infection without neutropenia	4	2	0	4

NCI-CTC National Cancer Institute-Common Toxicity Criteria, *Hb* hemoglobin

the RRs to second-line chemotherapy in phase II trials for gastric cancer were similar to those observed for other cancers which are more commonly treated after the failure of first-line chemotherapy. Furthermore, 2 Japanese randomized trials (i.e., SPIRITS [5] and JCOG9912 [6]) achieved a median OS of 13.0 months despite the relatively short median PFS of about 4–6 months. Although both JCOG9912 and our previous phase III study (JCOG9205 [19]) utilized 5-FU continuous infusion (c.i.) and 5-FU/CDDP, the obtained median PFS was 2 months and the OS in JCOG9912 was much longer than that in JCOG9205. In the present study, the proportion of patients who received second-line chemotherapy was >70%, which is higher than that obtained in our previous study (53%). The results of previous phase II trials consistently suggest that patients treated with second-line chemotherapy may survive longer than those provided with BSC, although the survival benefit of the second-line chemotherapy has not yet been clarified.

According to the 26 prospective phase II studies reported in the literature, obtained using the search expressions "gastric cancer" and "second-line chemotherapy" in PubMed, the average and median RRs were 18.8 and 20.0% (0–34.6%), respectively [18, 20–44]. Although the present study did not disprove the null hypothesis about RR, it is suggested that MMC/CPT-11 therapy with an RR of 28.9% may possess some antitumor activity as second-line chemotherapy.

As for survival, the present study showed a median survival time of 10.1 months (95% CI, 7.3–12.9 months), and a 1-year survival proportion of 38%. These data are similar to those obtained in the first-line chemotherapy setting and appeared to be better than those obtained using several other regimens, showing a survival period of 3.5–13 months compared with the reported median survival period of 7–10 months in untreated patients. However, it is very difficult to compare phase II studies due to differences in patient background and subsequent therapy. One reason for improved survival may be good clinical selection of a patient. At the baseline evaluation, the median age of the patients in the present study was 64 years (range, 36–75), and all the patients had a good PS of 0 or 1. Another reason for the improved survival was the high proportion of tumor stabilization (66.7%) after the administration of the MMC/CPT-11 regimen. Therefore, it is considered that MMC/CPT-11 therapy may provide some survival benefit.

The toxicity of the MMC/CPT-11 regimen can be considered tolerable and manageable. Hematological toxicity was within the expected range, including grade 4 neutropenia, observed in 13 patients (29%) and grade 3 febrile neutropenia in 4 patients (9%). According to a Japanese prospective pharmacogenomic study of CPT-11, homozygotes and double heterozygotes of *6 and *28 (*6/*6, *28/*28 and *6/*28) were significantly associated with severe neutropenia. The UGT1A1 gene test prior to receiving this regimen may be useful to decide the starting dose of CPT-11 or to decide whether the patient should receive CPT-11 and MMC combination chemotherapy or CPT-11 monotherapy [45]. Although treatment-related death was observed in 1 patient (2%) in the present study, the occurrence of adverse events was similar to that in JCOG9911-DI, a phase II study of the same regimen for colon cancer; thus, MMC/CPT-11 therapy was considered tolerable. In the present study, the proportion of patients with toxicity was similar to that of patients where MMC/CPT-11 therapy was used as second-line treatment against colorectal cancer [16].

From the above results, the present phase II study of MMC/CPT-11 therapy for FU-based chemotherapy-refractory gastric cancer is judged to be negative on the basis of the decision rule defined in the protocol. This may be due to the threshold RR being set very high owing to the lack of data as the basis for setting the threshold level and expected RR, because of the small number of phase II studies of second-line treatment when this protocol was developed. In fact, the RR cannot be considered poor compared with that in phase II studies performed in other treated patients (as shown in Table 2), with a favorable

survival time of 10 months. In conclusion, the MMC/CPT-11 regimen might be one treatment option for pretreated AGC in patients with good PS.

Acknowledgments This study was supported by a Grant-in-Aid for Cancer Research from the Japanese Ministry of Health, Labour and Welfare. We thank Ms. M. Kobayashi and Ms. M. Shinogi for data management and Ms. H. Orita for her secretarial assistance.

Conflict of interest None.

Appendix

Investigators in participating institutions: Yamagata Prefectural Central Hospital, H. Saito; Tochigi Cancer Center, H. Fuji; Saitama Cancer Center, K. Yamaguchi; National Cancer Center Hospital East, T. Doi; Chiba Cancer Center Hospital, T. Denda; National Cancer Center Hospital Tokyo, Y. Shimada; Kitasato University East Hospital, W. Koizumi; Aichi Cancer Center Hospital, Y. Inaba; Nagoya Medical Center, H. Iwase; Osaka Medical College, H. Takiuchi; National Hospital Organization Shikoku Cancer Center, J. Nasu; Kumamoto Regional Medical Center Hospital, M. Yoshida.

References

1. Statistics of Cancer, Center for Information Services, National Cancer Center, Japan. http://ganjoho.ncc.go.jp/public/statistics/pub/update.html.
2. Glimelius B, Hoffman K, Haglund U, Nyren O, Sjoden PO. Initial or delayed chemotherapy with best supportive care in advanced gastric cancer. Ann Oncol. 1994;5:189–90.
3. Murad A, Santiago F, Petroianou A. Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. Cancer. 1993;72:37–41.
4. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M. Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. Br J Cancer. 1995;71:587–91.
5. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol. 2008;9:215–21.
6. Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, et al. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. Lancet Oncol. 2009;10:1063–9.
7. Thuss-Patience PC, Kretzschmar A, Deist T, Hinke A, Bichev D, Lebedinzew B, et al. Irinotecan versus best supportive care (BSC) as second-line therapy in gastric cancer: a randomized phase III study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). J Clin Oncol. 2009;27:abstr 4540.
8. Futatsuki K, Wakui A, Nakao I, Sakata Y, Kambe M, Shimada Y, et al. Late phase II study of irinotecan hydrochloride (CPT-11) in advanced gastric cancer. CPT-11 Gastrointestinal Cancer Study Group. Gan To Kagaku Ryoho. 1994;21:1033–8.
9. Boku N, Ohtsu A, Shimada Y, Shirao K, Seki S, Saito H, et al. Phase II study of a combination of irinotecan and cisplatin against metastatic gastric cancer. J Clin Oncol. 1999;17:319–23.
10. Ajani JA, Baker J, Pisters PW, Ho L, Mansfield PF, Feig BW, et al. CPT-11 plus cisplatin in patients with advanced, untreated gastric or gastroesophageal junction carcinoma: results of a phase II study. Cancer. 2002;94:641–6.
11. Ilson DH, Saltz L, Enzinger P, Huang Y, Kornblith A, Gollub M, et al. Phase II trial of weekly irinotecan plus cisplatin in advanced esophageal cancer. J Clin Oncol. 1999;17:3270–5.
12. Pozzo C, Barone C, Szanto J, Padi E, Peschel C, Bukki J, et al. Irinotecan in combination with 5-fluorouracil and folinic acid or with cisplatin in patients with advanced gastric or esophageal-gastric junction adenocarcinoma: results of a randomized phase II study. Ann Oncol. 2004;15:1773–81.
13. Dank M, Zaluski J, Barone C, Valvere V, Yalcin S, Peschel C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. Ann Oncol. 2008;19:1450–7.
14. Kano Y, Suzuki K, Akutsu M, Suda K, Inoue Y, Yoshida M, et al. Effects of CPT-11 in combination with other anti-cancer agents in culture. Int J Cancer. 1992;50:604–10.
15. Yamao T, Shirao K, Matsumura Y, Muro K, Yamada Y, Goto M, et al. Phase I-II study of irinotecan combined with mitomycin-C in patients with advanced gastric cancer. Ann Oncol. 2001;12:1729–35.
16. Yamada Y, Shirao K, Hyodo I, Arai Y, Denda T, Ambo T, et al. Phase II study of biweekly irinotecan and mitomycin C combination therapy in patients with fluoropyrimidine-resistant advanced colorectal cancer. Cancer Chemother Pharmacol. 2003;52:125–30.
17. Green S, Benedetti J, Crowley J. Clinical trials in oncology (interdisciplinary statistics). 2nd ed. Boca Raton: Chapman & Hall/CRC; 2002.
18. Cascinu S, Graziano F, Cardarelli N, Marcellini M, Giordani P, Menichetti ET, et al. Phase II study of paclitaxel in pretreated advanced gastric cancer. Anticancer Drugs. 1998;9:307–10.
19. Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H, et al. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG9205). J Clin Oncol. 2003;21:54–9.
20. Schmitz SH, Voliotis DL, Schimke J, Diehl V. Continuous 5-fluorouracil and leucovorin as a second-line therapy for advanced gastric carcinoma. Oncology. 1994;51:502–6.
21. Vanhoefer U, Wilke H, Weh HJ, Clemens M, Harstrick A, Stahl M, et al. Weekly high-dose 5-fluorouracil and folinic acid as salvage treatment in advanced gastric cancer. Ann Oncol. 1994;5:850–1.
22. Hartmann JT, Kanz L, Bokemeyer C. Phase II study of continuous 120-hour-infusion of mitomycin C as salvage chemotherapy in patients with progressive or rapidly recurrent gastrointestinal adenocarcinoma. Anticancer Res. 2000;20:1177–82.
23. Ajani JA, Baker J, Pisters PW, Ho L, Mansfield PF, Feig BW, et al. Irinotecan/cisplatin in advanced, treated gastric or gastroesophageal junction carcinoma. Oncology (Huntingt). 2002;16:16–8.
24. Kim DY, Kim JH, Lee SH, Kim TY, Heo DS, Bang YJ, et al. Phase II study of oxaliplatin, 5-fluorouracil and leucovorin in previously platinum-treated patients with advanced gastric cancer. Ann Oncol. 2003;14:383–7.

25. Chun JH, Kim HK, Lee JS, Choi JY, Lee HG, Yoon SM, et al. Weekly irinotecan in patients with metastatic gastric cancer failing cisplatin-based chemotherapy. Jpn J Clin Oncol. 2004;34:8–13.
26. Park SH, Kang WK, Lee HR, Park J, Lee KE, Lee SH, et al. Docetaxel plus cisplatin as second-line therapy in metastatic or recurrent advanced gastric cancer progressing on 5-fluorouracil-based regimen. Am J Clin Oncol. 2004;27:477–80.
27. Giuliani F, Molica S, Maiello E, Battaglia C, Gebbia V, Di Bisceglie M, et al. Irinotecan (CPT-11) and mitomycin-C (MMC) as second-line therapy in advanced gastric cancer: a phase II study of the Gruppo Oncologico dell' Italia Meridionale (prot. 2106). Am J Clin Oncol. 2005;28:581–5.
28. Kim ST, Kang WK, Kang JH, Park KW, Lee J, Lee SH, et al. Salvage chemotherapy with irinotecan, 5-fluorouracil and leucovorin for taxane- and cisplatin-refractory, metastatic gastric cancer. Br J Cancer. 2005;92:1850–4.
29. Kunisaki C, Imada T, Yamada R, Hatori S, Ono H, Otsuka Y, et al. Phase II study of docetaxel plus cisplatin as a second-line combined therapy in patients with advanced gastric carcinoma. Anticancer Res. 2005;25:2973–7.
30. Park SH, Choi EY, Bang SM, Cho EK, Lee JH, Shin DB, et al. Salvage chemotherapy with irinotecan and cisplatin in patients with metastatic gastric cancer failing both 5-fluorouracil and taxanes. Anticancer Drugs. 2005;16:621–5.
31. Nguyen S, Rebischung C, Van Ongeval J, Flesch M, Bennamoun M, Andre T, et al. Epirubicin–docetaxel in advanced gastric cancer: two phase II studies as second and first line treatment. Bull Cancer. 2006;93:E1–6.
32. Barone C, Basso M, Schinzari G, Pozzo C, Trigila N, D'Argento E, et al. Docetaxel and oxaliplatin combination in second-line treatment of patients with advanced gastric cancer. Gastric Cancer. 2007;10:104–11.
33. Hartmann JT, Pintoffl JP, Al-Batran SE, Quietzsch D, Meisinger I, Horger M, et al. Mitomycin C plus infusional 5-fluorouracil in platinum-refractory gastric adenocarcinoma: an extended multicenter phase II study. Onkologie. 2007;30:235–40.
34. Kodera Y, Ito S, Mochizuki Y, Fujitake S, Koshikawa K, Kanyama Y, et al. A phase II study of weekly paclitaxel as second-line chemotherapy for advanced gastric cancer (CCOG0302 study). Anticancer Res. 2007;27:2667–71.
35. Jeong J, Jeung HC, Rha SY, Im CK, Shin SJ, Ahn JB, et al. Phase II study of combination chemotherapy of 5-fluorouracil, low-dose leucovorin, and oxaliplatin (FLOX regimen) in pretreated advanced gastric cancer. Ann Oncol. 2008;19:1135–40.
36. Lee JL, Ryu MH, Chang HM, Kim TW, Yook JH, Oh ST, et al. A phase II study of docetaxel as salvage chemotherapy in advanced gastric cancer after failure of fluoropyrimidine and platinum combination chemotherapy. Cancer Chemother Pharmacol. 2008;61:631–7.
37. Park SH, Kim YS, Hong J, Park J, Nam E, Cho EK, et al. Mitomycin C plus S-1 as second-line therapy in patients with advanced gastric cancer: a noncomparative phase II study. Anticancer Drugs. 2008;19:303–7.
38. Shin SJ, Jeung HC, Ahn JB, Choi HJ, Cho BC, Rha SY, et al. Capecitabine and doxorubicin combination chemotherapy as salvage therapy in pretreated advanced gastric cancer. Cancer Chemother Pharmacol. 2008;61:157–65.
39. Sym SJ, Chang HM, Kang HJ, Lee SS, Ryu MH, Lee JL, et al. A phase II study of irinotecan and docetaxel combination chemotherapy for patients with previously treated metastatic or recurrent advanced gastric cancer. Cancer Chemother Pharmacol. 2008;63:1–8.
40. Takiuchi H, Goto M, Imamura H, Furukawa H, Imano M, Imamoto H, et al. Multi-center phase II study for combination therapy with paclitaxel/doxifluridine to treat advanced/recurrent gastric cancer showing resistance to S-1 (OGSG 0302). Jpn J Clin Oncol. 2008;38:176–81.
41. Zhong H, Zhang Y, Ma S, Ying JE, Yang Y, Yong D, et al. Docetaxel plus oxaliplatin (DOCOX) as a second-line treatment after failure of fluoropyrimidine and platinum in Chinese patients with advanced gastric cancer. Anticancer Drugs. 2008;19:1013–8.
42. Baize N, Abakar-Mahamat A, Mounier N, Berthier F, Caroli-Bosc FX. Phase II study of paclitaxel combined with capecitabine as second-line treatment for advanced gastric carcinoma after failure of cisplatin-based regimens. Cancer Chemother Pharmacol. 2009;64:549–55.
43. Leary A, Assersohn L, Cunningham D, Norman AR, Chong G, Brown G, et al. A phase II trial evaluating capecitabine and irinotecan as second line treatment in patients with oesophagogastric cancer who have progressed on, or within 3 months of platinum-based chemotherapy. Cancer Chemother Pharmacol. 2009;64:455–62.
44. Lorizzo K, Fazio N, Radice D, Boselli S, Ariu L, Zampino MG, et al. Simplified FOLFIRI in pre-treated patients with metastatic gastric cancer. Cancer Chemother Pharmacol. 2009;64:301–6.
45. Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, et al. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. Pharmacogenet Genomics. 2007;17:497–504.