Table 3. Best RECIST-defined tumour response by independent review and investigator assessment

Response	First-line population ($n = 25$)		Pretreated population ($n = 26$)		Total population ($n = 51$)	
	Independent review	Investigator assessment	Independent review	Investigator assessment	Independent review	Investigator assessment
Objective response, % (95% CI)	48.0 (27.8–68.7)	48.0 (27.8–68.7)	46.2 (26.6–66.6)	46.2 (26.6–66.6)	47.1 (32.9–61.5)	47.1 (32.9–61.5)
Complete response, *n*	1	0	0	0	1	0
Partial response, *n*	11	12	12	12	23	24

CI, confidence interval.

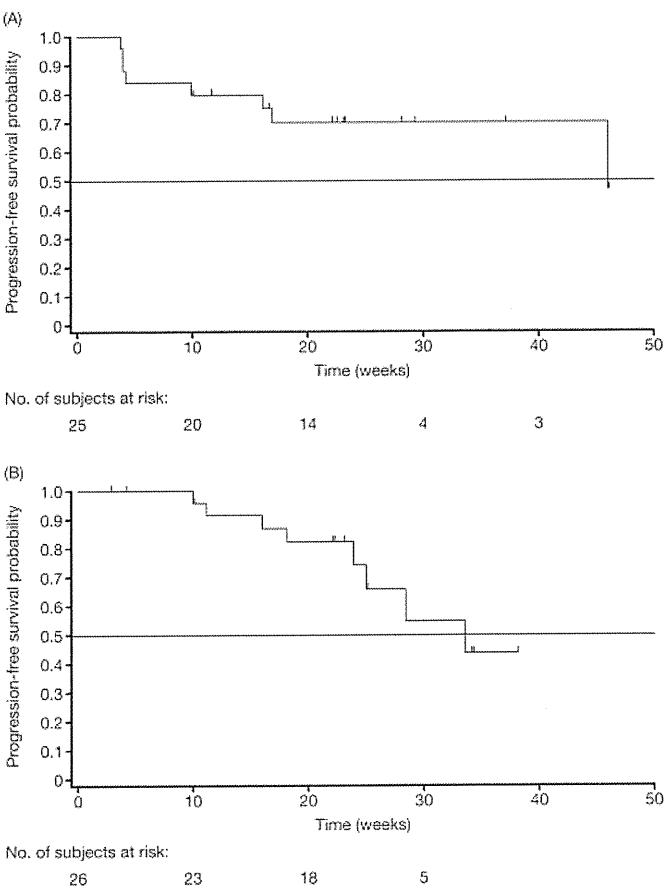

Figure 1. Kaplan–Meier estimates of progression-free survival in the (A) first-line and (B) pretreated populations.

Treatment changes (dose reductions, interruptions and/or extensions of off-treatment periods) owing to treatment-related adverse events were reported in 40 patients (78%); overall, 17 patients (68%) in the first-line population, and 23 patients (88%) in the pretreated population. Treatment changes owing to decreased platelet counts were reported in approximately 50% of patients.

QT-corrected interval prolongation occurred in two patients (4%) overall, but was not clinically significant and resolved without treatment changes. Reduced left ventricular ejection fraction (LVEF) was reported as a serious adverse event in one patient, but abated after treatment discontinuation.

Five patients (20%) in the first-line population and four patients (15%) in the pretreated population experienced treatment-related adverse events that led to discontinuation. The adverse events most commonly leading to discontinuation were hypertension and decreased LVEF, each reported in two patients (4%) overall. No discontinuations owing to haematological or biochemical laboratory abnormalities were reported.

Health-related Quality of Life

For the EQ-5D index score (data not shown), the range of mean change at each endpoint from baseline was from −0.1573 to 0.0375 in the first-line population and from −0.0974 to 0.0513 in the pretreated population. For EQ-VAS score (Figure 2), the range of mean change at each endpoint from baseline was from −12.35 to 2.71 in the first-line population and from −11.82 to 4.17 in the pretreated population. Both scores tended to decline during treatment with sunitinib and subsequently recovered during the off-treatment periods in both populations.

Pharmacokinetics

Median trough plasma concentrations of total drug reached therapeutic levels (>50 ng/ml) (23) on day 14 of cycle 1 in both populations, and levels were sustained throughout treatment during the dosing periods without clinically relevant differences in concentrations between populations. Median trough plasma concentrations of total drug on days 14 and 28 of cycle 1 were comparable to those observed on day 28 of cycles 2 and 3 (Figure 3).

Potential associations between body weight and systemic exposure (area under the plasma-concentration curve; AUC) to sunitinib, SU12662 and total drug were explored in Japanese and Caucasian subjects (Figure 4). The population for this analysis comprised 13 Western studies, one of which included Japanese subjects, and a Japanese study (25). No clear correlations between weight and exposure were observed in either population.

DISCUSSION

Results from this open-label, multicentre phase II trial demonstrated that sunitinib 50 mg, self-administered orally

Table 4. Treatment-related adverse events and laboratory abnormalities reported in >25% of patients in the first-line ($n = 25$) and pretreated ($n = 26$) populations

Adverse event	Maximum NCI CTCAE[a] Grade[b]				
	Grade 1, *n* (%)	Grade 2, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)	Total, *n* (%)
First-line population					
Anorexia	4 (16)	10 (40)	3 (12)	0	17 (68)
Skin discoloration	16 (64)	0	0	0	16 (64)
Diarrhoea	8 (32)	3 (12)	4 (16)	0	15 (60)
Pyrexia	9 (36)	6 (24)	0	0	15 (60)
Nausea	7 (28)	5 (20)	2 (8)	0	14 (56)
Stomatitis	8 (32)	4 (16)	1 (4)	0	13 (52)
Dysgeusia	8 (32)	4 (16)	0	0	12 (48)
Rash	11 (44)	1 (4)	0	0	12 (48)
Fatigue	4 (16)	5 (20)	3 (12)	0	12 (48)
Hand-foot syndrome	3 (12)	4 (16)	4 (16)	0	11 (44)
Hypertension	3 (12)	5 (20)	3 (12)	0	11 (44)
Vomiting	2 (8)	5 (20)	1 (4)	0	8 (32)
Face oedema	6 (24)	2 (8)	0	0	8 (32)
Malaise	6 (24)	1 (4)	1 (4)	0	8 (32)
Laboratory abnormality					
Decreased platelet count	7 (28)	3 (12)	12 (48)	2 (8)	24 (96)
Decreased white blood cell count	4 (16)	12 (48)	5 (20)	0	21 (84)
Decreased lymphocyte count	2 (8)	9 (36)	8 (32)	2 (8)	21 (84)
Decreased neutrophil count	4 (16)	4 (16)	8 (32)	2 (8)	18 (72)
Increased lactate dehydrogenase	11 (44)	5 (20)	1 (4)	0	17 (68)
Increased lipase	5 (20)	3 (12)	4 (16)	3 (12)	15 (60)
Increased creatinine	7 (28)	6 (24)	1 (4)	0	14 (56)
Increased aspartate aminotransferase	10 (40)	1 (4)	2 (8)	1 (4)	14 (56)
Increased alanine aminotransferase	8 (32)	2 (8)	3 (12)	0	13 (52)
Decreased haemoglobin	7 (28)	3 (12)	1 (4)	0	11 (44)
Increased amylase	6 (24)	3 (12)	2 (8)	0	11 (44)
Increased alkaline phosphatase	7 (28)	2 (8)	1 (4)	0	10 (40)
Increased bilirubin	3 (12)	3 (12)	1 (4)	0	7 (28)
Decreased calcium	5 (20)	2 (8)	0	0	7 (28)
Decreased phosphorus	0	1 (4)	6 (24)	0	7 (28)
Pretreated population					
Skin discoloration	20 (77)	1 (4)	0	0	21 (81)
Fatigue	8 (31)	3 (12)	6 (23)	1 (4)	18 (69)
Anorexia	9 (35)	5 (19)	0	0	14 (54)
Dysgeusia	12 (46)	2 (8)	0	0	14 (54)
Rash	13 (50)	1 (4)	0	0	14 (54)
Hand-foot syndrome	4 (15)	6 (23)	3 (12)	0	13 (50)
Hypertension	1 (4)	9 (35)	3 (12)	0	13 (50)
Pyrexia	4 (15)	6 (23)	1 (4)	0	11 (42)
Epistaxis	11 (42)	0	0	0	11 (42)
Stomatitis	6 (23)	3 (12)	1 (4)	0	10 (38)
Oedema peripheral	8 (31)	0	1 (4)	0	9 (35)
Eyelid oedema	8 (31)	1 (4)	0	0	9 (35)
Nausea	3 (12)	6 (23)	0	0	9 (35)
Cheilitis	6 (23)	2 (8)	0	0	8 (31)
Malaise	4 (15)	2 (8)	2 (8)	0	8 (31)
Face oedema	6 (23)	0	1 (4)	0	7 (27)
Diarrhoea	6 (23)	0	1 (4)	0	7 (27)
Pain in extremity	2 (8)	2 (8)	3 (12)	0	7 (27)
Laboratory abnormality					
Decreased platelet count	4 (15)	5 (19)	12 (46)	2 (8)	23 (88)
Decreased neutrophil count	3 (12)	3 (12)	15 (58)	1 (4)	22 (85)
Decreased white blood cell count	3 (12)	16 (62)	3 (12)	0	22 (85)
Increased lactate dehydrogenase	17 (65)	2 (8)	0	0	19 (73)
Increased aspartate aminotransferase	14 (54)	2 (8)	1 (4)	0	17 (65)
Increased lipase	1 (4)	3 (12)	10 (38)	3 (12)	17 (65)
Decreased lymphocyte count	2 (8)	5 (19)	5 (19)	2 (8)	14 (54)
Increased alanine aminotransferase	11 (42)	2 (8)	0	0	13 (50)
Increased amylase	7 (27)	2 (8)	3 (12)	0	12 (46)
Decreased haemoglobin	9 (35)	1 (4)	1 (4)	0	11 (42)
Increased creatinine	4 (15)	6 (23)	0	0	10 (38)
Increased bilirubin	3 (12)	5 (19)	0	0	8 (31)
Increased alkaline phosphatase	6 (23)	1 (4)	0	0	7 (27)

[a]National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.
[b]No grade 5 adverse events or laboratory abnormalities were reported.

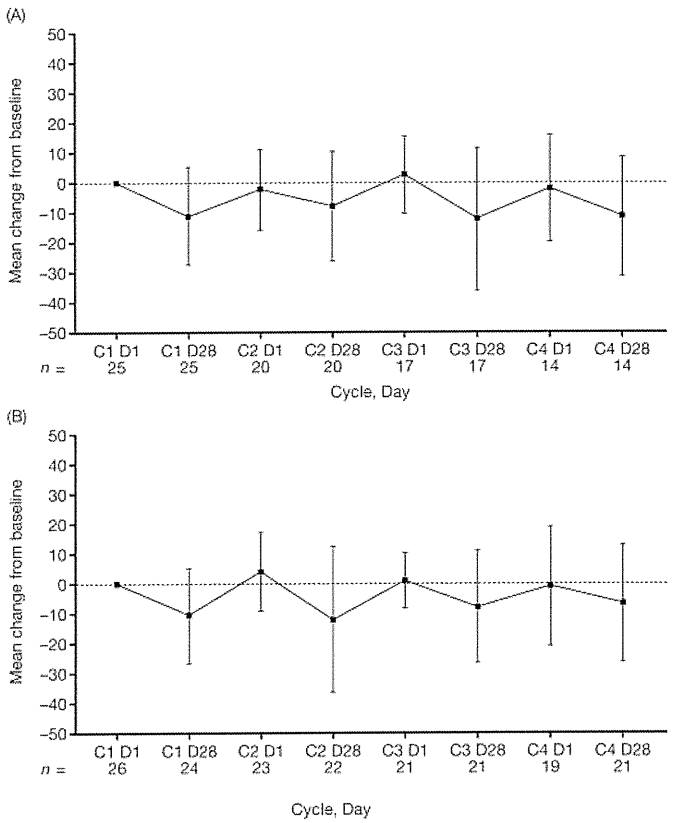

Figure 2. Mean change from baseline in EQ-VAS scores in the (A) first-line and (B) pretreated populations. EQ-VAS, EuroQol visual analogue scale; C, cycle; D, day (e.g. C1D1, cycle 1, day 1). Error bars represent standard deviations.

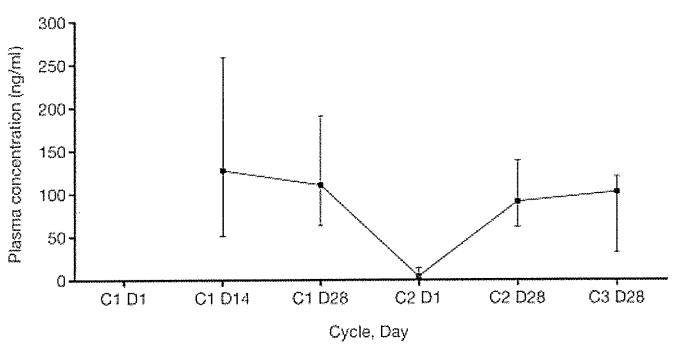

Figure 3. Median trough plasma concentrations of total drug (sunitinib + SU12662) for all patients receiving a sunitinib starting dose of 50 mg during cycles 1–3. Vertical bars represent ranges of median values. C, cycle; D, day (e.g. C1D1, cycle 1, day 1). Note: Data represent median plasma concentration (lowest to highest) in patients who started with sunitinib 50 mg in each treatment cycle.

on Schedule 4/2, was effective and well-tolerated for the first- and second-line treatment of metastatic RCC in Japanese patients. This is the first such study of this magnitude of patients in Japan.

Data revealed similar ORRs in the two patient populations, 48.0% and 46.0% in the first-line and pretreated patients, respectively, with identical findings reported by investigator and independent reviewers. Although cross-study comparisons should be interpreted with care owing to differences in methodology, these results compare favourably with those from previous, larger trials of sunitinib in treatment-naïve (47%) (17) and cytokine-refractory patients (33%) (14–16) with metastatic RCC, as well as with the ORR of 12.4% recently reported for sorafenib in a phase II study of Japanese patients with cytokine-refractory metastatic RCC (24). In addition, as reported in previous trials (14–17), sunitinib was associated with substantially longer PFS in the first-line population compared with the pretreated population (46.0 vs. 33.6 weeks, respectively).

All patients experienced treatment-related adverse events, the majority of which were grade 1 or 2 in severity. Most patients were able to resume therapy following treatment changes, with only five patients (20%) in the first-line population and four patients (15%) in the pretreated population having discontinued because of adverse events. The most commonly reported grade 3 adverse events and laboratory abnormalities were diarrhoea, hand-foot syndrome and decreased platelets in the first-line population, and fatigue and decreased neutrophils and platelets in the pretreated population. In particular, the incidence of grade 3/4 haematological toxicities such as decrease of neutrophils and platelets seems to be higher in this trial when compared with the previous worldwide data, more than 50% versus around 10%, respectively.

There were modest declines in health-related quality of life during the sunitinib treatment periods as measured by EQ-5D and EQ-VAS, which readily recovered during the subsequent off-treatment periods. In addition, overall scores were slightly lower at the end of treatment compared with baseline, and the data suggested a slight, non-significant trend towards higher scores in the pretreated over the first-line population.

Preclinical studies have demonstrated that sunitinib is effective at plasma concentrations $\geq$50 ng/ml (23). In the current study, therapeutically effective levels of the drug were reached after 2 weeks of treatment during cycle 1, levels which, although cyclic, reflecting the dosing schedule, were sustained for the duration of treatment in both populations. Repeated dosing was not associated with accumulation of sunitinib in plasma.

Houk et al. (25) analysed merged data from 13 Western studies (including Japanese subjects in one study) and one Japanese study using a population-PK approach, and demonstrated that tumour type (i.e. metastatic RCC, gastrointestinal stromal tumours or other solid tumours) as a covariate contributed the largest effect on the PK of sunitinib and SU12662. Gender, body weight and ECOG PS score had less of an impact, and Japanese patients showed similar PK to Caucasians. They concluded that no starting sunitinib dose adjustments are recommended based on the magnitude of predicted changes owing to any covariate studied. Correlations between body weight and AUC values of sunitinib, SU12662 and total drug in Japanese and Caucasian subjects from the above 13 Western studies and one Japanese study were further investigated, and no clear correlations

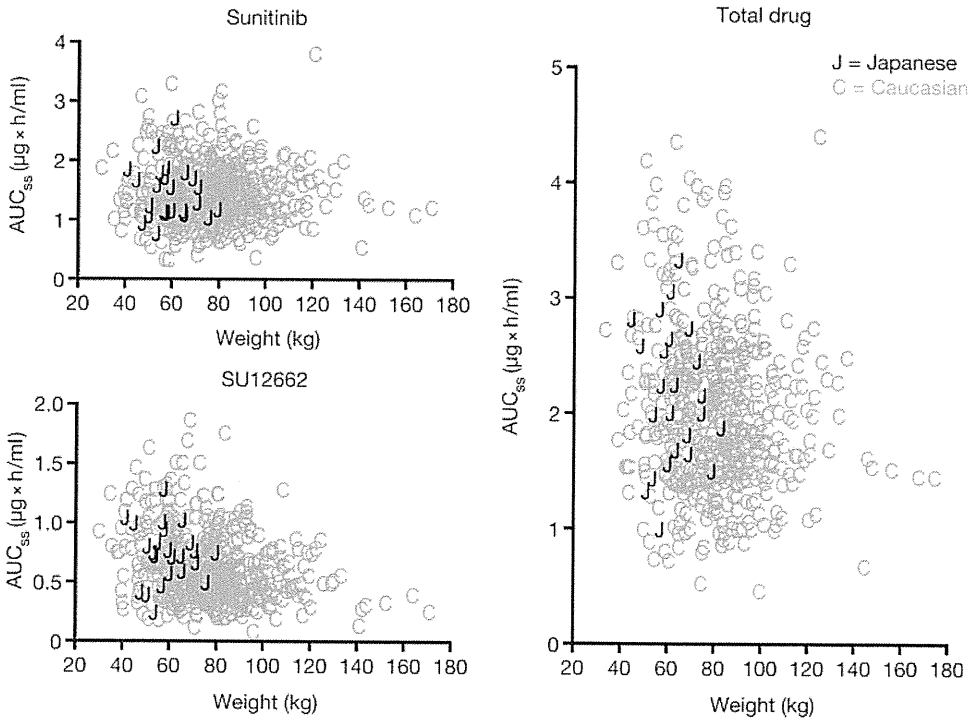

Figure 4. Correlations between the steady state AUC values (sunitinib, SU12662 and total drug) and the body weight of individual Caucasian (C) and Japanese (J) subjects from 13 Western studies (including Japanese subjects in one study) and one Japanese study described in reference (25).

between the AUC values and body weight were identified in either population (Figure 4). Importantly, although the body weights of Japanese subjects were generally lower when compared with those of Caucasian subjects, the AUC values seen in Japanese subjects were within the range of those seen in Caucasian subjects, indicating no substantial difference in the AUC of sunitinib, SU12662 or total drug between these two populations.

The incidence of haematological adverse events was numerically higher in this trial with Japanese patients (n = 51) compared with those previously reported for sunitinib in Western patients (n = 375) (17). Better understanding of the mechanisms underlying the side effects of sunitinib is of great interest and may help explain this difference. Since the AUC values of sunitinib and SU12662 were similar between Japanese and Caucasian subjects (Figure 4), the likely disparities in the adverse event profiles may be explained by differences in the relative importance (e.g. expression levels and activity) of sunitinib-sensitive kinases that are involved in the homeostatic regulation of the haematopoietic system. However, given the small number of Japanese patients in this trial (n = 51), it is still to be statistically determined whether substantial racial/ethnic differences exist in the pharmacological properties of sunitinib.

CONCLUSION

Sunitinib 50 mg administered on Schedule 4/2 is effective and well-tolerated for the treatment of Japanese patients with metastatic RCC in both the first- and second-line treatment settings. It is also of note that there was a trend towards a greater antitumour efficacy and higher incidence of haematological adverse events in Japanese patients compared with the mostly Western patients who participated in prior trials, warranting further investigation.

AUTHORSHIP CONTRIBUTIONS

All authors discussed the results and commented on the manuscript. Specifically, each author contributed to the study and manuscript as follows: Hirotsugu Uemura designed this study, conducted patient treatment and wrote/edited the manuscript; Nobuo Shinohara, Takeshi Yuasa, Yoshihiko Tomita, Hiroyuki Fujimoto, Soichi Mugiya, Masashi Niwakawa designed this study, conducted patient treatment and edited the manuscript; Tsuneharu Miki designed this study and edited the manuscript; Norio Nonomura, Masayuki Takahashi, Yoshihiro Hasegawa designed this study, conducted patient treatment and edited the manuscript; Naoki Agata analysed the data and wrote/edited the manuscript; Brett Houk analysed the data and edited the manuscript; Seiji Naito designed this study, conducted patient treatment and edited the manuscript; and Hideyuki Akaza designed this study, conducted patient treatment, edited the manuscript and serves as the corresponding author for this paper.

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Conflict of interest statement

The authors, Naoki Agata Ph.D. and Brett Houk Ph.D., are employees of Pfizer Inc.

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Original Article

Overall Survival and Updated Results from a Phase II Study of Sunitinib in Japanese Patients with Metastatic Renal Cell Carcinoma

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Background: In a phase II, open-label, multicentre Japanese study, sunitinib demonstrated antitumour activity and acceptable tolerability in metastatic renal cell carcinoma patients. Final survival analyses and updated results are reported.

Methods: Fifty-one Japanese patients with a clear-cell component of metastatic renal cell carcinoma (25 treatment-naïve; 26 cytokine-refractory) received sunitinib 50 mg orally, once daily (Schedule 4/2). Overall and progression-free survivals were estimated by the Kaplan–Meier method. Objective response rate (per Response Evaluation Criteria in Solid Tumours) and safety were assessed with an updated follow-up.

Results: First-line and pretreated patients received a median 6.0 and 9.5 treatment cycles, respectively. Investigator-assessed, end-of-study objective response rate was 52.0, 53.8 and 52.9% in first-line, pretreated and overall intent-to-treat populations, respectively. The median progression-free survival was 12.2 and 10.6 months in first-line and pretreated patients, respectively. Fourteen patients per group died (56 and 54%), and the median overall survival was 33.1 and 32.5 months, respectively. The most common treatment-related Grade 3 or 4 adverse events and laboratory abnormalities were fatigue (24%), hand-foot syndrome (18%), decreased platelet count (55%), decreased neutrophil count (53%) and increased lipase (49%). No Grade 5 treatment-related adverse events occurred. Forty patients (78%) required dose reduction, and 13 (25%) discontinued, due to treatment-related adverse events.

Conclusions: With the median overall survival benefit exceeding 2.5 years, and acceptable tolerability, in first-line and pretreated Japanese metastatic renal cell carcinoma patients with Eastern Cooperative Oncology Group performance status 0/1, sunitinib showed a favourable risk/benefit profile, similar to Western studies. However, there was a trend towards greater efficacy and more haematological adverse events in Japanese patients.

Key words: Japanese – phase II – renal cell carcinoma – sunitinib

INTRODUCTION

Sunitinib is an orally administered, multitargeted tyrosine kinase inhibitor of vascular endothelial growth factor (VEGF) receptors 1–3 and platelet-derived growth factor (PDGF) receptors α and β (1). Data from Western studies of patients with treatment-naïve and cytokine-pretreated renal cell carcinoma (RCC) have demonstrated consistent benefit with single-agent sunitinib therapy, with investigator-assessed objective response rates (ORRs) of up to 47 and 49% and median overall survival (OS) times of 26.4 and 23.9 months, for first- and second-line treatment, respectively (2–5).

We previously reported interim results of the first Japanese phase II study of single-agent sunitinib in 51 patients with metastatic RCC, demonstrating comparable efficacy and tolerability to that observed in Western patients (6). In this study, the primary endpoint of ORR, based on independent review, was 48.0% in treatment-naïve patients, 46.2% among pretreated patients and 47.1% in the overall intent-to-treat (ITT) population. Median progression-free survival (PFS) was 46.0, 33.6 and 46.0 weeks (10.6, 7.8 and 10.6 months) in the same patient groups, respectively. As a result of these findings, multinational approvals of sunitinib for treatment of first- and second-line advanced RCC now include approval in Japan for patients with RCC not indicated for curative resection and patients with metastatic RCC.

Although the primary endpoint of the Japanese phase II study was met at the time of interim analysis, the median OS had not yet been reached. Here we report the final OS analysis, as well as updated efficacy and safety findings.

METHODS

Patients

Patients with histologically proven RCC with a clear-cell component and metastases were included in the study. No prior systemic therapy was permitted in the first-line population, and previous treatment with only one cytokine-based regimen (that could include multiple cytokines) was permitted in the pretreated group. Additional eligibility and exclusion criteria [e.g. all patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1] have been reported previously (6).

Study Design and Treatment

This was a multicentre, open-label, non-randomized, single-arm, phase II study of sunitinib (SUTENT®; Pfizer, New York, NY, USA) in patients with treatment-naïve or cytokine-pretreated metastatic RCC. The study was carried out in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and was approved by the institutional review board at each participating centre. All patients provided written informed consent.

Sunitinib was administered orally at a starting dose of 50 mg once daily in the morning, without regard to meals. Treatment was given in repeated 6-week cycles consisting of 4 weeks on therapy, followed by 2 weeks off (Schedule 4/2). Intrapatient dose reductions or interruptions were permitted to manage adverse events (AEs), according to the protocol. Treatment was continued until disease progression, requirement for additional anticancer therapy, development of left ventricular systolic dysfunction or withdrawal of consent.

Assessments

The primary endpoint was ORR in the ITT population based on independent review, and secondary endpoints included the investigator-assessed ORR, duration of response, time to tumour response, PFS, OS and safety. Tumour assessments were based on the Response Evaluation Criteria in Solid Tumours (RECIST) (7), with computed tomography or MRI scans (with or without X-rays) obtained every 6 weeks by investigators. Safety and tolerability were monitored as previously described (6), and AEs were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). A post-study survival survey was conducted once per year and included all patients who received at least one dose of sunitinib.

Statistical Methods

In the primary efficacy analysis of the ITT population, the ORR and 95% confidence intervals (CIs) were calculated based on independent review, with the same analysis performed for investigators' assessments. Sample sizes of 26 and 25 patients were required for the pretreated and first-line populations, respectively, to provide a power of 80% with an alpha level of 2.5%. These sample size calculations were based on the threshold values for response rates (5% for pretreated patients and 10% for the first-line population) considered to be clinically ineffective for each population. The efficacy of sunitinib would be confirmed if the lower limit of the 95% CI of the observed ORR was greater than or equal to the threshold rate for each population. Time-to-event endpoints were estimated using the Kaplan–Meier method (8).

Independent review of imaging scans was discontinued after the interim analysis in February 2007 when the primary endpoint was met; therefore, only updated investigator-assessed results are reported herein.

RESULTS

Patient Characteristics and Disposition

A total of 51 patients were enrolled at 12 study centres in Japan. The first-line population consisted of 25 patients with a mean age of 56.6 years (range, 33–76), and the pretreated

population comprised 26 patients with a mean age of 61.1 years (range, 34–77). At baseline, the ECOG PS of all patients was 0 or 1, and the lung was the most prevalent site of metastases. Additional patient baseline characteristics have been described previously (6).

All 51 patients received at least one dose of sunitinib. At the time of analysis, the first-line and pretreated groups received a median 6.0 and 9.5 treatment cycles, respectively. No patients continued to receive sunitinib after disease progression in the first-line group; however, 9/18 patients (50%) with progressive disease in the pretreated group continued to receive sunitinib after progression because of continuing benefit. Ten patients (20%) had completed the study (6 patients in the first-line group and 4 patients in the pretreated group) and 41 (80%) had discontinued (19 and 22 patients, respectively). Reasons for discontinuation included disease progression (13 patients in each group) and treatment-related AEs (6 and 7 patients, respectively, including 1 pretreated patient with a laboratory abnormality); in addition, within the pretreated group, 1 patient died and 1 patient had discontinued due to AEs unrelated to treatment.

Efficacy

At the end of the study, the investigator-assessed ORR (95% CI) was 52.0% (31.3, 72.2) in the first-line population, 53.8% (33.4, 73.4) in the pretreated population and 52.9% (38.5, 67.1) in the overall ITT population (Table 1). An additional 13 patients (6 in the first-line population and 7 in the pretreated group) had a best response of stable disease ≥6 weeks. The median time to tumour response was 10.0 and 10.5 weeks, and the duration of response was 25.8 and 8.8 months in the first-line and pretreated groups, respectively.

Median PFS was 12.2 months (95% CI: 7.8, 48.8) in the first-line population and 10.6 months (95% CI: 6.6, 24.2) in the pretreated population (Figure 1). A total of 14 patients in each arm died (56 and 54%, respectively), and the median OS was 33.1 months (95% CI: 14.8, not reached) and 32.5 months (95% CI: 19.8 months, not reached) for the first-line and pretreated groups, respectively (Figure 2).

Safety

The most frequently reported treatment-related AEs of all grades were anorexia (72%), and skin discolouration and diarrhoea (both 64%) in the first-line population (Table 2), and skin discolouration and fatigue (both 81%), as well as anorexia and hypertension (both 65%) in the pretreated population (Table 3). In all patients, the majority of AEs were mild to moderate (Grades 1 or 2) in severity. Commonly reported Grade 3, treatment-related AEs in the first-line and pretreated groups, respectively, included diarrhoea (16 and 15%), fatigue (16 and 27%), hand-foot syndrome (16 and 19%) and hypertension (12 and 19%). A total of three Grade 4, treatment-related AEs were reported overall (hypomagnesaemia, dyspnoea and fatigue; all $n = 1$), and no Grade 5 events occurred.

Laboratory abnormalities were frequently reported in both patient populations (Tables 2 and 3). The most common abnormalities were decreased counts for platelets (96%), white blood cells (88%), lymphocytes (84%) and neutrophils (76%) in the first-line population (Table 2) and decreased counts for platelets (88%), white blood cells (85%) and neutrophils (85%) and increased lipase (77%) in the pretreated population (Table 3). Grade 3 or 4 laboratory abnormalities

Table 1. Best RECIST-defined tumour response at study end (investigator assessment)

Response	First-line population ($n = 25$)	Pretreated population ($n = 26$)	Total population ($n = 51$)
Objective response rate, %	52.0%	53.8%	52.9%
(95% CI)	(31.3, 72.2)	(33.4, 73.4)	(38.5, 67.1)
Partial response, *n*	13	14	27
Stable disease ≥ 6 weeks, *n*	6	7	13

CI, confidence interval; RECIST, Response Evaluation Criteria in Solid Tumours.

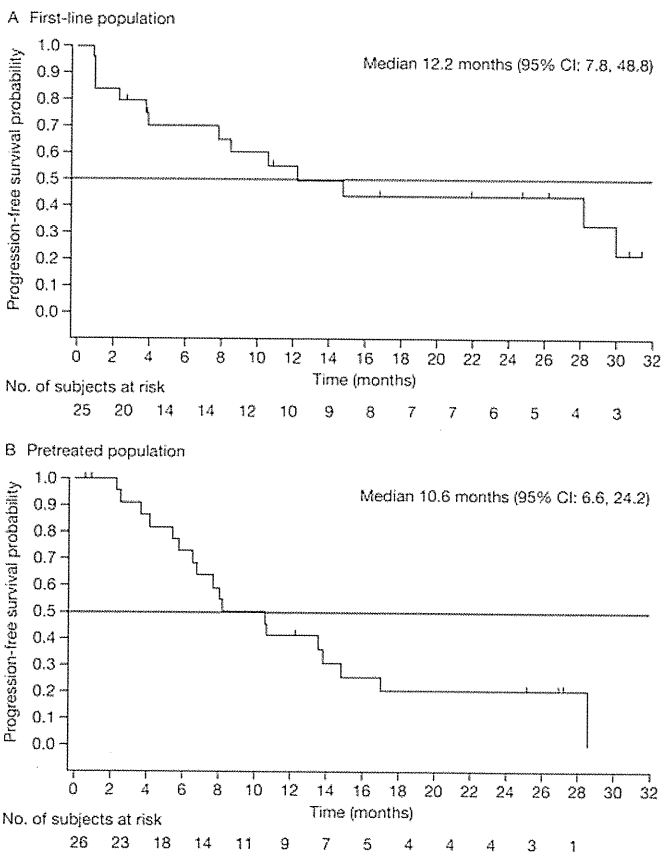

Figure 1. Kaplan–Meier estimates of progression-free survival in the (A) first-line and (B) pretreated patient populations. CI, confidence interval.

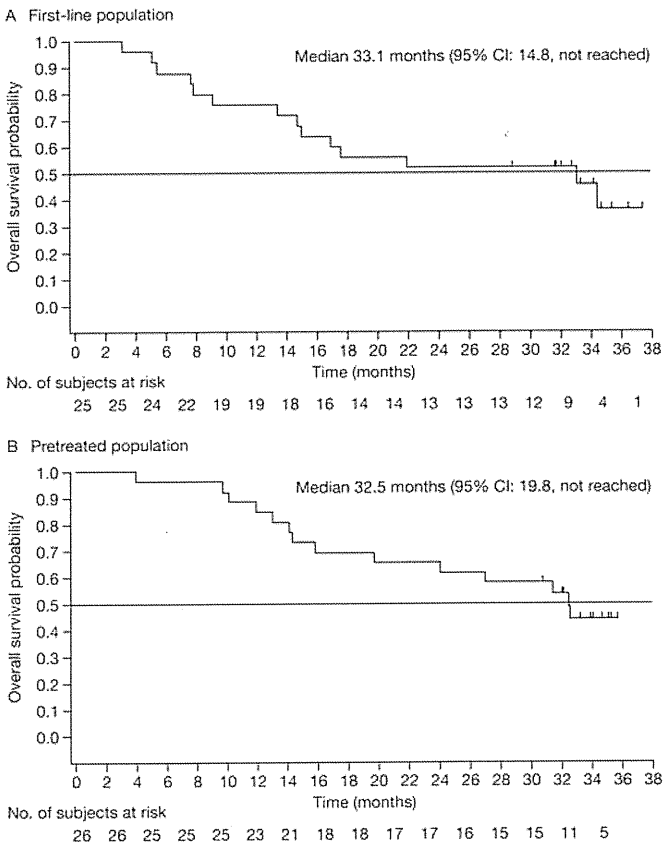

Figure 2. Kaplan–Meier estimates of overall survival in the (A) first-line and (B) pretreated patient populations. CI, confidence interval.

reported in at least 50% of patients in the first-line and pretreated populations, respectively, included decreased counts for platelets (56 and 54%) and neutrophils (44 and 62%) and increased lipase (32 and 65%). Decreased lymphocyte count and increased lipase were the most frequently reported Grade 4 laboratory abnormalities in the first-line population (each $n = 3$; 12%), while increased lipase was the most common Grade 4 abnormality in the pretreated population ($n = 5$; 20%). No Grade 5 laboratory abnormalities were observed.

The overall incidences of QT-corrected interval prolongation ($n = 2$; 1 patient each with Grades 1 and 2 severity) and decreased left ventricular ejection fraction (LVEF; $n = 2$; 1 patient each with Grades 2 and 3 severity) were low (both 4%) with neither condition existent at baseline. Both cases of QT-corrected interval prolongation were not clinically significant and resolved without treatment changes. One case of decreased LVEF was reported as a serious AE and resolved following treatment discontinuation.

Treatment-related AEs led to dose modifications (dose reduction or temporary discontinuation) in a total of 46 patients (90%), comprising 22 and 24 patients in the first-line and pretreated populations, respectively. Overall, the most frequent AEs leading to treatment changes were decreased counts for platelets ($n = 26$; 51%), neutrophils ($n = 23$; 45%) and white blood cells ($n = 15$; 29%), as well as fatigue ($n = 13$; 26%) and hand-foot syndrome ($n = 11$; 22%). The majority of these events resolved, either with or without standard treatment, and did not result in sunitinib discontinuation. Discontinuation due to treatment-related AEs occurred among 13 patients (25%), and the most common AEs involved were fatigue, decreased LVEF and hypertension.

DISCUSSION

In this phase II, open-label, multicentre study of sunitinib 50 mg/day (Schedule 4/2) in Japanese patients with first-line and cytokine-refractory metastatic RCC, sunitinib showed significant antitumour activity, including a median OS benefit exceeding 2.5 years, and was generally well tolerated. The primary endpoint of ORR was met at the first interim analysis (February 2007 data cutoff) (6), and increased in each patient population as the study continued to produce a final ORR of 52.9% in all patients, 52.0% in the first-line population and 53.8% in the pretreated population.

Final results for the median PFS (12.2 and 10.6 months for first-line and pretreated patients, respectively) and the median OS (33.1 and 32.5 months, respectively) indicate that sunitinib provides substantial survival benefits in both treatment-naïve and cytokine-pretreated Japanese patients with metastatic RCC. Notably, the results cannot answer whether sunitinib should be used in treatment-naïve or cytokine-pretreated patients, since there may be possible differences in clinical backgrounds of each population.

Although cross-study comparisons should be interpreted with care due to methodological issues, both the median PFS and OS were longer in this study, compared with sunitinib trials in Western patients with metastatic RCC. In a recent phase III trial of first-line therapy, the median PFS was 11 months and the median OS was 26.4 months in Western patients (5) (compared with 12.2 and 33.1 months, respectively, in the present study), while, in two consecutive phase II trials of second-line therapy, the median PFS was 8.7–8.8 months with the median OS of 23.9 months in the latter trial (3,4) (compared with 10.6 and 32.5 months, respectively, in the present study). Similarly, investigator-assessed ORRs were ~5% higher in Japanese patients in this trial compared with Western patients (2–5). Preliminary findings from an ongoing, expanded-access trial of sunitinib in patients with metastatic RCC found that efficacy was comparable between patients in Asia-Pacific and Western countries (9). However, the expanded access trial included both treatment-naïve and pretreated patients, as well as patients from a greater geographical range than the present study, which may have affected the outcome. Finally, the >50% ORR reported in our study also compares favourably with the ORR of 14.7% reported in a phase II study of sorafenib in Japanese patients with unresectable RCC (10).

Safety findings from the present study as well as the ongoing expanded access trial (9) indicate that the safety profile of sunitinib is generally similar in Asian and

Table 2. Treatment-related adverse events and laboratory abnormalities reported in at least 25% of patients in the first-line population

	Maximum NCI CTCAE[a] grade[b] ($n = 25$)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total
Adverse event, *n* (%)					
Anorexia	5 (20)	10 (40)	3 (12)	0	18 (72)
Skin discolouration	15 (60)	1 (4)	0	0	16 (64)
Diarrhoea	8 (32)	4 (16)	4 (16)	0	16 (64)
Pyrexia	9 (36)	6 (24)	0	0	15 (60)
Nausea	6 (24)	7 (28)	2 (8)	0	15 (60)
Stomatitis	8 (32)	5 (20)	1 (4)	0	14 (56)
Hypertension	2 (8)	9 (36)	3 (12)	0	14 (56)
Dysgeusia	4 (16)	9 (36)	0	0	13 (52)
Hand-foot syndrome	2 (8)	7 (28)	4 (16)	0	13 (52)
Rash	10 (40)	2 (8)	0	0	12 (48)
Fatigue	2 (8)	6 (24)	4 (16)	0	12 (48)
Malaise	7 (28)	1 (4)	1 (4)	0	9 (36)
Face oedema	6 (24)	3 (12)	0	0	9 (36)
Vomiting	3 (12)	4 (16)	2 (8)	0	9 (36)
Oedema peripheral	6 (24)	2 (8)	0	0	8 (32)
Laboratory abnormality, *n* (%)					
Decreased platelet count	7 (28)	3 (12)	12 (48)	2 (8)	24 (96)
Decreased white blood cell count	2 (8)	14 (56)	5 (20)	1 (4)	22 (88)
Decreased lymphocyte count	1 (4)	10 (40)	7 (28)	3 (12)	21 (84)
Decreased neutrophil count	2 (8)	6 (24)	9 (36)	2 (8)	19 (76)
Increased lactate dehydrogenase	11 (44)	5 (20)	1 (4)	0	17 (68)
Increased lipase	5 (20)	3 (12)	5 (20)	3 (12)	16 (64)
Increased aspartate aminotransferase	10 (40)	2 (8)	2 (8)	1 (4)	15 (60)
Increased creatinine	7 (28)	6 (24)	2 (8)	0	15 (60)
Increased alanine aminotransferase	7 (28)	3 (12)	3 (12)	0	13 (52)
Decreased haemoglobin	6 (24)	5 (20)	1 (4)	0	12 (48)
Increased amylase	6 (24)	3 (12)	2 (8)	0	11 (44)
Increased alkaline phosphatase	6 (24)	3 (12)	1 (4)	0	10 (40)
Increased bilirubin	4 (16)	3 (12)	1 (4)	0	8 (32)
Decreased phosphorus	1 (4)	1 (4)	6 (24)	0	8 (32)
Decreased calcium	5 (20)	2 (8)	0	0	7 (28)

[a]National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.
[b]No Grade 5 adverse events or laboratory abnormalities were reported.

non-Asian patients. The majority of treatment-related AEs observed in this study were Grades 1 or 2 in severity, were manageable with prespecified dose changes or standard medical treatment and did not lead to study withdrawal. The previously published analysis from this study found that patients experienced modest declines in the health-related quality of life during sunitinib treatment periods, followed by recovery during subsequent off-treatment periods (6). In combination with the AE data reported here, these results indicate that sunitinib therapy was well tolerated overall. The most frequently reported Grade 3, treatment-related AEs and laboratory abnormalities in the first-line group were diarrhoea, fatigue and hand-foot syndrome and decreased counts for platelets, neutrophils and lymphocytes, and, in the pretreated group, fatigue, hand-foot syndrome and hypertension and decreased counts for neutrophils and platelets and

Table 3. Treatment-related adverse events and laboratory abnormalities reported in at least 25% of patients in the pretreated population

	Maximum NCI CTCAE[a] grade[b] ($n = 26$)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total
Adverse event, *n* (%)					
Skin discolouration	20 (77)	1 (4)	0	0	21 (81)
Fatigue	9 (35)	4 (15)	7 (27)	1 (4)	21 (81)
Anorexia	8 (31)	6 (23)	3 (12)	0	17 (65)
Hypertension	1 (4)	11 (42)	5 (19)	0	17 (65)
Dysgeusia	11 (42)	4 (15)	0	0	15 (58)
Rash	13 (50)	2 (8)	0	0	15 (58)
Face oedema	13 (50)	0	1 (4)	0	14 (54)
Diarrhoea	10 (38)	0	4 (15)	0	14 (54)
Hand-foot syndrome	3 (12)	6 (23)	5 (19)	0	14 (54)
Oedema peripheral	12 (46)	0	1 (4)	0	13 (50)
Epistaxis	11 (42)	0	0	0	11 (42)
Stomatitis	6 (23)	4 (15)	1 (4)	0	11 (42)
Pyrexia	4 (15)	6 (23)	1 (4)	0	11 (42)
Nausea	3 (12)	7 (27)	1 (4)	0	11 (42)
Malaise	4 (15)	3 (12)	3 (12)	0	10 (38)
Eyelid oedema	7 (27)	2 (8)	0	0	9 (35)
Dyspepsia	6 (23)	2 (8)	0	0	8 (31)
Cheilitis	5 (19)	3 (12)	0	0	8 (31)
Pain in extremity	2 (8)	2 (8)	3 (12)	0	7 (27)
Headache	6 (23)	1 (4)	0	0	7 (27)
Laboratory abnormality, *n* (%)					
Decreased platelet count	4 (15)	5 (19)	12 (46)	2 (8)	23 (88)
Decreased white blood cell count	3 (12)	15 (58)	4 (15)	0	22 (85)
Decreased neutrophil count	2 (8)	4 (15)	14 (54)	2 (8)	22 (85)
Increased lipase	0	3 (12)	12 (46)	5 (19)	20 (77)
Increased lactate dehydrogenase	16 (62)	3 (12)	0	0	19 (73)
Increased aspartate aminotransferase	13 (50)	4 (15)	2 (8)	0	19 (73)
Increased amylase	6 (23)	5 (19)	4 (15)	0	15 (58)
Decreased lymphocyte count	3 (12)	5 (19)	5 (19)	2 (8)	15 (58)
Increased alanine aminotransferase	9 (35)	4 (15)	0	0	13 (50)
Increased creatinine	6 (23)	6 (23)	1 (4)	0	13 (50)
Decreased haemoglobin	5 (19)	4 (15)	3 (12)	0	12 (46)
Decreased albumin	4 (15)	4 (15)	1 (4)	0	9 (35)
Increased alkaline phosphatase	5 (19)	3 (12)	0	0	8 (31)
Increased bilirubin	3 (12)	5 (19)	0	0	8 (31)

[a]National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.
[b]No Grade 5 adverse events or laboratory abnormalities were reported.

increased lipase. In the preliminary report by Lee et al. (9), Asian patients treated at Asian sites had a higher frequency of leukopenia, thrombocytopenia, stomatitis and hand-foot syndrome and a lower incidence of diarrhoea, compared with non-Asian patients. Our findings are generally comparable, although the incidence of diarrhoea in our study was similar to or higher than that previously reported for Western patients (3–5), and the frequency of Grade 3 or 4

thrombocytopenia was ~25% higher among Japanese patients in our study versus the Asian patients treated at Asian sites in the expanded access trial (9). Grade 3 and 4 decreases in counts for neutrophils and platelets were also considerably more common in Japanese patients in the present study than in Western patients in prior studies.

At the current time, it is not clear why the tolerability and efficacy of sunitinib may differ to some extent in Japanese and Western populations. Analysis of sunitinib pharmacokinetic parameters from 13 Western studies and one Japanese study showed similar plasma exposure for sunitinib and its active metabolite SU12662 between the two populations (11), and we have previously reported a lack of correlation between sunitinib and SU12662 systemic exposure and body weight in Japanese and Caucasian subjects in an analysis that included pharmacokinetic data from the present study (6). Further studies are needed to validate and investigate the basis of potential sunitinib tolerability and efficacy differences in these populations. In this respect, analysis of mature data from Asian and non-Asian patients in the sunitinib expanded access trial will be of interest although, there are inherent limitations to using data from such trials.

In conclusion, sunitinib 50 mg/day on Schedule 4/2 has a favourable risk/benefit profile in Japanese patients with metastatic RCC. The median OS benefit exceeded 2.5 years in both first-line and pretreated patients, in whom ECOG PS was 0 or 1, and was accompanied by acceptable tolerability. Overall, the safety and efficacy of sunitinib was similar to that reported in Western studies, although there was a trend towards greater efficacy and an increased incidence of haematological AEs in Japanese patients.

AUTHORS' ROLES

All authors discussed the results and commented on the manuscript. Specifically, each author contributed to the study and manuscript as follows: Y.T. designed this study, conducted patient treatment and wrote/edited the manuscript; N.S. designed this study, conducted patient treatment and edited the manuscript; T.Y. designed this study, conducted patient treatment and edited the manuscript; H.F. designed this study, conducted patient treatment and edited the manuscript; M.N. designed this study, conducted patient treatment and edited the manuscript; S.M. designed this study, conducted patient treatment and edited the manuscript; T.M. designed this study and edited the manuscript; H.U. designed this study, conducted patient treatment and edited the manuscript; N.N. designed this study, conducted patient treatment and edited the manuscript; M.T. designed this study, conducted patient treatment and edited the manuscript; Y.H. designed this study, conducted patient treatment and edited the manuscript; N.A. analysed the data and wrote/edited the manuscript; B.H. analysed the data and edited the manuscript; S.N. designed this study, conducted patient treatment and edited the manuscript; and H.A. designed this study, conducted patient treatment, edited the manuscript and serves as the corresponding author for this paper.

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Conflict of interest statement

The authors, Naoki Agata and Brett Houk, are employees of Pfizer Inc.

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Circulating tumor cells as a surrogate marker for determining response to chemotherapy in patients with advanced gastric cancer

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The purpose of this study was to quantify circulating tumor cells (CTCs) in advanced gastric cancer (AGC) patients, and to demonstrate the role of CTCs in cancer therapy. This study investigates the hypothesis that CTCs can predict clinical outcomes in patients with AGC. From November 2007 to June 2009, 52 patients with AGC were enrolled into a prospective study. The chemotherapy regimen was an S-1-based regimen (S-1 with or without cisplatin) or paclitaxel. CTCs of whole blood at baseline, 2 weeks, and 4 weeks after initiation of chemotherapy, were isolated and enumerated using immunomagnetics. Patients with ≥4 CTCs at 2-week points and 4-week points had a shorter median progression-free survival (PFS) (1.4, 1.4 months, respectively) than those with the median PFS of <4 CTCs (4.9, 5.0 months, respectively) (log-rank test; $P < 0.001$, $P < 0.001$, respectively). Patients with ≥4 CTCs at 2-week points and 4-week points had shorter median overall survival (OS) (3.5, 4.0 months, respectively) than those with the median PFS of <4 CTCs (11.7, 11.4 months, respectively) (log-rank test; $P < 0.001$, $P = 0.001$, respectively). In conclusion, this study demonstrates that CTC measurement may be useful as a surrogate marker for determining response to S-1-based or paclitaxel regimens in AGC. (*Cancer Sci* 2010; 101: 1067–1071)

Gastric cancer is more prevalent in Asia, Eastern Europe, and Central and South America than in other areas. In Japan, this cancer is one of the most common causes of cancer-related mortality, despite dramatic advances in diagnosis and treatment. Outcomes are extremely poor in patients with unresectable gastric cancer, with the median survival ranging from 3 to 5 months with the best supportive care.[1–3] The ability to identify patients with the worst prognoses or those destined to progress quickly could have broad clinical applications.

Circulating tumor cells (CTCs) or disseminated tumor cells (DTCs) in bone marrow and peripheral blood from patients with cancers have been documented.[4–6] Braun *et al.*[7,8] reported that ~30% of women with primary breast cancer have DTCs in bone marrow, and a 10-year follow-up of these patients revealed a significantly decreased disease-free survival and overall survival (OS) when compared with patients without DTCs. However, aspiration of bone marrow is time consuming and, in many cases, uncomfortable for the patients precluding multiple samplings for therapy monitoring studies. Therefore, recent efforts have concentrated on the detection of CTCs in the peripheral blood of cancer patients. Cristofanilli *et al.*[9,10] showed in a prospective study that CTC detection provided significant prognostic information for patients with metastatic breast cancer. Cohen *et al.*[11] showed that the number of CTCs before and during treatment was an independent predictor of PFS and OS in patients with metastatic colorectal cancer. It is not clear whether CTC detection using this system provides prognostic information for patients with advanced gastric cancer. We initiated this study to evaluate whether CTCs could serve as a prognostic and/or predictive marker in patients with AGC.

Materials and Methods

Patients. All patients were enrolled using institutional review board-approved protocols at the Cancer Institute Hospital at the Japanese Foundation for Cancer Research and provided informed consent. The study population consisted of patients aged 18 years or older with histologically proven AGC. Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 2; adequate organ function; and S-1-based (S-1 with or without cisplatin) or paclitaxel chemotherapy regimen. The subjects were five patients treated with S-1 (40 mg/m^2, twice daily, days 1–28, repeated every 6 weeks), 26 patients treated with S-1 plus CDDP (S-1 40 mg/m^2, twice daily, days 1–21, CDDP 60 mg/m^2, day 8, repeated every 5 weeks), and 21 patients treated with paclitaxel (80 mg/m^2, weekly).

Sample preparation for isolation of CTCs from blood. Blood was drawn from advanced gastric cancer patients into 10 mL of evacuated blood for CTC in a Cell Save Preservative Tube (Veridex, Raritan, NJ, USA). Blood was always drawn from cancer patients before treatment initiation (baseline), 2 weeks, and 4 weeks after the administration of an S-1-based or paclitaxel regimen. The CellSearch system (Veridex) consists of the CellPrep system, the CellSearch Epithelial Cell Kit (for the measurement of CTC), and the CellSpotter Analyzer. The CellPrep system is a semi-automated sample preparation system, and the CellSearch Epithelial Cell Kit consists of ferrofluids coated with epithelial cell-specific EpCAM antibodies to immunomagnetically enrich epithelial cells; a mixture of two phycoerythrin-conjugated antibodies that bind to cytokeratin 8, 18, and 19; an antibody to CD45 conjugated to allophycocyanin; nuclear dye 4′,6- diamidino-2-phenylindole (DAPI) to fluorescently label the cell; and buffers to wash, permeabilize, and resuspend the cells. Sample processing and evaluation were done as described by Allard *et al.* Briefly, 7.5 mL of blood for CTCs were mixed with 6 mL of buffer, centrifuged at 800g for 10 min, and then placed on the CellPrep system. After aspiration of the plasma and buffer layer by instrument, ferrofluids were added. After incubation and subsequent magnetic separation, unbound cells and remaining plasma were aspirated. The staining reagents were then added in conjunction with a permeabilization buffer to fluorescently label the immunomagnetically labeled cells. After incubation in the system, the magnetic separation was repeated, and

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excess staining reagents were aspirated. In the final processing step, the cells were resuspended in the MagNest Cell Presentation Device (Veridex). This device consists of a chamber and two magnets that orient the immunomagnetically labeled cells for analysis using the CellSpotter Analyzer.

Sample analysis. The MagNest was placed on the CellSpotter Analyzer, a four-color semi-automated fluorescence microscope. Image frames covering the entire surface of the cartridge for each of the four fluorescent filter cubes were captured. The captured images containing objects that met predetermined criteria were automatically presented in a web-enabled browser from which final selection of cells was made by the operator. The criteria for an object to be defined as a CTC include round to oval morphology, a visible nucleus (DAPI positive), positive staining for cytokeratin, and negative staining for CD45. Results of cell enumeration are always expressed as the number of cells per 7.5 mL of blood for CTCs.

Statistical analysis. Progression-free survival (PFS) was defined as the elapsed time from blood collection to progression. Kaplan–Meier survival plots were generated based on CTC levels each time blood was collected, and the curves were compared using a log-rank testing. A P-value <0.05 was considered significant. Cox proportional hazards regression was used to determine univariate and multivariate hazard ratios for selected potential predictors of PFS and OS. The distribution of patients above and below the CTC threshold and clinical response was compared using Fisher's exact test.

Results

Patient characteristics. A total of 52 patients were enrolled. Patients' characteristics at baseline are summarized in Table 1. Patients' characteristics were as follows: median age, 62 years (range, 24–78 years); PS 0/1/2, 39/12/1; primary tumor +/−, 33/19; and regimen S-1/S-1 with cisplatin/paclitaxel, 5/26/21. Thirty-five patients had diffuse-type histology (67.3%). Seventeen patients (32.7%) had intestinal type. Among 52 patients, the best response rates were 28.8% (complete response [CR]/partial response [PR]/stable disease [SD]/progressive disease [PD]: 0/15/19/18). Of 31 patients treated with the S-1-based regimen (S-1 alone or S-1/cisplatin [CDDP]) assessable for response, we observed 14 PR (45.2%), 11 patients (35.5%) with SD, and six patients (19.4%) with PD during treatment. The overall response rate was 45.2%. On the other hand, of 21 patients treated with the weekly paclitaxel regimen assessable for response, we observed one PR (4.8%), eight patients (38.1%) with SD, and 12 patients (57.1%) with progression of disease during treatment, for an overall response rate (RR) of 4.8% (Table 2).

Table 1. Patient demographics

Demographic	Number or median (range)
Median age (range)	62 (24–78)
Male/female	44/8
PS: 0/1/2	39/12/1
S1-based/PAC regimen	31/21
Line: 1st/2nd	34/18
Histopathology: diffuse/intestinal type	35/17
Primary tumor: +/−	33/19
Sites of metastasis: +/−	
Liver	24/28
Lung	3/49
Bone	1/51
Peritoneum	22/30
Lymph node	37/15

Table 2. Objective response

	S1-based regimen (31) S1 alone (5), S1/CDDP (26) 1st line (31)	PAC (21) Weekly PAC (21) 1st line (3), 2nd line (18)
CR	0	0
PR	14	1
SD	11	8
PD	6	12

CDDP, cisplatin; CR, complete response; PAC, paclitaxel; PD, progressive disease; PR, partial response; SD, stable disease.

Stratification according to CTC levels. To select a level of circulating tumor cells that most clearly distinguished patients with a response of chemotherapy, thresholds of 1 to 88 cells for 2-week point were systematically correlated with PFS for 26 of the 30 patients in the training set. The median PFS among patients with levels above or below each threshold differed at the level of one circulating tumor cell per 7.5 mL of blood, and reached a plateau at approximately four cells per 7.5 mL of blood. At the latter level, the Cox proportional hazards ratio signifying the difference between slow and rapid progression of disease also reached a plateau. Thus, a cut-off of four circulating tumor cells per 7.5 mL of blood was chosen to distinguish patients.[12] The Kaplan–Meier circulating tumor-cell counts were available at a 2-week point for 26 of the thirty patients in the training set and for 21 of the 22 patients in the validation set. Neither PFS nor OS was significantly different in the two sets (data not shown). Because the two sets of data were nearly identical, they were combined for the estimation of PFS and OS for the entire population.

CTCs and imaging to assess response to therapy. Thirty-four (65.4%) of 52 patients were classified as having non-progressive disease (non-PD), with 24 of these patients (46.2%) having <4 CTCs and 10 patients (19.2%) having ≥4 CTCs before the initiation of therapy. Ten (19.2%) of 52 patients were classified as having PD, with 11 of these patients (21.2%) having <4 CTCs and seven patients (13.4%) having ≥4 CTCs before the initiation of therapy. The difference between the clinical responses and CTC levels were not significant. In contrast, 33 (64.7%) of 51 patients were classified as having non-PD, with 33 of these patients (64.7%) having <4 CTCs and no patients (0%) having ≥4 CTCs at 2 weeks. Eighteen (35.3%) of 51 patients were classified as having PD, with 11 of these patients (21.6%) having <4 CTCs and seven patients (13.7%) having ≥4 CTCs at 2 weeks. The difference between the clinical responses and CTC levels was highly significant. (P = 0.001, Fisher's exact test). Thirty-two (64%) of 48 patients were classified as having non-PD, with 31 of these patients (64.6%) having <4 CTCs and one patient (2.0%) having ≥4 CTCs at 4 weeks. Sixteen (33.3%) of 48 patients were classified as having PD, with eight of these patients (16.7%) having <4 CTCs and eight patients (16.7%) having ≥4 CTCs at 4 weeks. The difference between the clinical responses and CTC levels were highly significant (P < 0.001, Fisher's exact test) (Table 3).

Analysis of PFS according to CTC level. Figure 1 shows the Kaplan–Meier plots for prediction of PFS using the baseline CTC counts (Fig. 1a), at 2 weeks (Fig. 1b), and at 4 weeks (Fig. 1c). Seventeen of the patients (32.7%) had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with that of patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.4 months; 95% confidence interval [CI], 1.2–1.6) than the median PFS of <4 CTCs at 2 weeks (4.9 months; 95% CI, 4.0–5.8) (P < 0.001) (Fig. 1b). Patients with ≥4 CTCs at the 4-week point had a shorter median

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Table 3. CTCs and correlation with response assessment by imaging

	Non-PD			PD			Fisher's exact *P*-values
	No. of patients	CTCs <4 (%)	CTCs ≥4 (%)	No. of patients	CTCs <4 (%)	CTCs ≥4 (%)	
Baseline	34	24 (46.2)	10 (19.2)	18	11 (21.2)	7 (13.4)	0.544
2 week	33	33 (64.7)	0 (0)	18	11 (21.6)	7 (13.7)	0.001
4 week	32	31 (64.6)	1 (2.0)	16	8 (16.7)	8 (16.7)	<0.001

CTCs, circulating tumor cells; PD, progressive disease.

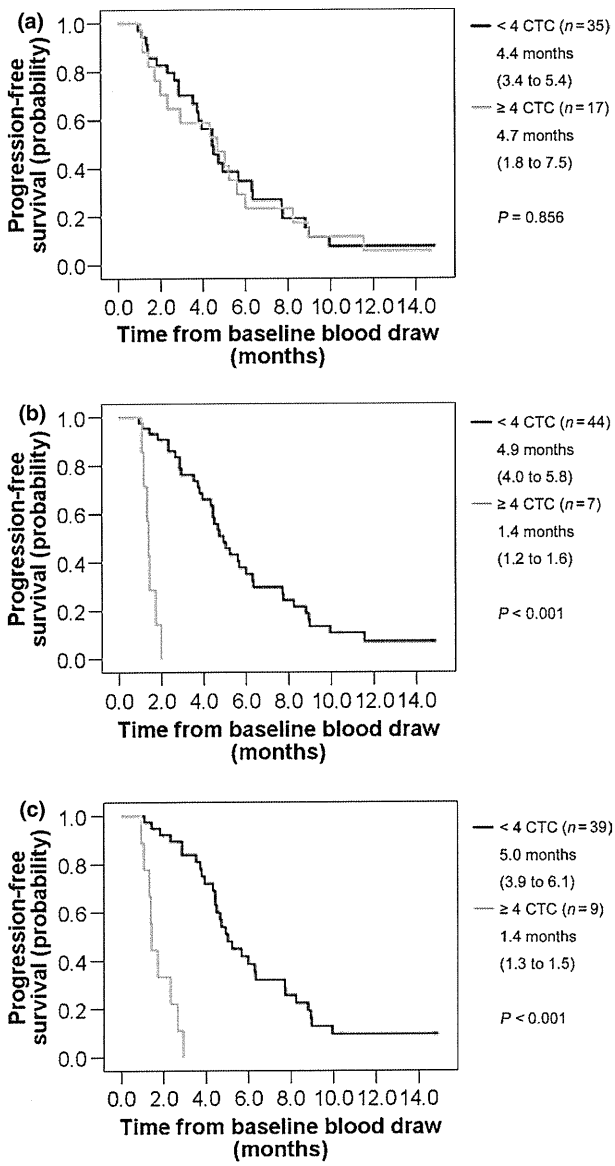

Fig. 1. Kaplan–Meier plots of progression-free survival (PFS) in advanced gastric cancer patients with less than four circulating tumor cells (CTCs) or ≥4 CTCs at baseline (a), 2 weeks (b), and 4 weeks (c).

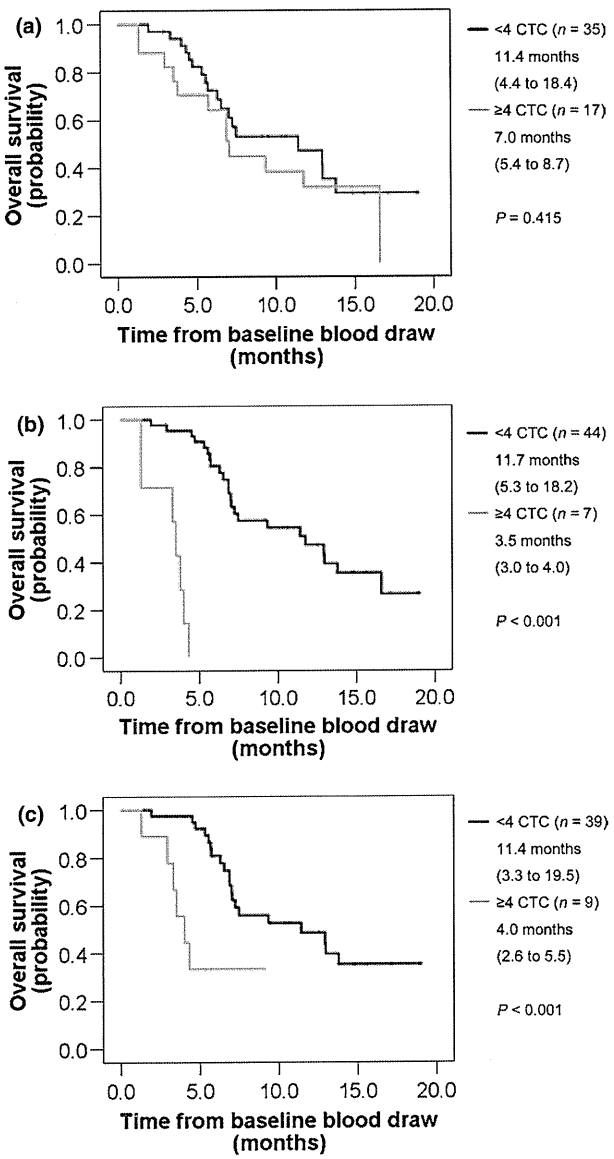

Fig. 2. Kaplan–Meier plots of overall survival (OS) in advanced gastric cancer patients with less than four circulating tumor cells (CTCs) or ≥4 CTCs at baseline (a), 2 weeks (b), and 4 weeks (c).

PFS (1.4 months; 95% CI, 1.3–1.5) than the median PFS of <4 CTCs at 4 weeks (5.0 months; 95% CI, 3.9–6.1) ($P < 0.001$) (Fig. 1c). With the S-1-based regimen, 10 patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with 21 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.2 months) than the median PFS of <4 CTCs at 2 weeks (6.0 months; 95% CI, 4.3–7.7) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median PFS (2.3 months; 95% CI, 0.7–3.9) than the median PFS of <4 CTCs at 4 weeks (6.3 months; 95% CI, 3.0–9.7) ($P < 0.001$). With the paclitaxel regimen, seven patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significantly different PFS compared with 14

patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median PFS (1.4 months; 95% CI, 1.4–1.5) than the median PFS of <4 CTCs at 2 weeks (4.3 months; 95% CI, 3.5–5.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median PFS (1.4 months; 95% CI, 1.0–1.8) than the median PFS of <4 CTCs at 4 weeks (4.4 months; 95% CI, 3.6–5.3) ($P < 0.001$).

Analysis of OS according to CTC level. Figure 2 shows the Kaplan–Meier plots for prediction of OS using baseline CTC counts (Fig. 2a), at 2 weeks (Fig. 2b), and at 4 weeks (Fig. 2c). Seventeen of the patients (32.7%) with ≥4 CTCs per 7.5 mL of blood at baseline had no significant different OS compared with patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (3.5 months; 95% CI, 3.0–4.0) than the median OS of <4 CTCs at 2 weeks (11.7 months; 95% CI, 5.3–18.2) ($P < 0.001$) (Fig. 2b). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (4.0 months; 95% CI, 2.6–5.5) than the median OS of <4 CTCs at 4 weeks (11.4 months; 95% CI, 3.3–19.5) ($P = 0.001$) (Fig. 2c). With the S-1 based regimen, 10 patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significant different OS compared with 21 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (1.3 months) than the median OS of <4 CTCs at 2 weeks (13.8 months; 95% CI, 9.4–18.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (4.0 months; 95% CI, 2.3–5.7) than the median OS of <4 CTCs at 4 weeks (>11.7 months) ($P = 0.031$). With the paclitaxel regimen, seven patients had ≥4 CTCs per 7.5 mL of blood at baseline. These patients had no significant different OS compared with 14 patients with <4 CTCs per 7.5 mL of blood at baseline. Patients with ≥4 CTCs at the 2-week point had a shorter median OS (3.5 months; 95% CI, 3.1–4.0) than the median OS of <4 CTCs at 2 weeks (6.5 months; 95% CI, 5.9–7.2) ($P < 0.001$). Patients with ≥4 CTCs at the 4-week point had a shorter median OS (3.5 months; 95% CI, 2.3–4.7) than the median OS of <4 CTCs at 4 weeks (6.5 months; 95% CI, 5.5–7.5) ($P = 0.013$).

Univariate and multivariate analysis of predictors of PFS and OS. Univariate and multivariate Cox proportional hazards regression was performed to assess the association between factors of interest and PFS or OS. In univariate analysis, PS, treatment regimen, line of chemotherapy, and CTC levels (cut-off, 4) at 2 and 4 weeks predicted PFS and OS (Table 4). In order to evaluate the independent predictive effect of chemotherapy, multivariate Cox regression analysis was carried out (Table 5). CTC levels at 2 and 4 weeks were the strongest predictors.

Discussion

The CellSearch system is designed to enrich and enumerate CTCs from peripheral blood. Furthermore, it is the first system to validate the clinical use of CTCs in patients with advanced gastric cancer. Our results show that the system is a suitable tool for assessment of CTCs in these patients, enabling reliable detection of CTCs in whole blood.

Approaches for isolation of CTCs in a research setting range from enrichment of tumor cells using density-gradient centrifugation[13–15] and flow cytometry.[16,17] CTC number as quantified by the CellSearch methodology[18–21] has been shown to have prognostic significance, and post-therapy decreases and increases in CTC number are associated with a superior and inferior survival, respectively, in patients with breast cancer, prostate cancer, and colorectal cancer. In this study, a finding of <4 CTCs in 7.5 mL of peripheral blood at 2 and 4 weeks after initiation of chemotherapy was associated with significantly longer PFS and OS as compared with these patients with ≥4 CTCs in 7.5 mL of peripheral blood. The results of this analysis demonstrated that the presence of four or more CTCs in 7.5 mL of blood before initiation of chemotherapy is not associated with PFS and OS. But the levels of CTCs at 2 and 4 weeks after initiation of chemotherapy are predictive of treatment efficacy, PFS,

Table 4. Univariate Cox regression analysis of independent parameters for prediction of PFS and OS

Parameter	No. of patients	PFS				OS			
		HR	95% CI	*P*-values	χ^2	HR	95% CI	*P*-values	χ^2
ECOG, 2 *vs* 1 *vs* 0	52	1.817	1.010–3.268	0.046	0.042	2.795	1.416–5.516	0.003	0.002
Treatment regimen	52	0.422	0.225–0.792	0.007	0.006	0.239	0.106–0.538	0.001	<0.001
Line of therapy	52	3.155	1.577–6.311	0.001	0.001	4.527	2.031–10.088	<0.001	<0.001
CTCs at the 2nd week	51	22.633	6.214–82.429	<0.001	<0.001	42.796	8.382–218.515	<0.001	<0.001
CTCs at the 4th week	48	15.947	5.380–47.271	<0.001	<0.001	4.699	1.751–12.609	0.002	0.001

CI, confidence interval; CTCs, circulating tumor cells; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

Table 5. Multivariate Cox regression analysis for prediction of PFS and OS

Parameter	PFS				OS			
	No. of patients	HR	95% CI	*P*-values	No. of patients	HR	95% CI	*P*-values
No. of patients	51				51			
Line of therapy, 1st *vs* 2nd		0.463	0.219–0.977	0.043		0.307	0.129–0.731	0.008
Lymph node metastasis		0.458	0.214–0.980	0.044				
CTCs at the 2nd week		0.049	0.012–0.199	<0.001		0.037	0.007–0.191	<0.001
Model χ^2			<0.001				<0.001	
No. of patients	48				48			
Line of therapy, 1st *vs* 2nd		0.412	0.192–0.880	0.022		0.217	0.089–0.504	<0.001
CTCs at the 4th week		0.082	0.027–0.224	<0.001		0.216	0.077–0.607	0.004
Model χ^2			<0.001				<0.001	

CI, confidence interval; CTCs, circulating tumor cells; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

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and OS. The presence of at least four CTCs at 2 and 4 weeks is a strong independent prognostic factor for inferior PFS and OS. These data demonstrate that CTC measurement may be a useful biomarker for monitoring response to therapy in AGC.

Outcomes are extremely poor in patients with ≥4 CTCs at 2 and 4 weeks, with the median OS ranging from 2 to 5 months. These data suggest the value of this technology in the identification of chemotherapy-resistant patients who could benefit from early treatment change and/or more investigational. Further study should prospectively address whether a change of treatment based on ≥4 CTCs at 2 or 4 weeks after initiation of chemotherapy early in the course of treatment will result in improvement in OS. CTC levels drawn at 2 and 4 weeks, before typical imaging intervals, may have the potential to suggest treatment choices and spare unnecessary toxicity by suggesting that an early change in therapy is warranted. Because the CellSearch system has not been approved in Japan, the price of one sample costs about ¥80 000 as in the case of the extra laboratory in the clinical trial. Several prospective trials led to the FDA approval of CTC counts for monitoring of patients with breast, colorectal, and prostate cancer. We expect CTC counts for monitoring of patients with gastric, breast, colorectal, and prostate cancer to be approved in Japan.

In conclusion, this study demonstrates the independent predictive value of CTCs for patients initiating chemotherapy for AGC. The data obtained in this clinical trial of the CellSearch system were for enumeration of CTCs in AGC. Our study was not designed to assess whether a change in therapy based on ≥4 CTCs is beneficial. However, clinical trials to explore this hypothesis are warranted.

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Disclosure Statement

The authors have no conflict of interest.

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Phase I study of inotuzumab ozogamicin (CMC-544) in Japanese patients with follicular lymphoma pretreated with rituximab-based therapy

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Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapeutic agent composed of an anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic, specifically targets the CD22 antigen present in >90% of B-lymphoid malignancies, rendering it useful for treating patients with B-cell non-Hodgkin lymphoma (B-NHL). This phase I study evaluated the safety, tolerability, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients. Eligible patients had relapsed or refractory CD22-positive B-NHL without major organ dysfunction. Inotuzumab ozogamicin was administered intravenously once every 28 days (dose escalation: 1.3 and 1.8 mg/m^2). All 13 patients had follicular lymphoma, were previously treated with ≥1 rituximab-alone or rituximab-containing chemotherapy, and were enrolled into two dose cohorts (1.3 mg/m^2, three patients; 1.8 mg/m^2, 10 patients). No patient had dose-limiting toxicities, and the maximum tolerated dose, previously determined in non-Japanese patients (1.8 mg/m^2), was confirmed. Drug-related adverse events (AEs) included thrombocytopenia (100%), leukopenia (92%), lymphopenia (85%), neutropenia (85%), elevated AST (85%), anorexia (85%), and nausea (77%). Grade 3/4 drug-related AEs in ≥15% patients were thrombocytopenia (54%), lymphopenia (31%), neutropenia (31%), and leukopenia (15%). The AUC and C_{max} of inotuzumab ozogamicin increased dose-dependently with pharmacokinetic profiles similar to non-Japanese. Seven patients had complete response (CR, 54%) including unconfirmed CR, four patients had partial response (31%), and two patients had stable disease (15%). The overall response rate was 85% (11/13). Inotuzumab ozogamicin was well tolerated at doses up to 1.8 mg/m^2 and showed preliminary evidence of activity in relapsed or refractory follicular lymphoma pretreated with rituximab-containing therapy, warranting further investigations. This trial was registered in ClinicalTrials.gov (NCT00717925). (*Cancer Sci* 2010; 101: 1840–1845)

The successful use of monoclonal antibodies (mAbs) in the treatment of human diseases has been growing steadily in the past decade. Rituximab, a human-mouse chimeric anti-CD20, unconjugated antibody, was approved in 1997 in the USA as the first mAb for antilymphoma therapy. It is now most commonly used in combination with chemotherapy for first and subsequent lines of therapy in B-cell non-Hodgkin lymphoma (B-NHL), such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).[1–6] However, a subgroup of patients does not respond, and early relapses occur in patients with initial response, thus indicating rituximab resistance. This indicates a clear unmet need to explore alternative antibodies non-cross resistant to rituximab as a therapy for B-NHL. One alternative is inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent that specifically targets CD22. Inotuzumab ozogamicin is composed of a recombinant engineered humanized IgG4 anti-CD22 antibody G544 conjugated to calicheamicin, a potent cytotoxic antibiotic derivative.[7]

CD22 is a potential therapeutic target for B-NHL because it is expressed in >90% of B-NHL cells.[8] In addition, CD22 is expressed in mature B cells, but not in their precursor or memory B cells, which may potentially minimize the adverse effect of CD22-targeted treatment on long-term immune function. Moreover, when antibodies bind to the CD22 antigen, the antigen is internalized, that is it is not shed into the extracellular environment.[9]

Both inotuzumab ozogamicin and unconjugated calicheamicin showed potent cytotoxic activity *in vitro* against CD22-positive B cells in preclinical studies.[7] In addition, the unconjugated form of inotuzumab ozogamicin, G544, did not demonstrate any antitumor activity in preclinical studies.[7] Inotuzumab ozogamicin inhibited the growth and the establishment of B-cell lymphomas and induced the regression of large B-cell lymphomas in mouse xenograft models.[7] Furthermore, in preclinical models of disseminated B-NHL in which rituximab was ineffective, treatment with inotuzumab ozogamicin lead to a significant tumor regression and an improvement in survival.[10] This potent cytotoxic activity in preclinical murine models of B-cell lymphomas in which rituximab had failed as a therapeutic agent[11] establishes support for the clinical investigation of inotuzumab ozogamicin for the treatment of CD22-positive B-NHL.

A phase I dose escalation study was previously conducted in the USA and the European Union in patients with relapsed or refractory B-NHL (both FL and DLBCL).[12] In this study, intravenous administration of the drug demonstrated clinical activity in patients with relapsed or refractory B-NHL with clinically manageable thrombocytopenia as the main toxicity. The maximum tolerated dose (MTD) in this non-Japanese patient population was determined to be 1.8 mg/m^2 once every 4 weeks.

The objectives of the present study were to assess the safety, toleralility, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients with relapsed or refractory B-NHL who had received prior treatment with rituximab.

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Materials and Methods

Study design. The present trial was an open-label multicenter phase I study in which inotuzumab ozogamicin was administered intravenously (IV) as a single agent to patients with CD22-positive B-NHL once every 28 days (±2 days, 1 cycle) for at least four doses provided that the drug was well tolerated with no evidence of progressive disease (PD). The protocol was approved by the Institutional Review Board of each participating institution, and it conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo, 2004). All the patients gave written informed consent.

Patients. Patients were eligible for enrollment if they had a diagnosis of CD22-positive B-NHL, according to the World Health Organization (WHO) classification, version 3.[13] Patients were included if they had progressed after at least one prior chemotherapy regimen for indolent B-NHL, or after one or two chemotherapy regiments, which included anthracyline or anthraquinone for aggressive B-NHL. Other inclusion criteria were age ≥20 and <75 years, a performance status of one or better on the Eastern Cooperative Oncology Group Scale, life expectancy ≥12 weeks, an absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L, serum creatinine ≤1.5 × upper limit of normal (ULN), urine protein-to-creatinine ratio of ≤0.2, total bilirubin ≤1.5 × ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN, and at least one measurable lesion ≥1.5 cm in at least one dimension by computer tomography (CT) at inclusion, in an area of no prior radiation therapy, or clear progression in an area that had been previously irradiated.

Dose escalation and toxicity criteria. Dose escalation decisions were based on the toxicities observed in the first 28 days after the administration of the first dose. Patients (three and 10 patients per cohort) could receive more than the four planned doses of inotuzumab ozogamicin if they experienced at least stable disease and tolerated treatment. The starting dose was 1.3 mg/m^2 administered IV once every 28 days, and dose escalation was performed up to the MTD of 1.8 mg/m^2 administered IV once every 28 days. Both the starting dose and the MTD were based on information from a previous clinical trial.[12] The dose escalation in subsequent cohorts was based on the toxicity assessed in the first 28 days after the first dose. Dose escalation continued until three or more patients in a cohort experienced a dose-limiting toxicity (DLT).

A DLT was defined as any of the following that were at least possibly related to inotuzumab ozogamicin during the first 28 days after the first dose: any grade 3 or 4 (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTC], version 3.0) nonhematologic toxicity (except grade 3 alopecia, nausea, or vomiting unless the patient was receiving optimal medical therapy); febrile neutropenia (grade 4 ANC ≥3-day duration and temperature ≥38.0°C); grade 4 ANC ≥7-day duration; grade 4 thrombocytopenia ≥3-day duration, or any bleeding episode requiring platelet transfusion; or delayed recovery (to grade 1 or baseline, except alopecia or grade 2 nausea or vomiting unless the patient was receiving optimal medical therapy) from a toxicity related to inotuzumab ozogamicin that delayed the initiation of the next dose by more than 3 weeks. Patients who experienced a DLT had the subsequent doses of inotuzumab ozogamicin reduced by one dose level, the maximum allowed dose reduction per patient. Patients who experienced toxicities other than DLTs could receive additional doses of inotuzumab ozogamicin at the same dose if they met the following criteria: recoveries to ≤grade 1 (nonhematologic), or baseline toxicity except alopecia; ANC $\geq 1.5 \times 10^9$/L; platelet count $\geq 75 \times 10^9$/L; serum creatinine ≤1.5 × ULN, and urine protein-to-creatinine ratio of ≤0.2. The maximum number of doses of inotuzumab ozogamicin was 8 for 1.3 mg/m^2 and 7 for 1.8 mg/m^2.

Pharmacokinetics. Timed blood samples for pharmacokinetic analysis were collected for cycles 1–3 at 0 (pre-dose), 1, 4 (cycles 1 and 3 only), 24, 48, 120, 168, 216, 336, and 504 h relative to the start of infusion for each dosing period and at pre-dose only for cycle 4. If the patient received four doses, then the sample had to be drawn before cycle 5. The serum concentrations of inotuzumab ozogamicin and total calicheamicin were determined using a validated enzyme-linked immunosorbent assay.

The noncompartmental pharmacokinetic parameters of inotuzumab ozogamicin and total calicheamicin were estimated using the WinNonlin (version 4.1) program. The parameters which were determined included the following: end-of-infusion peak concentration (C_{max}), area under the concentration-time curve (AUC), clearance (CL), apparent steady-state volume of distribution (V_{ss}), and the terminal-phase elimination half-life ($t_{1/2}$).

Safety. An AE was considered to be treatment emergent if its onset occurred between the first and the last dose, plus a lag of 28 days provided the following criteria were met: (i) the AE was not present before the start of the first dose and did not occur in the patient as a chronic condition; (ii) the AE was present before the start of the first dose or was part of the patient's medical history, but the severity or frequency increased after the start of the first dose.

Efficacy. Patients were evaluable for efficacy if they received ≥2 doses of inotuzumab ozogamicin, had a baseline tumor CT scan and had undergone at least one tumor assessment for response after baseline assessment. In addition, patients with documented PD prior to receiving two doses of inotuzumab ozogamicin were considered evaluable for efficacy. Tumor response was assessed according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma.[14] The overall response rate (ORR) was defined as the percentage of patients meeting the criteria for complete response (CR), unconfirmed complete response (CRu), or partial response (PR). Stable disease (SD) was measured from the start of the treatment until the criteria for PD were met, taking as the reference the smallest measurements recorded since the initiation of treatment.

Statistical analysis. The sample size for this study was determined by clinical rather than statistical considerations. The probabilities of detecting at least one AE of grade ≥3 with six patients receiving inotuzumab ozogamicin were 0.469, 0.822, and 0.984 when the true rates were 0.10, 0.25, and 0.50, respectively. The probabilities of detecting at least one such event in 10 patients receiving treatment were 0.651, 0.944, and 0.999, respectively.

With cohort sizes of three to six patients, if the true underlying rates of DLT were 0.1, 0.2, 0.3, 0.4, and 0.5, there would be a 0.985, 0.905, 0.754, 0.558, and 0.359 chance, respectively, of escalating to the next full dose. The ORR was estimated using an exact confidence interval (CI) approach.

Results

Patients. From March 2007 to July 2008, a total of 13 patients were enrolled in the study; three patients enrolled in the 1.3 mg/m^2 dose cohort and 10 patients in the 1.8 mg/m^2 dose cohort. The summary of demographic and other baseline characteristics for all patients is presented in Table 1. There were seven males and six females, all with a median age of 49 years (range, 43–72 years). All 13 patients had FL. The median number of prior treatment regimens was 1 (range, 1–13). All 13 patients had previous rituximab treatment (monotherapy or in combination with chemotherapy). Patients were categorized in low (38.5%), intermediate (42%), and high (15%) risk groups according to Follicular Lymphoma International Prognostic Index (FLIPI).[15]

Table 1. Demographic and baseline characteristics, safety population

Characteristics	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
Median age, years (range)	57 (51–66)	48 (43–72)	49 (43–72)
Sex, n (%)			
Female	2 (67)	4 (40)	6 (46)
Male	1 (33)	6 (60)	7 (54)
ECOG performance status, n (%)			
0	3 (100)	10 (100)	13 (100)
Primary diagnosis, n (%)			
Follicular lymphoma	3 (100)	10 (100)	13 (100)
FLIPI risk groups, n (%)			
Low	2 (67)	3 (30)	5 (39)
Intermediate	1 (33)	5 (50)	6 (46)
High	0	2 (20)	2 (15)
Number of prior chemo-/immunotherapy regimens, n (%)			
1	2 (67)	6 (60)	8 (62)
2	0	0	0
3	0	1 (10)	1 (8)
≥4	1 (33)	3 (30)	4 (31)

ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index.

Safety. In dose escalation, no patients had DLTs, and the MTD previously determined in non-Japanese patients (1.8 mg/m^2) was confirmed for Japanese patients in this study. The most common drug-related AEs were thrombocytopenia (100% patients); leukopenia (92%); neutropenia, elevated AST, anorexia, and lymphopenia (85%, each); elevated blood fibrinogen (69%); nausea (77%); elevated ALT, elevated alkaline phosphatase, and decreased hemoglobin (54%, each); malaise, elevated blood bilirubin, and headache (46%, each; Table 2(a)).

A summary of drug-related grade 3 or higher AEs is shown in Table 2(b). At least one drug-related grade ≥3 AEs was reported in nine of the 13 (69%) patients. Drug-related grade ≥3 AEs were thrombocytopenia (7 patients, 54%), lymphopenia and neutropenia (4, 31% each), leukopenia (2, 15%), and elevated blood bilirubin and hypokalemia (1, 8% each). Although neither lymphopenia nor leukopenia was reported for the 1.3 mg/m^2 cohort, the overall incidence of drug-related grade ≥3 AEs was comparable between the two cohorts. There were no patients who died during the study.

A total of four patients experienced dose delays, one (33%) patient in the 1.3 mg/m^2 cohort and three (30%) patients in the 1.8 mg/m^2 cohort (Table 3). Each had one delay. The AEs leading to dose delays were neutropenia (3 patients, 23%) and thrombocytopenia (2, 15%). Two (20%) patients in the 1.8 mg/m^2 cohort had one dose reduction (Table 4). Adverse events (AEs) leading to the dose reduction were thrombocytopenia and pleural effusion (1 patient, 8% each). There were no dose reductions in the 1.3 mg/m^2 cohort.

Seven patients discontinued treatment due to AEs: one patient because of grade 2 rash, one patient because of grade 2 urticaria, and five patients because of AEs that required treatment delays of >3 weeks (two patients with prolonged thrombocytopenia, one patient with prolonged thrombocytopenia and neutropenia, one patient with neutropenia and elevated alkaline phosphatase, and one patient with prolonged neutropenia and elevated total bilirubin).

Pharmacokinetics. Pharmacokinetic data after the first dosing were obtained for all 13 patients. The two patients who received 1.8 mg/m^2 inotuzumab ozogamicin and had a dose reduction after cycle 1 were excluded from pharmacokinetic assessments for cycle 2 and thereafter. The mean ± SD serum concentrations of inotuzumab ozogamicin and total calicheamicin *versus* time

Table 2. Inotuzumab ozogamicin-related adverse events, (a) all grades in ≥4 patients (b) grades ≥3

Adverse event, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
(a) all grades in ≥4 patients			
Thrombocytopenia	3 (100)	10 (100)	13 (100)
Leukopenia	3 (100)	9 (90)	12 (92)
Lymphopenia	3 (100)	8 (80)	11 (85)
Neutropenia	3 (100)	8 (80)	11 (85)
Aspartate aminotransferase increased	3 (100)	8 (80)	11 (85)
Anorexia	3 (100)	8 (80)	11 (85)
Nausea	3 (100)	7 (70)	10 (77)
Blood fibrinogen increased	2 (67)	7 (70)	9 (69)
Alanine aminotransferase increased	1 (33)	6 (60)	7 (54)
Blood alkaline phosphatase increased	1 (33)	6 (60)	7 (54)
Hemoglobin decreased	1 (33)	6 (60)	7 (54)
Malaise	3 (100)	3 (30)	6 (46)
Blood bilirubin increased	2 (67)	4 (40)	6 (46)
Headache	2 (67)	4 (40)	6 (46)
Constipation	1 (33)	4 (40)	5 (39)
Influenza	1 (33)	4 (40)	5 (39)
Blood lactate dehydrogenase increased	2 (67)	3 (30)	5 (39)
Fibrin D dimer increased	0	5 (50)	5 (39)
Hyperglycemia	1 (33)	4 (40)	5 (39)
Stomach discomfort	1 (33)	3 (30)	4 (31)
Fatigue	0	4 (40)	4 (31)
Hypercholesterolemia	1 (33)	3 (30)	4 (31)
Hypokalemia	2 (67)	2 (20)	4 (31)
Somnolence	2 (67)	2 (20)	4 (31)
Epistaxis	0	4 (40)	4 (31)
Rash	1 (33)	3 (30)	4 (31)
(b) grades ≥3			
Thrombocytopenia	2 (67)	5 (50)	7 (54)
Lymphopenia	0	4 (40)	4 (31)
Neutropenia	1 (33)	3 (30)	4 (31)
Leukopenia	0	2 (20)	2 (15)
Blood bilirubin increased	1 (33)	0	1 (8)
Hypokalemia	1 (33)	0	1 (8)

Table 3. Number (%) of patients reporting adverse events leading to dose delays, safety population

Parameter, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
No. of patients with dose delays			
No dose delays	2 (67)	7 (70)	9 (69)
One or more dose delays	1 (33)	3 (30)	4 (31)
No. of dose delays per patient*			
One	1 (100)	3 (100)	4 (31)
Any adverse event leading to dose delay†	1 (33)	3 (30)	4 (31)
Neutropenia	1 (33)	2 (20)	3 (23)
Thrombocytopenia	1 (33)	1 (10)	2 (15)

*Percentages are based on number of patients with ≥1 inotuzumab ozogamicin dose delay in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

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