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ORIGINAL ARTICLE

No inhibitory effect on P-glycoprotein function at blood–brain barrier by clinical dose of clarithromycin: a human PET study with [^{11}C]verapamil

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Abstract
Objective To investigate the effects of the clinical dose of clarithromycin, a substrate of P-glycoprotein (P-gp), on P-gp function using positron emission tomography (PET) with [^{11}C]verapamil.
Methods Two PET scanning with [^{11}C]verapamil were performed before and after administration of 400 mg/day of clarithromycin on each of four healthy male subjects. The rate constant of transfer from plasma to brain (K_1) was estimated by integration plot method.
Results K_1 values of [^{11}C]verapamil before administration of clarithromycin were 0.042–0.070 mL/cm^3/min (0.054 ± 0.012) and those after administration were 0.037–0.066 mL/cm^3/min (0.055 ± 0.013). No significant change in K_1 values of [^{11}C]verapamil was observed between before and after administration of clarithromycin ($P = 0.85$).
Conclusion K_1 values of [^{11}C]verapamil were not changed by clinical dose administration of clarithromycin, suggesting that a clinical dose of clarithromycin does not affect P-gp function at the blood–brain barrier.

Keywords Blood–brain barrier · Clarithromycin · [^{11}C]Verapamil · P-glycoprotein · Positron emission tomography

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Introduction

P-glycoprotein (P-gp) is a membrane protein that is expressed in several organs such as the liver, kidney and intestines [1, 2]. P-gp also exists at the blood–brain barrier (BBB), and its function is an efflux pump to prevent toxic substrates from entering the brain [3, 4]. It has been reported that co-administration of P-gp substrate increases the brain toxicity of drugs. For example, respiratory depression by loperamide occurred with pre-administration of quinidine [5]. This can be interpreted as inhibition of P-gp function by quinidine causing increased brain concentration of loperamide. Another case study reported that P-gp inhibition by verapamil at BBB might cause tetraparesis associated with colchicine [6]. Because many drugs are known as substrates of P-gp [7], evaluation of the effects on P-gp function by such drugs is clinically important.

[^{11}C]verapamil, a radioligand of the substrate of P-gp, was used for in vivo measurement of P-gp function at BBB, based on the fact that brain penetration of [^{11}C]verapamil reflects P-gp function [8]. No differences in multidrug resistance gene (MDR1) polymorphism on P-gp function have been reported using this radioligand [9, 10]. Possible changes in P-gp function in neurologic disorders such as Parkinson's disease [11–13] or epilepsy [14] were suggested using this radioligand and drug–drug interactions at P-gp in BBB were evaluated in primates and humans [15, 16].

Clarithromycin, a macrolide antibacterial, is also known to be a substrate of P-gp. Some in vitro studies have indicated that clarithromycin inhibits P-gp function [17, 18]. However, the effect of clarithromycin on P-gp function at BBB in living human brain has not been evaluated. In this study, we investigated the inhibitory effects of the clinical dose of clarithromycin on P-gp function by

measuring changes of the brain uptake of [^{11}C]verapamil using positron emission tomography (PET).

Materials and methods

Subjects

Four healthy men (age range 21–37 years, 31.0 ± 7.3, mean ± SD) participated in this study. All subjects were free of somatic, neurological and psychiatric disorders as determined by their medical history and T1- and T2-weighted magnetic resonance (MR) imaging of the brain. They had no history of current or previous drug abuse. They were administered orally 400 mg/day of clarithromycin (twice a day) for 2 days. PET scans were performed before and on the day after the last administration of clarithromycin. Venous blood samplings were performed just before radioligand injection to determine the plasma concentration of clarithromycin. Plasma concentrations of clarithromycin were determined using a validated liquid chromatography coupled to mass spectrometry/mass spectrometry (LC–MS/MS) method (Taisho Pharmaceutical Co. Ltd). After complete description of this study, written informed consent was obtained from all subjects. The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan.

PET procedure

[^{11}C]verapamil was synthesized from norverapamil (Eisai Co. Ltd) as described previously [19]. The racemic mixture of verapamil was used. A PET scanner system, ECAT EXACT HR + (CTI-Siemens, Knoxville, TN, USA), was used for all measurements. A head fixation device was used to minimize head movement. A transmission scan for attenuation correction was performed using a ^{68}Ge–^{68}Ga source before each scan. A dynamic PET scan was performed for 60 min after intravenous bolus injection of 734–783 MBq (745 ± 16 MBq, mean ± SD) of [^{11}C]verapamil. The specific radioactivity of [^{11}C]verapamil was 106–258 GBq/μmol (157 ± 48 GBq/μmol) at the time of injection. Total amount of verapamil was 1.3–3.2 μg (2.3 ± 0.6 μg). Radiation dose of each subject was 4.47–4.60 mSv estimated by our animal dosimetry experiment (0.003 mSv/MBq, unpublished data). MR images of the brain were acquired with a 1.5 Tesla MR imaging system, Gyroscan NT (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE 9.2 ms, TR 21 ms, flip angle 30°, field of view 256 mm, acquisition matrix 256 × 256, slice thickness 1 mm).

Arterial blood sampling and metabolite analysis

To obtain the arterial input function, a series of arterial blood samples was taken manually 29 times (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165 s, 3, 3.5, 4, 5.5, 7, 10, 16, 22, 27, 33, 39, 45, 51, 57 min) from an arterial catheter during the PET scanning. Each blood sample was centrifuged to obtain plasma and blood cell fractions, and the concentrations of radioactivity in whole blood and plasma were measured. Seven of the plasma samples (3, 7, 12, 22, 33, 45, 57 min) were deproteinized with acetonitrile and then centrifuged. The supernatant was analyzed for radioactive components using a high-performance liquid chromatography system (PU-610A series; GL Sciences) with a coupled bismuth germanate positron detector to measure the plasma [^{11}C]verapamil metabolites. Isocratic elution was performed with a reversed-phase semipreparative Waters Bondpak C18 column [7.8 mm (inner diameter) × 300 mm]. The mobile phase consisted of a mixture of acetonitrile and 0.1 mol/L ammonium acetate (70:30, v/v). The percentage of parent compound was determined from the parent radioactivity with respect to the total radioactivity in the chromatogram. The arterial plasma input function was defined as the radioactivity of plasma multiplied by the percentage of unchanged radioligand.

Data analysis

All T1-weighted MR images were co-registered to each PET image using the Statistical Parametric Mapping system (SPM2; Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College of London, London, UK). Regions of interest (ROIs) were drawn manually on summated PET images with reference to co-registered MR images. ROIs were defined for the frontal, temporal, parietal, occipital and cerebellar cortex (Fig. 1). Regional radioactivities of all ROIs were averaged. Regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time.

Integration plot

The influx rate constant from plasma to brain (K_1) was estimated by the integration plot method previously reported [8].

$$\frac{C_t(t)}{C_w(t)} = (1 - \mathrm{BV})K_1 \times \frac{\int_0^t C_p(s)\mathrm{d}s}{C_w(t)} + \mathrm{BV},$$

where $C_p(t)$ and $C_w(t)$ are the radioactivity concentrations in plasma and whole blood, $C_t(t)$ is the radioactivity concentration in the brain, and BV is blood volume in the

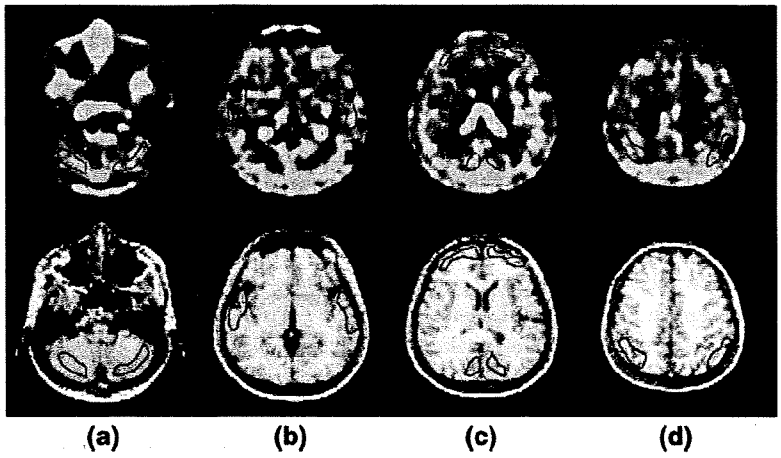

Fig. 1 Typical summated images of [^{11}C]verapamil and co-registered MR images. Regions of interest (ROI) are shown at the level of cerebellum (**a**), temporal cortex (**b**), frontal and occipital cortex (**c**), and parietal cortex (**d**)

brain. In this method, the data from 20 s to 3 min after the start of scanning were used.

Statistics

The difference in K_1 values between pre- and post-administration of clarithromycin was estimated using the paired t test. Correlations between changes in K_1 [(post–pre)/pre] and plasma concentration of clarithromycin were also evaluated using Pearson's correlation coefficient. In all analyses, $P < 0.05$ was considered significant.

Results

None of the participants complained of side effects after clarithromycin administration. The percentage of unchanged [^{11}C]verapamil was 97.4 ± 1.0% (mean ± SD) at 3 min and 34.6 ± 5.1% at 57 min after the start of scanning. In the integration plot, the brain and plasma concentration data up to about 3 min were approximately linear (Fig. 2). K_1 values of [^{11}C]verapamil before and after clarithromycin administration were 0.042–0.070 mL/cm^3/min (0.054 ± 0.012 mL/cm^3/min, mean ± SD) and 0.037–0.066 mL/cm^3/min (0.055 ± 0.013 mL/cm^3/min), respectively (Fig. 3). The plasma concentration of clarithromycin was 81.3–1,496 ng/mL (626 ± 612 ng/mL (0.84 ± 0.82 μmol/L), mean ± SD). There was no significant difference in K_1 values of [^{11}C]verapamil between before and after administration of clarithromycin ($P = 0.85$). There was no significant correlation between changes in K_1 and plasma concentrations of clarithromycin ($P = 0.70$).

Discussion

In this study, the K_1 value of [^{11}C]verapamil did not change with a clinical dose of clarithromycin. Moreover, no

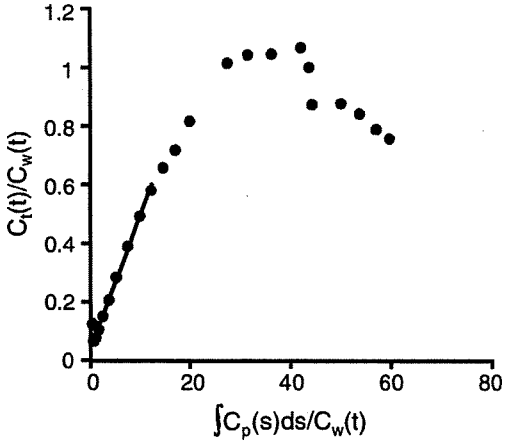

Fig. 2 Integration plot of one subject. K_1 value was estimated by time points between 20 s and 3 min

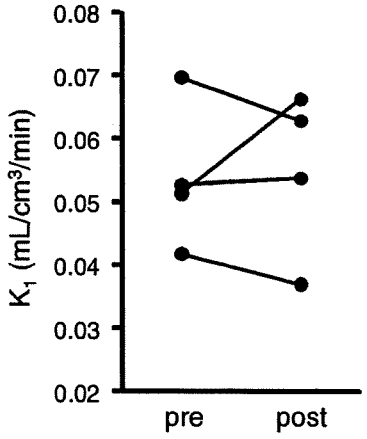

Fig. 3 K_1 values of each subject between pre- and post-administration of clarithromycin. There was no significant difference between the two measurements

correlation was observed between changes in K_1 and plasma concentrations of clarithromycin. Previous PET studies reported that an increase in brain uptake of [^{11}C]verapamil was observed after the administration of PSC833 in primate brain [16] and cyclosporine in human brain [15]. These studies indicated that substrates of P-gp, such as cyclosporine and PSC833, inhibit the efflux function of P-gp. However, P-gp function at BBB was not affected by clarithromycin given at a clinical dose in the present study. In a previous human PET study, cyclosporine increased brain uptake of [^{11}C]verapamil by 88% when the plasma concentration of cyclosporine was 2.8 ± 0.4 μmol/L [15]. This concentration of cyclosporine showed threefold greater intracellular uptake of vinblastine (P-gp substrate) when compared with the control state in mouse leukemia cells, which overexpressed P-gp [17]. In that study, clarithromycin showed no effect at 1 μmol/L, which was of the same order as in the present study (mean plasma concentration; 0.84 μmol/L), although the uptake of vinblastine increased twofold with 100 μmol/L of clarithromycin. Another in vitro study showed increased uptake of nimodipine (P-gp substrate) in cultured cells by both cyclosporine and clarithromycin, but the concentration of clarithromycin was 100 μmol/L whereas that of cyclosporine was 0.1 μmol/L [18]. In pharmacokinetics studies, maximum plasma concentration (C_{max}) of clarithromycin at a clinical dose was reported as about 2–3 mg/L (2.7–4.0 μmol/L) [20, 21]. On the other hand, C_{max} of cyclosporine in clinical treatment for transplant patients was reported as about 0.7–2.0 μg/mL (0.6–1.7 μmol/L) [22, 23]. Considering its low potency as P-gp substrate (100–1,000 times lower than cyclosporine) and the plasma concentration at a clinical dose (2–6 times higher than cyclosporine), the inhibition of P-gp function by clinical dose-level clarithromycin would be expected to be fairly low, although the results of in vitro studies may not directly apply to living human brain.

The variability of plasma concentrations of clarithromycin was very large (81.3–1,496 ng/mL). P-gp at the small intestine may affect the absorption of clarithromycin [24]. Moreover, there may be differences in metabolism by cytochrome P450 (CYP) 3A [25]. However, because the genotype of P-gp and CYP3A of subjects was not evaluated, these effects on the pharmacokinetics could not be clarified.

In this study, the function of P-gp was evaluated in terms of K_1 values obtained by integration plot method. This method needs only PET measurements data of the initial few minutes. The main metabolite of verapamil is also known as a substrate of P-gp at BBB, and therefore the effects of metabolites of [^{11}C]verapamil on brain radioactivity should be considered in the later part of the scanning duration [8]. In the present study, the mean percentage of unchanged [^{11}C]verapamil was 97.4% at 3 min and 34.6% at 57 min. Because only the initial 3 min of PET data were used in the integration plot method, the effects of metabolites of [^{11}C]verapamil could be negligible.

In conclusion, K_1 values of [^{11}C]verapamil did not change with a clinical dose of clarithromycin. This suggested that this dose of clarithromycin did not affect P-gp function at BBB in terms of drug–drug interaction.

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Conflict of interest statement This study was supported by a consignment expense for the Molecular Imaging Program on "Research Base for PET Diagnosis" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government. None of the authors has any conflicts of interest.

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Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study

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Summary

Background The best chemotherapy regimen for metastatic gastric cancer is uncertain, but promising findings have been reported with irinotecan plus cisplatin and S-1 (tegafur, 5-chloro-2,4-dihydropyrimidine, and potassium oxonate). We aimed to investigate the superiority of irinotecan plus cisplatin and non-inferiority of S-1 compared with fluorouracil, with respect to overall survival, in patients with metastatic gastric cancer.

Methods We undertook a phase 3 open label randomised trial in 34 institutions in Japan. We enrolled patients aged 20–75 years or younger, who had histologically proven gastric adenocarcinoma, and randomly assigned them by minimisation to receive either: a continuous infusion of fluorouracil (800 mg/m² per day, on days 1–5) every 4 weeks (n=234); intravenous irinotecan (70 mg/m², on days 1 and 15) and cisplatin (80 mg/m², on day 1) every 4 weeks (n=236); or oral S-1 (40 mg/m², twice a day, on days 1–28) every 6 weeks (n=234). The primary endpoint was overall survival. Analyses were done by intention to treat. This study is registered with Clinicaltrials.gov, number NCT00142350, and with UMIN-CTR, number C000000062.

Findings All randomised patients were included in the primary analysis. Median overall survival was 10·8 months (IQR 5·7–17·8) for individuals assigned fluorouracil, 12·3 months (8·1–19·5) for those allocated irinotecan plus cisplatin (hazard ratio 0·85 [95% CI 0·70–1·04]; p=0·0552), and 11·4 months (6·4–21·3) for those assigned S-1 (0·83 [0·68–1·01]; p=0·0005 for non-inferiority). Three treatment-related deaths occurred in the irinotecan plus cisplatin group and one was recorded in the S-1 group.

Interpretation S-1 is non-inferior to fluorouracil and, in view of the convenience of an oral administration, could replace intravenous fluorouracil for treatment of unresectable or recurrent gastric cancer, at least in Asia. Irinotecan plus cisplatin is not superior to fluorouracil in this setting.

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Introduction

Gastric cancer is the second leading cause of death from malignant disease worldwide.[1] The prognosis of unresectable or recurrent tumours is dismal: with best supportive care, median survival is about 4 months, and with chemotherapy it is around 8 months.[2–4]

During the early 1990s, several randomised trials for gastric cancer were undertaken of anthracyclines, mitomycin C, fluorouracil, methotrexate, and cisplatin.[5–31] At that time, the standard treatment for this malignant disease had not been established. When planning our current trial, no meta-analysis had been published of chemotherapy for advanced gastric cancer. Data from three phase 3 trials did not show a survival benefit of fluorouracil plus cisplatin over fluorouracil alone.[11–13] We reported previously that fluorouracil plus cisplatin caused more toxic effects and did not extend survival compared with continuous infusion of fluorouracil alone, despite a higher response rate and longer progression-free survival.[13] We concluded that continuous infusion of fluorouracil would be a standard arm in any subsequent phase 3 study.

In the late 1990s, new antitumour agents were developed for gastric cancer. In a phase 2 trial, combination chemotherapy with irinotecan plus cisplatin showed a response rate of 59% and median survival time of 322 days with grade 4 neutropenia (57%) and grade 3 or 4 diarrhoea (20%).[14] These efficacy measures were the best compared with those of other phase 2 trials. Although this regimen showed substantial toxic effects, they were deemed manageable, with dose reduction in some patients.

S-1 is a new oral fluoropyrimidine, consisting of tegafur, 5-chloro-2,4-dihydropyrimidine, and potassium oxonate. Data of two phase 2 studies of S-1 alone[15,16] showed a response rate of 45% and 2-year survival of 17%, in association with 5% or lower frequencies of grade 3 or 4 toxic effects. Furthermore, treatment could be administered on an outpatient basis.

With these findings in mind, we planned a three-arm phase 3 study of two pair-comparisons. On behalf of the gastrointestinal oncology study group of Japan Clinical Oncology Group (GIOSG/JCOG), we aimed to investigate

superiority of irinotecan plus cisplatin, and non-inferiority of S-1, compared with continuous infusion of fluorouracil for metastatic gastric cancer.

Methods

Patients

We undertook a three-arm, phase 3, randomised trial in 34 institutions in Japan. We used the following eligibility criteria to screen patients for inclusion: histologically proven gastric adenocarcinoma; unresectable or recurrent disease; adequate self-supported nutritional intake; age-range 20–75 years; Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less; no history of chemotherapy, radiation therapy, or both (however, adjuvant chemotherapy with an oral fluoropyrimidine other than S-1, not exceeding 1-year duration, completed more than 6 months before entry, was allowed); preserved organ functions; white-blood-cell count of 3·0–12·0×10⁹/L; number of platelets 100×10⁹/L or more; aspartate aminotransferase and alanine aminotransferase concentrations of 99 U/L or less; total bilirubin 25·65 μmol/L or lower; creatinine concentration 132·6 μmol/L or less; and creatinine clearance of 50 mL/min or faster. Having a target lesion or lesions according to response evaluation criteria in solid tumours was not mandatory. We excluded patients with severe peritoneal metastasis such as ileus or sub-ileus, ascites beyond the pelvic cavity, or narrowing of the colon detected by barium enema.

All eligible patients provided written informed consent to participate. The study was approved by the institutional review board of every participating institution. The JCOG data and safety monitoring committee (standing committee) monitored patients' safety, adverse events, and progress of the trial.

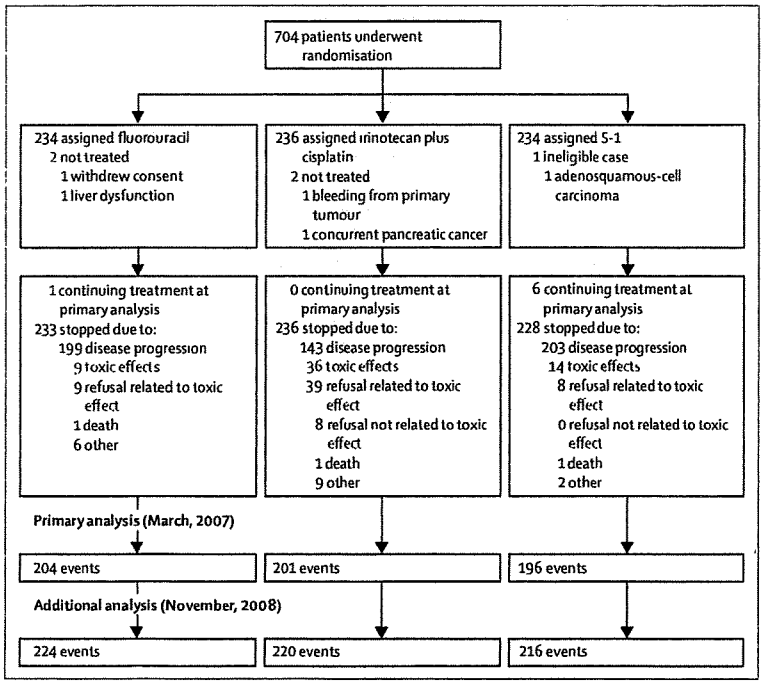

Figure 1: Trial profile

Randomisation and masking

We communicated patient's details to the data centre by fax or telephone. Staff in data centre entered these details into the computer to check eligibility, complete registration if appropriate, and randomly allocate the patient to a treatment group. Staff at the JCOG data centre randomly assigned every patient to either continuous infusion of fluorouracil, irinotecan plus cisplatin, or S-1, using the minimisation method,[17] with an algorithm (concealed to the investigators) that balanced institution, ECOG performance status (0, 1, or 2), and previous treatment (none, curative surgery alone, curative surgery and adjuvant chemotherapy). The treatment allocation was then communicated to the appropriate investigator by fax or telephone. The investigators participating in this trial treated their patients and took care of them all through the clinical course. Because the three treatment methods studied were quite different, the treatment allocation could not be masked from the investigators or patients. All data in case-report forms were sent to the JCOG data centre and checked by central data managers.

Procedures

Patients assigned fluorouracil received 800 mg/m² daily as a continuous infusion for 5 days, repeated every 4 weeks. Those assigned irinotecan plus cisplatin received an infusion of 70 mg/m² irinotecan on days 1 and 15 and 80 mg/m² cisplatin as a drip infusion on day 1 with adequate hydration, repeated every 4 weeks. After six cycles, the same dose of irinotecan alone was continued every 2 weeks. Individuals assigned S-1 received 40 mg/m² twice a day orally for 4 weeks, followed by a 2-week rest.

We delayed every treatment cycle until non-haematological toxic effects had recovered to grade 1 or lower, body temperature was 38°C or less, white-blood-cell count was 3·0–12·0×10⁹/L, platelets were 100×10⁹/L or more, aspartate aminotransferase and alanine aminotransferase concentrations were 99 U/L or less, total bilirubin was 25·65 μmol/L or lower, and creatinine concentration was 132·6 μmol/L or less. We reduced the treatment dose if, during the previous cycle, one of the following events had arisen: grade 4 leucopenia (less than 1·0×10⁹/L); thrombocytopenia (less than 10·0×10⁹/L); haemoglobin (less than 65g/L); grade 3 or higher non-haematological toxic effect; irinotecan not given on day 15; or S-1 or fluorouracil administration was suspended. The dose of cisplatin was reduced if the amount of creatinine was 106·1–132·6 μmol/L. We discontinued treatment if disease progression was diagnosed clinically or by imaging, if a serious adverse

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event arose, if a treatment cycle was delayed due to an adverse event continuing for longer than 2 weeks, if an adverse event meant a subsequent dose reduction was needed after the first reduction, if the patient refused treatment, or if judged necessary by the treating doctor for other reasons.

We did physical examinations and laboratory tests at least once every 2 weeks, and we assessed all adverse events according to the National Cancer Institute's common toxicity criteria (version 2.0). The JCOG data and safety monitoring committee reviewed serious adverse events and judged whether an adverse event was attributable to treatment. We assessed tumour response every 2 months according to RECIST (version 1.0). CT and endoscopic images of responders taken every 2 months independently of the treatment schedule were reviewed centrally at a trial group meeting; reviewers were unaware of treatment allocations at this time. We calculated response rates without interval confirmation.

The primary endpoint was overall survival. Secondary endpoints were time to treatment failure, non-hospitalised survival, adverse events, and response rate in patients with target lesions. We measured overall survival from the date of randomisation to the date of death and censored at the date of last contact for a surviving patient. We calculated progression-free survival to the date disease progression was detected, or death, and censored at the date on which progression-free status was verified. We deemed time to treatment failure to be the date when the doctor decided to discontinue treatment for any reason, and we censored at the date of last contact. We calculated non-hospitalised survival by subtracting the sum of all days in hospital from overall survival.

Statistical analysis

We estimated 6-month and 1-year survival with a continuous infusion of fluorouracil as 50% and 30%. The initial sample size was 450 in total, which allowed detection of a 10% increase in overall survival for irinotecan plus cisplatin and a 5% margin of non-inferiority for S-1, with a study-wide one-sided α level of 0·05 and a power of 70% for each pair comparison. Non-inferiority with a 5% margin corresponds to a hazard ratio of 1·16. We adjusted for multiplicity due to two pair-comparisons with the Bonferroni method, with a one-sided α level of 0·025 for each comparison keeping a study-wide α error of 0·05. We planned an interim analysis when 300 patients had been accrued, using the O'Brien and Fleming type α spending function.

We calculated 1-year survival for all randomised patients when initial accrual was almost complete and it was much higher than anticipated. Therefore, in March, 2005, we recalculated the sample size along with an increase of power from 70% to 80%, and the final sample size was 690. To raise statistical efficiency, we amended the method for adjustment of multiplicity in February, 2007, to that of Holm.[18] According to Holm's method, the pair with the largest difference is compared at first with an α of 0·025 and, if significant, then the other is compared with an α of 0·05. If non-inferiority of S-1 is confirmed, superiority is tested with the same significance level. We planned these amendments in a masked way and they were approved by the data and safety monitoring committee before the primary analysis.

We did the primary analysis in March, 2007, of all randomised patients, based on data up to 1 year after the last patient was enrolled. We analysed overall survival with the stratified log-rank test, and we estimated every hazard ratio (HR) with stratified Cox's proportional-hazards model. We did these stratified analyses with the balancing factors used for randomisation, except for institution. For analyses of progression-free survival, time to treatment failure, and non-hospitalised survival, and for subgroup analyses, we used the log-rank test and estimated the hazard ratio with the Cox model, assuming a common baseline hazard without balancing factors. All subgroup analyses were exploratory and details were not prespecified in the protocol. We revised the protocol to undertake additional analyses of overall survival, progression-free survival, and non-hospitalised survival after 2 years of follow-up, in November, 2008.

	Fluorouracil (n=234)	Irinotecan plus cisplatin (n=236)	S-1 (n=234)
Age (years)	63·5 (57–69)	63 (59–68)	64 (58–69)
Sex (male)	176	180	175
ECOG performance status			
0	152	151	151
1	79*	81	80
2	3	4	3
Surgery			
Unresectable	189	190	188
Recurrent	45	46	46
Previous adjuvant chemotherapy	1	1	1
Macroscopic type†			
0	5	5	5
1, 2	63	73	68
3, 4, 5	164	155	161
Histological type‡			
Intestinal	111	102	110
Diffuse	121	134	124
Target lesions§	175	181	175
Metastatic sites			
0, 1	103	100	102
≥2	131	136	132
Peritoneal metastasis	87	76	69

Data are median (range) or number of patients, with the exception of age (median; IQR). *Includes one patient who underwent random allocation as ECOG performance status 1, but was later found to be 0. This patient was treated as performance status 1 in all analyses. †Japanese classification of gastric carcinoma; no data available for two patients assigned fluorouracil and three assigned irinotecan plus cisplatin. ‡Assessed with Lauren classification; no data available for two patients assigned fluorouracil and for one in the S-1 arm with adenosquamous-type cancer. §Assessed with the RECIST; target lesions larger than double the size of a CT slice.

Table 1: **Baseline characteristics**

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We did all analyses by intention to treat using SAS version 9.1. Unless otherwise specified, we present one-sided p values for superiority. This study is registered with ClinicalTrials.gov, number NCT00142350, and UMIN-CTR, number C000000062.

For UMIN-CTR see http://www.umin.ac.jp/ctr

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 13, 2000, and Jan 20, 2006, 704 patients underwent randomisation: 234 were allocated continuous infusion of fluorouracil, 236 irinotecan plus cisplatin, and 234 S-1 (figure 1). Baseline characteristics were well balanced between the three treatment groups (table 1). Nearly all individuals had an ECOG performance status of 0 or 1. Only one patient in every group had received previous adjuvant chemotherapy. About 75% (531/704) of participants had a target lesion or lesions.

Table 2 shows adverse events recorded within 6 months. For patients assigned continuous infusion of fluorouracil, grade 3 or 4 adverse events with frequencies greater than 10% were haemoglobin (<80 g/L) and anorexia. For individuals assigned irinotecan plus cisplatin, grade 3 or 4 leucopenia and neutropenia had the highest frequencies and were associated with febrile neutropenia and infection with neutropenia. Frequencies of grade 3 or 4 adverse events in patients assigned S-1 were similar to those seen with continuous infusion of fluorouracil, except for a higher rate of diarrhoea. Three treatment-related deaths were reported in the group assigned irinotecan plus cisplatin and one in the S-1 group.

	Fluorouracil (n=232)*	Irinotecan plus cisplatin (n=234)*	S-1 (n=234)
Leucocytes (<2·0×10⁹/L)	0	97 (41)	2 (1)
Neutrophils (<1·0×10⁹/L)	3 (1)†	152 (65)	13 (6)
Haemoglobin (<80 g/L)	36 (16)	92 (39)	30 (13)
Febrile neutropenia	0	22 (9)	0
Infection with neutropenia	0	18 (8)	1 (<1)
Infection without neutropenia	9 (4)	9 (4)	13 (6)
Aspartate aminotransferase (≤99 U/L)	11 (5)	6 (3)	11 (5)
Alanine aminotransferase (≤99 U/L)	8 (3)	6 (3)	8 (3)
Bilirubin (≤25·65 µmol/L)	7 (3)	3 (1)	10 (4)
Creatinine (≤132·6 µmol/L)	0	5 (2)	2 (1)
Hyponatraemia	15 (6)‡	53 (23)	12 (5)‡
Fatigue	4 (2)	24 (10)	12 (5)
Anorexia	29 (13)	77 (33)	29 (12)
Diarrhoea	1 (<1)	21 (9)	18 (8)
Nausea	16 (7)	48 (21)	13 (6)
Stomatitis	7 (3)	0	4 (2)
Hand-foot syndrome	0	0	3 (1)
Neuropathy—motor	0	1 (<1)	2 (1)
Neuropathy—sensory	0	1 (<1)	0
Treatment-related death§	0	3 (1)	1 (<1)

Data are number of patients (%). *Two patients were not treated in each group. †Data for one patient not available. ‡Data for two patients not available. §Judged by data and safety monitoring committee.

Table 2: Adverse events (grade 3 or higher) recorded within 6 months

At the time of the primary analysis (March, 2007), 601 (85%) events had been recorded (figure 1). Median overall survival in patients assigned continuous infusion of fluorouracil was 10·8 (IQR 5·7–17·8) months, in individuals allocated irinotecan plus cisplatin it was 12·3 (8·1–19·5) months, and in those assigned S-1 it was 11·4 (6·4–21·3) months. Irinotecan plus cisplatin was not superior to continuous infusion of fluorouracil (HR 0·85 [95% CI 0·70–1·04]; p=0·0552). Non-inferiority of S-1 to a continuous infusion of fluorouracil was confirmed (0·83 [0·68–1·01]; p=0·0005), but S-1 was not superior to fluorouracil (p=0·0336; one-sided α=0·025).

At the time of the additional analysis (November, 2008), the number of events had risen to 660 (94%; figure 1). Actual 2-year overall survival was 14% in patients assigned continuous infusion of fluorouracil, 18% in individuals allocated irinotecan plus cisplatin, and 21% in those assigned S-1 (figure 2). Irinotecan plus cisplatin was not superior to continuous infusion of fluorouracil (HR 0·82 [95% CI 0·68–0·99]; p=0·0194), whereas S-1 was non-inferior to fluorouracil (0·83 [0·68–1·00]; p=0·0002 for non-inferiority, p=0·0233 for superiority). All HR calculated by multivariate analyses with baseline factors were essentially the same as those measured by univariate analyses (data not shown).

The median time to treatment failure was 2·3 (IQR 1·4–5·4) months for patients assigned continuous infusion of fluorouracil, 3·7 (1·9–5·6) months for those allocated irinotecan plus cisplatin (HR 0·85 [95% CI 0·71–1·02]; p=0·0430), and 4·0 (2·0–6·3) months for individuals assigned S-1 (0·73 [0·61–0·88]; p=0·0004). More than 85% of patients who were allocated either continuous infusion of fluorouracil or S-1 discontinued treatment because of disease progression; a third of those allocated irinotecan plus cisplatin stopped because of toxic effects (figure 1). Median non-hospitalised survival was 7·2 (IQR 2·7–13·3) months for individuals assigned continuous infusion of fluorouracil, 9·5 (4·9–15·7) months for those allocated irinotecan plus cisplatin (0·81 [0·67–0·97]; p=0·0115), and 9·3 (4·2–18·0) months for those assigned S-1 (0·77 [0·63–0·92]; p=0·0025).

Second-line chemotherapy was given to 194 (83%) patients assigned continuous infusion of fluorouracil, 183 (78%) allocated irinotecan and cisplatin, and 173 (74%) assigned S-1 (data not available for 31 individuals). Of those assigned continuous infusion of fluorouracil, 70 crossed over to irinotecan plus cisplatin and 20 moved to S-1. Of those allocated irinotecan plus cisplatin,

127 moved to S-1 and seven to continuous infusion of fluorouracil. Finally, of those in the S-1 arm, two patients crossed over to continuous infusion of fluorouracil and 68 moved to irinotecan plus cisplatin.

Median progression-free survival was 2·9 (IQR 1·7–5·7) months for patients assigned continuous infusion of fluorouracil, 4·8 (2·3–8·2) months for those allocated irinotecan plus cisplatin (HR 0·69 [95% CI 0·58–0·83]; p<0·0001), and 4·2 (2·2–7·1) months for individuals assigned S-1 (0·77 [0·64–0·93]; p=0·0027; figure 2). In patients with a target lesion or lesions, response rates were 9% (15/175) for those assigned continuous infusion of fluorouracil, 38% (68/181) for those allocated irinotecan plus cisplatin, and 28% (49/174, data not available for one patient) for individuals assigned S-1. In this subgroup, median progression-free survival was 2·2 (1·4–5·3) months for patients assigned continuous infusion of fluorouracil, 4·8 (2·3–8·1) months for those allocated irinotecan plus cisplatin (0·56 [0·45–0·69]; p<0·0001) and 3·8 (2·0–5·6) months for those assigned S-1 (0·80 [0·65–0·98]; p=0·0174).

Findings of exploratory subgroup analyses of overall survival (figure 3) showed favourable results for S-1 compared with continuous infusion of fluorouracil for all subgroups except recurrent cases. In the subgroup with target lesions, median survival was 9·0 (IQR 5·4–15·2) months for patients assigned continuous infusion of fluorouracil (n=175), 12·1 (8·1–19·0) months for those allocated irinotecan plus cisplatin (n=181; HR 0·73 [0·59–0·91]; p=0·0022), and 10·5 (5·6–19·2) months for those assigned S-1 (n=175; 0·84 [0·68–1·05]; p=0·0590). In the subgroup without target lesions, median survival was 13·5 (7·9–23·4) months for patients assigned continuous infusion of fluorouracil (n=59), 14·4 (9·0–20·7) months for those allocated irinotecan plus cisplatin (n=55; 1·12 [0·76–1·65]; p=0·7219), and 18·1 (10·5–26·6) months for those assigned S-1 (n=59; 0·79 [0·53–1·16]; p=0·1101).

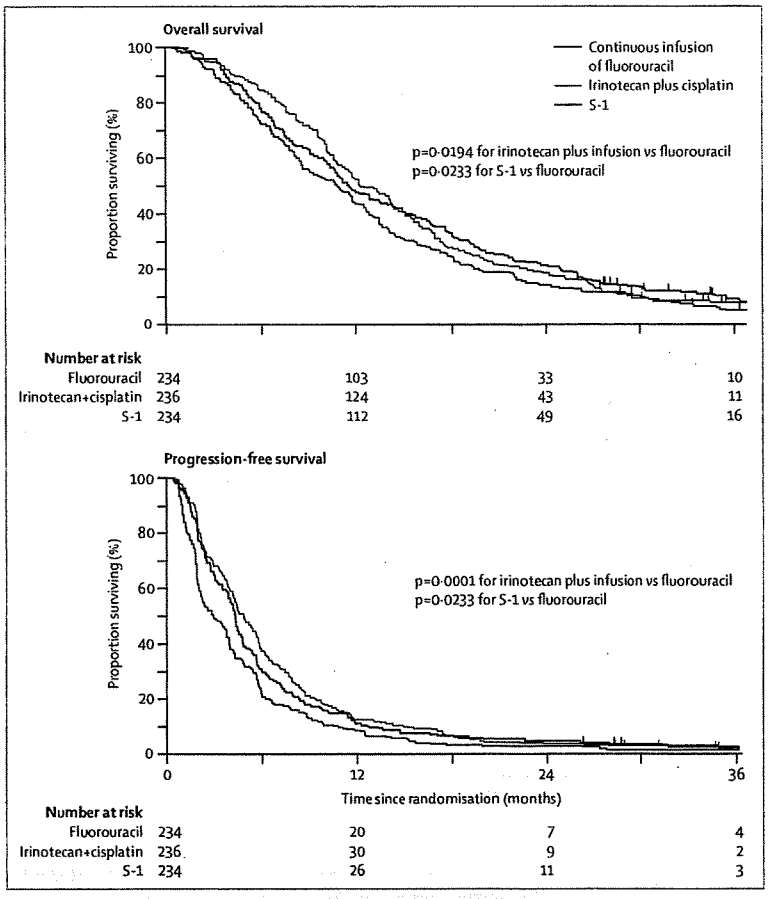

Figure 2: **Survival curves of all randomised patients (November, 2008)**

Discussion

Our findings show that S-1 is non-inferior to continuous infusion of fluorouracil with respect to overall survival. Although S-1 was not superior with respect to overall survival at the primary analysis, patients assigned S-1 had a 7% higher 2-year overall survival rate than those allocated a continuous infusion of fluorouracil. Furthermore, other measures of effectiveness of S-1, such as response rate and progression-free survival, were better than those obtained with continuous infusion of fluorouracil. These findings for S-1 are consistent with those reported in two phase 3 trials containing an S-1 alone arm.[19,20] Drug development for gastric cancer has been focused on replacement of intravenous fluorouracil with oral agents.[21,22] Taken together with our findings, S-1 might have some advantages over continuous infusion of fluorouracil.

Any new treatment, even if non-inferior to standard treatment, should have some benefits, such as for quality of life, cost, or safety. In our study, compared with continuous infusion of fluorouracil, S-1 was associated with almost equivalent safety and longer non-hospitalised survival. Additionally, in Japan, the cost of S-1 (about ¥76 000 per month [about US$834]) is cheaper than that of continuous infusion of fluorouracil (about ¥140 000 per month [US$1537]). In view of the effectiveness, safety, convenience, and cost, continuous infusion of fluorouracil could be replaced by S-1 for first-line chemotherapy of metastatic gastric cancer.

Findings of a meta-analysis of chemotherapy for advanced gastric cancer[23] indicated that survival was slightly better with combination chemotherapy than with a single agent. In the SPIRITS trial,[19] in which S-1 plus cisplatin was compared with S-1 alone for recurrent or unresectable gastric cancer, the combination showed a survival benefit over S-1 alone. In a previous study by us,[13] fluorouracil plus cisplatin could not prolong survival compared with a continuous infusion of fluorouracil, and our findings in this current study suggest that S-1 is non-inferior to continuous infusion of fluorouracil. Therefore, these data support the rationale for S-1 to be a control arm in the SPIRITS trial.[19] Several studies of

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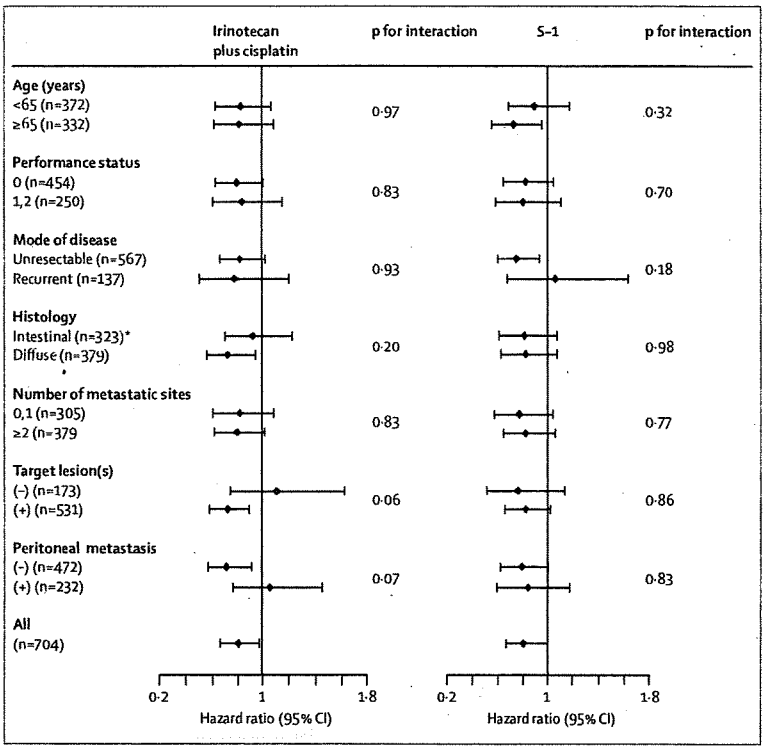

Figure 3: **Forest plot of subgroup analyses**
For every analysis, continuous infusion of fluorouracil is compared with irinotecan plus cisplatin (left) and S-1 (right). *Unknown types were excluded from the analysis.

combination chemotherapy based on S-1 plus cisplatin, including molecular target agents, are ongoing.

Toxic effects of S-1 have been reported to be more severe in individuals from the USA than in Asian patients, resulting in different recommended doses in these populations.[24,25] Since similar discrepancies in toxic effects have been noted with tegafur and uracil,[26] ethnic variations would seem to be a factor with these dihydropyrimidine dehydrogenase inhibitory fluoropyrimidines. In a trial from China,[27] S-1 plus cisplatin was superior to continuous infusion of fluorouracil plus cisplatin. Outside Asia,[28] despite differences in dose and schedule of S-1 from Asian trials, S-1 plus cisplatin was associated with fewer toxic effects, had slightly better survival, and showed non-inferiority compared with fluorouracil plus cisplatin. S-1 plus cisplatin, with an equitoxic dose to fluorouracil plus cisplatin, should be investigated in European and North American populations.

The toxic effects of irinotecan plus cisplatin were the most severe of the three treatment groups in our study, and the rate of treatment failure due to toxic effects was the highest, resulting in a shorter time to treatment failure than that obtained with S-1. In the subgroup with target lesions, of the three treatment groups, irinotecan plus cisplatin showed the best response rate, progression-free survival, survival within 1 year, and overall survival. In North America, divided doses of irinotecan and cisplatin have been investigated,[29] which are associated with a similar response rate to, and fewer toxic effects than, the regimen in our study. Since control of toxic effects of irinotecan plus cisplatin is a big problem, divided doses of irinotecan and cisplatin should be investigated in future phase 3 trials.

Some chemosensitivity-related markers have been suggested to be prognostic factors for irinotecan plus cisplatin treatment.[30] Expression of specific chemosensitivity-related genes is currently being investigated in patients enrolled in our study, and preliminary data suggest that dihydropyrimidine dehydrogenase expression could be a predictive marker for whether irinotecan plus cisplatin or S-1 (plus cisplatin) would be the better treatment in a given patient.[31] We postulate that some populations would benefit from irinotecan plus cisplatin even though chemotherapy regimens containing irinotecan have not shown a survival benefit in phase 3 trials.[20,32] Because clinical behaviour and pathogenesis of gastric cancer are heterogeneous, treatment strategies tailored for optimum chemotherapy according to a patient's clinical and genetic background should be established in the near future, and irinotecan plus cisplatin could then serve as one of the options.

Although median progression-free survival of S-1 and irinotecan plus cisplatin in our study were similar to those reported in other phase 3 trials, median overall survival was somewhat extended.[21,22,32–34] Moreover, median progression-free survival—both in this study and in our previous phase 3 trial[13]—was 2 months for patients who received continuous infusion of fluorouracil. Overall survival of patients with target lesions in this current study was about 2 months longer than that reported by us previously. The proportion of patients who received second-line chemotherapy in our study was more than 70%, which is higher than in our previous study (53%).[13] Since irinotecan and taxanes were approved in the late 1990s in Japan, available active agents for subsequent chemotherapy differed between this current study and our previous study. We postulate that second-line chemotherapy might have contributed to the favourable overall survival in this study, although a survival benefit of second-line chemotherapy has not yet been clarified.

Contributors
NB, HF, and SY wrote the protocol and designed the trial based on discussion with, and agreement from, all authors. All authors (except SY and HF) recruited patients to the study. HF directed the data centre. SY and HF did the statistical analysis. NB wrote the report with revisions from all other authors.

Conflicts of interest
The authors declared no conflicts of interest.

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PHASE II STUDIES

Phase I/II study of sunitinib malate in Japanese patients with gastrointestinal stromal tumor after failure of prior treatment with imatinib mesylate

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Summary *Purpose:* To establish a recommended sunitinib dosing schedule in Japanese patients with imatinib-resistant/intolerant gastrointestinal stromal tumor (GIST) and to evaluate the efficacy, safety/tolerability, pharmacokinetics, and pharmacodynamics of sunitinib using this schedule. *Patients and methods:* In the phase I part of this open-label phase I/II trial, Japanese GIST patients received 25, 50, or 75 mg/day of sunitinib on Schedule 4/2 (4 weeks on treatment; 2 weeks off treatment) following imatinib failure. In phase II, patients received the recommended (maximum tolerated) dose on this schedule; the primary endpoint was clinical benefit rate (CBR; percent objective responses or stable disease [SD] ≥22 weeks). Additional efficacy, safety, pharmacokinetic, and biomarker analyses were performed. *Results:* In phase I (12 patients), the recommended dose was determined to be 50 mg/day. Sunitinib pharmacokinetics were similar to those observed in studies with Western patients. In the phase II part (36 patients), the CBR was 39% (95% CI: 23–57%; 11% partial responses, 28% SD ≥22 weeks). The most common treatment-related non-hematologic adverse events (AEs) were hand–foot syndrome (86%) and fatigue (67%). A trend towards a correlation between decreases from baseline in plasma soluble KIT levels and improved CB was found. *Conclusions:* The pharmacokinetics observed and clinical outcomes achieved in Japanese GIST patients on sunitinib (50 mg/day, Schedule 4/2) after imatinib failure appeared similar to those of Western patients in previous sunitinib trials. Although some serious AEs were observed, AEs were generally manageable using dose interruption/modification and/or standard medical treatments.

Keywords Sunitinib · GIST · Japanese patients · Pharmacokinetics · Biomarkers

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Introduction

Gastrointestinal stromal tumor (GIST) is a soft-tissue sarcoma that most commonly arises in the stomach or small intestine, and less frequently in the large bowel or other gastrointestinal sites [1,2]. Greater than 80% of GISTs are associated with activating mutations of KIT (stem-cell-factor receptor, CD117) [3,4], and another 5–7% express activating mutations of platelet-derived growth factor receptor alpha (PDGFR-α) [4,5].

Imatinib mesylate—a small-molecule tyrosine kinase inhibitor with selectivity for KIT and PDGFRs—is the current mainstay of treatment for metastatic or unresectable GIST. However, approximately 11–14% of GISTs are initially resistant to imatinib [6,7] and another 40–50% acquire resistance within 18–24 months of initial therapy [7,8].

Sunitinib malate (SUTENT®) is an oral multitargeted tyrosine kinase inhibitor with activity against KIT and PDGFRs, as well as vascular endothelial growth factor receptors (VEGFRs), glial cell line-derived neurotrophic factor receptor (REarranged during Transfection; RET), colony-stimulating factor 1 receptor (CSF-1R), and FMS-like tyrosine kinase-3 receptor (FLT3) (Pfizer Inc., data on file) [9–13]. Sunitinib received multinational approval for the treatment of GIST after failure of imatinib due to resistance or intolerance, based largely on the interim results of an international, randomized, double-blind, placebo-controlled phase III trial [14].

The clinical safety and efficacy of both imatinib and sunitinib in GIST have primarily been established in Western patients residing in the USA or Europe and have not been thoroughly studied in Asian patients. Fifty-six centers in 11 countries participated in the phase III trial of sunitinib in GIST, but only 15 of the 312 patients were of Asian descent (10 and 5 in the sunitinib and placebo groups, respectively). An open-label, phase I/II trial was therefore undertaken to establish a recommended dosing schedule for sunitinib in Japanese GIST patients after imatinib failure and to better evaluate the efficacy and safety of sunitinib in this patient population. In addition, the pharmacokinetic profiles of sunitinib and its active metabolite were assessed, and an initial evaluation of potential biomarkers of sunitinib activity in this patient population was performed.

Patients and methods

Patients

Japanese patients, 20–75 years of age, were required to have histologically proven metastatic or unresectable malignant GIST and confirmed failure of prior imatinib therapy, as demonstrated by disease progression (based on Response Evaluation Criteria in Solid Tumors [RECIST] [15]) or discontinuation of imatinib due to toxicity. Additional eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate cardiac, hepatic, renal, coagulation, and hematologic function. Key exclusion criteria included lack of recovery from the acute toxic effects of previous anticancer therapy or imatinib treatment, discontinuation of imatinib therapy within 2 weeks or of any other approved or investigational drug for GIST within 4 weeks prior to starting sunitinib treatment, clinically significant cardiovascular events or disease in the previous 12 months, diabetes mellitus with clinical evidence of peripheral vascular disease or diabetic ulcers, or a diagnosis of any second malignancy within the previous 5 years. All patients provided written informed consent to participate in the study.

Study design

This was an open-label, single-arm, non-randomized, multicenter, dose-escalation, phase I/II trial performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines. In the phase I part of the study, patients received one 6-week treatment cycle of sunitinib (4 consecutive weeks on treatment, followed by 2 weeks off treatment; Schedule 4/2). Successive cohorts of three to six patients received doses of 25, 50, or 75 mg administered orally once daily in the morning. Patients were enrolled in the subsequent cohort if less than one-third of patients in the initial or preceding cohort had experienced a dose-limiting toxicity (DLT). A DLT was defined as a grade 4 hematologic toxicity of ≥7 days' duration or complicated by fever ≥38°C; a grade 3/4 hematologic toxicity complicated by infection, hemorrhage, or requiring blood product support, including a hematopoietic growth factor; a grade 3/4 non-hematologic event (except for asymptomatic increases in serum amylase or lipase, or nausea/vomiting or diarrhea manageable with antiemetic and antidiarrheal drugs); or evidence of left ventricular dysfunction, defined as development of congestive heart failure or decline in left ventricular ejection fraction (LVEF) by an absolute value of ≥20% from baseline and to less than the lower limit of normal (LLN). Patients experiencing a DLT during the phase I part of the study were withdrawn from sunitinib treatment but could resume dosing if the toxicity resolved and there was evidence of clinical benefit. A primary objective of the phase I part of the study was to determine a recommended dose for the phase II part by identifying the maximally tolerated dose (MTD) of sunitinib, defined as the highest dose below the dose at which the proportion of patients experiencing DLTs was ≥33.3%.

Patients continuing in the phase II part of the study from the phase I part switched to/continued on the recommended dose (50 mg/day) on Schedule 4/2 after it was determined. Newly enrolled patients in the phase II part received the recommended dose on Schedule 4/2. Any drug-related grade 3/4 adverse events developing during the phase II part were managed using standard medical treatments and/or by discontinuing drug temporarily until the event resolved sufficiently, followed by dose reduction by 12.5–25 mg for grade 3/4 non-hematologic events or grade 4 hematologic events. Criteria for permanent drug withdrawal were a need to reduce the sunitinib dose to <25 mg/day, evidence of RECIST-defined disease progression, or evidence of left ventricular dysfunction (as previously defined).

Study endpoints and assessments

The primary endpoints of the phase I part were measures of safety and pharmacokinetic parameters of sunitinib and its principal active metabolite, SU12662. Adverse events were assessed by type, grade, and relationship to study drug, with grading determined using National Cancer Institute Common Toxicity Criteria version 2.0 [16]. Serious adverse events were defined as any untoward medical occurrences that resulted in death, were life-threatening, required or prolonged hospitalization, or resulted in persistent or significant disability/incapacity or a congenital anomaly/birth defect. Safety assessments included vital signs, ECOG performance status, 12-lead electrocardiogram, echocardiogram or multiple-gated acquisition scan, and laboratory analysis of blood and urine. Pharmacokinetic parameters were determined using blood samples collected pre-dose on days 1, 2, 7, 14, 21, 28, and end of treatment (or withdrawal), and post-dose at 1, 2, 4, 6, 8, and 10 h on days 1 and 28 as well as at 24 and 48 h on day 28. Plasma concentrations of sunitinib, SU12662, and total drug (sunitinib + SU12662) were determined using a liquid chromatography/mass spectrometry method with a lower limit of detection of 0.1 ng/ml [17].

The primary endpoint of the phase II part was the clinical benefit rate, defined as the percentage of patients with RECIST-defined objective response (confirmed complete response [CR] or partial response [PR]) or stable disease (SD) ≥22 weeks. Best overall response was evaluated by the investigators and an independent extramural review committee, with the evaluations of the latter group used for the primary efficacy analysis. Secondary efficacy endpoints included objective response rate, disease control rate (proportion of patients with confirmed CR or PR, or SD ≥10 weeks), time to tumor progression (TTP), and progression-free survival (PFS). As in the phase I part, measures of safety were also recorded. In the phase II part, blood samples for pharmacokinetic analysis were collected pre-dose on days 1, 14, and 28 of cycles 1–4 (or cycles 2–4 for patients entering phase II following completion of phase I).

Plasma concentrations of soluble KIT (sKIT), soluble VEGFR-2 (sVEGFR-2), and VEGF were determined using blood samples collected pre-dose on days 1, 14, and 28 of phase I and cycles 1–4 of phase II (cycles 2–4 for patients completing phase I) and evaluated as potential biomarkers for sunitinib activity using quantitative performance-validated enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) run under Good Laboratory Practice conditions as described [18].

Statistical methods

Statistical analyses were performed on three patient populations: DLT-analysis, intention-to-treat (ITT), and per-protocol. The DLT-analysis population, used in phase I of the study, consisted of patients who experienced DLTs or who received ≥85% of the planned total dose. The ITT population, defined as all patients who received at least one dose of study drug, was used as the primary analysis set for efficacy, safety, pharmacokinetic, and biomarker analyses. The per-protocol population consisted of the ITT population after exclusion of patients because of serious deviation from inclusion/exclusion criteria, administration of prohibited concomitant medications, administration of study drug on <75% of the planned dosing days before confirming clinical benefit (CR, PR, or SD≥22 weeks) or cycle 4, or no evaluation of objective tumor response after sunitinib dosing. The per-protocol population was used as a secondary set for analysis of the primary endpoint.

Time-to-event data were assessed using Kaplan–Meier methods. TTP was defined as the period between the day of initial study treatment and the day of initial confirmation of progressive disease (PD). Data from patients who were not confirmed to have PD either during the study or before the initiation of another antitumor therapy were censored at the final confirmation of absence of PD during the study. PFS was defined as the period between the day of initial study treatment and the day of initial confirmation of PD or of death due to any cause. Data from patients who were not confirmed to have PD or to have died during the study or before the initiation of another antitumor therapy were censored at the final confirmation of absence of PD during the study. Descriptive statistics were used to evaluate pharmacokinetic parameters and potential soluble biomarkers. Relationships between changes in plasma levels of biomarkers and the antitumor effects of sunitinib were evaluated using the Wilcoxon rank-sum test.

Results

Phase I results

Patient disposition and identification of recommended dose A total of 12 patients were enrolled in the phase I part of the study: three in the 25-mg cohort, six in the 50-mg cohort, and three in the 75-mg cohort. None of the initial three patients in the 25-mg cohort or the subsequent six patients in the 50-mg cohort experienced any DLTs in the first cycle of sunitinib treatment. However, two of the three patients in the 75-mg cohort experienced DLTs leading to termination of treatment after 12 and 15 days at that dose, and the third patient's treatment was subsequently terminated on day 7 as per recommendation of the Independent Safety Data Monitoring Committee. One of the patients' DLTs included grade 3 fatigue, anorexia, hypophosphatemia, and skin reaction, while the other patient's DLTs were grade 3 decreased platelet count (complicated by a need for blood product support) and grade 3 increased aspartate transaminase (AST). Therefore, 50 mg/day was identified as the MTD and as the recommended dose for use on Schedule 4/2 in the phase II part of the trial. All three patients who received 75 mg/day of sunitinib during the phase I part resumed dosing during cycle 2 of the phase II part at the recommended dose of 50 mg/day (the two patients who had experienced DLTs resumed after 18 and 28 days, respectively). There were no other dosing interruptions or any dose reductions due to toxicity during the phase I part of the trial, although initiation of cycle 2 was delayed by 14 days in one patient on 50 mg/day due to adverse events.

Pharmacokinetics Plasma concentration–time profiles and pharmacokinetic parameters for sunitinib, SU12662, and total drug are presented in Fig. 1 and Table 1, respectively. Exposure (maximum concentration [C_{max}] and area under the concentration–time curve from 0 to 24 h [AUC_{0-24}]) to sunitinib, SU12662, and total drug increased approximately linearly with dose on day 1 (25, 50, and 75 mg) and day 28 (25 and 50 mg). By day 28, AUCs for sunitinib, SU12662, and total drug were approximately 4, 11, and 5 times as high as those on day 1, respectively. Sunitinib was absorbed slowly after administration, with a median time to C_{max} of 6–10 h. Trough plasma concentrations of sunitinib and SU12662 appeared to reach steady state by 7–14 and 14–21 days after administration, respectively (data not shown). Trough concentrations of sunitinib and SU12662 at steady state (on day 28) closely correlated with the corresponding AUC_{0-24} and C_{max} values (r^2=0.80~0.90). The oral clearance (CL/F) of sunitinib did not show dose-dependency (data not shown).

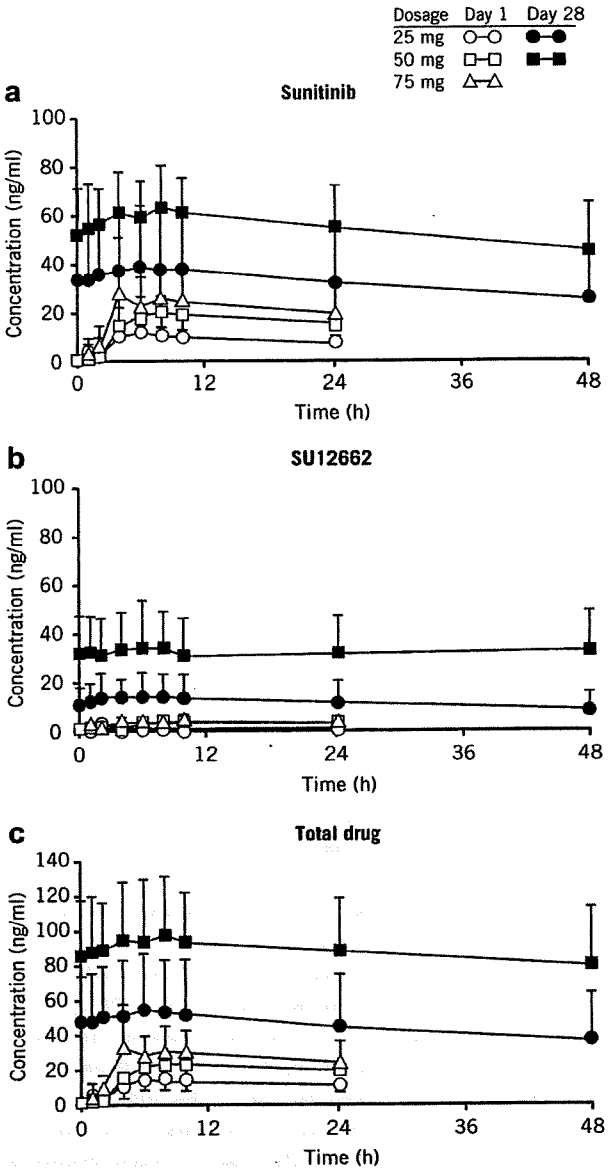

Fig. 1 Mean plasma concentration–time profiles of **a** sunitinib, **b** its active metabolite SU12662, and **c** total drug (sunitinib plus SU12662) by treatment cohort on days 1 and 28 in the phase I part of the study

Phase II results

Patient baseline characteristics, disposition, and study drug exposure Thirty-six patients were enrolled in the phase II part of the study and received sunitinib 50 mg/day on Schedule 4/2, three of whom initially received sunitinib 25 mg/day and three of whom initially received sunitinib 75 mg/day during the phase I part of the study. The ITT population comprised all 36 patients, while the per-protocol

Table 1 Pharmacokinetic parameters (mean ± standard deviation) of sunitinib, SU12662, and total drug by treatment cohort in the phase I part of the study

Parameter		Sunitinib			SU12662			Total drug		
		25 mg (n=3)	50 mg (n=6)	75 mg (n=3)	25 mg (n=3)	50 mg (n=6)	75 mg (n=3)	25 mg (n=3)	50 mg (n=6)	75 mg (n=3)
Day 1	C_{max}	12.1	22.8	32.3	2.0	4.1	4.8	14.1	26.7	37.0
	(ng/ml)	±4.9	±6.4	±20.8	±1.3	±0.9	±2.5	±6.1	±7.4	±22.1
	AUC_{0-24}	199	374	508	30.9	70.0	91.1	230	444	599
	(ng·h/ml)	±89.4	±68.9	±259	±20.6	±14.4	±45.3	±108	±82.8	±287
	T_{max}[b] (h)	6 (4–8)	7 (6–24)	8 (4–10)	6 (4–8)	9 (6–24)	10 (4–10)	6 (4–8)	7 (6–24)	8 (4–10)
Day 28[a]	C_{max}	39.5	69.3	–	15.2	38.8	–	54.0	105	–
	(ng/ml)	±25.0	±18.9		±10.2	±15.9		±32.2	±35.1	
	AUC_{0-24}	858	1,406	–	324	772	–	1,183	2,178	–
	(ng·h/ml)	±600	±364		±223	±358		±734	±702	
	T_{max}[b] (h)	10 (6–10)	6 (1–24)	–	4 (2–8)	3 (0–48)	–	6 (4–8)	6 (0–24)	–

AUC area under the concentration–time curve, C_{max} maximum concentration, T_{max} time to C_{max}

[a] Day 28 data were not collected for the 75-mg cohort due to early termination of this cohort following occurrence of dose-limiting toxicities

[b] Median (range)

population consisted of 30 patients. Six patients, comprising all of the patients in the 75-mg/day cohort and three in the 50-mg/day cohort, were excluded from the per-protocol population due to insufficient dosing.

Baseline patient characteristics, patient disposition, and exposure to study drug over both phases of the study are summarized in Table 2. Tumor progression was the primary reason for termination of imatinib therapy in 92% of patients, with the other 8% having discontinued due to imatinib intolerance. At the time of data cutoff, patients had received a median of four cycles of sunitinib (range: 2–12) at a median dose of 50 mg/day and dose intensity of 89%. Sixteen patients (44%) had dose reductions, 15 (42%) due to adverse events. Twenty-two patients (61%) discontinued sunitinib treatment, two (6%) due to adverse events and 20 (56%) due to PD. Adverse events causing discontinuation of sunitinib treatment were grade 2 decreased LVEF (one patient) and grade 4 decreased neutrophil count (one patient) that persisted despite dose reduction to 25 mg.

Efficacy The clinical benefit rate (percent objective responses or SD ≥22 weeks) based on extramural assessment of the ITT population (the primary endpoint) was 39% (95% CI: 23–57) and 40% (95% CI: 23–59) in the 50-mg cohort (Table 3). Analysis of the per-protocol population yielded similar results (data not shown). Based on extramural assessment, four patients (all in the 50-mg cohort) exhibited a RECIST-defined objective response (all PRs), yielding an objective response rate of 11% in the ITT population and 13% in the 50-mg cohort. The disease control rate (percent objective responses or SD ≥10 weeks) was 61% and 57% in the ITT population and 50-mg cohort, respectively. The median TTP was 28.3 weeks (95% CI: 22.0–39.3) in the ITT population and 27.9 weeks (95% CI: 22.0–39.3) in the 50-mg cohort; TTP and PFS were equivalent (data not shown).

Safety/tolerability All 36 patients in the ITT population experienced at least one adverse event that was considered to be treatment-related. Most adverse events were mild to moderate in intensity: 84% of all treatment-related adverse events were grade 1/2. Toxicities experienced in the study were generally manageable and reversible through careful dosing interruption, dose modification, and/or standard medical treatment. Among treatment-related non-hematologic adverse events, the most common events of any grade reported were hand–foot syndrome (86%); fatigue (67%); and diarrhea, anorexia, and skin discoloration (64%; Table 4), and the most common grade 3 events reported were hand–foot syndrome (31%) and hypertension (25%). No grade 4 events were reported among the non-hematologic adverse events listed in Table 4, and among all treatment-related non-hematologic events, only one grade 4 event (nephrotic syndrome) was reported. This event resolved after a dosing interruption of 52 days and dose reduction from 37.5 mg to 25 mg.

Hematologic and non-hematologic laboratory abnormalities are also presented in Table 4. Two incidents of grade 4 hematologic laboratory abnormalities were reported: one reduced hemoglobin concentration and one reduced neutrophil count; the former resolved after a dosing interruption of 14 days and dose reduction to 37.5 mg; the latter resulted in discontinuation, as mentioned above. The most

Table 2 Summary of patient demographics, imatinib treatment history, disposition, and study drug exposure across the phase I and II parts of the study through data cutoff

Characteristic	Treatment cohort			
	25 mg (n=3)	50 mg (n=30)	75 mg (n=3)	Total (N=36)
Median age (range), years	36 (33–54)	56 (41–74)	66 (50–68)	56 (33–74)
Sex, n (%)				
Male	2	19 (63)	3	24 (67)
Female	1	11 (37)	0	12 (33)
Median weight (range), kg	56 (48–64)	51 (40–79)	56 (51–58)	52 (40–79)
ECOG PS, n (%)				
0	3	18 (60)	2	23 (64)
1	0	12 (40)	1	13 (36)
Tumor immunohistochemistry, n (%)				
KIT-positive	3	29 (97)	3	35 (97)
CD34-positive	2	16 (53)	2	20 (56)
Imatinib treatment history				
Median duration of treatment (range), months	31 (19–32)	26 (2–46)	32 (22–38)	26 (2–46)
Primary reason for termination, n (%)				
Tumor progression	3	27 (90)	3	33 (92)
Intolerance	0	3 (10)	0	3 (8)
Most common metastatic sites, n				
Liver	3	22	3	28
Peritoneum	3	16	3	22
Lung	1	1	1	3
Ascites	2	1	0	3
Sunitinib treatment				
Median number of cycles completed (range)	10 (3–12)	4 (2–10)	5 (3–5)	4 (2–12)
Median daily dose (range), mg	25 (25–31)	50 (33–50)	52 (43–59)	50 (25–59)
Median dosing days/cycle (range)	28 (27–28)	26 (20–28)	20 (14–22)	26 (14–28)
Median dose intensity (range), %	100 (95–124)	89 (47–100)	44 (39–49)	89 (39–124)
Discontinuations, n (%)	1	18 (60)	3	22 (61)
Due to an adverse event	0	2 (7)	0	2 (6)
Due to PD	1	16 (53)	3	20 (56)
Dose reductions, n (%)	0	13 (43)	3	16 (44)
Due to an adverse event	0	13 (43)	2	15 (42)

ECOG PS Eastern Cooperative Oncology Group performance status, *PD* progressive disease

common non-hematologic laboratory abnormalities were increased AST levels (72%) and decreased albumin levels (61%). The most common grade 3/4 non-hematologic laboratory abnormalities were increased lipase (19%), increased uric acid (19%), and increased AST (11%). None of the cases of increased lipase were associated with additional signs or symptoms of pancreatitis.

In addition to hypertension, cardiovascular adverse events included a prolongation of the QTc interval to 450–<480 ms in two patients and a maximum change of 30–<60 ms from baseline QTc interval in two patients. These were not clinically significant and resolved without treatment changes. LVEF was below the LLN in three patients, two of whom experienced an absolute ≥20% decrease from baseline, which resulted in discontinuation in one patient, as mentioned above. The LVEF decrease in this latter patient ultimately abated after discontinuation. This patient also experienced cardiomyopathy, which was diagnosed by echocardiogram and magnetic resonance imaging after discontinuation and resolved after completion of the study. Two patients in the study experienced hypothyroidism (grade 1).

Nine patients (25%) experienced one or more serious treatment-related adverse events (which were abdominal pain, cardiomyopathy, gastric ulcer, hand–foot syndrome, hemorrhage, hypoglycemia, hypoproteinemia, myalgia, nephrotic syndrome, perianal abscess, reduced platelet

Table 3 Clinical response to sunitinib treatment across the phase I and II parts of the study through data cutoff

Response parameter	Treatment cohort			
	25 mg (n=3)	50 mg (n=30)	75 mg (n=3)	Total (N=36)
Tumor response, n (%)				
PR	0	4 (13)	0	4 (11)
SD	3 (100)	15 (50)	3 (100)	21 (58)
≥10 weeks	3 (100)	13 (43)	2 (67)	18 (50)
≥22 weeks	1 (33)	8 (27)	1 (33)	10 (28)
Objective response rate	0	13	0	11
95% CI		4–31		3–26
Disease control rate[a]	100	57	67	61
95% CI	–	37–75	–	44–77
Clinical benefit rate[b]	33	40	33	39
95% CI	–	23–59	–	23–57

PR partial response, *SD* stable disease

[a] Disease control rate, percent PRs + SD ≥10 weeks

[b] Clinical benefit rate, percent PRs + SD ≥22 weeks

count, and reflux esophagitis). All were reported in one patient each, except reduced platelet count, which was experienced by two patients. None of these events led to discontinuation, although the patient who experienced cardiomyopathy had already discontinued treatment due to LVEF decrease as described above. No treatment-related grade 5 events were reported in the study.

Pharmacokinetics Among patients receiving sunitinib 50 mg/day, median trough concentrations of sunitinib, SU12662, and total drug ranged from 42.3 to 59.5 ng/ml, 18.7 to 29.7 ng/ml, and 62.4 to 84.9 ng/ml, respectively, while on treatment (days 14 and 28) in cycles 1–4. Following 2 weeks off treatment, pre-dose total drug concentrations in cycles 2–4 were low, but measurable in many patients (medians: 3.0–4.0 ng/ml). Trough concentrations of sunitinib and SU12662 varied over time in a manner similar to those of total drug, corresponding to periods on and off treatment, and repeated dosing was not found to result in accumulation of plasma drug levels across four treatment cycles (data not shown).

Biomarkers Plasma levels of sKIT, sVEGFR-2, and VEGF changed in response to treatment, but only sKIT appeared to show sustained changes (data not shown). During sunitinib dosing, plasma concentrations of VEGF increased while concentrations of sVEGFR-2 decreased, but plasma concentrations of both of these biomarkers tended to return to near-baseline levels after the 2-week off-treatment period. Conversely, plasma concentrations of sKIT showed a trend for sustained decrease across both on-treatment and off-treatment periods.

Percent changes in VEGF, sVEGFR-2, and sKIT levels from baseline were compared among patient groups categorized by best overall response. There was no apparent difference in percent change of VEGF and sVEGFR-2 among the patient groups at any time point. Results for cycle 4, day 28 are shown in Table 5. Patients with an objective response and those achieving clinical benefit (objective responses or SD ≥22 weeks) showed a trend of decline in sKIT levels across cycles 1–4, while patients without objective responses exhibited only modest decreases or increases in sKIT levels (p values for clinical benefit versus no clinical benefit for cycle 4, day 28: sKIT, 0.238; VEGF, 0.459; sVEGFR-2, 0.484).

Discussion

The results of this trial of sunitinib in Japanese GIST patients following imatinib failure were highly consistent with those obtained in a number of previous sunitinib trials. The phase I part of the study yielded the same recommended dosing schedule (50 mg/day on Schedule 4/2) as that identified in prior studies of sunitinib in largely Western patients with a variety of tumor types [19–21]. In the phase II part of the study, sunitinib demonstrated similar efficacy to that previously reported in a double-blind, placebo-controlled, phase III trial also involving predominantly Western patients with imatinib-resistant/-intolerant GIST [14]. In the 50-mg cohort in the current study, 13% and 40% of patients experienced objective responses (PRs) or clinical benefit from sunitinib, respectively. By way of

Table 4 Toxicities occurring across the phase I and II parts of the study through data cutoff

Adverse event/laboratory abnormality	Treatment cohort					
	25 mg (n=3)	50 mg (n=30)			75 mg (n=3)	Total (N=36)
	Any grade[a] n	Grade 1/2 n (%)	Grade 3 n (%)	Any grade[a] n (%)	Any grade[a] n	Any grade[a] n (%)
Treatment-related non-hematologic adverse events ≥25%[b]						
Hand–foot syndrome	2	17 (57)	9 (30)	26 (87)	3	31 (86)
Fatigue	2	19 (63)	1 (3)	20 (67)	2	24 (67)
Diarrhea	2	19 (63)	0 (0)	19 (63)	2	23 (64)
Anorexia	1	19 (63)	1 (3)	20 (67)	2	23 (64)
Skin discoloration	0	21 (70)	0 (0)	21 (70)	2	23 (64)
Stomatitis	2	17 (57)	1 (3)	18 (60)	2	22 (61)
Nausea	1	13 (43)	0 (0)	13 (43)	2	16 (44)
Hypertension	0	7 (23)	7 (23)	14 (47)	2	16 (44)
Dysgeusia	1	11 (37)	0 (0)	11 (37)	2	14 (39)
Rash	1	12 (40)	0 (0)	12 (40)	1	14 (39)
Gingivitis	0	12 (40)	0 (0)	12 (40)	0	12 (33)
Abdominal pain	2	9 (30)	0 (0)	9 (30)	0	11 (31)
Cheilitis	1	9 (30)	0 (0)	9 (30)	1	11 (31)
Edema	0	9 (30)	0 (0)	9 (30)	1	10 (28)
Pigmentation disorder	0	7 (23)	0 (0)	7 (23)	2	9 (25)
Hematologic laboratory abnormalities						
Neutrophils	3	15 (50)	11 (37)	27 (90)[c]	3	33 (92)[c]
Leukocytes	3	21 (70)	5 (17)	26 (87)	3	32 (89)
Platelets	2	21 (70)	6 (20)	27 (90)	3	32 (89)
Hemoglobin	2	9 (30)	10 (33)	20 (67)[c]	3	25 (69)[c]
Lymphocytes	1	11 (37)	9 (30)	20 (67)	2	23 (64)
Non-hematologic laboratory abnormalities ≥40%[b]						
AST	2	19 (63)	3 (10)	22 (73)	2	26 (72)
Albumin	1	19 (63)	0 (0)	19 (63)	2	22 (61)
Total bilirubin	1	13 (43)	0 (0)	13 (43)	3[c]	17 (47)[c]
Alkaline phosphatase	1	9 (30)	3 (10)	12 (40)	3	16 (44)
ALT	0	12 (40)	2 (7)	14 (47)	1	15 (42)
Hyperglycemia	1	10 (33)	1 (3)	11 (37)	3	15 (42)
Phosphate	2	10 (33)	0 (0)	10 (33)	3	15 (42)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

[a] No grade 4 events were reported among the treatment-related non-hematologic adverse events listed; the only grade 4 events reported among those listed were one reduced hemoglobin concentration and one reduced neutrophil count in the 50-mg cohort and one increased total bilirubin in the 75-mg cohort, as noted

[b] Based on the total population

[c] Includes one grade 4 event

comparison, the objective response and clinical benefit rates were 7% and 24%, respectively, in the phase III trial [14]. Likewise, the median TTP for the 50-mg cohort in the current study was 27.9 weeks, compared with 27.3 weeks in the phase III trial [14]. Except for ethnicity and the smaller sample size in the current study, patients in the two studies were generally comparable in terms of demographic characteristics, GIST histology, and duration of prior imatinib treatment and primary cause for discontinuation. The benefit derived from sunitinib by Japanese patients after imatinib failure is important because there are no other approved and effective second-line treatments for GIST: sunitinib remains the only approved treatment multinationally for patients with GIST after imatinib failure.

Sunitinib-related adverse events experienced by patients across both phases of the current study were predominantly