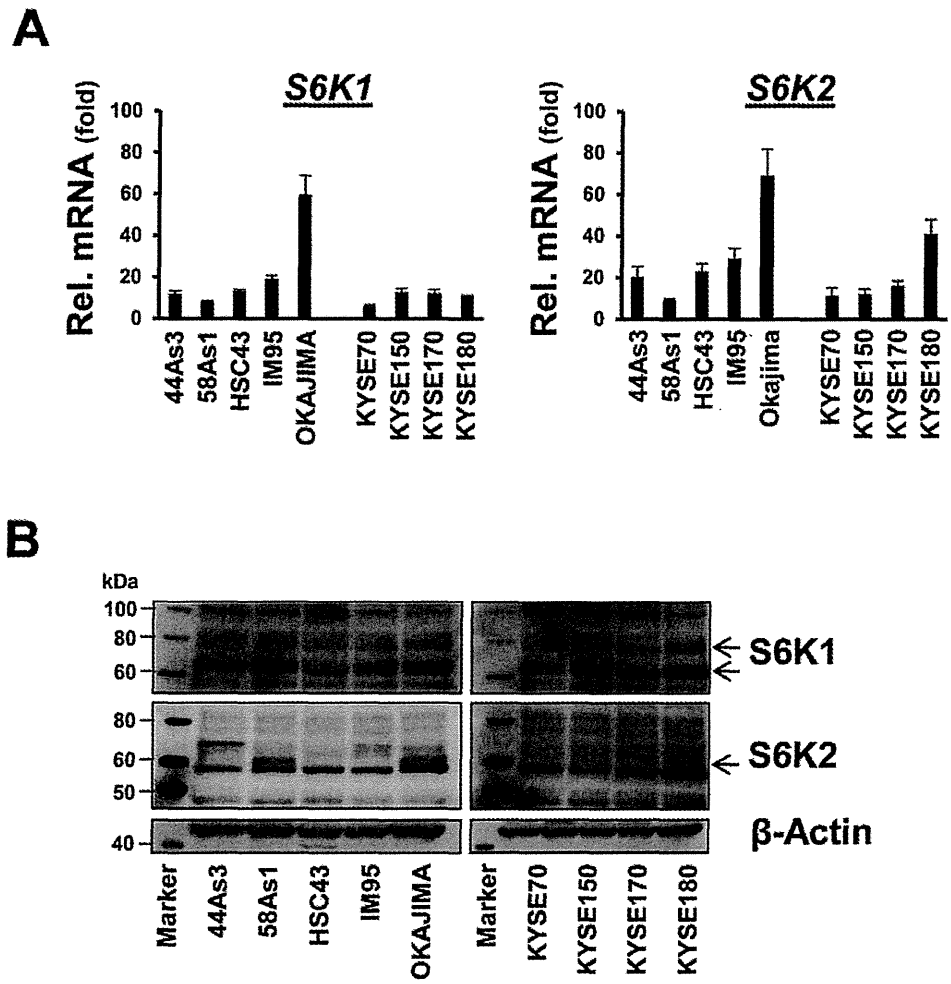

Figure 2. *S6 kinase-1 (S6K1) and S6K2 expression in cancer cell lines. A: The mRNA expression levels of S6K1 and S6K2 were determined using real-time RT-PCR. Rel. mRNA: Relative mRNA expression levels (target genes/GAPD $\times 10^3$). B: Western blot analysis for S6K1 and S6K2 protein. The arrows indicate the target proteins. β-actin was used as an internal control.*

percentages of the pathological stages were as follows: stage I, 8%; stage II, 12%, stage III, 29% and stage IV, 51%. Intestinal-type gastric cancer was observed in 42% (89/213) of the histologically-examined gastric carcinomas. The patient age, sex, pathological stage, and histology were not significantly associated with *S6K2*-amplification (Table I). The characteristics of the S6K2-amplified gastric carcinomas are summarized in Table II.

Finally, we examined the prognostic impact of *S6K2* amplification on OS after surgery. Although the sample size was relatively small (n=108), *S6K2* amplification was associated with a significantly shorter OS, compared with non-amplified cases, among patients with stage IV gastric cancer (log rank test, p=0.02; Figure 3C). Thus, *S6K2* amplification may be a novel prognostic factor for gastric cancer.

Discussion

Accumulating evidence demonstrates that the level of phosphorylation of S6K1 protein as detected using immunohistochemistry, which reflects activated mTORC1-S6K1 signaling, is increased in various types of cancer, including breast, lung, melanoma, hepatocellular carcinoma and glioma (3). Most of these studies showed that an increased phospho-S6K1 level was positively correlated with a poor prognosis, such as nodal metastasis and overall survival (3, 13-14). Therefore, the activation and overexpression of S6K1 enhances the malignant potential of the cancer cells. Regarding the gene amplification of *S6K1*, amplification is mostly observed in breast cancer, with a detection frequency of 6% to 14% using a Southern blot

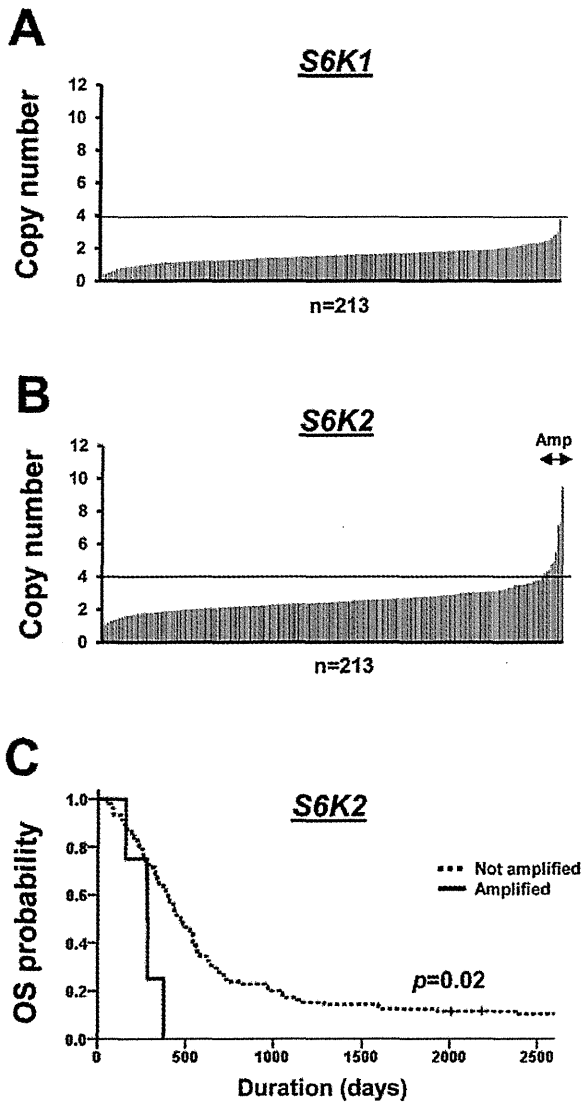

Figure 3. *Gene amplification of S6K1 and S6K2 in clinical gastric cancer samples. S6K1 (A) amplification and S6K2 (B) amplification were determined using the DNA copy number assay for 213 gastric cancer samples. S6K2 amplification over four copies was observed in 10 cases. C: Kaplan-Meier curves for overall survival (OS) in patients with stage IV gastric cancer. Patients were divided into two groups according to the S6K2 amplification. Amp, Gene amplification.*

Table I. *S6 kinase-2 (S6K2) amplification and clinicopathological features in gastric cancer.*

			S6K2 amplification		
		Total n=213	+ n=10	– n=203	*p*-Value
Age (years)	Range	31-91	45-75	31-91	0.94
	Median	63	63	63	
Gender	Male	147	7	140	0.78
	Female	66	3	63	
pStage	I	18	0	18	0.70*
	II	25	3	22	
	III	61	3	58	
	IV	108	4	104	
	Unknown	1	0	1	
Histology	Tub1	32	1	31	0.66**
	Tub2	44	1	43	
	Pap	5	0	5	
	Muc	8	1	7	
	Sig	11	2	9	
	Poor1	25	0	25	
	Poor2	88	5	83	

Tub, Tubular adenocarcinoma; Pap, papillary adenocarcinoma; Muc, mucinous adenocarcinoma; Sig, signet ring-cell carcinoma; Poor, poorly-differentiated adenocarcinoma; pStage, pathological stage. *Comparison between pStage I+II and III+IV. **Comparison between intestinal (Tub1, Tub2, Pap and Muc) and others. *p*-Values were calculated using the *t*-test for age and the χ^2 test for the other variables.

analysis, CGH, and fluorescence *in situ* hybridization (15-19). Thus, breast cancer is widely recognized to harbor a *S6K1* amplification in around 10% of samples. For other types of cancers, *S6K1* amplification was observed in three out of 16 (19%) medulloblastomas, in seven out of 38 (18%) diffuse large B-cell lymphoma combinations, and in two out of 17 (8%) ovarian cancer cell lines (20-22). In this study, we found that the 44As3 gastric cancer cell line harbored *S6K1* amplification, but amplification was not detected in clinical gastric cancer samples, suggesting that *S6K1* amplification is relatively rare in gastric cancer.

A limited number of studies have focused on the molecular and biological function of S6K2, despite the great number of studies that have examined S6K1 to date. Similarly, the dysregulation of S6K2 in cancer remains largely unknown. A recent study demonstrated that *S6K2* amplification was observed in nine out of 207 (4%) breast carcinomas, whereas the *S6K1* amplification was observed in 22 out of 206 (11%) (23). In addition, the *S6K2* amplification was correlated with a high mRNA expression level and was associated with a poor prognosis (23). Of note, another study from the same research group showed that the chromosomal region of 11q13, which includes the *S6K2* gene, was frequently co-amplified with the chromosomal region of 8p12, which includes another key downstream molecule of mTOR signaling, the eukaryotic translation initiation factor 4E binding protein-1 gene (24). Further study on such co-amplification is needed to understand the dysregulation of mTOR signaling. In addition, *S6K2* amplification may alter sensitivity to mTOR inhibitors, and thus further studies are warranted.

Table II. *Summary of patients with S6 kinase-2 (S6K2)-amplified gastric cancer.*

No.	Age	Gender	Macroscopic type*	Histology	pStage	OS (days)	S6K1 (CN)	S6K2 (CN)
1	45	M	2	Poor2	IIIa	394	1.5	4.1
2	64	M	4	Sig	IV	283	2.5	4.2
3	63	F	0-IIc	Sig	IIIb	4732+	1.8	4.3
4	52	M	2	Tub2	II	3935+	1.2	4.4
5	64	M	3	Tub1	II	1907	1.8	4.8
6	66	M	3	Poor2	IV	157	1.9	4.9
7	71	F	3	Poor2	II	835	1.3	5.5
8	75	M	3	Poor2	IV	280	2.0	7.1
9	73	M	4	Poor2	IV	373	1.7	7.3
10	57	F	3	Muc	IIIb	2742	1.6	9.5

No., Sample number; pStage, pathological stage; OS, overall survival; CN, copy number; *classification is based on the definitions of the Japanese Research Society for Gastric Cancer; + for OS, indicates the patient was still alive at the time of writing. Tub, Tubular adenocarcinoma; Muc, mucinous adenocarcinoma; Sig, signet ring-cell carcinoma; Poor, poorly-differentiated adenocarcinoma; pStage, pathological stage.

In conclusion, we found that the *S6K2* amplification was observed in gastric cancer at a frequency of 4.7%, and its amplification was related to a poor outcome. Our results may provide an insight into the dysregulation of mTOR signaling in gastric cancer.

Conflicts of Interest

None.

Acknowledgements

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MET amplification as a potential therapeutic target in gastric cancer

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ABSTRACT:

Our aim was to investigate both the prevalence of MET amplification in gastric cancer as well as the potential of this genetic alteration to serve as a therapeutic target in gastric cancer. MET amplification was assessed by initial screening with a PCR-based copy number assay followed by confirmatory FISH analysis in formalin-fixed, paraffin-embedded specimens of gastric cancer obtained at surgery. The effects of MET tyrosine kinase inhibitors (MET-TKIs) in gastric cancer cells with or without MET amplification were also examined. The median MET copy number in 266 cases of gastric cancer was 1.7, with a range of 0.41 to 21.3. We performed FISH analysis for the 15 cases with the highest MET copy numbers. MET amplification was confirmed in the four assessable cases with a MET copy number of at least 4, whereas MET amplification was not detected in those with a gene copy number of <4. The prevalence of MET amplification was thus 1.5% (4 out of 266 cases). Inhibition of MET by MET-TKIs resulted in the induction of apoptosis accompanied by attenuation of downstream MET signaling in gastric cancer cell lines with MET amplification but not in those without this genetic change. MET amplification identifies a small but clinically important subgroup of gastric cancer patients who are likely to respond to MET-TKIs. Furthermore, screening with a PCR-based copy number assay is an efficient way to reduce the number of patients requiring confirmation of MET amplification by FISH analysis.

INTRODUCTION

Gastric cancer is the third most common cause of death from malignant disease in men (fifth in women) worldwide [1]. The prognosis for patients with unresectable advanced or recurrent gastric cancer remains poor, with a median survival time of less than 1 year in individuals receiving conventional therapy [2-8]. The combination of trastuzumab, an antibody targeted to HER2, with chemotherapy has yielded a survival benefit for patients with HER2-positive gastric or gastro-esophageal junction cancer [7], with HER2-positive tumors accounting for 7 to 17% of all gastric cancers [9-11]. Further research is thus warranted to identify new therapeutic targets for gastric cancer patients.

The *MET* proto-oncogene encodes the receptor tyrosine kinase c-MET. The binding of its ligand, hepatocyte growth factor, to MET results in tyrosine phosphorylation of the receptor and activation of downstream signaling molecules. Oncogenic activation of *MET* suppresses apoptosis and promotes cell survival, proliferation, migration, and differentiation as well as gene transcription and angiogenesis [12]. In gastric cancer, such activation of *MET* has been attributed to

gene amplification [13-15]. However, the prevalence of *MET* amplification has varied among studies [13-21], possibly as a result of differences in the methods applied. This uncertainty led us to determine the prevalence of *MET* amplification in 266 formalin-fixed, paraffin-embedded (FFPE) specimens of gastric cancer obtained during surgery. To ensure the efficient detection of *MET* amplification, we adopted a sequential approach involving PCR-based determination of gene copy number followed by confirmatory FISH analysis. Moreover, to assess the potential of *MET* amplification as a therapeutic target in gastric cancer, we investigated its impact on cell survival and signal transduction.

RESULTS

MET amplification in gastric cancer cell lines

We first applied FISH (Figure 1A) and a real-time PCR–based method (Figure 1B) to examine *MET* copy number in gastric cancer cell lines whose *MET* amplification status was previously determined [22]. In gastric cancer cell lines negative for *MET* amplification, including KATO III, SNU1, SNU216, MKN1, MKN7, HSC39, MKN28, and NUGC3, the copy number of *MET* as determined by the PCR-based assay ranged between 1.3 and 3.3. In contrast, cell lines positive for *MET* amplification, including Hs746T, MKN45, and SNU5, showed *MET* copy numbers of 21.3, 21.3, and 17.9, respectively. The PCR-based assay thus revealed a high copy number for *MET* only in gastric cancer cell lines previously shown to be positive for *MET* amplification by FISH.

MET amplification in gastric cancer specimens

To determine the prevalence of *MET* amplification in advanced gastric cancer, we examined 266 FFPE specimens of surgically resected primary gastric tumors. Most of the patients were male (68.8%) and had undifferentiated-type gastric cancer (62.8%), including mucinous adenocarcinoma, signet ring cell adenocarcinoma, and poorly differentiated

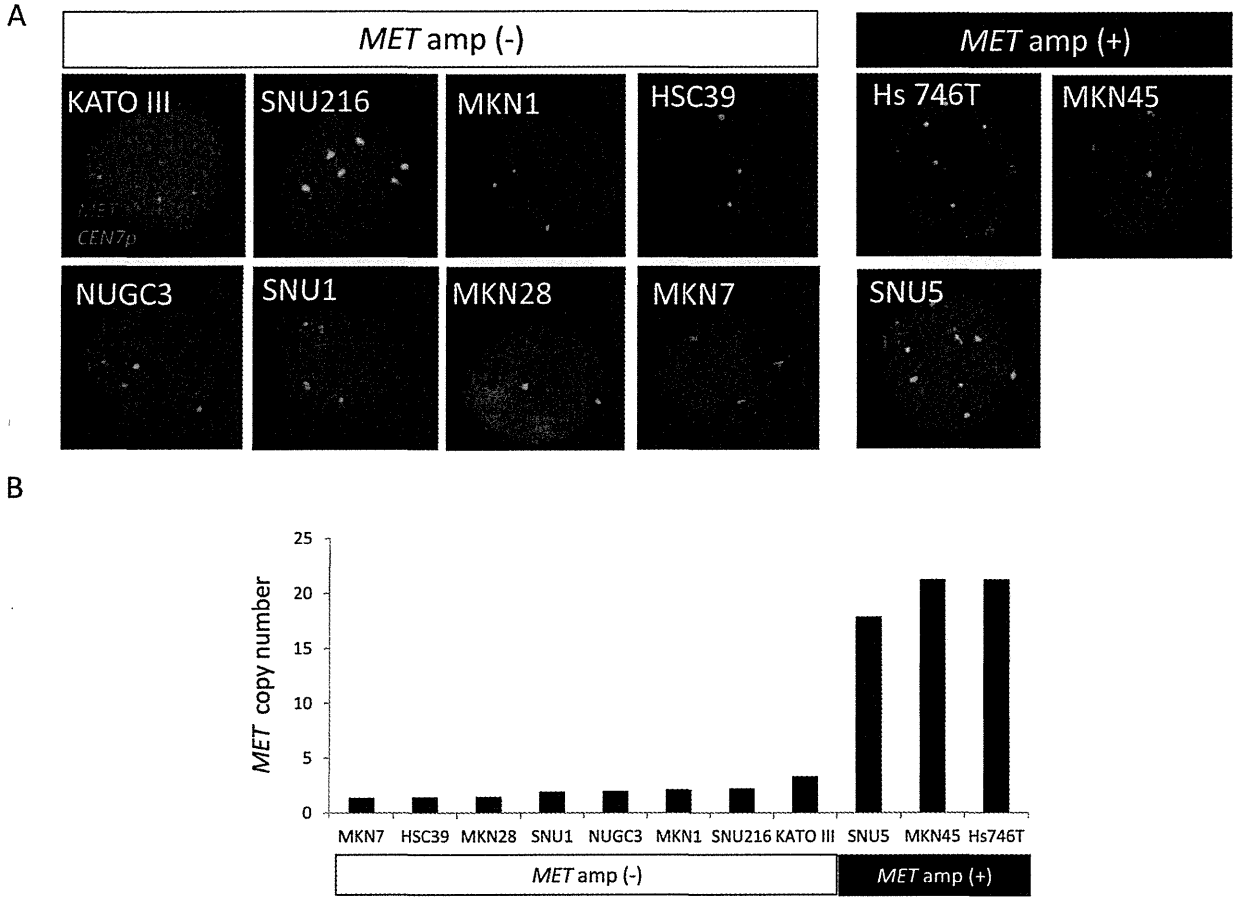

Figure 1: Amplification of *MET* in gastric cancer cell lines. *A*, FISH analysis of cell lines positive or negative for *MET* amplification (amp). Each image shows a single cancer cell, with green and red signals corresponding to CEN7p and the *MET* locus, respectively. *B*, Evaluation of *MET* copy number in gastric cancer cell lines with a PCR-based assay.

Table 1: Characteristics of the 266 study patients

Characteristic	n
Sex	
Male	183 (68.8%)
Female	83 (31.2%)
Pathological stage	
I	25 (9.4%)
II	31 (11.7%)
III	77 (28.9%)
IV	133 (50.0%)
Histology	
Differentiated type	99 (37.2%)
Undifferentiated type	167 (62.8%)

The patients had a median age of 63 years (range, 31 to 91 years).

adenocarcinoma (Table 1). The median age was 63 years, with a range of 31 to 91 years.

The PCR-based assay revealed that the median *MET* copy number for the 266 cases was 1.7, with a range of 0.41 to 21.3 copies (Figure 2A). Given that gastric cancer cell lines with *MET* amplification have been found to have a high copy number for *MET* [23], we arranged all cases in the order of *MET* copy number and performed FISH analysis for the 15 cases with the highest copy numbers (Table 2). *MET* amplification was detected by FISH in four of these cases (G72, G289, G322, and G181), which had a *MET* copy number of at least 4, whereas six cases

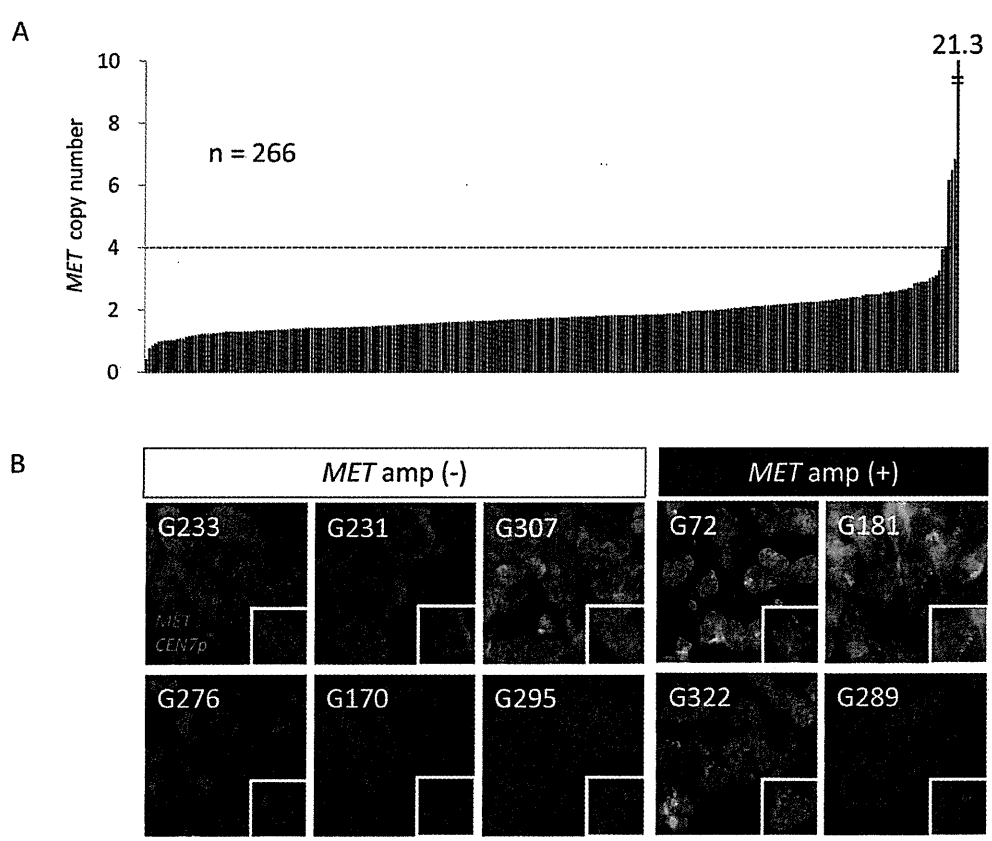

Figure 2: Amplification of *MET* in surgical specimens of gastric cancer. *A*, *MET* copy number determined with a PCR-based assay for 266 FFPE surgical specimens of gastric cancer. A *MET* copy number of >4 was observed in five cases. *B*, FISH analysis of gastric cancer specimens among the 15 samples with the highest *MET* copy numbers as determined with the PCR-based assay. Green and red signals correspond to CEN7p and the *MET* locus, respectively. Higher magnification images of individual cancer cells are shown in the insets. The specimens are grouped into those determined to be positive of negative for *MET* amplification by FISH.

(G276, G233, G295, G170, G307, and G231) with a copy number of less than 4 did not exhibit *MET* amplification (Figure 2B, Table 2). The remaining five cases (G331, G223, G217, G118, and G42) were not assessable by FISH analysis because of a lack of hybridization signals.

We thus identified four out of 266 gastric cancer patients (1.5%) as having *MET* amplification. The clinical features of patients with or without *MET* amplification are shown in Tables 2 and 3. All four patients with *MET* amplification had undifferentiated-type gastric cancer. We further examined the prognostic impact of *MET* amplification for all 266 patients but found that OS after surgery did not differ significantly between those with or without *MET* amplification (log-rank test, $P = 0.3$).

MET amplification is associated with increased sensitivity to MET-TKIs in gastric cancer cell lines

To investigate the biological impact of *MET* amplification in gastric cancer, we first examined the effects of two highly selective MET receptor tyrosine kinase inhibitors (MET-TKIs), JNJ38877605 and SGX523, on the growth of gastric cancer cell lines positive or negative for *MET* amplification. The IC_{50} values of JNJ3887605 and SGX523 for inhibition of cell growth were 0.02 to 0.05 μM and 0.06 to 0.07 μM, respectively, for cells positive for *MET* amplification, whereas they were >10 μM for *MET* amplification–negative cells (Figure 3A). An annexin V binding assay revealed that both MET-TKIs induced a substantial level of apoptosis in *MET* amplification–positive cells but were largely without

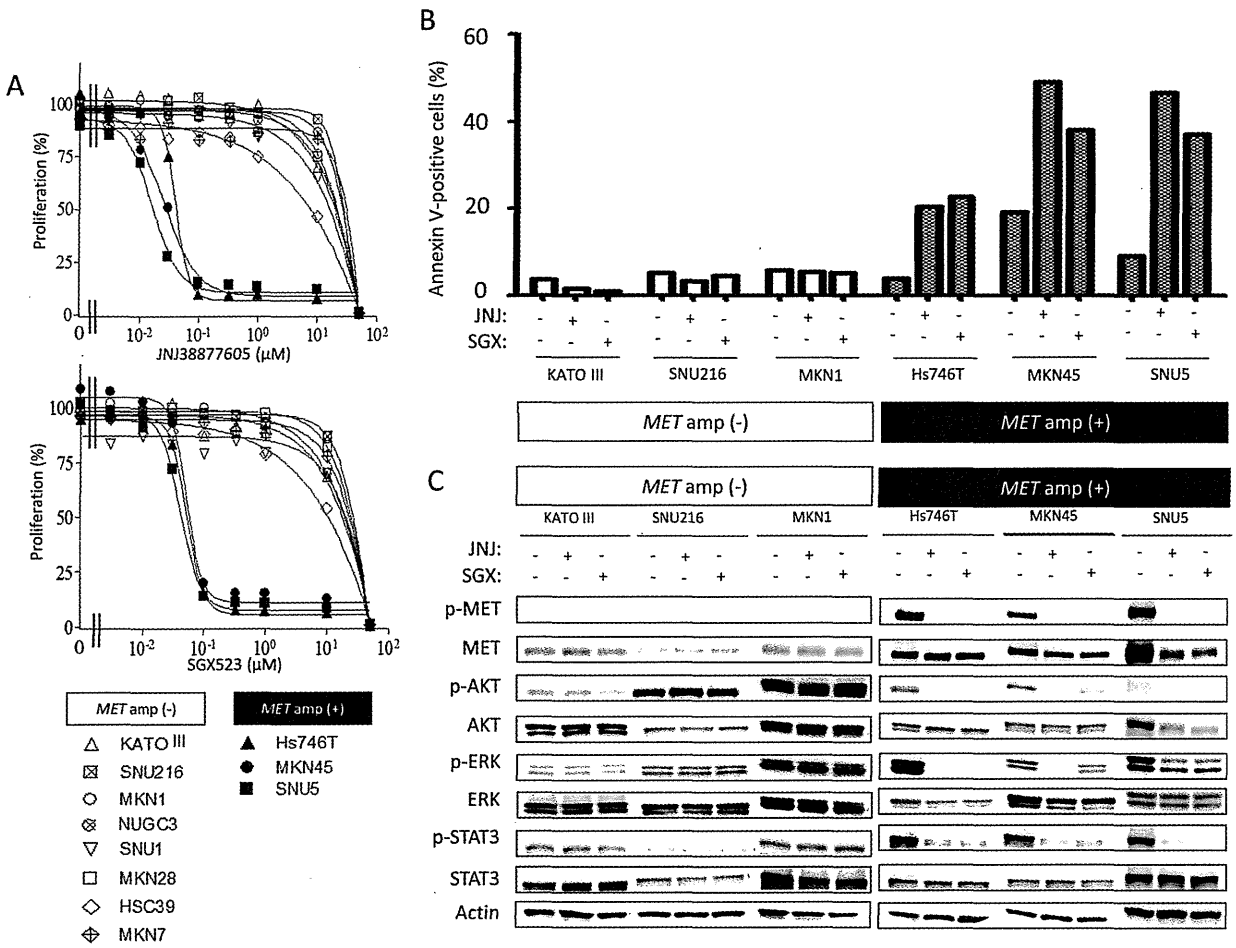

Figure 3: Effects of MET-TKIs in human gastric cancer cell lines classified according to *MET* amplification status. *A*, Effects of JNJ38877605 and SGX523 on cell growth as determined with the MTT assay. Data are means of triplicates from representative experiments. *B*, Cells were incubated in the absence or presence of 0.10 μM JNJ38877605 or 0.10 μM SGX523 for 48 h, after which the number of apoptotic cells was determined by staining with annexin V followed by flow cytometry. *C*, Cells were incubated in the absence or presence of 0.10 μM JNJ38877605 or 0.10 μM SGX523 for 48 h, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of MET, AKT, ERK, or STAT3 or with those to β-actin (loading control).

Table 2: Characteristics of the 15 patients with the highest MET copy numbers

Case no.	MET copy number	MET/ CEN7p	Sex	Age (years)	Histology	Stage	OS (days)
G72	21.3	5.9 (+)	M	66	U	IV	157
G289	6.84	5.2 (+)	F	48	U	IV	483
G322	6.45	7.2 (+)	F	70	U	IV	84
G331	6.14	ND	M	76	U	II	>2764
G181	4.02	6.6 (+)	F	52	U	IIIb	>1977
G223	3.92	ND	M	62	U	IIIa	>3340
G276	3.23	1.2 (–)	M	70	U	Ib	1089
G233	3.09	1.2 (–)	F	63	U	IIIb	>4732
G217	3.02	ND	F	69	U	IIIa	>3827
G118	2.97	ND	M	66	U	IIIa	2650
G295	2.89	2.0 (–)	M	74	U	IV	539
G170	2.88	1.7 (–)	M	53	D	Ia	>2088
G42	2.87	ND	M	64	D	II	1907
G307	2.85	1.3 (–)	M	60	U	IV	824
G231	2.83	1.9 (–)	M	67	D	IIIa	>2921

Abbreviations: ND, signals not detected; U, undifferentiated type; D, differentiated type. (+) or (–) denote positive or negative for MET amplification on the basis of the MET/CEN7p ratio; > for OS indicates the patient was still alive.

effect in cell lines without *MET* amplification (Figure 3B). Immunoblot analysis showed that the MET-TKIs inhibited the phosphorylation of MET, AKT, ERK, and STAT3 in gastric cancer cells with *MET* amplification, whereas they had no effect on signaling events in those negative for *MET* amplification (Figure 3C). These findings thus indicated that gastric cancer cells with *MET* amplification are predominantly dependent on MET signaling for their growth and survival and are therefore rendered hypersensitive to MET-TKIs.

DISCUSSION

Activation of MET signaling promotes tumor cell growth, survival, migration, and invasion as well as tumor angiogenesis [24]. In gastric cancer, gain-of-function mutations of *MET* are exceedingly rare [25-27], with MET activation having been attributed mostly to gene amplification [13-15]. Previous studies based on FISH analysis have detected *MET* amplification in up to 4% of patients with gastric cancer [14, 16, 20]. On the other hand, an increase in *MET* copy number was found in 10 to 20% of gastric cancer patients by Southern blot analysis [17-19] or with a PCR-based assay [28, 29]. An increase in gene copy number in malignant tumors can result from at least two genetic mechanisms, gene amplification and polysomy. Gene amplification refers to a copy number gain for a specific gene (or group of genes) on a given chromosome arm without a change in copy number for genes located in other regions of the chromosome [30], whereas polysomy gives rise to a copy number gain for a given gene as a result of the presence of extra copies of the entire chromosome. Southern blot analysis and PCR-based copy number assays recognize a gain in gene copy number regardless of the underlying cause and are thus unable to distinguish gene amplification from polysomy, a limitation that is sometimes overlooked, with consequences for determination of the true prevalence of *MET* amplification in gastric cancer.

FISH analysis is a semiquantitative method that can be performed with two probes for determination of the number of signals for a target gene and for the centromeric

Table 3: Clinical and pathological characteristics of gastric cancer patients classified according to MET amplification status

Characteristic	MET amplification(+) (n = 4)	MET amplification(–) (n = 262)	P
Median age (range), years	59 (48–70)	63 (31–91)	0.976
Sex, n			
Male	1 (25.0%)	182 (69. 5%)	0.091
Female	3 (75.0%)	80 (30. 5%)	
Pathological stage, n			
I	0	25 (9.5%)	0.582[a]
II	0	31 (11.8%)	
III	1 (25.0%)	76 (29.0%)	
IV	3 (75.0%)	130 (49.6%)	
Histology, n			
Differentiated type	0	99 (37.8%)	0.300[b]
Undifferentiated type	4 (100%)	163 (62.2%)	

[a]Comparison between stages I + II and III + IV. [b]Comparison between intestinal-type and diffuse-type gastric cancer. P values were calculated with Student's two-tailed t test for age and the chi-square test for the other variables.

portion of the corresponding chromosome. Given that the number of centromeric signals directly reflects the copy number of the chromosome, FISH analysis yields the copy number gain for the target gene from the ratio of the copy number of the gene to that of the chromosome. FISH is thus the gold standard for detection of gene amplification. However, the identification and counting of FISH signals are hampered by many factors including cutting artifacts, nuclear overlap, and heterogeneity of tumor specimens. Moreover, FISH is expensive and time-consuming, and it requires technical expertise [31]. The efficient determination of gene amplification in a large number of tumor specimens would thus benefit from the availability of a high-throughput screening assay. In this regard, PCR-based assays for determination of gene copy number are simple to perform and rapidly yield quantitative and reproducible results. Given that, among tumors showing a gain in gene copy number, those confirmed to be positive for gene amplification show the greatest increases in gene copy number [21, 23, 32, 33], we investigated the potential of a PCR-based assay for screening in order to select cases of gastric cancer for confirmation of *MET* amplification by FISH. We thus performed PCR-based screening for *MET* copy number in 266 surgically resected specimens of gastric cancer and then applied FISH analysis to the 15 cases showing the highest gene copy numbers. *MET* amplification was confirmed by FISH in four cases among the five with a *MET* copy number of at least 4; the remaining case (G331) was not assessable by FISH because of a lack of hybridization signals. *MET* amplification was not detected in the cases with a gene copy number of <4. We therefore identified *MET* amplification at a frequency of 1.5% (4 out of 266 cases), consistent with values determined by FISH analysis in recent studies of gastric cancer [16, 20]. Our results thus suggest that screening for *MET* amplification with a PCR-based assay is an efficient means with which to reduce the number of specimens requiring evaluation by FISH analysis. As mentioned above, one specimen (G331) in the present study showed a high *MET* copy number (6.14 copies) but could not be confirmed positive for *MET* amplification because of the lack of a FISH result. The issue of how to identify *MET* amplification status in such cases remains to be resolved.

We examined the biological impact of *MET* amplification in gastric cancer cells by comparing the effects of the MET-TKIs JNJ38877605 and SGX523 between gastric cancer cell lines positive for *MET* amplification and those negative for this genetic alteration. In gastric cancer cells with *MET* amplification, the MET-TKIs markedly inhibited AKT, ERK, and STAT3 signaling and triggered apoptosis, whereas such effects were not evident in cells without *MET* amplification. To investigate whether attenuation of MET signaling by the MET-TKIs is related to the induction of apoptosis, we transfected gastric cancer cell lines with an siRNA specific for MET mRNA. Such transfection inhibited MET signal transduction as well as induced apoptosis in gastric cancer cell lines with *MET* amplification but not in those without it (data not shown). Our observations thus indicate that gastric cancer cell lines positive for *MET* amplification depend predominantly on constitutive activation of the encoded growth factor receptor for their survival and thus show high sensitivity to cell killing by MET-TKIs. Targeting of MET signaling by MET-TKIs is therefore a potentially valuable therapeutic approach for patients with *MET* amplification–positive gastric cancer. Indeed, the MET-TKI crizotinib (PF-02341066) was recently found to induce a radiographic response (partial response) and rapid clinical improvement in patients with advanced

gastric cancer who were found to be positive for *MET* amplification by FISH [16]. Further investigation of the efficacy of MET-TKIs in patients with advanced gastric cancer positive for *MET* amplification is thus warranted.

Given the potential of MET-targeted therapy for gastric cancer with *MET* amplification, it is important to determine the prevalence of such gene amplification in patients with unresectable advanced gastric cancer, most of whom are currently treated with systemic chemotherapy. Our present study was limited to gastric cancer patients who underwent gastrectomy, and so further studies will be needed for patients with unresectable advanced tumors. Given the apparent low prevalence of *MET* amplification in gastric cancer, implementation of a sequential approach including screening with a PCR-based copy number assay followed by confirmatory FISH analysis should facilitate the identification of *MET* amplification in a large cohort of patients with unresectable advanced gastric cancer.

MATERIALS AND METHODS

Cell culture

The human gastric cancer cell lines SNU1, SNU5 and Hs746T were obtained from American Type Culture Collection (Manassas, VA); MKN1, MKN7, MKN45, and NUGC3 were from the Health Science Research Resources Bank (Japan Health Sciences Foundation, Tokyo, Japan); KATO III, MKN28, and HSC39 were from Immuno-Biological Laboratories (Gunma, Japan); and SNU216 was from the Korean Cell Line Bank (Seoul National University, Seoul, Korea). All of the cell lines were maintained under a humidified atmosphere of 5% CO_2 at 37°C in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated FBS (Gibco BRL, Grand Island, NY), penicillin, and streptomycin.

Patients

A total of 267 patients with histologically confirmed gastric cancer who had undergone surgery at the National Cancer Center Hospital (Tokyo, Japan) between 1996 and 2006 were included in the study. All the patients had an Eastern Cooperative Oncology Group performance status of 0 to 2. One patient was subsequently excluded as a result of an insufficient quantity of DNA extracted from the corresponding tissue specimen. The specimens from the remaining 266 patients were thus analyzed. The present study was approved by the Institutional Review Board of the National Cancer Center Hospital, and informed consent was obtained from all subjects.

Isolation of genomic DNA

Macrodissection of the surgical specimens preserved as FFPE tissue was performed after removal of paraffin in order to select a region of cancer tissue. Genomic DNA was extracted from the cancer tissue with the use of a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). The DNA concentration of the extracts was determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA).

PCR-based determination of MET copy number

The copy number of *MET* was determined with the use of a TaqMan Copy Number Assay [32] and the Hs05005660_cn (intron 16) primer (Applied Biosystems, Foster City, CA). The *TERT* locus was used as the internal reference, and DNA from noncancerous FFPE tissue was used as a normal control. Real-time PCR was performed in a total volume of 20 μL per well containing 10 μL of TaqMan genotyping master mix, 20 ng of genomic DNA, and each primer. The amplification protocol included an initial incubation at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The resulting products were detected with the use of ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Data were analyzed with SDS 2.2 software and Copy Caller software (Applied Biosystems).

FISH

MET copy number per cell was determined by FISH with the use of the c-met / CEN7p Dual Color FISH Probe (GSP Laboratory, Kawasaki, Japan) [22], where CEN7p is the centromeric region of chromosome 7p. The signals were detected by fluorescence microscopy and were evaluated by independent observers (H.K. and I.O.). After screening all entire sections, images of tumor cells were captured and recorded and the signals for 60 random nuclei were counted for an area where individual cells were recognized in at least 10 representative images. Nuclei with a disrupted boundary were excluded from the analysis. Gene amplification was strictly defined by a mean *MET*/CEN7p copy number ratio of >2.2, corresponding to a previous definition of *MET* amplification [16]. The presence of polysomy or an equivocal *MET*/CEN7p ratio (1.8 to 2.2) were thus scored as negative for amplification.

Immunoblot analysis

Immunoblot analysis was performed as described previously [22]. Rabbit polyclonal antibodies to phosphorylated human MET (pY1234/pY1235), to total AKT, to phosphorylated AKT, to phosphorylated

extracellular signal–regulated kinase (ERK), to phosphorylated or total forms of STAT3 were obtained from Cell Signaling Technology (Danvers, MA); those to total ERK were from Santa Cruz Biotechnology (Santa Cruz, CA); those to total MET were from Zymed/ Invitrogen (Carlsbad, CA); and those to β-actin were from Sigma. All antibodies were used at a 1:1000 dilution, with the exception of those to β-actin (1:200).

Cell growth inhibition assay

Cells were transferred to 96-well flat-bottomed plates and cultured for 24 h before exposure to various concentrations of JNJ38877605 (Janssen Pharmaceutica NV, Beerse, Belgium) or SGX523 (SGX Pharmaceuticals, San Diego, CA) for 72 h. Tetra Color One (5 mmol/L tetrazolium monosodium salt and 0.2 mmol/L 1-methoxy-5-methyl phenazinium methylsulfate; Seikagaku Kogyo, Tokyo, Japan) was then added to each well, and the cells were incubated for 3 h at 37°C before measurement of absorbance at 490 nm with a Multiskan Spectrum instrument (Thermo Labsystems, Boston, MA). Absorbance values were expressed as a percentage of that for nontreated cells, and the IC_{50} values of JNJ38877605 and SGX523 for inhibition of cell growth were determined.

Annexin V binding assay

The binding of annexin V to cells was measured with the use of an Annexin-V-FLUOS Staining Kit (Roche, Basel, Switzerland). Cells were harvested by exposure to trypsin-EDTA, washed with PBS, and centrifuged at 200 × *g* for 5 min. The cell pellets were resuspended in 100 μL of Annexin-V-FLUOS labeling solution, incubated for 10 to 15 min at 15° to 25°C, and then analyzed for fluorescence with a flow cytometer (FACSCalibur) and Cell Quest software (Becton Dickinson, Franklin Lakes, NJ).

Statistical analysis

Overall survival (OS) curves were estimated with the Kaplan-Meier method and compared with the log-rank test. Other statistical analysis was performed with Student's two-tailed *t* test or the chi-square test. A *P* value of <0.05 was considered statistically significant.

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Conflict of interest statement

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Phase I study of cediranib in combination with cisplatin plus fluoropyrimidine (S-1 or capecitabine) in Japanese patients with previously untreated advanced gastric cancer

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Abstract
Purpose The primary objective of this Phase I study was to assess the safety and tolerability of the vascular endothelial growth factor signalling inhibitor cediranib in combination with cisplatin plus an oral fluoropyrimidine, in Japanese patients with previously untreated advanced gastric cancer.
Methods Patients received continuous, once-daily oral doses of cediranib 20 mg in combination with either cisplatin (60 mg/m^2 iv day 1) plus S-1 (40–60 mg bid, days 1–21) every 5 weeks for a maximum of eight cycles [Arm A]; or cisplatin (80 mg/m^2 iv, day 1) plus capecitabine (1,000 mg/m^2 bid, days 1–14) every 3 weeks for a maximum of six cycles [Arm B]. In both arms, the assessment period for dose-limiting toxicities (DLTs) was the first 21 days of cycle 1.
Results Fourteen patients (Arm A, $n = 6$; Arm B, $n = 8$) were enrolled and received at least one dose of cediranib. One patient in each arm experienced a DLT (Arm A; decreased appetite, grade 3; Arm B, decreased appetite, fatigue and hyponatraemia, all grade 3). Overall, the most common adverse events were decreased appetite, fatigue and nausea (all $n = 13$ [92.9%]). Preliminary efficacy evaluation showed one confirmed (Arm A) and three unconfirmed (Arm A, $n = 1$; Arm B, $n = 2$) partial responses that were ongoing at data cut-off.
Conclusions Cediranib 20 mg/day in combination with cisplatin and S-1 or capecitabine was tolerable, with no new toxicities identified, and showed preliminary evidence of antitumour activity.

Keywords Cediranib · VEGF signalling · Phase I · Gastric cancer · Japanese

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Introduction

Gastric cancer is the most common malignancy in Japan. GLOBOCAN figures revealed that in 2008, there were 102,040 new cases of gastric cancer, and 50,156 deaths were attributed to this disease in Japan [1]. The only curative treatment is surgery, however, over half of patients present with inoperable tumours. For those patients with unresectable tumours and receiving best supportive care, outcomes are extremely poor with median survival times ranging from 3 to 5 months [2–4].

Combination chemotherapy regimens with platinum-based cisplatin plus an oral fluoropyrimidine are commonly used as first-line treatment for advanced gastric cancer in Japan [5]. This treatment regimen is based on early-phase clinical trials that showed cisplatin in combination with 5-fluorouracil (5-FU) or oral fluoropyrimidines yielded overall response rates of approximately 40% and median survival times of 7–13 months [6–10].

Vascular endothelial growth factor (VEGF) plays an essential role in the formation and maintenance of tumour vasculature [11]. The addition of bevacizumab, an anti-VEGF-A antibody, to standard chemotherapy has demonstrated clinical benefit in patients with advanced colorectal cancer [12–14] and non-small-cell lung cancer [15].

Cediranib is an oral, highly potent VEGF signalling inhibitor with activity against all three VEGF receptors [16, 17]. Initial clinical evaluation of cediranib monotherapy demonstrated that it is suitable for once-daily oral dosing in Japanese [18] and Western [19] patients, with biological activity at doses ≥20 mg/day [19]. Subsequent Phase I studies showed that cediranib 30 mg/day was generally well tolerated in combination with various standard anti-cancer treatments, with encouraging preliminary evidence of antitumour activity [20–23]. However, when the protocol for the present study was being developed, emerging data from Phase II and III trials indicated that cediranib 20 mg was the highest tolerable dose suitable for chronic once-daily dosing in combination with chemotherapy, with higher doses not considered to be more effective [24, 25]. Consequently, the dose of cediranib selected for this combination study was 20 mg/day. The primary objective of the current Phase I study (ClinicalTrials.gov, number NCT00960349) was to assess the safety and tolerability of cediranib 20 mg/day in combination with capecitabine/cisplatin or S-1/cisplatin in Japanese patients with previously untreated advanced gastric cancer.

Methods

Patients

Japanese patients ≥20 years of age with histologically or cytologically confirmed previously untreated recurrent or metastatic unresectable gastric adenocarcinoma were eligible for inclusion. Patients were required to have a life expectancy ≥12 weeks and a World Health Organization performance status of 0 or 1. The main exclusion criteria were as follows: significant respiratory, cardiac, hepatic or renal dysfunction; unstable brain metastases; poorly controlled hypertension; significant haemorrhage (>30 ml bleeding/episode in the previous 3 months) or haemoptysis (>5 ml fresh blood in the previous 4 weeks); arterial thromboembolic events in the previous 12 months; history of other malignancies within the previous 5 years; any unresolved toxicity according to Common Terminology Criteria for Adverse Events (CTCAE) from prior radiotherapy; recent (<14 days) major thoracic or abdominal surgery; and incomplete recovery from prior surgery. All patients provided written informed consent. The study was approved by the institutional review board at each participating centre and conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and the AstraZeneca policy on Bioethics [26].

Study design

This was a multicentre, open-label, non-randomized, Phase I study. Eligible patients received cediranib 20 mg/day orally (starting on day 2 in cycle 1) in combination with either cisplatin (60 mg/m^2 intravenous [iv], day 1) plus S-1 (40–60 mg orally twice daily, days 1–21) [Arm A] or cisplatin (80 mg/m^2 iv, day 1) plus capecitabine (1,000 mg/m^2 orally twice daily, days 1–14) [Arm B] (Fig. 1). One cycle of treatment in Arm A was 5 weeks, and one cycle of treatment in Arm B was 3 weeks. The rest periods in Arms A (2 weeks) and B (1 week) were consistent with standard clinical practice for administration of S-1 and capecitabine, respectively. The chemotherapy treatments in Arms A and B were continued for a maximum of eight and six cycles, respectively. Thereafter, treatment of cediranib plus S-1/capecitabine could be continued until a discontinuation criterion was met. Patients were initially entered into Arm A. Following enrolment of six patients into Arm A, patients were then entered into Arm B.

The primary study objective was to assess the safety and tolerability of cediranib in combination with S-1/cisplatin or capecitabine/cisplatin. After entry of six evaluable patients in each arm, a safety review committee (SRC) discussed whether the regimen was tolerated. The treatment was considered tolerable if ≤1 of the six patients experienced a DLT. If 2–3 of the six patients experienced a DLT, either the SRC recommended the combination was tolerated or the cohort was expanded to include three further evaluable patients. If ≥4 patients experienced a DLT, the treatment was considered intolerable.

In both arms, a DLT was any toxicity considered related to study drug that commenced within the first 21 days of cycle 1 and met any of the following criteria: hypertension or diarrhoea that required cessation of cediranib treatment; an absolute neutrophil count <500/mm^3 for ≥5 days despite growth factor support; a platelet count <50,000/mm^3 for ≥5 days; a dose delay to starting any chemotherapy agent in cycle 2 for longer than 14 days; dose reductions of cediranib due to cediranib-related toxicity; a single increase from baseline in the QT interval corrected for heart rate

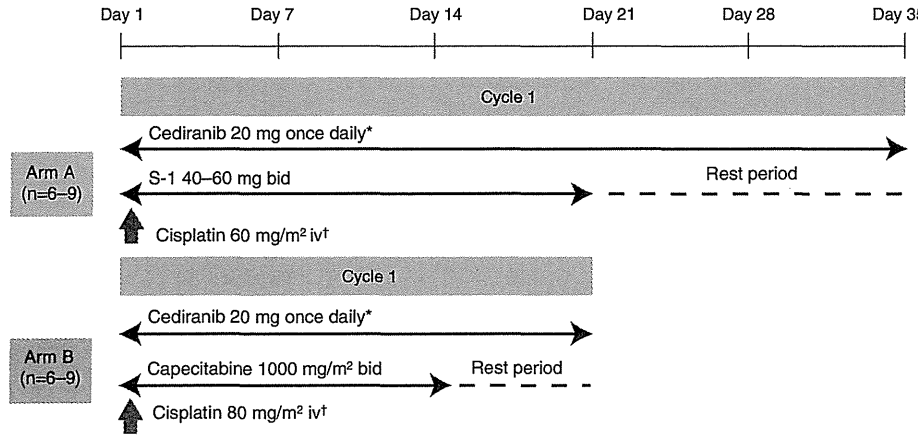

Fig. 1 Study design

*Cediranib initiated on day 2 in cycle 1
†Cisplatin continued for a maximum of eight cycles in Arm A and six cycles in Arm B
Safety assessments were performed on days 1, 8, 21 and 35 (Arm A only)

(QTc) of 60 ms that results in a QTc of at least 460 ms; two QTc measurements >490 ms taken at least 24 h apart; and any other CTCAE grade ≥3 that was, in the opinion of the investigator and the SRC, not clearly related to disease progression, clinically significant and related to the study drug.

Secondary objectives were to determine the steady-state pharmacokinetics (PK) of cediranib alone and in combination with chemotherapy and to investigate the potential effect of cediranib on the PK of the chemotherapy components (cisplatin and S-1/capecitabine [5-FU]). An exploratory objective was to assess the preliminary efficacy of the combination regimens by measurement of tumour response according to the Response Evaluation Criteria In Solid Tumours (RECIST version 1.0) [27].

Assessment of safety and tolerability

After a full physical examination at enrolment, toxicity was monitored throughout the study by the assessment of adverse events (AEs), which were graded according to CTCAE version 3.0. Vital signs (blood pressure [BP], pulse rate and body temperature) were measured, electrocardiograms recorded and samples taken for clinical chemistry, haematology assessment and urinalysis at the screening visit and on days 1, 8 and 21 in both arms; patients in Arm A repeated these assessments on day 35.

Pharmacokinetic assessment

To evaluate steady-state cediranib PK, blood samples were taken immediately before and 1, 2, 4, 6, 8 and 24 h after cediranib treatment on the final day of cycle 1 (cediranib alone) and day 1 of cycle 2 (presence of chemotherapy). To evaluate S-1/capecitabine (5-FU) PK, blood samples were collected immediately before and 0.5, 1, 2, 4, 6 and 8 h after S-1/capecitabine treatment on day 1 of cycle 1 (absence of cediranib) and day 1 of cycle 2 (presence of cediranib). To evaluate cisplatin PK, blood samples were taken pre-dose; 5 min before the end of the 2-h iv infusion; and 2.5, 3, 4, 6, 8 and 24 h post start of infusion on day 1 of cycle 1 (absence of cediranib) and day 1 of cycle 2 (presence of cediranib).

Plasma concentrations of cediranib, capecitabine (5-FU only), S-1 (5-FU only) and cisplatin (total platinum equivalents) were determined using high-performance liquid chromatography with mass spectrometry (LC–MS/MS). PK parameters were calculated using standard non-compartmental analysis.

Assessment of tumour response

Objective tumour assessments determined by RECIST were performed every 12 weeks from the start of treatment until disease progression, death or discontinuation of cediranib due to any other reason.

Results

Patient characteristics

Between August and December 2009, 14 patients were recruited into Arm A ($n = 6$) or Arm B ($n = 8$). Patient demographic and baseline characteristics are summarized in Table 1. At data cut-off (4 January 2010), three patients in Arm A and five patients in Arm B were still receiving cediranib, and one patient in Arm B continued to receive capecitabine and cisplatin. The reasons for discontinuation of cediranib treatment were clinical disease progression

Table 1 Patient demographics and baseline characteristics

Characteristics	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Age, years			
Median	59.5	60.5	60.5
Range	53–71	27–72	27–72
Sex, *n* (%)			
Male	4 (66.7)	5 (62.5)	9 (64.3)
Female	2 (33.3)	3 (37.5)	5 (35.7)
WHO performance status, *n* (%)			
0	3 (50.0)	4 (50.0)	7 (50.0)
1	3 (50.0)	4 (50.0)	7 (50.0)
Number of metastatic sites (%)			
1	1 (16.7)	0	1 (7.1)
>1	5 (83.3)	8 (100.0)	13 (92.9)
Recurrence, *n* (%)	0	1 (12.5)	1 (7.1)
Stage IV, *n* (%)	6 (100)	7 (87.5)	13 (92.9)
Measurable target lesion, *n* (%)	5 (83.3)	6 (75.0)	11 (78.6)
Histology, *n* (%)			
Adenocarcinoma (intestinal)	1 (16.7)	3 (37.5)	4 (28.6)
Adenocarcinoma (diffuse)	1 (16.7)	0	1 (7.1)
Tubular adenocarcinoma	3 (50.0)	2 (25.0)	5 (35.7)
Signet ring carcinoma	1 (16.7)	3 (37.5)	4 (28.6)

WHO World Health Organization

(Arms A and B, $n = 1$), AEs (Arms A and B, $n = 1$) and withdrawal of consent (Arm A, $n = 1$). One patient in Arm B was revealed ineligible at cycle 2 due to a pulmonary embolism at baseline; this patient discontinued study treatment but was included in safety analyses.

Safety and tolerability

All patients received at least one dose of cediranib and were therefore evaluable for safety. The median (range) daily cediranib dose was 16.0 (12.9–20.0) mg in Arm A and 15.9 (13.7–20.0) mg in Arm B, and median (range) duration of actual exposure to cediranib was 72.5 days (13–127) for Arm A and 38.5 days (13–62) for Arm B. The median (range) number of chemotherapy cycles received was 2.5 (1–4) for both arms.

Overall, 12 (86%) [Arm A, $n = 5$; Arm B, $n = 7$] patients experienced one or more cediranib dose interruptions, with one patient from each arm having a dose reduction to 15 mg/day. All six patients in Arm A experienced a dose reduction or interruption of S-1 and seven patients (87.5%) in Arm B experienced a dose reduction or interruption of capecitabine. Five patients in each arm (Arm A, 83.3%; Arm B, 62.5%) had a dose reduction or dose delay of cisplatin. Two patients in Arm A (alopecia, $n = 1$; diarrhoea, stomatitis, fatigue, decreased appetite and hyponatraemia, $n = 1$) and one patient in Arm B (diarrhoea, fatigue, decreased appetite and hypomagnesaemia) experienced AEs that led to permanent discontinuation of cediranib treatment.

Table 2 Most common adverse events (incidence > 30% in total population)

AE, preferred term	All grades, *n* (%)		
	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Decreased appetite	5	8	13 (92.9)
Fatigue	5	8	13 (92.9)
Nausea	5	8	13 (92.9)
Constipation	3	7	10 (71.4)
Diarrhoea	5	5	10 (71.4)
Stomatitis	4	6	10 (71.4)
Hypertension	3	6	9 (64.3)
Weight decreased	5	4	9 (64.3)
Neutropenia	5	3	8 (57.1)
Vomiting	3	5	8 (57.1)
Alopecia	2	4	6 (42.9)
Dysphonia	2	4	6 (42.9)
Hiccups	1	4	5 (35.7)
Leukopenia	3	2	5 (35.7)
Proteinuria	3	2	5 (35.7)

AE adverse event

DLTs were reported in one patient in Arm A (decreased appetite, grade 3) and one patient in Arm B (decreased appetite, fatigue and hyponatraemia; all grade 3). In Arm A, the investigator assessed that decreased appetite was related to S-1 and/or cisplatin. In Arm B, the investigator judged decreased appetite and hyponatraemia related to cediranib, S-1 and cisplatin, and stomatitis related to cediranib and S-1. The SRC decided neither DLT warranted cohort expansion for further evaluation of safety.

The most commonly reported AEs were decreased appetite, fatigue and nausea (all $n = 13$ [92.9%]) [Table 2]. Five (83%) patients in Arm A and six (75%) patients in Arm B experienced AEs grade ≥3 (Table 3). Hypertension was reported as an AE in nine patients (Arm A, $n = 3$; Arm B, $n = 6$), only one (Arm B) of which was

Table 3 Any CTCAE grade ≥3 adverse events

	Grade	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Neutropenia	3	3	2	5 (35.7)
Hypokalaemia	3	0	3	3 (21.4)
Hyponatraemia	3	1	2	3 (21.4)
Decreased appetite	3	1	1	2 (14.3)
Fatigue	3	0	2	2 (14.3)
Anaemia	3	0	1	1 (7.1)
Diarrhoea	3	1	0	1 (7.1)
Haemoglobin decreased	3	1	0	1 (7.1)
Hyperbilirubinaemia	3	0	1	1 (7.1)
Hyperglycaemia	3	0	1	1 (7.1)
Hypertension	3	0	1	1 (7.1)
Hypomagnesaemia	3	0	1	1 (7.1)
Platelet count decreased	3	1	0	1 (7.1)
Pulmonary embolism	4	0	1	1 (7.1)
Stomatitis	3	1	0	1 (7.1)
Syncope	4	1	0	1 (7.1)
White blood cell count decreased	3	1	0	1 (7.1)
Wound infection	3	1	0	1 (7.1)

grade 3; no action was taken regarding dose adjustment. One patient in Arm A experienced grade 4 transient syncope on day 6, cycle 2. A head computed tomography (CT) scan showed no cerebral haemorrhage and the syncope resolved on the same day it appeared. The investigator considered this event to be related to cediranib, S-1 and cisplatin. One patient from Arm B experienced a grade 4 pulmonary embolism that was identified on day 18, cycle 2 after the patient complained of chest pain. After careful review of the baseline CT scan, the pulmonary embolism was found to be pre-existing at study entry. The investigator judged the event as worsening of the pulmonary embolism related to cediranib, capecitabine and cisplatin. Increases in thyroid stimulating hormone were observed in both arms, but free T4 and T3 remained within normal limits for the majority of these patients. Increases were observed in alanine aminotransferase and aspartate aminotransferase in both arms, but most values were generally within the normal ranges. There were no clinically relevant results related to electrocardiogram, physical findings or other safety observations.

Five serious AEs (SAEs) were reported in three patients in Arm A (decreased appetite, $n = 2$; hyponatraemia, $n = 1$; stomatitis, $n = 1$; syncope, $n = 1$), and in addition to the pulmonary embolism in one patient, three other SAEs were reported in a separate patient in Arm B (decreased appetite, hyponatraemia and fatigue). All SAEs, except for the pulmonary embolism, had resolved by data cut-off. There were no deaths in the period to data cut-off in either arm.

Pharmacokinetics

A summary of PK parameters for cediranib, cisplatin and S-1/capecitabine is shown in Table 4. Only six patients (Arm A, $n = 2$; Arm B, $n = 4$) were evaluable for PK analysis, having completed the planned sampling schedule; therefore, limited data were available for within-patient comparison. In Arm A ($n = 2$), the PK parameters for S-1 in combination with both cediranib and cisplatin were similar to those for S-1 when administered with cisplatin alone, and the PK parameters for cediranib were similar in the presence and absence of chemotherapy; however, there were insufficient data to draw meaningful conclusions on the PK in Arm A. Based on limited data from Arm B ($n = 4$), the cediranib PK parameters were similar in the absence and presence of capecitabine/cisplatin. The PK profile of capecitabine was generally similar in the absence and presence of cediranib; one patient (patient 4 in Table 4) had a higher exposure in the presence of cediranib, but the reason for this is not clear as no interaction would be expected. In all patients (Arms A and B), slight increases in exposure to cisplatin (total platinum equivalents; maximum plasma concentration [C_{max}] and area under plasma concentration–time curve from time zero to 8 h [AUC_{0-8h}]) were observed when cediranib was administered with chemotherapy compared with chemotherapy alone; however, samples collected in the absence of cediranib were obtained following single-dose cisplatin, whereas those collected in the presence of cediranib were obtained following multiple-dose cisplatin.

Efficacy

Seven patients (Arm A, $n = 4$; Arm B, $n = 3$) had a post-baseline scan and were therefore evaluable for efficacy. Tumour shrinkage was observed in five of these patients (Fig. 2); the mean largest change from baseline was −41.8% in Arm A ($n = 4$) and −26.3% in Arm B ($n = 3$). One patient in Arm A had a partial response that was ongoing at data cut-off (duration >79 days). Among the four patients with stable disease ($n = 2$ in each arm), three had unconfirmed partial responses at data cut-off. One patient in each arm had a best response of progressive disease.

Table 4 Summary of pharmacokinetic parameters

Analyte	Patient	Combination	C_{max}, ng/ml	AUC, ng h/ml
Arm A				
Cediranib	Patient 1	Cediranib alone	25.5	378
		Cediranib + S-1 + cisplatin	51.3	598
	Patient 2	Cediranib alone	153	2,640
		Cediranib + S-1 + cisplatin	192	2,780
5-FU	Patient 1 (60 mg S-1)	S-1 + cisplatin	58.6	302
		Cediranib + S-1 + cisplatin	92.1	446
	Patient 2 (50 mg S-1)	S-1 + cisplatin	182	908
		Cediranib + S-1 + cisplatin	130	644
Cisplatin	Patient 1	S-1 + cisplatin	2,740	12,700
		Cediranib + S-1 + cisplatin	3,040	14,100
	Patient 2	S-1 + cisplatin	2,400	10,400
		Cediranib + S-1 + cisplatin	2,790	12,600
Arm B				
Cediranib	All patients ($n = 4$)	Cediranib alone	77.5 (32.9–99.9)	1,180 (479–1,800)
	All patients ($n = 4$)	Cediranib + capecitabine + cisplatin	86.3 (50.2–115)	1,220 (687–1,850)
5-FU	Patient 3 (1,600 mg capecitabine)	Capecitabine + cisplatin	130	283
		Cediranib + capecitabine + cisplatin	284	421
	Patient 4 (1,750 mg capecitabine)	Capecitabine + cisplatin	132	187
		Cediranib + capecitabine + cisplatin	983	889
	Patient 5 (1,450 mg capecitabine)	Capecitabine + cisplatin	167	305
		Cediranib + capecitabine + cisplatin	105[a]	335[a]
	Patient 6 (1,600 mg capecitabine)	Capecitabine + cisplatin	287	518
		Cediranib + capecitabine + cisplatin	392[b]	647[b]
Cisplatin	All patients ($n = 4$)	Capecitabine + cisplatin	3,430 (2,720–3,840)	16,900 (13,500–18,900)
	All patients ($n = 4$)	Cediranib + capecitabine + cisplatin	4,620 (3,230–5,720)	21,700 (16,600–23,600)

AUC_{0-24h} was calculated for cediranib; AUC_{0-4h} for capecitabine (5-FU); and AUC_{0-8h} for cisplatin and S-1 (5-FU)

In Arm B, cediranib and cisplatin parameters are expressed as mean (min–max); all other data are individual patient values as there are insufficient data to summarize by mean value

AUC area under the plasma concentration–time curve, C_{max} maximum plasma (peak) drug concentration

[a] Dose of 1,300 mg capecitabine administered: data dose normalized to 1,450 mg

[b] Dose of 1,200 mg capecitabine administered: data dose normalized to 1,600 mg

Discussion

The impact of conventional chemotherapy on advanced gastric cancer remains modest, with median survival times reaching a plateau of 7–13 months [6–8]. More effective treatment options are needed. In this Phase I study, we evaluated the VEGF signalling inhibitor cediranib in combination with cisplatin and S-1 or capecitabine in Japanese patients with previously untreated locally advanced or metastatic unresectable gastric adenocarcinoma. Treatment was tolerable, with only one patient in each arm experiencing a DLT. Overall, the safety profile of each regimen was consistent with previous studies of the individual agents in patients with advanced cancer [8, 9, 18, 19, 23, 28–30], and no new toxicities were identified. The most commonly reported AEs were decreased appetite, fatigue and nausea. There were no reports of severe hypertension as a SAE, and the overall incidence of hypertension was consistent with that reported in a Phase I study of cediranib monotherapy in Japanese patients [18].

Insufficient PK data preclude any meaningful conclusions relating to Arm A. Based on the limited PK data from Arm B, there was no clear indication of a consistent interaction between cediranib and cisplatin/capecitabine. This is not unexpected as it is considered unlikely that cisplatin, capecitabine or S-1 would affect cediranib routes of metabolism [31]. The slight increases in cisplatin exposure observed in all patients when cediranib was administered with chemotherapy compared to chemotherapy alone may be due to an accumulation of platinum following multiple dosing.

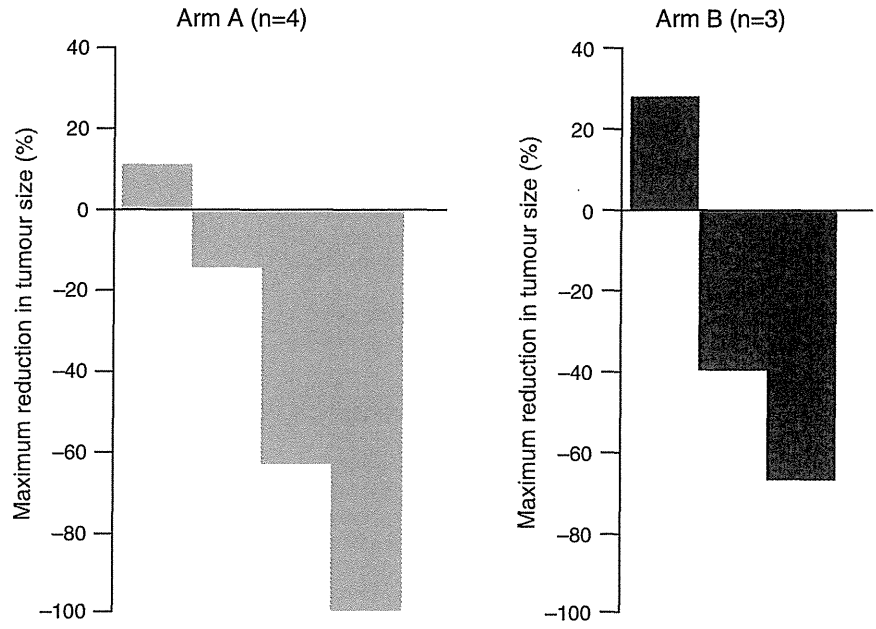

Fig. 2 Waterfall plot for best change in tumour size in each patient

In this small Phase I study, tumour shrinkage was observed in five of seven evaluable patients. This preliminary evidence of antitumour activity is consistent with the efficacy findings observed in an early-phase dose-finding study of sorafenib, a multi-targeted kinase inhibitor with activity versus VEGFR-2 and -3, in combination with capecitabine and cisplatin as a first-line treatment for patients with advanced gastric cancer [32]. However, targeting VEGF signalling with bevacizumab, an anti-VEGF-A monoclonal antibody, in patients with advanced gastric cancer met with disappointing results in the recently reported Phase III AVAGAST study [33]. This first-line study failed to meet its primary endpoint of improved overall survival with the addition of bevacizumab to cisplatin plus capecitabine/5-FU, although an efficacy analysis by geographical region revealed that, for both arms, median overall survival was greatest for patients who enrolled in the Asia/Pacific region. Despite the primary outcome of the AVAGAST study, the bevacizumab regimen showed significant advantages for the secondary efficacy endpoints of progression-free survival and overall response rate, suggesting that anti-VEGF treatment strategies are worthy of continued investigation in advanced gastric cancer.

In conclusion, cediranib 20 mg plus cisplatin and S-1 or capecitabine had a manageable tolerability profile as a first-line treatment in Japanese patients with advanced gastric cancer and showed preliminary evidence of antitumour activity.

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Conflict of interest X.S. and K.H.B. are employees of AstraZeneca and own stock. T.S., Y.Y, K.M., H.H., Y.S., D.T., K.T., T.E.N. and N.B. declare no conflicts of interest.

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