Original Article

Clinical significance of *MET* gene amplification in metastatic or locally advanced gastric cancer treated with first-line fluoropyrimidine and platinum combination chemotherapy

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Abstract

Objective: To investigate the clinical significance of *MET* gene amplification in patients with gastric cancer in the palliative setting.

Methods: *MET* amplification was assessed using fluorescence *in situ* hybridization (FISH) in 50 patients and quantitative polymerase chain reaction (qPCR) in 326 patients; 259 patients treated with first-line fluoropyrimidine and platinum were included for survival analysis.

Results: The results of FISH and qPCR indicated that the c-*MET*/CEP7 ratio was correlated with gene copy number. The optimal cutoff value for the copy number using qPCR to detect *MET* gene amplification with FISH was 5 (κ=0.778, P<0.001). Twenty-one out of 326 patients (6.4%) were identified as *MET* amplification with a copy number of >5 detected by qPCR. *MET*-amplified gastric cancer was associated with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of ≥2 (33.3% *vs*. 10.5% P=0.007), peritoneal metastasis (76.2% *vs*. 46.2%, P=0.008), and elevated bilirubin levels (28.6% *vs*. 7.3%, P=0.006). The median overall survival (OS) and progression-free survival (PFS) were 11.9 and 5.6 months, respectively. *MET*-amplified gastric cancer was not associated with survival outcomes [hazard ratio (HR)=0.68, 95% confidence interval (95% CI): 0.35−1.32, P=0.254 for PFS; HR=0.68, 95% CI: 0.35−1.32, P=0.251 for OS].

Conclusions: qPCR can be used to detect *MET* gene amplification. *MET* amplification was not a predictor of poor prognosis in patients with metastatic or unresectable gastric cancer.

Keywords: *MET*; amplification; advanced gastric cancer; prognosis; quantitative real-time polymerase chain reaction

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Introduction

Despite improvements in outcomes with targeted agents, including trastuzumab (1), ramucirumab (2) and apatinib (3), the prognosis of unresectable or metastatic gastric cancer remains unfavourable. There are unmet needs to discover novel treatments for advanced gastric cancer.

Dysregulation of c-MET signaling pathway has been implicated not only in gastric cancer (4), but also in other malignancies, including breast, lung, pharynx, colorectal, and cervical cancers (5-9). The aberrant c-MET signaling pathway including gene mutation, gene amplification, overexpression of the ligand and/or receptor, autocrine signaling, and paracrine signaling has been indicated as a

potential mechanism in carcinogenesis (10). The activation of c-MET signaling pathway in gastric cancer has been associated mainly with gene amplification (11-13), whereas gain of function mutations in *MET* are rare (14). *MET* proto-oncogene amplification activates the MET/hepatocyte growth factor pathway to promote cell proliferation, anti-apoptotic activities, cell detachment, migration, and invasion for metastasis (15,16).

Fluorescence *in situ* hybridization (FISH) is the standard method to detect gene amplification. Because of the high cost and long turnaround time for obtaining FISH results, a real-time quantitative polymerase chain reaction (qPCR)-based gene copy number assay has been considered as an alternative method for gene amplification. However, the cutoff value of proper copy number for predicting *MET* gene amplification has not yet been established. The frequencies of *MET* gene copy number gain in gastric cancer ranged from 1.5% to 21.2% depending on the cutoff points (17-19). In addition, the concordance between *MET* gene amplification using qPCR and FISH is controversial (17-20).

Little is known about the clinicopathologic features and prognosis of *MET*-amplified gastric cancer, although studies suggested worse survival outcomes for *MET*-amplified gastric cancer patients based on resected tissue obtained during curative surgery (11,17,18,20). Based on these findings, there are some limitations for patients with metastatic or unresectable gastric cancer who are indicated for palliative chemotherapy.

In this study, we investigated the efficacy of qPCR to screen *MET* gene amplification and determined the clinicopathologic features and prognosis of *MET* gene copy number gain in patients with locally advanced unresectable or metastatic gastric cancer.

Materials and methods

Study population and data collection

Two types of registries were used in this study. The first one was a retrospective registry consisting of 552 patients with locally advanced or metastatic gastric cancer who were treated with a first-line fluoropyrimidine and platinum (FP) regimen between June 2006 and June 2011. After histological review, 193 cases with ≥70% tumor cells in the formalin-fixed paraffin-embedded (FFPE) tissues were selected. The second one was a prospective registry that consisted of 815 patients with locally advanced or metastatic gastric cancer between September 2012 and December 2014, who were not categorized according to chemotherapy regimen. All collected FFPE tissues were examined to define the tumor cell proportion and gene copy number using qPCR. There were 133 patients with gene amplification based on qPCR results in patients with ≥70% tumor proportion. Among them, only 66 patients received a first-line FP regimen. In addition, we randomly selected 50 patient samples from a prospective gastric cancer registry to assess *MET* gene amplification using both FISH and qPCR. Tumor microdissections were conducted by a pathologist (Y Park), when the sample did not have sufficient tumor cells (<70%) before conducting FISH and qPCR.

This study adhered to the guidelines established by the Declaration of Helsinki, and was approved by the Institutional Review Board at Asan Medical Center.

FISH

For FISH, 2-μm sections from each paraffin block were prepared. Deparaffinization, pretreatment and protease digestion procedures were performed following an established protocol using a D7S522 probe and CEP7 purchased from Abbott Vysis (Des Plaines, IL, USA). Probes were hybridized at 37 °C for 14–18 h. After hybridization, slides were washed in 2× saline-sodium citrate/0.3% NP-40 at 72 °C for 5 min, air-dried, and counterstained with 4',6-diamidino-2-phenylin-dole (DAPI). The slides were examined under a fluorescence microscope equipped with Spectrum Texas Red, fluorescein isothiocyanate, and DAPI filters. The slides were stored at −20 °C until examination. The cMET/CEP7 ratio was established after counting at least 40 tumor cells. A cMET/CEP7 ratio of ≥2 and ≥10% tumor cells with a *MET* gene copy number >4 were considered as *MET* gene amplification.

Real-time qPCR-based determination of gene copy number

Genomic DNA was extracted from biopsies or surgical FFPE tissues using a QIAamp DNA FFPE Tissue kit or QIAamp DNA Mini kit (Qiagen, Hilden, Germany). The DNA concentration was measured using a NanoDrop2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Predesigned Applied Biosystems TaqMan copy number assays were performed (Thermo Scientific, Waltham, MA, USA) to determine the gene copy number of *MET*. A total volume of 10 μL of the Master mix

contained 10 ng of genomic DNA, 5 μL of the TaqMan genotyping Master mix, and each primer for real-time PCR. The primer ID was Hs02884964_cn. The telomerase reverse transcriptase gene and human genomic DNA (Takara) were used as the internal reference for the copy number and the normal control, respectively. The thermal cycling conditions were 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The results were analyzed using the ABI PRISM 7900HT Sequence Detection System (Thermo Scientific, Waltham, MA).

Statistical analysis

The Spearman's rank correlation coefficient was used to assess the correlation between FISH and qPCR data. A receiver operating characteristic (ROC) curve was plotted and the area under curve (AUC) was estimated to set the cutoff value of the highest sensitivity and specificity by calculating the κ values using concordance evaluation.

Progression-free survival (PFS) was defined as the time between the start of FP chemotherapy to tumor progression or death by any cause. Overall survival (OS) was calculated from the initiation date of first-line FP to death by any cause. Data were censored if patients were free from progression or alive at the last follow-up. Categorical variables were evaluated using the Chi-square test or Fisher's exact test, as appropriate. The Kaplan-Meier method was used to estimate PFS and OS. Survival curves were compared using a log-rank test, according to *MET* amplification. A Cox proportional hazard model was used to estimate hazard ratio (HR) for survival outcomes.

All statistical analyses were performed using the IBM SPSS Statistics (Version 21; IBM Corp., NewYork, USA) for the Social Sciences and statistical software package R version 3.0.2 (http://www.r-project.org/). All tests were two-sided with 5% defined as the level of significance.

Results

Correlation of copy numbers evaluated using qPCR and FISH

The analysis of FFPE specimens with both qPCR and *MET* FISH revealed a good correlation between the c-MET/CEP7 ratios and the copy numbers with a value of 0.443 (P=0.002) based on Spearman's rank correlation coefficient. Five cases (10%) showed *MET* gene amplification with a c-MET/CEP7 ratio of greater than 2 (*Figure 1*). One patient without *MET* amplification had a c-MET/CEP7 ratio of 0.82 and a copy number of 2.98 (*Figure 2A*). Among the 5 patients with *MET* gene amplification, the range of cMET/CEP7 ratio was from 3.75 to 9.35 and the copy numbers ranged from 7.18 to 9.03 (*Figure 2B*,*C*). To determine the cutoff point of copy numbers to assess *MET* gene amplification, we plotted a ROC curve with AUC 0.953 (*Supplementary Figure S1*). The value of the highest sensitivity and specificity was identified at 5.22 copy numbers. We calculated the κ values at the cutoff point of various copy numbers. The κ values for 3, 4, 5, 6, and 7 copy number were 0.248 (P=0.008), 0.462 (P<0.001), 0.778 (P<0.001), 0.730 (P<0.001), and 0.730 (P<0.001), respectively.

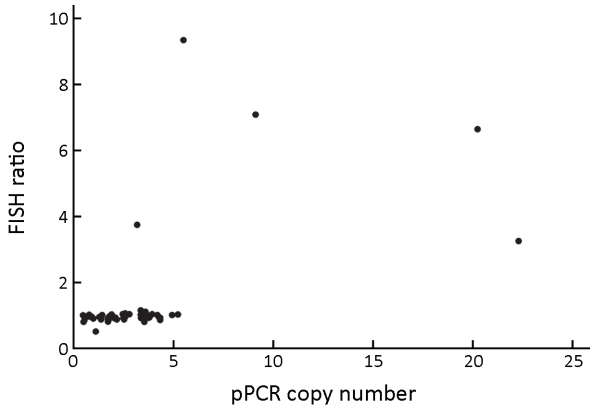

Figure 1 Correlation between *MET* gene copy numbers and c-MET/CEP7 ratios in 50 metastatic gastric cancer patients. FISH, fluorescence *in situ* hybridization; qPCR, quantitative polymerase chain reaction.

Patient characteristics

A total of 326 samples (64 samples from surgical resection and 262 samples from biopsy) were suitable for assessing the association between *MET* amplification and clinicopathologic factors. Among them, 259 patients treated with first-line FP were used for the survival analysis.

The median age of patients was 58 (range, 23−85) years old. Among all patients, 65.9% of patients had initially metastatic disease, and others presented with recurrence and locally advanced unresectable disease. At the time of diagnosis, 287 (88.0%) patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0−1 (*Table 1*). The distant lymph nodes and peritoneum were the most common metastatic sites, and more than half of the patients (62.3%) presented with poorly differentiated histology. 122 (37.4%) patients did human epidermal

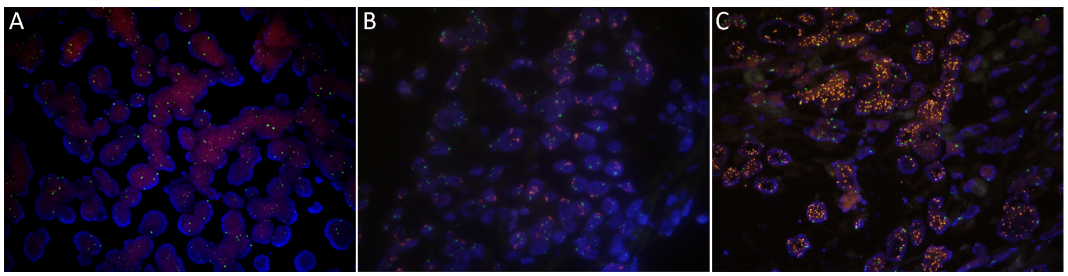

Figure 2 *MET* gene amplification was evaluated using fluorescence *in situ* hybridization showing representative image of non-amplification (A), low-level amplification (B), and high-level amplification (C).

growth factor receptor 2 (HER2) immunohistochemistry (IHC) and/or silver *in situ* hybridization, and 103 (31.6%) patients were HER2 negative. Nineteen (5.8%) HER2+ patients were determined based on HER2 positivity with IHC 3+ or IHC 2+ gene amplification by *in situ* hybridization.

The median copy number of MET using qPCR was 1.69 with a range of 0.18–206.30. When applying the optimal cutoff point of 5 copy numbers based on qPCR results to detect *MET* amplification using FISH, the frequency of *MET* amplification was 6.4% (n=21).

Association of MET amplification with clinicopathologic features

Clinical characteristics were compared between patients with and without *MET* amplification using qPCR with a cutoff point of >5 copy numbers. The *MET* amplification group had an ECOG PS of ≥2 (33.3% *vs*. 10.5%, P=0.007), lower albumin level (52.4% *vs*. 31.6%, P=0.051), and elevated total bilirubin (28.6% *vs*. 7.4%, P=0.006) compared with those of the non-amplification group. When we applied our previously developed prognostic model for metastatic or recurrent gastric cancer (21), *MET* amplification was associated with the poor prognostic group (47.6% *vs*. 14.2%, P=0.001) (*Table 2*). This prognostic model divided patients into three risk groups according to the sum of scores (good; 0–1, moderate; 2–3 and poor; ≥4) which was based on 8 clinical features; ECOG PS (score 2), no gastrectomy history, peritoneal metastasis, bone metastasis (score 2), lung metastasis, elevated alkaline phosphatase, decreased albumin level and increased bilirubin level.

Association of MET amplification with survival outcome

Among 259 patients treated with an FP regimen, 171 patients presented with measurable lesions, and 81 (47.4%) out of 171 patients achieved objective responses. The overall response rate showed no significant difference between the *MET* amplification group and the non-amplification group [8 of 15 (53.3%) *vs*. 73 of 156 (46.8%), respectively, P=0.628]. Overall, 94.6% patients died at the time of analysis. With a median follow-up of 12.6 (range, 0.7–104.2) months, the median OS and PFS were 11.9 [95% confidence interval (95% CI): 10.2–13.6)] months and 5.6 (95% CI: 4.5–6.7) months, respectively.

In the univariate analysis, both PFS and OS were similar between patients with *MET*-amplified gastric cancer and those with non-amplified gastric cancer (*Figure 3*). The median OS of the *MET*-non-amplified and the *MET*-amplified groups were 12.6 (95% CI: 10.9–14.4) months and 11.3 (95% CI: 2.2–20.5) months, respectively, and their median PFS were 5.5 (95% CI: 4.4–6.6) months and 5.6 (95% CI: 1.4–9.8) months, respectively. PFS and OS were even worse in patients who had not undergone gastrectomy and had poor PS, Borrmann type IV disease, lung metastasis, bone metastasis, a low albumin level, and an elevated alkaline phosphatase level (*Table 3*). Using our previous prognostic model, these risk factors showed good discriminative function to predict OS (*Table 3*).

Since *MET* amplification was not a significant prognostic factor, multivariate analysis was not conducted with other risk factors. When we conducted the multivariate analysis with the risk groups using our prognostic model and amplification, *MET* amplification was not significantly associated with either PFS (HR=0.71, 95% CI: 0.39–1.28; P=0.252) or OS (HR=0.67, 95% CI: 0.37–1.20; P=0.189).

Discussion

We assessed *MET* gene amplification using a qPCR-based gene copy number assay. *MET* gene amplification was observed in 6.4% patients with metastatic or locally advanced unresectable gastric cancers who received palliative chemotherapy. *MET* amplification was associated with poor pre-treatment PS, peritoneal metastasis, and

Table 1 Baseline characteristics (N=326)

Characteristics	n	%	Median (range)
Male	225	69.0	
Age (≥65 years)	83	25.5	58 (23–85)
ECOG PS			
0–1	287	88.0	
2–3	39	12.0	
Borrmann type			
I	17	5.2	
II	71	21.8	
III	177	54.3	
IV	50	15.3	
Early gastric cancer	10	3.1	
Not available	1	0.3	
Histology			
WD/MD	117	35.9	
PD/SRC/mucinous	203	62.3	
Others	6	1.8	
No gastrectomy	211	64.7	
Disease status			
Initially metastatic	215	66.0	
Recurred	98	30.1	
Locally advanced	13	4.0	
Metastatic organ			
Peritoneum	157	48.2	
Liver	94	28.8	
Lung	20	6.1	
Intraabdominal distant LN	154	47.2	
Extraabdominal distant LN	31	9.5	
Bone	27	8.3	
Hemoglobin ≤UNL*,**	222	68.1	11.7 (6.7–17.4)
WBC≥10,000/mm^3**	47	14.4	6,900 (2,200–48,700)
Platelet ≤150×10^3/mm^3**	38	11.7	265 (14–630)×10^3
Albumin <3.3 g/dL***	104	31.9	3.6 (1.7–5.3)
ALP>120 IU/L**	70	21.5	79 (29–1,294)
Total bilirubin >1.2 mg/dL**	28	8.6	0.6 (0.2–6)
Asan medical center prognostic model**			
Good (0–1)	154	47.2	
Moderate (2–3)	111	34.0	
Poor (≥4)	52	16.0	
MET qPCR >5 gCN	21	6.4	2.68 (0.73–513.04)

ECOG PS, Eastern Cooperative Oncology Group performance status; WD, well differentiated; MD, moderate differentiated; PD, poorly differentiated; SRC, signet ring cell carcinoma; LN, lymph node; UNL, upper normal limit; WBC, white blood cells; ALP, alkaline phosphatase; qPCR, quantitative polymerase chain reaction; gCN, gene copy number; *, hemoglobin ≤12 g/dL for women and ≤13 g/dL for men; **, initial complete blood count, alkaline phosphatase and bilirubin levels, and scores from the Asan Medical Center prognostic model were not available in 9 patients (2.8%); ***, albumin level was not available in 11 patients (3.4%).

Table 2 Relationships between c-*MET* amplification and clinicopathologic features (N=326)

Variables	*MET* qPCR <5 in gCN	*MET* qPCR ≥5 in gCN	P
Sex			
Male	214 (70.2)	11 (52.4)	0.088
Female	91 (29.8)	10 (47.6)	
Age (year)			
<65	226 (74.1)	17 (81.0)	0.486
≥65	79 (25.9)	4 (19.0)	
ECOG PS			
0–1	273 (89.5)	14 (66.7)	0.007
2–3	32 (10.5)	7 (33.3)	
Bormann type			
I/II/III	247 (84.0)	18 (85.7)	1.000
IV	47 (16.0)	3 (14.3)	
Histology			
WD/MD	111 (37.1)	6 (28.6)	0.432
PD/SRC/mucinous	188 (62.9)	15 (71.4)	
HER2 expression			
Negative	98 (32.1)	5 (23.8)	0.594
Positive	17 (5.6)	2 (9.5)	
Not available	190 (62.3)	14 (66.7)	
Disease status			
Initially metastatic	198 (64.9)	17 (81.0)	0.263
Recurred	94 (30.8)	4 (19.0)	
Locally advanced	13 (4.3)	0 (0)	
Peritoneal metastasis			
No	164 (53.8)	5 (23.8)	0.008
Yes	141 (46.2)	16 (76.2)	
Liver metastasis			
No	218 (71.5)	14 (66.7)	0.804
Yes	87 (28.5)	7 (33.3)	
Lung metastasis			
No	287 (94.1)	19 (90.5)	0.804
Yes	18 (5.9)	2 (9.5)	
Distant LN metastasis			
No	157 (51.5)	8 (38.1)	0.236
Yes	148 (48.5)	13 (61.9)	
Bone metastasis			
No	282 (92.5)	17 (81.0)	0.084
Yes	23 (7.5)	4 (19.0)	
Hemoglobin*,**			
>UNL	91 (30.7)	4 (19.0)	0.258
≤UNL	205 (69.3)	17 (81.0)	

Table 2 (*continued*)

Table 2 (*continued*)

Variables	*MET* qPCR <5 in gCN	*MET* qPCR ≥5 in gCN	P
WBC (mm^3)**			
<10,000	253 (85.5)	17 (81.0)	0.751
≥10,000	43 (14.5)	4 (19.0)	
Platelet ($\times 10^3/mm^3$)**			
>150	260 (87.8)	19 (90.5)	0.761
≤150	36 (12.2)	2 (9.5)	
Albumin (g/dL)***			
>3.3	201 (68.4)	10 (47.6)	0.051
≤3.3	93 (31.6)	11 (52.4)	
ALP (IU/L)**			
≤120	234 (79.1)	13 (61.9)	0.098
>120	62 (20.9)	8 (38.1)	
Total bilirubin (mg/dL)**			
≤1.2	274 (92.6)	15 (71.4)	0.006
>1.2	22 (7.4)	6 (28.6)	
Asan medical center prognostic model**			
Good (0–1)	147 (49.7)	7 (33.3)	0.001
Moderate (2–3)	107 (36.1)	4 (19.0)	
Poor (≥4)	42 (14.2)	10 (47.6)	

ECOG PS, Eastern Cooperative Oncology Group performance status; WD, well differentiated; MD, moderate differentiated; PD, poorly differentiated; SRC, signet ring cell carcinoma; LN, lymph node; UNL, upper normal limit; WBC, white blood cells; ALP, alkaline phosphatase; qPCR, quantitative polymerase chain reaction; gCN, gene copy number. *, hemoglobin ≤12 g/dL for women and ≤13 g/dL for men; **, initial complete blood count, alkaline phosphatase and bilirubin levels, and scores from the Asan Medical Center prognostic model were not available in 9 patients (2.8%); ***, albumin level was not available in 11 patients (3.4%).

elevated bilirubin levels. We found that *MET* gene amplification was not associated with the prognosis of patients with metastatic or locally advanced unresectable gastric cancers who were treated with palliative FP chemotherapy. To the best of our knowledge, our study is the largest one to investigate the clinical significance of *MET* amplification in metastatic or locally advanced unresectable gastric cancers.

To detect the activation of the c-*MET* pathway in gastric cancer, various methods have been applied, such as FISH or silver *in situ* hybridization for gene amplification (12,18,20,22,23), real-time PCR to assess amplification or messenger RNA expression level (17,19,23), and immunohistochemistry for protein level (11,20,23-26). Among them, the ideal surrogate marker to assess the c-MET pathway is inconclusive. Although FISH is the standard method for detecting gene amplification, qPCR-based copy number assays to detect *MET* gene amplification was explored in our study because FISH is expensive and time-consuming, and it requires technical expertise (27). There were no definite conclusions regarding the appropriate cutoff value for the gene copy number, and the range were 2–5 (17-20). Lee *et al*. reported that the concordance rate between *MET* amplification assessed by qPCR and FISH was only 58.1% in 309 tissue samples, when the cutoff value for the copy number was 4 (18). Another study reported a strong correlation between the results of qPCR and silver *in situ* hybridization with a cutoff value for the copy number >2 in 26 tissue samples (20). Our results suggest that qPCR have similar ability to evaluate *MET* amplification with FISH with gene copy number of >5. Because the qPCR results depend on the tumor proportion of the samples, we performed tumor microdissection to maintain the tumor cell proportion at more than 70% to minimize the dilution of tumor cells by normal cells, which could explain the good concordance rates between the qPCR and FISH results.

Previous data from patients with gastric cancer who underwent curative resection showed MET protein overexpression in 22.0%–82.4% cases using IHC

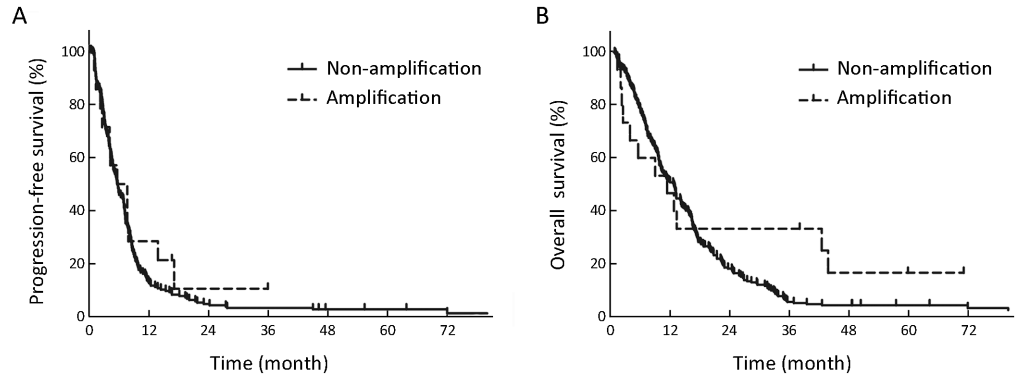

Figure 3 Progression-free survival (A) (P=0.368) and overall survival (B) (P=0.169) according to *MET* gene amplification.

Table 3 Univariate analysis of association of clinicopathologic factors with PFS and OS (n=259)

Variables	PFS			OS		
	HR	95% CI	P	HR	95% CI	P
Female	1.04	0.78–1.40	0.785	0.98	0.74–1.30	0.904
Age ≥65 years	0.96	0.70–1.31	0.782	1.26	0.95–1.68	0.108
ECOG PS 2–4	1.70	1.12–2.58	0.013	2.60	1.80–3.77	<0.001
Borrmann type: IV	1.89	1.32–2.70	<0.001	1.75	1.24–2.46	0.001
Histology: PD/SRC/mucinous	1.15	0.86–1.53	0.343	1.11	0.85–1.45	0.434
No gastrectomy	0.61	0.45–0.82	0.001	0.54	0.41–0.72	<0.001
Peritoneal metastasis	0.97	0.74–1.27	0.828	1.18	0.92–1.51	0.206
Liver metastasis	1.17	0.87–1.57	0.299	1.13	0.86–1.48	0.377
Lung metastasis	1.90	1.06–3.41	0.031	1.82	1.02–3.26	0.044
Distant LN metastasis	1.00	0.77–1.30	0.990	0.92	0.71–1.16	0.429
Bone metastasis	2.33	1.47–3.68	<0.001	3.36	2.17–5.20	<0.001
Hemoglobin ≤UNL	0.82	0.61–1.09	0.167	0.99	0.75–1.31	0.957
WBC ≥10,000/mm^3	1.00	0.67–1.48	0.988	1.16	0.80–1.68	0.427
Platelet ≤150×10^3/mm^3	1.01	0.67–1.54	0.955	1.11	0.75–1.64	0.609
Albumin ≤3.3 g/dL	1.52	1.15–2.01	0.003	2.14	1.65–2.79	<0.001
ALP >120 IU/L	1.90	1.38–2.63	<0.001	1.94	1.43–2.62	<0.001
Total bilirubin >1.2 mg/dL	1.27	0.81–1.98	0.293	1.24	0.81–1.89	0.315
Risk groups by AMC prognostic model						
Good	1.00			1.00		
Moderate	1.14	0.85–1.53	0.369	1.38	1.04–1.83	0.024
Poor	2.14	1.45–3.15	<0.001	3.19	2.23–4.57	<0.001
MET qPCR ≥5 gCN	0.77	0.43–1.37	0.766	0.66	0.37–1.20	0.172

PFS, progression-free survival; OS, overall survival; ECOG PS, Eastern Cooperative Oncology Group performance status; PD, poorly differentiated; SRC, signet ring cell carcinoma; LN, lymph node; UNL, upper normal limit; WBC, white blood cells; ALP, alkaline phosphatase; AMC, Asan Medical Center; qPCR, quantitative polymerase chain reaction; gCN, gene copy number; HR, hazard ratio; 95% CI, 95% confidence interval.

(11,24,25), whereas the incidence of *MET* gene amplification was found to be 1.5%–10.0% using FISH (19,22) and 10.0%–21.2% using qPCR (17,18,23). The inconsistency may be from a poor correlation between high protein expression and *MET* gene amplification (18,23,24) or from *MET* gene heterogeneity in surgical tissue (28), although one report demonstrated a good correlation between *MET* protein expression and gene amplification (20). Various antibodies, different definitions of positivity or cutoff of scoring systems could contribute to the

inconsistent results (11,20,23-25). In contrast, the frequency of *MET* amplification in patients with metastatic or recurrent gastric cancer was 8.3% using FISH (26) and 10.3% using qPCR (29). The frequency of *MET* amplification detected by qPCR in our study was 6.4%, which is lower than those of previous studies.

Our study revealed that *MET* gene amplification was associated with poor performance, peritoneal metastasis, and elevated bilirubin levels. One study demonstrated that *MET* amplification is associated with poor performance and poorly differentiated histology in metastatic gastric cancer (26), and MET protein level is associated with liver metastasis (30). As mentioned above, most of these factors are in turn associated with poor prognosis in metastatic or recurrent gastric cancers (21,31,32). Furthermore, in resectable cases, MET protein level is associated with an advanced disease stage and lymph node metastasis (11,25), and *MET* gene amplification is also associated with an advanced disease stage (22) and progression to peritoneal metastasis (13). These findings suggest that *MET* gene amplification might be related to metastatic disease progression in resectable gastric cancer.

Studies have delineated the association between poor OS and MET protein overexpression (11,20) or *MET* gene amplification (17,18,22) in patients who underwent curative surgery for resectable gastric cancer. In terms of metastatic or unresectable gastric cancer, 2 studies have indicated a poor clinical outcome in *MET*-amplified gastric cancer patients who received palliative chemotherapy (26,29), whereas biomarker analysis of the RILOMET-1 trial revealed no significant relationship between *MET* amplification and treatment outcome in a palliative setting (33). Although An *et al*. used both FISH assay and IHC for detecting *MET* amplification, they analyzed 232 inoperable gastric cancer patients who were treated with various first-line fluoropyrimidine-based regimens, which may have influenced treatment outcomes. A univariate analysis was conducted for OS and PFS, and only 170 patients were included in the PFS analysis without any information regarding *MET* amplification status and treatment regimens, which may cause biased results (26). Matsusaka *et al*. reported that *MET* gene amplification was associated with OS, but not with PFS in 150 patients who were uniformly treated with S-1 and cisplatin for metastatic or recurrent gastric cancer, which needs careful interpretation since patients received the same regimen and the *MET* amplification was assessed by real-time PCR (29). These studies applied an arbitrary threshold ≥5 copy number for identifying amplification without considering samples' tumor proportion. Having enough tumor tissues for the amplification assessment was important for accurate results by reducing the risk of normal cell dilution. With these limitations and results from RILOMET-1 trial, the prognostic impact of *MET* amplification has not been established, and our results suggest that *MET* amplification is not a prognostic predictor in patients with unresectable or recurrent gastric cancer who were treated with palliative FP.

MET inhibitor monotherapy, including tivantinib and foretinib, showed modest efficacy in unselected patients with metastatic gastric cancer (34-36). In a phase II study of tivantinib, there was no obvious relationship between drug efficacy and biomarkers, including *MET* gene amplification, and expression of c-MET, p-MET, and hepatocyte growth factor (34). AMG337 monotherapy showed remarkable response rate (5 of 10; 50%) in patients with *MET*-amplified gastroesophageal cancer in a phase I trial (37), however, the phase II study was terminated early due to efficacy and safety issues. The combination of chemotherapy and monoclonal antibodies blocking the c-MET pathway could not meet their primary endpoints in patients with MET-positive disease according to IHC (33,38,39). *MET* gene aberrations might not be the single driver of oncogene addiction, or other appropriate biomarkers might exist for MET inhibitor. Our results showed no prognostic value of *MET* gene amplification, which supports these hypotheses. However, given the clear association between clinical aggressiveness and *MET* amplification or protein overexpression in resectable gastric cancer, the MET pathway could have a pivotal role in the development of metastasis or recurrence from resectable diseases.

Our study may have some possible limitations because it is a retrospective single centre study, although part of our cohort was prospectively collected. Furthermore, selecting patients who had enough tumor tissues to assess and received uniform chemotherapy may have led to potential bias, even though these processes were essential for accurate results. However, after excluding inappropriate patients for analysis, our study investigated the largest dataset assessing *MET* amplification in metastatic or locally advanced unresectable gastric cancer. For applying our results in cases with unsuitable tumor proportion, tumor microdissection is essential; we believe that this could make our results more reliable and useful in clinical applications.

Conclusions

We found that *MET* amplification was not a prognostic predictor in patients with unresectable or recurrent gastric cancer who were treated with palliative FP, indicating that aberrant *MET* signaling pathway might not be the main driver in locally advanced or metastatic gastric cancer. Further validation is warranted to determine the clinical significance of *MET* amplification in patients with gastric cancer in the palliative setting.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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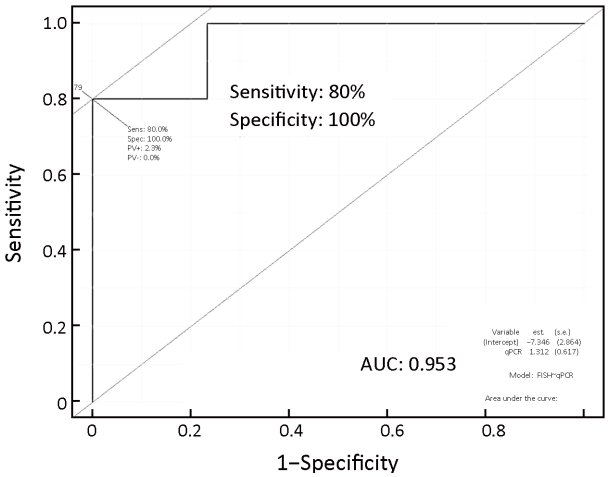

Figure S1 Receiver operating characteristic (ROC) curve. AUC, area under curve.

