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Cancer Biology and Signal Transduction

Molecular Cancer Therapeutics

Tumor MET Expression and Gene Amplification in Chinese Patients with Locally Advanced or Metastatic Gastric or Gastroesophageal Junction Cancer

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Abstract

MET and its sole ligand, hepatocyte growth factor (HGF), are promising targets in gastric and gastroesophageal junction cancer. We evaluated whether MET protein expression or *MET* gene amplification is prognostic for overall survival (OS) in Chinese patients with advanced gastric or gastroesophageal junction cancer. Archival formalin-fixed, paraffin-embedded tumor samples from patients with unresectable locally advanced or metastatic gastric or gastroesophageal junction cancer enrolled in clinical trials at Peking University Cancer Hospital from 2008 to 2010 were assessed for MET and phospho-MET (p-MET) expression by immunohistochemistry and *MET* amplification by FISH. MET-positive expression was defined as membrane protein staining in ≥25% of tumor cells. *MET* amplification was defined as *MET*:centromere 7 ratio >2.0. We tested the association of MET status with clinical characteristics and OS, and also evaluated the association between expression and amplification. One hundred sixty-eight patients were eligible. Of the evaluable samples, 53 of 137 (39%) were MET positive, eight of 134 (6%) were p-MET positive, and eight of 113 (7%) were *MET* amplified. Neither MET expression nor *MET* amplification were associated with clinical characteristics, except Lauren classification ($P = 0.04$); *MET* amplification was associated with diffuse type. No significant OS difference was observed between MET-positive and MET-negative populations, regardless of first-line chemotherapy received. In 95 evaluable patients, MET expression was significantly associated with *MET* amplification ($P < 0.001$); all *MET*-amplified tumor samples showed some MET expression. In 96 evaluable patients, p-MET positivity was significantly associated with *MET* amplification ($P < 0.001$). Further evaluation in larger and independent sample sets is warranted to confirm our findings. *Mol Cancer Ther; 14(11); 2634–41. ©2015 AACR.*

Introduction

More than 40% percent of the world's gastric cancer cases occur in China. Gastric cancer is the third most common cancer in China, with 405,000 estimated new cases in 2012 (1). Furthermore, gastric cancer causes more than 325,000 deaths annually in China (1). Despite recent advancements in therapy, median survival of patients with advanced gastric cancer remains poor at approximately 9 to 12 months (2–9).

Hepatocyte growth factor (HGF), also known as scatter factor, and its receptor MET appear to be promising therapeutic targets in oncology (10). Activation of the HGF/MET signaling pathway promotes the proliferation, migration, and survival of tumor cells (10). It has also been shown to be associated with cancer pathogenesis, invasion, and metastasis (11).

Potential prognostic and predictive biomarkers in advanced gastric cancer have been identified. For example, overexpression of HER2 has been shown to be predictive of outcomes in patients with advanced gastric cancer treated with trastuzumab (2). Furthermore, *MET* gene amplification and increased MET protein expression have been associated with advanced disease and poor prognosis in metastatic gastric cancer (12–16).

Although others have explored the relationship between MET expression or *MET* amplification and prognosis in gastric cancer, most of these studies evaluated early-stage patients or mixed-stage populations (13–20), so the prognostic effect of MET in late-stage disease remains unclear. Cancer stage may be an important factor when studying the prognostic effect of MET, as MET is associated with tumor invasion (11). Moreover, prior studies primarily evaluated samples from resected tissue (13–20). Biopsy samples are more relevant in advanced disease, and limited data about MET status in biopsy samples were available in these studies.

In the current study, we obtained biopsy samples from patients with locally advanced or metastatic gastric or gastroesophageal junction cancer who were treated at a large cancer center in China, and we retrospectively tested the samples for MET expression, phospho-MET (p-MET) expression, and *MET* gene amplification.

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Our objective was to evaluate whether MET expression and *MET* amplification are prognostic for overall survival (OS).

Materials and Methods

Patients

Eligible patients had unresectable locally advanced or metastatic gastric or gastroesophageal junction cancer and were enrolled in multicenter phase II/III clinical trials between 2008 and 2010 at the Peking University Cancer Hospital in Beijing, China. Trials included the following, as identified by their ClinicalTrials.gov identifiers: NCT00548548, NCT01041404, NCT00887822, NCT00678535, NCT01015339, and NCT00842491. Biopsy tumor samples were obtained from all patients by endoscopy from the primary gastric cancer site, prior to chemotherapy. The number of metastatic sites was determined by the number of involved organs at trial enrollment. Tissues were fixed with formalin and paraffin-embedded in blocks, which were prepared at the time of biopsy. Patients with available baseline characteristics and clinical data were included in the analysis. All patients provided written informed consent for the trial participation, and the trials were approved by the Institutional Review Board of the Peking University Cancer Hospital. All patients were followed until December 31, 2013.

Analytical methods

MET protein expression, *MET* gene amplification, phospho-MET (p-MET) expression, and HER2 expression were retrospectively evaluated.

MET expression was determined by an automated MET immunohistochemistry (IHC) Investigational Use Only (IUO) assay using antibody clone MET4 (Dako), as previously described (21). Samples were defined as MET positive if $\geq$25% of tumor cells had membrane protein staining at any intensity (21). *MET* amplification was analyzed by FISH using the Research Use Only (RUO) *MET*/CEN-7 IQFISH Probe Mix assay (Dako). Samples were defined as *MET* amplified in this analysis if the *MET*:centromere 7 ratio was >2.0. Several cutoffs have been probed to define *MET* amplification using the Colorado scoring system as a guide (22). One cutoff that appears to define focal amplification is a *MET*:centromere 7 ratio >2.0 (21). This cutoff also mirrors the HER2 amplification cutoff, which is widely used in gastric cancer. Prior to assay implementation, technicians and pathologists were trained and certified as proficient by Dako.

Phospho-MET expression was analyzed by IHC using a research grade assay that utilized the monoclonal p-MET antibody (Tyr1234/1235; D26; Cell Signaling Technology, Inc.) Immunopositive cases were defined as those exhibiting membrane protein staining in >10% of tumor cells in the sample.

HER2 expression was analyzed by IHC using the PATHWAY anti-HER-2/neu (4B5) rabbit monoclonal primary antibody (Ventana Medical Systems, Inc.) HER2 positivity was defined as IHC 3+ staining alone or the combination of IHC 2+ staining and FISH positive. In cases of IHC 2+ staining, FISH was performed with PathVysion (Abbott Laboratories). Tumor specimens with a *HER2*:centromere 17 ratio >2.0 were considered *HER2* FISH–positive.

All samples were analyzed and evaluated in the Department of Pathology at Peking University Cancer Hospital. Pathologists were blinded to the clinical and molecular characteristics of the patients.

Statistical analysis

The Fisher exact test was used to evaluate the following: (i) association between MET expression and *MET* amplification, (ii) association of MET expression and *MET* amplification with clinical characteristics, and (iii) association between MET expression or *MET* amplification and p-MET expression. Survival curves for OS were plotted using the Kaplan–Meier method and were compared using the log-rank test. The HR for OS between MET-positive and MET-negative patients was evaluated using the Cox proportional hazards model. Statistical significance was defined as $P < 0.05$. For the 95% confidence interval (CI) of a proportion, a binomial exact CI was provided.

Results

Patient characteristics

Overall, 168 patients were eligible for the study. Of these, 155 patients had available tumor samples for testing: 137 patients had samples evaluable for MET IHC, 113 patients had samples evaluable for *MET* FISH, and 134 patients had samples evaluable for p-MET IHC. A patient flow diagram is shown in Fig. 1. Patient demographics, disease characteristics, and treatment are shown in Table 1. All patients with available tumor samples received first-line systemic chemotherapy for metastatic disease. Besides one patient receiving paclitaxel alone, all other patients received doublet or triplet chemotherapy. Among the 137 patients with evaluable MET IHC, 60% received first-line platinum-based therapy (fluoropyrimidine/platinum or fluoropyrimidine/platinum/taxane), and 53% received first-line taxane-based therapy (fluoropyrimidine/taxane, fluoropyrimidine/taxane/platinum, or taxane alone; Table 1).

Incidence of MET protein expression and *MET* gene amplification

Of the 137 patients evaluable for MET IHC, 53 patients (39%; 95% CI, 30%–47%) had MET-positive tumors. Forty-one patients (30%) had tumors with no MET membrane staining of any intensity and 43 patients (31%) had tumors with >0% and <25% of cells with MET membrane staining of any intensity. Of the 113 patients evaluable for *MET* FISH, 8 patients (7%; 95% CI, 3%–13%) had tumors with *MET* amplification.

Association between MET expression/*MET* amplification and clinical characteristics

Among the 137 patients with evaluable IHC samples, no significant association was observed between MET expression and clinical characteristics (Table 1). Among the 113 patients with evaluable FISH samples, *MET* amplification was not associated with sex, primary tumor site, distant metastasis (liver vs. lung), or number of metastases (Table 1). However, among the 111 patients for whom Lauren classification data were available, Lauren classification was associated with *MET* amplification ($P = 0.04$; Table 1); seven of 46 diffuse samples (15%) were *MET* amplified, whereas one of 49 intestinal samples (2%) were *MET* amplified.

Association between MET expression and *MET* amplification

Ninety-five patients had samples evaluable for both MET IHC and *MET* FISH, and of these, eight patients had *MET*-amplified tumors. MET expression was significantly associated with *MET* amplification ($P < 0.001$). All eight tumor samples with *MET* amplification had $\geq$90% MET expression (Fig. 2).

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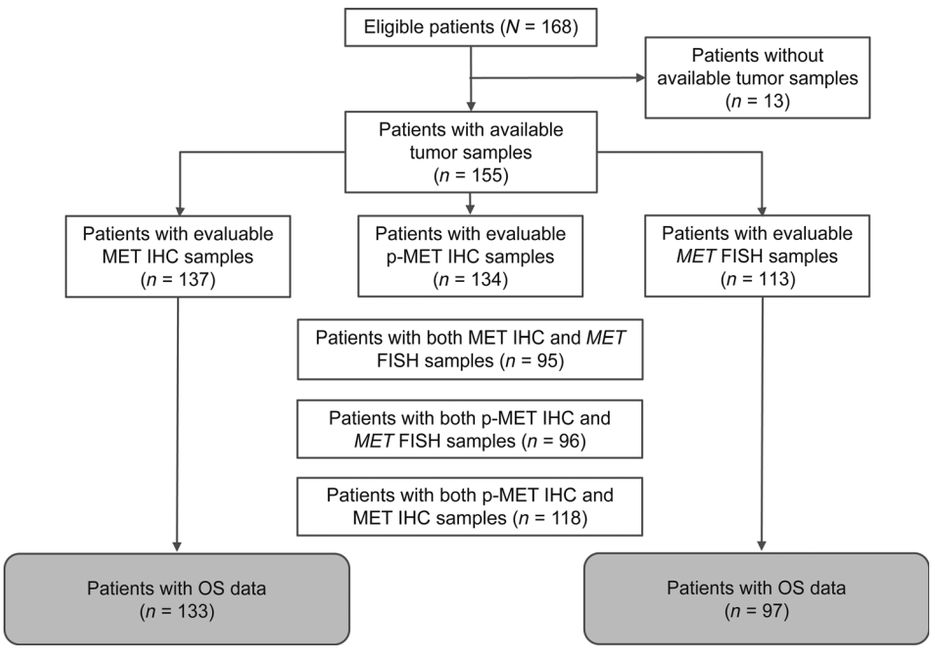

Figure 1.
Patient flow diagram. Eligible patients had unresectable locally advanced or metastatic gastric or gastroesophageal junction cancer. Tumor samples were obtained by biopsy, prior to chemotherapy.

Survival analyses

Of the 141 patients with available survival data, the median follow-up time was 10.9 months. Of 15 censored patients with available survival data, the median follow-up time was 35.0 months. Follow-up time was measured from the start date of chemotherapy for advanced gastric cancer.

Of the 137 evaluable patients for MET IHC, 133 had available survival data. In this population, no statistically significant

Table 1. Patient demographics, disease characteristics, and treatment by IHC and FISH subgroups

	IHC analysis				FISH analysis			
	All evaluable patients[a] (*n* = 137)	**MET-positive expression[b] (*n* = 53)**	**MET-negative expression[b] (*n* = 84)**	***P*[c]**	**All evaluable patients[a] (*n* = 113)**	***MET*-positive amplification[b] (*n* = 8)**	***MET*-negative amplification[b] (*n* = 105)**	***P*[c]**
Median age, yr	58	58	58		58	62	58	
Sex, *n* (%)				0.14				0.68
Male	106 (77)	45 (42)	61 (58)		89 (79)	6 (7)	83 (93)	
Female	31 (23)	8 (26)	23 (74)		24 (21)	2 (8)	22 (92)	
Primary tumor site, *n* (%)				0.85				1.00
Stomach	91 (66)	36 (40)	55 (60)		80 (71)	6 (8)	74 (92)	
Gastroesophageal junction	46 (34)	17 (37)	29 (63)		33 (29)	2 (6)	31 (94)	
Lauren classification[d], *n* (%)				0.45				0.04
Intestinal	57 (43)	20 (35)	37 (65)		49 (44)	1 (2)	48 (98)	
Diffuse	54 (40)	24 (44)	30 (56)		46 (41)	7 (15)	39 (85)	
Mixed	23 (17)	7 (30)	16 (70)		16 (14)	0 (0)	16 (100)	
Number of organs with metastasis, *n* (%)				1.00				0.72
1–2	92 (67)	36 (39)	56 (61)		76 (67)	5 (7)	71 (93)	
≥3	45 (33)	17 (38)	28 (62)		37 (33)	3 (8)	34 (92)	
Distant metastasis, *n* (%)								
Liver	61 (45)	26 (43)	35 (57)		55 (49)	5 (9)	50 (91)	
Lung	10 (7)	5 (50)	5 (50)		9 (8)	1 (11)	8 (89)	
Treatment, *n* (%)								
First-line platinum-based therapy[e]	82 (60)	35 (43)	47 (57)		—	—	—	
First-line taxane-based therapy[e,f]	73 (53)	23 (32)	50 (68)		—	—	—	
First-line platinum + taxane (triplet therapy)	18 (13)	5 (28)	13 (72)		—	—	—	
Monotherapy	1 (1)	1 (100)	0		—	—	—	
Chemotherapy/trastuzumab	6 (4)	3 (50)	3 (50)		—	—	—	

[a]Percentages reflect each patient subgroup as the percentage of all evaluable patients (percentages calculated by column).
[b]Percentages reflect each MET subgroup as the percentage of the subset of patients in each patient subgroup (percentages calculated by row).
[c]Fisher exact test.
[d]Number of patients with Lauren classification available: IHC analysis – total, *n* = 134; MET-negative, *n* = 83; MET-positive, *n* = 51; FISH analysis – total, *n* = 111; *MET* amplification negative, *n* = 103; *MET* amplification positive, *n* = 8.
[e]Includes platinum- and taxane-based triplet (*n* = 18).
[f]Includes paclitaxel alone (*n* = 1).

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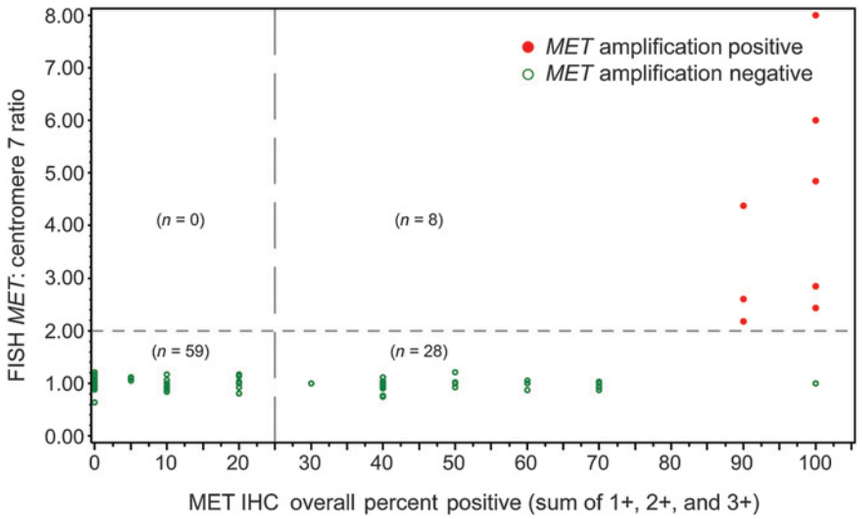

Figure 2.
Association between MET expression and *MET* amplification. Scatter plot shows the percentage of MET IHC overall positive versus FISH *MET*:centromere 7 ratio ($n = 95$). MET expression was significantly associated with *MET* amplification ($P < 0.001$).

difference in OS was observed between the MET-positive and MET-negative patients ($P = 0.80$; Fig. 3A). The HR (95% CI) was 1.049 (0.725–1.518), and the median OS times were 12.3 and 10.9 months in the MET-positive and MET-negative patients, respectively (Supplementary Table S1).

We also evaluated the prognostic value of MET expression according to treatment disposition. In patients receiving first-line platinum/fluoropyrimidine therapy ($n = 62$), there was a nonsignificant trend toward shorter OS in the MET-positive patients versus MET-negative patients ($P = 0.12$; Fig. 3B); the HR (95% CI) was 1.530 (0.892–2.625), and the median OS times were 10.6 and 11.9 months, respectively (Supplementary Table S1). In patients receiving first-line taxane-based therapy ($n = 71$), no statistically significant difference in OS was observed between the MET-positive patients and MET-negative patients ($P = 0.26$; Fig. 3C); the HR (95% CI) was 0.736 (0.431–1.256), and the median OS times were 12.6 and 10.8 months, respectively (Supplementary Table S1). In patients receiving first-line platinum-based therapy ($n = 80$), no statistically significant difference in OS and no apparent trends were observed between the MET-positive and MET-negative patients ($P = 0.37$; Fig. 3D); the HR (95% CI) was 1.243 (0.774–1.996), and the median OS times were 11.3 and 11.8 months, respectively (Supplementary Table S1).

Association between MET expression/*MET* amplification and p-MET expression

Of the 134 patients evaluable for p-MET IHC, eight (6%; 95% CI, 3%–11%) had p-MET–positive tumors. Representative p-MET IHC images are shown in Fig. 4A.

Ninety-six patients were evaluable for both p-MET IHC and *MET* FISH, and of these patients, seven had p-MET–positive tumors. Of the seven patients with p-MET–positive tumors, all had *MET* amplification; only one patient with a *MET*-amplified tumor was not p-MET–positive (Table 2, Fig. 4B). There was a significant association between p-MET expression and *MET* amplification ($P < 0.001$).

Of 118 patients evaluable for both p-MET IHC and MET IHC, 8 had p-MET–positive tumors (Fig. 4C). Of the 8 patients with p-MET–positive tumors, all had MET expression. There was a significant association between p-MET expression and MET expression ($P < 0.001$).

Association between MET expression/*MET* amplification and HER2

Seventy patients were evaluable for HER2 status and MET expression. Of these, 12 (17%) were HER2-positive and 58 (83%) were HER2-negative (Table 3). MET expression was not associated with HER2 status ($P = 0.75$). Of the 12 HER2-positive tumors, 6 (50%) showed MET-positive expression.

Forty-four patients were evaluable for HER2 status and *MET* amplification. Of these, 11 (25%) were HER2-positive and 33 (75%) were HER2-negative; no HER2-positive tumors were *MET* amplified (Table 3). Likewise, no association was observed between *MET* amplification and HER2 status ($P = 0.56$).

Discussion

To our knowledge, this study is the first to evaluate MET protein expression and *MET* gene amplification in biopsy samples from Asian patients with advanced gastric or gastro-esophageal junction cancer by the MET4 IHC IOU and *MET*/CEN-7 IQFISH Probe Mix RUO assays. To date, most studies in Chinese patients have employed small sample sizes with patients of variable baseline characteristics (23–25). Our study included 155 patients with available tumor samples, of whom 137 patients were evaluable for MET IHC, and 113 patients were evaluable for *MET* FISH, all with advanced cancer. Currently, there is no globally recognized standard testing platform for MET. Interpretation of IHC results across studies is hindered by lack of uniform scoring criteria for the different IHC testing methods or *MET* FISH (14). Recent studies have evaluated MET expression by the Ventana assay (12, 14, 16, 26–29), with MET-positive expression defined as IHC 3+ or 2+ staining in variable percentages of tumor cells. Our study showed a MET-positive expression prevalence rate of 39% by IHC in biopsy samples from Chinese advanced gastric or gastroesophageal junction patients when MET-positive expression was defined as ≥25% tumor cells with membrane protein staining at any intensity using the MET4 antibody.

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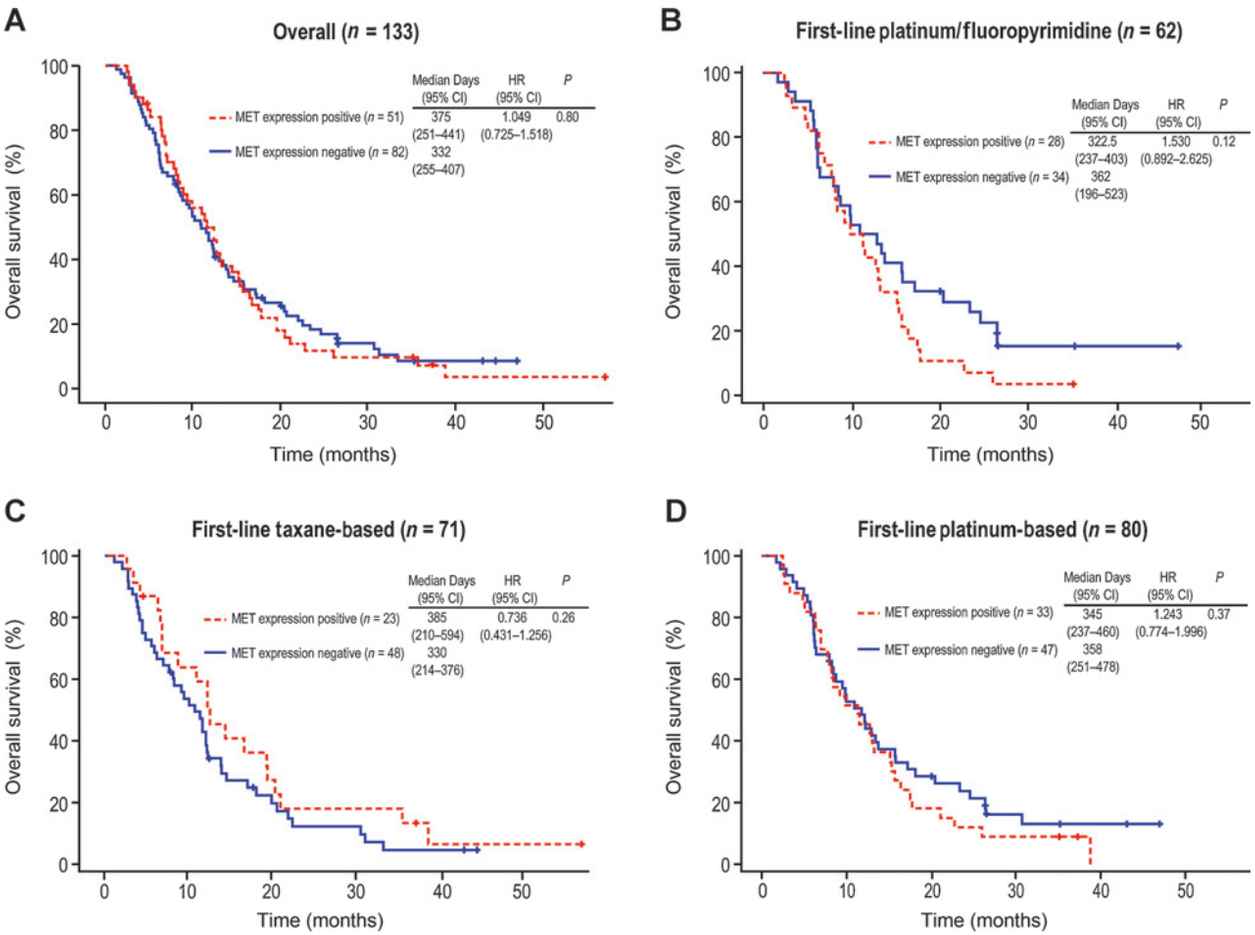

Figure 3.
OS of MET expression–positive patients versus MET expression–negative patients. MET expression–positive was defined as ≥25% of tumor cells with membrane staining at any intensity. A, in all patients who had a follow-up date or death date and MET status ($n = 133$), no statistically significant difference in OS was observed between the MET expression–positive and MET expression–negative patients ($P = 0.80$). B, in patients receiving first-line platinum/fluoropyrimidine without taxanes ($n = 62$), there was a nonsignificant trend toward shorter OS in the MET expression–positive patients versus MET expression–negative patients ($P = 0.12$). C, in patients receiving first-line taxane-based therapy with or without platinum ($n = 71$), no statistically significant difference in OS was observed between the MET expression–positive patients and MET expression–negative patients ($P = 0.26$). D, in patients receiving first-line platinum-based regimens with or without taxanes ($n = 80$), no statistically significant difference in OS and no apparent trends were observed between the MET expression–positive and MET expression–negative patients ($P = 0.37$).

For the HGF/MET pathway, gene amplification may be a crucial biomarker for MET-targeted agents, especially for small-molecule MET inhibitors. The prevalence of *MET* amplification in gastric and gastroesophageal junction cancer has been reported in several studies by different methodologies, including Southern blot analysis, quantitative PCR (qPCR), and *in situ* hybridization (ISH) technologies [FISH or silver ISH (SISH); refs. 12–14, 16, 26–32]. Earlier studies using Southern blot analysis or qPCR reported relatively high rates of *MET* amplification of approximately 10% (13, 30, 31), but recent studies using FISH or SISH found that *MET* amplification rates were lower, varying from 2% to 8% (12, 26, 27, 29, 31, 32). In our study, the *MET* amplification rate was 7%, which is consistent with the 8% rate reported by An and colleagues who evaluated Chinese patients with locally advanced or metastatic gastric or gastroesophageal junction cancer using a similar FISH method (12). The prevalence of *MET* amplification may be related to disease stage; our *MET* amplification rate (7%) and that reported by An and colleagues (8%) are relatively high compared with other studies in which the majority of patients had stage I–III disease. For example, a large cohort study by Lee and colleagues, with only 12% of patients having stage IV disease, found *MET* amplification by SISH in 13 of 381 patients (3%) (26). However, in the same study, *MET* amplification was observed in four of 41 patients with stage IV disease (10%). Recent studies of AMG 337, INC280, and crizotinib have shown promising tumor response in *MET*-amplified gastric or non–small cell lung cancers (33–35).

A notable finding of our study was the strong association between MET-positive expression and *MET* amplification in the biopsy tumor samples ($P < 0.001$), consistent with other studies (12, 26). In the small number of samples with *MET* amplification ($n = 8$), all had ≥90% MET expression. Furthermore, all samples with ≥90% MET expression and

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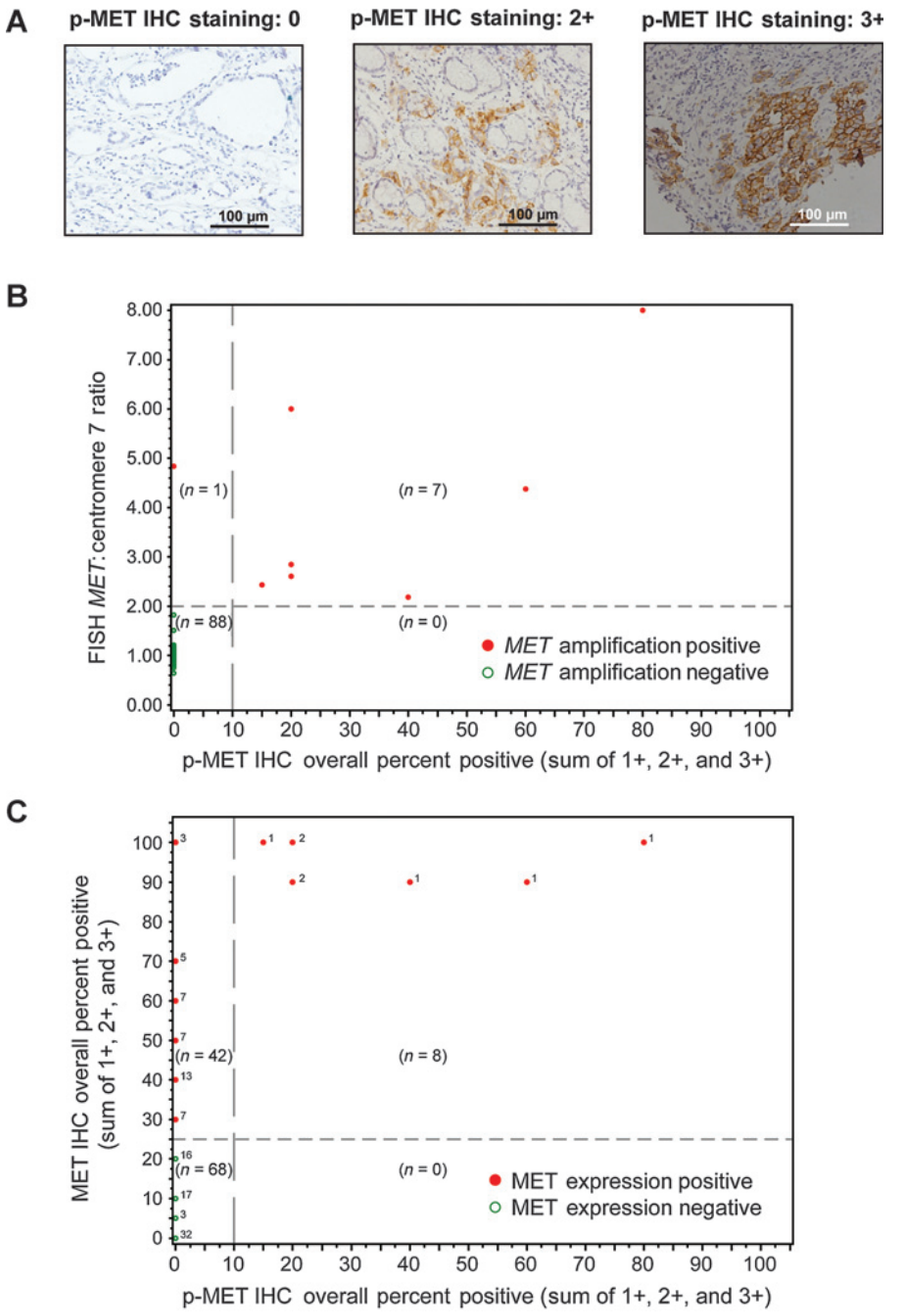

Figure 4.
Phospho-MET results. A, representative p-MET immunohistochemistry staining images. B, scatter plot indicating p-MET IHC overall percent positive versus FISH *MET*: centromere 7 ratio ($n = 96$). There was a significant association between p-MET expression and *MET* amplification ($P < 0.001$). C, scatter plot indicating p-MET IHC overall percent positive versus MET IHC overall percent positive ($n = 118$). There was a significant association between p-MET expression and MET expression ($P < 0.001$). Numbers indicate the number of patients represented at each data point.

valid FISH results showed *MET* amplification, except one. However, the number of *MET*-amplified samples is too small to draw strong conclusions. As *MET* amplification may be an important biomarker for MET-targeted agents, it is crucial to find a convenient and cost-effective approach to detect *MET* amplification. Further research is needed to investigate whether IHC may act as a potential prescreening method for *MET* amplification.

We further evaluated p-MET protein expression and the association between p-MET IHC and *MET* FISH. Phospho-MET expression indicates activation of the MET pathway. Therefore, inhibiting the MET pathway in patients with positive p-MET expression might be a viable therapeutic approach. Moreover, p-MET staining is more convenient compared with *MET* FISH and may act as a substitute method for choosing patients for anti-MET treatment. To date, several studies have evaluated p-MET expression in gastric cancer (36–39), and the results were inconsistent. The p-MET–positive rate of 6% (8/134) observed in our study is similar to the rate reported by Janjigian and colleagues, who also used the same antibody that recognizes p-MET at Y1234/1235 (37). Of interest, we found that there was a strong association between p-MET expression (IHC) and *MET* amplification (FISH). All p-MET–positive samples with valid FISH results showed

Table 2. p-MET IHC results in the *MET*-amplified patients[a]

Patient	p-MET IHC status	IHC score	*MET*:centromere 7 ratio
1	Positive	100	2.85
2	Positive	90	2.18
3	Negative	100	4.84
4	Positive	90	2.60
5[b]	Positive	100	6
6	Positive	90	4.38
7[b]	Positive	100	8
8	Positive	100	2.43

[a]Valid results for both p-MET IHC and *MET* FISH were available for 96 samples. [b]*MET*:centromere 7 ratios for patients 5 and 7 were imputed to 6 and 8, respectively, due to the clustering of FISH signals.

MET amplification, and all samples with *MET* amplification had valid p-MET results and were p-MET positive, except one. To our knowledge, we are the first to show the potentially strong association between p-MET IHC and FISH in patients with advanced gastric or gastroesophageal junction cancer. More studies are needed to confirm whether p-MET IHC may be an alternative and cost-effective method to FISH to select *MET*-amplified patients as a prescreening tool.

In the current study, no significant association was found between MET expression and survival, a result that is inconsistent with observations reported by others that patients with MET-positive gastric cancer have poorer outcomes than those with MET-negative disease (14, 21). When we evaluated OS by different treatment subgroups, there was a nonsignificant trend toward shorter OS for MET-positive patients versus MET-negative patients receiving first-line platinum/fluoropyrimidine therapy ($n = 62$; 10.6 months vs. 11.9 months, $P = 0.12$).

We did not observe a significant association between HER2 status and *MET* amplification in our study, which is consistent with other studies (40). Of note, among the 44 patients with samples evaluable for both HER2 status and *MET* amplification, all four *MET*-amplified samples were HER2 negative. Our interpretation of this finding is limited by the small sample size, but alteration of either one of these genes may be sufficient to support tumor growth.

In our study, *MET* amplification but not MET expression was associated with Lauren classification. A majority of *MET*-amplified samples (seven of eight, 88%) were found in patients with diffuse gastric cancer according to the Lauren classification. These results do not align with the HER2-positive status, which is found predominantly in tumors of intestinal classification (2, 41). The difference in Lauren classification between HER2-positive tumors and *MET*-amplified tumors provides early evidence that these two tumor types may represent different molecular subtypes.

Table 3. Association between HER2 status and MET expression/*MET* amplification

	Total	HER2$^+$	HER2$^-$	*P*[a]
MET expression, *n*	70	12	58	
MET positive, *n* (%)	30 (43)	6 (50)	24 (41)	0.75
MET negative, *n* (%)	40 (57)	6 (50)	34 (59)	
MET amplification, *n*	44	11	33	
Yes, *n* (%)	4 (9)	0 (0)	4 (12)	0.56
No, *n* (%)	40 (91)	11 (100)	29 (88)	

[a]Fisher exact test.

Several limitations of this study should be noted. This was a retrospective study, and the sample size was small, especially in the subgroups. Tumor biopsy samples were not available for all eligible patients, and all samples were not evaluable for IHC and/or FISH. Moreover, the study was limited by the variable first-line treatments among patients. Conclusions regarding outcomes based on MET expression may be confounded by differences in subsequent therapies and selection bias for patients with available samples.

Conclusion

In our study, no statistically significant survival difference was observed between the MET-positive and MET-negative populations, based on MET protein expression, in Chinese patients with advanced gastric or gastroesophageal junction cancer. A significant association was found between *MET* gene amplification and MET protein expression as well as between *MET* gene amplification and p-MET protein expression. Prospective and larger studies where patients receive uniform treatment are needed to further evaluate potential prognostic markers in Chinese patients with advanced gastric or gastroesophageal junction cancer.

Disclosure of Potential Conflicts of Interest

K.S. Oliner has ownership interest (including patents) in Amgen, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Z. Peng, J. Gao, K.S. Oliner, Y.-J. Hei, L. Shen
Development of methodology: Z. Peng, K.S. Oliner
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Z. Peng, Z. Li, J. Gao, M. Lu, J. Gong, K.S. Oliner, L. Shen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Z. Peng, J. Gao, M. Liu, E.-T. Tang, J. Gong, K.S. Oliner, Y.-J. Hei
Writing, review, and/or revision of the manuscript: Z. Peng, Z. Li, J. Gao, M. Lu, J. Gong, E.-T. Tang, K.S. Oliner, Y.-J. Hei, H. Zhou, L. Shen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Z. Peng, Z. Li, J. Gao, M. Lu, J. Gong, E.-T. Tang, L. Shen
Study supervision: Z. Peng

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References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013 accessed 6 Aug 2014. Available from http://globocan.iarc.fr.
2. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687–97.
3. Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, et al. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. Lancet Oncol 2009;10:1063–9.
4. Dank M, Zaluski J, Barone C, Valvere V, Yalcin S, Peschel C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. Ann Oncol 2008;19:1450–7.
5. Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, et al. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. Ann Oncol 2009;20:666–73.
6. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 2008;9:215–21.
7. Ohtsu A, Shah MA, VanCutsem E, Rha SY, Sawaki A, Park SR, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol 2011;29:3968–76.
8. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol 2006;24:4991–7.
9. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2008;358:36–46.
10. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. Nat Rev Cancer 2012;12:89–103.
11. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010;11:834–48.
12. An X, Wang F, Shao Q, Wang FH, Wang ZQ, Wang ZQ, et al. MET amplification is not rare and predicts unfavorable clinical outcomes in patients with recurrent/metastatic gastric cancer after chemotherapy. Cancer 2014;120:675–82.
13. Nakajima M, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, et al. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. Cancer 1999;85:1894–902.
14. Peng Z, Zhu Y, Wang Q, Gao J, Li Y, Li Y, et al. Prognostic significance of MET amplification and expression in gastric cancer: a systematic review with meta-analysis. PLoS ONE 2014;9:e84502.
15. Taniguchi K, Yonemura Y, Nojima N, Hirono Y, Fushida S, Fujimura T, et al. The relation between the growth patterns of gastric carcinoma and the expression of hepatocyte growth factor receptor (c-met), autocrine motility factor receptor, and urokinase-type plasminogen activator receptor. Cancer 1998;82:2112–22.
16. Erichsen R, Oliner KS, Kelsh MA, Nielsen KB, Froslev T, Laenkholm A-V, et al. Prognostic impact of tumor MET expression among patients with stage IV gastric cancer: a Danish cohort study. J Clin Oncol 2014;32:abstr 43.
17. Chi F, Fu D, Zhang X, Lv Z, Wang Z. Expression of the c-Met proto-oncogene and Integrin alpha5beta1 in human gastric cardia adenocarcinoma. Biosci Biotechnol Biochem 2012;76:1471–6.
18. Drebber U, Baldus SE, Nolden B, Grass G, Bollschweiler E, Dienes HP, et al. The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. Oncol Rep 2008;19:1477–83.
19. Hong S, Ahn JR, Zhang X, Lee H-Y, Kim HR, Lee C-K, et al. Receptor tyrosine kinases (RTKs) profiling and its role in advanced gastric cancer. Mol Cancer Ther 2011;11:abstr B34.
20. Kuboki Y, Fuse N, Kuwata T, Shinozaki E, Machida N, Yuki S, et al. Prognostic impact of c-MET status on overall survival of patients with advanced gastric cancer treated with standard chemotherapy: a Japanese multicenter collaborative retrospective study. Eur J Cancer 2013;49:abstr 2646.
21. Iveson T, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, et al. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. Lancet Oncol 2014;15:1007–18.
22. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Nat Cancer Inst 2005;97:643–55.
23. Li Y, Chen CQ, He YL, Cai SR, Yang DJ, He WL, et al. Abnormal expression of E-cadherin in tumor cells is associated with poor prognosis of gastric carcinoma. J Surg Oncol 2012;106:304–10.
24. Shi J, Yao D, Liu W, Wang N, Lv H, He N, et al. Frequent gene amplification predicts poor prognosis in gastric cancer. Int J Mol Sci 2012;13:4714–26.
25. Zhao J, Zhang X, Xin Y. Up-regulated expression of Ezrin and c-Met proteins are related to the metastasis and prognosis of gastric carcinomas. Histol Histopathol 2011;26:1111–20.
26. Lee HE, Kim MA, Lee HS, Jung EJ, Yang HK, Lee BL, et al. MET in gastric carcinomas: comparison between protein expression and gene copy number and impact on clinical outcome. Br J Cancer 2012;107:325–33.
27. Liu YJ, Shen D, Yin X, Gavine P, Zhang T, Su X, et al. HER2, MET and FGFR2 oncogenic driver alterations define distinct molecular segments for targeted therapies in gastric carcinoma. Br J Cancer 2014;110:1169–78.
28. Yu S, Yu Y, Zhao N, Cui J, Li W, Liu T. C-Met as a prognostic marker in gastric cancer: a systematic review and meta-analysis. PLoS ONE 2013;8:e79137.
29. Li J, Wang C, Su Q, Wang Y, Shi Y, Sun M, et al. The prognostic significance of MET overexpression in Chinese patients with gastric adenocarcinoma. J Clin Oncol 2014;3253.
30. Carneiro F, Sobrinho-Simoes M. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. Cancer 2000;88:238–40.
31. Graziano F, Galluccio N, Lorenzini P, Ruzzo A, Canestrari E, D'Emidio S, et al. Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. J Clin Oncol 2011;29:4789–95.
32. Jardim DL, de Melo Gagliato D, Falchook GS, Janku F, Zinner R, Wheler JJ, et al. MET aberrations and c-MET inhibitors in patients with gastric and esophageal cancers in a phase I unit. Oncotarget 2014;5:1837–45.
33. Hong DS, LoRusso P, Hamid O, Beaupre DM, Janku F, Khan R, et al. First-in-human study of AMG 337, a highly selective oral inhibitor of MET, in adult patients (pts) with advanced solid tumors. J Clin Oncol 2014;32:abstr 2508.
34. Bang Y-J, Su W-C, Nam D-H, Lim W-T, Bauer TM, Brana I, et al. Phase I study of the safety and efficacy of INC280 in patients with advanced MET-dependent solid tumors. J Clin Oncol 2014;32: abstr 2520.
35. Camidge DR, Ou S-HI, Shapiro G, Otterson GA, Villaruz LC, Villalona-Calero MA, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). J Clin Oncol 2014;32:abstr 8001.
36. Inoue T, Kataoka H, Goto K, Nagaike K, Igami K, Naka D, et al. Activation of c-Met (hepatocyte growth factor receptor) in human gastric cancer tissue. Cancer Sci 2004;95:803–8.
37. Janjigian YY, Tang LH, Coit DG, Kelsen DP, Francone TD, Weiser MR, et al. MET expression and amplification in patients with localized gastric cancer. Cancer Epidemiol Biomarkers Prev 2011;20:1021–7.
38. Lee J, Seo JW, Jun HJ, Ki CS, Park SH, Park YS, et al. Impact of MET amplification on gastric cancer: possible roles as a novel prognostic marker and a potential therapeutic target. Oncol Rep 2011;25:1517–24.
39. Wu JG, Yu JW, Wu HB, Zheng LH, Ni XC, Li XQ, et al. Expressions and clinical significances of c-MET, p-MET and E2f-1 in human gastric carcinoma. BMC Res Notes 2014;7:6.
40. Ali SM, Sanford EM, Klempner SJ, Rubinson DA, Wang K, Palma NA, et al. Prospective comprehensive genomic profiling of advanced gastric carcinoma cases reveals frequent clinically relevant genomic alterations and new routes for targeted therapies. Oncologist 2015;20:499–507.
41. Fan XS, Chen JY, Li CF, Zhang YF, Meng FQ, Wu HY, et al. Differences in HER2 over-expression between proximal and distal gastric cancers in the Chinese population. World J Gastroenterol 2013;19:3316–23.

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