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ORIGINAL REPORT

# *MET* Amplification Identifies a Small and Aggressive Subgroup of Esophagogastric Adenocarcinoma With Evidence of Responsiveness to Crizotinib

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See accompanying articles on pages 4789 and 4837

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## ABSTRACT

### Purpose
Amplification of the *MET* proto-oncogene in gastroesophageal cancer (GEC) may constitute a molecular marker for targeted therapy. We examined a GEC cohort with follow-up and reported the clinical response of four additional patients with *MET*-amplified tumors to the small molecule inhibitor crizotinib as part of an expanded phase I cohort study.

### Patients and Methods
From 2007 to 2009, patients with GEC were genetically screened as a consecutive series of 489 tumors (stages 0, I, and II, 39%; III, 25%; IV, 36%; n = 222 esophageal, including n = 21 squamous carcinomas). *MET*, *EGFR,* and *HER2* amplification status was assessed by using fluorescence in situ hybridization.

### Results
Ten (2%) of 489 patients screened harbored *MET* amplification; 23 (4.7%) harbored *EGFR* amplification; 45 (8.9%) harbored *HER2* amplification; and 411 (84%) were wild type for all three genes (ie, negative). *MET*-amplified tumors were typically high-grade adenocarcinomas that presented at advanced stages (5%; n = 4 of 80). *EGFR*-amplified tumors showed the highest fraction of squamous cell carcinoma (17%; n = 4 of 23). *HER2*, *MET*, and *EGFR* amplification were, with one exception (*MET* and *EGFR* positive), mutually exclusive events. Survival analysis in patients with stages III and IV disease showed substantially shorter median survival in *MET*/*EGFR*-amplified groups, with a rank order for all groups by median survival (from most to least aggressive): *MET* (7.1 months; $P < .001$) less than *EGFR* (11.2 months; $P = .16$) less than *HER2* (16.9 months; $P = .89$) when compared with the negative group (16.2 months). Two of four patients with *MET*-amplified tumors treated with crizotinib experienced tumor shrinkage (−30% and −16%) and experienced progression after 3.7 and 3.5 months.

### Conclusion
*MET* amplification defines a small and aggressive subset of GEC with indications of transient sensitivity to the targeted MET inhibitor crizotinib (PF-02341066).

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## INTRODUCTION

The global health burden of gastroesophageal cancer (GEC) has been compared with that imposed by lung cancer,[1] and, despite improvements in surgical approaches and chemoradiotherapy,[2-4] benefits from combining chemotherapy with radiation in the early and locally advanced setting have reached a plateau.[5] The prognosis for advanced GEC remains extremely poor, and overall 5-year survival rates are approximate 15%.[1,6] Cytotoxic chemotherapy remains the mainstay of treatment for patients with incurable disease,[7] and targeted therapeutics are assuming an increasingly important role.[4,8] The ToGA (Trastuzumab for Gastric Cancer) trial, for example, confirmed that, in *HER2*-positive inoperable gastric and gastroesophageal junction cancers, trastuzumab plus cisplatin and either capecitabine or fluorouracil resulted in improved overall survival (OS) compared with chemotherapy alone.[9] Consequently, this strategy has been approved as the standard regimen in those approximately 20% of patients with metastatic GEC who demonstrate *HER2*

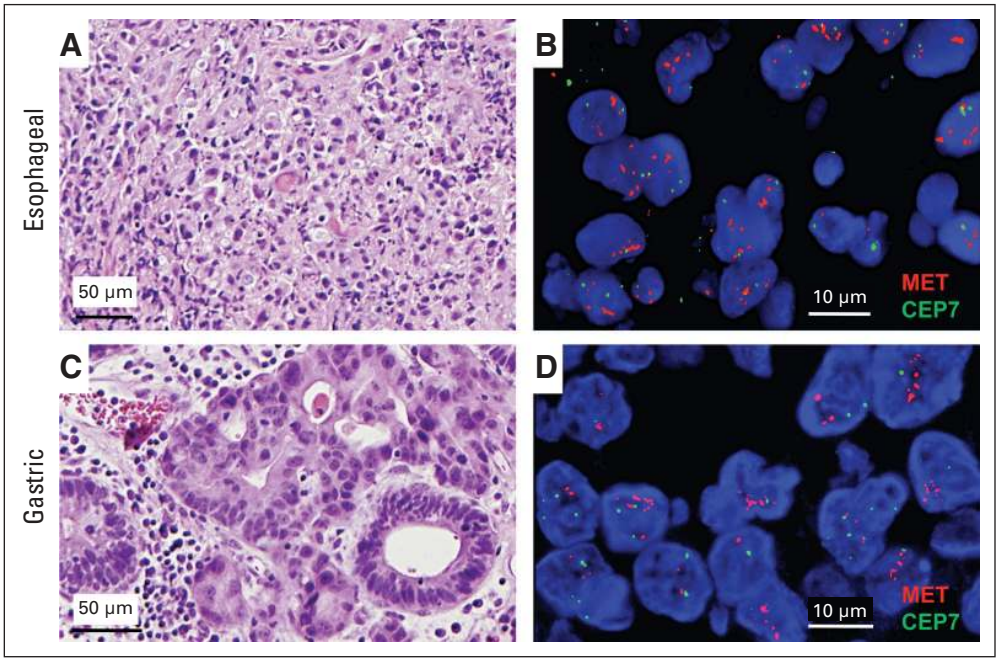


**Fig 1.** Diagnostic features of *MET*-amplified gastroesophageal carcinoma. Hematoxylin and eosin staining of a representative (A) high-grade esophageal and (C) gastric adenocarcinoma with corresponding fluorescence in situ hybridization that demonstrate *MET* gene-to-chromosome ratios of (B) greater than five and (D) approximately four.

positivity.[7] Additional targeted agents are undergoing clinical evaluation in advanced GEC, including the *EGFR* antibodies cetuximab and panitumumab[10-12] and the angiogenic inhibitors bevacizumab and sorafenib.[13-15]

One promising subset may include tumors with *MET* gene amplification resulting in overexpression and constitutive activation of the encoded receptor tyrosine kinase MET.[16,17] In a large-scale preclinical screening approach, we have previously identified *MET* amplification in approximately 20% of gastric cancer cell lines and have demonstrated that this amplification confers extraordinary susceptibility to apoptosis induction by the selective MET inhibitor PHA-665752 (Pfizer, La Jolla, CA).[18] Recently, crizotinib (PF-02341066, Pfizer) was identified as an orally bioavailable, potent, ATP-competitive small-molecular inhibitor of the catalytic activity of *MET* kinase.[19,20] The crizotinib expanded phase I cohort study allowed us to test whether in vitro responses to MET inhibition can be replicated in patients with *MET*-amplified metastatic disease.

The potential for amplified *MET* to act as an oncogenic driver[8,16-18] (http://www.vai.org/met/), the availability of MET inhibitors[8,16] and variability in reported prevalence (3.9% to 25%),[21-24] led us to perform a focused examination of the biologic behavior, as well as the demographic and tumor-associated features in a large cohort of GEC patients. We evaluated *MET* amplification and its relationship to *EGFR* and *HER2* status. Further, we report results of four patients with *MET*-amplified tumors participating in the crizotinib phase I study. Our findings underscore the prognostic and potentially predictive value of *MET* amplification as well as the challenges in identifying GEC subgroups that might benefit from targeted therapies.

## PATIENTS AND METHODS

*Study population.* The study included an institutional review board–approved retrospective analysis of a consecutive series of patients with GEC and with biopsy-proven carcinoma (all types) seen at Massachusetts General Hospital (MGH) Cancer Center/Dana Farber Harvard Cancer Center. Patients enrolled on targeted therapy trials, patients with insufficient tissue for genetic testing, or patients for whom fluorescence in situ hybridization (FISH) was inconclusive were excluded from survival analysis.

*Data collection and survival analysis.* Medical records were reviewed to extract data on clinicopathologic characteristics and outcomes. The primary end point was OS, measured from the date of diagnosis until the date of death. Patients were censored if they were lost to follow-up, if they experienced death unrelated to GEC, or if they were alive and well. For patients on the crizotinib trial, responses were classified by using standard RECIST (Response Evaluation Criteria in Solid Tumors), version 1.1.[25]

*Tumor pathology and staging.* No a priori selection by tumor type was performed, allowing unbiased examination of genetic associations. Hematoxylin and eosin staining was performed on 5-$\mu$m sections from formalin-fixed paraffin-embedded tumor tissue (Fig 1). All tumors were evaluated by two pathologists, were classified by using WHO criteria,[26] and were staged according to updated 2010 American Joint Committee on Cancer/TNM criteria.[27] Tumor location was classified on the basis of the epicenter as either esophageal, junctional, or gastric, and we analyzed gene amplification and outcomes by site to allow comparison with prior studies.

*Genetic analysis.* FISH was performed to identify gene copy number (CN) on formalin-fixed paraffin-embedded tissue by using two separate hybridizations for *MET*/*EGFR*/centromere 7 (CEP7) and *HER2*/CEP17. The triple-target hybridization employed two bacterial artificial chromosome clones specific to *MET* (CTD-1013N12) and *EGFR* (CTD-2113A18) in combination with a CN control corresponding to CEP7 (Abbott-Vysis 06J54-027; Abbott Laboratories, Des Plaines, IL). The separate dual target hybridization employed an *HER2*/CEP17 probe combination (PathVysion Kit No. 32-801200; Abbott Laboratories). Amplification followed strict definitions as established for *HER2* testing.[28] Briefly, we used a strict definition for defining gene amplification as a gene-to-CN control probe ratio G:CN of greater than 2.2 scored in 50 tumor nuclei, which was extrapolated from established *HER2* criteria.[28] Specifically, polysomy, high polysomy, or equivocal G:CN ratio (ie, 1.8 to 2.2) were scored as negative for amplification.

### Statistical Analysis

Statistical analysis consisted of Fisher's exact test (association of genotype with dichotomous factors), $\chi^2$, or $t$ test (comparison of means). The Kaplan-Meier method was used to estimate OS, and

  

**Table 1.** Demographic and Clinical Characteristics of Genetically Screened Patients With Gastroesophageal Cancer

| Characteristic | Patients (N = 489) No. | % |
|---|---|---|
| Age, years | | |
| Median | 64 | |
| Range | 22-96 | |
| Sex | | |
| Male | 367 | 75 |
| Female | 122 | 25 |
| Pathology | | |
| Adenocarcinoma | 460 | 94 |
| Intestinal | 370 | 76 |
| Diffuse | 79 | 16 |
| Mixed | 4 | 0.8 |
| Mucinous | 5 | 1 |
| Medullary | 2 | 0.4 |
| Adenosquamous | 1 | 0.2 |
| Squamous | 21 | 4.3 |
| Neuroendocrine | 7 | 1.4 |
| Differentiation | | |
| Well | 26 | 5 |
| Moderate | 210 | 43 |
| Poor | 248 | 51 |
| Undifferentiated | 5 | 1 |
| Stage* | | |
| 0 | 36 | 7 |
| I | 69 | 14 |
| II | 85 | 17 |
| III | 121 | 25 |
| IV | 178 | 37 |

*Substaging for esophageal (American Joint Committee on Cancer 2010: esophageal + junctional) and gastric lesions is provided in Fig 2; findings by anatomic location (esophageal *v* junctional *v* gastric) are tallied in the Data Supplement.

differences between genotypes were compared by using the log-rank method. Data analysis was conducted by using Prism 5.0b (GraphPad Software, San Diego, CA), and significance was defined as $P < .05$.

*Crizotinib trial.* Our study also included preliminary data of clinical responses in four patients (Cr1-4; from Seoul National University Hospital, Seoul, South Korea; Peter MacCallum Cancer Centre, Melbourne, Australia; and MGH, Boston, MA) enrolled on an open-label, multicenter, trial of the *MET/ALK* tyrosine kinase inhibitor, crizotinib. The trial was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee at each participating institution; patients on the crizotinib trial were required to give written informed consent before enrolling on that study.

## RESULTS

On the basis of the results of our cell line–based screening approach,[18] we established screening of patient samples between November 2007 and July 2009, and we tested 489 patients with GEC for *MET*, *EGFR*, and *HER2* amplification. Patients were selected for genetic screening from routine diagnostic workflow, and we excluded precursor lesions (eg, high-grade dysplasia) or focal intramucosal carcinoma (eg, endoscopic mucosal resections). Thus, despite coverage of the entire spectrum of histologic tumor types (eg, including 21 patients with esophageal squamous carcinomas), the cohort was enriched for patients with either stage III or IV disease (Table 1). Furthermore, genetic screening was performed primarily on biopsy samples (n = 414; approximately 84%) taken from routine surgical pathology workflow, which reduced bias regarding sample referral; however, we cannot exclude selection bias on the basis of referral or presentation in our oncology clinics.

### *Prevalence of* MET, EGFR, *and* HER2 *Gene Amplification in GEC*

Of the 489 tumors screened, 10 patients (2%) harbored *MET* amplification (*MET* positive; Data Supplement), 23 patients (4.7%) harbored *EGFR* amplification (*EGFR* positive), 44 patients (8.9%) harbored *HER2* amplification (*HER2* positive), and 411 patients (84%) were wild type for *MET*/*EGFR*/*HER2* (designated as negative). A single tumor with abundant *MET* amplification (G:CN ratio > 5) also demonstrated low-level *EGFR* amplification (G:CN ratio approximately 2.5). Thus, the overall detection rate for an amplification event was approximately 16% (n = 77 of 489).

Comparison of clinicopathologic features by anatomic location validates most recent staging guidelines[29] and is provided in the Data

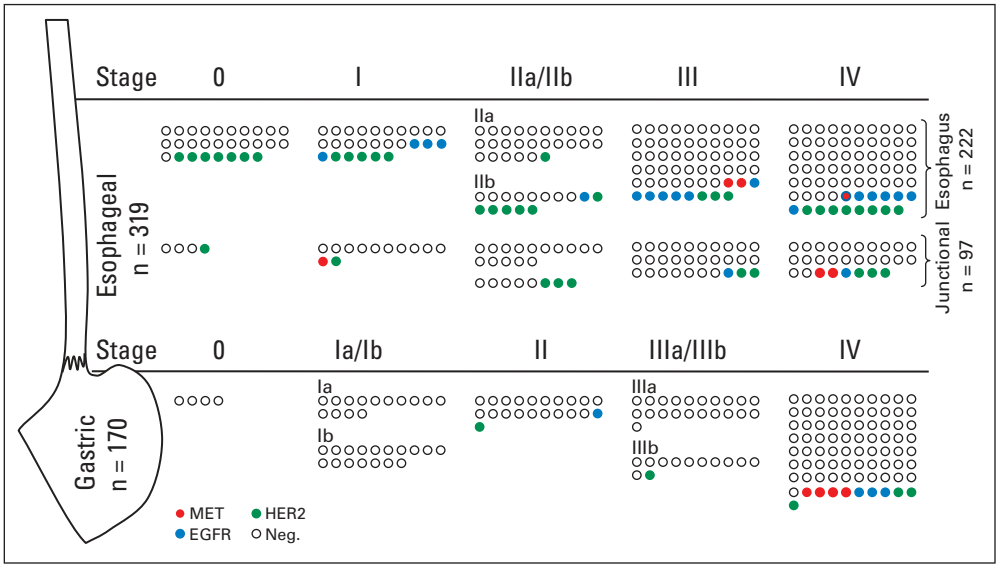


**Fig 2.** Frequency of genetic subtypes by location and anatomic stage; Neg., no *MET*/*EGFR*/*HER2* amplification.

Supplement. Anatomic and stage-based distribution is provided in Figure 2. The highest frequency of *MET* positivity was observed in junctional tumors (3%; 3/97) whereas *EGFR* positivity and *HER2* positivity were most frequently found in the esophageal tumors (*EGFR* positive, 8%; n = 18 of 222; *HER2* positive, 13.5%; n = 30 of 222). Review of the amplification frequency by anatomic location (Data Supplement) indicates that, in the unselected GEC population, amplification of *MET* is a rare event.

### *Clinicopathologic Characteristics of* MET-, EGFR-, *and* HER2-*Amplified GEC*

Examination of clinicopathologic features demonstrated the following genetic associations: *MET*-positive tumors were uniformly adenocarcinomas with significantly higher grades ($P = .02$) and advanced stages at presentation ($P = .04$; subgroup analysis provided in Table 2). *EGFR*-positive tumors showed the highest proportion of squamous cell carcinomas (*EGFR* positive, 17% *v EGFR* negative, 3.9%; $P = .025$) and the highest proportion of female patients (*EGFR* positive, 40% *v EGFR* negative, 25%; $P = .14$) and also demonstrated significantly higher tumor grades when compared with the negative group ($P < .001$). *HER2*-positive tumors showed the greatest spectrum of histologic tumor types (Table 2) and, in agreement with prior reports,[9,30-32] showed a significant number of patients with history of intestinal metaplasia/Barrett's metaplasia (*HER2* positive, 51% *v* negative, 10%; $P < .001$),

**Table 2.** Demographic and Clinical Characteristics of Genotype-Specific Subsets of Patients With Gastroesophageal Cancer

| Characteristic | *MET* (n = 10) | | *EGFR* (n = 23) | | *HER2* (n = 45) | | Negative (n = 411) | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | *MET v* Negative | *EGFR v* Negative | *HER2 v* Negative |
| Age, years | | | | | | | | | .71 | .71 | .68 |
| Median | 66 | | 63 | | 63 | | 65 | | | | |
| Range | 33-83 | | 34-84 | | 36-92 | | 22-96 | | | | |
| Sex | | | | | | | | | 1.0 | .14 | .36 |
| Male | 8 | 80 | 14 | 61 | 37 | 82 | 308 | 75 | | | |
| Female | 2 | 20 | 9 | 39 | 8 | 18 | 103 | 25 | | | |
| Site | | | | | | | | | | | |
| Esophageal | 3 | 30 | 18 | 78 | 30 | 67 | 171 | 42 | | | |
| Junctional | 3 | 30 | 1 | 4 | 10 | 22 | 83 | 20 | | | |
| Gastric | 4 | 40 | 4 | 17 | 5 | 11 | 157 | 38 | | | |
| IM* | | | | | | | | | .38 | .78 | < .001 |
| Prior positive | 3 | 30 | 4 | 17 | 23 | 51 | 42 | 10 | | | |
| Prior negative | 4 | 40 | 15 | 65 | 17 | 38 | 118 | 29 | | | |
| NA | 3 | 30 | 4 | 17 | 5 | 11 | 251 | 61 | | | |
| Pathology | | | | | | | | | .52 | 1.0 | .75 |
| Adenocarcinoma | 10 | 100 | 19 | 83 | 44 | 98 | 387 | 93 | | | |
| Intestinal | 8 | 80 | 18 | 78 | 41 | 92 | 303 | 74 | | | |
| Diffuse | 1 | 10 | 1 | 4 | 2 | 4 | 75 | 18 | | | |
| Mixed | | | | | | | 4 | 1 | | | |
| Mucinous | 1 | 10 | | | | | 4 | 1 | | | |
| Medullary | | | | | 1 | 2 | 1 | 0.2 | | | |
| Adenosquamous | | | | | | | 1 | 0.2 | | | |
| Squamous | | | 4 | 17 | 1 | 2 | 16 | 3.9 | | | |
| Neuroendocrine | | | | | | | 7 | 1.7 | | | |
| Differentiation | | | | | | | | | .02 | < .001 | < .001 |
| Well | | | | | 12 | 27 | 14 | 4 | | | |
| Moderate | 1 | 10 | 7 | 30 | 16 | 36 | 186 | 45 | | | |
| Poor | 9 | 90 | 16 | 70 | 17 | 38 | 206 | 50 | | | |
| Undifferentiated | | | | | | | 5 | 1 | | | |
| Stage† | | | | | | | | | .04 | .69 | .04 |
| 0 | | | | | 8 | 18 | 28 | 7 | | | |
| I | 1 | 10 | 4 | 17 | 6 | 13 | 58 | 14 | | | |
| II | | | 2 | 9 | 11 | 24 | 72 | 17 | | | |
| III | 2 | 20 | 7 | 30 | 6 | 13 | 106 | 26 | | | |
| IV | 7 | 70 | 10 | 43 | 14 | 31 | 147 | 36 | | | |

NOTE. Negative indicates no *MET*/*EGFR*/*HER2* amplification. *P* values from Fisher's exact test for dichotomous variables: intestinal versus diffuse cancer type; low-grade (well plus moderate) versus high-grade (poor plus undifferentiated) or $c^2$ for stage comparisons (taking all categories into account).

Abbreviations: IM, intestinal metaplasia; NA, not applicable.

*Samples with histologically confirmed IM, defined as Barrett's esophagus (esophageal or junctional samples) or chronic atrophic gastritis with IM (gastric samples) in at least one prior biopsy (ie, prior positive) were tallied versus patients with prior biopsies without evidence of IM (ie, prior negative); NA refers to patients without previous biopsies.

†Staging including subcategories for esophageal and gastric lesions is provided in Fig 2; findings by anatomic location (esophageal *v* junctional *v* gastric) are tallied in the Data Supplement.

  

significantly better tumor differentiation ($P < .001$), and significantly lower stages of disease at presentation ($P = .04$).

On the basis of the low frequency of amplification events in GEC, we investigated two approaches to increase the detection of amplification events. First, we attempted immunohistochemical screening for *MET* amplification. Such strategies have been reported for *HER2*-overexpressing tumors[9,30-34]; however, our attempts to correlate *MET* status with immunohistochemical staining pattern were unsuccessful (data not shown). Briefly, we used a mouse-anti-MET antibody (catalog No. 370100; Invitrogen, Carlsbad, CA) and observed strong baseline staining, even in normal esophageal and gastric epithelium, that precluded meaningful assessment.[35,36] A second approach was recalculation of detection rates that was based on a priori selection by using clinicopathologic criteria. For example, assuming that only high-grade stages III and IV esophageal (ie, esophageal + junctional) adenocarcinomas would have been screened for *MET* amplification, the detection rate would increase to 5% (ie, four of 80 patients). When we applied similar criteria to *EGFR* (for squamous carcinoma), 20% (ie, four of 20 patients) were positive; for *HER2* (for well-differentiated tumors), 46% (ie, 12 of 26 patients) were positive.

### *Genotype-Specific Clinical Outcome*

We focused on patients potentially amenable to targeted treatment on clinical trials (ie, stages III and IV; n = 299 patients); at the time of data review, the median follow-up time of patients was 13 months. At that time, 204 patients had died as a result of disease, and 95 patients were either alive or lost to follow-up. OS analysis comparing contribution of the subgroup of squamous carcinoma showed minimal differences, and inclusion of these patients did not alter the conclusions drawn from the statistical analysis (Data Supplement). Similarly, there were no statistically significant differences in median survival by site (esophageal, 17.33 months; junctional, 14.2; gastric, 14.47; *P* range, .22 to .66). Additional comparisons by stage, location, and genotype are provided in the Data Supplement. In the absence of OS differences in advanced tumors by anatomic location ($P = .46$), the data were analyzed via separate categories by using current staging guidelines (esophageal + junctional, Fig 3A *v* gastric, Fig 3B) as well as a merged esophagogastric category (ie, GEC; Fig 3C). OS in patients with amplified tumors (either *MET*, *EGFR*, or *HER2*) was significantly shorter (11.3 months) when compared with the negative group (16.2 months; $P = .03$). Analyzed by gene and location, the median survival times for esophageal (ie, esophageal + junctional) carcinoma were 7.16 months (*MET* positive; $P = .0006$), 11.8 months (*EGFR* positive; $P = .45$), and 16.9 months (*HER2* positive; $P = .97$) versus 17.6 months (negative), whereas the median survival times for gastric carcinoma were 6.1 months (*MET* positive; $P = .01$), 2.3 months (*EGFR* positive; $P = .0003$), and 14.1 months (*HER2* positive; $P = .59$) versus 14.5 months (negative; Fig 3B). These findings indicate that gene amplification, and especially *MET* positivity and *EGFR* positivity, are associated with an aggressive disease phenotype. In the esophagus and junctional mucosa, *MET* amplification identified the most aggressive subset (Fig 3A), whereas *EGFR*-amplified and *MET*-amplified tumors represented the most aggressive subtypes in the gastric samples (Fig 3B; stage IV median survival: 6.2 months for *MET* positive *v* 2.3 months for *EGFR* positive; $P = .93$); stage comparison (stage III *v* IV) by location and genotype is provided in the Data Supplement. The shortest OS, and thereby the most aggressive molecular subgroup, in the GEC cohort was *MET* (Fig 3C). The rank order for all groups by median survival (from most to least aggressive phenotype) was as follows: *MET* (7.1 months; $P < .005$) less than *EGFR* (11.2 months; $P = .16$) less than *HER2* (16.9 months; $P = .89$) when compared with the negative group (16.2 months).

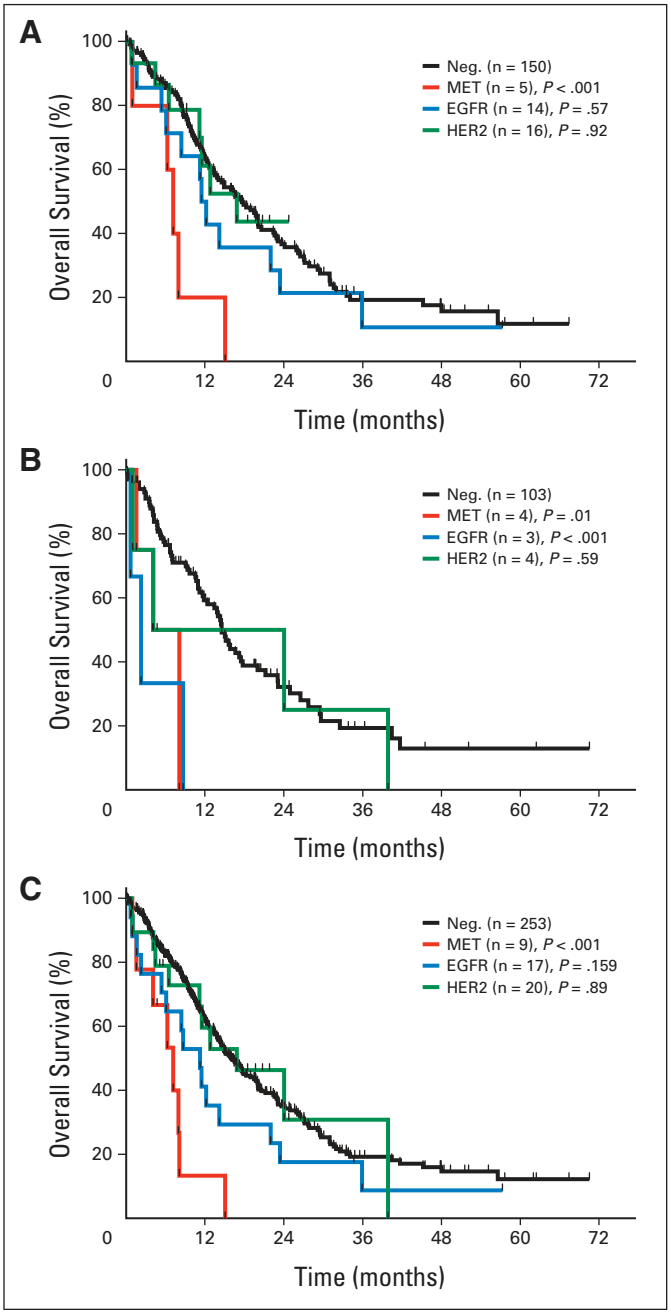


**Fig 3.** Overall survival (OS) of patients with stage III to IV *MET*-amplified tumors compared with patients who have *EGFR*-amplified, *HER2*-amplified, or no *MET*/*EGFR*/*HER2* (negative [Neg]) amplification. Kaplan-Meier survival plots of OS in (A) esophageal + junctional cancer, (B) gastric cancer, and (C) the entire cohort. *P* values, log-rank test.

### *Clinical Responses to Crizotinib in* MET*-Amplified GECs*

Because crizotinib inhibits MET kinase activity at a half maximal inhibitory concentration of 8 nmol/L, an objective of the phase I

clinical trial was enrollment of patients with *MET*-driven tumors into an expanded molecular cohort. A shared clinical observation by the authors has been that recruitment of patients with *MET*-amplified GEC onto the clinical trial has been challenging. Specifically, the unexpected rarity, in combination with the aggressive nature and resulting inability to meet clinical entry criteria, has limited the number of enrolled patients. Several patients experienced progression even during the lag time from genetic identification to crizotinib initiation, and supportive care measures had to be initiated. None of 10 patients in our initial cohort were enrolled; however, four separate patients (Cr1-4) with *MET*-amplified GEC were treated on study with crizotinib 250 mg administered twice daily. Two of these patients (Cr1, Cr2) with advanced gastric cancer were enrolled at Seoul National University Hospital. FISH demonstrated focal amplification in Cr1 (3.3) and a *MET*/CEP7 of greater than 5 in (Cr2), and both showed rapid progression before first study restaging (time to progression on crizotinib, 43 and 27 days for Cr1 and Cr2, respectively). However, two additional patients with *MET*/CEP7 of greater than 5 who were enrolled at Peter MacCallum Cancer Center (Cr3) and MGH (Cr4) experienced clinical benefit. Specifically, both patients had stage IV junctional GEC; after 1 week of crizotinib, patient Cr3 experienced rapid symptomatic response with improvement in appetite, reduction of pain, and improvement in performance status. A computed tomography scan at the end of cycle 2 (8 weeks) showed a partial response to treatment with a 39% reduction in tumor measurements, which was confirmed at 12 weeks (41% reduction; Fig 4). Scans performed at 16 weeks, however, showed disease progression, and the patient was taken off study; time to progression on crizotinib was approximately 112 days. Patient Cr4 also showed rapid clinical improvement, with decreased pain and improved performance status after 1 week of crizotinib. After two cycles of treatment, restaging computed tomography scans demonstrated tumor reduction of 16% in multiple target lesions (stable disease by RECIST). Treatment continued, and restaging after an additional 55 days demonstrated progression (44%; progressive disease by RECIST); time to progression on crizotinib was 105 days. Crizotinib was discontinued, and the patient reported a prompt increase in pain. After two additional cycles of standard chemotherapy, supportive care measures were initiated, and the patient died after 4 months. OS for Cr4 was 21 months. Additional patient details are provided in the Data Supplement.

## DISCUSSION

Here, we report that MET amplification identifies a rare and highly aggressive subset of GEC with early evidence of at least partial sensitivity to the *MET* inhibitor crizotinib.

Most *MET*-amplified tumors are high-grade, high-stage adenocarcinomas with significantly shorter OS (7.2 months for *MET* positive *v* 16.2 months for negative; $P < .001$). In addition to implications as a prognostic marker, the phenotype differs from that observed in the *EGFR*, *HER2,* and negative subpopulations (Table 2). The notion of distinct biologic entities is supported by our findings that gene amplification of *MET*, *EGFR*, and *HER2* are, with one exception, mutually exclusive events (Fig 2). The aggressive biology of *MET*-amplified tumors is plausible, given the role of *MET* as an oncogenic driver in these tumors and the wide-ranging downstream effects with implications in tumor growth, invasiveness, tumor angiogenesis, epithelial-mesenchymal transition, and metastasis.[8,16-18]

An important finding of this study is that *MET* amplification is rare. We found it in 2% of all GEC patients or in 5% of high-grade adenocarcinomas that presented in advanced stages. In contrast, literature values derived from smaller series or cell lines range up to 25% for GEC.[21-24] It seems plausible that the cellular consequences of *MET* amplification render tumors better sources for sustainable cell lines, which may explain why we found 20% *MET* amplification in gastric cancer cell lines.[18] However, additional reasons for the lower prevalence of *MET* amplification may contribute in tumors. First, there may be geographic and epidemiologic differences in the populations under examination.[37-40] For example, it is well established that a completely different set of precursor lesions, as well as histologies of GEC, exist in the Asian population.[37] Comparisons of reported *MET* amplification frequency in recent US- and Asian-based case series[41,42] support the validity of our findings that *MET* amplification is less frequent than previously reported,[21,22] at least in the Westernpopulation. Second, we applied strict American Society of Clinical Oncology/College of American Pathologists guidelines for the definition of gene amplification,[28] and the overall lower frequency of *MET* amplification may be related to differences in scoring criteria employed in previous studies,[21-24,43] which may also contribute to the somewhat lower detection rates for *EGFR* (4.7%) and *HER2* (8.9%) in comparison to prior reports.[9,44-47] Third, mechanisms of *MET* activation other than amplification may contribute to subsets of GEC (eg, mutations in the

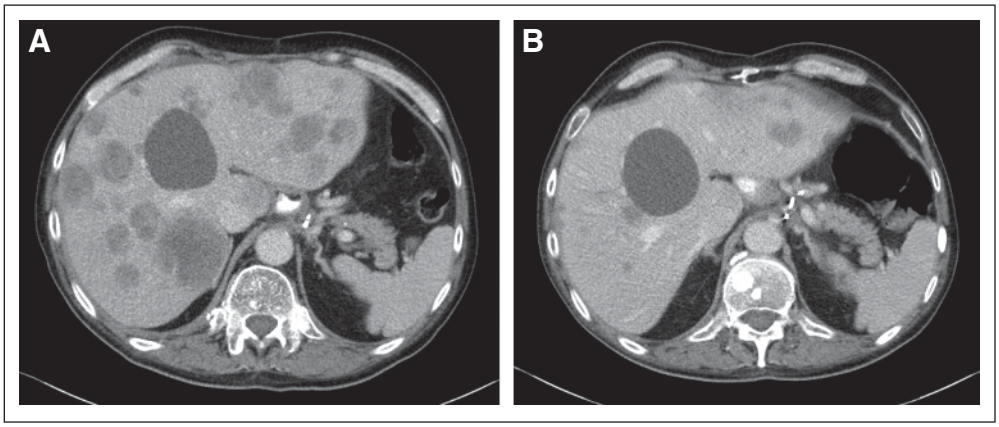


**Fig 4.** Response of patient Cr3 with metastatic *MET*-amplified gastroesophageal cancer to the MET inhibitor crizotinib. (A) Pretreatment image and (B) partial response after two cycles of crizotinib (250 mg twice daily for a total of 8 weeks).

  

juxtamembranous domain, autocrine loops, or failed degradation). Finally, there may be an ascertainment bias regarding the referral practice in our tertiary hospital setting, and patients may present with higher stages of disease. However, because *MET*-amplified tumors tend to present at a higher stage (Fig 2), our case mix should be enriched for *MET*-amplified tumors but clearly is not, which also argues for rarity of this particular subset.

Although *MET* amplification is individually rare, given that amplifications of *MET*, *EGFR*, and *HER2* are largely exclusive events, our combined approach led to amplification detection in one of six patients screened, which may represent a feasible approach to implement clinical testing. Interestingly, our results are overall supportive of the 2010 American Joint Commission on Cancer staging updates (see Results Data Supplement),[27] and the distinct molecular phenotypes (Table 2), including differences in survival (Fig 3; Data Supplement), hold up within this representative cohort. This argues for incorporation of amplification status as a novel determinant into existing prognostication schemes.

The overall rarity and aggressive nature of *MET*-amplified GEC will make clinical trials of *MET* inhibitors challenging to design, conduct, and complete. We report preliminary responses in two of four such patients treated with crizotinib. Findings indicate that *MET* amplification has the potential to act as an oncogenic driver in vivo and renders at least a subset of *MET*-amplified tumors responsive to crizotinib. Mechanistically, these findings confirm our in vitro data[18] in patients with metastatic disease; however, the response rate, with the caveat of small numbers, is not as impressive as the exquisite sensitivity to MET inhibition observed in amplified cell lines.[18] This discrepancy may be based on the level of gene amplification, on tumor heterogeneity (one patient, Cr1, had focal *MET* amplification), or on the evolution of complete dependence on MET signaling during in vitro passage of cell lines. Nonetheless, the clinical responses in patients Cr3 and Cr4 suggest that MET is an important target. However, the transient nature of the responses indicates in vivo adaptation. This notion is supported by recent in vivo studies that implicate additional mutations in the MET activation loop (Y1230),[48] autocrine activation of the EGFR axis,[48] or amplification and overexpression of wild-type *KRAS*[49] as some of the underlying mechanisms in acquired resistance to MET inhibition. Although these findings suggest that combined strategies may result in synergistic effects, a larger cohort of patients with *MET*-amplified GEC will be necessary to understand the observed response patterns.

Our efforts highlight the practical hurdle imposed by the low prevalence of *MET* amplification in GEC. These efforts suggest that implementation of larger-scale, genome-wide assays—which would include assessment of *MET* copy number as well as other infrequent gene amplifications—may be an effective approach to identify multiple rare subgroups that might benefit from targeted therapies.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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