Increased MET and HGF gene copy numbers are associated with trastuzumab failure in HER2-positive metastatic breast cancer

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BACKGROUND: To investigate whether copy number gain of MET or hepatocyte growth factor (HGF) affect trastuzumab sensitivity in HER2-positive metastatic breast cancer (MBC).

METHODS: We analysed 130 HER2-positive MBC treated with trastuzumab-based therapy. MET and HGF gene copy numbers (GCN) were assessed by fluorescence in situ hybridisation (FISH) in primary breast cancer samples. Receiver operating characteristic analysis was applied to find the best cutoff point for both MET and HGF GCN.

RESULTS: MET FISH-positive cases (N = 36, mean GCN ≥ 3.72) had a significantly higher trastuzumab failure rate (44.4% vs 16.0%; P = 0.001) and a significantly shorter time to progression (5.7 vs 9.9 months; HR 1.74; P = 0.006) than MET FISH-negative cases (N = 94, mean GCN < 3.72). Hepatocyte growth factor GCN was evaluated in 84 cases (64.6%). Receiver operating characteristic analysis identified 33 HGF FISH-positive patients (mean HGF GCN ≥ 3.01). HGF FISH-positive status was significantly associated with higher risk of failure (30.3% vs 7.8%; P = 0.007) as compared with HGF FISH-negative cases (N = 51, mean GCN < 3.01). MET and HGF FISH-positive status was highly correlated (P < 0.001) and combination of both biomarkers did not increase predictive value of either considered separately.

CONCLUSION: High GCNs of MET and HGF associate with an increased risk of trastuzumab-based therapy failure in HER2-positive MBC.

Keywords: breast cancer; HER2-positive; HGF; MET

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor that belongs to the epidermal growth factor receptor (EGFR) family. Approximately 20–25% of breast cancers are characterised by HER2 protein overexpression or gene amplification, and such events are associated with poor prognosis (Slamon et al, 1987).

The huminsed recombinant monoclonal antibody trastuzumab (Herceptin, Genentech, Inc., San Francisco, CA, USA) was the first HER2-targeting agent approved for clinical use in breast cancer (Carter et al, 1992). In HER2-overexpressing or -amplified (HER2-positive) breast cancer patients, large phase III trials demonstrated that trastuzumab in combination with chemotherapy was significantly more effective than chemotherapy alone both in advanced disease and in adjuvant setting (Slamon et al, 2001; Piccart-Gebhart et al, 2005; Romond et al, 2005; Robert et al, 2006; Slamon et al, 2011). Although trastuzumab-based treatments represent today the standard approach for HER2-positive breast cancer, not all patients benefit from this therapy. In metastatic breast cancer (MBC) patients with high degree of HER2 expression, single-agent trastuzumab resulted in 35% response rate (Vogel et al, 2002), indicating that there is a considerable proportion of individuals potentially refractory to HER2 inhibition even in presence of the drug target. Moreover, one of the major clinical problems encountered with trastuzumab treatment is that MBC patients who initially respond to trastuzumab, show disease progression within 1 year from treatment initiation. A better knowledge of mechanisms responsible for primary and acquired resistance may improve prediction of trastuzumab sensitivity. Several mechanisms of resistance have been described to date, including co-expression of the truncated p95HER2 receptor (Scaltriti...
By fluorescence in situ was defined as positive in presence of gene amplification detected in Italy and Poland. The HER2 status was determined locally and combination with chemotherapy or as a single agent in 13 centres 130 HER2-positive MBC patients treated with trastuzumab in This retrospective study was conducted in a consecutive series of patients.

**PATIENTS AND METHODS**

**Patient selection**

This retrospective study was conducted in a consecutive series of 130 HER2-positive MBC patients treated with trastuzumab in combination with chemotherapy or as a single agent in 13 centres in Italy and Poland. The HER2 status was determined locally and was defined as positive in presence of gene amplification detected by fluorescence in situ hybridisation (FISH) or in presence of high degree of expression (3 +) by immunohistochemistry according to criteria described elsewhere (Hammond et al, 2011). MET and HGF GCN were evaluated on primary breast tumour tissue obtained at the time of surgery before any trastuzumab-based therapy. Main inclusion criteria adopted for patient selection included availability of primary breast cancer tumour tissue, possibility to verify the response according to RECIST criteria, and availability of clinical data including survival. The study was approved by the ethics committees of all local hospitals and was conducted in accordance with ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines on good clinical practice, whichever represented the greater protection of the individuals.

**Fluorescence in situ hybridisation analyses**

Unstained 4–5 μm sections were subjected to a tri-colour FISH assay, using a probe cocktail including HGF sequences (RP11-554M24 labelled in Spectrum Gold), MET sequences (RP 11-95I20 labelled in Spectrum Red) and centromere 7 sequences (CEP7, labelled in Spectrum Green, Abbott Molecular, Denver, CO, USA). The FISH assays were performed according to previously described protocol (Cappuzzo et al, 2009), including pre-treatment with 2 × SSC at 75 °C and digestion with Proteinase K for 5–20 min each, co-denaturation at 85 °C for 15 min, hybridisation for approximately 36 h and rapid post-hybridisation washes with 2 × SSC/0.1% NP40. Signals were enumerated in at least 50 tumour nuclei per specimen, using epifluorescence microscope with single interference filters at the following excitation/emission wavelengths: 350/460 for blue, 492/530 for green, 530/580 for gold and 572/625 for red, as well as dual (red/green) and triple (blue, red, green) band pass filters. For each slide, the mean and s.d. of copy number per cell of each tested DNA sequence, the percentage of cells with ≤2, 3 and ≥4 copies of each target and the ratio of MET/CEP7 and HGF/CEP7 were calculated. For documentation, images were captured using a CCD camera and merged using dedicated software (Leica Microsystems, Denver, CO, USA) (Figure 1).

MET FISH analysis was successfully performed in all 130 cases. Fluorescence in situ hybridisation analysis of HGF was only performed in 84 cases (64.6%), as adequate material was not available in 46 cases.

Lack of additional tumour sections did not allow us to perform additional biomarker analyses.

**Statistical analyses**

The primary end point of the study was to assess whether increased MET and HGF GCNs affect sensitivity to trastuzumab in

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**Figure 1** Hybridisation with the probe mix MET (Spectrum Red) CEP7 (Spectrum Green) and HGF (Spectrum Gold) showing both MET and HGF low GCN in (A) and high GCN in (B). The color reproduction of this figure is available at the British Journal of Cancer online.
terms of failure rate. Patients were dichotomised into sensitive (complete or partial response and disease stabilisation) and refractory (evidence of progressive disease at the first imaging assessment). The cutoff for \( \text{MET} \) and \( \text{HGF} \) GCN discriminating between a positive or negative result was determined using a receiver operating characteristic analysis. Time to progression (TTP) was calculated from the date of first administration of trastuzumab to the date of progression or last assessment. Overall survival was calculated from the date of first administration of trastuzumab to the date of death or last contact. Differences in failure rate were compared by Fisher’s exact test or \( \chi^2 \) test. Time to progression, OS and the 95% confidence intervals for the groups with negative and positive biomarker were evaluated by survival analysis using Kaplan–Meier method (Kaplan and Meier, 1985), and compared using the log-rank test. Statistical significance was set at <0.05 for each analysis. Multivariable analysis was performed using logistic regression analysis with a step-down procedure method, with response considered as independent variable. The model was built only on clinical variable, which were significantly associated with response rate at bivariate analysis.

**RESULTS**

**Patient characteristics**

A total of 130 HER2-positive MBC patients were included in this analysis. The vast majority of patients (\( N=109, 83.8\% \)) received trastuzumab in combination with chemotherapy in first-line setting (\( N=82, 63\% \), most frequently in association with taxanes (paclitaxel 32.3%, docetaxel 20.8%). The remaining 21 patients (16.1%) received single-agent trastuzumab (Table 1). In the entire study population, response rate, including complete and partial response was 49.2%, median TTP was 9.4 months (range: 8.3–10.5 months) and median OS was 28.3 months (range: 22.6–33.9 months).

**\( \text{MET} \) FISH results**

No \( \text{MET} \) gene amplification (defined as ratio mean \( \text{MET} \)/mean CEP 7 > 2) was detected and median mean \( \text{MET} \) GCN was 2.96 (range, 1.66–8.40 copies per cell). In two cases, an equivocal range (\( \text{MET} \)/CEP 7 ratio between 1.8 and 2) was observed (\( \text{MET} \)/CEP 7 ratio of 1.84 and 1.82, respectively). As illustrated in Figure 2A, receiver operating characteristic analysis identified a mean of 3.72 \( \text{MET} \) GCN as the optimal cutoff value for discriminating between sensitive and refractory patients. A total of 36 cases (27.7%) had mean \( \text{MET} \) /CEP 7 ratio ≥ 3.72 (\( \text{MET} \) FISH positive) and 94 cases (72.3%) had mean \( \text{MET} \) /CEP 7 ratio < 3.72 (\( \text{MET} \) FISH negative). As shown in Table 2, \( \text{MET} \) FISH status was not associated with any clinical or biological characteristic. However, \( \text{MET} \) FISH-positive patients had a significantly higher failure rate (44.4% vs 16.0%; \( P=0.001 \)) and a significantly shorter TTP (median 5.7 vs 9.9 months; HR: 1.74; 95% CI 1.16–2.62; \( P=0.006 \)) than \( \text{MET} \) FISH-negative patients (Figure 3A). \( \text{MET} \) FISH-positive patients had slightly shorter OS than \( \text{MET} \) FISH-negative patients (median 26.4 vs 29.1 months), but the difference was not statistically significant (HR: 1.12; 95% CI 0.65–1.93; \( P=0.681 \); Figure 3B). Importantly, a difference between \( \text{MET} \) FISH positive and \( \text{MET} \) FISH negative was observed in the small subgroup (\( N=21 \)) of individuals treated with trastuzumab alone. In such subgroup, failure rate (20.0% vs 50.0%) and TTP (median 9.5 vs 19.0 months) were in favour of \( \text{MET} \) FISH-negative patients, even if, probably because of the small numbers, differences were not statistically significant (\( P=0.3 \) and \( P=0.2 \), respectively).

**Fluorescence in situ hybridisation results of \( \text{HGF} \)**

Median mean \( \text{HGF} \) GCN was 2.80 (range, 1.14–6.90 copies per cell). As illustrated in Figure 2B, receiver operating characteristic analysis identified a mean of 3.01 \( \text{HGF} \) GCN as the optimal cutoff value for discriminating between sensitive and refractory patients. This cutoff split 33 cases (39.3%) as \( \text{HGF} \) FISH positive (mean \( \text{HGF} \) GCN ≥ 3.01) and 51 cases (60.7%) as \( \text{HGF} \) FISH negative (mean \( \text{HGF} \) GCN <3.01). As summarised in Table 3, \( \text{HGF} \) FISH status was not associated with any clinical characteristics, whereas there was a strong association between \( \text{HGF} \) and \( \text{MET} \) FISH status (\( P<0.001 \)): all \( \text{MET} \) FISH-positive cases resulted \( \text{HGF} \) FISH positive and all \( \text{HGF} \) FISH-negative cases were \( \text{MET} \) FISH negative. Patients who were \( \text{HGF} \) FISH positive had a significantly higher failure rate (30.3% vs 7.88%; \( P=0.007 \)) and a non-significantly shorter TTP (median 9.9 vs 10.5 months, HR 1.10 95% CI 0.70–1.74, \( P=0.665 \)) than \( \text{HGF} \) FISH-negative patients (mean <3.01; Figure 4A). Patients who were \( \text{HGF} \) FISH positive had a not statistically significant longer OS (median 35.2 vs 26.1 months, HR 0.83 95% CI 0.44–1.56, \( P=0.567 \)) than \( \text{HGF} \) FISH-negative patients (Figure 4B).

### Table 1: Patient characteristics

| Characteristics | Total | % |
|-----------------|-------|---|
| Total no. of patients | 130 | 100 |
| Median age, years (range) | 55 (33–80) | |
| Menopausal status | Available/not available | 104/26 | 80/20 |
| | Premenopausal/postmenopausal | 18/86 | 17.3/82.7 |
| Histology | Invasive ductal carcinoma | 114 | 87.7 |
| | Invasive lobular carcinoma | 8 | 6.1 |
| | Other types | 8 | 6.1 |
| Grade | 2 | 47 | 36.1 |
| | 3 | 68 | 52.3 |
| | Not defined | 15 | 11.5 |
| Hormonal status (IHC) | ER value ≥ 10% | 52 | 40 |
| | PgR value ≥ 10% | 43 | 33 |
| **MB1/Ki67** | Available/not available | 47/83 | 36.1/63.8 |
| | Value ≥ 10% | 44 | 93.6 |
| HER2 + (IHC/FISH) | IHC 3 + and FISH not done | 76 | 58.5 |
| | IHC 2 + and FISH amplified | 6 | 4.6 |
| | IHC 3 + and FISH amplified | 48 | 36.9 |
| Treatment | Trastuzumab monotherapy | 21 | 16.1 |
| | Trastuzumab with chemotherapy | 109 | 83.8 |
| **Line of treatment** | First line | 82 | 63 |
| | Second line | 37 | 28.5 |
| | Third or subsequent lines | 11 | 8.5 |
| **Drug combined with trastuzumab** | None | 21 | 16.1 |
| | Paclitaxel | 42 | 32.3 |
| | Docetaxel | 27 | 20.8 |
| | Vinorelbine | 25 | 19.2 |
| | Other | 15 | 11.5 |

**Abbreviations:** MBC = metastatic breast cancer; ER = oestrogen receptor; PgR = progesterone receptor; HER2 + = human epidermal growth factor receptor 2 positive (overexpression and/or amplification); IHC = immunohistochemistry; FISH = fluorescent in situ hybridisation.
Clinical Studies

**Figure 2** (A) Receiver operating characteristic (ROC) analysis identified a mean of 3.72 MET GCN as the optimal cutoff value discriminating sensitive and resistant population, associated with a sensitivity of 51.6% and a specificity of 79.8%. AUC (area under the curve) value was 0.679. (B) ROC analysis identified a mean of 3.01 HGF GCN as optimal cutoff value, associated with a sensitivity of 71.4% and a specificity of 67.1%. AUC (area under the curve) value was 0.663.

**Table 2** Association between MET gene copy number and clinical and biological characteristics in HER2-positive metastatic breast cancer patients (N = 130)

| Characteristic | MET FISH + (N/%) | MET FISH - (N/%) | P-value |
|---------------|-----------------|-----------------|---------|
| All           | 36/27.7         | 94/72.3         |         |
| Age ≥55 years | 17/47.2         | 47/50.0         | 0.777   |
| Age <55 years | 19/52.8         | 47/50.0         |         |
| Prenopausal   | 6/19.4          | 12/16.4         | 0.719   |
| Postmenopausal| 25/80.6         | 61/83.6         |         |
| Invasive ductal carcinoma | 33/91.7 | 81/86.2 | 0.554 |
| Other histology | 3/83          | 13/13.8         |         |
| Grade 2       | 9/29.0          | 38/45.2         | 0.117   |
| Grade 3       | 22/71.0         | 46/54.8         |         |
| IHC ER value ≥10% | 15/41.7     | 37/39.4         | 0.810   |
| IHC ER value <10% | 21/58.3       | 57/60.6         |         |
| IHC PgR value ≥10% | 12/33.3      | 31/33.0         | 0.969   |
| IHC PgR value <10% | 24/66.7      | 63/67.0         |         |
| MiB1/Ki67 value ≥10% | 35/97.2    | 92/97.9         | 1.000   |
| MiB1/Ki67 value <10% | 1/28          | 2/21.2          |         |

Abbreviations: MET = mesenchymal-epithelial transition factor; HER2 = human epidermal growth factor receptor 2; ER = oestrogen receptor; PgR = progesterone receptor; IHC = immunohistochemistry; FISH = fluorescent in situ hybridisation.

**MET/HGF FISH combination**

To further investigate the impact of combined MET and HGF GCNs, we analysed the outcome of the 84 patients in whom both biomarkers were assessable. As illustrated in Table 4, overall results confirmed that failure rate was significantly lower in the population negative for both MET and HGF (P = 0.007), with the percentage of progressing patients not significantly different that with detected with a single biomarker assay (failure rate: 7.8% in MET and HGF negative, 7.8% in HGF negative only and 16.0% in MET negative only).

**Univariate and multivariate analysis**

To define which variables were predictive of trastuzumab sensitivity, clinical and biological characteristics, such as age (<55 vs ≥55 years), menopausal status (pre vs post), grade (2 vs 3), oestrogen receptor status (<10% vs >10%), progesterone receptor status (<10% vs >10%), proliferative activity (MiB1 <10% vs >10%), MET GCN (<3.72 vs >3.72) and HGF GCN (<3.01 vs >3.01) were evaluated in a univariate analysis, using trastuzumab failure rate as end point. Variables found significant in the univariate analysis (MET and HGF GCNs) were included in the multivariate model. Because of the strong correlation between MET and HGF, multivariable model did not include both biomarkers at the same time. When MET was excluded, increased GCN of HGF resulted in a odds ratio of 5.87 (95% CI: 1.21–28.39, P = 0.028). When HGF was not included, increased GCN of MET resulted in a odds ratio of 6.02 (95% CI: 2.24–16.8, P < 0.001).

**DISCUSSION**

The present study, the first evaluating the role of MET and HGF GCN in a large cohort of HER2-positive MBC patients treated with trastuzumab-based therapy, provides important evidence for the critical role of HGF/MET signalling pathway for sensitivity to anti-HER2 agents. Increased MET or HGF GCNs were detected in approximately one-fourth cases of HER2-positive breast cancer and were significantly associated with higher risk of treatment failure, supporting a role of anti-MET strategies in breast cancer. Predictive value of MET and HGF GCN was similar and combining both biomarkers did not increase sensitivity of the assay.

MET is a plasma membrane protein that relays signals from the extracellular environment into the cytoplasm, activated when its extracellular domain binds to HGF, also known as scatter factor. Recent data demonstrated that increased MET GCN represents a negative prognostic factor in human malignancies including lung (Cappuzzo et al, 2009) and gastric cancer (Graziano et al, 2011). Preclinical and limited clinical data showed that MET amplification is an event responsible for resistance to agents interfering with the EGFR family. In lung cancer, two studies demonstrated that MET amplification occurs in approximately 15–20% of EGFR-mutant non-small-cell lung cancers with acquired resistance to the reversible EGFR tyrosine kinase inhibitors, gefitinib or erlotinib (Bean et al, 2007; Engelman and Jänne, 2008). In non-small-cell lung cancer, combination of anti-MET agents with anti-EGFR tyrosine kinase inhibitors (EGFR-TKIs) seems to be one of the most promising strategies to overcome acquired resistance to such agents (Engelman et al, 2007). Although in breast cancer MET is generally not focally amplified (Shattuck et al, 2008), recent studies...
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Figure 3  Time to progression (A) and survival (B) in MET FISH-positive and -negative patients, according to the cutoff of 3.72 GCN identified with the receiver operating characteristic (ROC) analysis. MET FISH-positive patients ($N = 36$, 27.7%) had a significantly shorter time to progression (median 5.7 vs 9.9 months, HR 1.74; $P = 0.006$) and a non-significant shorter survival (median 26.4 vs 29.1 months, HR 1.12; $P = 0.681$) than MET FISH-negative ($N = 94$, 72.3%).

Table 3  Association between HGF gene copy number and clinical and biological characteristics in HER2-positive metastatic breast cancer patients ($N = 84$)

| Characteristic | HGF FISH + (N %) | HGF FISH – (N %) | P-value |
|---------------|------------------|------------------|---------|
| All           | 33/39.3          | 51/60.7          |         |
| Age $\geq 55$ years | 17/51.5          | 26/51.0          | 0.962   |
| Age $< 55$ years          | 16/48.5          | 25/49.0          |         |
| Premenopausal          | 0               | 4/11.1           | 0.287   |
| Postmenopausal          | 22/100          | 32/88.9          |         |
| Invasive ductal carcinoma | 28/84.8         | 43/84.3          | 0.947   |
| Other histology         | 5/15.2          | 8/15.7           |         |
| Grade 2               | 9/32.1          | 23/51.1          | 0.112   |
| Grade 3               | 19/67.9         | 22/48.9          |         |
| IHC ER value $\geq 10\%$ | 13/39.4         | 19/37.3          | 0.844   |
| IHC ER value $< 10\%$     | 20/60.6         | 32/62.7          |         |
| IHC PgR value $\geq 10\%$ | 9/27.3          | 17/33.3          | 0.557   |
| IHC PgR value $< 10\%$   | 24/72.7         | 36/66.7          |         |
| MIB1/Ki67 value $\geq 10\%$ | 33/100          | 50/98.0          | 1.000   |
| MIB1/Ki67 value $< 10\%$ | 0               | 1/2.0            |         |
| MET FISH +          | 21/63.6         | 0                | $<0.001$ |
| MET FISH –          | 12/36.4         | 51/100           |         |

Abbreviations: HGF = hepatocyte growth factor; FISH = fluorescent in situ hybridisation; MET = mesenchymal-epithelial transition factor; HER2 = human epidermal growth factor receptor 2; ER = oestrogen receptor; PgR = progesterone receptor; IHC = immunohistochemistry.

Table 4  Associations between MET and HER2 FISH amplification and clinicopathological characteristics in HER2-positive metastatic breast cancer patients ($N = 84$)

| Characteristic                  | MET FISH + (N %) | MET FISH – (N %) | P-value |
|--------------------------------|------------------|------------------|---------|
| All                            | 39/47.0          | 45/53.0          |         |
| Age $\geq 55$ years            | 17/51.5          | 26/51.0          | 0.962   |
| Age $< 55$ years               | 22/61.5          | 29/38.5          |         |
| Premenopausal                  | 22/65.8          | 12/34.2          | 0.001   |
| Postmenopausal                 | 17/51.5          | 26/48.5          |         |
| Invasive ductal carcinoma      | 25/71.4          | 32/28.6          | 0.121   |
| Other histology                | 5/15.2           | 8/15.7           |         |
| Grade 2                        | 9/32.1           | 23/51.1          | 0.112   |
| Grade 3                        | 19/67.9          | 22/48.9          |         |
| IHC ER value $\geq 10\%$       | 14/41.2          | 18/58.8          | 0.432   |
| IHC ER value $< 10\%$          | 23/69.7          | 25/30.3          |         |
| IHC PgR value $\geq 10\%$      | 9/27.3           | 17/33.3          | 0.557   |
| IHC PgR value $< 10\%$         | 26/72.7          | 34/66.7          |         |
| MIB1/Ki67 value $\geq 10\%$    | 33/100           | 50/98.0          | 1.000   |
| MIB1/Ki67 value $< 10\%$       | 0                | 1/2.0            |         |
| MET FISH +                      | 21/63.6          | 0                | $<0.001$ |
| MET FISH –                      | 12/36.4          | 51/100           |         |

Abbreviations: MET = mesenchymal-epithelial transition factor; HER2 = human epidermal growth factor receptor 2; FISH = fluorescent in situ hybridisation; HR = hazard ratio; CI = confidence interval.

Our findings are in agreement with previous studies confirming that MET amplification is generally absent in breast cancer and that high MET GCN increases the risk of treatment failure (Shattuck et al, 2008). Response and TTP favoured patients with no MET GCN gain, both in patients treated with trastuzumab plus chemotherapy and in patients treated with trastuzumab alone, supporting the potential therapeutic impact of anti-MET agents in MBC, particularly in combination with anti-HER2 agents.

Recently, Previdi et al (2012) showed that ARQ 197 (tivantinib), a c-MET inhibitor, significantly delays the onset and progression of bone metastases in in vitro and in vivo models, strongly suggesting that targeting c-MET may have therapeutic value in the treatment of MBC. In another study, Liu et al (2011) characterised MET and HER expression and signalling in a panel of human tumour cell lines and demonstrated the differential susceptibility of these cell lines to single agents or combinations of foretinib, a multi-kinase MET inhibitor, with HER-targeted agents, erlotinib or lapatinib. Interestingly, MET-amplified lines with EGFR or HER2 amplification were more sensitive to the combination of foretinib with lapatinib or erlotinib. Overall, these data suggest that therapy including a combination of anti-MET and anti-HER-targeted agents should be tested as a treatment option in HER2-positive patients with MET-amplified or -overexpressing tumours. The idea of combining trastuzumab with other targeted agents is not a new concept in breast cancer, as demonstrated in recent studies comparing trastuzumab with the combination of trastuzumab and pertuzumab, a novel anti-HER2 monoclonal antibody (Baselga et al, 2012; Gianni et al, 2012) or single-agent lapatinib with the association of trastuzumab and lapatinib in trastuzumab-refractory patients (Blackwell et al, 2010).

Another interesting finding in our study was the strong association of MET and HGF GCN. To the best of our knowledge, this is the first study reporting such association in breast cancer. Moreover presence of increased GCN of both MET and HGF in the same tumours explains why a single test was equally predictive than the combination of both assays. Although MET activation can either occur through ligand-independent or ligand-dependent mechanisms (Kang et al, 2003), our findings suggest that probably ligand-dependent MET activation could represent a relevant mechanism in HER2-positive breast cancer, indicating a potential role for anti-MET monoclonal antibodies in breast cancer. In a recent study, Xie et al (2012) demonstrated that HGF autocrine expression correlated with phospho-MET levels in HGF autocrine cell lines and these cell lines showed high sensitivity to MET inhibition. Our findings together with that of Xie et al (2012) data suggest that MBC patients with high HGF and MET levels could result particularly sensitive to MET therapeutics.

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**Figure 4** Time to progression (A) and survival (B) in HGF FISH-positive and -negative patients, according to the cutoff of 3.01 GCN identified with the receiver operating characteristic (ROC) analysis. HGF FISH-positive patients (N = 33, 39.3%) had a not significant shorter time to progression (median 9.9 vs 10.5 months, HR 1.10; P = 0.665) and a longer, not statistically significant, survival (median 35.2 vs 26.1 months, HR 0.83; P = 0.567) than HGF FISH-negative patients (N = 51, 60.7%).

**Table 4** Outcome according to MET and HGF GCN

| Biomarker | N   | Failure rate (N/%) | TTP (months) | OS (months) |
|-----------|-----|-------------------|-------------|-------------|
| A         | 21  | 6/28.6            | 9.2         | 26.4        |
| B         | 31  |                   |             |             |
| C         | 12  | 4/33.3            | 10.3        | 48.8        |
| D         | 51  | 4/78.8            | 10.5        | 26.1        |

P-value: A vs C = 0.007 0.7 0.6, A vs D = 0.054 0.2 0.9, A + C vs D = 0.007 0.7 0.6.

Table 4: Outcome according to MET and HGF GCN.

In conclusion, this large retrospective study showed that HGF/MET signalling pathway interferes with trastuzumab-based therapy sensitivity in HER2-positive MBC. These data, together with previous preclinical and clinical studies support further expansion of related studies and clinical development of anti-MET agents in combination with anti-HER2 compounds in HER2-positive MBC.

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**Conflict of interest**

The authors declare no conflict of interest.

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In conclusion, this large retrospective study showed that HGF/MET signalling pathway interferes with trastuzumab-based therapy sensitivity in HER2-positive MBC. These data, together with previous preclinical and clinical studies support further expansion of related studies and clinical development of anti-MET agents in combination with anti-HER2 compounds in HER2-positive MBC.

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**Conflict of interest**

The authors declare no conflict of interest.

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