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ORIGINAL ARTICLE

HER2 Status in Gastric Cancer: Comparison between Primary and Distant Metastatic Disease

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Abstract HER2 (human epidermal growth factor receptor-2) assessment in histological samples of gastric cancer is essential to determine which patients might benefit from trastuzumab therapy. HER2 is often evaluated in primary tumor even if trastuzumab therapy is used to treat metastatic disease. However, the exact relationship in terms of HER2 status between primary and metastatic tumors has not been fully clarified. We aimed to evaluate the HER2 status concordance between primary gastric cancer and corresponding distant metastasis. HER2 status was evaluated by IHC (immunohistochemistry) and/or FISH (fluorescence in situ hybridization) in 41 patients in primary gastric cancer and in paired metastasis. HER2 was assessed according scoring criteria applied in clinical approach. HER2 positivity was found in 14,6 % primary tumors and in 24,4%corresponding metastasis. HER2 concordance rate between primary and metastasis was 80,5 % (K-value = 0,388). Eight/41 (19,5 %)cases resulted discordant: 6 patients with metastatic HER2 positive lesions were found HER2 negative in primary cancers while 2 patient HER2 positive in primary lesion showed a negative conversion in metastasis. Our results showed a good concordance in terms of HER2 status between primary and metastatic lesions, as well as in biopsy and surgical removed specimens. However, the higher rate of HER2 positive status found in metastatic lesions underlined the importance of HER2 assessment in all samples obtained from different sites of gastric cancer disease.

Keywords HER2 · Gastric cancer · FISH · Immunoistochemistry · Metastatic disease

Abbreviations

HER2	Human Epidermal Growth Factor Receptor
FISH	Fluorescence in situ hybridization
IHC	Immunoistochemistry
FFPE	formalin fixed-paraffin embedded

Introduction

Pharmacological cancer therapy for decades was performed with non-targeted mostly DNA-interacting cytostatic drugs [1]. One of the main disadvantages of those substances is that they do not specifically target cancer cells but all (also benign) rapidly dividing cells [2]. This non-specific mechanism of action was the rationale to develop specifically targeted anticancer TK (tyrosine kinase) molecules. For instance Trastuzumab is approved in combination with chemotherapy as a new standard option for patients with HER2 positive advanced gastric or gastro esophageal cancer [3]. However, the benefit of trastuzumab was limited to patients with a score of immunohistochemistry (IHC) 3+ or 2+ and FISH-positive [3, 4]. In gastric adenocarcinoma, HER2 (Human Epidermal

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Growth Factor Receptor) gene amplification and/or protein overexpression is found in 7–34 % primary tumors [5–9]. After the approval of trastuzumab for HER2 positive metastatic gastric and gastro-esophageal junction tumors in clinical practice, HER2 status assessment in histological samples has become essential to determine which patients might benefit from trastuzumab therapy. Although trastuzumab-based therapy is used to treat metastatic disease, the evaluation of HER2 status is mostly performed in the primary tumor, because metastatic sites are rarely biopsied before treatment [3]. A number of studies showed a high concordance rate in terms of HER2 status in primary lesions and in their corresponding locoregional lymph node metastases. However, the relationship between primary gastric cancer and distant metastatic disease has not been fully clarified. Previously, in a small court of patients, we reported that HER2 amplification status in primary gastric cancer correlated with corresponding metastatic disease [10].

Aim of this study is to assess the concordance rate of HER2 status in primary gastric cancer and distant metastatic lesions in a relative large cohort of patients. Moreover differences between endoscopic biopsy and surgical resection were also evaluated.

Materials and Methods

Clinicopathological Data and Histological Specimens

Forty-one metastatic gastric cancers were retrieved from the archives of Pathology Unit at Campus Bio-Medico, Rome consecutively selected on the basis of availability of primary and metastatic tissue. Clinical pathological staging (TNM) of the primary tumors was determined according to the International Union Again Cancer [11], histological type according to Lauren's classification [12] and differentiation grade according to WHO classification [13]. Both surgical and gastric biopsy specimens of primary tumor lesions were available for 21 patients while only biopsy or surgical specimens were available in 5 and 15 cases respectively. The median number of endoscopic biopsy fragments per patients was 7 (range 5–9). As regards metastatic lesions, 27/41 (65,8 %) were surgical samples and 14/41 (34,2 %) were bioptic ones. Patients underwent to neoadjuvant chemotherapy treatments were not included for the present study. Clinicopathological features are summarized in Table 1.

For each case, formalin fixed-paraffin embedded (FFPE) tissue blocks were selected on the basis of quality and representativeness of tumor. Three micron consecutive slides were obtained from each FFPE block in order to perform IHC and/or FISH (Fluorescence in situ hybridization) analysis. The study protocol, conformed to the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the local Ethics Committee.

Table 1 Clinical and pathological features

Feature		n (%)
Gender	Male	20(49)
	Female	21(51)
Age	≤ 50	8(19,5)
	>50	33(80,5)
pT*	T1	0
	T2	6(16,7)
	T3	17(47,2)
	T4	13(36,1)
pN*	N-	4 (11,1)
	N+	32 (88,9)
Grade	Moderate	10(24,4)
	High	31(75,6)
Histotypes	Intestinal	23 (56,1)
	Diffuse	18 (43,9)
Metastatic site	Omentum	21 (51,2)
	Distant lymph nodes	11(26,8)
	Liver	4(9,7)
	Bowel	4(9,8)
	Skin	1(2,4)

pT tumour size; *pN* regional lymph nodes

*pT and pN were obtained from original pathology reports of surgical resection specimens

HER2 Assessment – IHC

Immunohistochemistry was carried using Autostainer LINK48 (Dako). In brief, after deparaffinisation, endogenous peroxidase was blocked by 3 % hydrogen peroxide and antigen retrieval was performed by PT Link (Dako) with citrate buffer. Polyclonal rabbit anti-human c-erbB-2 oncoprotein was used as primary antibody (Dako Corp, Carpinteria,CA, USA) at 1/400 dilution. Sections were incubated with Envision Flex Mini Kit. Diaminobenzidine was used for color development and hematoxylin was used for counterstaining (Fig. 1). The normal gastric epithelium was used as internal control. Negative control slides, processed without primary antibody, were included for each staining. In case of surgical specimens (both in primary and metastatic lesions), at least two paraffin blocks were analyzed and the higher HER2 value was considered for HER2 status definition.

HER2 immunostaining was scored according four tiered scoring system proposed for gastric cancer adenocarcinoma: score 0 indicates no stain or membrane stain in less than 10 % of cells; score 1, faint/barely perceptible membrane stain in 10 % or more of cells (cells are only stained in part of their membrane); score 2, weak to moderately complete or baso-lateral stain in 10 % or more of tumor cells; and score 3, moderate to strong complete or baso-lateral stain in 10 % or more of tumor cells [8]. Samples that scored 3+ were considered positive, while samples that scored 0 or 1+ were considered HER2 negative

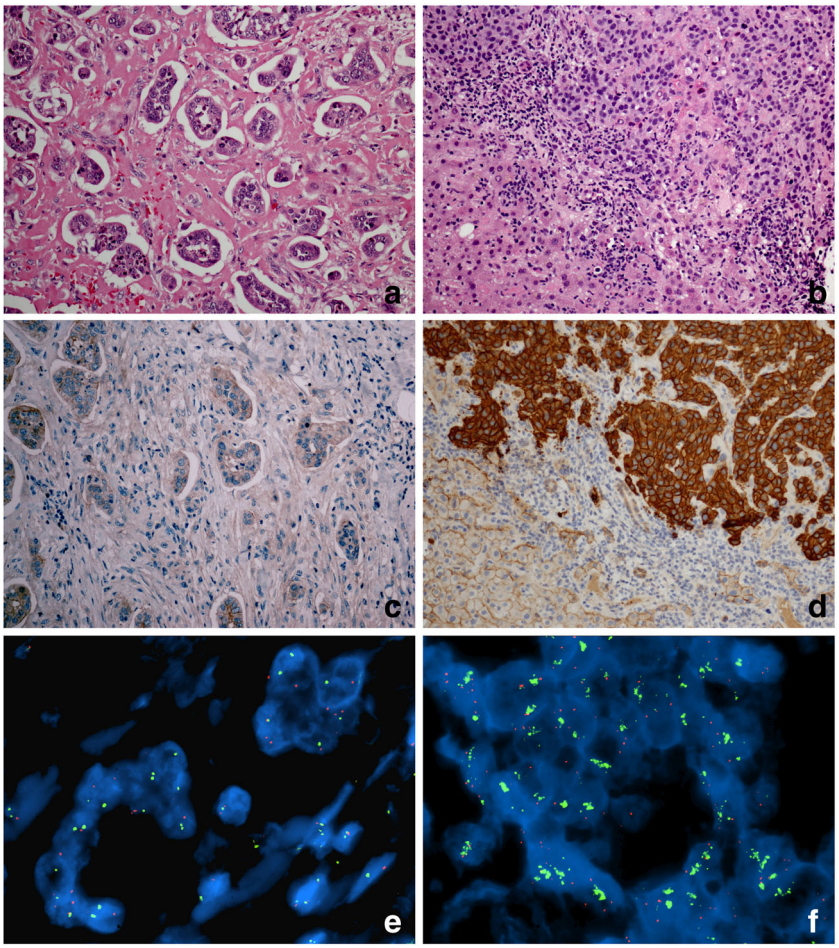

Fig. 1 HER2 status between primary and corresponding metastatic liver gastric cancer. **a, b**) Hematoxylin-eosin staining; **c, d**) c-erbB2 immunohistochemical staining; **e, f**) HER2 FISH staining. **a, c, e**) Primary intestinal type gastric cancer. **b, d, f**) Corresponding metastatic liver disease. Figure shows discordant HER2 status (immunohistochemistry and FISH) between primary gastric cancer and corresponding distant metastatic lesions. In particular, **c** c-erbB2 membranous immunostaining of primary gastric cancer was faint and incomplete in <10 % of tumor cells (IHC score = 0) while (**d**) strong and complete in >10 % of tumor cells (IHC score = 3) in corresponding liver metastasis. Consistently, FISH analysis demonstrated HER2 amplification status (cluster pattern) in metastatic disease (**f**) whereas normal HER2 status in primary gastric lesion (**e**). Original magnification 200X (**a, b, c, d**);1000X (**e, f**)

(Table 2). Cases with 2+ HER2 score were considered equivocal and were evaluated by FISH. In case of biopsy (not surgery) samples with cohesive either IHC 3+ and/or FISH + clones are considered positive irrespective of percentage [8].

Slides were examined blinded by 2 different investigators without knowledge of the corresponding clinicopathological data. Agreement in immunohistochemical evaluation between the 2 observers was more than 90 %. In cases of disagreement, a final score was determined by consensus after reexamination.

HER2 Assessment – FISH

HER2 amplification was assessed by ZytoLight® FISH-Tissue Implementation Kit: spectrum Green fluorophore-labeled DNA probe for HER2 gene locus and a Spectrum Orange fluorophore-labeled α-satellite DNA probe for chromosome 17 (SPEC HER2/CEN 17 Dual Color Probe Kit, ZytoVision). Slides were hybridized using the Hybrite denaturation/hybridization system (Fig. 1). FISH images were processed with an Olympus MX60 fluorescence microscope (Olympus, Hamburg, Germany) equipped with a 100-W mercury lamp. The HER2 gene was considered amplified when HER2/CEN17 ratio was ≥2.0 or HER2 cluster signal was observed [8].

Statistical Analysis

Concordance between HER2 status on primary vs metastatic sites was calculated as the ratio of concordant cases to total cases. Cohen's kappa coefficient (K-value) was used as statistical measure of inter-rater agreement in terms of HER2 status in different histopathological samples; k-values <0 as indicating no agreement and 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect agreement [14]. The clinicopathological variables included sex, age, grading, pT and pN factors, and Her2 status values <0.05 were regarded as statistically significant in two-tailed Spearman tests. SPSS software (Version 13.00, SPSS, Chicago, IL) was used for statistical analysis.

Results

Clinicopathological Features and HER2 Status

The HER2 status was evaluated by IHC and/or FISH in 41 patients in primary gastric cancer lesions (biopsy or surgical specimens) and in their corresponding metastatic disease. In

Table 2 c-erbB2 immunohistochemistry expression in primary (bioptic and/or surgical) and metastatic lesions

Case	Biopsy specimen					Surgical specimen					Metastatic lesion				
	% c-erbB2 positive cells				IHC Score	% c-erbB2 positive cells				IHC Score	% c-erbB2 positive cells				IHC Score
	0	1+	2+	3+		0	1+	2+	3+		0	1+	2+	3+	
1	75	20	15	0	2+	100	0	0	0	0	100	0	0	0	0
2	5	15	80	0	2+	90	5	5	0	0	0	20	80	0	2+
3	80	20	0	0	1+	100	0	0	0	0	100	0	0	0	0
4	50	30	20	0	2+	30	70	0	0	1+	100	0	0	0	0
5	80	0	20	0	2+	30	40	30	0	2+	100	0	0	0	0
6	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0
7	100	0	0	0	0	–	–	–	–	–	100	0	0	0	0
8	70	30	0	0	1+	100	0	0	0	0	100	0	0	0	0
9	–	–	–	–	–	0	10	40	50	3+	0	0	20	80	3+
10	0	0	40	60	3+	100	0	0	0	0	0	0	30	70	3+
11	10	40	50	0	2+	–	–	–	–	–	70	30	0	0	1+
12	70	30	0	0	1+	95	0	5	0	0	30	50	20	0	2+
13	30	40	30	0	2+	40	60	0	0	1+	80	20	0	0	1+
14	100	0	0	0	0	–	–	–	–	–	100	0	0	0	0
15	100	0	0	0	0	–	–	–	–	–	100	0	0	0	0
16	90	0	10	0	2+	95	0	5	0	0	50	30	20	0	2+
17	60	10	30	0	2+	100	0	0	0	0	100	0	0	0	0
18	100	0	0	0	0	–	–	–	–	–	0	20	80	0	2+
19	–	–	–	–	–	100	0	0	0	0	100	0	0	0	0
20	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0
21	–	–	–	–	–	100	0	0	0	0	100	0	0	0	0
22	40	30	30	0	2+	20	30	50	0	2+	70	30	0	0	1+
23	–	–	–	–	–	100	0	0	0	0	100	0	0	0	0
24	–	–	–	–	–	70	0	30	0	2+	20	30	50	0	2+
25	–	–	–	–	–	100	0	0	0	0	50	20	30	0	2+
26	–	–	–	–	–	80	20	0	0	1+	80	20	0	0	1+
27	–	–	–	–	–	100	0	0	0	0	100	0	0	0	0
28	–	–	–	–	–	30	65	5	0	1+	0	10	90	0	2+
29	20	80	0	0	1+	20	20	10	50	3+	0	0	50	50	3+
30	–	–	–	–	–	60	20	20	0	2+	80	0	20	0	2+
31	20	40	40	0	2+	30	10	10	50	3+	80	0	20	0	2+
32	60	40	0	0	1+	30	70	0	0	1+	0	30	70	0	2+
33	–	–	–	–	–	0	20	80	0	2+	0	100	0	0	1+
34	0	100	0	0	1+	100	0	0	0	0	0	100	0	0	1+
35	–	–	–	–	–	20	10	70	0	2+	0	100	0	0	1+
36	0	20	80	0	2+	100	0	0	0	0	20	30	50	0	2+
37	–	–	–	–	–	100	0	0	0	0	0	100	0	0	1+
38	–	–	–	–	–	0	10	90	0	2+	100	0	0	0	0
39	0	20	80	0	2+	0	20	80	0	2+	0	20	40	40	3+
40	100	0	0	0	0	70	30	0	0	1+	20	30	50	0	2+
41	–	–	–	–	–	0	80	20	0	2+	0	80	20	0	2+

21 cases, gastric biopsies and surgical resection specimens from same primary lesion were available. Therefore, a total of 103 tissue samples were assessed in term of HER2 status (Table 3).

Table 3 HER2 status assessment in 41 primary gastric cancer (bioptic and surgical specimens) and corresponding distant metastatic lesions

	Tot	IHC 0 (%)	IHC 1+ (%)	IHC2+ (%)		IHC 3+ (%)	HER2+ (%)
				FISH-	FISH+		
Surgical specimens	36	18 (50)	6 (16,7)	6 (16,7)	3 (8,3)	3 (8,4)	6 (16,7)
Biopsy	26	7 (26,9)	6 (23,1)	10 (38,5)	2 (7,7)	1 (3,8)	3 (11,5)
Metastatic lesions	41	16 (39)	8 (19,5)	7 (17)	6 (14,6)	4 (9,7)	10 (24,4)
Tot	103	41	20	22	11	8	19

IHC immunohistochemistry; *FISH* fluorescence in situ hybridation

To define HER2 status on primary lesions, biopsy specimens were considered if patient was not underwent to surgical resection. Otherwise, surgical specimens were used for HER2 status evaluation. In our series 6/41 (14,6 %) of primary lesions showed HER2 positivity: 5 intestinal and 1 diffuse histotype. In terms of HER2 status, significant statistical difference was found between intestinal and diffuse histologic types (p = 0,024). No differences were found according sex, age, differentiation grade, pT and pN status.

HER2 Status: Primary Vs Metastatic Gastric Cancer

HER2 positivity (IHC 3+ and IHC 2+/FISH+) was observed in 6/41 (14,6 %) primary tumors (surgical or biopsy specimens). Differently, HER2 positive status was found in 10/41 (24,4 %) specimens of metastatic lesions. A significant statistical correlation in terms of HER2 status between primary and metastatic lesions was found (Spearman test; p = 0.008; r = 0,408) with a concordance rate of 80,5 % (k-value =0,388). However, 6 patients with metastatic HER2 positive disease resulted HER2 negative in the respective primary lesions and 2 patient with primary HER2 positive gastric cancer were found negative in metastatic disease: take together, 8/41 (19,5 %) cases resulted discordant (Table 4). As regard metastatic lesions, 33/41 (80,5 %) were synchronous and 8/41 (19,5 %) metachronous disease. In 1/8 (12,5 %) metachronous lesion a discordant HER2 status (negative conversion) was found. Moreover, 7/33 (21,2 %) synchronous metastasis showed a discordant HER2 status (6 positive and 1 negative conversion).

HER2 Status: Endoscopic Biopsies Vs Surgical Specimens

HER2 status was analyzed in 21 cases both in endoscopic biopsies and surgical specimens of primary gastric lesions. Concordance value was 85,7 % (k-value was 0,66). Three/21 (14,3 %) cases resulted discordant: 2 HER2 positive biopsy resulted negative in surgical specimens and 1 HER2 negative biopsy resulted positive in respectively surgical specimen. Interestingly, these three patients resulted HER2 positive on their metastatic disease (Table 5).

Discussion

The present study confirms a limited concordance between primary and metastatic distant lesions in terms of HER2 status (concordance rate of 80,5 %; K-value =0,388). In fact, our data underline a higher rate of HER2 positive status in distant metastatic disease (24,4 %) rather than in the related primary cancers (14,6 %).

Based on TOGA trials, HER2 evaluation acquired high relevance in the management of patients with advanced gastric or gastro-esophageal junction cancer. To date, a HER2 accurate assessment is crucial to identify patients who could benefit trastuzumab therapy. Frequently, metastatic lesions cannot be biopsied or surgically resected and HER2 status is assessed in primary gastric cancer (surgical gastric resection or endoscopic biopsy). This approach is based on the notion that the HER2 status does not undergo significant change during dis-

Table 4 HER2 status in 41 primary gastric cancer and corresponding distant metastases

HER2 in primary cancer	HER2 in metastases		Tot
	HER2- (%)	HER2+ (%)	
HER2-	29 (82,9)	6 (17,1)	35
HER2+	2 (33,3)	4 (66,7)	6
Tot	31	10	41

HER2 human epidermal grown factor receptor 2

Table 5 HER2 status on 21 biopsies and corresponding surgical specimens

HER2 in biopsy	HER2 in surgical specimens		Tot
	HER2-	HER2+	
HER2-	17 (94,4)	1 (5,6)	18
HER2+	2 (66,6)	1 (33,3)	3
Tot	19	2	21

HER2 human epidermal grown factor receptor 2

ease progression as also reported in breast cancer pathology [15, 16]. Similar results were reported on gastric cancer indicating a good concordance of HER2 status between primary gastric cancer and metastatic disease. For example, Marx et al. reported that gastric carcinoma showed identical HER2 status (no discordant cases were described) in 49 primary gastric cancer and their corresponding lymph node metastases [9]. Similarly, Pagni et al. showed that HER2 status in primary gastric cancer is generally maintained in metastatic lymph node although a discordant HER2 status was found in 4/34 (11,8 %) cases [17]. Consistent with this findings, Kochi M et al. showed a significant correlation between primary gastric cancer lesions and lymph node metastases. In this study, 10/102 (9.8 %) discordant cases were found: 4/102(3.9 %) cases HER2 positive status was observed in primary cancer with negativity conversion in the lymph node metastases, while 6 (5.9 %) HER2 negative in primary gastric cancer showed positive conversion in corresponding lymph node metastases [18]. In a preliminary study, we treated this issue [10], confirming a good concordance between primary and metastatic disease in terms of HER2 status: however, 3 cases on 27 studied were found discordant. Therefore, the question about HER2 concordance status between primary and metastatic cancer remains unclear.

Here, we examined HER2 status in a relative large court of primary gastric cancer patients and, differently by above cited studies, corresponding distant metastases collected for clinical intent. In the present study, 8/41 (19,5 %) cases were found discordant in term of HER2 status between primary and corresponding distant metastatic disease: 6 patients resulted HER2 positive in distant metastatic lesions were found HER2 negative in the respective primary cancer and 2 patients classified as HER2 positive in primary lesions showing HER2 negativity in corresponding distant metastatic disease. Our data demonstrated a higher rate of HER2 positive status in distant metastatic disease rather than in the related primary cancers due to a positive conversion of HER2 status. Similar data was obtained by Gumusay et al. evaluating HER2 status in an Asian court of 74 primary gastric cancer (12 endoscopic biopsies and 62 resection specimens) and paired metastasis by immunohistochemical staining (IHC) and dual-color silver in situ hybridation (SISH). HER2 discordance status were detected in 16.2 % of the patients ($N = 12$). Positive conversion was more frequent than negative one (10.8 % vs. 5,4 %) [19]. These results were also confirmed by other recent reports conducted on Asiatic patient cohorts [20–22].

One of the possible causes of HER2 status discordance could be ascribed to HER2 heterogeneity. Intratumoral HER2 heterogeneity is a common feature in gastric cancer disease [8, 20, 23, 24]. Also in our cohort, gastric cancer HER2 heterogeneity was documented (Table 2). This heterogeneity may arise via random genetic alteration with clonal progression, likely resulting in genetic subclones of cells within the primary gastric cancer. In vitro evidences demonstrated that HER2 amplification increases the metastatic potential in murine and human cancer cell lines [25, 26] and induces metastases in transgenic animal models suggesting that gastric cancer subclones with HER2 amplification could have metastatic advantage [27, 28]. In this direction, the positive HER2 conversion in 6/8 (75 %) paired primary vs metastatic disease was found suggesting, also in clinical sample, that HER2 positive distant metastasis may arise from a small undetectable HER2 positive sub-clone within primary disease. In this direction, we found a number of cases in which small clone of cancer cells (<10 %) show different HER2 expression compared to the whole tumor bulk (Table 2).

Moreover, our data showed a high concordance rate between biopsy and surgical samples in term of HER2 status (concordance value 85,7 %; k-value was 0,66) in line with previous published data [29–31]. Nevertheless, 3 HER2 discordant cancer pairs were found: 2 HER2 positive endoscopic cancer biopsy resulted negative in surgical specimens while 1 HER2 negative biopsy sample resulted positive on respectively surgical specimen. Also in this circumstance it is suggested that intratumoral heterogeneity is likely to affect the accuracy of HER2 status evaluation and is likely to be the main reason of discordance between endoscopic biopsy and excisional tumor specimens [32]. Interesting, the two patients HER2 positive in biopsy and HER2 negative in gastric resection specimens, resulted HER2 amplified in metastatic disease.

The limitations of our study include its retrospective nature and the limited number of cases per year due to the rarity of recruitment of paired primary and metastatic tissue samples of gastric cancer disease.

In conclusion our data underline that the HER2 status should be reassessed in all samples obtained from different sites of gastric cancer disease available for the patient (surgical and bioptic specimens of primary lesion and metastatic disease). In the era of targeted therapy, an accurate definition of HER2 status in patients who can benefit by anti-HER2 drugs represents a pivotal commitment in the clinical management of metastatic gastric cancer patients.

Authors' Contributions MA, GP conceived and participated in its design and coordination. DR, CP performed the experiments. MA, GP, DR analyzed the data and wrote the paper. CR, DS contributed clinical data to the study. PC, RC, DC, FDM contributed surgical specimens to the study. GT, DS, AOM article revision and corrections. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Disclosure/Conflict of Interest The authors declare no conflict of interest.

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