

# HER2/neu Expression in Gastric and Esophagogastric Junction Adenocarcinoma

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## Abstract

**Background:** Gastric cancer is one of the most commonly diagnosed cancer and one of the most common causes of cancer-related deaths worldwide. HER-2/neu, a well-recognized factor, is indicated in the development of certain human solid tumors, notably breast cancer. High expression of HER2/neu has correlated with poor prognosis. Studies reveal combination of treatment with antibody targeting HER-2/neu protein (Trastuzumab) and chemotherapy significantly improves overall survival in patients with gastric and esophagogastric junctional (EGJ) adenocarcinomas with HER-2/neu protein overexpression. We aimed to investigate the frequency of Her2/neu expression and association with clinicopathological parameters in our gastric and EGJ adenocarcinoma series to make a contribution to the emerging data on this oncogene.

**Materials and Methods:** A total of 100 cases, including biopsy and resection specimens between November 2012 and December 2014, were selected. Her2/neu expression was determined by immunohistochemistry (IHC) on tumor tissues. Samples with IHC 2+ scores/equivocal cases were tested using fluorescent *in situ* hybridization (FISH).

**Result:** We found 8% positivity for HER2/neu protein expression (IHC 3+ and IHC 2+/FISH +). Gene amplification in 5 cases revealed 100% concordance with IHC 3+. The findings of our study are in concordance with international data. We also observed a relationship of intestinal phenotype, EGJ tumors, and well-differentiated adenocarcinoma with HER2 overexpression.

**Conclusion:** The findings of our study suggest a relationship of EGJ tumors, intestinal phenotype, and well-differentiated adenocarcinoma with HER2 overexpression.

**Key words:** Gastric adenocarcinoma, HER2/neu, Esophagogastric junction adenocarcinoma, Prognostic relevance of HER2/neu in gastric tumors

## INTRODUCTION

Gastric carcinoma is one of the most common causes of cancer-related deaths worldwide, second after lung cancer. Genetic alterations of tumor suppressor genes and proto-oncogenes lead to gastric adenocarcinoma.<sup>1,2</sup>

Surgical resection is the mainstay of therapy for both gastric and esophagogastric junction (EGJ) carcinomas. However, the most patients are diagnosed in an unresectable stage.

For these patients, chemotherapy may prolong life, but survival rates remain low. Given the poor prognosis, and the fact that most gastric and esophageal cancers are diagnosed at an advanced or unresectable stage, new therapeutic strategies and treatment options and novel therapeutic targets are the need of the hour.<sup>3,4</sup>

Overexpression of HER2/neu occurs in 10-34% of invasive breast cancer patients and it is associated with aggressive behavior, resistance to treatment with chemotherapeutics and poor response to endocrine treatment. HER2/neu overexpression has been observed in various types of cancers including colon, bladder, ovary, endometrium, lung, uterine cervix, head and neck, esophagus, and stomach carcinomas.<sup>3-5</sup>

The prognostic role of HER2 overexpression in gastric cancer remains controversial.<sup>6</sup>

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Predictably, however, patients with tumors have HER2/neu overexpression benefit from trastuzumab therapy. It is therefore essential to determine the HER2/neu status of tumors to use trastuzumab as an additional agent in treatment of related cancers.<sup>7,8</sup>

## MATERIALS AND METHODS

This hospital-based prospective study was conducted at the Kidwai Memorial Institute of Oncology, Bengaluru, South India. Patients diagnosed with gastric and EGJ adenocarcinoma cancer in our Institute as well as those referred from other hospitals from November 2012 to December 2014 were included in the study.

The study protocol was approved by the hospital's Institutional Review Board and Ethical Committee.

The clinical data in our study were limited due to the high percentage of patients lost to follow up attributable to various factors such as advanced stage of disease, apathy, and poor economic conditions among others.

For fresh specimens received in the laboratory, fixation in 10% neutral buffered formalin for 6 to 48 h was ensured. The cases were initially evaluated by routine hematoxylin and eosin stained sections.

Based on histomorphology, the cases were categorized according to the WHO 2012 classification into well, moderately, and poorly differentiated adenocarcinoma and into intestinal, diffuse, and mixed according to Lauren's classification.<sup>9</sup>

Histopathological, immunohistochemical (IHC), and *in situ* hybridization studies were done on formalin-fixed, paraffin-embedded tissue blocks in the Department of Pathology, Kidwai Memorial Institute of Oncology, Bengaluru. Biopsy specimens were graded and also evaluated by alcian blue in cases with signet ring cell morphology for confirmation of the cell type. Blocks of biopsy specimens were also evaluated for adequacy of tumor tissue for IHC.

HER2 scoring was done in accordance with CAP/ASCO guidelines.

Samples with IHC 2+ scores/equivocal cases were tested using fluorescent *in situ* hybridization (FISH).

### IHC (n = 100)

A one step polymer-horseradish peroxidase detection system was used.

## Procedure

3  $\mu$  tissue sections on poly-L-lysine-coated slides were dewaxed, treated with an antigen retrieval solution, blocked with 2% skimmed milk blocking solution and then incubated with the primary antibody. The primary antibody binds to the antigen of interest. This was followed by incubation with the secondary antibody conjugated with horseradish peroxidase polymer and color development using 3,3'-diaminobenzidine substrate. When adequate color development was seen, the slides were washed in water to stop the reaction, counterstained with hematoxylin and covered with a mounting medium. The details of interpretation of the immunostaining are given in Table 1.

### Fluorescence *in Situ* Hybridization (n = 5)

The standard operational protocol for FISH probe use of solid tissue was followed. The probe used was those of the PathVysion HER2/neu DNA probe kit which is FDA approved.

The results of FISH are expressed as the ratio between the number of copies of the HER2 gene and the number of copies of chromosome 17 within the nuclei counted in 20 cancer cells.

The definition of FISH positivity in gastric or EGJ cancer is an HER2: Chromosome 17 ratio of >2.0. The details of interpretation of the FISH are given in Table 2.

### Statistical Analysis

Data collected were subjected to statistical analysis using "R" software (free download). Frequency tables were generated on clinical profile parameters. Correlation of HER2 expression with clinical parameters was done using Spearman's rank coefficient  $\rho$ . Any  $\rho$  value  $\leq 0.05$  was considered statistically significant.

## RESULTS

Mean patient age was 53.57. Out of the 100 cases studied 64 were male, 36 female.

The distribution of cases by site included 72 cases with epicenter of the tumor in the stomach and 28 with epicenter of the tumor in the EGJ. A total of 76 of the 100 cases were of biopsy specimens and 24 of resected specimens were taken in the study.

Out of 100 cases, 57 were diagnosed with intestinal type and a subset of 43 patients were diagnosed with diffuse type, as per Lauren's histological subtype. On the basis of degree of differentiation of the 100 cases, 5 were of Grade I, 50 of Grade II, and 45 of Grade III. In IHC examination,

**Table 1: Interpretation of immunohistochemistry<sup>10</sup>**

Surgical specimen staining pattern	Surgical specimen staining pattern	HER2 overexpression assessment	Score
No reactivity or membranous reactivity in <10% of tumor cells	No reactivity in any tumor cell	Negative	0
Faint or barely perceptible membranous reactivity in 10% or more of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster * with faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative	1
Weak to moderate complete, basolateral or lateral membranous reactivity in 10% or more of tumor cells	Tumor cell cluster * with weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal	2
Strong complete, basolateral or lateral membranous reactivity in 10% or more of tumor cells	Tumor cell cluster* with strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Positive	3

\*≥5 cells

**Table 2: Interpretation of FISH<sup>11</sup>**

Gastric resection 20 cohesive tumor cells showing highest gene count (count additional 20 if ratio 1.8-2.2)	HER2/CEP17 ratio≥2.0 HER2 signals>6	Positive
Gastric biopsy 20 cohesive tumor cells showing highest gene count (count additional 20 if ratio 1.8-2.2)	HER2/CEP17 ratio<2.0 HER2 signals<6	Negative
	HER2/CEP17 ratio≥2.0 HER2 signals>6	Positive
	HER2/CEP17 ratio<2.0 HER2 signals<6	Negative

FISH: Fluorescent *in situ* hybridization

90 of 100 cases were negative for HER2/neu expression (score 0) and 2 cases showed incomplete membranous staining in <10% of tumor cells, and they were scored as 1(+) (Figure 1). A total of 92 cases consisting of IHC score 0 and 1(+) were negative. A total of 5 cases revealed strong complete membranous staining in more than 10% of tumor cells (IHC score 3+) (Figures 2-4). In the rest 3 cases, there was weak to moderate staining of the entire or basolateral membrane staining in >10% of the tumor cells (IHC score 2+) (Figure 3). These 3 cases were scored as 2(+) IHC. FISH assay was carried out in all IHC 2(+) cases as per ASCO/CAP guidelines and also in 1 IHC 1(+) and 1 IHC 3(+) case (Table 3). All cases of IHC score 2(+) and the case of IHC 3(+) for which FISH was performed which showed gene amplification. The case of IHC 1(+) did not show gene amplification. After IHC and FISH results, the total HER2/neu positivity rate was 8 cases (8%).

Patient demographics and pathological tumor characteristics with respect to HER2/neu expression results in our study group are summarized in Table 4. Totally, 7 out of 8 of the HER2/neu positive cases (12.28%) tumors were of intestinal type gastric/EGJ adenocarcinomas, whereas 1 (2.32%) was of diffuse type adenocarcinoma. Three of 28 (10.71%) cases of EGJ adenocarcinoma were HER2/neu positive, with 5 of 72 (6.94%) cases of gastric tumors being positive. We did not find statistical significance in evaluating HER2/neu expression with Lauren's histological subtype ( $P = 0.3469$ ) or site of the tumor ( $P = 0.4479$ ). However, we noted a strong association with these variables, with approximately 5 times more positivity in intestinal tumors than diffuse type. Furthermore, we did

**Table 3: FISH**

Number of cases analyzed	IHC score	FISH score HER2/neu: CEP	Result
1/3	1+	<2	Not amplified
3/3	2+	>2	Amplified
1/5	3+	>2	Amplified

FISH: Fluorescent *in situ* hybridization

not observe an association between patient age and gender with HER2/neu expression (Figures 6 and 7).

## DISCUSSION

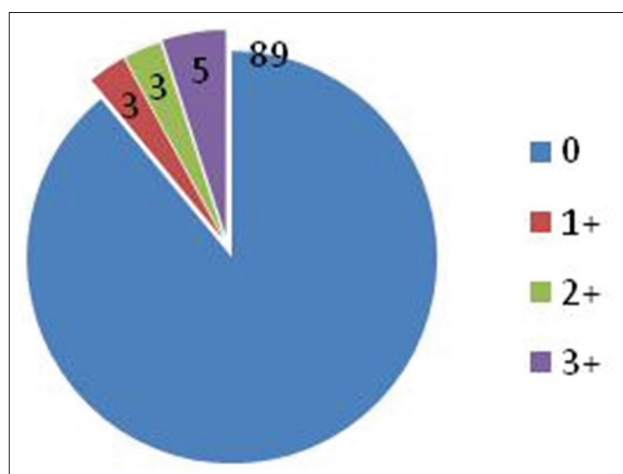
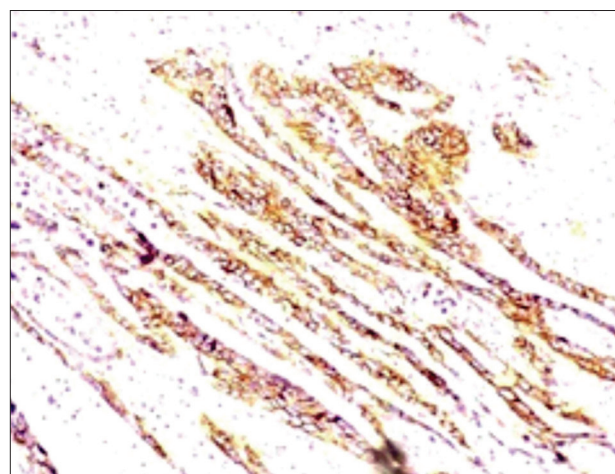
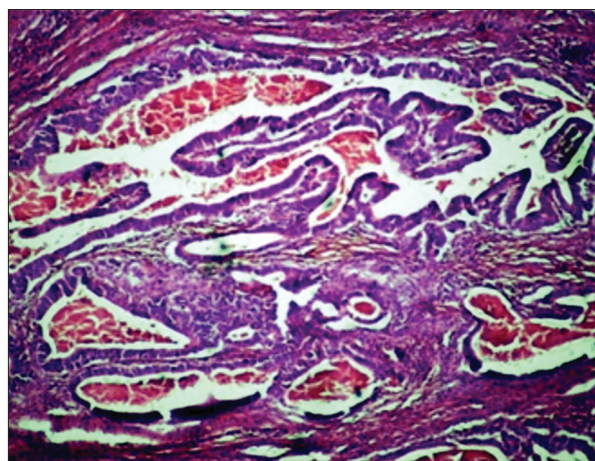
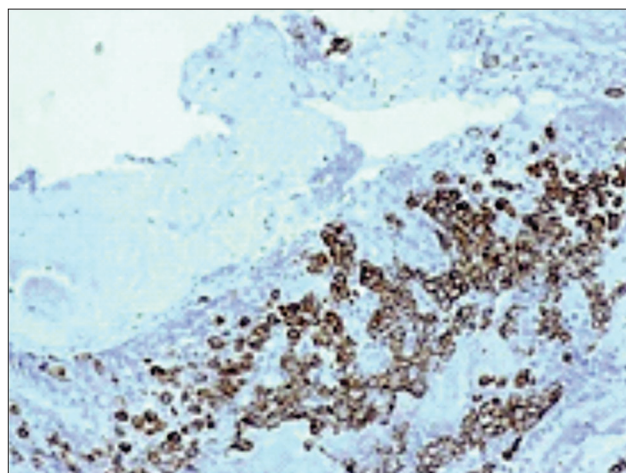
The incidence and mortality rates of gastric cancer are decreased worldwide, but despite the recent decline, gastric cancer remains the fourth most common cancer and the second leading cause of cancer-related mortality.<sup>12,13</sup>

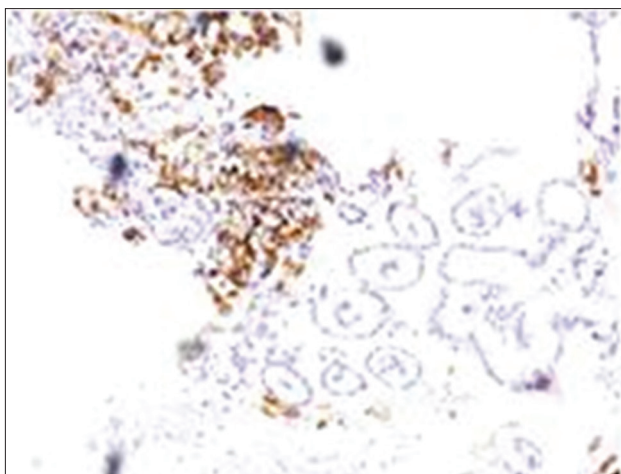
In the recent years, interest has grown in understanding the relationship between the biological characteristics of gastric cancer and the association of these characteristics with the clinical outcomes of the disease (Figure 8). HER-2/neu a well-recognized factor is indicated in the development of certain human solid tumors, notably breast cancer. High expression of HER2/neu has correlated with poor prognosis. Studies reveal combination of treatment with antibody targeting HER-2 protein (Trastuzumab) and chemotherapy significantly improves overall survival in patients with gastric and EGJ adenocarcinomas with HER-2/neu protein overexpression.<sup>14</sup>



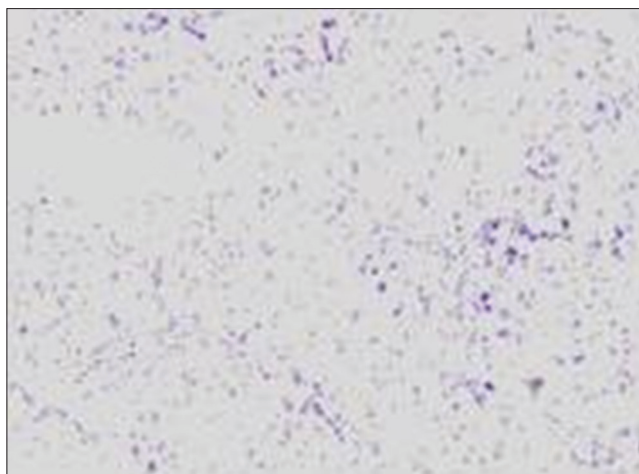
**Table 4: Correlation of HER2 score with other variables**

Parameter	HER2/neu score				P value
	0 (%)	1+	2+	3+	
Age					
<60 years (n=65)	58 (89.2)	2 (3.07)	2 (3.07)	3 (4.61)	0.9286
≥60 years (n=35)	32 (91.4)	0 (0)	1 (2.85)	2 (5.71)	
Gender					
Male (n=64)	59 (92.1)	1 (1.56)	1 (1.56)	3 (4.68)	0.7026
Female (n=36)	31 (86.11)	1 (2.77)	2 (5.55)	2 (5.55)	
Type of specimen					
Biopsy (n=76)	68 (89.47)	1 (1.31)	3 (3.94)	4 (9.26)	0.801
Resected (n=24)	22 (91.6)	1 (4.16)	0 (0)	1 (4.16)	
Location of tumor					
Stomach (n=72)	65 (90.27)	2 (2.77)	1 (1.38)	4 (5.55)	0.4479
EGJ (n=28)	25 (89.28)	0 (0)	2 (7.14)	1 (3.57)	
Histological subtype: Lauren's					
Intestinal (n=57)	49 (85.9)	1 (1.75)	3 (5.26)	4 (7.01)	0.3469
Diffuse (n=43)	41 (95.3)	1 (2.32)	0 (0)	1 (2.32)	
Mixed (n=0)	0	0	0	0	
Degree of differentiation					
Grade I (n=5)	4 (80)	0 (0)	0 (0)	1 (20)	0.2428
Grade II (n=50)	43 (86)	1 (2)	3 (6)	3 (6)	
Grade III (n=45)	43 (95.5)	1 (2.22)	0 (0)	1 (2.22)	

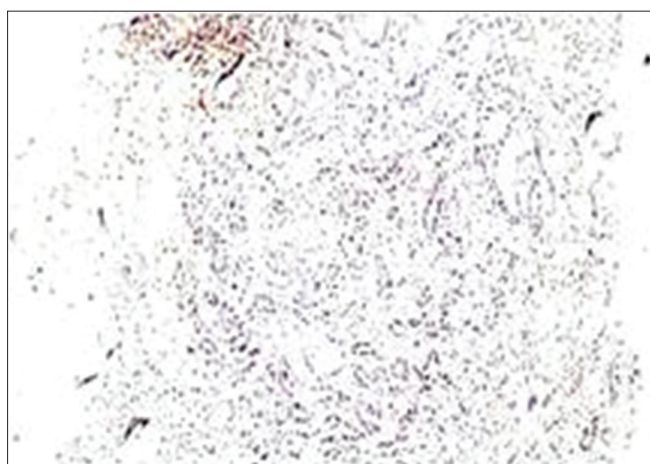
**Figure 1: Distribution of cases by HER2/neu score****Figure 3: HER2/neu immunostains (x10): 3+ resected specimen****Figure 2: Adenocarcinoma Grade I/intestinal differentiation, resected specimen, H and E (x40)****Figure 4: HER2/neu immunostains 3+, biopsy**



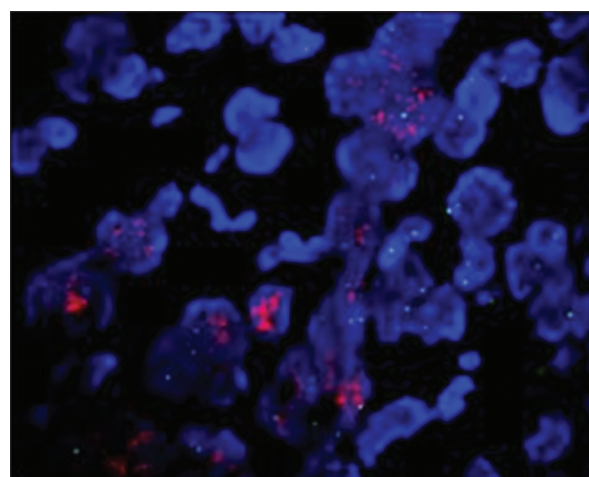
**Figure 5: HER2/neu immunostain (x4): 2+ with normal mucosa**



**Figure 7: HER2/neu immunostains (x10): 0, intestinal type**



**Figure 6: HER2/neu immunostains (x4): 1+, intestinal type**



**Figure 8: Fluorescent *in situ* hybridization score HER2/neu: CEP >2 gene amplification**

Many studies have evaluated HER-2/neu status in GC, with ranges of HER2/neu positivity varying from 4% to 53%.<sup>15</sup> However, fewer studies have been conducted in India to evaluate this prognostic marker. There has been considerable interest in HER2/neu as a prognostic marker. In our study, we assessed the HER2/neu expression pattern and correlated this with other histopathological features and available clinical data.

The type of specimen in our study included a higher percentage of biopsies (76%) versus surgically resected specimens (24%). In the ToGA trial, it was suggested that the type of specimen does not affect the IHC study for HER2/neu protein expression. In the study by Janjigian *et al.*,<sup>16</sup> there was no difference between HER2/neu positive rates between resection and biopsy specimens.

In our study, 8 of 100 cases (8%) of gastric and OGJ adenocarcinoma cases were positive for HER2/neu (IHC3+ and IHC 2+/FISH +). Takehana *et al.*,<sup>12</sup> Tereshima *et al.*,<sup>12</sup> Shan *et al.*,<sup>17</sup> Yildirim *et al.*,<sup>1</sup> Barros Silva *et al.*,<sup>12</sup> have reported positivity rates of 8%, 9%, 9.8%, 11.5%, 8%, and 9.3%,

respectively, in their studies comparable with our study. Other studies have reported higher rates (Yk *et al.* 42%)<sup>18</sup> and this variation may be explained by analytical limitations by fixation, sample size, number of intestinal, diffuse and mixed tumors, usage of different antibody clones, heterogeneous staining of tumor tissue, and also subjective testing by semi-quantitative IHC testing. Racial differences have not been reported in the ToGA trial. A study of subjects in the Indian population by Sekaran *et al.* showed positive rates of 44.2%.<sup>19</sup>

A total of 5 of 8 patients who were HER2/neu positive were below the age of 60. No gender predilection was noted in our study with 4 cases of males and 4 female patients with HER2/neu positivity.

HER 2/neu positivity rates were varied by tumor site with higher rates of HER2/neu positivity in EGJ adenocarcinoma than in stomach cancer in this study (10.71% vs. 6.94%, respectively). This is consistent with the results of other studies for EGJ versus GC adenocarcinoma: Tanner *et al.*<sup>20</sup> 24% versus 12.2%, Janjigian



**Table 5: Comparison of HER2/neu positivity with other studies<sup>12</sup>**

Parameter	Our study	ToGA trial	Tanner <i>et al.</i>	Gravalos <i>et al.</i>	Lordick <i>et al.</i>
n	100	3667	231	166	1527
HER2/neu positivity					
IHC 3(+)/IHC 2(+) and FISH+	8%	22.1	36.6	13	22
Histological subtype					
Intestinal	12.28	32.2	21.5	16	34
Diffuse	2.32	6.1	2	7	6
Mixed	-	20.4	5	14	20
Location of tumor					
Stomach	6.94	20.9	12	9.5	18
EGJ	10.71	33.2	24	25	32

FISH: Fluorescent *in situ* hybridization

*et al.*<sup>16</sup> 25% versus 18%, Shan *et al.*<sup>17</sup> 14.6% versus 7% and in the ToGA trial<sup>10</sup> 33.2% versus 20.9% (Table 5).

We noted a strong association of intestinal subtype with 7 of 8 positive cases displaying intestinal differentiation. Nearly, 12.28% of intestinal and 2.32% of diffuse tumors showed HER2/neu overexpression. This is in correlation with other studies who report a strong correlation of intestinal phenotype with HER2/neu expression compared to diffuse subtype: Tanner *et al.*<sup>20</sup> (21.5% vs. 2.2%), Grاسبach *et al.*<sup>21</sup> (3.8% vs. 0%), Liu *et al.*<sup>21</sup> (11% vs. 8%), Yildirim *et al.*<sup>1</sup> (21.6% vs. 4%), and Janjigian *et al.*<sup>16</sup> (33% vs. 8%) (Table 5).

FISH analysis for gene amplification was carried out in all IHC 2+ (equivocal) cases (3/3) and one IHC 3+ case (1/5) and one IHC 1+ case (1/2). All cases with score of IHC 2+ and IHC 3+ showed gene amplification. The case of IHC 1+ did not show gene amplification. The concordance rate between IHC and FISH was 100%. ToGA trial reports a concordance rate of 87.2% between IHC and FISH. Yk *et al.* reported an HER2 + rate by FISH of 20.3% and by IHC of 42%.

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