Chapter 3

HER2 Amplification or Overexpression in Upper GI Tract and Breast Cancer with Clinical Diagnosis and Treatment

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Additional information is available at the end of the chapter

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1. Introduction

EGFR and HER2 family with signal pathway and carcinogenesis: The human epidermal growth factor receptors (HER-2) gene is localized to chromosome 17q and encodes a transmembrane tyrosine kinase receptor protein. Numerous studies were done from basic mechanism of HER family for cell proliferation and oncogenesis, HER2 overexpression or amplification in various solid tumors to clinical treatment of breast cancer, gastro-esophageal cancer by trastuzumab in many recent reviews [1-8].

HER2 belongs to a family including epidermal growth factor receptor (EGFR), HER2, HER3 and HER4, which are a group of transmembrane glycoproteins, collectively named receptor tyrosine kinases (RTKs), whose cytoplasmic domains harbor an enzymatic activity, namely tyrosine-specific phosphorylation [9]. The family of epidermal growth factor molecules, which comprises different ligands sharing a 50–60 amino acid receptor-binding domain, bind with subtype RTKs. Each receptor consists of an extracellular ligand-binding domain, a transmembrane domain, and a tyrosine kinase portion [10]. Upon ligand binding, the otherwise inactive monomeric receptors form active homodimers or heterodimers, thereby leading to receptor phosphorylation and signaling via various biochemical pathways (Fig.1), such as the mitogen-activated protein kinase (MAPK), the phosphatidylinositol 3-kinase (PI3K), phospholipase C-γ, and transcription factors like the signal transducers and activators of transcription (STATs) or SMAD proteins [1]. These modules of cellular activation and the respective growth factors (GFs)s are co-opted in several phases of tumor progression.

HER-2 gene amplification in breast cancer has been associated with increased cell proliferation, cell motility, tumor invasiveness, progressive regional and distant metastases, accelerated angiogenesis, and reduced apoptosis [11]. Overexpression of HER2 in human

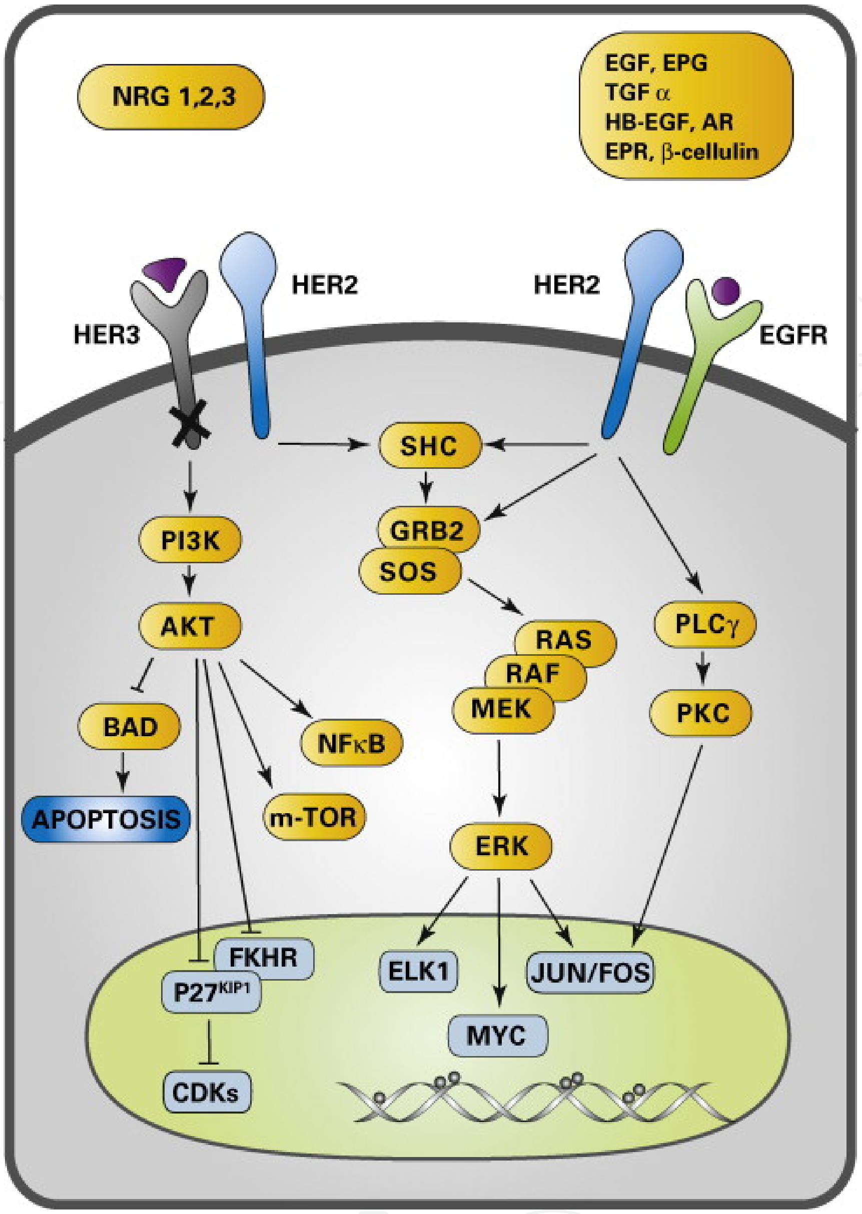

Figure 1. Signal transduction pathways instigated by HER2, co-receptors and EGF-like growth factors. Heterodimers of HER2/ErbB-2 and either EGFR/ErbB-1 or the kinase-defective ErbB-3/HER3 (note the letter X that symbolizes a defective cytoplasm-facing kinase domain) are shown, along with the growth factor ligands they bind. All ligands share an epidermal growth factor (EGF) motif of 50–60 amino acids. They include, in addition to EGF, epiregulin (EPG), transforming growth factor alpha (TGF-alpha), heparin-binding epidermal growth factor-like factor (HB-EGF), amphiregulin (AR), epiregulin (EPR) and betacellulin. Another group includes four classes of neuregulins (NRGs). Note that HER2 is unable to bind a ligand. Nevertheless, HER2 takes part in signaling via its own constitutive phosphorylation, as well as by trans-activation of its heterodimerization partners. Tyrosine phsphorylated receptors are coupled to several biochemical cascades, including the phosphoinositide-3-kinase (PI3K) pathway and the extracellular signal-regulated kinase (ERK), which belongs to the MAPK family. Activation of ERK/MAPK is mediated via the RAS-RAF-MEK pathway and leads to cellular proliferation via the activation of a number of nuclear targets, including the AP1 (FOS and JUN) complex, MYC, which regulates cell cycle progression, and ELK1, a member of the ETS family of transcription factors. SHC and GRB2 are adaptor proteins sharing the ability to bind each other, as well

as tyrosine phosphorylated receptors. The EGFR/HER2 heterodimer also couples to phospholipase C (PLC) and the downstream protein kinase C. On the other hand, ErbB-3/HER3-containing heterodimers strongly activate another kinase, AKT, via a lipid kinase, PI3K, leading to activation of mTOR (mechanistic target of rapamycin). Activation of AKT blocks signaling via BAD, a BH3-only protein, which contributes to tissue homeostasis by regulating initiation of apoptosis. Activation of AKT inhibits FKHR and the cyclin-dependent kinase inhibitor $p27^{KIP}$. The forkhead box O1 (FKHR, FOXO1) transcription factor is a member of the FOXO family of transcription factors, involved in tumor suppression and cell death. (From Emde A, et al. Crit Rev Oncol/Hematol (2010), http://dx.doi.org/10.1016/j.critrevonc.2010.09.002, Permitted by Elsevier Limited).

mammary epithelial cells induces proliferative advantage, transformed characteristics, tumorigenic growth, and induces proliferative and anti-apoptotic changes that mimic early stages of epithelial cell transformation [12]. HER2 amplification is also seen in early in situ ductal carcinomas without any evidence of invasive disease [13, 14]. HER2 status is maintained during progression to invasive disease, nodal metastasis and distant metastasis [14, 15]. HER2 overexpression has been shown to activate multiple signaling complexes, which results in a striking dysregulation of the global transcriptome [1].

Clinical treatment targeting on HER2 receptor: It took a long journey to develop monoantibody to target HER2. Murine origin of mAb to HER2 limits their clinical application since immunoglobulin molecules are immunogenic. When injected into humans, it shortens their half-lives in circulation. Winter and colleagues (1988) generated a mouse–human chimeric antibody [16]. Later transgenic mice whose immunoglobulin loc have been genetically inactive, was used to produce the first fully human antibody, Panitumumab, an antibody to EGFR. Then, trastuzumab which carry all human immunoglobulin genes, a monoclonal antibody to HER2, was approved for clinical use in lymphoma and in breast cancer [17]. So far, only two drugs that target HER2, Trastuzumab and a kinase inhibitor called Lapatinib/Tykerb, are approved for clinical application in breast cancer, but several novel drugs are in development (see figure 2).

Trastuzumab, monoclone antibody on HER2: Trastuzumab, a monoclonal antibody that targets HER2, induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signaling and prevents cleavage of the extracellular domain of HER2 [12]. Based on multi-centers and countries clinical trial for HER2 positive breast cancer, [18,19,20] trastuzumab was significantly improve the prognosis of breast cancer. Therefore, it was initially approved for treatment of patients with HER2 overexpressing metastatic breast cancer. Because Trastuzumab also enhances the efficacy of adjuvant chemotherapy in operable or locally advanced HER2-positive tumors [21], the antibody currently represents the standard of care for patients with early or advanced stages of HER2-overexpressing breast cancer.

Since breast cancer showed better prognosis with trastuzumab treatment for HER2 positive breast cancer patients and similar HER2 positive cancers were identified in gastric and gastro-esophageal cancer, clinical trial ToGA was performed in gastric carcinoma. ToGA (Trastuzumab for Gastric Cancer) was an open-label, international, phase 3, randomized controlled trial undertaken in 122 centers in 24 countries [22]. Clinical trial ToGA used trastuzumab combined with standard chemotherapy for HER2 positive gastric cancer and gastro-esophageal junction cancer which demonstrated a significant improvement of gastric cancer survival. Now, trastuzumab is approved for treatment of gastric cancer in European, United States, Japan and other multiple countries.

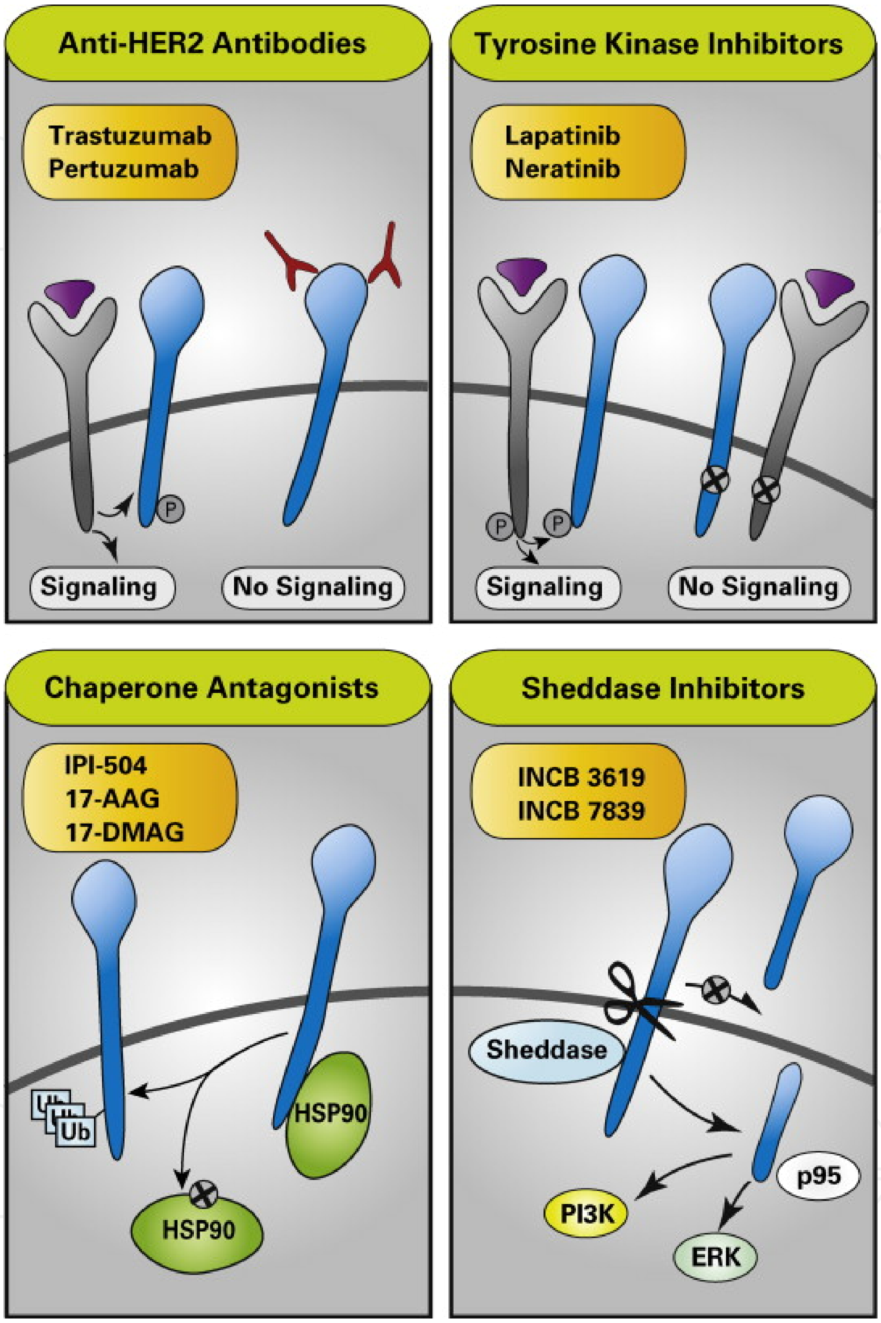

Figure 2. Clinically approved and experimental therapeutic strategies targeting ErbB-2/HER2 in carcinomas. Trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of HER2, is approved for the treatment of HER2-overexpressing breast cancer. The antibody recruits immune effector mechanisms and can induce apoptosis, block angiogenesis and inhibit tumor cell proliferation. Similarly, Pertuzumab is able to prevent heterodimerization of HER2 with other family members. Unlike the ultimate specificity of Trastuzumab and Pertuzumab to HER2, tyrosine kinase inhibitors like the reversible inhibitor Lapatinib (approved for treatment of breast cancer) and the irreversible inhibitor Neratinib variably inhibit a broad range of tyrosine kinases. The drug has

completed phase II clinical trials. HSP90 is a molecular chaperone required for proper folding of protein kinases like HER2. Hence, HSP90 inhibitors, such as 17-AAG, which block the ATP/ADP binding pocket of HSP90 and target HER2 for proteasomal degradation are in clinical trials. A naturally occurring truncated form of HER2, p95-HER2, has been implicated as a mechanism conferring resistance to Trastuzumab. Its formation is mediated by processing of the membrane bound HER2 by matrix metalloproteinases (MMPs) of the ADAM (*a d*isintegrin *a*nd *m*etalloproteinase) family. INCB3619 and INCB7839 are potent inhibitors of ADAM10 and ADAM17. ADAM10 is the principle sheddase for different molecules associated with tumor cell proliferation, whereas ADAM17 is the main sheddase for the EGFR ligands TGF-alpha, AR, NRGs, and HB-EGF. These similar inhibitors may effectively block truncation of HER2 and onset of patient resistance to Trastuzumab, but clinical testing has not been completed. (From Emde A, et al. Crit Rev Oncol/Hematol (2010), http://dx.doi.org/10.1016/j.critrevonc.2010.09.002, Permitted by Elsevier Limited).

The clinical efficacy of Trastuzumab likely entails a combination of immunological and non-immunological mechanisms [1]. The ability of Trastuzumab to elicit antibody-dependent cellular cytotoxicity critically influences the efficacy of Trastuzumab-based therapies. Non-immunological mechanisms of Trastuzumab action include the inhibition of HER2 activation and downstream signaling. Alternatively, Trastuzumab may act by removing HER2 from the cell surface. Because it binds to an epitope near the cleavage site of HER2's extracellular domain, Trastuzumab inhibits HER2 activation by metalloproteinase-mediated shedding of the extracellular domain. The resulting interference with HER2-mediated downstream signaling processes shuts down cell proliferation, angiogenesis, invasive growth, resistance to apoptosis, and DNA repair, thus sensitizing tumor cells to conventional therapeutic modalities such as chemotherapy, endocrine treatment and radiotherapy.

Lapatinib, small molecule kinase inhibitor: Lapatinib, binding either reversibly or irreversibly to the nucleotide-binding cleft of their target kinases, is a highly specific, reversible inhibitor that blocks the catalytic action of both HER2 and EGFR[23]. Experiments in vitro and xenograft models, established the ability of Lapatinib to inhibit both the intact form of HER2 and the truncated intracellular form (p95-HER2), which is not recognized by Trastuzumab.

Similar to Trastuzumab, Lapatinib combined with chemotherapy was found to be better effect than capecitabine alone in HER2-positive women with advanced breast cancer that progressed after treatment with regimens that included Trastuzumab, an anthracycline and a taxane [24]. In addition, Lapatinib demonstrated clinical activity and was well tolerated as first-line monotherapy in HER2-amplified, locally advanced or metastatic breast cancer [25, 26]. Recently, laptinib showed a synergistic effect with trastuzumab in vitro and in vivo to inhibit HER2 amplified human gastric cancer cells and animal model [23]. Clinical phase II trial of lapatinib as first line therapy in patients with advanced or metastatic cancer showed well tolerated, which will be another potential drug to target HER2 receptors.

Lapatinib response correlated with EGFR and HER2 expression levels in patients' tumors, and associated with increased pre-treatment expression of phosphorylated-HER2 (p-

HER2)[27]. Lapatinib is able to induce apoptosis of Trastuzumab-resistant breast cancer cells via alteration of IGF-1 signaling, [28, 29] and also block NRG-induced p95-HER2/HER3 heterodimers formation [30].

2. HER2 in gastric adenocarcinoma

Gastric cancer is the fourth most common cancer worldwide and the second most common cause of cancer-related death in the world [31, 32]. The incidence of gastric cancer varies substantially worldwide, with the highest rates (>20 per 100,000) occurring in Japan, China, Eastern Europe, and South America, but the lowest rates (<10 per 100,000) finding in North America, southern Asia, North and East Africa, Australia, and New Zealand. In addition, it is more common in men than in women (10.9 vas 5.5 per 100,000). Although the survival of gastric cancer is improved in recently years in Western countries the 5 year survival is still around 5-20%. The multimodality treatments including surgery and neoadjuvent chemotherapy have a limited effect on the overall survival. In breast cancer, HER2 overexpression and amplification were reported around 25% and associate with poorer prognosis [2]. Trastuzumab treatment of HER2 positive breast cancer patient improved survival. HER2 overexpression and amplification were reported in gastric and gastro-esophageal junction (GEJ) tumors from 6-43%. In addition, trastuzumab were found to inhibit tumor growth in gastric carcinoma cell lines, animal model and xenograft models [33-35]. Recently international large scale phase III clinical trial called ToGA showed that trastuzumab added to standard chemotherapy significantly improved the response rate, median progression-free survival, and overall survival of gastric adenocarcinoma[22]. Trastuzumab combined with standard chemical therapy (such as capecitabine or 5-fluorouracil and cisplatin) now is approved by European Medicines Agency, United States and Japan etc. for the treatment of patients with HER2 overexpression or amplification. Thus clinical tests for HER2 overexpression and amplification in gastric adenocarcinoma patients become a key to recruit eligible patients for clinical treatment and evaluation of treatment effect.

IHC studies on HER2 overexpression: HER 2 overexpression was reported from 7-34% by many studies [3]. For clinical trial and treatment, it is very important to develop a standard HER2 test to recruit eligible patients for trastuzumab treatment. Before clinical trial ToGA, Hofmann and colleagues (2008)[36] first set up an IHC criteria based on HER2 IHC test on 168 gastric and GEJ resection patients (see Table 2). Based on the standard HER2 test on the breast cancer, they further proposed that strong incompletely membranous stain with basolateral “U” shape in gastric cancer was positive for HER2 overexpression. In addition, the HER2 expression showed higher heterogeneity about 4.8% in gastric samples than about 1.4% in breast cancer. They modified breast criteria in several points including incomplete membranous stain pattern and percentage of cells (≥ 10% cut off), which improved the concordance level between IHC and FISH tests to 93.5%. For ToGA clinical trial, Bang et al [22] reported that HER2 positive rate was a 22.1%. In addition, they found that HER2-

Gastric Cancer	Breast Cancer	Score/classification
IHC score criteria		
No reactivity or membranous reactivity in <10% of cells; Biopsy specimens < 5 Cells	No reactivity or membranous reactivity in <10% of cells	**0/negative**
Faint/barely perceptible membranous reactivity in ≥10% of cells; biopsy specimens≥5 Cells	Faint membranous reactivity in >10% of cells;	**1+/negative**
Weak to moderate complete or basolateral membranous activity in ≥10% of tumor cells; biopsy specimens≥5 Cells	Weak to moderate complete membrane staining in >10% of tumor cells	**2+/equivocal**
Moderate to strong complete or basal/lateral membranous activity in ≥10% of resection tumor cells; biopsy specimens≥5 Cells	Strong complete membrane staining in >10% of tumor cells	**3+/positive**
FISH HER2/CEP 17		
≥ 2 At least 20 evaluable, non-overlapping cells in the invasive component	**> 2.2** At least 20 evaluable, non-overlapping cells in the invasive component	**Amplification**
	1.8-2.2	**Equivocal**
<2 At least 20 evaluable, non-overlapping cells in the invasive component	**<1.8** At least 20 evaluable, non-overlapping cells in the invasive component	**negative**

Table 1. Consensus panel recommendations on HER2 scoring for gastric cancer

positive rate were higher in GEJ cancer than in gastric cancer (33% vs 21%) and in intestinal than diffuse or mixed cancer (32.2% vs 6.1% vs 20.4%). The concordance between IHC and FISH was 87.5%. Ruschoff and colleaguesl[37, 38](2010, 2012) further validate the HER2 test procedure to determine whether pathologists from different sits were able to reproduce the method of gastric cancer HER2 status evaluation as it was used by Ruschoff within the ToGA study. They validated the HER2 status testing procedure in terms of inter-laboratory and inter-observe consensus for IHC scoring a series of 547 gastric cancer tissue samples on a tissue microarray. They published a practical approach of HER2 test in gastric carcinoma. Based on multiple laboratories and 8 pathologists HER2 test results, they further confirmed the HER2 positive rate of 22.8% which is close to 22.1% from Hoffman's score system. In addition, they compared Daco (HecepTest) and Ventana (Pathway HER2 antibody, 4B5). They found that HercepTest had a higher inter-laboratory discordance than 4B5. Furthermore, Ruschoff and a group of international pathologist reviewed previous HER2 studies; they built up new detailed criteria for gastric and gastro-esophageal HER2 tests (see Table 3; Ruschoff 2012). In their practical procedure for gastric cancer HER2 test, the surgical specimen cutoff is complete, basolateral, or lateral membranous reactivity in ≥10% of cells; the biopsy specimen cutoff is complete, basolateral, or lateral membranous reactivity in ≥5 clustered cells; the borderline cutoff is immunohistochemistry 1+/immunohistochemistry 2+ or focal staining in <10% cells which recommend for FISH or SISH tests. This new score system further improved Hoffmann's score system, but it still need further proved in future HER2 tests, especially the results mostly based on European laboratories. The large scale HER2 studies in Asia are need to build up an optimal HER2 test system in gastric cancer since the incidence of gastric cancer is much higher in Asian countries.

a. Immunohistochemistry

Testing recommendations

- Representative surgical samples or an adequate number of viable biopsy specimens (ideally six to eight) are required
    - If few biopsies are available, all viable specimens should be tested
- Immunohistochemistry should be the initial HER2 testing methodology for gastric cancer and bright-field methodologies are preferred wherever possible

  HER2-positive per European Medicines Agency license: immunohistochemistry 3+ or immunohistochemistry 2+/fluorescence *in situ* hybridization-positive or immunohistochemistry 2+/silver *in situ* hybridization-positive

  Borderline immunohistochemistry 1+/immunohistochemistry 2+ cases and samples with focal and intense membranous reactivity in <10% cells may also be retested with fluorescence *in situ* hybridization or silver *in situ* hybridization (scores for both assays should be indicated separately on the report)
- Validated immunohistochemistry HER2 assays should be used

Scoring recommendations

- Due to the tumor heterogeneity (focal areas of positivity) and incomplete membrane staining commonly seen in gastric cancer, the gastric cancer-specific scoring criteria should be adhered to:
    - Surgical specimen cutoff: complete, basolateral, or lateral membranous reactivity in ≥10% of cells
    - Biopsy specimen cutoff: complete, basolateral, or lateral membranous reactivity in ≥5 clustered cells
- The 'magnification rule' should be used in conjunction with the scoring criteria
- Borderline cases (immunohistochemistry 1+/immunohistochemistry 2+ or focal staining in <10% cells) that score fluorescence *in situ* hybridization-positive or silver *in situ* hybridization-positive may be considered HER2-positive (scores for both assays should be indicated separately on the report)

b. In situ hybridization

Testing recommendations

- Tumor samples classified as immunohistochemistry 2+ should be retested by fluorescence in situ hybridization or silver in situ hybridization to assess HER2 status

• Silver *in situ* hybridization is a more suitable methodology than fluorescence *in situ* hybridization for assessing HER2 status in gastric tumor samples as it is a bright-field methodology and thus allows for rapid identification of HER2-positive tumor foci within a heterogeneous sample
Validated *in situ* hybridization HER2 assays should be used
Scoring recommendations
• The definition of fluorescence *in situ* hybridization or silver *in situ* hybridization positivity in gastric or gastro–esophageal junction cancer is a HER2:chromosome 17 ratio of ≥2.0
• The entire case should be screened for amplified regions (particularly important for fluorescence *in situ* hybridization samples where a bright-field image is not available)
• At least 20 evaluable, non-overlapping cells in the invasive component should be counted initially
• In borderline amplification cases, ~20 additional cells should be recounted or scoring should be performed in an alternative area of tissue
The overall *HER2* gene count is important:
• >6 *HER2* gene copies using single probe: considered positive
• Four to six *HER2* gene copies: dual probe test advised and the ratio should be recalculated by counting an additional 20 cells
Ensuring quality and timely HER2 testing results
• The use of validated immunohistochemistry and *in situ* hybridization tests is strongly recommended and appropriate controls should be included in each run
• Turnaround time from initial diagnosis to reporting of results should ideally not exceed 5 working days and a multidisciplinary approach is required
• Centralized testing is recommended wherever possible and all laboratories should participate in validated quality assurance programs

Table 2. Human epidermal growth factor receptor 2 (HER2) testing recommendations in gastric cancer, (a) immunohistochemistry and (b) *in situ* hybridization (From Ruschoff J, Hanna W, Bilous M, et al. HER2 testing in gastric cancer: a practical approach. Mod Pathol 2012)

HER2 immunohistochemistry features	**Score**
No reactivity or very faint membranous stain in <10% of cells; biopsy specimens<5 Cells	**0**
Faint membranous stain in >10% of cells; biopsy specimens≥5 Cells	**1+**
Weak to moderate complete or baso/lateral membranous stain in >10% of tumor cells; biopsy specimens≥5 Cells	**2+/positive**
Strong complete or basal/lateral membranous stain in >10% of tumor cells; biopsy specimens≥5 Cells	**3+/positive**
HER2 FISH/chromogenic in situ hybridization test	
Ratio of average *HER2*/CEP17 ≥2.0	**Positive**
Ratio of average *HER2*/CEP17 <2.0	**Negative**

Table 3. Modified score criteria of HER2 immunohistochemical stain and FISH/chromogenic in situ hybridization for esophageal adenocarcinoma

Recently, in Asia, several IHC HER2 tests focused on comparing the HER2 antibodies from various companies. Cho and colleagues [39] used four different HER2 antibodies compared to standard FISH test. They found the various positive rates with HercepTest (14%), A0485 (16%), 4B5 (14%), and CB11 (9%). The sensitivity and specificity of IHC compared to FISH was 78.9%/96% for HercepTest, 86.5%/94.4% for A0485, 76.3%/95.6% for 4B5 and 60.5%/98.4% for CB11. Compare to FISH, there was no significantly differences in the sensitivity and specificity among the four IHC tests. However, CB11 had a highest specificity (98%), but a lowest sensitivity (61%). Park et al [39] (2012) compared HercepTest with 4B5, only 41 cases showed discrepancies, yielding a 96.1% concordance rate. However, HER2 positive rate with both methods are very low: HecepTEST, 5.9% and 4B5, 6.4%.

In addition, the standard breast HER2 test was compared with modified gastric carcinoma HER2 test (Table2). Sever studies used breast cancer score rule [40-42]. Barros-Silva et al. [40] found 3.9% as IHC2+ and 5.4% as IHC3+ from resection 463 gastric adenocarcinomas using the breast cancer scoring rules. Using breast cancer scoring, Park et al. (2012)[41] found that HER2 positive rate are very low with two antibodies: HecepTEST, 5.9% and 4B5, 6.4%. The similar result also was presented in TMA data which were classified as IHC2+ (1.6%) or IHC3+ (3.2%) if breast cancer scoring was applied[42]. As the same group also tested gastric cancer TMAs using gastric cancer specific scoring [36]the corresponding rates were 4% IHC2+ and 13% for IHC3+, demonstrating an about fourfold increase of HER2 positivity rate[42]. Therefore, Rushcoff concluded that it is supposed that application of breast cancer scoring to gastric cancer may produce an up to 50% false-negative rate if IHC is used as the primary test platform as favored by EMEA[37].

FISH, CISH and SISH studies on HER2 amplification: HER2 amplification was first reported in gastric cancer in 1986[43]. Since then, HER2 amplification in gastric cancer was extensively studied (see Table 3). Kimura et al. [44] first set criteria of FISH test as HER2/CEP17 ≥ 2.0 which is modified from breast standard HER2 FISH test with 83% of concordance between IHC 2+ and 3+ samples. Hoffman et al.[36] proved that these FISH criteria for gastric cancer showed a higher concordance (93%) between HER2 amplification and overexpression in gastric cancer. Ruschoff et al. [38, 45] (2010, 2012) further validate the HER2 test procedure to determine whether pathologists from different sits were able to reproduce the method of gastric cancer HER2 status evaluation as it was used by Ruschoff within the ToGA study. HER2 amplification was determined by FISH assays, using either HER2 FISH pharmDX™ (Dako Denmark A/S) or PathVysion® (Abbott Laboratories, Des Plaines, IL, USA). Automated brightfield dual-color silver in situ hybridization (SISH) assay (BDISH; Inform™, Ventana Medical Systems SA) was used to determine gene amplification at three of the participating sites. Based on their experience and previous studies, a new practical procedure for HER2 FISH, CISH or SISH tests were established. The positivity of HER2 FISH, CISH or SISH tests in gastric or gastro-esophageal junction cancer is a HER2/Chromosome 17 ratio ≥ 2.0 and > 6 *HER2* gene copies using single probe. At least 20 evaluable, non-overlapping cells in the invasive component should be counted initially. If the results are borderline (four to six *HER2* gene copies or HER2/Chromosome 17 ratio 1.8-2.2), [20] additional cells should be recounted or scoring should be performed in an alternative area of tissue. However, they also concluded that silver *in situ* hybridization is a more suitable methodology than fluorescence *in situ* hybridization for assessing HER2 status in gastric tumor samples as it is a bright-field methodology and thus allows for **rapid** identification of HER2-positive tumor foci within a heterogeneous sample.

Comparing FISH and SISH methods for HER2 test in gastric cancer was also reported by several studies. Park et al [41] (2012) compared both SISH and FISH HER2 tests in Korea gastric adenocarcinoma 588 cases. They found only 9 cases with discrepancy, yielding a 98.3% concordance rate. Garcia-Garcia et al [46] (2011) compared both SISH and FISH HER2 tests in Spanish gastric adenocarcinoma in 166 cases. They found 96% concordance rate. Long et al [47] (2011) compared both SISH and FISH HER2 tests in China gastric adenocarcinoma 80 cases. They found only one case with discrepancy, yielding a 99% concordance rate. From above studies, FISH and SISH showed similar positive rates. The only difference between two methods is that SISH is much easier to count the HER2 signals.

HER2 amplification or overexpression in primary tumor vs metastatic tumor was also reported. Bozzetti et al [48] (2011) tested HER2 status with both FISH and IHC. The found that concordance of HER2 status between primary and metastatic tumor is 98.2% by FISH and 94.9% by IHC. They concluded that HER2 status is maintained in most cases unchanged during the metastatic process.

HER2 amplification or overexpression correlating with patient survival and clinicopathological features: In breast cancer, HER2 amplification or overexpression is clearly associated with poorer prognosis and aggressive disease. However, the prognosis of HER2 amplification or overexpression in gastric cancer is controversial. In addition, the association of HER2 positive gastric cancer with clinicopathological features are also not consistent.

Yonemura et al [49] (1991) first reported HER2 overexpression in 260 primary gastric cancer. Patients with erbB-2 protein-positive tumors had 5-fold greater relative risk of death, as compared with those with erbB-2 protein-negative tumors. erbB-2 protein expression was associated with serosal invasion, lymph node metastasis, and lymphatic invasion. Later, their results were confirmed by Nakajima et al (1999). Nakajima et al. [50](1999) also reported HER2 overexpression in 16.4% of gastric cancer, which was associated with significantly poorer survival. However, Kim et al. [51] (1994) studied the HER2 overexpression in 152 Korea gastric carcinoma patients. They reported that the survival analysis of 104 patients with stage III gastric carcinoma revealed no significant association between c-erbB-2 staining status and survival duration. The 5-year survival rates of the c-erbB-2 positive group and its negative group were 21% and 28%, respectively. In addition, there was little association between staining of c-erbB-2 protein and clinicopathological findings such as age, sex, location, histology, gross type, lymph node status, depth of invasion, and stage. However, other Korea studies found HER2 positive gastric cancer had a poor prognosis [41,52]. Park et al [41] reported that HER-2/neu overexpression and amplification in 182 gastric cancer Korea patients was examined with IHC. Twenty-nine of 182 patients expressed the HER-2/neu protein by IHC. Tumors with HER-2/neu amplification were associated with poor mean survival rates (922 vs 3243 days) and 5-year survival rates (21.4% vs 63.0%; $P < 0.05$). Age, TNM stage, and amplification of HER-2/neu were found to be independently related to survival by multivariate analysis. In another Korea study with 1,414 cases and 595 tissue microarray cases, HER2-positivity was detected in 12.3% of whole-tissue sections and 17% of TMAs [53]. They found that HER2-positivity was correlated with age, histological type, lymphovascular invasion, and lymph node metastasis. Multivariate analyses of the differentiated gastric carcinoma subgroup revealed that HER2-positivity was an independent poor prognostic.

Zhang et al (2009) studied the HER2 and HER3 overexpression in Chinese gastric cancer with 102 cases. Overexpression of HER2 and HER3 was detected around 18.6% and 13.7%. HER2 and HER3 overexpression was correlated with a significantly worse survival ($p = 0.046$ and 0.024, respectively). The overexpression rates of HER2 and HER3 in phase III-IV (TNM stage) disease were significantly higher than that in phase I-II disease (24.0% vs. 7.7%, $p < 0.05$ and 22.0% vs. 5.8%, $p < 0.05$, respectively). They proposed that HER3 may become another molecular target.

In European and United States, Tanner et al [54] (2005) found that HER2 amplification was present in 12.2% of the 131 gastric cancer and 24% of the 100 GEJ adenocarcinomas in Finland which was associated with poor carcinoma-specific survival. In contrast, Kunz [55](2012) reported that twelve of 99 (12%) gastric carcinomas were positive for HER2 and seven of 70 (10%) gastroesophageal junction carcinomas were positive for HER2. HER2 status or primary tumor site did not correlate with patient survival.

Recently, Jorgensen and Hersom [56] (2012) reviewed previous studies with more than 100 patients and analysis of association between the HER2 status and survival or relevant clinicopathological characteristics. Forty-two publications with a total of 12,749 patients fulfilled the two criteria and were reviewed in detail. The majority of the publications (71%) showed that a HER2-postive status measured either by IHC or ISH was associated with poor survival and/or clinicopathological characteristics, such as serosal invasion, lymph node metastases, disease stage, or distant metastases. Based on the current analysis a clear trend towards a potential role for HER2 as a negative prognostics factor in gastric cancer was shown, suggesting that HER2 overexpression and/or amplification is a molecular abnormality that might be linked to the development of gastric cancer

Trastuzumab or other HER2 related medication on treatment of HER2 amplification gastric adenocarcinoma: Trastuzumab, a monoclonal antibody that targets HER2, induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signaling, and prevents cleavage of the extracellular domain of HER2[12]. Trastuzumab were found to inhibit tumor growth in gastric carcinoma cell lines, animal model and xenograft models[23, 33, 57, 58]. Fujimoto-Ouchi (2007) used trastuzumab as a single agent inhibited the tumor growth in both of the HER2-overexpressing models but not in the HER2-negative models, GXF97 and MKN-45. In any combination with capecitabine, cisplatin, irinotecan, docetaxel, or paclitaxel, trastuzumab showed more potent antitumor activity than the anticancer agents alone. A three-drug combination of capecitabine, cisplatin, and trastuzumab showed remarkable tumor growth inhibition. Since breast cancer showed better prognosis with trastuzumab treatment for HER2 positive breast cancer patients, clinical trial was also performed in gastric carcinoma. ToGA (Trastuzumab for Gastric Cancer) was an open-label, international, phase 3, randomised controlled trial undertaken in 122 centers in 24 countries[22]. Patients with gastric or gastro-esophageal junction cancer were eligible for inclusion if their tumors showed overexpression of HER2 protein by immunohistochemistry or gene amplification by fluorescence in-situ hybridization. Participants were randomly assigned in a 1:1 ratio to receive a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin given every 3 weeks for six cycles or chemotherapy in combination with intravenous trastuzumab. 594 patients were randomly assigned to study treatment (trastuzumab plus chemotherapy, n=298; chemotherapy alone, n=296). Median follow-up was 18·6 months in the trastuzumab plus chemotherapy group and 17·1 months in the chemotherapy alone group. Median overall survival was 13·8 months in those assigned to trastuzumab plus chemotherapy compared with 11·1 months in those assigned to chemotherapy alone (hazard ratio 0·74).

Although the survival improvement about 3 months, it is a great breakthrough for gastric carcinoma treatment since the survival of these cancer has not change for a decade. After ToGA clinical trial, trastuzumab combined with standard chemical therapy (such as capecitabine or 5-fluorouracil and cisplatin) now is approved by European Medicines Agency, United States and Japan etc. for the treatment of patients with HER2 overexpression or amplification. In addition, laptinib showed a synergistic effect with trastuzumab in vitro and in vivo to inhibit HER2 amplified human gastric cancer cells and animal model [23]. Clinical phase II trial of lapatinib as first line therapy in patients with advanced or metastatic cancer showed well tolerated, which will be another potential drug to target HER2 receptors[59].

3. HER2 in esophageal adenocarcinoma

EAC incidence has increased 6 folds in United States and Western countries in the last three decades and the prognosis is usually very poor with 5-year survival rates ranging from 14-22%[60-63]. While surgical treatment of EAC can offer cure, many patients first present as a disseminated disease and require systemic therapy. Current chemotherapy regimens provide only minimal survival benefit, predominantly when used in combination with surgery or radiation. Recently clinical trial (ToGA) in Asian and European countries showed that anti-HER2 monoclonal antibody trastuzumab treatment significantly improved the survival of patients with gastric adenocarcinoma and HER2 overexpression and amplification. The clinical trial of trastuzumab to treat esophageal adenocarcinoma patients are approved in United States and European countries. Here is a comprehensive review of HER2 overexpression and amplification in esophageal adenocarcinoma.

IHC studies on HER2 overexpression: In esophageal adenocarcinoma, *HER2* overexpression and amplification recently has been reported at frequencies similar to those observed in breast cancer. Based on most reports from English literature, the frequency of

HER-2 immunohistochemistry shows an average of 12%. The current problems for IHC test for HER2 overexpression is the standard score criteria of the intensity of IHC stain. Recently, Zhou and his colleagues (2011) set up a new score criteria which is modified from Hoffman's gastric adenocarcinoma score system (Table 3). In our modified score criteria, IHC 2+ will be counted as positive HER2 overexpression since all IHC 2+ case had HER2 amplification with CISH test. However, the recent Mayo Clinic study reported that only 15% of IHC2+ cases showed HER2 amplification with FISH tests with breast HER2 criteria. It is difficult to compare their criteria since there are no pictures in their reports.

FISH and CISH studies on HER2 amplification: In esophageal adenocarcinoma, HER2 amplification recently has been extensively studies. Reichelt *et al*. found that 15% (16/110) of tumors had *HER2* gene amplification with FISH. Similarly, Brien *et al*. showed that 19% (12/63) of esophageal adenocarcinomas had *HER2* gene amplification. In addition, with 3-dimentional FISH method in thick slides (16 μm, n=124), Rauser et al. [64] found that *HER2* amplification was 10.5% in high-level amplification (≥ 6.0 signals) and 60% in low-level copy number change (≥ 2.5-4.0 signals). However, in thin slides (4 μm, n=123), *HER2* amplification was found in 9 % in high-level amplification (≥ 6.0 signals) and 6 % in low-level copy number change (≥ 2.5-4.0 signals). However, there is a huge difference between traditional FISH in thin section (6%) and three-dimensional FISH in thick section (60%) to detect the low-level *HER2* amplification. They considered that the tumor cell nuclei were truncated due to standardized thin tissue sectioning. Therefore, three dimension FISH need to be further evaluated to help better understand any prognostic significance. In our study, we found that *HER2* amplification was 18% (21/116) detected by CISH and 16.4% (19/116) by high definition microarray in cases of esophageal adenocarcinoma. In addition we found no evidence of *HER2* amplification in low grad dysplasia, Barrett's esophagus, columnar cell metaplasia or normal esophageal squamous epithelium. Thus, the frequency of *HER2* amplification in esophageal adenocarcinoma appears to be consistent between studies with a range of 15-19% and this event appears not to occur prior to the development of high grade dysplasia. Radu et al[65] (2012) compared HER2 antibodies with FISH tests. They used CAP definition of HER2 amplification to evaluate the FISH results. They found that the very high HER2 amplification rate (30/103, 29%) with HER2/CEP17 ≥ 2.2 and (32/102, 31%) with HER2/CEP17 ≥ 2.0. From their slides, they used 5 μm instead of 3-4 μm routine section. Actually the similar phenomenon was reported in esophageal adenocarcinoma cases [64]. Using 16 μm *vs* 4 μm sections for HER2 FISH tests, they found that 16 μm sections showed higher HER2 amplification than 4 μm. Higher HER2 amplification from Radu may be caused by thicker section.

HER2 amplification or overexpression correlating with patient survival and clinicopathological factors: In esophageal adenocarcinoma, the relationship between *HER2* amplification and prognosis is limited and controversial [66, 67]. Brien et al. [66] found that patients with *HER2* amplification (n=11) had shorter survival durations than did patients without amplification (n=43). In contrast, Reichelt et al.[67] found no survival difference between the *HER2* amplification (n=16) and no *HER2* amplification groups (n=90)(p=0.953).

In addition, Rauser et al.[28] found that *HER2* gene amplification was associated with increased disease-specific mortality on 3-dimensional fluorescence in situ hybridization (FISH) analysis in thick slides (16 µm), but not on FISH and immunohistochemical analyses in thin (4 µm) sections. Our results [68] indicate no association of *HER2* amplification with patient survival in a large cohort studies (total 232 patients) by both CISH and high density DNA microarrays methods although HER2 amplification group shows better prognosis (23 months vs 25 months). However, Yoon et al[69] (2012) found that HER2 amplification significantly associated with improved overall survival (n=713) with 35% of HER2 positive patients alive at 5 years as compared with 26% of HER2 negative patients. It is interesting that they divided the HER2 positive EAC into two groups: EAC with and without adjacent BE. They found that HER2 positive EAC with BE significantly associated with disease specific survival and overall survival, but HER2 positive EAC without BE was not significantly associated with disease-specific-survival and overall survival. The prognosis of HER2 positive EAC patients still cannot be concluded. At present, we can say HER2 positive EAC patients do not show worse prognosis.

The association between *HER2* amplification and these clinicopathological factors were controversial. First, Brien [66] 2000 reported that HER2 amplification was not significantly associated with any clinicopathological features such as depth of tumor invasion, lymph node metastasis, differentiation and pathological stage. Reichelt et al [67] (2007) found that HER2 amplification was not associated with pathological staging (TNM) and grade. In our study [68], 21 of 116 EAC patients had *HER2* amplification. Nineteen were male, and 2 female (M:F ratio, 10:1), with a mean age of 63 years (range, 51 to 74 years). The remaining patients (85 males and 10 females [M:F ratio, 9:1]; mean age, 65 years [34 to 85 years]) had no amplification. A Fisher's exact test shows that there is no significant association between *HER2* and gender (p=1.0), age (p =0.188,), the stage (p =0.325), and the number of metastatic lymph nodes (p =0.234). However, the frequency of HER2 amplification was found to be significantly higher (p=0.004) in moderately differentiated tumors (13/22) compared with poor or well differentiated tumors (1/6 and 7/61 respectively). Yoon [69](2012) study supported our finding that HER2 amplification cases were significantly associated with better differentiation, but HER2 amplification cases were not associated with age and gender. However, they also showed that HER2 amplification was associated with lower depth of tumor invasion (T stage), fewer malignant nodes, and absence of signet ring cells.

In summary, the association of HER2 amplification with survival and clinicopathological features is not very clear. At least HER2 amplification was not associated with worse prognosis in most large cohort studies. In addition, the HER2 amplification may be associated better differentiation, but not associated with age and sex. The large, multi-institute study is needed to confirm current studies.

Trastuzumab or other HER2 related medication on treatment of HER2 amplification esophageal adenocarcinoma: Safran et al [70, 71] (2004, 2007) first reported clinical trial with trastuzumab, paclitaxel, cisplatin and radiation for locally advanced esophageal adenocarcinoma patients with HER2 overexpression. They took patients with histologically documented EAC with T3, T4 or lymph nodal disease. They used IHC 2+ and 3+ with more than 10% cells as HER2 positive overexpression and set FISH ratio greater than 2 as HER2 amplification. The median survival for all 19 patients is 24 months, which is similar to prior studies. Esophagitis, nausea, dehydration, and neutropenia were the most common toxicity. However, toxicity was modest with only 2 patients (10%) having grade 3-4 esophagitis. Therefore, trastuzumab does not increase toxicities when added to chemoradiation for patients with esophageal cancer.

ToGA clinical trials in patients with gastric adenocarcinoma (trial *vs* control: 236 *vs* 243 patients) and gastroesophageal junction adenocarcinoma (trial *vs* control: 58 *vs* 48 patients) have shown a significant survival benefit for patients treated with a combination of trastuzumab and standard chemotherapy.[22,72] Now Safran and colleagues started Phase III clinical trial to study Radiotherapy, Paclitaxel, and Carboplatin with versus without Trastuzumab in patients with HER2-overexpressing esophageal adenocarcinoma (RTOG-1010, and NCI web site: http://www.cancer.gov/ncicancerbulletin/062811/page6). Their primary goal is to determine whether trastuzumab increases disease-free survival when combined with radiotherapy, paclitaxel, and carboplatin followed by surgery in patients with HER2-overexpressing esophageal adenocarcinoma. It is interesting to follow up their results.

4. HER2 in breast cancers

Among new breast cancer patients, 15% to 20% will develop tumors that harbor a genomic alteration involving the *HER2* gene locus. This alteration results in amplification of an amplicon on chromosome 17 that contains the HER2 proto-oncogene[73, 74]. Gene amplification is the primary mechanism that drives HER2 receptor protein over-expression in this important subset of breast cancers. HER2 over-expression resulting from gene amplification dramatically increases the likelihood of receptor activation and signaling, contributing to a more aggressive tumor biology and is associated with worse clinical outcome including higher rates of early, predominantly visceral and central nervous system recurrence and mortality. [75, 76] In addition to the prognostic impact, HER2 over-expression in breast cancer is highly correlated with a younger age at presentation, higher tumor grade as well as a higher tumor burden compared with HER2 negative disease [77]. HER2 over-expression in breast cancer was recognized early on as being an ideal target for therapy, given the location of the receptor on the surface of tumor cells and its role in driving the clinical course of disease for the subset of patients with the HER2 alteration [78], [79]. The drug Trastuzumab was developed as a targeted biologic therapeutic against the HER2 receptor protein. Trastuzumab is a humanized monoclonal antibody that combines the mouse recognition sequence of a monoclonal antibody (clone 4D5) against an extracellular epitope of the receptors with a human IgG1[74]. Trastuzumab demonstrates a high affinity and specificity for the HER2 receptor and in preclinical studies was shown to be effective at inhibiting the growth of HER2 over-expressing breast cancer cells.[80]

In numerous clinical trials, targeting HER2 has been shown to be remarkably effective against HER2 positive breast cancer in both the metastatic and the adjuvant settings, particularly in combination with cytotoxic chemotherapy. Treatment with the drug Trastuzumab has been shown to improve response rates, time to progression, and even survival when used alone[18] or added to chemotherapy in metastatic setting .[78] The success of therapeutically targeting HER2 in the metastatic setting led to several international, prospective randomized trials that have demonstrated that adjuvant trastuzumab reduces the relative risk of recurrence by half and mortality by one third in early-stage breast cancer.[81-84] The data from these clinical trials highlights the importance of accurate HER2 testing for every newly diagnosed breast cancer patient in order to help select those patients who will be the most suitable candidates for HER2 targeted therapy. [85]

Clinical assays to assess the HER2 status include IHC, which detects protein over-expression, or FISH, which detects gene amplification. [85-87] Both assays have been clinically validated in the above mentioned prospective randomized clinical trials and have received FDA approval for predicting a clinical response and patient benefit from HER2-targeted treatment. Published data from these clinical trials suggest that only those patients whose breast cancer demonstrates protein over-expression and/or gene amplification by the above assays are likely to benefit from therapy with Trastuzumab. [88] Since the results of HER2 assays stand alone in determining which breast cancer patients will be the most appropriate for HER2-targeted therapy, accurate, reliable and reproducible results are a high priority for ensuring optimal patient treatment.

The ASCO/CAP task force, has published recommendations for HER2 testing, in which the panel has concluded that both tests were equally efficient in identifying patients who are candidates for HER2-targeted therapy, as long as the assays have been properly validated and all aspects of the testing is performed in a highly standardized fashion with a rigorous quality assurance program. [89] This task force also recognized the importance of standardizing pre-analytical variables including tissue handling and fixation to improve the quality of clinical samples for predictive factor analysis. [90] The IHC and FISH methodologies for evaluating the HER2 status in breast cancer are complementary in nature. [91] These tests examine different aspects of the biology that underlies HER2 driven breast tumors. FISH evaluates the status of the *HER2* gene in the nucleus and is a surrogate for protein expression, while IHC directly evaluates over-expression of the receptor protein at the surface of the cell. In the majority of HER2-positive cancers, HER2 protein over-expression is the result of gene amplification, thus *HER2* gene/protein status should be highly correlated in most cases. Consequently, HER2 gene/protein discordant results in the majority of cases are related to technical issues. However, unusual *HER2* genotypes such as polysomy for chromosome 17 and genomic heterogeneity can lead to discrepant non-correlating cases that may be clinically important. [92, 93] For such cases, the assessment of both the gene and the protein may be necessary in order to sort out the most appropriate HER2 status for the purpose of determining therapy.

Despite the remarkable clinical efficacy of HER2 targeted therapy, not all patients respond and de novo as well as acquired resistance remains an important clinical issue. Currently there are no clinically validated factors that can be used to predict resistance to HER2 targeted therapy in breast cancer. Preclinical data and more recent clinical studies have suggested a number of potential mechanisms of resistance including reduction of antibody affinity and binding due to steric hindrance from MUC4 over-expression, constitutively active downstream signaling involving p27 Kip1, PTEN, PI3K, mTOR, and Akt as well as cross-talk with other signaling pathways including EGFR and IGFR-1, that can by-pass HER2-blockade. [94, 95]

		HER2 amplified		**HER2 non-amplified**		*p* value
Age		63 (51-74)		65 (34-85)		0.188
Gender		MALE	FEMALE	MALE	FEMALE	1.0
		19	2	85	10	
		POS	NEG	POS	NEG	
Lymph node METASTASIS		13	8	69	26	0.234
pStaging						0.325
	I	3		10		
	II	8		25		
	III	10		60		
Median survival (months)		25 (7-71)		23 (0.03-108)		0.19
Differentiation						0.004
	poor	7		61		
	moderate	13		22		
	well	1		6		

Table 4. Association of *HER2* amplified group and *non-HER2* amplified group with multiple clinical factors (From Hu Y, Bandla S, Godfrey TE, et al. HER2 amplification, overexpression and score criteria in esophageal adenocarcinoma. Mod Pathol 2011;24:899-907)

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5. References

[1] Emde A, Kostler WJ, Yarden Y. Therapeutic strategies and mechanisms of tumorigenesis of HER2-overexpressing breast cancer. *Crit Rev Oncol Hematol* 2010 http://dx.doi.org/10.1016/j.critrevonc.2010.09.002.

[2] Ross JS. Update on HER2 testing for breast and upper gastrointestinal tract cancers. *Biomark Med* 2011; 5(3):307-18.

[3] Albarello L, Pecciarini L, Doglioni C. HER2 testing in gastric cancer. *Adv Anat Pathol* 2011; 18(1):53-9.

[4] Fornaro L, Lucchesi M, Caparello C, *et al.* Anti-HER agents in gastric cancer: from bench o bedside. *Nat Rev Gastroenterol Hepatol* 2011; 8(7):369-83.

[5] Hicks DG, Whitney-Miller C. HER2 testing in gastric and gastroesophageal junction cancers: a new therapeutic target and diagnostic challenge. *Appl Immunohistochem Mol Morphol* 2011; 19(6):506-8.

[6] Tsang RY, Finn RS. Beyond trastuzumab: novel therapeutic strategies in HER2-positive metastatic breast cancer. *Br J Cancer* 2012; 106(1):6-13.

[7] Rexer BN, Arteaga CL. Intrinsic and Acquired Resistance to HER2-Targeted Therapies n HER2 Gene-Amplified Breast Cancer: Mechanisms and Clinical Implications. *Crit Rev Oncog* 2012; 17(1):1-16.

[8] Petrelli F, Barni S. Role of HER2-neu as a prognostic factor for survival and relapse in pT1a-bN0M0 breast cancer: a systematic review of the literature with a pooled-analysis. *Med Oncol* 2012.

[9] Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. *Physiology Bethesda)* 2010; 25(2):85-101.

[10] Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 2000; 19(13):3159-67.

[11] Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene* 2007; 26(45):6469-87.

[12] Moulder SL, Yakes FM, Muthuswamy SK, *et al.* Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo. *Cancer Res* 2001; 61(24):8887-95.

[13] Liu E, Thor A, He M, *et al.* The HER2 (c-erbB-2) oncogene is frequently amplified in in situ carcinomas of the breast. *Oncogene* 1992; 7(5):1027-32.

[14] Park K, Han S, Kim HJ, Kim J, Shin E. HER2 status in pure ductal carcinoma in situ and in the intraductal and invasive components of invasive ductal carcinoma determined by fluorescence in situ hybridization and immunohistochemistry. *Histopathology* 2006; 48(6):702-7.

[15] Carlsson J, Nordgren H, Sjostrom J, *et al.* HER2 expression in breast cancer primary tumours and corresponding metastases. Original data and literature review. *Br J Cancer* 2004; 90(12):2344-8.

[16] Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. *Nature* 1988; 332(6162):323-7.

[17] Ben-Kasus T, Schechter B, Sela M, Yarden Y. Cancer therapeutic antibodies come of age: targeting minimal residual disease. *Mol Oncol* 2007; 1(1):42-54.

[18] Cobleigh MA, Vogel CL, Tripathy D, *et al.* Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999; 17(9):2639-48.
[19] Vogel CL, Cobleigh MA, Tripathy D, *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002; 20(3):719-26.
[20] Slamon D, Pegram M. Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. *Semin Oncol* 2001; 28(1 Suppl 3):13-9.
[21] Untch M, Rezai M, Loibl S, *et al.* Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. *J Clin Oncol* 2010; 28(12):2024-31.
[22] Bang YJ, Van Cutsem E, Feyereislova A, *et al.* Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; 376(9742):687-97.
[23] Wainberg ZA, Anghel A, Desai AJ, *et al.* Lapatinib, a dual EGFR and HER2 kinase inhibitor, selectively inhibits HER2-amplified human gastric cancer cells and is synergistic with trastuzumab in vitro and in vivo. *Clin Cancer Res* 2010; 16(5):1509-19.
[24] Geyer CE, Forster J, Lindquist D, *et al.* Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 2006; 355(26):2733-43.
[25] Baselga J, Bradbury I, Eidtmann H, *et al.* Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2012; 379(9816):633-40.
[26] Gravalos C, Gomez-Martin C, Rivera F, *et al.* Phase II study of trastuzumab and cisplatin as first-line therapy in patients with HER2-positive advanced gastric or gastroesophageal junction cancer. *Clin Transl Oncol* 2011; 13(3):179-84.
[27] Spector NL, Xia W, Burris H, 3rd, *et al.* Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J Clin Oncol* 2005; 23(11):2502-12.
[28] Nahta R, Yuan LX, Du Y, Esteva FJ. Lapatinib induces apoptosis in trastuzumab-resistant breast cancer cells: effects on insulin-like growth factor I signaling. *Mol Cancer Ther* 2007; 6(2):667-74.
[29] Nahta R, Shabaya S, Ozbay T, Rowe DL. Personalizing HER2-targeted therapy in metastatic breast cancer beyond HER2 status: what we have learned from clinical specimens. *Curr Pharmacogenomics Person Med* 2009; 7(4):263-74.
[30] Xia W, Liu LH, Ho P, Spector NL. Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. *Oncogene* 2004; 23(3):646-53.
[31] Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 2006; 24(14):2137-50.
[32] Patel SH, Kooby DA. Gastric adenocarcinoma surgery and adjuvant therapy. *Surg Clin North Am* 2011; 91(5):1039-77.

[33] Tanner M, Hollmen M, Junttila TT, *et al.* Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. *Ann Oncol* 2005; 16(2):273-8.

[34] Matsui Y, Inomata M, Tojigamori M, *et al.* Suppression of tumor growth in human gastric cancer with HER2 overexpression by an anti-HER2 antibody in a murine model. *Int J Oncol* 2005; 27(3):681-5.

[35] Tanaka S, Mori M, Akiyoshi T, *et al.* Coexpression of Grb7 with epidermal growth factor receptor or Her2/erbB2 in human advanced esophageal carcinoma. *Cancer Res* 1997; 57(1):28-31.

[36] Hofmann M, Stoss O, Shi D, *et al.* Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; 52(7):797-805.

[37] Ruschoff J, Dietel M, Baretton G, *et al.* HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 2010; 457(3):299-307.

[38] Ruschoff J, Hanna W, Bilous M, *et al.* HER2 testing in gastric cancer: a practical approach. Mod Pathol 2012 25(5):637-50.

[39] Cho EY, Srivastava A, Park K, *et al.* Comparison of four immunohistochemical tests and FISH for measuring Her2 expression in gastric carcinomas. *Pathology* 2012; 44(3):216-20.

[40] Barros-Silva JD, Leitao D, Afonso L, *et al.* Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients. *Br J Cancer* 2009; 100(3):487-93.

[41] Park YS, Hwang HS, Park HJ, *et al.* Comprehensive analysis of HER2 expression and gene amplification in gastric cancers using immunohistochemistry and in situ hybridization: which scoring system should we use? *Hum Pathol* 2012; 43(3):413-22.

[42] Tapia C, Glatz K, Novotny H, *et al.* Close association between HER-2 amplification and overexpression in human tumors of non-breast origin. *Mod Pathol* 2007; 20(2):192-8.

[43] Yamamoto T, Ikawa S, Akiyama T, *et al.* Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. *Nature* 1986; 319(6050):230-4.

[44] Kimura M, Tsuda H, Morita D, *et al.* Usefulness and limitation of multiple endoscopic biopsy sampling for epidermal growth factor receptor and c-erbB-2 testing in patients with gastric adenocarcinoma. *Jpn J Clin Oncol* 2005; 35(6):324-31.

[45] Ruschoff J, Nagelmeier I, Baretton G, *et al.* [Her2 testing in gastric cancer. What is different in comparison to breast cancer?]. *Pathologe* 2010; 31(3):208-17.

[46] Garcia-Garcia E, Gomez-Martin C, Angulo B, *et al.* Hybridization for human epidermal growth factor receptor 2 testing in gastric carcinoma: a comparison of fluorescence in-situ hybridization with a novel fully automated dual-colour silver in-situ hybridization method. *Histopathology* 2011; 59(1):8-17.

[47] Long XY, Bu H, Wei B, *et al.* [Dual-color silver-enhanced in-situ hybridization and fluorescence in-situ hybridization for determination of HER2 gene status in gastric carcinoma]. *Zhonghua Bing Li Xue Za Zhi* 2011; 40(5):300-3.

[48] Bozzetti C, Negri FV, Lagrasta CA, *et al.* Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. *Br J Cancer* 2011; 104(9):1372-6.

[49] Yonemura Y, Ninomiya I, Ohoyama S, *et al.* Expression of c-erbB-2 oncoprotein in gastric carcinoma. Immunoreactivity for c-erbB-2 protein is an independent indicator of poor short-term prognosis in patients with gastric carcinoma. *Cancer* 1991; 67(11):2914-8.

[50] Nakajima M, Sawada H, Yamada Y, *et al.* The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer* 1999; 85(9):1894-902.

[51] Kim JP, Oh ST, Hwang TS, Chi JG. The prognostic significance of c-erbB-2 and p53 protein expressions in gastric carcinoma--a multivariate analysis of prognostic factors. *J Korean Med Sci* 1994; 9(3):248-53.

[52] Kim JW, Im SA, Kim M, *et al.* The Prognostic Significance of HER2 Positivity for Advanced Gastric Cancer Patients Undergoing First-line Modified FOLFOX-6 Regimen. *Anticancer Res* 2012; 32(4):1547-53.

[53] Kim KC, Koh YW, Chang HM, *et al.* Evaluation of HER2 protein expression in gastric carcinomas: comparative analysis of 1,414 cases of whole-tissue sections and 595 cases of tissue microarrays. *Ann Surg Oncol* 2011; 18(10):2833-40.

[54] Tanner SM, Li Z, Perko JD, *et al.* Hereditary juvenile cobalamin deficiency caused by mutations in the intrinsic factor gene. *Proc Natl Acad Sci U S A* 2005; 102(11):4130-3.

[55] Kunz PL, Mojtahed A, Fisher GA, *et al.* HER2 expression in gastric and gastroesophageal junction adenocarcinoma in a US population: clinicopathologic analysis with proposed approach to HER2 assessment. *Appl Immunohistochem Mol Morphol* 2012; 20(1):13-24.

[56] Jorgensen JT, Hersom M. HER2 as a Prognostic Marker in Gastric Cancer - A Systematic Analysis of Data from the Literature. *J Cancer* 2012; 3:137-44.

[57] Fujimoto-Ouchi K, Sekiguchi F, Yasuno H, *et al.* Antitumor activity of trastuzumab in combination with chemotherapy in human gastric cancer xenograft models. *Cancer Chemother Pharmacol* 2007; 59(6):795-805.

[58] Yamashita-Kashima Y, Iijima S, Yorozu K, *et al.* Pertuzumab in combination with trastuzumab shows significantly enhanced antitumor activity in HER2-positive human gastric cancer xenograft models. *Clin Cancer Res* 2011; 17(15):5060-70.

[59] Wainberg ZA, Lin LS, DiCarlo B, *et al.* Phase II trial of modified FOLFOX6 and erlotinib in patients with metastatic or advanced adenocarcinoma of the oesophagus and gastro-oesophageal junction. *Br J Cancer* 2011; 105(6):760-5.

[60] Gamliel Z, Krasna MJ. Multimodality treatment of esophageal cancer. *Surg Clin North Am* 2005; 85(3):621-30.

[61] Luketich JD, Alvelo-Rivera M, Buenaventura PO, *et al.* Minimally invasive esophagectomy: outcomes in 222 patients. *Annals of surgery* 2003; 238(4):486-94; discussion 94-5.

[62] Swanson SJ, Batirel HF, Bueno R, *et al.* Transthoracic esophagectomy with radical mediastinal and abdominal lymph node dissection and cervical esophagogastrostomy for esophageal carcinoma. *Ann Thorac Surg* 2001; 72(6):1918-24; discussion 24-5.

[63] Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005; 97(2):142-6.

[64] Rauser S, Weis R, Braselmann H, *et al.* Significance of HER2 low-level copy gain in Barrett's cancer: implications for fluorescence in situ hybridization testing in tissues. *Clin Cancer Res* 2007; 13(17):5115-23.
[65] Radu OM, Foxwell T, Cieply K, *et al.* HER2 Amplification in Gastroesophageal Adenocarcinoma: Correlation of Two Antibodies Using Gastric Cancer Scoring Criteria, H Score, and Digital Image Analysis With Fluorescence In Situ Hybridization. *Am J Clin Pathol* 2012; 137(4):583-94.
[66] Brien TP, Odze RD, Sheehan CE, McKenna BJ, Ross JS. HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. *Human pathology* 2000; 31(1):35-9.
[67] Reichelt U, Duesedau P, Tsourlakis M, *et al.* Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus. *Mod Pathol* 2007; 20(1):120-9.
[68] Hu Y, Bandla S, Godfrey TE, *et al.* HER2 amplification, overexpression and score criteria in esophageal adenocarcinoma. *Mod Pathol* 2011; 24(7):899-907.
[69] Yoon HH, Shi Q, Sukov WR, *et al.* Association of HER2/ErbB2 expression and gene amplification with pathologic features and prognosis in esophageal adenocarcinomas. *Clin Cancer Res* 2012; 18(2):546-54.
[70] Safran H, Dipetrillo T, Akerman P, *et al.* Phase I/II study of trastuzumab, paclitaxel, cisplatin and radiation for locally advanced, HER2 overexpressing, esophageal adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2007; 67(2):405-9.
[71] Safran H, DiPetrillo T, Nadeem A, *et al.* Trastuzumab, paclitaxel, cisplatin, and radiation for adenocarcinoma of the esophagus: a phase I study. *Cancer Invest* 2004; 22(5):670-7.
[72] Jorgensen JT. Target HER2 treatment in advanced gastric cancer *Oncology* 2010; 78(1):26-33.
[73] Hicks DG, Tubbs RR. Assessment of the HER2 status in breast cancer by fluorescence in situ hybridization: a technical review with interpretive guidelines. *Hum Pathol* 2005; 36(3):250-61.
[74] Ross JS. Breast cancer biomarkers and HER2 testing after 10 years of anti-HER2 therapy. *Drug News Perspect* 2009; 22(2):93-106.
[75] Winstanley J, Cooke T, Murray GD, *et al.* The long term prognostic significance of c-erbB-2 in primary breast cancer. *Br J Cancer* 1991; 63(3):447-50.
[76] Hicks DG, Yoder BJ, Short S, *et al.* Loss of breast cancer metastasis suppressor 1 protein expression predicts reduced disease-free survival in subsets of breast cancer patients. *Clin Cancer Res* 2006; 12(22):6702-8.
[77] Crowe JP, Patrick RJ, Rybicki LA, *et al.* A data model to predict HER2 status in breast cancer based on the clinical and pathologic profiles of a large patient population at a single institution. *Breast* 2006; 15(6):728-35.
[78] Slamon DJ, Leyland-Jones B, Shak S, *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344(11):783-92.
[79] Slamon DJ, Clark GM, Wong SG, *et al.* Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235(4785):177-82.
[80] Yarden Y. Biology of HER2 and its importance in breast cancer. *Oncology* 2001; 61 Suppl 2:1-13.

[81] Slamon DJ, Romond EH, Perez EA. Advances in adjuvant therapy for breast cancer. *Clin Adv Hematol Oncol* 2006; 4(3 Suppl 7):suppl 1, 4-9; discussion suppl 10; quiz 2 p following suppl

[82] Joensuu H, Kellokumpu-Lehtinen PL, Bono P, *et al.* Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006; 354(8):809-20.

[83] Romond EH, Perez EA, Bryant J, *et al.* Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005; 353(16):1673-84.

[84] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, *et al.* Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005; 353(16):1659-72.

[85] Hicks DG, Kulkarni S. Trastuzumab as adjuvant therapy for early breast cancer: the importance of accurate human epidermal growth factor receptor 2 testing. *Arch Pathol Lab Med* 2008; 132(6):1008-15.

[86] Tubbs RR, Hicks DG, Cook J, *et al.* Fluorescence in situ hybridization (FISH) as primary methodology for the assessment of HER2 Status in adenocarcinoma of the breast: a single institution experience. *Diagn Mol Pathol* 2007; 16(4):207-10.

[87] Powell WC, Hicks DG, Prescott N, *et al.* A new rabbit monoclonal antibody (4B5) for the immunohistochemical (IHC) determination of the HER2 status in breast cancer: comparison with CB11, fluorescence in situ hybridization (FISH), and interlaboratory reproducibility. *Appl Immunohistochem Mol Morphol* 2007; 15(1):94-102.

[88] Yoder BJ, Tso E, Skacel M, *et al.* The expression of fascin, an actin-bundling motility protein, correlates with hormone receptor-negative breast cancer and a more aggressive clinical course. *Clin Cancer Res* 2005; 11(1):186-92.

[89] Wolff AC, Hammond ME, Schwartz JN, *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; 131(1):18-43.

[90] Hicks DG, Kushner L, McCarthy K. Breast cancer predictive factor testing: the challenges and importance of standardizing tissue handling. *J Natl Cancer Inst Monogr* 2011; 2011(42):43-5.

[91] Hicks DG, Kulkarni S. HER2+ breast cancer: review of biologic relevance and optimal use of diagnostic tools. *Am J Clin Pathol* 2008; 129(2):263-73.

[92] Downs-Kelly E, Yoder BJ, Stoler M, *et al.* The influence of polysomy 17 on HER2 gene and protein expression in adenocarcinoma of the breast: a fluorescent in situ hybridization, immunohistochemical, and isotopic mRNA in situ hybridization study. *Am J Surg Pathol* 2005; 29(9):1221-7.

[93] Vance GH, Barry TS, Bloom KJ, *et al.* Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. *Arch Pathol Lab Med* 2009; 133(4):611-2.

[94] Khoury T, Mojica W, Hicks D, *et al.* ERBB2 juxtamembrane domain (trastuzumab binding site) gene mutation is a rare event in invasive breast cancers overexpressing the ERBB2 gene. *Mod Pathol* 2011; 24(8):1055-9.

[95] Gallardo A, Lerma E, Escuin D, *et al.* Increased signalling of EGFR and IGF1R, and deregulation of PTEN/PI3K/Akt pathway are related with trastuzumab resistance in HER2 breast carcinomas. *Br J Cancer* 2012; 106(8):1367-73.