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HER2 in Resected Gastric Cancer: Is there Prognostic Value?

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ABSTRACT

HER2 in Resected Gastric Cancer: Is There Prognostic Value?

Sarah B. Fisher

Introduction: Human epidermal growth factor receptor 2 (HER2) is a receptor tyrosine kinase whose amplification results in protein over-expression and tumorigenesis. Most studied in breast cancer, HER2 amplification and over-expression has been associated with resistance to cytotoxic chemotherapy, presence of adverse pathologic characteristics, and poor overall prognosis. Although documented in gastric cancer, the prevalence and prognostic value of HER2 in patients with early stage/resectable gastric cancer is controversial.

Methods: 111 pts underwent curative intent resection of gastric adenocarcinoma between 1/00-6/11 and had tissue available for analysis. Immunohistochemistry (IHC) for HER2 was performed on banked tumor specimens and graded by two pathologists blinded to outcomes. An IHC score of 0+ or 1+ was regarded as negative, 3+ as positive. Fluorescence in-situ hybridization (FISH) for HER2 was performed on equivocal (2+) IHC samples, and in cases of inter-pathologist disagreement. HER2 status was compared with the presence of known adverse prognostic factors and evaluated as a prognostic marker for overall survival.

Results: The demographics and clinical characteristics of the patient population were representative of patients undergoing resection for gastric cancer, with a median overall survival of 27.2 months. HER2 expression as measured by IHC was negative in 61 (55%), equivocal in 37 (33.3%), and positive in 13 (11.7%) cases. Of the 37 equivocal cases, FISH was positive in 8, for a total of 21 HER2-positive cases (18.9%, 95% C.I. 11.6%-26.2%) and 90 HER2-negative cases (81.1%, 95% C.I. 73.8%-88.3%). Patients with HER2-positive tumors were less likely to have signet ring cell features (23.8% vs 53.9%, $p=0.008$). HER2 status was not associated with tumor size, presence of perineural or lymphovascular invasion, margin status, nodal metastases, or stage ($p>0.05$). HER2 status was not associated with overall survival ($p=0.385$).

Conclusions: HER2 over-expression/amplification is present in a measurable amount but does not appear to be associated with adverse prognostic factors or survival in patients with resected gastric cancer. Our results, combined with the growing body of evidence from others suggest HER2 is not prognostic for patients with early stage gastric cancer.

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INTRODUCTION

Each year, gastric cancer is diagnosed in more than 21,000 people and causes more than 10,000 deaths in the United States.[1] Worldwide, gastric cancer is among the top five most frequently diagnosed cancers, and unfortunately remains in the top five causes of cancer-related deaths, with significant burden in developing countries.[2] The prevalence of gastric cancer varies substantially with geography, with higher incidences in East Asian, South American, and Western European countries.[3] In addition to the differences in incidence, variability in disease biology,[4,5] standard treatment modalities,[3,6,7] and prognosis[8] exist between the East and the West, such that findings that rely mainly on Eastern patients may not be applicable to Western patients.

In the West, the prognosis for most patients with gastric cancer is poor, with 5 year survival for all patients less than 30%.[9] For those patients presenting with early stage disease, surgical resection is the mainstay of curative therapy. In addition to surgery, multiple randomized controlled trials have demonstrated a survival advantage with multimodality therapy (chemotherapy with or without radiation therapy before or after surgical resection).[10-14] Yet even with the most aggressive combinations of surgery, chemotherapy, and radiation for patients with early stage/resectable disease, recurrences are common and five-year survival for remains a dismal 30 to 50%.[3]

Given the disappointing results of standard therapy, a paradigm shift in oncology is occurring that focuses on tailoring therapy to the individual patient. Improved understanding of the molecular pathways associated with tumor cell growth, proliferation, invasion, and metastasis have led to the identification and development of drugs specific to tumor-related molecular targets. One example of the success of targeted

therapy involves human epidermal growth factor receptor 2 (HER2) in patients with breast cancer.

HER2 is a transmembrane tyrosine kinase receptor expressed by normal and cancerous cells.[15-17] HER2 dimerizes with either itself or one of three other family members (HER1, HER3, HER4) to activate a cascade of intracellular signaling via the mitogen activated protein kinase and phosphatidylinositol pathways, which results in cell proliferation, growth, and cell survival.[18] Research has focused on HER2 in particular as it is thought to be the preferred binding partner for all members of the HER family. The specific ligand for HER2 is unknown; HER2 is thought to be capable of constitutive activity such that amplification of the gene and the resulting over-expression of the receptor itself is sufficient for cell growth, proliferation, and survival.[16,18] In breast cancer, HER2 amplification and over-expression have been associated with tumorigenesis, poor response to standard cytotoxic therapies, and poor overall prognosis.[19] The development of the monoclonal antibody trastuzumab, which targets HER2-related tumor proliferation specifically, has improved treatment options and prognosis for the 20-30% of breast cancer patients with HER2 positive tumors.[17,19,20] HER2 amplification and over-expression have been detected in multiple other human malignancies, including gastric cancer, as well as cervical, colorectal, endometrial, esophageal, lung, ovarian, pancreas, prostate, and urothelial cancers.[16,17,20,21]

The prevalence and prognostic value of HER2 in patients with gastric cancer is less established than in breast cancer. Estimates of HER2 prevalence range from 9-38% in most studies,[16] although values as low as 2.5%[22] to as high as 91%[23] have been reported. Similar to its prevalence, determinations of the prognostic value of HER2 are

also controversial, with some studies identifying it as a negative prognostic factor for survival, some as a positive prognostic factor, and others failing to find a relationship.[16,17,24] This variability may in part be explained by differences in study populations but is likely due to differences in the method(s) of testing and interpretation. Current best practice for assessing HER2 in gastric cancer involves immunohistochemistry (IHC) for HER2 in all specimens followed by molecular analysis of *HER2/neu* gene amplification, most often by fluorescent in situ hybridization (FISH), in select samples.[18, 19] This methodology was not widely accepted until 2010.[25,26]

A clearly defined estimate of prevalence and improved understanding of the prognostic value of HER2 should be obtained before initiating clinical evaluations of trastuzumab for patients with early stage gastric cancer. In the current study we use reliable and reproducible methodology (IHC and FISH) with scoring systems developed specifically for gastric cancer[25,26] to evaluate the prognostic value of HER2 in a Western population of patients with early stage/resected gastric cancer. To accomplish this we will measure HER2 expression and amplification and evaluate its association with overall survival, controlling for other prognostic factors such as stage. We hypothesize that HER2 will be expressed and amplified in a measurable quantity, will be associated with the presence of established adverse prognostic factors, and will be an independent marker for poor overall survival.

BACKGROUND

In gastric cancer, reports of HER2 prevalence are variable, likely due to heterogeneity in testing. In breast cancer, standardized and validated methods of assessing HER2 involve IHC applied to all samples and scored as 0+ (negative), 1+ (negative), 2+ (equivocal), or 3+ (positive), followed by FISH for equivocal samples to assess HER2 amplification.[27] Regardless of cancer type, IHC is subjective and at risk for interpreter variability. Scoring guidelines, which are aimed at diminishing variability, further complicate historical comparisons as they have changed over time. Historically, the breast cancer scoring system for HER2 was used in gastric cancer. However, experts estimate that the breast cancer IHC scoring algorithm is inaccurate in up to 20% of breast cancer specimens,[28] and may underestimate the prevalence of HER2 in up to 50% of gastric cancer specimens, due to tumor heterogeneity unique to gastric cancer.[26,29]

Previous studies examining HER2 in gastric cancer used either the breast cancer scoring guidelines or unique and individualized methods of assessment.[22,23,30-32] In 2010 a gastric cancer specific scoring system for IHC was developed[27,33] and tested in combination with FISH in the randomized phase III ToGA (trastuzumab for gastric cancer) trial.[34] In this multi-institutional international study, 22.1% of screened patients with advanced or metastatic gastric cancer were HER2-positive and randomized to either therapy with a HER2-targeted monoclonal antibody (trastuzumab) in addition to standard chemotherapy or standard chemotherapy alone. The trial demonstrated a significant increase in median overall survival of 2.7 months for patients receiving trastuzumab (13.8 vs 11.1 months, HR 0.74, 95% CI [0.60-0.91], p=0.0046)[34] and led to the FDA approval of the drug as part of first line therapy for patients with metastatic gastric cancer.[24] The

ToGA trial was noteworthy both in its successful introduction of a new therapeutic agent for patients with advanced/metastatic gastric cancer and in its contribution to the standardization of HER2 testing in all stages of gastric cancer.

Prior to initiation of the trial, Hofmann et al[27] examined HER2 in 168 gastric and gastroesophageal adenocarcinoma specimens with specific attention to discrepancies between IHC and FISH to insure a reliable and reproducible method of HER2 assessment. Based on their results, an expert consensus panel recommended modifications to the breast cancer IHC scoring system to account for tumor heterogeneity and gastric cancer specific patterns of membranous staining. Initially, both IHC and FISH were recommended for all samples to identify patients with HER2-positive tumors, and both methods were applied to the 3,665 patients screened in the ToGA trial. After trial completion, analyses suggested that patients with IHC-negative/FISH-positive gastric cancer were unlikely to benefit from trastuzumab, and recommendations evolved such that the current best practice for HER2 evaluation in gastric cancer is to perform molecular analysis (FISH) only for equivocal samples (IHC 2+).[25,26]

In addition to variation in the testing method used, tumor heterogeneity represents a source of variability likely to influence HER2 testing in gastric cancer.[35-38] This has significant implications when examining either a biopsy specimen or a tissue-microarray specimen (TMA, in which small cores of representative tumor are chosen for analysis). In a series of 454 patients undergoing resection for gastric cancer, Warneke et al[35] demonstrated a high false negative rate of 24% (and false positive rate of 2%) when comparing whole slide examination to TMAs from the same specimen, primarily related to sampling error. Thus studies relying on interpretation of TMAs for HER2 evaluation

may be inaccurate due to tumor heterogeneity and sampling error. The authors extrapolated that studies relying on biopsy would also be subject to tumor heterogeneity and sampling error.[35]

Since the introduction of the ToGA trial in 2010, several investigators have used the gastric-cancer specific methodology to investigate the prevalence and prognostic value of HER2 in gastric cancer. Despite this, debate regarding the prognostic value of HER2 still exists and differences in methodology remain. Some of the recent studies used TMA (as opposed to the more comprehensive whole slide technique) for IHC, and some relied on biopsy specimens versus surgical specimens. Additionally, the majority of the recent reports have studied patients from Eastern countries, and questions of applicability to a Western population exist.[4,5] Further differences between studies include variations in the inclusion or exclusion of patients with metastatic disease (who have a distinctly different prognosis than patients with early stage/resectable disease[8]) and inclusion of patients with non-gastric cancers (i.e. esophageal cancer).

We therefore aimed to evaluate the prevalence and prognostic value of HER2 status in a Western population of patients with early stage/resectable gastric cancer. Our study uses representative whole slide sections of tissue from the surgical specimen and follows current testing guidelines, increasing the chances of an accurate evaluation of HER2. Accurately characterizing HER2 prevalence and its association with survival will further our understanding of this potential biomarker in gastric cancer and lay the groundwork for future trials with drugs that target HER2 specifically.

SPECIFIC AIMS & HYPOTHESES

Gastric cancer, even when diagnosed at its earliest and most curable stage, is a devastating diagnosis. Cytotoxic chemotherapy combined with complete surgical resection and radiation therapy in select cases offers a chance of cure, but recurrence is common and less than half of early-stage patients survive five years after diagnosis.[3] Efforts to improve survival have led to a paradigm shift in oncology, in which molecular targeted therapy has become the focus. One example of targeted therapy is the inhibition of human epidermal growth factor receptor 2 (HER2) with a targeted monoclonal antibody, trastuzumab. HER2 is a receptor tyrosine kinase whose amplification results in protein over-expression and cell proliferation, growth, and survival. Most studied in breast cancer, HER2 over-expression or amplification has been associated with resistance to cytotoxic chemotherapy, presence of adverse pathologic characteristics, and poor overall prognosis.[19] Recently investigators demonstrated that for the 22.1% of patients with advanced/metastatic gastric cancer who overexpressed HER2, trastuzumab provided a survival benefit.[34] In patients with early stage/resectable gastric cancer, the role of HER2 (and subsequently HER2-directed therapy) is unclear, with estimates of its prevalence and function as a prognostic marker varying widely.[16,17,24] Establishing the expression profile and prognostic value of HER2 in patients undergoing resection for gastric cancer will lay the groundwork for future evaluations of trastuzumab in this patient population.

Our aim was to evaluate HER2 as a prognostic marker for survival in patients undergoing curative intent resection of early stage gastric cancer. To do this, we (1) assessed the prevalence of HER2 using current best practice methodology (IHC and

FISH) developed specifically for gastric cancer, (2) evaluated the association of HER2 with other negative prognostic factors (such as stage), and (3) examined the impact of HER2 on overall survival. We hypothesized that in patients with early stage gastric cancer, HER2 will be overexpressed/amplified in a measurable amount, will be associated with the presence of other adverse prognostic factors, and will be independently associated with reduced overall survival.

METHODS

Patients

HER2 status was measured in banked tumor samples from patients undergoing resection for gastric adenocarcinoma at Emory University Hospital between May 2000 and June 2011. The patients were identified from an Institutional Review Board approved clinical registry maintained by the Division of Surgical Oncology. Inclusion criteria were adult patients status post curative intent resection of gastric adenocarcinoma with tissue available for analysis and documented tissue bank consent. Patients without consent for use of banked tissue and patients undergoing palliative resections were excluded. For specific aims 1 and 2, banked tissue was examined for HER2 status and correlated with known prognostic factors documented within the medical record at the time of operation, resulting in a cross-sectional design. For specific aim 3, survival information was collected retrospectively, resulting in a retrospective cohort study design. Emory University's Institutional Review Board granted permission for the study prior to data collection. All data collection was performed in a manner compliant with The Health Insurance Portability and Accountability Act of 1996.

Patient characterization

A retrospective chart review was performed to identify patient demographics, comorbidities, perioperative details, pathologic and prognostic characteristics, and survival status. Specifically, patient age, gender, and race, smoking history, history of alcohol abuse, history of diabetes mellitus, and history of hypertension were obtained from documentation within the medical record. Smoking history was defined as current

or former use ≥10 pack-years. Alcohol abuse was determined by documentation of significant dependency in the medical record by the treating physician.

Regarding perioperative details, receipt of neoadjuvant (before-surgery) and adjuvant (after-surgery) chemotherapy or radiotherapy was recorded from the medical record. Exact regimens and duration of chemotherapy were not routinely available and were not a focus of the study; trastuzumab is not currently standard of care for patients with early stage/resected gastric cancer and was not administered to any patient. Operation type and approach (open versus laparoscopic), degree of nodal dissection, resection of additional organs, estimated operative blood loss, and transfusion requirement were identified from the dictated operative note.

The pathology report was used to verify histologic diagnosis and record tumor size, tumor grade, margin status, presence or absence of signet ring cell features, lymphovascular invasion, perineural invasion, or nodal metastases. Based on the pathologic and clinical information, patients were staged according to the American Joint Committee on Cancer (AJCC) 7th Edition Tumor-Node-Metastasis (TNM) staging system.

In addition to documentation within the medical record, the Social Security death index was used to supplement survival data. Overall survival (OS) was calculated as time elapsed between the date of surgery and date of death. Perioperative mortality was defined as death within 30 days of operation or during the same hospitalization; these patients were excluded from all survival analyses. Patients discharged to hospice were considered deceased in all survival analyses. Patients who were not deceased were

censored at the last known point of contact or upon completion of data collection on July 5, 2012.

HER2 testing

Representative sections of tumor from formalin-fixed paraffin embedded slides were identified by an experienced pathologist. A combination testing algorithm incorporating HER2 immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) was used to assess HER2 status (Figure 1). HER2 IHC was performed on all tumor samples; FISH was performed for select samples as described below per current guidelines for HER2 testing in gastric cancer.[25,26]

Immunohistochemistry

Tumor samples were stained with HER2 using the HercepTest™ assay (Dako, Carpinteria, CA, USA) per manufacturer instructions and counterstained with hematoxylin using a Dako Autostainer. Two independent pathologists blinded to patient outcomes scored the HER2 IHC according to the same criteria used in the ToGA trial, which was developed specifically for gastric cancer.[34] The scoring is summarized as follows: no reactivity or membranous reactivity in < 10% of tumor cells (0+, negative); faint incomplete membranous reactivity in ≥ 10% of tumor cells (1+, negative); weak to moderate complete basolateral or lateral membranous reactivity in ≥ 10% of tumor cells (2+, equivocal); strong complete basolateral or lateral membranous reactivity in ≥ 10% of tumor cells (3+, positive). Representative images each of the possible IHC scores are shown in Figure 2.

Fluorescence in situ hybridization

Dual color FISH for HER2 amplification was performed for all specimens scored as IHC 2+, and for any discordant specimens. Discordance was defined as a disagreement between the two pathologists that would alter the IHC scoring classification (negative, equivocal, or positive). Samples that were scored as 0+/1+ or 1+/0+ were classified as negative and FISH was not performed. FISH was performed with the PathVysion HER2 kit (Abbott Molecular, Abbott Park, IL, USA) according to manufacturer instructions. HER2 and centromere probe 17 (CEP17) signals were counted in 30 tumor nuclei for each case, and the sum of the signals was used to calculate a HER2:CEP17 ratio. Tumors were classified as negative when the HER2:CEP17 ratio was <1.8 and positive if the HER2:CEP17 ratio was ≥2.2. Specimens with a HER2:CEP17 ratio greater than 1.8 but less than 2.2 were scored as equivocal. A representative image of HER2 amplification by FISH is shown in Figure 3.

HER2 Status

Tumor samples were categorized as positive or negative based on the combined results of IHC and FISH (Table 1).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 20.0 software (IBM, Armonk, NY, USA) and SAS 9.3 software (SAS Institute, Cary, NC, USA) were used for analyses. Data were graphically displayed and examined for distribution and outliers before analysis. The patient population studied represents a convenience sample, so baseline characteristics of the population were examined to assess the representativeness of the population. To assess HER2 prevalence, descriptive statistics were used to determine the frequency of HER2 over-expression (as measured by IHC), amplification (as measured

by FISH), and final tumor evaluation category (positive versus negative, Figure 1), with the latter the primary measurement of interest. Baseline clinicopathologic characteristics were compared between patients with HER2-positive and HER2-negative tumors to evaluate for potential confounders. Prior to survival analysis, associations between known prognostic factors for survival (with stage representing the most predictive factor) and HER2 prevalence were evaluated. Comparisons of continuous variables with HER2 status were made with Student's *t*-test. Comparisons of categorical variables with HER2 status were made with Pearson chi-square and Fisher exact tests, as appropriate. Statistical significance was predefined using two-tailed tests at $\alpha=0.05$.

Survival Analyses

Kaplan-Meier log rank survival analyses were used to calculate overall survival (OS). Known prognostic factors such as margin status and stage and their relationship to survival in the study population were evaluated. As chemotherapy and radiation regimens were heterogeneous during the study period, survival was analyzed by time period to evaluate for confounding by differences in therapy over time. The small sample size precluded evaluation by individual year, so the study population was dichotomized based on the median year (2007) into early (2000-2006) and late (2007-2011) periods. The prognostic value of HER2 status, as determined by IHC and FISH, was evaluated. As some researchers have found a relationship between HER2 expression or amplification specifically, subset analyses examining HER2 by its amplification and expression levels were also performed. The log-rank test was used to evaluate the statistical significance of observed differences between survival curves. A multivariate Cox proportional hazards regression model was used to evaluate the independent prognostic value of HER2 status,

accounting for potential confounders identified in our data. Potential confounders were identified by demonstration of imbalanced distribution between HER2 negative and positive groups and an association with survival, either in our data or well accepted in current literature. Log-log curves were evaluated to check proportional hazards assumptions for each covariate in the model.

Power analysis

Power calculations were calculated using PASS11 software (NCSS, LLC. Kaysville, Utah, USA) and were restricted by the number of samples available for analysis. Estimating a prevalence of HER2 positive tumors at 20%, assuming non-differential loss to follow-up of 1% per year, and using the median survival of the HER2-negative sample population, the power to detect a survival difference with hazard ratios between 1.5 and 3.0 in increments of 0.25 with a two-tailed α=0.05 is shown in Table 2.

RESULTS

Patient Population

During the study period 132 patients underwent resection for gastric adenocarcinoma. Of these, 19 patients had not given consent for the use of banked tissue and were excluded. These 19 patients were previously characterized in non-tissue based study and were similar in demographics, comorbidities, and clinicopathologic parameters to the remainder of the study population. Two patients underwent palliative resections and were also excluded, leaving a final study population of 111 patients. Baseline demographics, comorbidities, and operative details are representative of a Western population of patients undergoing curative intent resection for gastric adenocarcinoma (Table 3). One patient died during the 30-day postoperative period; this patient was excluded from all survival analyses. The median overall survival for the study population was 27.2 months (95% C.I. 9.9-44.5 months, Figure 4).

HER2 Testing

IHC was negative (0+ or 1+) for HER2 expression in 61 (55.0%) patient samples, equivocal (2+) in 14 (12.6%), and positive in 13 (11.7%). Interpathologist discordance occurred in 23 patient samples (20.7%) and involved the following combinations: 0+/2+ (n=3), 1+/2+ (n=12), and 2+/3+ (n=8). FISH for HER2 amplification was performed for all equivocal and discordant samples (n=37 total) and was positive in 8 (21.6% of tested samples). No samples tested equivocal based on FISH. Of the eight cases that demonstrated amplification, two had IHC scores of 2+ and the remainder were scored as discordant (2+/3+: n=5, 1+/2+: n=1). Overall, 21 samples (18.9%, 95% C.I. [11.6%-26.2%]) were HER2-positive (Figure 5).

Comparison of HER2-Positive and HER2-Negative Patients

Baseline characteristics

Patients with HER2-positive tumors were similar to patients with HER2-negative tumors in age, gender, and most comorbidities (Table 3). Although not statistically significant, race was unequally distributed between the two groups, with a higher proportion of black patients in the HER2-positive group, and white patients in the HER2-negative group (Table 3). Patients with HER2-positive tumors had a higher prevalence of hypertension than patients with HER2-negative tumors (71.4% vs 38.9%, p=0.014). There were no differences in the frequencies of preoperative therapy, operation type or approach, degree of nodal dissection, blood loss, and transfusion requirement between the groups (Table 3).

Pathologic and prognostic characteristics

The pathologic and prognostic characteristics of the entire group are representative of a Western population of patients undergoing curative intent resection for gastric adenocarcinoma (Table 4). Patients with HER2-positive tumors were similar to patients with HER2-negative tumors in terms of mean tumor size, tumor location, margin status, presence of lymphovascular or perineural invasion, nodal disease, and stage. Signet ring cell features were less often present in patients with HER2-positive tumors (Table 4). Although not significant, patients whose tumors were HER2-positive were less likely to have poorly differentiated tumors.

Overall survival

On Kaplan Meier survival analysis, margin status and AJCC 7th edition TNM stage were significant predictors of OS (Figure 6A-B); the presence of signet ring cell

cell features and poor differentiation demonstrated trends towards reduced OS (Figure 7A-B). In this study population the presence of previously described adverse prognostic factors such as lymphovascular invasion, perineural invasion, nodal metastases (examined outside of stage), perioperative transfusion, and postoperative complicationswere not associated with survival ($p>0.05$).[9,39] Survival differences by time of operation (early versus late periods) were not detected ($p>0.05$). Given the possible imbalances between HER2-negative and HER2-positive groups, race, hypertension, smoking history, and receipt of neoadjuvant or adjuvant therapy were examined for association with survival; none had a significant impact ($p>0.05$).

Patients with HER2-positive tumors had similar survival (median 48.0 months, 95% C.I. [11.8 – 84.2]) as HER2-negative patients (median 25.2 95% C.I. [11.6 – 38.8], Figure 8). In a subset analysis limited by sample size, HER2 amplification status did not show a relationship with survival (Figure 9). Significant differences in prognosis for patients stratified on HER2 expression (negative versus equivocal versus positive) as measured by IHC (Figure 10) were not demonstrated. Further evaluation of overall survival based on the presence (IHC score 1+, 2+, or 3+) or absence (IHC score 0+) of HER2 expression demonstrated a trend towards improved survival for the HER2-positive group (Figure 11a), but this population was biased by the inclusion of more patients with a positive margin of resection in the HER2-negative group (16.1% vs. 3.8%, $p=0.039$). Differences in survival were not observed when comparing patients with an IHC score of 3+ to those with 0-2+ (Figure 11b).

After controlling for the effect of margin status, tumor stage, presence of signet ring cell features, and poor differentiation between the HER2-positive and -negative groups, HER2 status remained unrelated to overall survival (Table 5).

DISCUSSION

Of the many molecular targets in oncology today, HER2 is perhaps the most well known. A transmembrane tyrosine kinase receptor, HER2 dimerizes with either itself or a structurally similar HER family member to activate a cascade of cellular pathways that contribute to cell growth, proliferation, and survival.[15-17] In breast cancer, HER2 amplification and over-expression have been associated with tumorigenesis, poor overall prognosis, resistance to standard cytotoxic therapies, and susceptibility to HER2-targeted therapies such as trastuzumab[17,19-21] Thus, in breast cancer, HER2 is a biomarker that has both prognostic (information regarding outcome regardless of therapy) and predictive (information regarding response to a particular therapy) value. In gastric cancer the prognostic and predictive value of HER2 is less established. Given the poor prognosis of most patients undergoing resection of gastric cancer, investigation of the prevalence, prognostic value, and ultimately the predictive value of HER2 is warranted. The current study used reliable and reproducible methodology developed specifically for gastric cancer to examine the prevalence and prognostic value of HER2 in patients undergoing curative intent resection for gastric cancer.

Historically efforts to quantify HER2 prevalence and prognostic value in gastric cancer have been hampered by variable methodology. During the past year, after the promising results of the ToGA trial[34] and the development of a standardized testing algorithm which facilitates between-study comparisons,[27,33] several other investigators have examined the prevalence and prognostic value of HER2 in early stage gastric cancer.

The prevalence of HER2 as measured by IHC and FISH in the current study is comparable to that of other post-ToGA studies,[37,38,40-47] although a moderate range of estimates exist (8.7% to 28%, Table 6). One explanation for the varied range even in the studies following standardized methodology involves the choice of study specimen, as some studies have used TMA as opposed to whole slide for evaluation and/or biopsy specimens versus surgical specimens, which could be subject to sampling error (Table 6).[35] The current study reduced this source of variability by using whole slide sections representative of the surgical specimen as determined by an experienced pathologist. Furthermore, an added feature of this study was to buffering against the subjectivity of IHC as two pathologists independently scored the slides and any disagreements regarding score were solved by the more qualitative technique of FISH.

Another explanation for the range of estimates of HER2 prevalence is highlighted by Phillips et al,[44] who found the prevalence of HER2 was 23% overall in their study population, with 28% of gastroesophageal junction tumors and only 15% of esophageal tumors demonstrating HER2. Significant differences in level of HER2 expression by tumor location were not identified in the current study; however the current study focused solely on gastric cancer and included only 16 patients (14.4%) with tumors located at the gastroesophageal junction. It is possible the same pattern could be identified with a larger sample size.

In addition to proximal location, HER2 over-expression/amplification has also been associated with intestinal histologic subtype as characterized by the Laurén classification.[16,48] Laurén classification is not routinely recorded at our institution and the inability to assess the prevalence of HER2 with regard to intestinal versus diffuse

histologic subtype represents a limitation. Evaluation of tumor differentiation may be a useful surrogate for Laurén classification. Intestinal type gastric cancers are more likely to demonstrate well to moderate differentiation, whereas diffuse type gastric cancers are more likely to be poorly differentiated, although differentiation exists along a continuum in each type.[49] Several investigators have demonstrated that HER2-positive tumors are more likely to be well or moderately differentiated.[22,43,44,46,47] The results of the current study, in which HER2-positive tumors tended to be well or moderately differentiated, support these findings.

The relationships between HER2 status and other prognostic characteristics are more ambiguous. Individual investigators have described associations between HER2-positive tumors and node positive disease,[47] tumor size,[47] and stage,[46] whereas others have failed to show an association.[22,31] The current study was unable to detect any significant relationship between clinicopathologic prognostic factors and HER2 status with the exception of the lower frequency of signet ring cell features in HER2-positive tumors. Conflicting evidence regarding HER2 and signet ring cell features exists. Grabsch et al.[22] noted that 100% of the tumor samples in their study with signet ring cell features (n=112) were HER2-negative. In contrast, Cangiano et al.[50] noted that tumors with signet ring cell features uniformly overexpressed HER2 as measured by IHC, although the total number was not reported and determination of HER2 status relied on the breast cancer scoring system for IHC without molecular analysis (FISH). Future studies should make an effort to quantify the association between signet ring cell features and HER2 status.

The pressing clinical question, however, is whether or not HER2 status confers prognostic information. The majority of the studies that have evaluated the prognostic value of HER2 were conducted in Eastern populations (Table 6). Several suggest that HER2 is not related to survival,[45-47] but some suggest HER2 is associated with poor survival, either for patients testing positive only by IHC[40] or only by FISH,[31] or for those with more advanced gastric cancer (stages III/IV).[42] In addition to questions about applicability to Western patients, these studies often included patients with metastatic disease who would not have been considered for curative intent resection in the West. To date, three studies outside of the current study have examined the prognostic value of HER2 in Western populations using modern testing techniques (Table 6). Chan et al.[41] included patients with metastatic gastric cancer and found the prevalence of HER2 to be 24% and unrelated to survival. Phillips et al.[44] also included patients with metastatic disease, and included patients with esophageal adenocarcinoma as well. They examined a combination of biopsy specimens and surgical specimens and demonstrated a 23% prevalence of HER2 without an association with survival. Okines et al.[43] used TMA rather than whole-slide IHC to investigate a combination of biopsy and surgical specimens from a European population (the UK Medical Research Council's MAGIC trial) and found a prevalence of 10.4%. They were also unable to identify a relationship between HER2 status and survival.

In addition to evaluation of HER2 using the combination of IHC and FISH to establish HER2-positive versus HER2-negative, we also examined the individual components of testing. Some investigators have demonstrated reduced survival specifically in patients testing FISH-positive[31] or only in patients with an IHC score of

3+,[40] however we were unable to demonstrate a relationship. With only 37 patients tested by FISH in the current study, the analysis based solely on amplification status was severely limited. It is interesting that when expression was evaluated by absent versus at least some expression present (0+ versus all others), a trend towards better survival with HER2-expression existed; however, the rate of positive margins of resection was higher in the IHC 0+ group, which is likely driving the difference. Our results, combined with the growing body of evidence from others,[41-46] suggest HER2 is not prognostic for patients with early stage gastric cancer.

Limitations

The current study has several limitations. As alluded to earlier, Laurén classification was not evaluated as it was not routinely assessed in this patient population. The retrospective nature of the study and the heterogeneous blend of treatment regimens precludes any evaluation of the predictive value of HER2 status for response to traditional cytotoxic chemotherapy. Examination of banked tissue from clinical trials of patients on specific regimens would be more appropriate to address this question. Although representative of a Western population of patients undergoing curative intent resection for gastric cancer, the study population is a convenience sample. Finally, it is possible that HER2 does have prognostic value and this was undetected, as the study is potentially underpowered.

Strengths

The current study used the best practices available for evaluation of HER2 in gastric cancer. Attention to reducing variability by selecting whole slide sections representative of the surgical specimen and resolving IHC scoring disagreements with

FISH further enhance the value. It is interesting to note that discordance between pathologists occurred in 23 samples, highlighting the heterogeneity of gastric cancer and the subjectivity of IHC even in the setting of scoring guidelines. Future studies should incorporate evaluation of this discordant population with attention to improving IHC scoring. The current study represents a methodologically sound evaluation of HER2 focused on the population of interest: patients undergoing curative intent resection of gastric cancer in a Western setting.

Future directions

The current study examined the gene amplification and expression of HER2 and failed to identify any prognostic value for patients undergoing resection of gastric cancer. While the majority of recent literature focuses on HER2 amplification/over-expression assessed by the methodology presented in the current study, differences in baseline expression of other members of the HER2 family and/or their respective ligands could affect HER2 activation, thus resulting in a HER2-mediated tumorigenesis not directly reflected in measurement of HER2 amplification or expression.

Interestingly, in a major breast cancer adjuvant therapy trial (NSABP-B31), retrospective tissue bank studies in patients originally tested by IHC and FISH and thought to be HER2-positive revealed that 9.7% (n=174) were actually HER2-negative. The 82 HER2-negative patients randomized to receive trastuzumab still appeared to benefit, with improved progression free and overall survival.[51] Whether this represents imperfections in testing or a true benefit of trastuzumab in HER2-negative patients is unclear. Similar findings have been demonstrated in another breast cancer adjuvant therapy trial, adding credibility to the possibility of a therapeutic benefit.[52] The results of

these studies highlight two major questions relevant to the study of HER2 in gastric cancer. First, is the current division of HER2-positive versus HER2-negative tumors biologically appropriate, or are there patients with subclinical HER2 activity that may benefit from HER2-directed therapy? Although our evaluation of patient samples with absent versus present HER2 expression as measured by IHC was limited by sample size and biased by unequal distribution of other prognostic factors (margin status), it is possible that differences exist between HER2-negative patients and that these differences may be targeted. This requires consideration in future studies examining HER2-directed therapy. The second major question: are there other mechanisms of HER2-mediated tumorigenesis not related to HER2 gene amplification or receptor expression? For example, the presence of point mutations in the *HER2/Neu* kinase domain can lead to constitutive activation of the kinase signaling cascade.[53] These mutations, if present, could promote oncogenic signaling regardless of receptor prevalence, and would not be detected in IHC or FISH based evaluations.

The possibility of constitutively active forms of HER2, downstream effects mediated by HER2, or other signaling pathways that are responsible for trastuzumab efficacy that are not directly related to the level of HER2 as measured by IHC or FISH exists. If such situations occurred in gastric cancer, they would be undetectable in the current study. The role of constitutively active HER2, possibly mediated by activating point mutations in *HER2/neu* kinase domains as well as the contributions of HER1, HER3, and HER4 should be investigated in future studies.

Another potential mechanism for HER2-mediated tumorigenesis that would also not be detected in the current study involves soluble HER2. In breast cancer, the

extracellular ligand-binding domain of HER2 has been detected in serum after being shed by proteolytic cleavage.[54] Serum levels of HER2 have been proposed as a diagnostic tool for patients with suspected ovarian cancer[55] and by others as a prognostic and predictive biomarker in patients with breast cancer, yet a consistent relationship to outcomes has not been shown.[54] Evaluating HER2 by means of a blood draw as opposed to a tissue based assay is attractive, but it is unclear whether serum levels are reflective of HER2 tumor activity at this point in time. It is also not yet established whether serum HER2 levels can predict response to HER2-directed therapy. Recently serum based studies of HER2 in the GeparQuinto trial, which tests either trastuzumab or lapatinib (a combined HER1-/HER2-inhibitor) in addition to cytotoxic chemotherapy in patients with primary breast cancer, have suggested that high baseline levels of serum HER2 as well as an early decrease in serum HER2 level after initiation of therapy predict increased susceptibility to lapatinib.[56] Although these results are promising, serum HER2 testing in breast cancer and gastric cancer requires further development and validation.

Serum levels of biomarkers are also notoriously impacted by physiologic stress, which may be one reason for the wide range of prevalences and conclusions regarding the utility of serum testing for HER2 in breast cancer. This has unique implications for patients undergoing operation; future evaluations of serum HER2 should incorporate the role of operative stress. One interesting future approach might involve serial testing of serum HER2 in patients undergoing resection of gastric cancer and evaluation of the association of trends in HER2 levels with survival.

With regards to methodology, a more traditional future direction may be to evaluate HER2 in a larger population to more definitively characterize prevalence. Given

the results of this study and other recent studies suggesting transmembrane HER2 does not have prognostic ability, and in the setting of limited healthcare resources, further studies focused solely on the prevalence and prognostic value of transmembrane HER2 may not be clinically high yield. However, even in the absence of prognostic ability, a biomarker can still have predictive ability for response to therapy. Preclinical studies in gastric cancer cell lines and xenograft models have shown that trastuzumab suppresses tumor growth and enhances cytotoxicity of standard chemotherapies.[57] Trastuzumab therapy, in combination with standard therapy, confers significant survival benefits for patients with all stages of HER2-positive breast cancer.[58-63] In patients with advanced/metastatic gastric cancer, the ToGA trial demonstrated a survival benefit for those patients receiving trastuzumab.[34] Although reported anecdotally,[64] trastuzumab remains to be tested in randomized controlled trials for patients with early stage gastric cancer. It is plausible that, as in breast cancer, HER2 represents a predictive biomarker for response to trastuzumab for patients with all stages of gastric cancer.

Thus, while HER2 as measured by current best practices is likely not prognostic for patients with resected gastric cancer, HER2 in gastric cancer remains an interesting topic for future research.

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TABLES

Table 1: Summary scoring for HER2

Tumor samples were classified as HER2 positive or negative based on the presence of any of the following characteristics:

HER2 Positive	HER2 Negative
IHC score of 3+	IHC score of 0+ or 1+
IHC score of 2+ with positive FISH	IHC score of 2+ with negative FISH
IHC score discordant with positive FISH	IHC score discordant with negative FISH

HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, FISH: fluorescence in situ hybridization

Table 2: Sample size calculations

With a fixed sample size of 110, 1% per year loss to follow-up, and a two-tailed α=0.05, we estimated the prevalence of HER2 to be 20% and hypothesized that patients with HER2+ tumors would have decreased survival. The resulting power and the estimated median survival of the HER2+ group is shown.

Hazard ratio (comparing HER2+ to HER2-)	Power (%)	Estimated Median Survival for HER2 + patients (months)
1.50	38.3	16.0
1.75	59.8	13.7
2.00	75.2	12.0
2.25	85.0	10.7
2.50	90.9	9.6
2.75	94.4	8.7
3.00	96.5	8.0

HER2: human epidermal growth factor receptor 2

Table 3: Clinical characteristics of patients undergoing curative intent resection for gastric adenocarcinoma stratified by HER2 status

Characteristic	All patients (n=111) n (%) or median, [IQR]	HER2 negative (n=90) n (%) or mean	HER2 positive (n=21) n (%) or mean	p-value*
Demographics & Comorbidities				
Age (years)	63.6 [54.3-71.3]	61.8	60.9	0.785
Male gender	60 (54.1)	50 (55.6)	10 (47.6)	0.679
Race				
White	51 (45.9)	45 (50.0)	6 (28.6)	
Black	45 (40.5)	33 (36.7)	12 (57.1)	
Other	15 (13.5)	12 (13.3)	3 (14.3)	0.176
ASA score				
2	25 (22.5)	21 (23.3)	4 (19.0)	
3	83 (74.8)	66 (73.3)	17 (81.0)	
4	3 (2.7)	3 (3.3)	0	0.854
Hypertension	50 (45.0)	35 (38.9)	15 (71.4)	**0.014**
Diabetes mellitus	19 (17.1)	15 (16.7)	4 (19.0)	0.755
H. pylori positive	3 (2.7)	3 (3.3)	0	1.000
Smoking history	33 (29.7)	30 (33.3)	3 (14.3)	0.113
Alcohol abuse	1 (0.9)	1 (1.1)	0	1.000
Neoadjuvant chemotherapy	16 (14.4)	11 (12.2)	5 (23.8)	0.180
Neoadjuvant radiotherapy	3 (2.7)	2 (2.2)	1 (4.8)	0.474
Operative Characteristics				
Total (vs subtotal gastrectomy)	41 (36.9)	33 (36.7)	8 (38.1)	1.000
Additional visceral resection	13 (11.7)	11 (12.2)	2 (9.5)	1.000
Laparoscopic approach	6 (5.4)	4 (4.4)	2 (9.5)	0.317
D2 lymphadenectomy	89 (80.2)	74 (82.2)	15 (71.4)	0.360
Estimated blood loss (ml)§	150 [100-300]	206.1	178.6	0.499
Perioperative RBC transfusion	9 (8.1)	8 (8.9)	1 (4.8)	1.000

*p-value compares patients by HER2 status, $\alpha<0.05$, significant values in bold
§Estimated blood loss data available for 73 patients (14 HER2 negative patients and 59 HER2 positive patients)
HER2: human epidermal growth factor receptor 2, ASA: American Society of Anesthesiologists, RBC: red blood cell

Table 4: Pathologic characteristics of patients undergoing curative intent resection for gastric adenocarcinoma stratified by HER2 status

Characteristic	**All patients (n=111)** n (%) or median, [IQR]	**HER2 negative (n=90)** n (%) or mean	**HER2 positive (n=21)** n (%) or mean	**p-value***
Tumor size (cm)	4 [2.5-6.5]	4.8	4.1	0.309
Proximal tumor location	16 (14.4)	14 (15.6)	2 (10.0)	0.732
Positive margin	8 (7.2)	7 (7.8)	1 (4.8)	1.000
Poorly differentiated	75 (67.6)	65 (72.2)	10 (47.6)	0.056
Signet ring cell features	58 (52.3)	53 (58.9)	5 (23.8)	**0.008**
Lymphovascular invasion	39 (35.1)	28 (31.1)	11 (52.4)	0.113
Perineural invasion	26 (23.4)	22 (24.4)	4 (19.0)	0.777
Nodal metastases	68 (61.3)	56 (62.2)	12 (57.1)	0.856
AJCC 7th Edition TNM Stage				
I	24 (21.6)	19 (21.1)	5 (23.8)	
II	32 (28.8)	26 (28.9)	6 (28.6)	
III	55 (49.5)	45 (50.0)	10 (47.6)	0.962

*p-value compares patients by HER2 status, $\alpha<0.05$, significant values in bold
HER2: human epidermal growth factor receptor 2, ASA: American Society of Anesthesiologists, RBC: red blood cell, AJCC: American Joint Committee on Cancer, TNM: Tumor-Node-Metastasis

Table 5: Cox proportional hazards model assessing the association between HER2 status and overall survival controlling for potential confounders

Characteristic	Estimated Hazard Ratio	95% C.I.	p-value*
HER2-positive	0.740	0.36-1.55	0.423
Margin positive resection	5.36	2.32-12.36	<0.0001
Increasing AJCC TNM Stage	1.74	1.14-2.65	0.011
Signet ring cell features	0.694	0.30-1.62	0.400
Poorly differentiated	1.675	0.71-3.96	0.239

*****α=0.05, significant p-values are in bold
AJCC TNM stage is coded as 1=stage 1, 2=stage 2, and 3=stage 3. All other variables are coded as 0=absent, 1=present
AJCC: American Joint Committee on Cancer, TNM: tumor node metastasis (based on 7th edition)

Table 6: Comparison of recent literature evaluating HER2 in patients undergoing resection for gastric cancer: patient population, assessment technique(s), and results

Study & Year	Patient population						Technique				Results	
	Area	n	Stage*				Source	Slide type	IHC Scoring	FISH	Prevalence of HER2	Associated w/Survival
			I	II	III	IV						
Yano[32] 2005	Asia	200	42	55	67	36	S	WS	U	All	23.0% by IHC, 27.1% by FISH	--
Park[31] 2006	Asia	182	81	32	51	18	S	WS	U	2+/3+ IHC	15.9% by IHC	Amp associated with poor DSS
Choi[30] 2009**	Asia	443	163	91	115	77	S	TMA	U	All	4.5% by IHC, 4.7% by FISH	--
Grabsch[22] 2010: Germany ***	Eur	418	--	--	--	23	S	WS	U	None	5.7%	No association
Grabsch[22] 2010: England ***	Eur	506	--	--	--	96	S	TMA	U	None	2.4%	No association
Yan[40] 2010	Asia	128§	--	--	--	--	S	TMA	ToGA	All	11.7% by FISH, 9.4% by IHC (3+ only)	Poor OS with 3+ IHC score
Chan[41] 2012#	Eur	85	42 stage I/II		43 stage III/ IV		S	WS	ToGA	None	24%	No association
Kataoka[42] 2012	Asia	213	50	77	90	6	S	WS	ToGA	2+/3+ IHC	11.7%	Associated with poor OS in Stage III/IV only
Okines[43] 2012	Eur	402	--	--	--	--ɤ	Bx (244) S (337) both (179)	TMA	ToGA	All	10.4%	No association
Pirelli[38] 2012	Eur	61	--	--	--	--	Bx, S	WS	ToGA	All	13%	--
Phillips[44] 2012@	USA	135	9$	43	53	19	Bx (120) S (84)	WS	ToGA	All	23%	No association

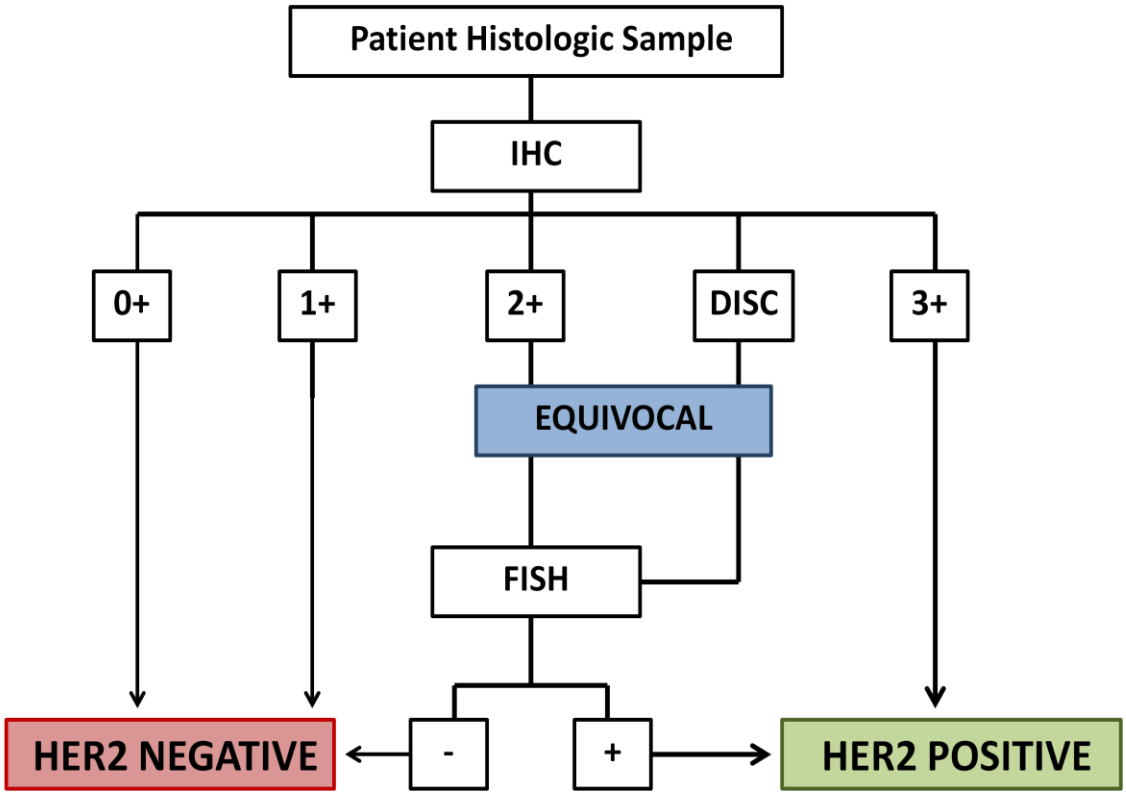
							both (69)					
Sukawa[45] 2012	Asia	231	49	45	82	51	S	WS	--	None	8.7%	No association
Terashima[46] 2012	Asia	829	0	372	457	0	S	WS	ToGA	All	13.6%	No association
Yang[37] 2012	Asia	148	35	47	40	26	Bx, S	WS	ToGA	All	25.6% by IHC	--
Zhou[47] 2012	Asia	227	46	58	123	0	S	TMA	ToGA	All	11.9%	No association
Fisher 2013 (present study)	USA	110	23	32	55	0	S	WS	ToGA	2+ IHC, disc	18.9%	No association

*As reported in original article
**Included a high proportion (30%) of patients with mucinous gastric adenocarcinomas
***Reported as results of 2 independent studies in a single manuscript
[§]Of 190 tested, 128 had IHC, FISH, and CISH
[#]Includes patients with esophageal cancer (18)
[@]Includes patients with esophageal cancer (53)
[$]Included patients receiving neoadjuvant therapy, 7 of whom had a complete pathologic response and were stage 0
ɤAlthough not directly reported, this study involved patients enrolled in the MAGIC trial, which required absence of evidence of metastatic disease for enrollment
--: Not reported
HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, FISH: fluorescent in situ hybridization, Eur: European, USA: United States of America, S: surgical, Bx: biopsy, WS: whole slide, U: Unique, Amp: amplification, TMA: tissue micro-array, DSS: disease-specific survival, N/A: not applicable, ToGA: trastuzumab for gastric cancer trial, OS: overall survival, GEJ: gastroesophageal junction

FIGURES

Figure 1: HER2 testing schematic

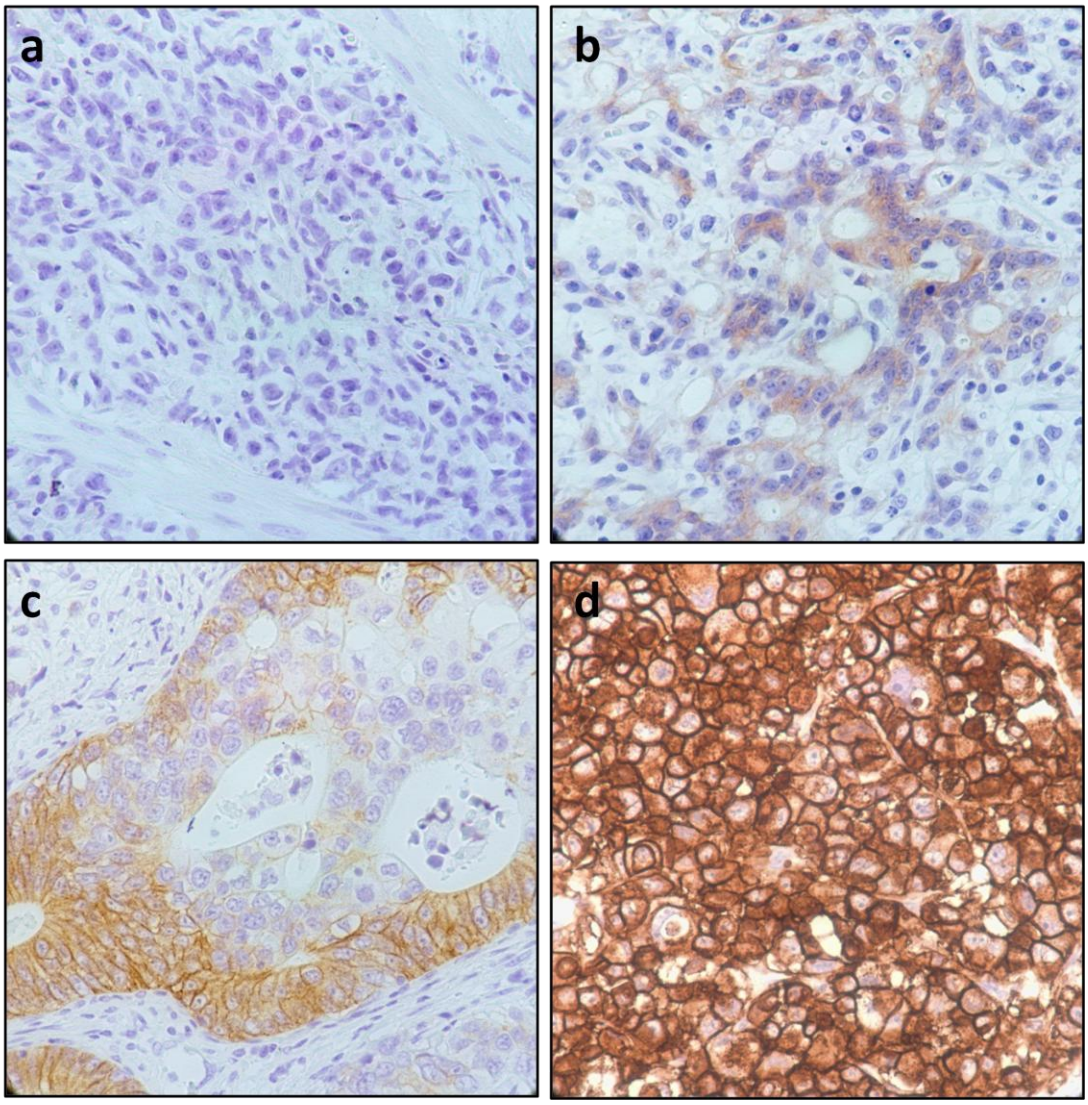
HER2 IHC is performed for all histologic samples. Samples scored as 0+ or 1+ are considered negative. Samples scored as 2+ or as discordant (inter-pathologist disagreement that would alter the defining score) are considered equivocal and proceed to FISH. Samples positive by FISH or scored on IHC as 3+ are considered positive.



HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, DISC: discordant, FISH: fluorescence in situ hybridization

Figure 2: Immunohistochemistry of HER2 in gastric adenocarcinoma

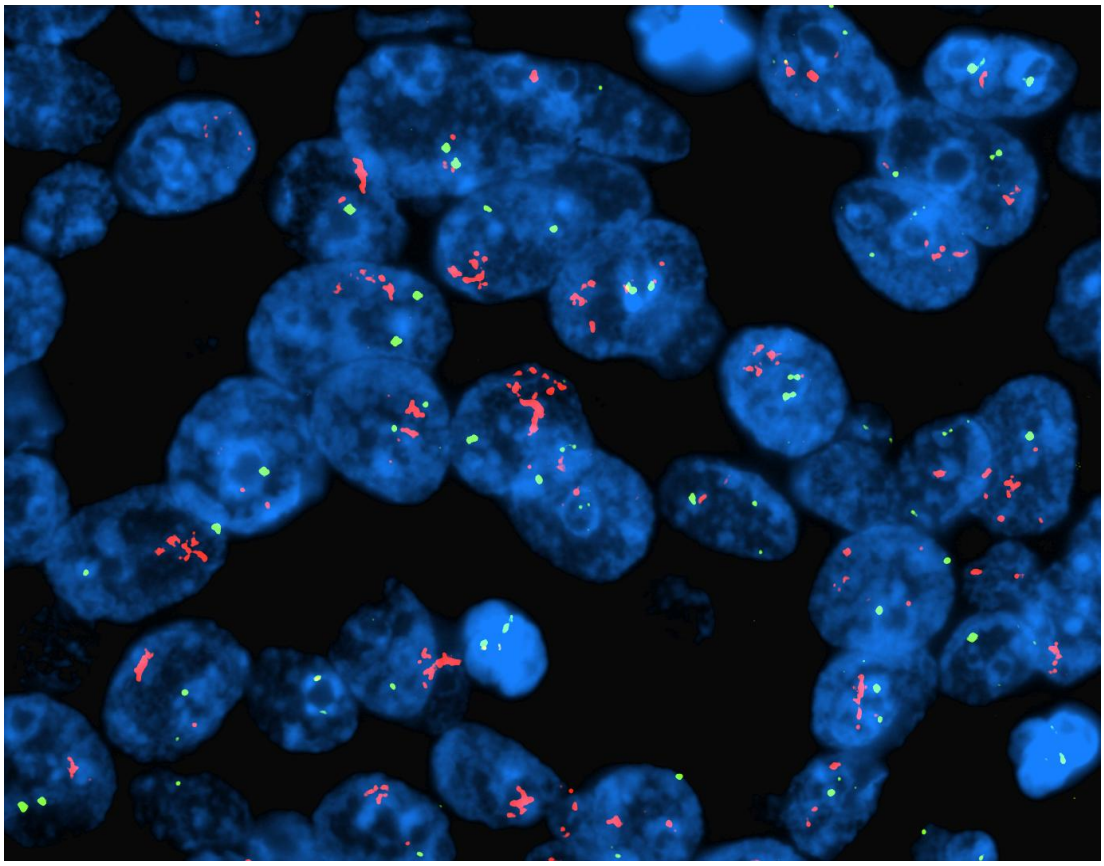
a) 0+ staining (negative), b) 1+ staining (negative), c) 2+ staining (equivocal), and d) 3+ staining (positive) (at 400x magnification)



HER2: human epidermal growth factor receptor 2

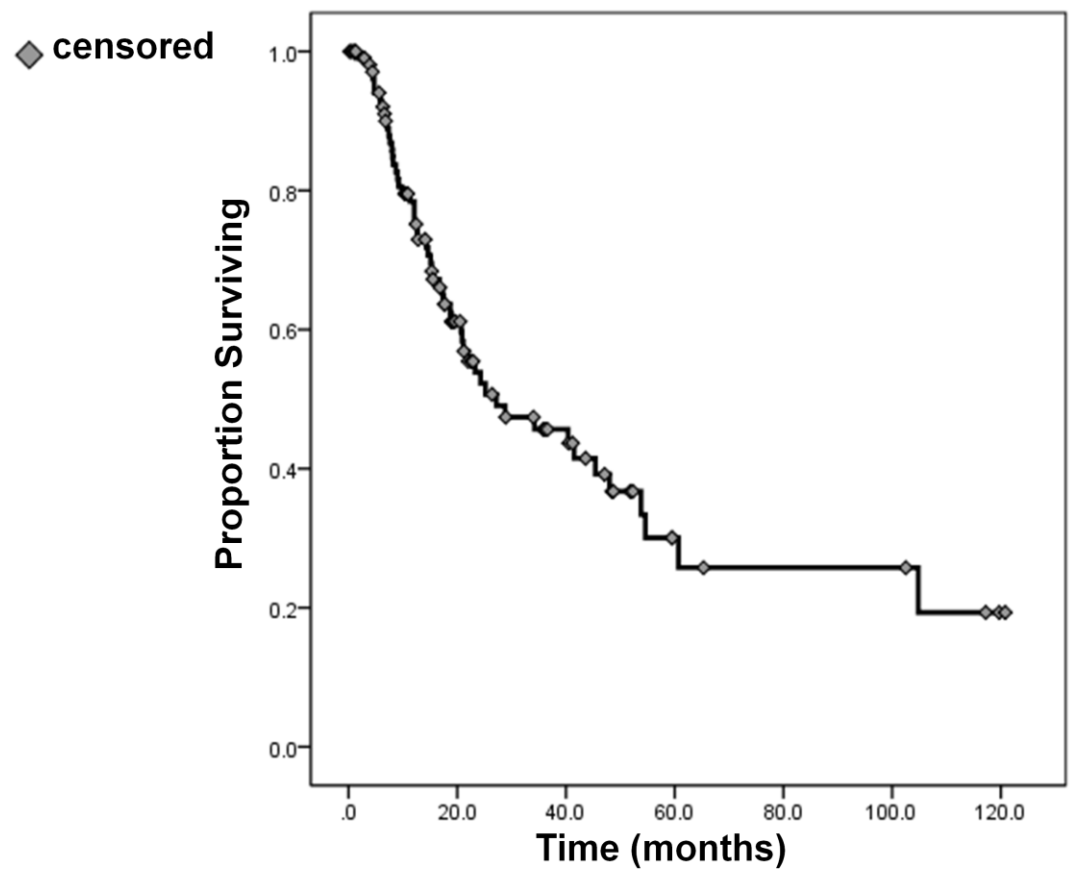
Figure 3: Amplification of HER2 in gastric adenocarcinoma demonstrated by fluorescence in situ hybridization

Tumor cell nuclei (blue) demonstrate HER2 signal amplification (red probe) relative to control (CEP17, green probe). (100x oil objective magnification)



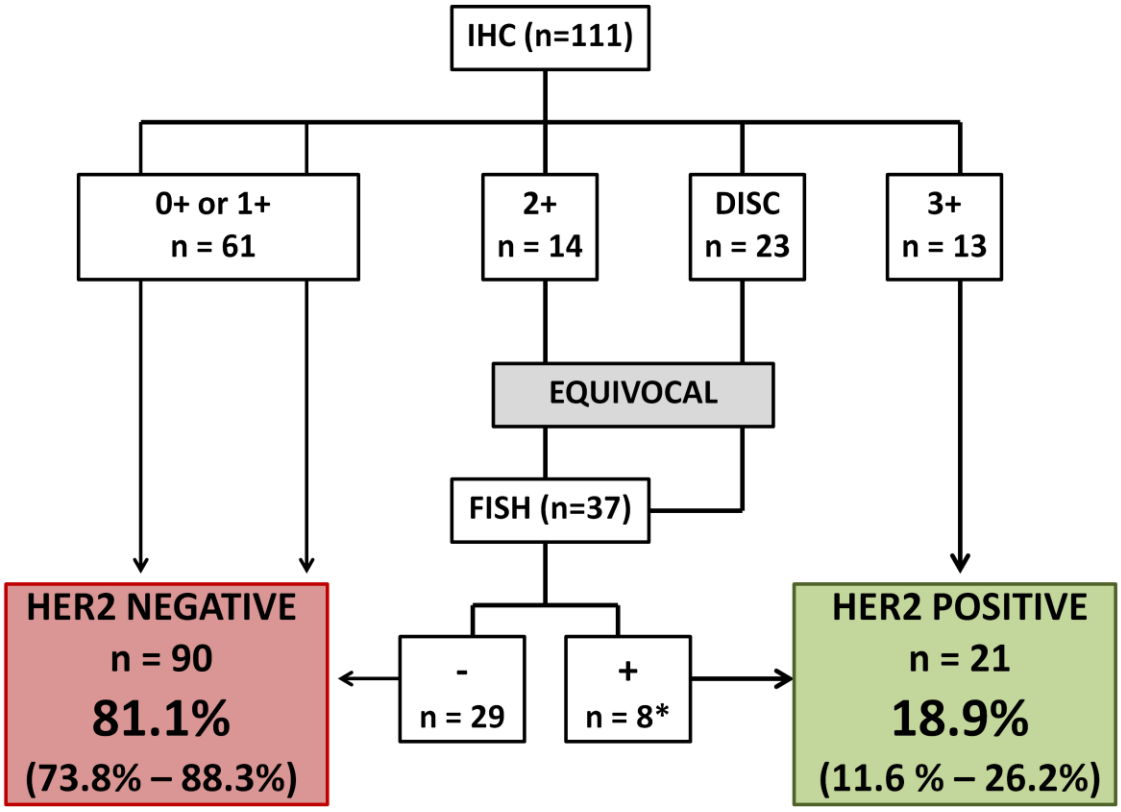
HER2: human epidermal growth factor 2, CEP17: centromere probe for chromosome 17

Figure 4: Follow-up and censorship for patients excluding perioperative mortality (n=110)



Cumulative Events	0	36	46	52	53	53	54
Cumulative Censored	0	30	41	51	52	52	55
Number at Risk	110	44	23	7	5	5	1

Figure 5: Results of IHC and FISH for HER2 in gastric adenocarcinoma by testing stage

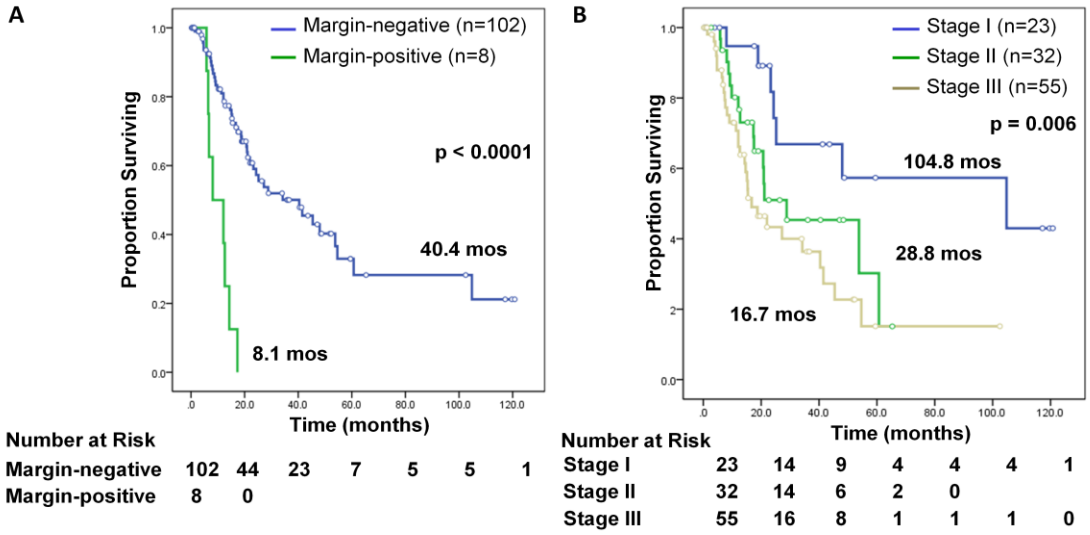


HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, DISC: discordant, FISH: fluorescence in situ hybridization

Figure 6A-B: Kaplan-Meier survival analysis for known adverse prognostic features and overall survival

A. Relationship between positive margin of resection and overall survival

B. Relationship between AJCC 7th edition TNM stage and overall survival

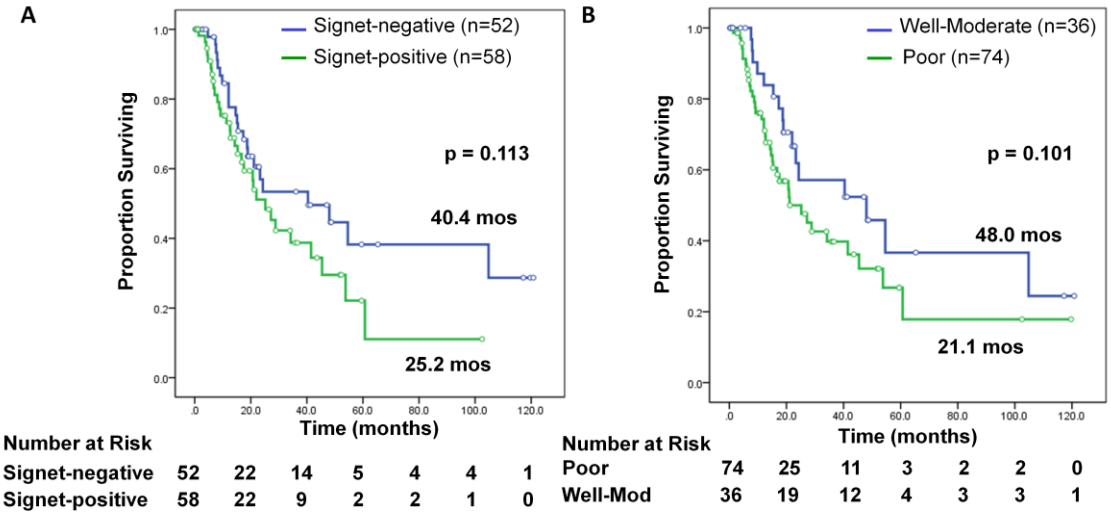


AJCC: American Joint Committee on Cancer, TNM: Tumor-Node-Metastasis

Figure 7A-B: Kaplan-Meier survival analysis for known adverse prognostic features and overall survival

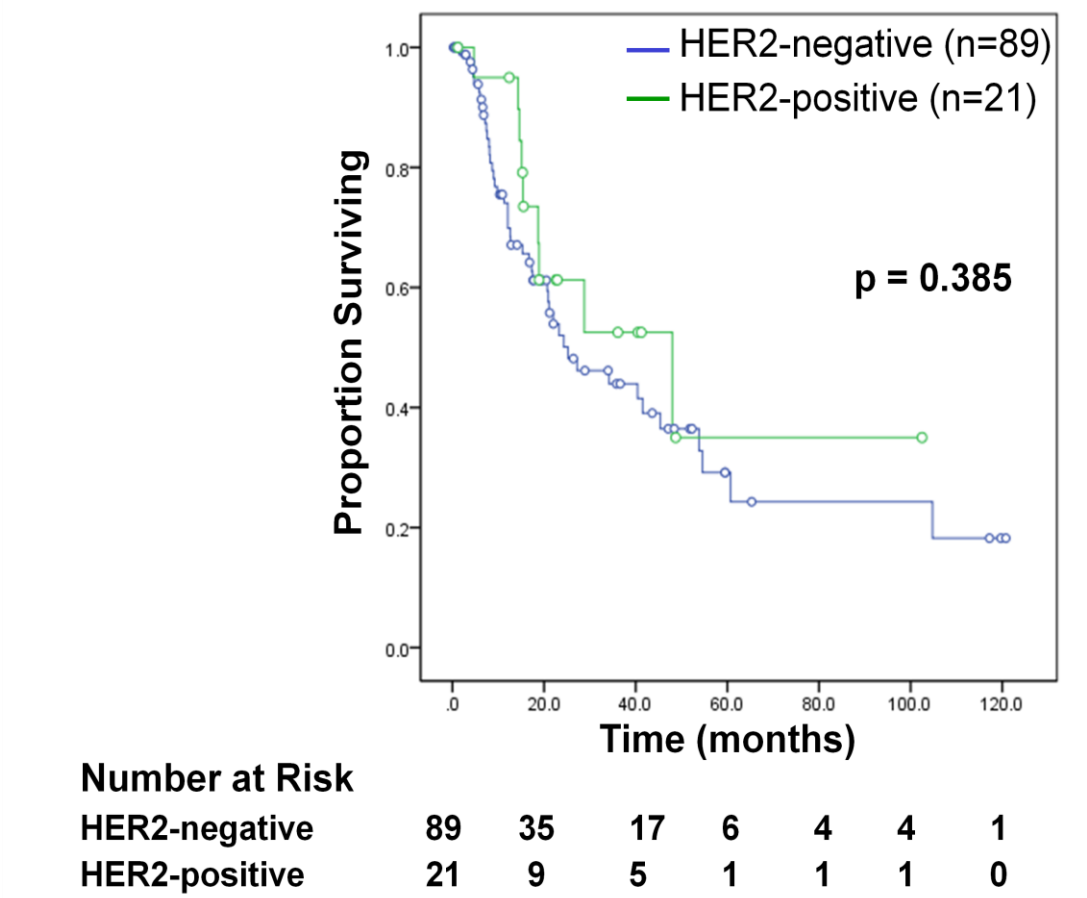
A. Relationship between presence of signet ring cell features and overall survival

B. Relationship between poor differentiation and overall survival



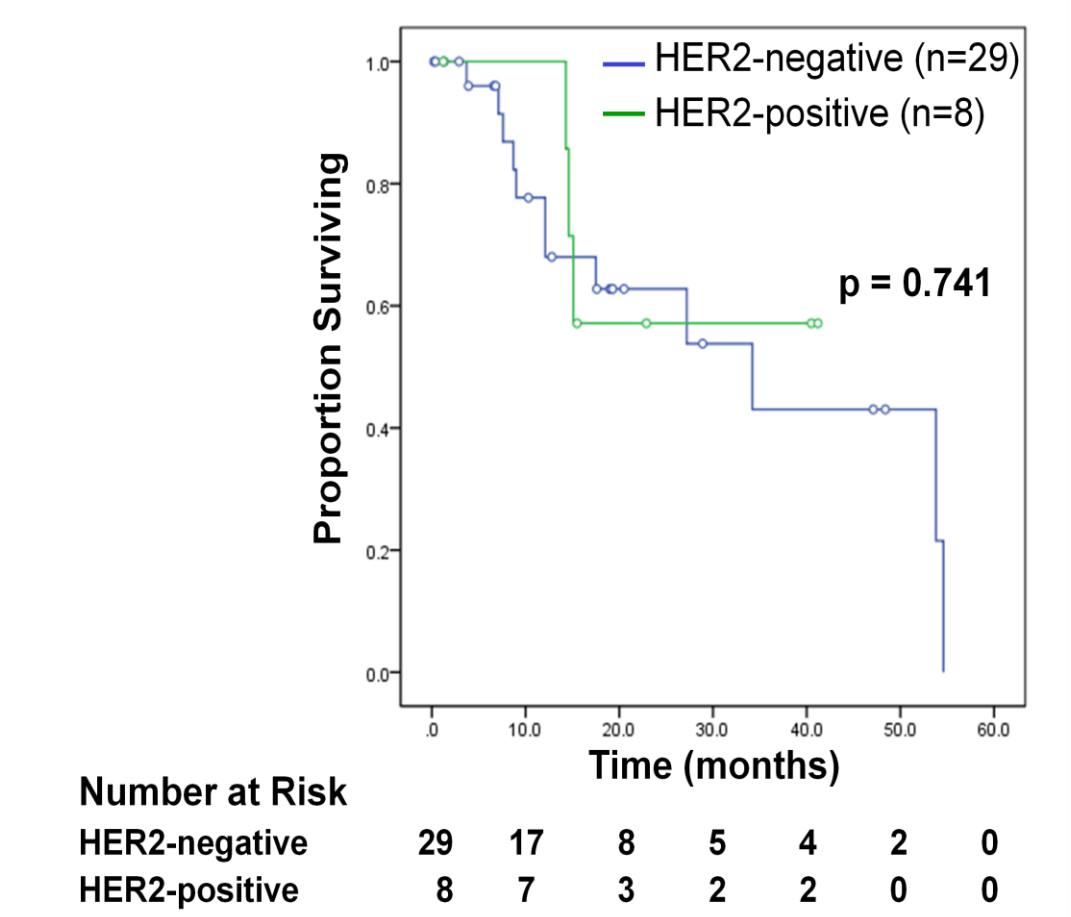
Well-Mod: tumors with well or moderate differentiation

Figure 8: Kaplan-Meier survival analysis for patients stratified by HER2 status (n=110)*



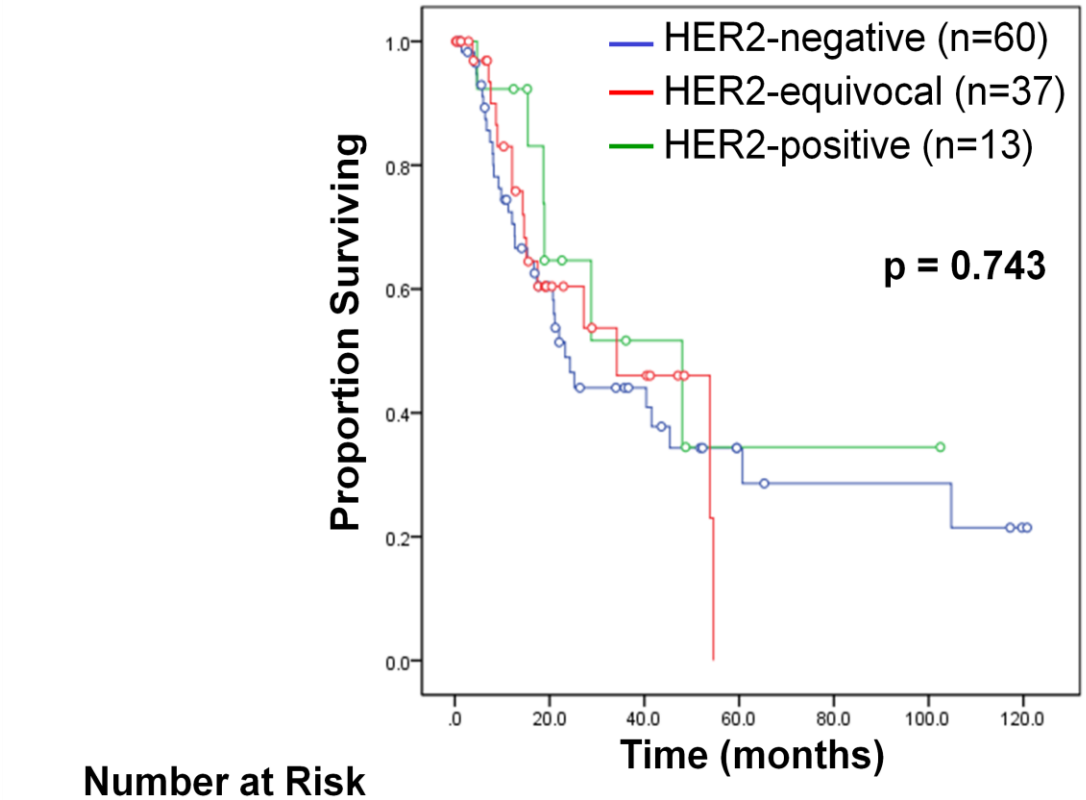
*Excludes 1 perioperative mortality
HER2: human epidermal growth factor receptor 2

Figure 9: Kaplan-Meier survival analysis for patients stratified by HER2 amplification (n=37)



HER2: human epidermal growth factor receptor 2

Figure 10: Kaplan-Meier survival analysis for patients stratified by HER2 expression by immunohistochemistry (n=110)*



Number at Risk

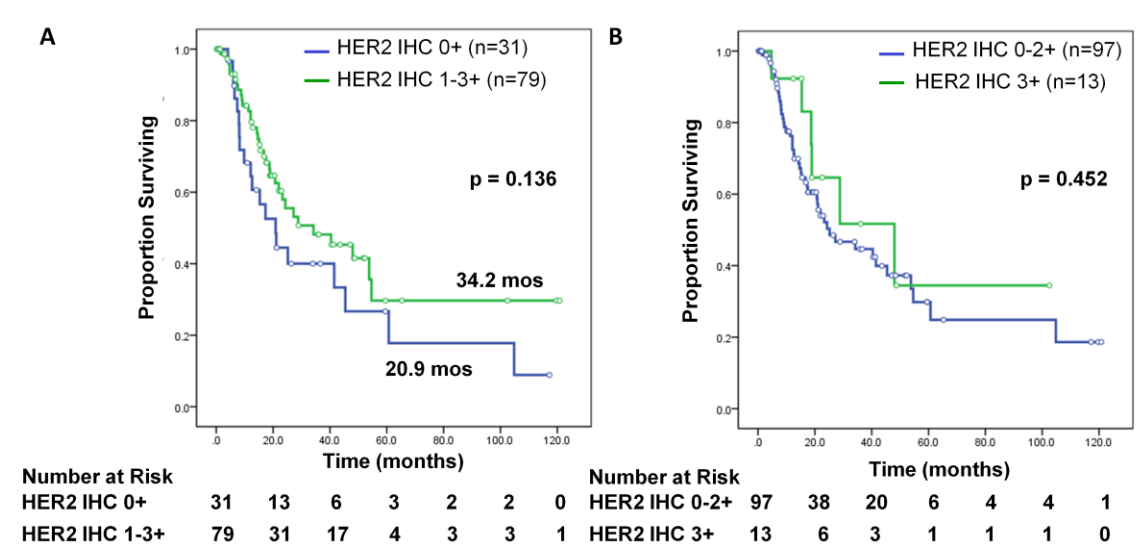
HER2-negative	60	27	14	6	4	4	1
HER2-equivocal	37	24	11	0	0	0	0
HER2-positive	13	6	3	1	1	1	0

*Excludes 1 perioperative mortality
HER2: human epidermal growth factor receptor 2

Figure 11: Kaplan-Meier survival analysis for patients stratified by HER2 expression by immunohistochemistry (n=110)*

A. Comparing absent (IHC 0+) to present (IHC 1=3+) expression

B. Comparing strongly present expression (IHC 3+) to all others (IHC 0-2+)



*Excludes 1 perioperative mortality
HER2: human epidermal growth factor receptor 2