Association of HER2/ErbB2 Expression and Gene Amplification with Pathologic Features and Prognosis in Esophageal Adenocarcinomas

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Abstract

**Purpose:** We examined the frequency, tumor characteristics, and prognostic impact of HER2 protein expression and gene amplification in patients with curatively resected esophageal adenocarcinoma (EAC).

**Experimental Design:** HER2 expression was analyzed by immunohistochemistry (IHC) in surgical EAC specimens (n = 713). Gene amplification was examined by FISH in a large subset (n = 344). Most tumors were T3–4 (66%) or node positive (72%); 95% were located in the esophagus or gastroesophageal junction. No patient received neoadjuvant therapy. Cox models were used.

**Results:** Overall, 17% of EACs were HER2 positive (i.e., IHC3+ or IHC2+ with amplification), with strong agreement between HER2 amplification (HER2/CEP17 ratio ≥ 2) and expression (κ = 0.83). HER2 positivity was significantly associated with lower tumor grade, less invasiveness, fewer malignant nodes, and the presence of adjacent Barrett’s esophagus (BE). EACs with BE had higher odds of HER2 positivity than EACs without BE, independent of pathologic features [OR = 1.8 (95% CI: 1.1–2.8), P = 0.014]. Among all cases, HER2 positivity was significantly associated with disease-specific survival (DSS) in a manner that differed by the presence or absence of BE (Pinteraction = 0.0047). In EACs with BE, HER2 positivity was significantly associated with improved DSS [HR = 0.54 (95% CI: 0.35–0.84), P = 0.0065] and overall survival (P = 0.0022) independent of pathologic features, but was not prognostic among EACs without BE.

**Conclusions:** HER2 positivity was shown in 17% of resected EACs and associated with reduced tumor aggressiveness. EACs with BE had nearly twice the odds of being HER2 positive and, within this subgroup, HER2 positivity was independently associated with improved survival. *Clin Cancer Res;* 18(2); 546–54. ©2012 AACR.

Introduction

HER2 (ErbB2) is a member of a family of transmembrane receptor tyrosine kinases [(HER1: epidermal growth factor receptor), HER3 (ErbB3), HER4 (ErbB4)] that are involved in the regulation of cellular processes that control cell growth, survival, differentiation, and migration (1). HER2 protein overexpression in the cellular membrane is induced primarily through gene amplification. HER2 plays a role in the development and progression of several types of human cancer (2, 3), including breast cancer in which it confers poor prognosis and predicts for response to HER2-targeted therapy (3). Recently, HER2 overexpression and/or amplification was detected in approximately 22% of advanced gastric cancers, and targeting the extracellular domain of HER2 in these patients was associated with clinical benefit compared with chemotherapy alone in a phase III trial (ToGA; ref. 4). As a result of this clinical trial, the anti-HER2 monoclonal antibody trastuzumab has been approved in the United States and Europe for the treatment of patients with metastatic carcinomas of stomach or gastroesophageal junction (GEJ; ref. 5). Although testing for HER2 protein overexpression or gene amplification in upper digestive cancer has become increasingly routine, the status of HER2 expression/amplification and its association with clinicopathologic features and clinical outcome in esophageal adenocarcinoma (EAC) remains unknown.

Adenocarcinoma of the esophagus, GEJ, or gastric cardia (referred to as EAC; ref. 6) are collectively among the fastest rising cancers in western countries (7) and are highly lethal malignancies (8). Many EACs are believed to arise either within Barrett’s epithelium or in the gastric cardiac mucosa of the distal esophagus. Barrett’s esophagus (BE) is a metaplastic change in the epithelium that is at risk for...
progression to dysplasia and cancer (9). Molecular features that can predict the progression of BE to esophageal or GEJ cancers are currently lacking. Whereas HER2 positivity in breast cancer is known to be associated with adverse clinicopathologic characteristics (10), data are limited in EAC and results have been inconsistent (11–16). Moreover, the frequency of HER2 positivity in the nonmetastatic, potentially curative setting has not been adequately studied, yet has substantial clinical relevance because such knowledge may guide HER2 testing for the identification of patients for HER2-targeted therapy. To date, studies in EAC have been limited by small sample sizes and inclusion of squamous cell carcinomas or subcardial gastric carcinomas in which epidemiology and biology are distinct from EAC (17–19).

We analyzed HER2 protein expression in a large cohort of curatively resected EAC in relation to HER2 gene amplification, clinicopathologic variables, and long-term cancer-specific outcomes. In contrast to some prior studies in gastric cancer in which HER2 was primarily analyzed in biopsy or tissue microarray specimens (4, 20), we examined surgical resection specimens. Due to the potential effects of chemotherapy on tumor viability and HER2 expression (21, 22), we focused on patients prior to routine use of neoadjuvant therapy (23).

Patients and Methods

Development of study cohort

The development of our study cohort, the Mayo Esophageal Cancer Outcomes Database, is detailed elsewhere (24). In brief, a query of the Mayo Clinic Tumor Registry and Surgical Index for all patients with neoplasms of the esophagus, GEJ, or gastric cardiag treated at Mayo Clinic, Rochester, Minnesota (January 1, 1980 to December 31, 1997) yielded 1,591 patients. Individual medical records were systematically reviewed by trained physicians with standardized intake forms. Inclusion criteria included tissue-confirmed adenocarcinoma of the esophagus, GEJ, or gastric cardiag (i.e., Siewert type 1 or II) and underwent surgical resection with curative intent at Mayo. Tumor location was determined through a combination of endoscopy, intraoperative findings, and/or histopathologic examination. Preoperative staging included computerized tomography scan of the chest and abdomen. Most exclusions were due to only nonadenocarcinoma histology \( n = 338 \), tumor not resected \( n = 212 \), or cancer-involved surgical margins \( n = 159 \). A total of 796 patients met all inclusion criteria and comprised the parent study cohort. Collected variables were related to BE adjacent to EAC, demography, pathology, tumor location, perioperative therapy, tumor recurrence, and cause of death. The presence or absence of BE in the EAC resection specimen was determined by initial pathology review at Mayo Clinic. Data on patient follow-up included data obtained by the Mayo Tumor Registry, which collects updated information on disease status and cause of death through annual questionnaires sent to patients. Independent review was carried out to ensure quality control of data collection. The American Joint Committee on Cancer (AJCC) 2009 criteria (7th edition) was used for staging.

Samples with invasive carcinoma were available in 718 (90.2%) of 796 patients. After 5 patients who received neoadjuvant chemotherapy and/or radiotherapy were excluded, 713 cases comprised the main study population. Eighty-eight percent of patients did not receive adjuvant therapy. In patients who received adjuvant therapy (12%), treatment consisted of chemotherapy plus concurrent radiotherapy in 50 (7%) patients, chemotherapy alone in 16 (2%), or radiotherapy alone in 22 (3%). Adjutant chemotherapies, delivered in various combinations, included 5-fluorouracil \( n = 53 \), doxorubicin \( n = 16 \), mitomycin C \( n = 5 \), cisplatin \( n = 7 \), carboplatin \( n = 1 \), etoposide \( n = 1 \), and unknown \( n = 7 \).

HER2 testing methods and criteria

HER2 tests approved by the U.S. Federal Drug Administration (FDA) were used. HER2 protein expression was assessed in invasive carcinoma cells by immunohistochemistry (IHC) in paraffin-embedded 5-μm tissue sections according to the manufacturer’s instructions (HercepTest; Dako; refs. 4, 25). Only cell membrane staining was considered positive. Each case was analyzed by 2 pathologists (T-W.T. and W.R.S.) blinded to clinical outcome with criteria specific to upper digestive cancer (4, 25) that include 2 parameters: (i) the intensity of complete, basolateral, or lateral membrane staining \( (0, \text{none}; 1, \text{faint}; 2, \text{weak to moderate}; \text{and} 3, \text{strong}) \) and (ii) the percentage of cancer cells with that staining intensity. These parameters were used to determine the IHC score as per ToGA as follows: high (IHC3±), strong intensity in 10% or more of cancer

Translational Relevance

HER2/ErbB2 is a receptor tyrosine kinase involved in regulating cell growth, survival, and differentiation. HER2 overexpression is induced mainly through gene amplification and plays a role in the development and progression of certain human cancers. The anti-HER2 monoclonal antibody, trastuzumab, was recently shown to benefit patients with advanced gastric cancer. We studied HER2 protein expression and gene amplification in resected esophageal adenocarcinomas (EAC). HER2 positivity was detected in 17% of cases and associated with favorable pathologic features. The frequency of HER2 positivity was nearly doubled in Barrett’s esophagus–associated EACs, and HER2 positivity in these tumors was associated with better survival. In summary, HER2 is associated with decreased tumor aggressiveness and exerts a favorable influence on the outcome of BE-related EACs. These data suggest differences between EACs arising in the presence or absence of BE and provide critical data to inform HER2 testing in this malignancy.
cells; medium (IHC2\(^+\)), weak-moderate intensity in 10% or more; low (IHC1\(^+\)), faint intensity in 10% or more; absent (IHC0; ref. 4).

HER2 gene amplification was assessed in all IHC2\(^+\) cases by FISH as described (26) on 5-\(\mu\)m tissue sections using the PathVysion HER-2 DNA Probe Kit [HER2 and centromere 17 (CEP17) probes; Abbott Molecular; ref. 26]. We also carried out FISH in 187 additional tumors (99 IHC0-1\(^+\) and 88 IHC3\(^+\)) selected without regard to clinicopathologic factors. Briefly, for each case, a parallel hematoxylin and eosin–stained slide was examined for regions of invasive carcinoma by a pathologist (W.R.S.). The complete tissue section was scanned by certified cytogenetic technologists to detect any subpopulation of amplified cells. A total of 60 representative nuclei from the invasive tumor were scored, with an overall evaluation conducted by a single cytogeneticist with extensive experience (R.B.J.; refs. 26, 27). A specimen with an overall evaluation conducted by a single cytogeneticist with extensive experience (R.B.J.; refs. 26, 27). A specimen with an HER2/CEP17 ratio of 2.0 or more in invasive cells was classified as HER2 amplified consistent with ToGA guidelines (4).

**Definition of HER2-positive status**

A case was considered HER2 positive if it was (i) IHC3\(^+\) or (ii) IHC2\(^+\) plus gene-amplified (4). Remaining cases (i.e., nonamplified IHC2\(^+\) or IHC0-1\(^+\)) were considered HER2 negative.

**Clinical endpoints and statistical analysis**

The Wilcoxon rank-sum and \(\chi^2\) test were used to compare variables between groups. \(k\) Statistic was used to evaluate agreement. Multivariate logistic regression analysis was conducted to assess the association between HER2 status and clinicopathologic factors of interest. ORs with 95\% CIs were reported. For survival analysis, outcome variables were overall survival (OS) and disease-specific survival (DSS). OS was defined as the time from surgery to death from any cause and was censored at the date of last contact for surviving patients. DSS was defined as the time from surgery to death related to index cancer and was censored at the date of death due to postoperative complications or other nonmalignant causes. Death beyond 5 years was censored. Kaplan–Meier methods and Cox proportional hazards models were used to assess the association between predictor variables and time to event outcomes. HRs were reported. All \(P\) values are 2-sided. \(P < 0.05\) was considered statistically significant. Analyses were conducted in SAS (version 9.1; SAS Institute). Study data were collected and managed, in part, by REDCap electronic data capture tools hosted at Mayo Clinic. The study was approved by the Mayo Clinic Institutional Review Board.

**Results**

**Study population**

The patient population in this study is from the Mayo Esophageal Cancer Outcomes Database (24). Table 1 shows key baseline clinicopathologic variables. In brief, all patients had adenocarcinomas; most were male (89\%) and had locally advanced tumor stage (66\% T3–4, 72\% node positive). Among 465 patients with T3–4 tumors, 437 were T3 and 28 were T4a. Among 464 patients with GEJ/cardia tumors, 427 tumors were located at the GEJ and 37 tumors were limited to the cardia. No patient received neoadjuvant therapy, and 88\% of patients did not receive postoperative adjuvant therapy. Median follow-up for vital status for surviving patients was 12.6 years. More specifically, of 708 patients with IHC and FISH data (described below), 510 and 475 patients experienced all-cause and disease-specific deaths within 5 years, respectively. Among 198 patients without an all-cause death event, only 7 (0.99\%) and 9 (1.3\%) patients had less than 3 and less than 5 years' follow-up, respectively. Among 233 patients without a disease-specific death event, only 39 (5.5\%) and 44 (6.2\%) patients had less than 3 and less than 5 years' follow-up, respectively.

**HER2 protein overexpression and gene amplification**

HER2 protein expression by IHC (Supplementary Fig. S1A–S1D) was high (IHC3\(^+\)) in 94 (13\%), medium (IHC2\(^+\)) in 172 (24\%), low (IHC1\(^+\)) in 159 (22\%), and absent (IHC0) in 288 (40\%) cases. HER2 staining was carried out by the U.S. FDA–approved HercepTest, and only cell membrane staining was considered positive. Staining was scored with criteria developed for the evaluation of upper digestive carcinoma (4, 25).

FISH was successfully completed in 96\% (344) of 359 tumors—that is, 93 of 99 IHC0-1\(^+\), 167 of 172 IHC2\(^+\), and 84 of 88 IHC3\(^+\) cases (Supplementary Fig. S1E–S1F). FISH was conducted in a larger number of IHC2\(^+\) (than IHC3\(^+\) or IHC0-1\(^+\)) cases, given data suggesting a weaker correlation between IHC and FISH in the IHC2\(^+\) subgroup (25, 28). A specimen with an HER2/CEP17 ratio of 2.0 or more in invasive cells was classified as HER2 amplified, consistent with eligibility criteria for ToGA (4). HER2 amplification was detected in 7\% (7 of 93) of IHC0-1\(^+\), 15\% (25 of 167) of IHC2\(^+\), and 90\% (76 of 84) IHC3\(^+\) cases. HER2 protein expression and gene amplification showed high agreement in the IHC0-1\(^+\) and IHC3\(^+\) groups [\(k = 0.83\) (95\% CI: 0.75–0.91), Table 2]. Furthermore, the level of gene amplification increased as protein expression increased: mean amplification level (HER2/CEP17 ratio) was 1.3 [median 1.1 (range: 0.8–4.6)] in IHC0-1\(^+\) cases, 1.9 [median 1.2 (0.7–15.8)] in IHC2\(^+\) cases, and 7.5 [median 7.7 (0.9–21.0)] in IHC3\(^+\) cases (\(P = 0.0010\) for IHC2\(^+\) vs. IHC0-1\(^+\); \(P < 0.0001\) for IHC3\(^+\) vs. IHC0-1\(^+\)). Within the subgroup of amplified cases (\(n = 108\)), mean amplification level (HER2/CEP17 ratio) was 2.8 [median 2.6 (range: 2.1–4.6)] in IHC0-1\(^+\) cases, 5.5 [median 4.5 (2.0–15.8)] in IHC2\(^+\) cases, and 8.1 [median 7.9 (2.2–21.0)] in IHC3\(^+\) cases (\(P = 0.018\) for IHC2\(^+\) vs. IHC0-1\(^+\); \(P = 0.0002\) for IHC3\(^+\) vs. IHC0-1\(^+\)).

We defined HER2 positivity as IHC3\(^+\) or IHC2\(^+\) with gene amplification, which is the group that derived greatest benefit from trastuzumab in ToGA (4), and the definition approved in Europe for trastuzumab eligibility (5). A total of 708 (99\%) of 713 cases had IHC data available or, if showing IHC2\(^+\) expression, were evaluable by FISH. Using
this group to determine HER2 status incorporating IHC and/or FISH data, the frequency of HER2 positivity was 17% (119 of 708). Specifically, there were 94 IHC3+ cases, 25 IHC2+ with and 142 IHC2+ without gene amplification, and 447 IHC0-1+. Five IHC2+ cases were unevaluable by FISH and were excluded from determining HER2 status.

**Association of HER2 with clinicopathologic features and patient outcome**

Using the composite definition of HER2 positivity (i.e., IHC3+ or IHC2+ plus gene amplified), HER2 positivity was more common in esophageal (vs. GEJ/cardia) tumors (21% vs. 15%, P = 0.03; Table 1). Among the limited number of gastric cardia tumors, 11% (4 of 37) showed HER2 positivity.

HER2-positive (vs. HER2-negative) cases were significantly associated with lower depth of tumor invasion (T stage), fewer malignant nodes, lower (better differentiated) histologic grade, and absence of signet ring cells (Table 1). The frequency of HER2 positivity was significantly increased in EACs with versus without adjacent BE (Table 1). Furthermore, the mean level of HER2 gene amplification was significantly higher in EACs with versus without BE (HER2/CEP17, ratio 4.0 vs. 2.6, P < 0.0001). A higher frequency of HER2 positivity in EACs with versus without BE was shown across EACs of varying tumor depth, nodal status, and histologic grade (Fig. 1). Only the presence of adjacent BE [OR = 1.8 (95% CI: 1.1–2.8), P = 0.014] and well to moderate differentiation [OR = 3.4 (95% CI: 2.1–5.7), P < 0.0001] were independently associated with HER2 positivity (Table 3). Of note, HER2 expression and/or amplification were assessed in invasive carcinoma cells, not

<table>
<thead>
<tr>
<th>Table 1. Clinicopathologic characteristics by HER2 status in resected EACs</th>
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<tbody>
<tr>
<td>Characteristic</td>
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<tr>
<td>Median age, y</td>
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<tr>
<td>Gender, n (%)</td>
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<td>Male</td>
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<td>T status, n (%)</td>
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<tr>
<td>T1–2</td>
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<td>T3–4</td>
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<tr>
<td>No. of positive nodes, mean (range)</td>
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<td>Histologic grade, n (%)</td>
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<td>G1–3</td>
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<td>G4</td>
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<td>Signet ring cells, n (%)</td>
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<tr>
<td>Absent</td>
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<tr>
<td>Present</td>
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<tr>
<td>Anatomic location, n (%)</td>
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<tr>
<td>Esophagus</td>
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<tr>
<td>GEJ or cardia</td>
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<tr>
<td>Adjacent Barrett’s, n (%)</td>
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<tr>
<td>Present</td>
</tr>
<tr>
<td>Absent</td>
</tr>
</tbody>
</table>

**Abbreviations:** IHC3+, high; IHC2+, medium; IHC0-1+, absent or low.

*Five cases with unevaluable FISH were excluded.

**Table 2. Association between HER2 protein overexpression and gene amplification in surgical EAC specimens**

<table>
<thead>
<tr>
<th>HER2 gene amplification</th>
<th>HER2 protein overexpression (N = 344)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHC3+ (n = 84)</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>76 (90)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>8 (10)</td>
</tr>
<tr>
<td>χ²</td>
<td>0.83 (95% CI: 0.75–0.91)</td>
</tr>
</tbody>
</table>

**Abbreviations:** IHC3+, high; IHC2+, medium; IHC0-1+, absent or low.

*For the IHC0-1+ and IHC3+ groups.*

**Association of HER2 with clinicopathologic features and patient outcome**

Using the composite definition of HER2 positivity (i.e., IHC3+ or IHC2+ plus gene amplified), HER2 positivity was more common in esophageal (vs. GEJ/cardia) tumors (21% vs. 15%, P = 0.03; Table 1). Among the limited number of gastric cardia tumors, 11% (4 of 37) showed HER2 positivity. HER2-positive (vs. HER2-negative) cases were significantly associated with lower depth of tumor invasion (T stage), fewer malignant nodes, lower (better differentiated) histologic grade, and absence of signet ring cells (Table 1). The frequency of HER2 positivity was significantly increased in EACs with versus without adjacent BE (Table 1). Furthermore, the mean level of HER2 gene amplification was significantly higher in EACs with versus without BE (HER2/CEP17, ratio 4.0 vs. 2.6, P < 0.0001). A higher frequency of HER2 positivity in EACs with versus without BE was shown across EACs of varying tumor depth, nodal status, and histologic grade (Fig. 1). Only the presence of adjacent BE [OR = 1.8 (95% CI: 1.1–2.8), P = 0.014] and well to moderate differentiation [OR = 3.4 (95% CI: 2.1–5.7), P < 0.0001] were independently associated with HER2 positivity (Table 3). Of note, HER2 expression and/or amplification were assessed in invasive carcinoma cells, not
HER2 status and improved DSS trended toward statistical significance (HR = 0.79, \( P = 0.066 \)). However, in a multivariable analysis, HER2 status was not significantly associated with OS (HR = 0.89, \( P = 0.35 \)) or DSS (HR = 0.84, \( P = 0.17 \)) after adjustment for pathologic tumor stage. The presence (vs. absence) of adjacent BE was associated with improved DSS [HR = 0.65 (95% CI: 0.54–0.80), \( P < 0.0001 \)] and OS (\( P < 0.0001 \)) in univariate analysis, but not after adjustment for pathologic features (\( P = 0.94 \) and 0.72, respectively).

Given the significant association between HER2 and adjacent BE, their univariate association with survival, and the limited data about their interrelationship, we determined whether an interaction was present between these variables. For DSS, the HER2 \( \times \) BE interaction term was significant (\( P = 0.0047 \)), indicating that the prognostic impact of HER2 positivity differed significantly based on BE status. Among EACs with BE (\( n = 240 \)), univariate analysis revealed that HER2 positivity was significantly associated with improved DSS and OS (Fig. 2). In multivariable analysis among EACs with BE, HER2 positivity was independently associated with improved DSS [HR = 0.54 (95% CI: 0.35–0.84), \( P = 0.0065 \)] and OS [HR = 0.50 (95% CI: 0.33–0.78), \( P = 0.0022 \)] after adjusting for tumor grade, depth of invasion, nodal status, and tumor location (Table 4). When considering EAC patients without BE (\( n = 468 \)), HER2 positivity was not significantly associated with DSS or OS in univariate or multivariable analysis (Fig. 2, Table 4).

Sensitivity analysis in the subgroup of patients (\( n = 625 \)) who did not receive adjuvant chemotherapy or radiotherapy showed stable results. Among EACs with BE, HER2 positivity was significantly associated with improved DSS [HR = 0.52 (95% CI: 0.31–0.87), \( P = 0.013 \)] after adjustment for covariates.

**Discussion**

We report the analysis of HER2 status in a large cohort of patients not treated with neoadjuvant therapy, thereby precluding the potential for confounding by preoperative treatment. HER2 positivity was detected in 17% of EACs with strong agreement between protein expression on cellular membranes and gene amplification. HER2 positivity was more frequent in esophageal than GEJ tumors. Furthermore, HER2 positivity was associated with favorable pathologic features including lower T and N stage and better tumor differentiation. HER2 positivity was higher in frequency in EACs with (vs. without) adjacent BE, and this pattern was maintained across tumors of varying invasiveness, nodal status, and histologic grade. Of note, the presence of adjacent BE and histologic grade predicted independently for HER2 positivity. When only HER2 amplification was examined, a significantly higher level was found in EACs with versus without BE. Although HER2 positivity was associated with more favorable survival in the full study cohort, it was not independent of covariates. Importantly, we found that the prognostic impact of HER2 positivity significantly differed based on BE status (\( P_{\text{interaction}} = 0.0047 \)). HER2 positivity was independently associated
with improved DSS and OS in EACs with BE, but not in EACs without BE.

Although BE is a premalignant lesion related to chronic gastroesophageal reflux disease, controversy exists as to whether all EACs originate from BE (29). Although not addressed in our study, metaplastic Barrett’s epithelium has been shown to lack HER2 expression and/or amplification, and the frequency of HER2 expression or amplification in BE-associated dysplasia are not well understood but may increase (30–32). In breast cancer, HER2 is overexpressed at a higher frequency in preinvasive lesions, such as ductal carcinoma in situ, as compared with invasive lesions (33, 34). Our findings of higher rates of HER2 positivity in adenocarcinoma cells from patients with adjacent BE and a better prognosis in these cases suggests that BE-associated EACs are distinct biologically from non-BE–associated cancers. In this regard, gene expression profiling has shown that EACs without BE show a greater number of overexpressed genes involved in tumor invasiveness and tissue remodeling compared with BE-associated EACs (35). Although the explanation for the association of HER2 with better clinical outcome remains unknown, a subtype of HER2-positive breast cancers is associated with an increased inflammatory or immune cell infiltration that showed a substantially improved prognosis as compared with other HER2-positive breast cancers, with potentially improved response to neoadjuvant trastuzumab-based chemotherapy (36, 37). Furthermore, recent population-based data of patients with inflammatory breast cancers suggest that HER2 positivity (vs. HER2 negativity) may have favorable prognostic impact

Table 3. Multivariable logistic regression model of tumor characteristics in relation to HER2 positivity

<table>
<thead>
<tr>
<th>Tumor characteristic</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasiveness, T1–2 vs. T3–4</td>
<td>1.1 (0.7–1.7)</td>
<td>0.75</td>
</tr>
<tr>
<td>Nodal status, negative vs. positive</td>
<td>0.9 (0.6–1.5)</td>
<td>0.73</td>
</tr>
<tr>
<td>Histologic grade, G1–3 vs. G4</td>
<td>3.4 (2.1–5.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site, esophagus vs. GEJ/cardia</td>
<td>0.9 (0.6–1.4)</td>
<td>0.77</td>
</tr>
<tr>
<td>Adjacent Barrett’s, yes vs. no</td>
<td>1.8 (1.1–2.8)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Abbreviations: IHC3⁺, high; IHC2⁺, medium.
*HER2 positivity was defined as IHC3⁺ or IHC2⁺ with gene amplification.
**Odds of being HER2 positive adjusted for all variables in the model.

Figure 2. Prognostic impact of HER2 positivity on DSS (A and B) and OS (C and D) in patients with EAC with (A and C) and without (B and D) adjacent BE who underwent curative surgical resection.
(38). In this context, it is noteworthy that a link in the development and/or progression of BE with chronic inflammation and the immune response has been reported (39–42).

Prognostic data for HER2 overexpression/amplification in upper digestive carcinoma are conflicting and, particularly for EAC, limited (11–16, 43). Data from ToGA in advanced gastric cancer patients suggests that HER2 overexpression correlates with favorable prognosis, given that median OS in the non-trastuzumab study arm was shown to improve with increasing protein expression (4). HER2 expression/amplification in gastric cancers has been frequently associated with intestinal-type histology as compared with diffuse-type histology (43). Intestinal-type gastric cancers are known to be associated with lower tumor grade and have a better prognosis as compared with diffuse (3).

Our data for HER2 is therapeutically relevant given recent clinical data showing a survival benefit in advanced gastric carcinomas with the use of the anti-HER2 monoclonal antibody, trastuzumab. Using the definition of HER2 positivity (i.e., IHC3+ or IHC2+ with gene amplification) shown in the ToGA trial to identify patients that derived greatest benefit from trastuzumab (4), we found that 17% of HER2 positive subset of EAC patients may benefit from HER2-targeted therapy. This question is being addressed in an ongoing phase III trial (RTOG-1010) in locally advanced HER2-overexpressing EAC, where the benefit of trastuzumab to preoperative chemoradiotherapy will be determined. Our findings contribute to the increasing appreciation that the therapeutic (predictive) value of a molecular target is significantly mediated by antibody-dependent cellular efficacy was seen in IHC0-1+ cases with amplification in contrast to IHC2+ cases in the ToGA trial (4, 44). The frequencies of overall HER2 positivity and/or IHC3+ observed in our study are similar to those described in literature (4, 11, 13, 14, 25, 45). However, our IHC2+ frequency (24%) was higher than reported in other upper digestive cancer studies (4, 13–15, 25, 45–47). Although comparisons of subgroup in subgroup frequencies are problematic in EAC given the paucity of studies and their limited sample size (13–15, 45), this higher IHC2+ frequency may partly explain the lower amplification rate we observed in the IHC2+ subgroup. This in context, it is important to note that, as described in the scoring criteria developed for upper digestive carcinoma, IHC2+ differs from IHC1+ only by the intensity of expression (i.e., “weak to moderate vs. faint” ref. 4). This difference underscores the importance of testing IHC2+ cases by FISH to determine trastuzumab eligibility, as recommended in Europe (5).

Importantly, our focus was on earlier stage disease where the potential for curative intervention exists. Our data raise the issue of whether the HER2 positive subset of EAC patients may benefit from HER2-targeted therapy. This question is being addressed in an ongoing phase III trial (RTOG-1010) in locally advanced HER2-overexpressing EAC, where the benefit of trastuzumab to preoperative chemoradiotherapy will be determined. Our findings contribute to the increasing appreciation that the therapeutic (predictive) value of a molecular target is significantly mediated by antibody-dependent cellular

| Table 4. Multivariable prognostic Cox models of HER2 positivity in EACs stratified by the presence or absence of adjacent BE

<table>
<thead>
<tr>
<th>Tumor characteristic</th>
<th>DSS HR (95% CI)</th>
<th>P</th>
<th>OS HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EACs with BE (n = 240)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2, positive vs. negative</td>
<td>0.54 (0.35–0.84)</td>
<td>0.0062</td>
<td>0.50 (0.32–0.77)</td>
<td>0.0020</td>
</tr>
<tr>
<td>Invasiveness, T3–4 vs. T1–2</td>
<td>1.71 (1.16–2.65)</td>
<td>0.0072</td>
<td>1.69 (1.16–2.47)</td>
<td>0.0066</td>
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<tr>
<td>Nodal status, positive vs. negative</td>
<td>5.06 (3.07–8.34)</td>
<td>&lt;0.0001</td>
<td>4.53 (2.83–7.24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Histologic grade, G1–3 vs. G4</td>
<td>1.58 (1.11–2.23)</td>
<td>0.0103</td>
<td>1.50 (1.07–2.0)</td>
<td>0.018</td>
</tr>
<tr>
<td>EACs without BE (n = 468)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2, positive vs. negative</td>
<td>1.17 (0.86–1.60)</td>
<td>0.31</td>
<td>1.13 (0.84–1.53)</td>
<td>0.42</td>
</tr>
<tr>
<td>Invasiveness, T3–4 vs. T1–2</td>
<td>1.32 (1.00–1.74)</td>
<td>0.054</td>
<td>1.29 (0.99–1.68)</td>
<td>0.063</td>
</tr>
<tr>
<td>Nodal status, positive vs. negative</td>
<td>3.44 (2.46–4.81)</td>
<td>&lt;0.0001</td>
<td>3.10 (2.27–4.24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Histologic grade, G1–3 vs. G4</td>
<td>1.15 (0.92–1.43)</td>
<td>0.23</td>
<td>1.16 (0.94–1.43)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Abbreviations: IHC3+, high; IHC2+, medium.

Pinteraction between HER2 and BE was 0.0047 for DSS and 0.0026 for OS.

HR for death adjusted for all variables in the model.

HER2 positive was defined as IHC3+ or IHC2+ with gene amplification.
toxicity, rather than exclusively by downregulation of HER2 signaling or by counteracting HER2’s direct tumor-promoting effects (1).

Strengths of our study include the interpretation of HER2 protein expression by 2 pathologists according to standard criteria developed for upper digestive cancer (4, 25). Although our design was retrospective, internal validity was preserved because ascertainment and collection of study variables (e.g., pathologic stage, BE, and vital status) did not differ by HER2 status. Our cohort was homogeneous in histology (excluded squamous cell carcinoma) and excluded subcardial gastric cancers. By numerous parameters—including distribution of age, gender, and stage—our study population is generalizable to other resected EAC patients in Western countries (8, 48). Tumor features were determined by pathologic examination of resected specimens, and we cannot exclude the possibility that EACs without apparent adjacent BE may represent short-segment BE that has been overgrown by tumor, or that metaplastic changes may have arisen in the gastric cardia and do not represent Barrett’s epithelium. In addition, it is important to note that our testing for interaction between HER2 and BE was not a priori planned and, therefore, these results require validation in an independent cohort.

In summary, we report a 17% frequency of HER2 positivity in surgically resected stage I to III EACs. HER2 positivity was associated with favorable pathologic features. Furthermore, HER2 overexpression/amplification was more common in BE-associated EAC and, within this subgroup, HER2 positivity was independently associated with improved survival.

Disclosure of Potential Conflicts of Interest

H.H. Yoon received a research grant from Genentech and has received honoraria for serving on advisory boards, Genentech.

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References


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