HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma

Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology

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• *Context.*—*ERBB2* (erb-b2 receptor tyrosine kinase 2 or *HER2*) is currently the only biomarker established for selection of a specific therapy for patients with advanced gastroesophageal adenocarcinoma (GEA). However, there are no comprehensive guidelines for the assessment of *HER2* in patients with GEA.

***Objectives.*—To establish an evidence-based guideline for HER2 testing in patients with GEA, to formalize the algorithms for methods to improve the accuracy of HER2 testing while addressing which patients and tumor specimens are appropriate, and to provide guidance on clinical decision making.**

***Design.*—The College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology convened an expert panel to conduct a systematic review of the literature to develop an evidence-based guideline with recommendations for optimal HER2 testing in patients with GEA.**

***Results.*—The panel is proposing 11 recommendations with strong agreement from the open-comment participants.**

***Recommendations.*—The panel recommends that tumor specimen(s) from all patients with advanced GEA, who are candidates for HER2-targeted therapy, should be assessed for HER2 status before the initiation of HER2-targeted therapy. Clinicians should offer combination chemotherapy and a HER2-targeted agent as initial therapy for all patients with HER2-positive advanced GEA. For pathologists, guidance is provided for morphologic selection of neoplastic tissue, testing algorithms, scoring methods, interpretation and reporting of results, and laboratory quality assurance.**

***Conclusions.*—This guideline provides specific recommendations for assessment of *HER2* in patients with advanced GEA while addressing pertinent technical issues and clinical implications of the results.**

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Gastroesophageal adenocarcinoma (GEA) is estimated to represent up to 43 280 cancer cases in the United States in 2016,[1] and represents the eighth (esophageal) and fifth (stomach) most common cancers worldwide.[2] Gastroesophageal adenocarcinoma is often diagnosed at an advanced stage, resulting in a poor prognosis. Most localized GEAs (stages II and III) are best treated with multimodality therapy, which can result in a five-year survival in ~40% of patients; however, once GEA is advanced (defined as unresectable local-regional, recurrent, or metastatic disease), therapies are limited and palliative with cure being extremely rare.

In 2010, results of an open-label, international, phase 3 randomized controlled trial (Trastuzumab for Gastric Cancer, ToGA), showed that the anti-HER2 humanized monoclonal antibody trastuzumab (Herceptin; Genentech, San Francisco, California) statistically significantly prolonged overall survival, compared with chemotherapy alone, in patients with HER2–positive advanced GEA.[3] *ERBB2* (also commonly known as *HER2*) is a proto-oncogene located on chromosome 17 that encodes a 185-kDa tyrosine kinase receptor belonging to the epidermal growth factor receptor family whose phosphorylation initiates signaling pathways that lead to cell division, proliferation, differentiation, and anti-apoptosis signaling.[4,5] Past investigations have estimated that between 7% and 38% of GEAs have amplification and/or overexpression of HER2.[3,6,7]

The frequency of overexpression of HER2 is slightly greater for cancers at the gastroesophageal junction in comparison to the stomach,[3,8–10] and overexpression in the stomach varies with histologic type (intestinal-type greater than diffuse-type) and differentiation (well and moderately differentiated greater than poorly differentiated).[11] In comparison to breast carcinomas, the heterogeneity of immunostaining is greater in GEA,[9,12] and the completeness of membrane staining required for positivity in mammary neoplastic cells is infrequent in GEA and often expression is seen in a basolateral pattern. Hofmann et al[13] proposed a 4-tier HER2 scoring system, also used in the ToGA trial, for GEA by applying an assessment area cutoff of at least 10% stained tumor cells for resection specimens and a small single cluster of cells (or at least 5 cells) for biopsy specimens.

Trastuzumab is a humanized monoclonal antibody that targets the extracellular domain of the HER2 receptor, inhibits downstream signal activation, and induces antibody-dependent cellular toxicity. The literature on *HER2* as a prognostic factor for patients with GEA is conflicting; not all studies have shown an association between HER2 overexpression and poor prognosis in GEA.[13,14] The National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Gastric Cancer and Esophageal and Esophagogastric Junction Cancers recommend assessment of HER2 overexpression using immunohistochemistry (IHC) and/or gene amplification using fluorescence in situ hybridization (FISH) or another in situ hybridization (ISH) method in tumor samples from patients with unresectable locally advanced, recurrent, or metastatic GEA for whom trastuzumab may be potentially beneficial.[11,15] Testing for *HER2* is primarily performed on formalin-fixed and paraffin-embedded biopsy or resection tumor tissue from the primary or metastatic site.

In 2007, a joint expert panel convened by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) met to develop guidelines for when and how to test for *HER2* in patients with breast cancer, which is amplified and/or overexpressed in up to 30% of cases.[16] In 2012, ASCO and CAP convened an Update Committee to conduct a comprehensive review of the peer-reviewed literature published since 2006 and to revise the guideline recommendations. The Update Committee developed new algorithms for testing and recommended quality assurance monitoring that would make HER2 testing less variable and ensure more analytic consistency among laboratories.[17]

Because there are important distinct differences in HER2 expression, scoring, and outcomes in GEA relative to breast carcinoma, the need for HER2 guidelines (that include critical clinical and laboratory considerations) was recognized. The CAP, American Society for Clinical Pathology (ASCP), and ASCO convened an international expert panel to systematically review published documents and to develop an evidence-based guideline to establish recommendations for HER2 testing in GEA.

PANEL COMPOSITION

The CAP Pathology and Laboratory Quality Center, ASCP, and ASCO convened an international expert panel consisting of practicing pathologists, oncologists, and a gastroenterologist with expertise and experience in GEA. Members included practicing clinicians and pathologists from the United States, Canada, and Europe. The CAP, ASCP, and ASCO approved the appointment of the project, cochairs, and expert panel members. In addition, a physician-methodologist experienced in systematic review and guideline development consulted with the panel throughout the project, and a patient advocate also participated to convey the patient experience.

CONFLICT OF INTEREST POLICY

Before appointment to the expert panel, potential members completed a joint conflict of interest (COI) disclosure process whose policy and form require disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations. The potential members completed the COI disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Potential conflicts were managed by the cochairs. All members were required to disclose conflicts before beginning the project and then continuously throughout the project's timeline. Disclosed conflicts of the expert panel members are listed in the Appendix. The CAP, ASCP, and ASCO provided funding for the administration of the project; no industry funding was involved in any aspect of the development of this guideline. All panel members volunteered their time and were not compensated for their involvement. Please see the supplemental digital content (SDC) (available at www.archivesofpathology.org in the December 2016 table of contents).

OBJECTIVE

The panel addressed the overarching questions "What is the optimal testing algorithm for the assessment of *HER2* status in patients with GEA?" and "What strategies can help ensure optimal performance, interpretation, and reporting of established assays in patients with GEA?"

This led to the following additional questions:

1. Should HER2 testing be performed in every patient diagnosed with GEA?
2. Which tumor specimen(s) is(are) the most appropriate to perform HER2 testing?

Table 1. Quality of Evidence Ratings in the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Framework[a]

GRADE	Definition
High	Further research is very unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain.

[a] Guyatt et al.[20] *The BMJ*. Adapted by permission from BMJ Publishing Group Limited. ©2008.

3. In patients with HER2-positive results, under what clinical scenario should HER2-targeted therapy be initiated?
4. Should HER2-directed therapy be delayed if HER2 status cannot be confirmed as positive or negative (ie, if an equivocal result is found with IHC)?
5. Under what circumstances should a patient's tumor specimen be retested for *HER2*?
6. What are the clinical performance characteristics of IHC and ISH?
7. What are the analytic performance characteristics of IHC and ISH?
8. What are the acceptable methodologies for HER2 IHC (different antibodies) and ISH (different probe platforms)?
9. What is the optimal testing algorithm for the assessment of HER2 status?
10. What are the steps/procedures needed to analytically validate a laboratory-developed *HER2* GEA assay before reporting results on patient tumor specimen(s)?
11. What is the best scoring method for IHC and ISH in GEA specimens?
12. How should *HER2* results be reported?
13. What is adequate tumor specimen handling for HER2 testing?
14. What is the appropriate morphologic correlation for interpretation of ISH?
15. What are the optimal quality assurance/quality control standards that all HER2 testing laboratories should adhere to?
16. Is there a role for *HER2* genomic testing?

METHODS

A detailed account of the methods used to create this guideline can be found in the SDC, including additional scope questions.

Systematic Literature Review and Analysis

A systematic literature search was completed for relevant evidence by using OvidSP, PubMed, and Scopus (January 1, 2008, to June 1, 2015). The search strategy included medical subject headings (MeSH) and text words to capture the general concepts of gastroesophageal neoplasms, human epidermal growth factor receptor 2 (*ERBB2/HER2*), targeted therapy, and laboratory testing methods. Database searches were supplemented with a search for unindexed literature, including a review of clinical trials and pertinent organizations' Web sites. All searches were limited to human studies. Expert panel recommendations and a review of reference lists of included articles for relevant reports completed the systematic literature review. Detailed information regarding the literature search strategy can be found in the SDC.

Eligible Study Designs

Eligible study designs were determined a priori on the basis of whether they were clinical or laboratory-based studies. Clinical studies were included if they were systematic reviews with or without meta-analyses, guidelines, consensus statements, or randomized controlled trials (except for phase I trials). Additional study types were included for laboratory-based studies owing to concern that relevant data would not otherwise be captured. Detailed information about included study designs is available in the SDC.

Inclusion Criteria

Published studies were selected for inclusion in the systematic review of evidence if they met the following criteria: (1) the study included human patients, (2) the study population consisted of patients with invasive GEA, (3) the study was published in English, (4) the study compared, prospectively or retrospectively, laboratory testing methodologies or potential testing algorithms for *HER2* testing, (5) the study addressed 1 of the key questions, and (6) the study included measurable data such as the negative predictive value or positive predictive value of ISH and IHC assays used to determine HER2 status, alone and in combination; negative and positive concordance across the platforms; sensitivity and specificity of individual tests and accuracy in determining HER2 status. Detailed information about the inclusion criteria is available in the SDC.

Exclusion Criteria

Articles were excluded from the systematic review if they were meeting abstracts that were not published in peer review journals; noncomparative or qualitative studies, including editorials, commentaries, and letters; animal studies; full text articles not available in English; studies that included patients with other tumor types, including esophageal squamous cell carcinoma, or patients with noninvasive tumors; studies that did not include relevant measurable data; and studies that did not address at least 1 of the key questions. Detailed information about the exclusion criteria is available in the SDC.

Quality Assessment

Study design aspects related to individual study quality, strength of evidence, strength of recommendations, and the risk of bias were assessed. Refer to the SDC for more information and for definitions of ratings for overall potential risk of bias.

Assessing the Strength of Recommendations

The guideline recommendations were crafted, in part, by using the GLIDES (Guidelines Into Decision Support) methodology[18] and accompanying BridgeWiz software (Yale University, New Haven, Connecticut).[19] Development of recommendations required that the panel review and identify evidence and make a series of key judgments (using procedures described in SDC). Additionally, the expert panel gave its recommendations with regard to potential clinical impact by assessing benefits and harms for each recommendation, and then rated the quality of evidence for the recommendations as high, intermediate, low, or insufficient. The Grading of Recommendations Assessment, Development and Evaluation, or GRADE method,[20] was used to rate the quality of the evidence. CAP uses a 3-tier system to rate the strength of recommendations instead of the traditional 2-tier approach of

Table 2. Strength of Recommendations[a]			
CAP Designation	**GLIDES Designation**	**Recommendation**	**Rationale**
Strong recommendation	Strong	Recommend for or against a particular practice (can include must or should)	Supported by high (convincing) or intermediate (adequate) quality of evidence and clear benefit that outweighs any harms
Recommendation	Moderate	Recommend for or against a particular practice (can include should or may)	Some limitations in quality of evidence (intermediate [adequate] or low [inadequate]), balance of benefits and harms, values, or costs but panel concludes that there is sufficient evidence and/or benefit to inform a recommendation
Expert consensus opinion	Weak	Recommend for or against a particular practice (can include should or may)	Serious limitations in quality of evidence (low [inadequate] or insufficient), balance of benefits and harms, values or costs, but panel consensus is that a statement is necessary
No recommendation	N/A	No recommendation for or against a particular practice	Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation

Abbreviations: CAP, College of American Pathologists; GLIDES, Guidelines Into Decision Support; N/A, not applicable.

[a] Data derived from Guyatt et al.[20]

strong or weak recommendations. This approach is consistent with prior CAP guidelines (Tables 1 and 2).

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original recommendations. If necessary, the entire panel will be reconvened to discuss potential changes. When appropriate, the panel will recommend revision(s) of the guideline to CAP, ASCP, and ASCO for review and approval.

Disclaimer

The CAP developed the Pathology and Laboratory Quality Center as a forum to create and maintain evidence-based practice guidelines and consensus statements. Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and consensus statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. CAP, ASCP, and ASCO make no warranty, express or implied, regarding guidelines and statements and specifically exclude any warranties of merchantability and fitness for a particular use or purpose. CAP, ASCP, and ASCO assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

RESULTS

A total of 969 studies met the search term requirements. A total of 116 articles were included for data extraction. This consisted of 1 systematic review, 2 meta-analyses, 2 randomized controlled trials, 27 prospective studies, 69 prospective-retrospective studies, and 15 retrospective studies. Excluded articles were available as discussion or background references. The expert panel met face-to-face on April 25, 2015, to develop the scope and the key questions, and on August 29, 2015, to draft recommendations and assess the quality of evidence. The panel met a total of 16 times via Web conference in small groups to review solicited feedback and finalize the recommendations. A nominal group technique was used by the panel for consensus decision making to encourage unique input with balanced participation among the group members. An open comment period was held from December 8, 2015, to January 11, 2016, during which draft recommendations were posted on the ASCP Web site. Twenty recommendations were drafted with strong agreement for each recommendation from the open-comment-period participants ranging from 82% to 95% (refer to Outcomes in SDC for full details). The Web site received a total of 294 comments.

Teams of 2 expert panel members were assigned to 2 key questions and 3 to 4 draft recommendations to review all the comments received and provide an overall summary to the rest of the panel. Following panel discussions and the final quality of evidence assessment, the panel members determined whether to maintain the original draft recommendations as is, or revise them with major content changes. The panel modified 1 draft recommendation and combined 4 draft recommendations from the feedback during the open comment period and the considered

Table 3. Guideline Statements and Strength of Recommendation

Guideline Statement	College of American Pathologists (CAP) Strength of Recommendation
1. In patients with GEA who are potential candidates for HER2-targeted therapy, the treating clinician should request HER2 testing on tumor tissue.	Strong recommendation
2. Treating clinicians or pathologist should request HER2 testing on tumor tissue in the biopsy or resection specimens (primary or metastasis) preferably before the initiation of trastuzumab therapy if such specimens are available and adequate. HER2 testing on FNA specimens (cell blocks) is an acceptable alternative.	Recommendation
3. Treating clinicians should offer combination chemotherapy and HER2-targeted therapy as the initial treatment for appropriate patients with HER2-positive tumors who have metastatic or recurrent GEA.	Recommendation
4. Laboratories/pathologists must specify the antibodies and probes used for the test and ensure that assays are appropriately validated for HER2 IHC and ISH on GEA specimens.	Strong recommendation
5. When GEA HER2 status is being evaluated, laboratories/pathologists should perform/order IHC testing first followed by ISH when IHC result is 2+ (equivocal). Positive (3+) or negative (0 or 1+) HER2 IHC results do not require further ISH testing.	Strong recommendation
6. Pathologists should use the Ruschoff/Hofmann method in scoring HER2 IHC and ISH results for GEA.	Strong recommendation
7. Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than 1 tissue block may be selected if different morphologic patterns are present.	Recommendation
8. Laboratories should report HER2 testing results in GEA specimens in accordance with the CAP "Template for Reporting Results of *HER2* (*ERBB2*) Biomarker Testing of Specimens From Patients With Adenocarcinoma of the Stomach or Esophagogastric Junction."	Strong recommendation
9. Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by IHC in GEA specimens for subsequent ISH scoring when required.	Strong recommendation
10. Laboratories must incorporate GEA HER2 testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to ensure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing GEA HER2 testing must participate in a formal proficiency testing program, if available, or an alternative proficiency assurance activity.	Strong recommendation
11. There is insufficient evidence to recommend for or against genomic testing in patients with GEA at this time.	No recommendation

Abbreviations: FNA, fine-needle aspirate; GEA, gastroesophageal adenocarcinoma; IHC, immunohistochemistry; ISH, in situ hybridization.

judgment process. Additionally, the panel decided that general recommendations about quality assurance, turnaround time, and specimen handling were best suited as part of the discussion, and would be included in the body of the final manuscript rather than as formal recommendations. Resolution of all changes was obtained by majority consensus of the panel, using nominal group technique (rounds of email discussion and multiple edited recommendations) among the panel members. The expert panel with a formal vote approved the final recommendations. The panel considered the risks and benefits throughout the entire process in their considered judgment process. Formal cost analysis or cost effectiveness was not performed. A summary of the final guideline statements and strength of recommendation is shown in Table 3.

Each organization instituted a review process to approve the guideline. The CAP convened an independent review panel representing the Council for Scientific Affairs to review and approve the guideline. The independent review panel was masked to the expert panel and vetted through the COI process. ASCP assigned the review of the guideline to a Special Review Panel at the discretion of the ASCP Executive Office and Board of Directors. The ASCO approval process required the review and approval of the Clinical Practice Guidelines Committee.

GUIDELINE STATEMENTS

1. Strong Recommendation.—In patients with advanced GEA who are potential candidates for HER2-targeted therapy, the treating clinician should request HER2 testing on tumor tissue.

(Quality of evidence: High; Strength of recommendation: Strong)

All patients who have documented advanced GEA and who are considered good candidates for combination chemotherapy plus trastuzumab therapy should have their tumor tissue tested for HER2 overexpression and/or amplification. In patients with HER2-positive GEA, the addition of trastuzumab can increase the response rate, prolong progression-free survival, and prolong overall survival. Other than providing guidance to the addition of trastuzumab to cytotoxic combination (when the tumor is HER2 positive), HER2 status provides little additional value such as prognostic or predictive information. Currently, there is no evidence of benefit of HER2-directed therapy in patients without advanced GEA.

In the ToGA trial, patients were randomly assigned to receive capecitabine plus cisplatin or fluorouracil plus cisplatin in combination with trastuzumab.[3,8] Of the 3803 patients originally screened for eligibility, 810 patients had IHC or FISH HER2-positive tumors but only 594 patients were randomly assigned to treatment. The HER2 positivity rate was 22.1% with similar rates between European and Asian patients (23.6% versus 23.9%). The eligible patients included those with advanced adenocarcinoma of the stomach or gastroesophageal junction; Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2; adequate organ function; and measurable or nonmeasurable disease. Patients were ineligible if they had congestive heart failure, baseline left ventricular ejection fraction less than 50%, transmural myocardial infarction, uncontrolled hypertension (systolic blood pressure > 180 mm Hg or diastolic blood pressure > 100 mm Hg), angina pectoris requiring medication, clinically significant valvular heart disease, high-risk arrhythmias, lack of physical integrity of the upper gastrointestinal tract or malabsorption syndrome, active gastrointestinal bleeding, and evidence of brain metastases. The median overall survival was 13.8 months for patients receiving trastuzumab plus chemotherapy, compared with 11.1 months for those receiving chemotherapy alone (hazard ratio [HR] = 0.74; 95% confidence interval [CI], 0.60–0.91; $P = .0038$). Patients with IHC of 3+ derived more benefit than those with IHC of 2+ (and concurrent *HER2* amplification by ISH). However, upon further follow-up of these patients, reanalyses demonstrated considerable reduction in patient benefit from the addition of trastuzumab (HR = 0.8; 95% CI, 0.67–0.97; $P = .019$). The difference in the median survival diminished to a mere 1.4 months.[21]

The cardiac adverse event rate was low (6%) and did not differ between the treatment groups. Trastuzumab was generally well tolerated, but the patients assigned to trastuzumab experienced slightly higher rates of diarrhea, stomatitis, anemia, thrombocytopenia, fatigue, and weight loss, but there was no difference between the groups in frequency of side effects, or grade 3 or 4 toxicities except for diarrhea.

NCCN Guidelines recommend systemic therapy, clinical trial participation, or palliative management for patients with a Karnofsky performance score greater or equal to 60%, or an ECOG performance score less than or equal to 2, and that trastuzumab should be added to active first-line combination chemotherapy for HER2-positive metastatic GEA (although the ToGA trial combined cisplatin and a fluoropyrimidine with trastuzumab).[11] Patients with a Karnofsky performance score less than 60%, or ECOG performance score greater than or equal to 3, are best managed with best supportive care.

Although the literature regarding *HER2* as a prognostic marker is conflicting, some studies[6,22–24] have demonstrated that *HER2* amplification or overexpression in GEA may be associated with a worse prognosis and is independent of other prognostic factors including age, sex, location, or stage. We briefly review only 2 large and representative studies that failed to correlate *HER2* status with prognosis. A retrospective study of 1006 Japanese patients with gastric cancer established HER2 overexpression in 11.7% of cases.[25] The HER2 status correlated with age, sex, grade, growth pattern, and nodal status; however, HER2 overexpression did not correlate with disease-specific survival or recurrence-free survival. Likewise, a combined analysis of 924 German and British patients who had undergone surgical resection demonstrated HER2 expression in less than 10% of tumor specimens with considerable intratumoral heterogeneity and no relationship between HER2 expression, patient survival, or stage.[26]

In summary, the evidence does not support the determination of HER2 status in patients who have a surgically resectable GEA, and HER2 status is not useful to prognosticate survival or similar endpoints. However, for patients with advanced GEA with a good performance status, low cardiac risk, and who would otherwise be candidates for systemic therapy including trastuzumab, HER2 testing should be performed and patients should be offered trastuzumab if GEA is HER2 positive.

2. Recommendation.—Treating clinicians or pathologists should request HER2 testing on tumor tissue in the biopsy or resection specimens (primary or metastasis) preferably before the initiation of trastuzumab therapy if such specimens are available and adequate. HER2 testing on fine-needle aspiration (FNA) specimens (cell blocks) is an acceptable alternative.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Recommendation/Moderate)

Tumor Specimens From the Primary GEA

Primary tumor specimens obtained either by biopsy or resection represent the principal sample type for assessment of HER2 status in a number of larger analyses that have included patients with resectable GEA. Of the 115 patient biopsy or resection specimens tested for *HER2* in the MAGIC (Medical Research Council Adjuvant Gastric Cancer Infusional Chemotherapy) trial, there was 92.9% (145 of 156) concordance between the 2 types of specimens.[27] In the ToGA trial, 2596 patients' tumors (68%) were acquired by a biopsy and 1199 patients' tumors (32%) were acquired from the surgical specimens. Of these, 579 biopsy specimens were HER2 positive, and 231 of the surgical specimens were HER2 positive. Overall positive rate was 23.2% for biopsy specimens and 19.7% for the surgical specimens. Of note, there was significant variability in staining intensities across tissue sections.[8]

In another collaborative effort on 381 patients with advanced GEA, 20% had HER2-positive tumors with higher rates in those with liver metastases and intestinal histology.[28] There was no difference in HER2 positivity between resections/biopsies of primary (biopsies 21% versus resection 19%, $P = .791$) or metastatic disease and no association with prognosis. In another study of 178 patients with GEA, there were 64 biopsy specimens and 60 gastrectomy specimens for HER2 testing. The overall positivity rate was 20.2%. There was a significantly higher percentage of patients with HER2 3+ expression in biopsy specimens than in gastrectomy specimens (31.2% versus 8.8%, $P = .0003$); however, the concordance of overall HER2 status was 74.1% between biopsy versus gastrectomy specimens. The biopsy specimens also included a higher proportion of intestinal-type tumors (70.3% versus 48.2%, $P = .003$).

Tumor Specimens From Resected GEA

In a Japanese study,[29] 207 surgically resected tumors and paired biopsy specimens from 158 patients with intestinal-type gastric cancers were analyzed for HER2 overexpression/amplification. In both specimen types, HER2 overexpression was observed in 17% of cases, whereas gene amplification was detected in 31% of the surgically resected tumors and 32% of biopsy specimens. Concordance between IHC and FISH was 90.9% in the surgically resected tumors and 90.2% in biopsy specimens. There was 72.7% concordance rate of FISH between the surgical and biopsy specimens.[29] Another analysis of endoscopic biopsy compared to surgical samples in evaluating HER2 status in GEA included 103 patients with matched specimens.[30] The concordance of IHC between biopsy and surgical samples was 80% and 95%, respectively. An Italian analysis of 61 consecutive pairs of biopsy specimens and surgical specimens noted a concordance of HER2 status of 91.8%.[12] Heterogeneous expression of HER2 protein in surgical specimens accounted for false-negative cases. In conclusion, there are limited studies that have compared matched pairs of biopsy versus resection specimen(s) for HER2 expression/amplification and the available results suggest a fair degree of concordance.

Tumor Specimens From Metastatic GEA

Multiple groups have investigated the status of HER2 expression in the primary tumor and metastatic lymph nodes in the same patient.[31–35] Qiu et al[31] examined 100 gastric cancers, in both primary tumors and corresponding malignant lymph node metastases, using IHC (scoring according to the criteria established by Hofmann et al[13]). HER2 overexpression (defined in this study as 2+ or 3+) was noted in 33.0% of primary specimens and 39.4% of the nodes. When HER2 status was compared in 2 or more nodes, there was 25.3% discordance. However, in a study that compared HER2 status in the metastatic lymph nodes compared to primary tumor, IHC and silver in situ hybridization (SISH) were used to compare HER2 status.[32] The SISH results were comparable with a concordance of 92.5%. The prevalence of HER2 discordance was significantly higher for tumors in the pN2 and pN3 categories ($P = .007$).

Some have compared HER2 status between the primary tumor specimen and synchronous metastatic specimens. In one such study of 41 paired samples with 5 HER2-positive tumors, there was a discrepancy observed in only 1 case.[36] Another study assessing HER2 status in 68 paired samples showed a 98.5% concordance of FISH results (n = 68) and 94.9% concordance of IHC results (n = 39).[37] Only 1 case was discordant, being negative in the primary tumor but positive in the metastatic peripancreatic lymph node. Others[38] have also shown good concordance between liver metastases (87.5%) and primary tumor. Thus, given the high degree of concordance, HER2 testing on the primary tumor or biopsy from a metastatic tumor deposit is appropriate.

Fine-Needle Aspiration or Cytology Specimen From Primary or Metastatic Tumor

There are occasions when resection or biopsy of the primary tumor or metastases may not be an option. Although not preferred, HER2 testing performed on the cell block of an FNA can be considered as an alternative. Bozzetti et al[37] compared metastatic FNA specimens and noted *HER2* amplification in 21% of specimens from the metastatic lesions sampled by histology and in 9% of cytology specimens. This difference was not ascribed to a bias of cytology given that FISH results were entirely concordant with those obtained on the histologic specimens of the corresponding primary tumors. It is likely that the discrepancy observed between the HER2-positive cases on cytology and on histology may be related to the small sample size.

Others[39] have assessed HER2 status on specimens obtained from malignant effusions by using both IHC and SISH. Cell blocks from 46 effusions obtained from patients with metastatic gastric carcinoma were examined. Immunohistochemistry was scored with the modified criteria of Hofmann et al.[13] Results were compared with histologic specimens to assess HER2 status concordance. Seven (15%) showed an IHC 2+/3+ reaction with a membranous pattern. Three (7%) showed *HER2* amplification on SISH. In 18 cases (39%), HER2 status was compared with histologic specimens, showing 100% concordance. The incidence of HER2 positivity (7% with SISH+ and IHC 2+/3+) was lower than reported in histologic samples.

Given the issue of intratumoral heterogeneity in GEA specimens, testing of multiple biopsy fragments (from a primary or metastatic site), or from the resected primary tumor, is preferred.[13,40] If this is not an option, testing a cytology specimen from an FNA cell block is acceptable. However, the specimens obtained in cytology specimens may not be truly representative given the limited sampling of the tumor. For biopsy specimens, current recommendations state that, when possible, a minimum of 5 biopsy specimens[41] and optimally, 6 to 8, should be obtained to account for intratumoral heterogeneity and to provide sufficient tumor specimens for diagnosis and biomarker testing, and this is also recommended by the NCCN Guidelines.[8,11,42] As well, if there is concern about the adequacy of the specimen, it is recommended that additional available primary or metastatic GEA tumor tissue be tested.

3. Strong Recommendation.—Treating clinicians should offer combination chemotherapy and HER2-targeted therapy as the initial treatment for appropriate patients with HER2-positive tumors who have advanced GEA.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

HER2-targeted therapy was established in 2010 as a new standard of care for the first-line treatment of patients with advanced GEA with HER2-positive tumors. The results of the ToGA trial (efficacy and safety) have been described above.[3] In addition, health-related quality of life (HRQoL) and quality-adjusted time without symptoms of disease or toxicity (Q-TWiST) were improved for patients who received trastuzumab, with a prolonged time to 10% definitive deterioration in all quality of life questionnaire (QLQ)–C30 and QLQ-STO22 scores and extended Q-TWiST by 2.42 months, compared with chemotherapy alone.[43] Thus, trastuzumab achieved a level-1 evidence for overall survival advantage in patients with HER2-positive advanced GEA in the first-line setting.[11,44]

In 2010, the US Food and Drug Administration (FDA) and European Medicines Agency approved trastuzumab in combination with cisplatin and a fluoropyrimidine (5-fluorouracil or capecitabine) for use in patients with *HER2*-positive GEAs. NCCN Guidelines, however, recommend the addition of trastuzumab to any active chemotherapy combination. In addition to the ToGA trial, smaller trials combining trastuzumab with weekly paclitaxel (in trastuzumab-naive patients) or capecitabine and oxaliplatin have documented some efficacy, but these results are supportive and not definitive.[45] When adding trastuzumab to a biweekly regimen (eg, oxaliplatin and a fluoropyrimidine), the loading dose should be 8 mg/kg and then 4 mg/kg every 2 weeks.

With the establishment of HER2 testing as a standard of care for patients with advanced GEA, it is important to note that the treating clinician should not offer HER2-targeted therapy until HER2 positivity is confirmed. Since patients with advanced GEA can be symptomatic, it is recommended to start combination cytotoxic therapy as soon as feasible while waiting for the establishment of HER2 status. This statement is based on expert opinion and based on the fact that in the ToGA trial, of 810 patients with HER2-positive tumors, 216 became ineligible (mainly due to deterioration of performance status) while waiting for HER2 test results. Once it is determined that GEA is HER2 positive, trastuzumab can be added to the chemotherapy combination. There is no documented benefit for starting HER2-

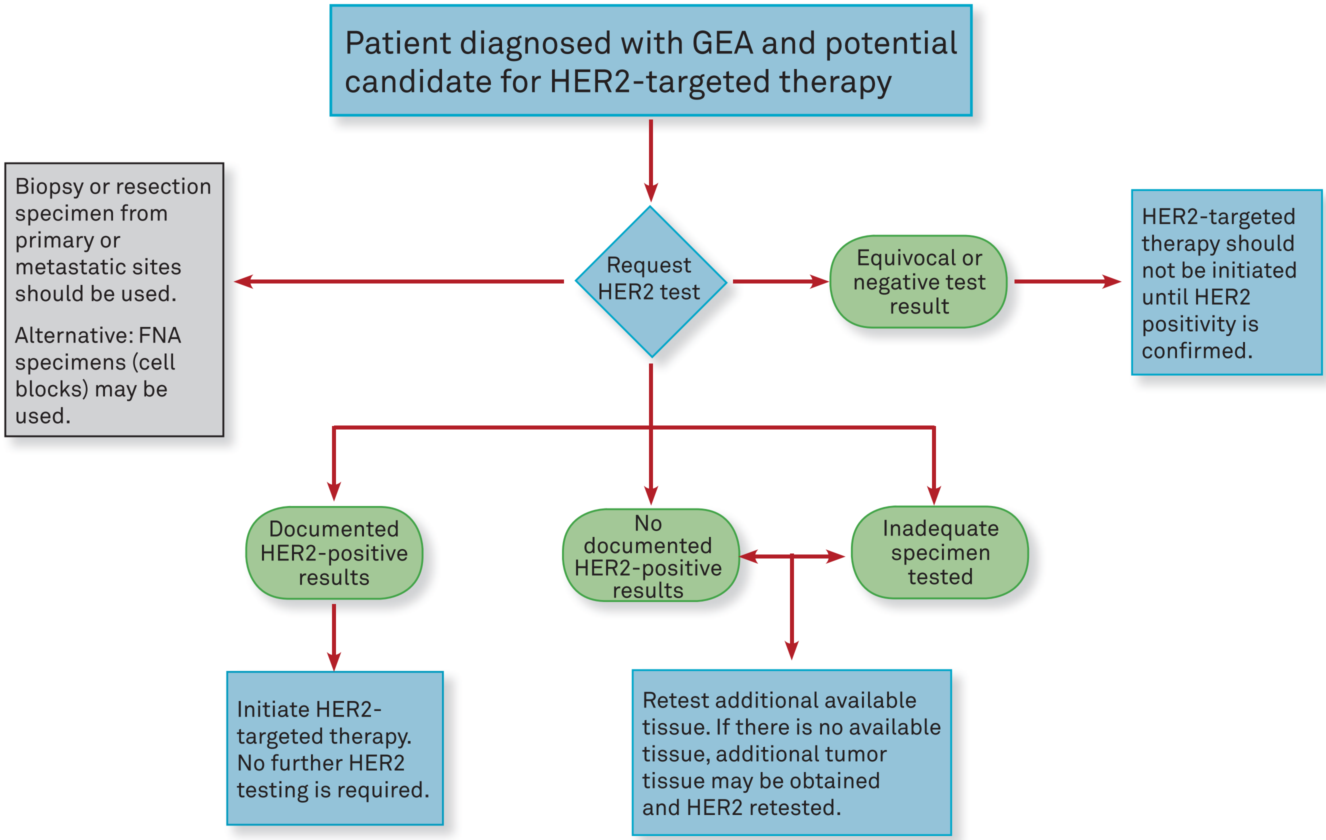

Figure 1. *Algorithm for clinicians. Abbreviations: FNA, fine-needle aspiration; GEA, gastroesophageal adenocarcinoma; HER2, human epidermal growth factor receptor 2.*

directed treatment in the absence of confirmed HER2 positivity, and there is an added potential for the patient to incur unnecessary side effects or costs. It is also recommended that if there is documentation of a HER2-positive result in any specimen (primary or metastatic tumor), the treating clinician does not need to request additional HER2 testing on additional tumor specimens. Conversely, if there is no documentation of a HER2-positive result, and there is no available tumor tissue, an attempt should be made to collect additional neoplastic tissue (primary or metastatic) for HER2 testing. In addition, there is currently no evidence to support repeating HER2 testing after evidence of progression following HER2-directed therapy (trastuzumab) combined with cytotoxic combination, and there is no evidence to support continuation of trastuzumab beyond progression in patients with GEA. In this regard, the TyTAN trial randomly assigned 262 patients with advanced *HER2*-amplified gastric adenocarcinoma, in the second-line setting, to lapatinib plus paclitaxel or paclitaxel alone and reported no advantage in overall survival for patients randomly assigned to lapatinib.[46] Additionally, the LOGiC trial that randomly assigned 545 patients with *HER2*-amplified advanced GEA to lapatinib or placebo plus capecitabine and oxaliplatin, in the first-line setting, demonstrated no overall survival advantage for patients who received lapatinib over those who received placebo.[47] Therefore, the efficacy of HER2-directed therapy is demonstrated by trastuzumab only restricted to the first-line setting. An algorithm for clinicians for HER2 testing in patients with GEA is presented in Figure 1.

In summary, randomized clinical data support the use of HER2-targeted therapy in combination with chemotherapy for patients who are fit and able to tolerate treatment. Addition of HER2-targeted therapy in patients with HER2-positive GEA results in improved survival and quality of life. Trastuzumab provides modest overall survival benefit for patients with HER2-positive advanced GEA in the first-line setting in combination with active cytotoxics.

4. Strong recommendation.—Laboratories/pathologists must specify the antibodies and probes used for the test and ensure that assays are appropriately validated for HER2 IHC and ISH on GEA specimens.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

Multiple antibodies are available for HER2 IHC (including but not limited to Ventana 4B5 [Tucson, Arizona], Thermo Fisher Scientific CB11 [Waltham, Massachusetts], Sigma-Aldrich SP3 [St Louis, Missouri], Dako AO485 and Dako HercepTest [Glostrup, Denmark]). Ventana 4B5, Thermo Fisher Scientific CB11, and Dako HercepTest are FDA approved.[48] The ToGA trial[3] used the HercepTest antibody, and many studies[30,49–53] have used 4B5 or CB11. There is generally moderate to good concordance between various antibodies,[54–58] though several articles note stronger staining for 4B5 than for other antibodies. However, no recommendation is made for the use of a specific antibody.

Likewise, multiple methods for ISH have been evaluated for *HER2* in GEA. The Dako pharmaDx (Glostrup, Denmark) *HER2* FISH kit was used for the ToGA trial,[3] and there is considerable experience with FISH in testing for

HER2 amplification in breast carcinomas. Development of brightfield ISH technologies has resulted in several other ISH methods, and 1 kit has obtained FDA approval (Dako *HER2* FISH pharmDx). One of these methods is SISH, where either 1 *HER2* slide, or 2 separate slides, are stained for *HER2* and chromosome enumeration probe (CEP) 17, both using silver as the chromogen. The other major brightfield ISH methods are chromogenic in situ hybridization and dual in situ hybridization, where either a nonsilver chromogen alone is used or is used in combination with a silver chromogen on a separate probe to mark both *HER2* and CEP17 on 1 slide. The authors of multiple studies[52,59–68] agree that these various ISH methods are comparable and effective for GEA *HER2* testing. There have been suggestions that brightfield ISH techniques have some advantages over FISH in that they can often be performed on automated stainers, do not require fluorescence microscopes, and allow for easier identification of tumor nuclei among normal tissues.[61,63] However, no recommendation is made regarding the use of any specific ISH method, as there is no major diagnostic advantage to one method over another.

While no recommendation regarding which specific antibody/ISH methodology is given, there is a strong recommendation regarding validation. If using a method other than the FDA-approved kit, pathologists and laboratories should carefully validate both IHC and ISH for HER2, and validation should be performed in the laboratory in which the assay will be used. The cases used for validation should be predominantly GEA cases as opposed to other tumors (ie, breast carcinomas) to allow those scoring to develop and maintain expertise with the different GEA tumor types and appearances. CAP and/or Clinical Laboratory Standards Institute guidelines should be followed for assay validation.[69–72]

The method of sampling for the validation specimens (ie, from resections or biopsies) should be similar to those expected in future sampling, and should use the same fixative. The CAP Laboratory Accreditation Program (ANP.22978) for HER2 validation for breast carcinomas proposes validation using 20 positive and 20 negative specimens for an FDA-approved test, and 40 positive/40 negative cases if the test is a laboratory-developed test.[73] If using a brightfield ISH assay kit, initial validation should be done by comparison to an FDA-approved FISH assay.[17] Records of validation must be maintained as per the CAP Laboratory Accreditation Program (ANP. 22750, ANP.22978, and ANP.22956).[73] Laboratories must also maintain good quality control. When reporting results, the final HER2 test reports should specify the antibody used for IHC and/or the probe used for ISH along with a brief description of the kit/methodology.

5. Strong Recommendation.—When GEA HER2 status is being evaluated, laboratories/pathologists should perform/order IHC testing first followed by ISH when IHC result is 2+ (equivocal). Positive (3+) or negative (0 or 1+) HER2 IHC results do not require further ISH testing.

(Quality of evidence: High; Strength of recommendation: Strong)

The ToGA trial demonstrated that the combination of trastuzumab plus chemotherapy significantly improved survival in patients with tumors showing high HER2 expression.[3] The latter was defined as HER2 score 3+ by IHC or HER2 score 2+ by IHC and *HER2* positivity (amplified) by FISH. *HER2*-positive results by FISH were observed in 11% of cases with IHC score 0 and 12% of cases with IHC score 1+.[3] Similarly, other studies[29,74–77] have shown *HER2* positivity by ISH in up to 14% to 24% of tumors with IHC scores of 0 or 1+. These patients did not significantly benefit from the addition of trastuzumab to the chemotherapy regimen in the ToGA trial.[3] Similar findings were reported in subsequent studies and reviews, demonstrating that ISH positivity alone does not correlate with response to trastuzumab therapy in GEA.[8,78]

NCCN Guidelines recommend that specimens with 2+ expression of HER2 by IHC should be additionally assessed by FISH or other ISH method. Specimens with 3+ overexpression by IHC or FISH positivity (*HER2*: CEP17 ratio $\geq$ 2) are considered positive.[11] Specimens having an IHC score of 0 or 1+ are considered negative and do not warrant further testing. The concordance between IHC 3+ and ISH positivity was high, with 94% concordance in the ToGA trial and 62% to 100% in the literature, with most reporting concordance of 90% or higher.[3,8,29,74–78] Since the benefit from the addition of HER2-directed therapy correlates with HER2 protein expression, initial HER2 testing should be performed by IHC. In situ hybridization should be reserved for IHC 2+ cases. In many studies,[8,29,74,76,77] ISH-positive results have been observed in 30% to 50% of IHC 2+ tumors. Of note, there can be interobserver variation in the interpretation of HER2 IHC, and the reproducibility of 1+ and 2+ scores can be low. If the IHC score is borderline and the distinction between 1+ and 2+ is challenging, *HER2* ISH can be considered. However, this approach is not recommended for cases that show an obvious 1+ IHC score. In most cases, ISH is not indicated if IHC scores are 0, 1+, or 3+. An algorithm for pathologists for HER2 testing in patients with GEA is presented in Figure 2.

6. Strong Recommendation.—Pathologists should use the Ruschoff/Hofmann method in scoring HER2 IHC and ISH results for GEA.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

The scoring system (Table 4) used in the ToGA trial and subsequently modified for biopsies has been used in many studies and has shown excellent correlation between IHC and gene amplification methods.[13,42] The IHC is subjectively scored by using the established criteria on a 4-tiered scale as 0, 1+, 2+, and 3+, with scores of 0 and 1+ considered negative, 3+ as positive, and 2+ as equivocal. Representative examples of IHC in GEA specimens are shown in Figure 3, A through D. Similar to breast cancer, only membranous staining, but not cytoplasmic staining, is considered for HER2 scoring, but unlike breast carcinoma, complete membranous staining is not required for positivity. Often the luminal surface of tumor cells fails to stain in *HER2*-amplified GEA. Only luminal surface staining in the absence of lateral and basal staining is considered negative. Assessment of IHC as weak, moderate, or strong for scoring is similar to that used for breast carcinoma and is subjective and thus can be a source of intraobserver and interobserver variability. Scoring using automated image analysis or virtual microscopy can be objective but has not been shown to improve reproducibility.[79] A few studies show good concordance of image analysis with a visual method for scoring HER2 IHC results; however, at this time there are limited data to make a specific recommendation for or against using image analysis for scoring HER2 in clinical practice.[34,79,80]

Various in situ visualization techniques used to evaluate *HER2* amplification include FISH and brightfield ISH using

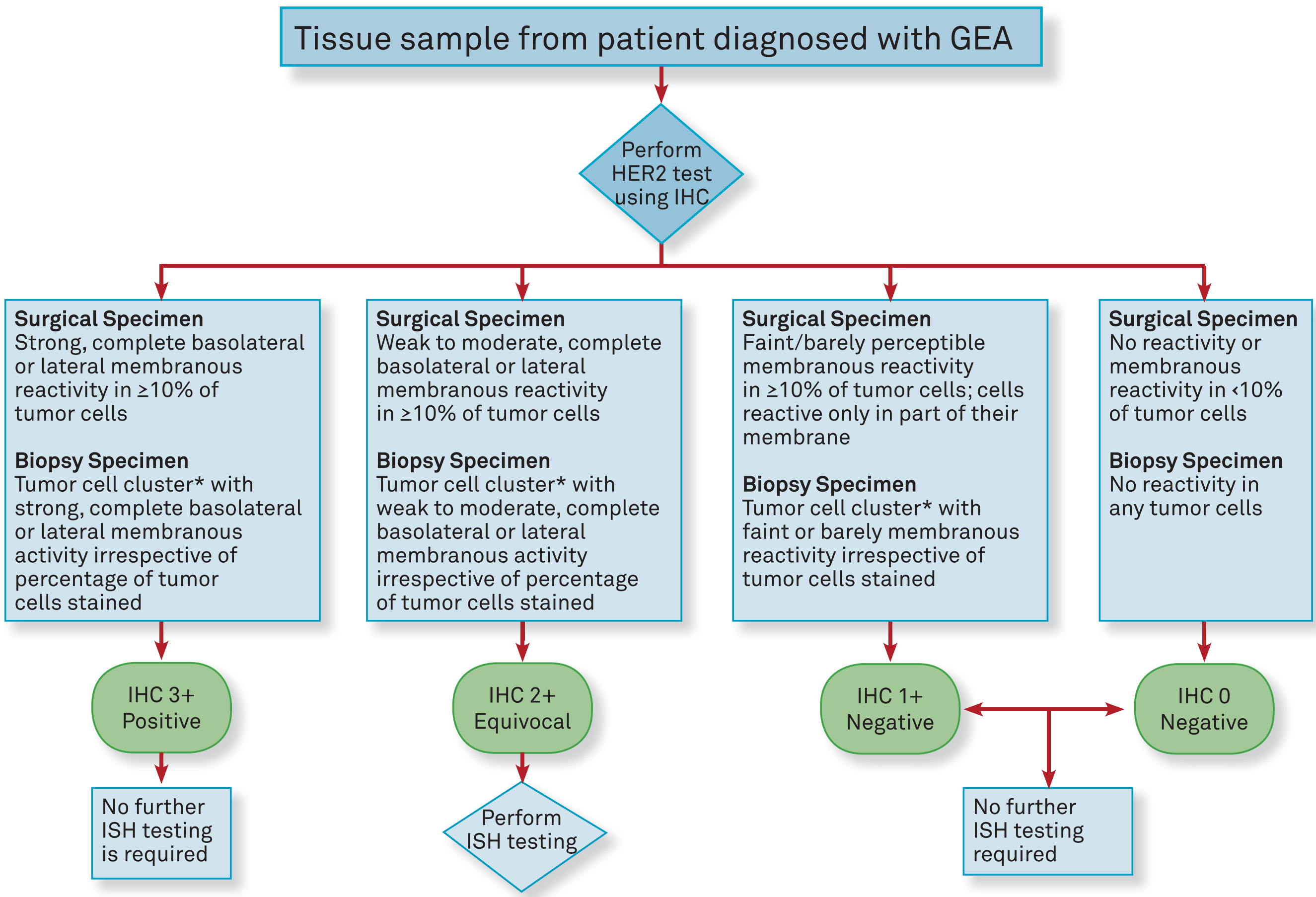

Figure 2. *Algorithm for pathologists. *Tumor cell cluster is defined as a cluster of 5 or more tumor cells. Additional recommendations: Pathologists should ensure that biopsy or resection specimens used for HER2 testing are rapidly placed in fixative, ideally within 1 hour (cold ischemic time) and are fixed in 10% neutral buffered formalin for 6 to 72 hours. Routine histology processing and HER2 testing should be performed according to analytically validated protocols. Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by IHC in the GEA specimen for subsequent scoring when ISH is required. Abbreviations: GEA, gastroesophageal adenocarcinoma; IHC, immunohistochemistry; ISH, in situ hybridization.*

Table 4. Scoring Guidelines for Interpretation of HER2 IHC in Gastric Carcinoma[a]

Surgical Specimen–Staining Pattern	Biopsy Specimen–Staining Pattern	Score	HER2 Expression Assessment
No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	0	Negative
Faint/barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster[b] with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	1+	Negative
Weak to moderate, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster[b] with a weak to moderate, complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	2+	Equivocal
Strong, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster[b] with a strong, complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	3+	Positive

Abbreviation: IHC, immunohistochemistry.

[a] Reprinted with permission from Hofmann et al.[13]
[b] Tumor cell cluster (≥5 neoplastic cells).

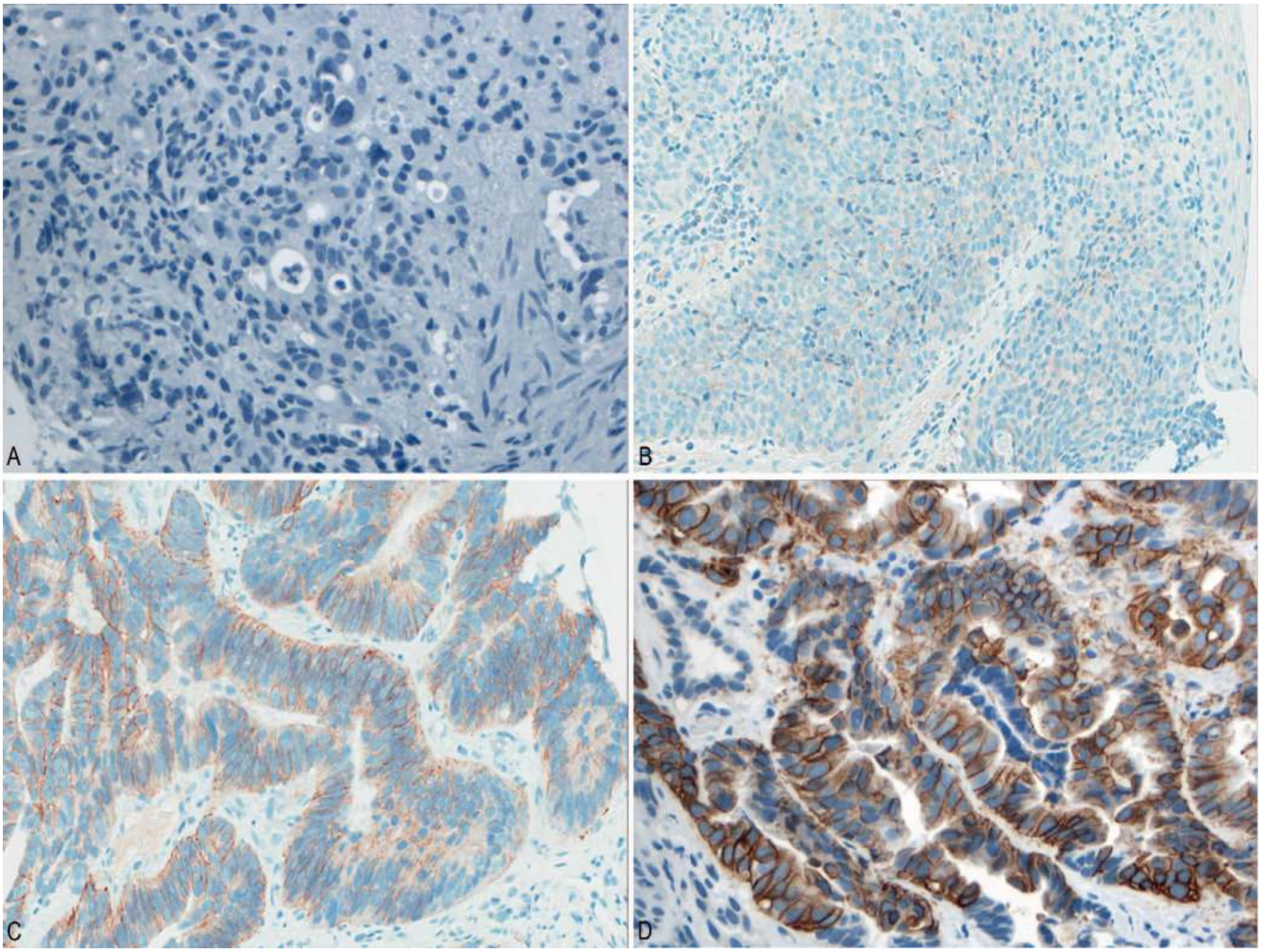

Figure 3. *HER2 immunochemistry showing representative cases for scoring. A, Negative 0: no reactivity, specifically no membranous reactivity is seen in any of the tumor cells. Any cytoplasmic staining is disregarded for scoring purposes. B, Negative 1+: tumor cells with faint/barely perceptible membranous staining. C, Equivocal 2+: tumor cells with weak to moderate, complete, basolateral and lateral membranous staining. Columnar cells that are sectioned tangentially tend to show a complete membranous staining pattern. D, Positive 3+: tumor cells with a strong, complete, basolateral and lateral membranous reactivity. Also note that cells showing a complete membranous staining pattern are often tangentially sectioned columnar cells (HER2, original magnifications ×40 [A, C, and D] and ×20 [B]).*

either *HER2* probe or dual *HER2* and centromere (CEP17) probes, and all are acceptable strategies. At least 20 nonoverlapping nuclei of tumor cells are evaluated for *HER2* probe and CEP17 probe signal enumeration (Figure 4, A through C). A ratio of *HER2* signal to CEP17 signal of 2.0 or greater is considered positive and a ratio of *HER2* signal to CEP17 signal below 2.0 is considered negative. To score ISH/FISH results, the scorer first scans the stained slide in all areas designated as invasive tumor to identify areas with higher level *HER2* amplification. In these areas, score both amplified and adjacent nonamplified cells that have cytomorphology consistent with malignant cells. Proceed to score in areas marked as strongest IHC intensity, if this information is available, since areas of overexpression may signify gene amplification in heterogeneous tumors.[8,42,81–83] Proceed to other invasive tumor areas until at least 20 cells are scored. Extra (3 or more) copies of CEP17, on average, were noted in 4.1% of gastric cancers in the ToGA trial. This phenomenon has been referred to as "polysomy" but technically is not polysomy in many cases, since the entire chromosome is not duplicated. Rather, the extra copies of CEP17 are due to an intrachromosomal segmental duplication overlapping the centromere of chromosome 17, typically also involving the *HER2* gene.[84] In such cases, there are often 4 to 6 copies of both *HER2* and CEP17 signals with a ratio below 2.0.

If IHC is 2+ and there are 3 or more CEP17 signals, on average, with a ratio below 2, then presence of more than 6 *HER2* signals, on average, is interpreted as positive for *HER2* amplification by ISH/FISH; fewer than 4 *HER2* signals, on average, is interpreted as negative for *HER2* amplification; and 4 to 6 signals, on average, indicates another 20 cells should be scored in a different target area. If additional scoring does not allow a definitive result to be rendered, then multiple options are feasible: (1) consultation between scorer and pathologist regarding selection of malignant cells or tumor areas for scoring; (2) switching out CEP17 for an alternative chromosome 17 probe in a retest to calculate the ratio with a new probe; (3) selecting a different tumor block for HER2 testing; (4) using genomics or an alternative analytic method to evaluate *HER2* amplification.

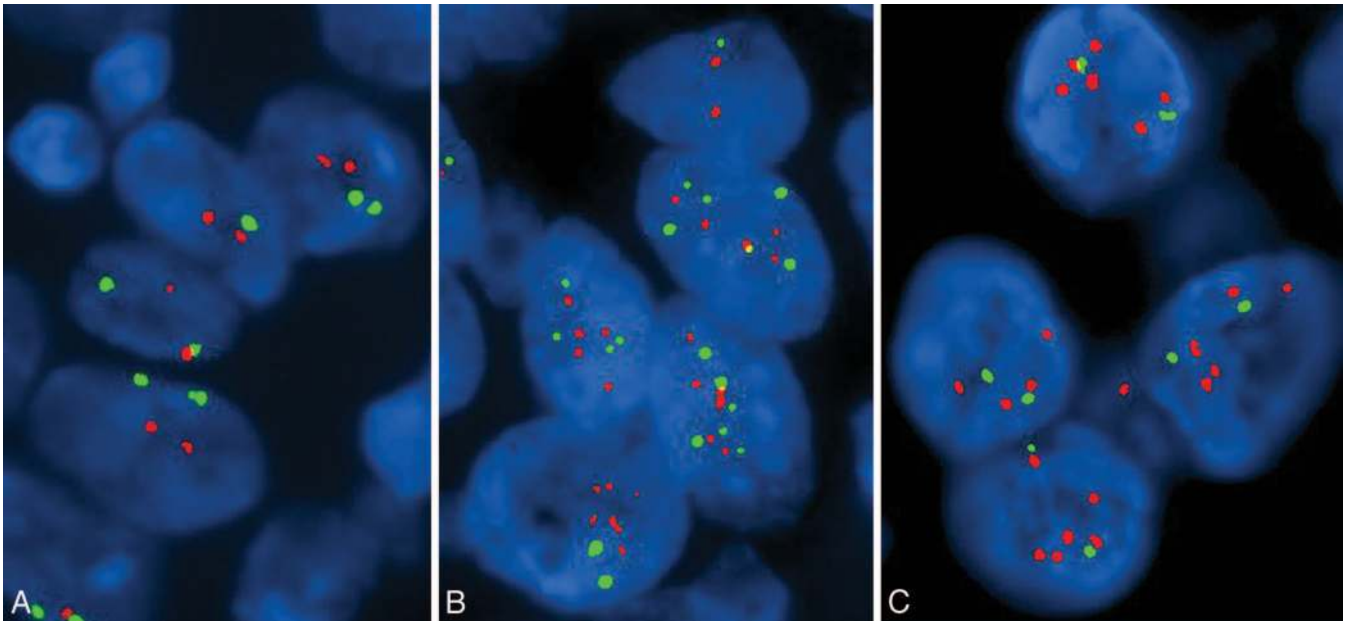

Figure 4. HER2 *and CEP17 FISH show scores of representative cases. A, Not amplified: ratio 1.0. Mean number of* HER2 *signals per cell is 1.9; mean number of CEP17 signals per cell is 1.8. B, Not amplified: ratio 1.3. Mean number of* HER2 *signals per cell is 3.4; mean number of CEP17 signals per cell is 2.7. Segmental duplication (or polysomy) likely accounts for signal numbers greater than 2 per cell. C, Amplified: ratio 3.0. Mean number of* HER2 *signals per cell is 5.2; mean number of CEP17 signals per cell is 1.7. Abbreviations: CEP, chromosome enumeration probe; FISH, fluorescence in situ hybridization.*

Of note, there are currently no definitive studies in the literature on interpreting monosomy of CEP17 in GEA. Furthermore, true monosomy for CEP17 is difficult to distinguish from truncated cells in thin sections, and there are no data on how to interpret CEP17 monosomy even if it were confirmed by orthogonal methods. Until further data are available, relying on the ratio of *HER2* to CEP17 signals remains a reasonable strategy for analyzing ISH/FISH results.

Each testing laboratory should specify the section thickness required for *HER2* ISH/FISH analysis. Section thickness is especially important for single-probe assays in which absolute counts per cell determine scoring, and is a major reason why single-probe ISH methods are not recommended. In contrast, dual-probe assays are recommended because they rely on a ratio of *HER2* to CEP17 signals, which are less affected by section thickness. We recommend 4-μm-thick paraffin sections unless validation studies demonstrate accurate results when using alternative specimen preparation, or if an FDA-approved kit specifies that another thickness be used. Thinner sections can yield greater sampling error, fewer cells that qualify for scoring by virtue of having at least 1 signal for each of the 2 probes, and less intense counterstain. Thicker sections can lead to the presence of overlapping nuclei and more difficulty with deparaffinization, protease digestion, and probe or detection reagent dispersion processes.

The exogenous control slide should be scored to ensure that the assay protocol performed as expected. In each patient specimen, ensure adequate staining and counterstaining without background interference, overdigestion, or other artifacts. Failure to detect probe signals in nonmalignant cells (fibroblasts, endothelial cells, inflammatory cells, benign epithelial cells) serves as an indicator of poor-quality hybridization. At least some of these nonmalignant cells are expected to have up to 2 copies per cell of *HER2* and CEP17 discrete signals serving as a quality check for DNA preservation, reagent perfusion, and sufficient signal to noise ratio. In malignant-appearing cells, discrete signals are enumerated, or an estimate of signal number is done when there are numerous overlapping signals (clusters). Correlation of the scored region(s) on the ISH slide with the tumor cell population marked on the IHC slide is essential to ensure that the scored cell population is tumor. In cases where it is difficult to demarcate the tumor cell population on the slide, direct pathologist review of the ISH slide and comparison with the morphology of the tumor on the IHC and hematoxylin-eosin–stained section is often necessary.

Interpret the *HER2* test result as indeterminate if technical issues prevent reporting as positive or negative. Examples of technical failures include improper specimen preparation or handling, quality checks outside acceptable limits, or artifact interfering with analysis or microscopy. Several manufacturers market reagents for *HER2* ISH, but as of the date of this publication only 1 manufacturer has FDA approval for GEA (Dako, eg, *HER2* IQFISH pharmDx). The *HER2* FISH pharmDx test used in the ToGA trial is no longer available. Manufacturer's instructions are often helpful for guiding analysis and interpretation of results. In the practice-changing ToGA trial, Hofmann et al[13] recommended modifications to the duration of pepsin and that temperature stability should be achieved during pretreatment.

7. Recommendation.—Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than 1 tissue block may be selected if different morphologic patterns are present.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Recommendation/Moderate)

As mentioned previously, studies show that HER2 overexpression is strongly associated with intestinal phenotype, and less frequently with diffuse (signet ring cell)

Table 5. Key Reporting Elements[a]

HER2 by immunohistochemistry result
___ Negative (score 0)
___ Negative (score 1+)
___ Equivocal (score 2+)
___ Positive (score 3+)
___ Indeterminate (explain): ________________________

HER2 (*ERBB2*) by in situ hybridization result
___ Negative (not amplified)
___ Positive (amplified)
___ Indeterminate (explain): ________________________

Number of cells counted: ________________________
___ Using dual-probe assay
HER2 (*ERBB2*) to CEP17 ratio: _______________
Average number of *HER2* (*ERBB2*) signals per cell: ________________________
Range of number of *HER2* (*ERBB2*) signals per cell: ________________________
___ Using single-probe assay
Average number of *HER2* (*ERBB2*) signals per cell: ________________________
Range of number of *HER2* (*ERBB2*) signals per cell: ________________________

HER2 (*ERBB2*) genomic test (specify findings, eg, gene amplification, nucleotide sequence of specific mutation[s])
___ Negative
___ Positive
___ Indeterminate (explain): ________________________

METHODS

HER2 protein expression by immunohistochemistry
___ FDA cleared (specify test/vendor): __________________
___ Laboratory-developed test

Specify primary antibody
___ 4B5
___ HercepTest
___ A0485
___ SP3
___ CB11
___ Other (specify): ____________________________

HER2 (*ERBB2*) gene amplification by in situ hybridization
___ FDA cleared (specify test/vendor): __________________
___ Laboratory-developed test (specify FISH or ISH, probes, major instrument): ____________________________
Number of observers: ____________________________

HER2 (*ERBB2*) genomic test for amplification or mutation
Laboratory-developed test method: ____________________

Abbreviations: CEP, chromosome enumeration probe; FDA, US Food and Drug Administration; FISH, fluorescence in situ hybridization; ISH, in situ hybridization.

[a] Reprinted from Bartley et al[92] with permission from *Archives of Pathology & Laboratory Medicine*. Copyright 2015 College of American Pathologists.

phenotype of GEA. The rates of HER2 positivity vary for intestinal (3%–23.5%), diffuse (0%–6%), and mixed histology (0%–20%) cancers.[36,85–88] Gastroesophageal adenocarcinoma has rare morphologic phenotypes that include adenosquamous, papillary, and neuroendocrine carcinomas,[74] but data regarding HER2 expression in such morphologic variants are limited. Most studies[8–10,57,86] have shown anatomic variation with HER2 expression/amplification being greater at the gastroesophageal junction than in the stomach (32.2% versus 21.4%). Correlation of HER2 expression and/or amplification with histologic grade is difficult to ascertain, as studies have used different methods including 2- to 4-tiered grading systems. Further, most studies do not specify the criteria used for grading, and grading is subjective. The American Joint Committee on Cancer recommends using a 3-tiered system of well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3). Rare undifferentiated carcinomas are classified as G3 in this system. HER2 positivity seems to be more strongly associated with low-grade than high-grade tumors and varies from 15% to 45% for low-grade, and 6% to 28% for high-grade, in different studies.[36,86,89–91] When choosing a tissue block, selecting one with the lower grade or intestinal morphology appears more likely to yield HER2-positive results and is thus recommended. If the cancer comprises substantially different grades or histologic patterns, it is reasonable to test different areas, which may require selection of more than 1 block.

8. Strong Recommendation.—Laboratories should report HER2 test results in GEA specimens in accordance with the CAP "Template for Reporting Results of *HER2 (ERBB2)* Biomarker Testing of Specimens From Patients With Adenocarcinoma of the Stomach or Esophagogastric Junction."[92]

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

The synoptic content of this template lists essential reporting elements. Element selection and the manner in which these elements are reported are at the discretion of the medical professional who issues the report. Key elements are listed in Table 5. The report should include a brief Methods section describing the kit or the critical reagents and instruments used. For a gene test, include the correct gene symbol (*ERBB2)* as approved by the Human Genome Organization Nomenclature Committee, following the colloquial symbol (*HER2* [*ERBB2*]). The "number of observers" refers to the number of laboratory professionals who performed scoring for ISH or the number who further interpreted the results of any automated scoring system. The reporting professional is responsible for assuring quality of the result via analytic interpretation of raw data and via use of validated protocols for preanalytic and analytic phases of testing.[93,94] Published guidance from the CAP describes general report elements promoting accurate communication of test results.[95,96]

9. Strong recommendation.—Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by IHC in GEA specimens for subsequent ISH scoring when required.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

This recommendation is intended to provide guidance on which parts of the slide to prioritize when scoring cells in ISH assays. Accurate ISH results scoring depends on 3 aspects of preanalytic histopathologic features that help localize regions to score: (1) areas of invasive tumor, (2) areas of intense HER2 overexpression as visualized on IHC, and (3) cytomorphology of the malignancy to help select individual cells for scoring. Areas of invasive carcinoma are identified on hematoxylin-eosin–stained sections adjacent to the unstained section used for hybridization. If there are distinct and separate histologic patterns of malignancy, different areas can be marked for ISH scoring, although there are few data to suggest that outcome is improved by separate scoring of each histologic subtype. More important is that invasive cancer is marked so that the scorer may scan these areas to identify regions enriched for amplification to prioritize for scoring.

Heterogeneity typically refers to intratumor variation in genotype or gene expression. In gastric cancers, this term is used when there is focal positivity by IHC or ISH. Ideally, a HER2 IHC stain of the same block used for ISH should be reviewed to find areas of maximum HER2 intensity irrespective of histologic subtype or grade. False positivity can be seen in areas of intestinal metaplasia, adjacent to ulcer sites, or in high-grade dysplasia, and these lesions should be avoided. Crush artifact and necrotic tissue also should be avoided. Areas with strongest IHC intensity may signify gene amplification in heterogeneous tumors.[8,42,81–83]

Good communication between the histopathologist and the scorer is critical for resolving difficult interpretations. If the proportion of malignant cells (as a proportion of all nucleated cells in the marked area) is low, the pathologist should communicate this to the ISH laboratory and mention this in the report, since low tumor cell content reduces confidence in the ISH results. The pathologist should also note the pattern of malignant cells (glands versus diffuse, sheets of tumor cells versus interspersed benign inflammatory/stromal cells) and the shape and relative size of the malignant cell nuclei (round versus oval, medium versus large) to assist scorers in identifying those malignant cells after hybridization and counterstaining. The goal is to maximize the proportion of malignant cells scored, while minimizing the proportion of nonmalignant cells scored. Morphologic evaluation of ISH stains helps resolve problematic interpretations due to overfixation or underfixation, delayed fixation with or without tissue-drying artifacts, inadequate deparaffinization, or predicting the value of repeating the test using shorter or longer protease digestion duration.

Tissue architecture and cytology are often better visualized in brightfield ISH than FISH, so in brightfield ISH the morphologic features of malignant cells are typically more distinguishable from those of benign cells, potentially improving the signal to noise ratio. However, compared to immunostains, brightfield ISH may suffer from less crisp histopathology because of the protease digestion required to promote probe dispersion into nuclei, and because of the near-boiling heat required to achieve DNA denaturation. The FISH signals are often brighter and easier to count than are brightfield ISH signals.[97] Nevertheless, as stated previously, ISH and FISH results are generally concordant in GEA,[59,61,65,98,99] and either method is considered acceptable.

10. Strong Recommendation.—Laboratories must incorporate GEA HER2 testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to ensure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing GEA HER2 testing should participate in a formal proficiency testing program, if available, or an alternative proficiency assurance activity.

(Quality of evidence: Moderate/Intermediate; Strength of recommendation: Strong)

While a HER2-expressing breast specimen may be initially used as the positive control,[17] validation of actual GEA specimens is preferred, when such appropriate specimens are available. Gastric cancer cell lines with HER2 expression may be used as the positive control[100] when a sufficient number of actual GEA specimens are unavailable, and the procedure should be specified and documented, since it may differ from those of breast. Checklists for recording positive and negative controls for each test should be incorporated into the laboratory quality improvement program (CAP or other available local programs). Given the heterogeneity of HER2 reactivity in GEA,[74,75,85,101–104] laboratories may consider tracking their own statistics of HER2 results in GEA, including interobserver reproducibility between pathologists and the histologic subtypes, which may facilitate a better understating of the relevant issues in HER2 testing in GEA.[105–109] Continuing education of pathologists who report on HER2 GEA specimens is important, especially in laboratories performing limited numbers of GEA specimens in comparison to breast specimens.

11. No Recommendation.—There is insufficient evidence to recommend for or against genomic testing in patients with GEA at this time.

In addition to IHC and ISH, other techniques have been used to determine *HER2* status. These technologies include polymerase chain reaction (PCR), single-nucleotide polymorphism chip, comparative genomic hybridization array, gene expression profiling by RNAseq or microarray, targeted/exome/whole genome sequencing, or proteomics.[84,110] Most studies comparing these technologies to standard *HER2* test methods have been carried out in breast cancer.[111] High concordance has been demonstrated for *HER2* status in GEA with droplet digital PCR, when compared with IHC and FISH.[112] Gene expression profiling using 8 transcripts has been shown to predict response to trastuzumab- and docetaxel-based chemotherapy in GEA with HER2 overexpression.[113]

Multiplex ligation-dependent probe amplification is a multiplex PCR technique that simultaneously quantifies several gene segments. This technique can be used to interpret whether the *HER2* region of the chromosome is amplified compared to control regions of chromosome 17.[114] However, the control regions are difficult to select given that segmental amplifications of chromosome 17, or polysomy 17, may or may not be present in a given tumor. Furthermore, when tissue is ground up to carry out nucleic acid extraction, varying proportions of nucleic acid from malignant and benign cells are represented in the assay, in comparison to IHC and ISH where cytologic and morphologic features may help limit interpretation of malignant cells. Thus, the criteria for interpreting gene amplification are difficult to set when using genomic technology. Ideally, the criteria for tissue selection for analysis, and for interpretation of genomic test results, would be validated with tissues from drug responders versus nonresponders.

At this time, the main utility for genomic testing is to help classify cases that are uninterpretable with standard IHC or ISH technology, such as in the setting of borderline amplification with or without extra centromere 17 signals by ISH.[114] Currently, however, there is insufficient evidence to provide recommendations for or against the routine use of genomic technologies for purposes of qualifying for HER2-targeted therapy.

OTHER GENERAL CONSIDERATIONS

Tissue Fixation and Processing

Pathologists should ensure that biopsy or resection specimens used for HER2 testing are rapidly placed in fixative, ideally within 1 hour (cold ischemic time) and are fixed in 10% neutral buffered formalin for 6 to 72 hours.

Routine histology processing, and HER2 testing, should be performed according to analytically validated protocols, and laboratories should establish policies to ensure efficient allocation and utilization of tissue for ancillary testing, particularly in small specimens. Validation studies must address preanalytic factors supporting the stated range of acceptable tissue preparations (eg, 10% neutral buffered formalin, alcohol fixatives, decalcification, air-dried smears, formalin–post fixation). Laboratories should test a sufficient number of GEA cases to ensure that assays consistently achieve expected results.

Gastroesophageal adenocarcinoma specimens need prompt fixation for ideal histology, IHC, and ISH testing. Biopsy specimens should be immediately placed into formalin in the endoscopy suite. Pathologists should communicate with gastroenterology colleagues to ensure prompt fixation and documentation. Surgical specimens require prompt specimen transport and opening of the specimen (by pathologist or appropriately trained personnel) to ensure prompt exposure of the tumor to adequate volumes of 10% neutral buffered formalin. Surgical specimens may need to first be inked, the tumor incised, and the specimen pinned on a cork or wax board to facilitate fixation. Pathologists should work with surgeons, nurses, and/or operating room personnel to facilitate recording of surgical specimen ischemic time and appropriate handling.[115]

Considerations regarding tissue ischemic and fixation time follow from principles of proteolytic degradation and fixation chemistry,[115,116] with data drawn mostly from the breast cancer literature.[16,17,115,117]

There is a need for direct data regarding the impact of ischemic time (time from specimen removal from the patient to fixation) and fixation time (time tumor is exposed to adequate volumes of formalin) on HER2 testing in GEA. One model using gastric cancer cell lines of known HER2 expression xenografted into mice demonstrated decreased IHC staining with delayed fixation of 6 and 24 hours with the Hercept test, and decreased *HER2* to CEP17 FISH ratios, as compared to immediate fixation.[100] This delayed fixation resulted in negative IHC and FISH interpretation for several samples with expected 2+ IHC staining and *HER2* to CEP17 ratio of 2.3 (SHC cell line). Unfortunately, ischemic intervals between 0 and 6 hours were not tested.[100] This same study demonstrated no effect of prolonged fixation of 5 and 7 days as compared to 24-hour fixation, but noted diminished IHC staining with 10-day fixation or use of fixatives other than 10% neutral buffered formalin.[100]

Full validation of the HER2 testing protocol should be performed for formalin-fixed and paraffin-embedded (FFPE) specimens, as described previously in Recommendation 4. Discussion of limited available data for alternatively fixed or decalcified specimens is provided below.

Regarding cytologic specimens, we are aware of a single small study (mentioned previously) of HER2 testing in gastric cancer effusion specimens (formalin-fixed plasma thrombin clots), which demonstrated concordance with tissue specimens in all of 18 cases, but acknowledged more granularity of HER2 staining, and difficulty in interpreting membrane staining in discohesive tumor preparations.[39] A sampling of studies comparing cytologic cell block preparations with FFPE breast carcinoma specimens evaluated for HER2 by immunohistochemistry demonstrates 87% to 100% positive agreement and 66% to 100% negative agreement (excluding 2+ equivocal scores)[39,118–124]; however, one small study exploring ethanol, cytolyte, and formalin-fixed cytologic breast cancer specimens is calculated to have only 14% to 40% positive agreement and 100% negative agreement with matched FFPE breast tissue samples (again excluding 2+ scores).[125] Several studies[39,120,122] reported false-positive interpretations, some attributed to cytoplasmic background staining.

There are wide differences in the handling and processing of cytologic preparations between studies and between laboratories (eg, proprietary fixative, alcohol-based fixative, alternative fixative followed by formalin fixation, direct formalin fixation),[39,118–122,125,126] and effects vary by antigen/antibody.[127] This further emphasizes the need for appropriate evaluation of HER2 staining of cytologic specimens in individual laboratories before testing and reporting patient samples. Nonformalin fixatives also have complexities for *HER2* ISH testing,[122,128,129] yet several studies[123,124,130] have shown good results with *HER2* FISH on cytologic breast cancer specimens.

Diminished IHC staining occurs after decalcification with a variety of antigen-antibody combinations,[127,131] yet studies of HER2 antibodies are lacking. Prolonged hydrochloric acid–based decalcification after formalin fixation was shown to have deleterious effects on the *HER2* ISH assay in a breast tumor and xenograft study.[129] Again, decalcification protocols vary widely among laboratories, reinforcing the need for local assay evaluation. While it remains impractical to fully validate every specimen variation (cytology, decalcification), laboratories should confirm test performance of HER2 assays on these types of specimens before reporting patient results (with testing paradigm to be determined by the laboratory director, based on local practices).

Turnaround Time

Laboratories must provide clinically appropriate turnaround times and optimal utilization of tissue specimens by using appropriate techniques (IHC and ISH) for HER2 in GEA. To inform therapeutic decision making, HER2 results should be reported promptly. The panel recommends a benchmark of 90% of reports available within 10 working days from the date of procedure or specimen acquisition. Laboratories that require send out of tests for HER2 testing in GEA should process and send specimens to reference laboratories in a timely manner. The panel suggests that a benchmark of 90% of specimens be sent to the reference laboratory within 3 working days of tissue processing.

CONCLUSIONS

Gastroesophageal adenocarcinoma continues to be a major health care burden throughout the world. Advanced GEA that is not amenable to effective local therapy remains incurable and patients have limited therapeutic options. Other than *HER2*, there is no biomarker available for selection of therapy for patients with advanced GEA. Trastuzumab is the only approved HER2-directed therapy that has resulted in modest but statistically significant prolongation of overall survival of patients with HER2-positive GEA.

Given the potential impact of HER2 status on therapeutic decision making in GEA, clear guidance is needed for medical oncologists and pathologists in testing for, and interpretation of, HER2 status. A guideline specific for GEA was needed because, although a comparable guideline exists for assessment of HER2 in breast cancer, the pattern of HER2 protein overexpression and/or gene amplification in GEA is distinctly different. Because of considerable heterogeneity of HER2 protein and gene expression in GEAs, scoring methodology for GEA is different than for breast cancer. To develop this evidence-based guideline for HER2 testing, the CAP, ASCP, and ASCO convened a multidisciplinary panel with broad expertise in the clinical and pathologic aspects of GEA. The panel developed a set of 11 recommendations that are pertinent to various aspects of establishing HER2 status. The guideline provides evidence-based recommendations for specimen identification, processing, testing methodology for IHC and ISH, interpretation of results, and the potential for clinical implementation.

The guideline recommends that HER2 status should be established in all patients with advanced GEA who are eligible for systemic (and especially HER2-directed) therapy. Tumor specimens from primary or metastatic GEA may be used for assessment. Testing should begin with IHC. If the result is negative (0 or 1+) or positive (3+), no further testing is required. If the result is equivocal (2+) by IHC, subsequent testing by ISH should be performed to determine amplification status. Patients whose tumor is considered HER2 positive (IHC 3+ or IHC 2+ and ISH positive/amplified) should be informed of the results, and HER2-directed therapy should be offered along with combination chemotherapy. Although the guideline recommends that HER2 status should be assessed in all patients with advanced GEA, it is acknowledged that some patients are not candidates for systemic therapy owing to poor general condition and poor performance status. In such patients, HER2 testing is not required. There are other circumstances where the HER2 status in a given patient is unclear owing to technical aspects (inadequate tumor or inability to adequately interpret the processed specimen) on a prior attempt. In these circumstances, collection of an additional tumor specimen is recommended but only when there are no major safety concerns associated with such a procedure.

Finally, as the fields of genomics, proteomics, and biotechnology continue to evolve, novel and more accurate methods of assessing HER2 status may become available. Similarly, as more clinical trials are conducted on HER2-directed therapy in GEA, changes in treatment algorithms may necessitate updates to these recommendations in the future.

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APPENDIX. Disclosed Interests and Activities January 2015–June 2016[a,b]

Name	Interest/Activity Type	Entity
Jaffer A. Ajani, MD	Consultancies	Lilly
		Amgen
		Celgene
	Grants	BMS
		Novartis
		Merck
		Roche
		Genentech
	Leadership in other associations	National Comprehensive Cancer Network Gastric and Esophageal Cancers Guideline Panel
		International Society of Gastrointestinal Oncology
Al B. Benson III, MD	Consultancies	Roche
		Merck
		Janssen
		Bayer
		Eli Lilly
	Leadership in other associations	Patient Advocate Foundation
		Association of Community Cancer Centers
		Debbie's Dream Foundation
		International Society of Gastrointestinal Oncology
		Eastern Cooperative Oncology Group and the American College of Radiology Imaging Network
Alfredo Carrato, MD, PhD	Consultancies	Roche
		Lilly
		Bayer
		Merck
		Janssen
Margaret L. Gulley, MD	Grants	Illumina
	Leadership in other associations	Association for Molecular Pathology
		The Cancer Genome Atlas
		Alliance for Clinical Trials in Oncology
Megan Troxell, MD, PhD	Speaker fees	Ventana
	Leadership in other associations	College of American Pathologists
Mary Kay Washington, MD, PhD	Leadership in other associations	American Joint Committee on Cancer

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[b] The information above reflects disclosures that were collected and reviewed by the College of American Pathologists, the American Society for Clinical Pathology, and the American Society of Clinical Oncology. The disclosures that appear in the individual journals of the societies may vary based on journal-specific policies and procedures.