HER2 Testing in Endometrial Serous Carcinoma

Time for Standardized Pathology Practice to Meet the Clinical Demand

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Context.—Endometrial serous carcinoma is an aggressive subtype of endometrial cancer with the highest rate of recurrence and mortality among all histotypes. A recent clinical trial showed prolonged progression-free survival in advanced-stage and recurrent human epidermal growth factor receptor 2 (HER2)–positive endometrial serous carcinoma when trastuzumab was added to the standard chemotherapy regimen. This targeted therapeutic approach was recently endorsed by the National Comprehensive Cancer Network clinical guidelines. There is a growing interest among clinicians to obtain HER2 testing in endometrial serous carcinoma, and pathologists need to be prepared to recognize the unique characteristics of HER2 protein expression and gene amplification in these tumors and apply specific HER2 scoring criteria.

Objective.—To provide a historical overview of targeted HER2 therapy in endometrial serous carcinoma and to summarize key findings from recent studies on the specific features of HER2 protein expression and gene amplification relative to other tumor types. Endometrial carcinoma–specific HER2 testing criteria are proposed based on evidence in the existing literature.

Data Sources.—Sources comprise review of the literature and personal experience of the author.

Conclusions.—HER2 protein overexpression and/or gene amplification is present in approximately 25% to 30% of endometrial serous carcinomas, providing an opportunity for targeted therapy. Pathologists play a key role in tumor HER2 testing and scoring to ensure appropriate patient selection and successful clinical outcome.


Endometrial cancer is the fourth most common malignancy among women in the United States, with an estimated 65 620 new diagnoses and 12 590 deaths in 2020. Although endometrial serous carcinoma represents only approximately 10% of all endometrial carcinomas, it has the highest recurrence rate (both local and distant) and is responsible for a disproportionate 40% of endometrial cancer mortality, with an overall 5-year survival rate of only 45%. In the era of precision medicine there is a growing demand for new targeted therapeutic approaches for this aggressive tumor type.

Since the mid-1990s there has been a considerable interest in potential anti–human epidermal growth factor receptor 2 (HER2) therapy for endometrial cancer, and several clinicopathologic studies have been published on the topic. However, in the absence of standardized HER2 testing and scoring methods, the HER2 protein overexpression rate varied significantly between 14% and 80%, and HER2 gene amplification detected by fluorescent in situ hybridization (FISH) was reported in 21% to 47% of tumors. In addition, most studies combined all histologic subtypes in their analyses, including low-grade endometrioid carcinomas. During the same time period a handful of case reports described successful targeted therapy in patients with HER2+ endometrial serous carcinomas. Villela et al reported 2 patients with advanced-stage HER2+ tumors: 1 patient showed complete response and the other patient had stable disease. Santin et al observed prolonged survival with stable disease in 2 patients. Despite the encouraging case studies, the Gynecologic Oncology Group published the results of the first phase 2 clinical trial (GOG 181B) in 2010, showing no clinical activity by single-agent trastuzumab in HER2+ advanced-stage or recurrent endometrial carcinoma. The trial results were received with criticism primarily because of the low enrollment numbers and poor patient selection criteria: most patients had endometrioid carcinoma, whereas only one-third had a diagnosis of serous carcinoma, and tumors with a 2+ HER2 immunohistochemical score and negative FISH were also included.

Following the failed GOG trial, a new multi-institutional, randomized phase 2 clinical trial was initiated in 2011 to evaluate the efficacy of carboplatin/paclitaxel versus carboplatin/paclitaxel/trastuzumab in advanced-stage and recurrent HER2+ endometrial serous carcinoma. Prolonged progression-free survival was observed in the trastuzumab arm by nearly 9 months in advanced-stage tumors, and by 3.2 months in recurrent endometrial serous carcinoma; an assessment of the overall survival data is currently in...
Current Criteria (Approved or Proposed) for HER2 Positivity by Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in Different Tumor Types

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>HER2 IHC (ASCO/CAP 2018)</th>
<th>HER2 FISH Amplification (HER2/CEP17)</th>
<th>HER2 IHC (ASCO/CAP 2016)</th>
<th>HER2 FISH Amplification (HER2/CEP17)</th>
<th>HER2 IHC (HERACLES Trial)</th>
<th>HER2 FISH Amplification (HER2/CEP17)</th>
<th>HER2 IHC (Fader et al Clinical Trial)</th>
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<tr>
<td>Breast</td>
<td>≥10% circumferential, strong, complete</td>
<td>HER2 signal ≥4.0 per nucleus OR ratio &lt;2.0 and HER2 signal ≥6.0 per nucleus (if IHC score 2+ or 3+)</td>
<td>≥10%, strong complete, or basolateral/lateral</td>
<td>HER2/CEP17 ratio ≥2.0 OR ratio &lt;2.0 and HER2 signal ≥6.0 per nucleus</td>
<td>≥50% strong complete, or basolateral/lateral</td>
<td>HER2/CEP17 ratio ≥2.0 in ≥50% of cells</td>
<td>&gt;30% strong complete or basolateral/lateral</td>
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<td>Gastric (HERACLES Trial)</td>
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<td>Endometrial Serous (Fader et al Clinical Trial)</td>
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Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists.

...progress. As a result, the National Comprehensive Cancer Network (NCCN) Uterine Neoplasm Guidelines endorsed the addition of trastuzumab to standard chemotherapy as the preferred regimen for the treatment of HER2+, advanced or recurrent endometrial serous carcinoma, leading to an increasing clinical demand for HER2 testing in pathology laboratories across the United States.

It has become clear in the recent literature that tumors in different organ systems have distinct characteristics of HER2 protein expression and gene amplification, which should be carefully studied to ensure appropriate patient selection for successful targeted therapy. In breast cancer, the pathology HER2 testing and scoring guidelines have continuously evolved for more than 2 decades based on the data from several clinical trials and clinicopathologic studies. Approximately 15% to 25% of breast carcinomas have been reported as being HER2+. Although HER2 testing of all breast carcinomas is performed regardless of tumor histologic subtype, a correlation between histotype and HER2 status does exist: for example, classic invasive lobular carcinomas and metaplastic carcinomas are typically HER2+, whereas some studies showed a higher proportion of HER2 positivity among invasive micropapillary carcinomas. In addition, invasive micropapillary breast carcinoma often shows a basal/basolateral HER2 immunostaining pattern, which is considered 2+ equivocal according to the current breast scoring guidelines. Heterogeneity of HER2 expression/amplification is generally uncommon in breast cancer, although higher rates have been reported for heterogeneity of gene amplification, with cluster and mosaic patterns of heterogeneity. In gastric cancer, the first set of specific HER2 testing guidelines has been developed using the patient selection criteria in the ToGA (trastuzumab for gastric cancer) clinical trial as the basic framework, recognizing and incorporating important differences from breast cancer. The overall HER2 positivity rate has been reported at 20% to 22% of all gastric carcinomas, with a higher rate among intestinal-type tumors and in tumors located at the gastroesophageal junction (up to 34%). Unlike in breast cancer, heterogeneity of HER2 protein expression is a common phenomenon, seen in approximately 50% of gastric carcinomas, and tumor cells often show a “U-shaped” (basolateral/lateral) membrane staining pattern. Most recently, yet another set of criteria has been put forward for metastatic colorectal cancer in the HERACLES trial, with a 50% strong membranous staining cutoff for HER2 protein overexpression (Table).

The unique characteristics of HER2 protein expression and gene amplification in endometrial serous carcinoma have been systematically evaluated and described in 2 pretrial pathology studies, which then helped guide patient enrollment in the clinical trial. Specifically, significant intratumoral heterogeneity of HER2 protein expression—defined as the presence of at least 2 degrees of difference in staining intensity involving at least 5% of tumor cells—was observed in more than 50% of HER2+ tumors by immunohistochemistry, which also correlated with a heterogeneous cluster HER2 gene amplification pattern by FISH in most cases (Figures 1 and 2). In addition, approximately 75% of HER2+ tumors lacked apical membrane staining, resulting in a basolateral/lateral membranous staining pattern, similar to that of gastric adenocarcinomas. Comparing 2 different tumor cell staining cutoffs, 10% versus 30% strong membranous expression, a higher immunohistochemistry (IHC)–FISH concordance was observed using the 30% cutoff in endometrial serous carcinoma. For patient enrollment in the clinical trial, tumor sections were first reviewed to confirm serous histology, followed by HER2 immunohistochemistry. Tumors with intense complete or lateral/basolateral membranous HER2 immunostaining in more than 30% of tumor cells were assigned a 3+ score, and 2+ score was assigned when intense complete or lateral/basolateral membranous staining was seen in ≤30%, or weak to moderate staining in ≥10%, of tumor cells (modified 2007 American Society of Clinical Oncology/College of American Pathologists [ASCO/CAP] breast criteria; Figure 3). FISH was performed only on tumors with a 2+ immunohistochemical score on a large tumor area in direct correlation with the HER2 immunostained slide, and a HER2/CEP17 ratio of ≥2.0 was considered amplified. The above-mentioned clinical trial criteria should serve as the basis for endometrial carcinoma–specific HER2 testing guidelines, to follow the example of guideline-development process in other tumor types.

Future studies are necessary to resolve a number of practical questions and to refine the testing algorithm. As an example, currently there are only limited data available on optimal specimen type (biopsy versus hysterectomy) for HER2 testing in this tumor type. In breast and gastric cancer, initial HER2 testing is typically performed on the biopsy specimen, given the frequent application of neoadjuvant chemotherapy. Repeat HER2 testing of the excision specimen is recommended in breast cancer for specific scenarios, and testing multiple specimens, including recurrent or metastatic tumors, is often preferred and requested by clinicians as it may result in a clinically significant change in HER2 status in approximately 20% of the cases. Repeat HER2 testing has also been shown to increase the rate of HER2 positivity in gastric cancer.
Huber et al. assessed the impact of specimen type on HER2 status in gastric and gastroesophageal junction adenocarcinomas, and they reported that in patients with only 1 specimen, 10.5% of tumors were HER2+, whereas the rate increased to 18.1% and 24.1% in patients with 2 or 3 specimens tested, respectively. Discordant HER2 status has also been observed between paired primary and metastatic tumors in close to 20% of breast, gastric, and most recently endometrial carcinomas. Furthermore, the optimal HER2 testing algorithm also depends on the correlation between the test type (IHC or FISH) and therapeutic response, although specific data for endometrial carcinoma are currently limited in the literature. In breast cancer, FISH and IHC are equally predictive of clinical response; hence, the current ASCO/CAP guidelines allow for either test to be performed primarily and recommend reflexing to the other method in cases with an equivocal result. In gastric cancer, on the other hand, HER2 protein expression showed the strongest association with efficacy of trastuzumab; therefore, the testing algorithm recommends HER2 IHC as the primary test. In the recent clinical trial for endometrial serous carcinoma, primary testing was performed by IHC, and only tumors with 2+ HER2 IHC score were subjected to FISH.

Regarding the HER2 FISH amplification criteria, adoption of the current breast or gastric recommendations may be considered for endometrial serous carcinomas to include tumors with a HER2/CEP17 ratio of <2.0 and an average HER2 copy number of ≥6 per nucleus in the HER2 amplified category. Additional clinicopathologic studies would also be essential in determining the clinical impact of intratumoral heterogeneity and the correlation of therapeutic response with HER2 protein expression and gene amplification. Previous studies in breast and gastric cancer showed that patients with homogeneous HER2 protein expression benefited significantly more from targeted trastuzumab therapy, both in progression-free and overall survival, compared with those with heterogeneous HER2 expression.

The increasing clinical demand for HER2 testing in endometrial serous carcinomas should also prompt pathology laboratories to implement specific protocols for specimen handling and fixation, using evidence from the breast literature and guidelines from breast and gastric carcinoma testing. In general, a cold ischemia time of less than 1 hour and fixation in 10% neutral buffered formalin...
for 6 to 72 hours should be ensured for both endometrial biopsy and hysterectomy specimens. Pathologists should also be aware of the added implications of endometrial carcinoma histologic subtype and of proper tissue block selection on HER2 testing and targeted therapy. The recent clinical trial and the NCCN guidelines limit targeted therapy to serous tumor subtype, either pure or as a component of mixed endometrial carcinoma. Up-to-date pathologic diagnostic criteria should be applied using ancillary tools if needed. In mixed carcinomas HER2 testing should be performed on a tissue block containing the most amount of serous component. There is a clinical interest in expanding targeted HER2 therapy to other high-grade gynecologic tumors in the future, and recent clinicopathologic studies identified similarities between HER2 protein expression/gene amplification in endometrial serous carcinoma and endometrial carcinosarcomas.

In summary, HER2 protein overexpression and/or gene amplification is present in approximately 25% to 30% of endometrial serous carcinomas, and patients with HER2+ tumors have been shown to have a significant survival benefit from targeted HER2 therapy. Pathologists play a

Figure 2. Heterogeneity of human epidermal growth factor receptor 2 (HER2) gene amplification in endometrial serous carcinoma by fluorescent in situ hybridization (FISH) in the form of “cluster amplification.” A large cluster of tumor cells shows HER2 amplification with a HER2/CEP17 ratio of 7.14 (A), in a background of nonamplified areas (B, HER2/CEP17 ratio = 1.25) (original magnification, ×600 [A and B]).

Figure 3. Proposed human epidermal growth factor receptor 2 (HER2) testing algorithm for endometrial serous carcinoma based on the clinical trial patient enrollment criteria. Abbreviations: FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.
crucial role in identifying the appropriate patient group by applying evidence-based tumor type-specific HER2 testing and scoring criteria.

References