ERBB2 (HER2/neu) (HercepTest) Testing

Both breast and gastric cancers are common causes of cancer-related deaths. Amplification of the ERBB2 (HER2) gene occurs in 15-20% of breast cancers and approximately 7-38% of gastric cancers. Trastuzumab (Herceptin) may improve the overall survival rate in individuals with HER2-positive breast or gastric cancer. Laboratory testing can determine ERBB2 status and aid in the prediction of response to HER2-directed therapy.

TYPICAL TESTING STRATEGY

Standard practice for evaluating primary, recurrent, and metastatic breast carcinoma, and gastric or gastroesophageal carcinoma:

**Breast Carcinoma**
- Assess ERBB2 status by immunohistochemistry (IHC) or in situ hybridization (ISH)/fluorescence in situ hybridization (FISH)
- Concordance between the methods can vary due to subjective interpretation
- If IHC equivocal (2+), confirm by ISH/FISH
- If ISH/FISH scores fall in Groups 2, 3, or 4 (formerly designated as equivocal), confirm by IHC with rescoring in area(s) of highest staining intensity

**Gastric Carcinoma**
IHC should be performed first, followed by FISH testing for equivocal results

DISEASE OVERVIEW

**Incidence**
- Breast cancer: ~268,600 cases diagnosed in the U.S.
- Gastroesophageal cancers: ~27,510 cases diagnosed in the U.S.

**Treatment Issues**
- Amplification of the ERBB2 gene occurs in 15-20% of breast cancers and approximately 7-38% of gastroesophageal adenocarcinomas and predicts poor prognosis in invasive breast cancer (Nitta, 2016; Bartley, 2017).
- Trastuzumab therapy inhibits HER2-positive cancers by directing antibodies against the extracellular portion of the HER2 protein. Trastuzumab may improve the overall survival rate in individuals with HER2-positive tumors.
- Trastuzumab has a potential for cardiac toxicity along with a high drug cost; therefore, tumors that demonstrate ERBB2 (HER2) gene amplification or protein overexpression (3+ IHC result) must be identified prior to the initiation of therapy.
- New therapies targeting HER2 include pertuzumab (Perjeta), T-DM1 (Kadcyla), and lapatinib (Tykerb); recent studies have shown that treatment with a combination of trastuzumab and pertuzumab was more effective than trastuzumab alone (in combination with docetaxel) in prolonging survival of breast cancer patients.

GENETICS

**Gene**

ERBB2
Function

Amplification of ERBB2 gene

- Increases membrane expression and activation of the HER2 protein
- Stimulates cell proliferation

**TEST INTERPRETATION**

**Gene Amplification**

**Breast**

<table>
<thead>
<tr>
<th>Result</th>
<th>Group</th>
<th>ERBB2/CEP17 ratio</th>
<th>Average ERBB2 copy number</th>
<th>Interpretationa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Group 1</td>
<td>≥2.0</td>
<td>≥4.0 signals/cell</td>
<td>Predicts favorable response to targeted therapy</td>
</tr>
<tr>
<td>Negative</td>
<td>Group 5</td>
<td>&lt;2.0</td>
<td>&lt;4.0 signals/cell</td>
<td>Predicts lack of response to targeted therapy</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Group 2</td>
<td>≥2.0</td>
<td>&lt;4.0 signals/cell</td>
<td>Perform concomitant HER2 IHC review</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>&lt;2.0</td>
<td>≥6.0 signals/cell</td>
<td>- IHC score of 3+ is considered positive and 0 or 1+ is considered negative</td>
</tr>
<tr>
<td></td>
<td>Group 4</td>
<td>&lt;2.0</td>
<td>≥4.0 and &lt;6.0 signals/cell</td>
<td>- For an IHC score of 2+, additional tumor nuclei are enumerated with FISH from area of highest IHC intensity by an individual blinded to the original results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Repeat scoring consistent with groups 2 and 4 is considered negative while scoring consistent with group 3 is considered positive</td>
</tr>
</tbody>
</table>

a It is uncertain whether patients with ≥4.0 and <6.0 average HER2 signals/cell and HER2/CEP17 ratio <2.0 benefit from HER2 targeted therapy in the absence of protein overexpression (IHC 3+)

**Gastric**

- Positive: ERBB2/CEP17 ratio ≥2.0 or ERBB2/CEP17 ratio <2.0 and average ERBB2 copy number ≥6.0 signals/cell
  - Predicts favorable response to targeted therapy
- Negative: ERBB2/CEP17 ratio <2.0 and average ERBB2 copy number <4.0 signals/cell
  - Predicts lack of response to targeted therapy
- If results are indeterminate, consider further testing with an alternate control probe or analytic method or follow-up testing on the resection specimen

**Limitations**

- Testing only validated for FFPE specimens; specimens fixed in other than 10% neutral buffered formalin have not been validated using this method
- Specimens placed in decal may have a false-negative result
- Assay is validated and FDA approved for invasive breast carcinoma and gastric cancers only
- Testing is interpreted according to ASCO/CAP 2018 Updated Guidelines for breast cancer and ASCO/CAP 2017 Guidelines for gastric cancer
- Repeat testing is recommended for discordant results

**Immunohistochemistry**

**ASCO/CAP 2018 HER2 IHC Scoring Criteria Used in the Interpretation of the HercepTest for Breast Cancer**

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
<th>Microscopic Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>No staining or membrane staining that is incomplete, faint/barely perceptible and within ≤10% of the invasive tumor cells</td>
</tr>
<tr>
<td>1+</td>
<td>Negative</td>
<td>Incomplete membrane staining that is faint/barely perceptible and within &gt;10% of the invasive tumor cells</td>
</tr>
<tr>
<td>2+</td>
<td>Equivocala</td>
<td>Weak to moderate complete membrane staining observed in &gt;10% of tumor cells</td>
</tr>
<tr>
<td>3+</td>
<td>Positiveb</td>
<td>Circumferential membrane staining that is complete, intense and in &gt;10% of invasive tumor cells</td>
</tr>
</tbody>
</table>

a Equivocal results (2+) should be confirmed by ISH testing

b Positive results (3+) indicate possible response to trastuzumab
### Biopsies of Gastric and Gastroesophageal Adenocarcinoma Using ERBB2 IHC Scoring

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
<th>Staining Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>No reactivity or no membranous reactivity in any tumor cell</td>
</tr>
<tr>
<td>1+</td>
<td>Negative</td>
<td>Tumor cell cluster (5 cells) with faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained</td>
</tr>
<tr>
<td>2+</td>
<td>Equivocal</td>
<td>Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained</td>
</tr>
<tr>
<td>3+</td>
<td>Positive</td>
<td>Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained</td>
</tr>
</tbody>
</table>

Hofmann, 2008

### Resections of Gastric and Gastroesophageal Adenocarcinoma Using ERBB2 IHC Scoring

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
<th>Staining Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>No reactivity or membranous reactivity in &lt;10% of tumor cells</td>
</tr>
<tr>
<td>1+</td>
<td>Negative</td>
<td>Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells. Cells are reactive only in part of their membrane</td>
</tr>
<tr>
<td>2+</td>
<td>Equivocal</td>
<td>Weak to moderate complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells</td>
</tr>
<tr>
<td>3+</td>
<td>Positive</td>
<td>Strong complete, basolateral or lateral membranous in ≥ 10% of tumor cells</td>
</tr>
</tbody>
</table>

Hofmann, 2008

**REFERENCES**


**RELATED INFORMATION**

Breast Cancer Biomarkers