A comparative study of p95-HER2 carboxy terminal fragment (CTF) detected by immunohistochemistry and VeraTag immunoassays in human breast tumors

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Abstract

BACKGROUND: C-terminally truncated forms of HER2 (HER2-CTF) have been reported to be highly oncogenic and correlate with node-positive breast cancer, poor prognosis, and resistance to treatment in patients with HER2-metastatic breast cancer (MBC). A quantitative, sensitive and specific immunoassay using the VeraTag™ technology has been developed to detect the active Met11-HER2 CTF (p95) in FFPE tumor samples and has been used to identify HER2+ patients who have a reduced response to trastuzumab in two independent cohorts. Recently, an IHC assay using an antibody directed to a similar p95 epitope was reported to detect a high percentage of HER2+ samples (79%) as p95 positive (W50, ASCO 2011).

METHODS: To verify the cellular specificity and accuracy of the p95 VeraTag assay, and evaluate the prevalence of p95 in breast tumor IHC assay was developed which utilizes the same p95 antibody as the VeraTag assay. The IHC and VeraTag assay formats were performed in 120 human breast tumor excisional and core biopsy samples submitted to central clinical reference lab testing, and having different levels of HER2 measured by HercepTest™ (Dako, Inc). IHC 0 (n=20); 1+(n=20), 2+(n=20), and 3+(n=60). Replicate slides were run in both VeraTag and IHC assays to demonstrate reproducibility. Nearly all p95 IHC signal was associated with tumor cells only.

RESULTS: Consistent with earlier p95 VeraTag data, a general correlation was observed between HER2 and p95 expression levels, with tumors expressing normal levels of HER2 rarely expressing high p95 levels. However, within the subset of HER2+ samples (HER2 3+ or p95 > 2.2; n=38), a ~30-fold range of p95 VeraTag signal was observed. A similar relationship was observed for samples assayed by p95 IHC. All samples measured as p95 IHC 3+(>10 cells with membrane staining) were HER2+ by central lab testing and represented nearly a third of the HER2+ samples. The p95 VeraTag assay showed a significant but weak correlation with p95 IHC.

CONCLUSIONS: These results indicate that the tumor-specific p95 signal detected by the p95 VeraTag assay is in general agreement with an IHC assay using the same antibody; however a number of cases with p95 VeraTag scores above the clinical cutoff (p95 VeraTag > 2.8) did not fall into the p95 IHC 3+ category. Additionally, the prevalence of p95 positivity by VeraTag assay and by IHC 3+ scores is consistent with previous peer-reviewed studies and lower than recently described in CCR, 16: 4226, 2010.

Methods - p95 VeraTag and p95 IHC

- **VeraTag detection of p95**
  - VeraTag method developed to detect the active Met11-HER2 CTF (p95).
  - VeraTag utilizes the same p95 antibody as the VeraTag assay.
- **HER2 IHC**
  - HER2 IHC assay was developed which utilizes the same p95 antibody as the VeraTag assay.
- **Binding blocked by HER2 ECD**
  - Solution is collected and analyzed by capillary electrophoresis.

**Cohort selection and study plan**

<table>
<thead>
<tr>
<th>HER2 IHC category</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC 0</td>
<td>0</td>
</tr>
<tr>
<td>IHC 1+</td>
<td>1</td>
</tr>
<tr>
<td>IHC 2+</td>
<td>2</td>
</tr>
<tr>
<td>IHC 3+</td>
<td>60</td>
</tr>
</tbody>
</table>

**p95 VeraTag standards**

- p95 VeraTag assay shows good reproducibility around working clinical cutoff of 2.8.
- Both p95 VeraTag and p95 IHC displayed high concordance between replicates.

**p95 IHC standards**

- Equivalent p95 IHC cutoff would be at 1+ or 2+ where reproducibility isn’t as high.
- All 16 VeraTag and 11 IHC batches performed as expected with tight clustering of standards.

**Assay Reproducibility**

- Spearman r = 0.89
- p95 VeraTag and p95 IHC may provide complementary measures of p95 expression.
- The clinical significance of using the p95 IHC assay to complement the p95 VeraTag score is being evaluated.