The HERmark® Breast Cancer Assay is a novel method to quantitatively measure HER2 total protein expression (H2T) in breast cancer in a multi-center Collaborative Biomarker Study (CBS) and correlates with clinical-pathological features.

**Results**

- The HERmark assay provides quantitative measurement of total HER2 expression (H2T) over a wide dynamic range (log2).
- HER mark showed good general concordance with routine “real-world” HER2 testing (IHC and FISH) (Table 1).
- However, as expected, concordance (excluding equivocal results) between HERmark and cental IHC was higher compared to that between HERmark and HER mark positive breast cancer patients.
- HER mark negative discordant cases were reported with local HER2 IHC when HER mark was positive.

**Table 1. Concordance of HERmark with local HER2 IHC, central HER2 IHC, local HER2 FISH, and site-reported (local) HER2 status**

<table>
<thead>
<tr>
<th>HERmark Status</th>
<th>Local HER2 IHC</th>
<th>Central HER2 IHC</th>
<th>Local HER2 FISH</th>
<th>Site-reported HER2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERmark Negative</td>
<td>63 (69%)</td>
<td>14 (15%)</td>
<td>14 (15%)</td>
<td>HERmark Negative 19 (79%)</td>
</tr>
<tr>
<td>HERmark Positive</td>
<td>6 (9%)</td>
<td>7 (11%)</td>
<td>52 (80%)</td>
<td>HERmark Positive 24 (13%)</td>
</tr>
</tbody>
</table>

**Methods**

The HERmark CBS was a retrospective biomarker study with the primary objective of comparing the quantitative H2T by HERmark with conventional immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) HER2 testing methods. Secondary objectives of the study included correlating HER2 results by HERmark versus local HER2 testing with clinicopathological outcomes, including outcomes in patients with discordant HER2 results by HER mark versus locally determined HER2 status.

**Tissue Sample**

Each site was instructed to identify approximately 50% HER2 positive and 50% HER2 negative breast cancer cases for the study. 238 FFPE breast cancer tissues collected between January 2008 and May 2010, were batched out and prepared as slides by 11 CBS study sites. HER2 testing by the HERmark assay and central laboratory IHC were performed in a parallel fashion. Local HER2 IHC and FISH results, site-reported clinical HER2 status (based on IHC or combination of IHC and FISH) (Figure 3), and HER mark and central HER2 IHC results were obtained in 162 cases for analysis. Patient demographics and tumor characteristics were documented for each patient after completion of the HERmark assay. Subsequent central HER2 IHC results were re-reviewed.

The HERmark® Breast Cancer Assay

H2T was quantified using the HERmark assay as previously described (Huang et al. J. Clin Pathol. 72:305, 2010). H2T was quantified using a novel fluorescent tag (FV) for "VeraTag® assay." (Figure 4) was conjugated to a HER2 monoclonal antibody (Ab). The antibody is paired with a fluorescent secondary HER2 mAb (Ab15). Upon illumination with red light, an avidin-linked fluorophore (PM) produces singlet oxygen (S1) which cleaves the tag (V) in close proximity. Signals (V) are captured by capillary electromigration and normalized to invasive tumor areas on the FV image section. The continuous H2T results are categorized as HER2 negative, HER2 equivocal, and HER2 positive with pre-defined "HER2" analytical cutoff values (Huang et al. J. Clin Pathol. 72:305, 2010) for the determination of HER2 positivity (Figure 5). A pre-defined HERmark clinical cutoff (HER2-T low, HER mark Cancer 2010;116:5168) was used to determine tumor HER2 low and HER2 high patient groups in overall survival analysis.

**Conclusions**

- Our study confirms prior reports that HER2 status determined by central lab testing appears to be more similar to local “real-world” HER2 results.
- Quantitative HER2 total protein expression (H2T) by HERmark enriched the identification of both HER2 negative and HER2 positive breast cancer patients in the study and may provide added clinical value to HER2 testing.
- Poor overall survival noted in the high H2T discordant cases which may identify a context for HER2 positive breast cancers that could benefit from HER2-targeted therapies. Future trials to test this hypothesis are warranted.