HERmark Assay Methodology

The HERmark assay specifically quantifies the full range of HER2 protein expression (H2T) levels in FFPE breast cancer tumor samples.

The "VeraTag" methodology uses two primary HER2-specific monoclonal antibodies and proximity-based conjugated fluorescent "VeraTag reporters" that are quantified using capillary electrophoresis. The amount of released "VeraTag reporters" is proportional to the amount of HER2 protein in the invasive tumor sample.

References


8. Scudiero DA, Fournier H, Paik S, et al. HER2-TARGETED THERAPY CANDIDATES FOR AIDS IN IDENTIFYING

HER2-Positiv...
The HERMARK® Breast Cancer Assay

HERMark is based on our proprietary VeraTag® technology that precisely quantifies HER2 proteins and protein complexes in formalin-fixed, paraffin-embedded (FFPE) tissue specimens."

- Produces direct and quantitative measurement of HER2 protein, the target of current anti-HER2 drug therapies.
- Provides a continuous, rather than semiquantitative, measurement of HER2 total protein over a 3-log dynamic range.
- Demonstrates a 2-to-3-fold improvement in sensitivity over IHC, with detection of HER2 levels down to ~250 pg per ml.

Clinical Significance of HERMark

HERMark Provides Accurate HER2 Assessment and prognostic Value

Accurate assessment of HER2 status in breast cancer is critical in identifying patients who may benefit from HER2-targeted therapy. Studies have shown that there is a statistically highly significant discordance level (19-37%) between central and local HER2 testing.

Representing “real-world” HER2 testing, a recent retrospective, multicenter Collaborative Biomarker Study (CBS) of breast cancer patients (not treated with trastuzumab) showed that the HERMark assay offers accurate quantification of HER2 protein expression that correlated well with central laboratory HER2 IHC re-testing, and less so with local laboratory HER2 IHC and FISH results.

- HERMark reclassified 15% of local HER2 status negative results as HERMark positives.
- HERMark offers a more accurate assessment of HER2 and may change therapy selection in approximately 20% of patients.
- HERMark has shown to be a better prognostic factor in overall survival patients for whom HER2 test results were discordant between HERMark and routine HER2 testing.

The difference in overall survival with the discordant groups did not appear to be due to clinicopathologic factors but more likely due to local HER2 misclassifications.

Comparison with Other HER2 Testing Methods

In validation studies involving more than 1,000 clinical tumor samples, HERMark demonstrated a high level of concordance with other HER2 testing methods performed in central reference laboratories.

Time to Brain Metastasis

HERMark® HER2 protein levels correlated with risk of brain metastases in HER2-positive metastatic breast cancer patients receiving trastuzumab therapy.

HERMark reclassified 15% of local HER2 status negative results as HERmark positives.

When to Use HERMark

HERMark provides an alternative HER2 testing method in order to identify candidates for HER2-targeted therapy. Consider HERMark:

- When a fully quantitative and validated HER2 protein measurement is desired.
- When HER2 status is inconclusive.
- Cases with discordant HER2 testing results in IHC and/or FISH (CISH).
- Cases with HER2 status “equivocal” by IHC and/or FISH (CISH).
- Cases that demonstrate significant heterogeneity in the expression of HER2 protein or gene amplification status of HER2.
- In cases with HER2 status “negative” by IHC and/or FISH(CISH), but the patient’s clinical picture (by physician’s assessment and experience) suggests an aggressively (possibly HER2-positive) disease.
HERmark is based on our proprietary VeraTag® technology that precisely quantifies HER2 proteins and protein complexes in formalin-fixed, paraffin-embedded (FFPE) tissue specimens.

- Produces direct and quantitative measurement of HER2 protein, the target of current anti-HER2 drug therapies.
- Provides an alternative to semiquantitative, measurement of HER2 tumor protein over a 3-log dynamic range.
- Demonstrates a 2- to 3-fold improvement in sensitivity over IHC, with detection of HER2 levels down to ~250 copies per cell.

**Clinical Significance of HERmark**

**HERmark Provides Accurate HER2 Assessment and Prognostic Value**

Accurate assessment of HER2 status in breast cancer is critical in identifying patients who may benefit from HER2-targeted therapy. Studies have shown that there still is a relatively high discordance level (16-27%) between central and local HER2 testing.

Representing "real-world" HER2 testing, a recent retrospective, multicenter Collaborative Biomarker Study (CBS) of breast cancer patients (not treated with trastuzumab) showed that the HERmark assay offers an accurate quantification of HER2 protein expression that correlated best with central laboratory HER2 IHC re-testing, and less so with local laboratory HER2 IHC and FISH results.

- HERmark reclassified 15% of local HER2 status negative results as HERmark positives.
- HERmark offers a more accurate assessment of HER2 status and may change therapy selection in approximately 20% of patients.
- HERmark has shown to be a better prognostic factor in overall survival patients for whom HER2 test result were discordant between HERmark and routine HER2 testing.
- The difference in overall survival with the discordant groups did not appear to be due to clinicopathologic factors but more likely due to local HER2 misclassifications.

**HERmark Provides Accurate HER2 Assessment and Prognostic Value**

HERmark measurements were performed in tumors of patients (n=330) enrolled in the NeoAdjuvant Trial. The NeoAdjuvant Trial evaluated combination treatment of HER2+ with trastuzumab vs. either treatment alone for HER2 positive early stage breast cancer. The results of this study include:

- HERmark provides a continuous, rather than semiquantitative, measurement of HER2 total protein.
- Higher HERmark values achieved better outcomes in patients with metastatic breast cancer treated with trastuzumab (n=102). Our HERmark clinical study showed HERmark outperformed FISH as a predictor of time to progression (TTP) in patients with metastatic breast cancer treated with trastuzumab (p<0.001).

**HERmark Provides Predictive Value**

HERmark-reclassified HER2-negative patients showed similar baseline characteristics to HER2-negative patients treated with trastuzumab, with the exception of a higher incidence of hormone receptor-negative status.

**Better Correlation with Clinical Outcomes on Trastuzumab**

Our HERmark clinical study showed HERmark outperformed FISH as a predictor of time to progression (TTP) in patients with metastatic breast cancer treated with trastuzumab (p<0.001). HERmark reclassified 15% of local HER2 status negative results as HERmark positives.

**HERmark Provides Prognostic Value**

HERmark-reclassified HER2-negative patients showed similar baseline characteristics to HER2-negative patients treated with trastuzumab, with the exception of a higher incidence of hormone receptor-negative status.

**HERmark’s Unique Value**

HERmark offers an alternative HER2 testing method in order to identify candidates for HER2-targeted therapy. Consider HERmark:

- When a fully quantitative and validated HER2 protein measurement is desired.
- When HER2 status is incoincidence.
- Cases with discordant HER2 testing results in IHC and/or FISH (CISH).
- Cases with HER2 status ‘equivocal’ by IHC and/or FISH.
- Cases that demonstrate significant heterogeneity in the expression of HER2 protein or gene amplification status of HER2.
- In cases with HER2 status ‘negative’ by IHC and/or FISH (CISH), but the patient’s clinical picture (by physician’s assessment and experience) suggests an aggressive (possibly HER2-positive) disease.
HER2 is a key receptor tyrosine kinase involved in cell proliferation and survival, whose overexpression can lead to aggressive breast cancer. HER2 testing is crucial for determining eligibility for HER2-targeted therapies, which can significantly improve patient outcomes.

**HER2 Testing Methods**

- **Central IHC**
- **Central FISH**
- **Central IHC and CISH**

Comparison with Other HER2 Testing Methods

In validation studies involving more than 1,000 clinical tumor samples, HERmark demonstrated a high level of concordance with other HER2 testing methods performed in central reference laboratories.

**HERmark Assay**

HERmark is a fully quantitative and validated HER2 protein measurement. It offers a more accurate assessment of HER2 status when compared to local laboratory methods, achieves a higher cutoff value for HER2-negative (trastuzumab- and lapatinib-responsive) patients, and can help in the selection of patients who may benefit from HER2-targeted therapy. HERmark results are reported as Patient HER2 Status (positive, negative, or equivocal) as well as the patient's precise quantitative HER2 level.

**HERmark Provides Accurate HER2 Assessment and Prognostic Value**

- HERmark provides accurate quantification of HER2 total protein expression that correlated best with central laboratory HER2 IHC re-testing, and less so with local laboratory HER2 IHC and FISH results.
- HERmark reclassified 15% of local HER2 status negative results as HERmark positive.
- HERmark measurements were performed in tumors of patients (n=324) enrolled in the NeoALTTO trial. The HERmark assay was predictive of pathologic complete response (pCR) when patients received trastuzumab and chemotherapy.
- HERmark positive patients had better outcomes and achieved a pCR of 39% vs. 11% for HER2-negative patients (Fisher exact test, p=0.01).
- Higher HERmark values achieved better outcomes and median time to progression (TTP) similar to chemotherapy plus trastuzumab alone.
- HERmark values unmasked benefit from combination treatment.
- HERmark-positive patients had better outcomes and achieved a pCR of 39% vs. 11% for HER2-negative patients (Fisher exact test, p=0.01).
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**HERmark Provides Predictive Value**

HERmark results were predictive of pathologic complete response (pCR) when patients received trastuzumab and chemotherapy. Patients with HER2 FISH-positive (HER2/CEP17 ratio ≥ 2.2) samples that were reclassified by HERmark as HER2-low (HER2-low) had similar TTP as patients with FISH-negative results (3.7 months and 4.5 months, respectively) (HR, 1.0; p=0.95).

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HERmark is a fully quantitative and validated HER2 protein measurement. It offers a more accurate assessment of HER2 status when compared to local laboratory methods, achieves a higher cutoff value for HER2-negative (trastuzumab- and lapatinib-responsive) patients, and can help in the selection of patients who may benefit from HER2-targeted therapy. HERmark results are reported as Patient HER2 Status (positive, negative, or equivocal) as well as the patient's precise quantitative HER2 level.

**When to Use HERmark**

- When a fully quantitative and validated HER2 protein measurement is desired.
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- Cases with discordant HER2 testing results in IHC and/or FISH (CISH).
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- Cases that demonstrate significant heterogeneity in the expression of HER2 protein or gene amplification status of HER2.

**HERmark Assay**

- Provides a continuous, rather than semiquantitative, measurement of HER2 total protein.
- Provides direct quantitative measurement of HER2 protein, the target of current anti-HER2 drug therapies.
- Generates a higher cutoff value for HER2-negative patients, which is more likely to select patients who may benefit from HER2-targeted therapy.
- HERmark reclassified 15% of local HER2 status negative results as HERmark positive.
- HERmark measurements were performed in tumors of patients (n=324) enrolled in the NeoALTTO trial. The HERmark assay was predictive of pathologic complete response (pCR) when patients received trastuzumab and chemotherapy.
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Specimen Requirement Options

Unsteamed slides
- 5 sections on positively-charged glass slides, 1 section per slide. A total of 5 unstained slides per patient is required (sections should contain at least 5 mm² invasive tumor).
- Freshly cut sections, stored at 4°C, and sent within 1 week.
- 1 paraffin-embedded tissue block (requires formalin-fixed tissue)
- If multiple blocks are available, select the tissue block with the highest amount of viable invasive tumor; submit only 1 block.

Reimbursement Assistance

Gateway Services can assist your office and your patients with obtaining coverage and reimbursement for HERmark. Gateway makes reimbursement assistance as easy as 1-2-3:
1. Call Gateway at 1-877-436-6243 prior to ordering a HERmark assay. The appropriate application forms will be sent to you via fax or e-mail.
2. Complete the application forms and return them to Gateway via fax, 1-888-249-0223.
3. Receive notification from Gateway of patient eligibility, typically within 24 to 48 hours.

In the event that verification/coverage cannot be established, Gateway can assist with the patient’s case and/or research alternative coverage.

References


Note:
- Invasive carcinoma of the breast is required cases with in situ disease only (e.g., DCIS or LCIS) are not acceptable.
- Fine needle aspiration (FNA) specimens are not acceptable.
- Excisional biopsy specimens are preferred; large core biopsies are also acceptable.

Gateway Services: 1-877-436-6243
Gateway Reimbursement Services: 1-888-249-0223
Gateway FAX: 1-888-369-0023
www.integratedoncology.com
HERMark Assay Methodology

The HERMark assay specifically quantifies the full range of HER2 protein expression (H2T) levels in FFPE breast cancer tumor samples.

The "VeriTag" methodology uses two primary HER2-specific monoclonal antibodies and proximity-based conjugated fluorescent "VeriTag reporters" that are quantified using capillary electrophoresis. The amount of released "VeriTag reporters" is proportional to the amount of HER2 protein in the invasive tumor sample.

HERMark breast cancer assay based on VeriTag® technology

- Direct, quantitative measure of the drug target, HER2 protein
- FISH, CISH, SISH, and microarrays are indirect assays of protein expression
- Dual antibody format enhances specificity, minimizes background, and results in greater signal/noise ratio

Reimbursement Assistance

Gateway Services can assist your office and your patients with obtaining coverage and reimbursement for HERMark. Gateway provides reimbursement assistance as easy as 1-2-3.

1. Call Gateway at 1-877-436-6243 prior to ordering a HERMark assay. The appropriate application forms will be sent to you via fax or e-mail.
2. Complete the application form and return it to Gateway via fax: 1-888-249-9223.
3. Receive notification from Gateway of patient eligibility, typically within 24 to 48 hours.

In the event that verification/coverage cannot be established, Gateway can assist with the patient’s case and/or research alternative coverage.

Specimen Requirement Options

- Unstained slides
  - 5 sections on positively-charged glass slides, 1 section per patient. A total of 5 unstained slides per patient is required (sections should contain ≥ 10 mm² invasive tumor)
  - Freshly cut sections, stored at 4°C, and sent within 1 week.
- On 1 paraffin-embedded tissue block (requires formalin-fixed tissue)
  - If multiple blocks are available, select the tissue block with the highest amount of viable invasive tumor—submit only 1 block.

Note:
- Invasive carcinoma of the breast is required (e.g., DCIS or IDC)
- Fine needle aspiration (FNA) specimens are not acceptable.
- Excision/skin biopsy specimens are preferred. Large core biopsies are also acceptable.

References

5. Dr. D. Yardley, D. et al, Quantitative measurement of HER2 expression in breast cancers: comparison with "real-world" routine HER2 testing in a multicenter collaborative biomarker study and correlation with overall survival. J Clin Oncol. 2010;28(15s, 2010 (suppl; abstr 1030).