Policy Statement

The assessment of human epidermal growth factor receptor 2 (HER2) status by quantitative total HER2 protein expression and HER2 homodimer measurement is considered **investigational**.

Policy Guidelines

Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

There is no specific CPT code for this testing. It would likely be reported using the following CPT code:

- **84999**: Unlisted chemistry procedure

Palmetto GBA’s MolDX® program instructs that for Medicare this test should be reported with the following unlisted code:

- **81479**: Unlisted molecular pathology procedure

Description

Novel assays that quantitatively measure total human epidermal growth factor receptor 2 (HER2) protein expression and homodimers have been developed to improve the accuracy and consistency of HER2 testing.

Related Policies

- N/A

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates [e.g., Federal Employee Program (FEP)] prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.
Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. HERmark® Breast Cancer Assay (Monogram Biosciences) is available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Rationale

Background

Human Epidermal Growth Factor Receptor 2

The human epidermal growth factor receptor (HER) family of receptor tyrosine kinases (EGFR/HER1, ErbB2/HER2, ErbB3/HER3, ErbB4/HER4) plays a major role in the pathogenesis of many solid tumors. In approximately 25% to 30% of breast cancers, overexpression of HER2 has been linked to shorter disease-free and overall survival, lack of responsiveness to tamoxifen antiestrogen therapy, and altered responsiveness to a variety of cytotoxic chemotherapy regimens.

Trastuzumab, a monoclonal antibody directed at the extracellular domain of HER2, has offered significant shorter disease-free and overall survival advantages in the metastatic and adjuvant settings in HER2-overexpressing patients, although not all patients respond. Fewer than 50% of patients with metastatic HER2-positive breast cancer show initial benefit from trastuzumab treatment, and many of those eventually develop resistance.1-3

Current methodologies for the selection of HER2-positive patients include immunohistochemistry (IHC) to detect HER2 protein overexpression and fluorescence in situ hybridization (FISH) to detect HER2 gene amplification. However, controversy still exists regarding the accuracy, reliability, and interobserver variability of these assay methods. IHC provides a semiquantitative measure of protein levels (scored as 0, 1+, 2+, and 3+) and the interpretation may be subjective. FISH is a quantitative measurement of gene amplification, in which the HER2 gene copy number is counted. However, FISH, which is considered to be more quantitative analytically, is not always representative of protein expression, and multiple studies have failed to demonstrate a relation between HER2 gene copy number and response to trastuzumab. Whereas patients who overexpress HER2 protein (IHC) or show evidence of HER2 gene amplification (FISH) have been shown to experience better outcomes on trastuzumab than those scored negative by these assays, differences in the degree of expression or amplification by these methods have generally not been shown to discriminate between groups with different outcomes. IHC and FISH testing may be affected by interlaboratory variability, and neither test provides quantitative data that reflect the activation state of signaling pathways in tumors, which may limit their utility in patient selection.4 Most laboratories in North America and Europe use IHC to determine HER2 protein status, with equivocal category results (2+) confirmed by FISH (or more recently by chromogenic in situ hybridization).

Typically, HER2 activates signaling pathways by dimerizing with ligand-bound epidermal growth factor receptor family members such as HER1 and HER3. A HER2 ligand has not been identified, but overexpressed HER2 is constitutively active. When HER2 is pathologically overexpressed, the receptor may homodimerize and activate signaling cascades in the absence of the normal regulatory control imposed by the requirement for ligand binding of its heterodimerization partners.

A novel assay (HERmark® Breast Cancer Assay) was developed to quantify total HER2 protein expression and HER2 homodimers in formalin-fixed, paraffin-embedded tissue samples.
Literature Review
See Appendix Table 1 for genetic testing categories.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose.

Quantitative Assay for Measurement of HER2 Total Protein Expression and HER2 Dimers
Clinical Context and Test Purpose
The purpose of assessment of human epidermal growth factor receptor 2 (HER2) status using quantitative total HER2 protein expression and HER2 homodimer measurement in patients who have breast cancer is to inform a decision whether to modify treatment strategies to include HER2-targeted therapy or not.

The question addressed in this evidence review is: Does an assessment of HER2 status using quantitative total HER2 protein expression and HER2 homodimer measurement in patients who have breast cancer result in an improved health outcome compared with assessment of HER2 status using immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH)?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest are individuals with breast cancer who are undergoing assessment of HER2 status.

Interventions
The relevant intervention is assessment of HER2 status using quantitative total HER2 protein expression and HER2 homodimer measurement.

Comparators
The relevant comparators of interest currently used to make decisions about assessment of HER2 status are: IHC and FISH.

Outcomes
The general outcomes of interest are overall survival, disease-free survival, test accuracy, and test validity.

The potential beneficial outcomes of primary interest would be improvements in OS and disease-specific survival.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to inappropriate clinical management with HER2-targeted therapy. False-negative test results can lead to absence or delayed HER2-targeted therapy.

Timing
The timeframe for outcome measures varies from immediately following testing diagnosis to long-term health outcomes subsequent to management changes.

Setting
Patients requiring treatment for breast cancer are managed by an oncologist and are likely to be tested in an outpatient setting. Referral for genetic counseling is important for the
Technically Reliable

The HERmark assay uses a proprietary, proximity-based platform (proximity ligation assay) to measure total human epidermal growth factor receptor 2 (HER2), HER2 protein (H2T) expression, and HER2 homodimers (H2D). Antibody binding to HER2 and other HER proteins releases fluorescent reporter tags (VeraTag, Monogram Biosciences). The proximity of a bound HER2 antibody to a different bound HER antibody indicates an H2T; proximity of 2 bound HER2 antibodies indicates an H2D. Quantification of fluorescence permits quantification of H2T and H2D.

The HERmark assay is currently commercially available only for quantification of H2T and H2D in breast cancer.5 HERmark assay uses VeraTag, a proprietary technology by Monogram Biosciences, for quantification of HER2 protein.

Shi et al (2009) conducted a study to characterize VeraTag assays for HER1 and HER2 protein expression and homodimerization in formalin-fixed, paraffin-embedded (FFPE) human cancer cell lines, and compare quantitative HER2 protein levels in breast tumor tissue with those measured by standard IHC methods.4 HER2 protein quantification was normalized to tumor area and compared with receptor numbers in 12 human tumor cell lines (determined by fluorescence-activated cell sorting and standard IHC) and to IHC categories in 170 human breast tumors.4 In contrast to conventional IHC test categories, HER2 protein levels determined by the VeraTag assay represent a continuous measurement over a dynamic range greater than 2 log10, and HER2 homodimer levels were consistent with crosslinking and immunoprecipitation results. VeraTag HER2 measurements of breast tumor tissue and cell lines correlated with standard IHC test categories (p<0.001), but the VeraTag HER2 expression levels overlapped with adjacent IHC categories indicating increased sample discrimination. The authors also performed reproducibility studies to assess variability within replicate samples. There was 10% to 20% observed variability for a single operator performing 10 separate VeraTag HER2 protein and HER2-HER2 homodimer assays. The normalization method was applied to large-scale testing by multiple operators, resulting in coefficients of variation ranging from 11% to 29%. A similar performance was observed for the HER2-HER2 homodimer assay.

Larson et al (2010) conducted analytic validation of HERmark assays measuring H2T and H2D expression in FFPE breast cancer tumors as well as cell line controls.6 The authors used breast cancer cell lines obtained from the American Type Culture Collection for this validation study. They evaluated the reproducibility (interassay variability) of the H2T and H2D assays by determining the variability between batches. A combination of 45 patient-derived FFPE samples and 12 cell line samples were grouped into 3 tumor sets and 1 cell line panel. Results were compared pairwise after each sample set was run in separate batches. Of the reportable values, 96% of the pairwise comparisons were within 2-fold in the H2T assay. For the H2D assay, 95% of the pairwise comparisons were within 2.2-fold. For the H2T assay, accuracy was assessed by measuring the HER2 total levels in 7 cell lines. Among 21 samples (3 samples for each of 7 cell lines), 100% (21/21 pairwise comparisons) of overall accuracy results matched expected results. Similarly, 100% (18/18 pairwise comparisons) of the results matched predicted H2D levels. Precision was evaluated by determining the variability within a single assay or batch. Measurements were done by testing 15 replicates per cell line per batch of 2 independent cell lines. For the H2T assay, 100% (210/210) pairwise comparisons were within 1.7-fold, and more than 95% of the pairwise comparisons were within 1.45-fold. For the H2D assay, 100% (210/210) of the pairwise comparisons were within 2.3-fold, and more than 95% of the pairwise comparisons were within 1.65-fold. For the H2T assay, 2 cell lines were selected for sensitivity assessment, and the samples were run in 1 batch of 15 samples, yielding 54 pairwise comparisons. All (54/54) of the pairwise comparisons were within set acceptance criteria. Similar sensitivity was reported for H2D assay.
Huang et al (2010) compared results of the HERmark assay with those of IHC and FISH centrally performed on 237 archived FFPE breast cancers. IHC had already been performed at the time of initial diagnosis in all of the cases but was repeated for this validation, and interpreted by 1 reviewer and scored as negative, equivocal, or positive use the American Society of Clinical Oncologists/College of American Pathologists guidelines. Reflex FISH for HER2 gene amplification had also been performed at the time of initial diagnosis on all 94 of the cases interpreted as 2+ by IHC. Repeat FISH was performed at the same laboratory and an overall evaluation performed by one pathologist. Of the 84 cases in the immunohistochemically negative subgroup, 80 (95%), 2 (2%), and 2 (2%) were classified as negative, equivocal, and positive by HERmark, respectively. Of the 101 cases in the immunohistochemically equivocal subgroup, 33 (32.7%), 31 (30.7%), and 37 (36.6%) were classified as negative, equivocal, and positive by HERmark, respectively. Of the 52 cases in the immunohistochemically positive subgroup, 1 (2%), 3 (6%), and 48 (92%) were classified as negative, equivocal, and positive by HERmark, respectively. Overall concordance was 67%, with a weighted κ of 63% (95% confidence interval [CI], 55% to 70%). When equivocal cases were excluded from the HERmark and IHC results, positive and negative concordance between HERmark and central IHC testing was 98%, and overall concordance was 98%, with a κ of 95% (95% CI, 89% to 100%).

Reflex FISH was performed on 94 breast cancers that had been determined as 2+ by IHC at the time of initial diagnosis. Variable H2T and H2D levels were correlated with corresponding results for the HER2/centromeric probe for chromosome 17 (HER2/CEP17) ratio. Of 94 cases that were 2+ by IHC, 62 (66%), 5 (5%), and 27 (29%) were determined at the same central laboratory as negative (<10.5), equivocal (10.5 ≤ H2T ≤ 17.8), and positive (>17.8) by FISH, respectively. (Units of H2T measurement are relative fluorescence [defined as relative peak area × illumination buffer volume] per mm² of the invasive tumor [RF/mm²].) Of 62 FISH-negative cases, 24 (39%), 21 (34%), and 17 (27%) were determined as negative, equivocal, and positive by HERmark, respectively. Of 5 FISH-equivocal cases, 1 (20%), 2 (40%), and 2 (40%) were determined as negative, equivocal, and positive by HERmark, respectively. Of 27 FISH-positive cases, 3 (11%), 6 (22%), and 18 (67%) were determined as negative, equivocal, and positive by HERmark, respectively.

Yardley et al (2015) conducted the retrospective, multicenter Collaborative Biomarker Study, which included women who had tumor tissues from routine surgical excision of invasive breast cancer between January 2000 and May 2005. A total of 232 FFPE breast cancer blocks were collected by the 11 multicenter Collaborative Biomarker Study sites. The study was designed to enroll approximately 50% cases with positive HER2 status and 50% with negative HER2 status as determined by study sites based on the combination of all available local HER2 results (IHC, FISH) for each case. H2T results by HERmark assay were determined, while testers were blinded to local HER2 results and other clinical information. The continuous H2T results were categorized as HERmark negative, HERmark equivocal, and HERmark positive with predefined H2T analytic cutoff values (<10.5, 10.5 to 17.8, and >17.8 RF/mm², respectively). The overall concordance of all 2 categories (negative, equivocal, positive) between HERmark and routine HER2 testing was 68% (κ=0.475) for local IHC, 69% (κ=0.481) for central IHC, and 73% (κ=0.510) for local HER2 status. After equivocal cases were excluded from all HER2 results, the concordance of positive and negative values between HERmark and routine HER2 testing was 84% (κ=0.676) for local IHC, 96% (κ=0.914) for central IHC, 85% (κ=0.705) for local FISH, and 84% (κ=0.682) for local HER2 status.

**Section Summary: Technically Reliable**

Existing evidence from validation studies has demonstrated that the HERmark assay sensitively and reproducibly measures quantitative H2T and H2D protein expression levels. Multiple retrospective studies have reported high agreement between HERmark test results and IHC and FISH findings.

**Clinically Valid**

Retrospective studies, discussed below, have reported on the association between H2T levels and survival outcomes (see Table 1).
## Table 1. Summary of Studies of HERmark Clinical Validity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cutoffs Used</th>
<th>Result</th>
<th>Favored Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bates et al (2011)</td>
<td>Low: &lt;13.8</td>
<td>Group with intermediate H2T levels experienced longest TTP and OS</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>High: &gt;68.5</td>
<td></td>
<td></td>
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<tr>
<td>Joensuu et al (2011)</td>
<td>NA</td>
<td>Patients with HER2-positive breast cancer with very high tumor HER2 content may benefit less from adjuvant trastuzumab than those whose cancer has more moderate HER2 content</td>
<td>● ●</td>
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<tr>
<td></td>
<td>≥125.4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toi et al (2010)</td>
<td>Low: &lt;median H2T</td>
<td>• Patients with higher H2T values (&gt;75% percentile) lived longer than those with lower H2T values in the high HER2-expressing group</td>
<td>● ●</td>
</tr>
<tr>
<td></td>
<td>High: ≥median H2T</td>
<td>• Patients with lower H2T values lived longer than those with higher H2T values in the low HER2-expressing group</td>
<td></td>
</tr>
<tr>
<td>Lipton et al (2010)</td>
<td>Low: &lt;13.8</td>
<td>Better response to trastuzumab at higher levels of HER2 total expression observed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High: &gt;68.5</td>
<td>Low H2T and high H2T correlated with shorter PFS and OS</td>
<td>●</td>
</tr>
<tr>
<td>Duchnowska et al (2012)</td>
<td>&lt;58c</td>
<td>Correlation between continuous H2T level and TTBM confirmed on multivariate analysis</td>
<td>● ●</td>
</tr>
<tr>
<td>Barros et al (2014)</td>
<td>Low: &lt;median heterodimer studied</td>
<td>Low heterodimer levels favored among unselected patients; no association among trastuzumab-treated or -naive HER2-positive patients</td>
<td>● ●</td>
</tr>
<tr>
<td>Yardley et al (2015)</td>
<td>Low: &lt;13.8</td>
<td>High H2T associated with significantly shorter OS vs low H2T</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>High: ≥13.8</td>
<td></td>
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</tbody>
</table>

HER2: human epidermal growth factor receptor 2; H2T: HER2 protein; Int: intermediate; NA: not applicable; OS: overall survival; PFS: progression-free survival; TTBM: time to brain metastasis; TTP: time to progression.

* Cutoff for very high H2T (≥22-fold the median H2T of cancers HER2-negative by chromogenic in situ hybridization [5.7]).
* Absolute values not reported.
* Median H2T level. H2T value of 50 was a better discriminator (smaller p value in Cox models).

Bates et al (2011) measured H2T in FFPE primary breast tumors from 98 women treated with trastuzumab-based therapy for metastatic breast cancer. Using subpopulation treatment effect pattern plots, the population was divided into H2T low (H2T <13.8), H2T high (H2T ≥68.5), and H2T intermediate (13.8 ≤H2T<68.5) subgroups. Kaplan-Meier analyses were used to compare groups for time-to-progression (TTP) and overall survival (OS). Cox multivariate analyses were used to identify correlates of clinical outcome. Bootstrapping analyses were used to test the robustness of results. TTP improved with increasing H2T until, at the highest levels of H2T, an abrupt decrease in the TTP was observed. Kaplan-Meier analyses demonstrated that patients with H2T low tumors (median TTP, 4.2 months; hazard ratio [HR], 3.7; p<0.001) or H2T high tumors (median TTP, 4.6 months; HR=2.7; p=0.008) had significantly shorter TTP than patients whose tumors were H2T intermediate (median TTP, 12 months). OS analyses yielded similar results. The authors concluded that patients with high levels of H2T may represent a subgroup with de novo resistance to trastuzumab but that these results were preliminary and required confirmation in larger controlled clinical cohorts.

Joensuu et al (2011) reported results of measurement of H2T using HERmark from FFPE tumor tissue of 899 (89%) women who participated in the FinHer trial (ISRCTN76560285) to determine if very high tumor H2T content influences outcome in early breast cancer treated with adjuvant trastuzumab plus chemotherapy. Using chromogenic in situ hybridization (CISH) test, 197 (21.9%) patients had HER2-positive cancer and were randomized to trastuzumab or control. Tumor H2T levels varied greatly, by 1808-fold. High H2T levels strongly correlated with a positive HER2 status by CISH (p<0.001). Patients with very high H2T (defined by ≥22-fold the median of HER2-negative cancers [5.7; range, 0.4-118.4]; 13% of CISH-positive cancers) did not benefit from...
adjuvant trastuzumab (HR for distant recurrence, 1.23; 95% CI, 0.33 to 4.62; p = 0.75), whereas the remaining patients with HER2-positive disease by CISH (87%) did benefit (HR for distant recurrence, 0.52; 95% CI, 0.28 to 1.00; p = 0.050). The authors concluded that patients with HER2-positive breast cancer with very high tumor HER2 content may benefit less from adjuvant trastuzumab than those whose cancer has more moderate HER2 content.

Toi et al (2010) investigated the relation between H2T or H2D and OS in 72 patients drawn from 6 oncology clinics in Japan who had metastatic breast cancer and had been treated with at least 1 chemotherapy regimen before receiving trastuzumab. Patients were originally selected for treatment with trastuzumab using IHC (88%) or FISH (12%). HERmark assay results were correlated with OS using univariate Kaplan-Meier, hazard function plots, and multivariate Cox regression analyses. Clinical outcome data were drawn from medical chart review. Measurements of H2T and H2D were tested for association with OS, defined as the time from start of trastuzumab treatment to cancer-associated death or end of follow-up (median, 18.2 months). The median duration of trastuzumab treatment was 14.6 months. The 2-year OS rate was 60.8% (95% CI, 48.4% to 73.2%). In univariate analyses, patients were classified into 4 subgroups defined by the 25th, 50th, and 75th percentiles for each of the 3 variables, H2T, H2D, and their ratio, H2D/H2T. Hazard function plots were estimated in the 4 H2T subgroups, and subgroups with the 25% highest and lowest H2T values had a substantially lower risk of death than the middle 2 subgroups. Dividing the cohort into high HER2-expressing (greater than or equal to the median value of H2T) and low HER2-expressing (less than the median value of H2T) subgroups and using Cox regression with the continuous H2T variable in each of subgroup, patients with higher HER2 values had longer survival than those with lower H2T values in the high HER2-expressing group (HR = 0.06; 95% CI, 0.01 to 0.51; p = 0.010). In contrast, in the low HER2-expressing group, the opposite trend (those with lower H2T values were favored) was observed (HR = 16.0; 95% CI, 1.64 to 155.9; p = 0.017). The authors concluded that there were 2 subpopulations in this cohort that behaved differently with respect to HER2 expression and OS and that the quantitative amount of HER2 expression measured by HERmark may be a useful new marker to identify a more relevant target population for trastuzumab treatment in patients with metastatic breast cancer.

Lipton et al (2010) used the HERmark assay to quantify HER2 expression and examined outcomes in 102 trastuzumab-treated metastatic breast cancer patients previously assessed as IHC 3+ by local but not central IHC, or FISH-positive, and then retested by central FISH. Of 102 metastatic breast cancer patients previously scored as 3+ (IHC) or 2+ (FISH)-positive and treated with trastuzumab-containing regimens, 98 had both central FISH and H2T values. Sixty-six (87%) of 76 central FISH-positive patients had high H2T levels (discordant positive), and 19 (86%) of 22 central FISH-negative patients were H2T low (discordant negative). Three (14%) of 22 central FISH-negative patients were H2T high (discordant H2T high), and 10 (13%) of 76 central FISH-positive patients were H2T low (discordant H2T low). The discordant positive group had a significantly longer TTP (median, 11.3 months) compared with the concordant negative group (median, 4.5 months; HR = 0.42; p < 0.001), and also compared with the discordant H2T low group (median, 3.7 months; HR = 0.43; p = 0.01). The discordant H2T low group behaved similarly compared with discordant negatives (HR = 1; p = 0.99). In analyses restricted to central FISH-positive patients only (n = 77), Cox proportional hazards multivariate regression identified H2T as an independent predictor of TTP (HR = 0.29; p < 0.001) and OS (HR = 0.19, p < 0.001). The authors concluded that a subset of patients with HER2 gene amplification by FISH express low levels of HER2 protein and have reduced response to trastuzumab-containing therapy, similar to FISH-negative.

In a subsequent retrospective analysis of this cohort, Lipton et al (2013) examined progression-free survival (PFS) and OS in subgroups defined by expression of HER3 (H3T) and p95HER2 (p95), a truncated form of HER2 that does not bind trastuzumab and is a marker of trastuzumab resistance. HER3 and p95 were quantified using VeraTag platforms. Results of the H3T analysis were available for 89 patients; of these, 61 (69%) were H2T-high (>13.8). Within this group, median PFS was 12.1 months in patients with low H3T (≤3.5) and 5.0 months in patients with high H3T (>3.5;
HR=2.7; p=0.002). Median PFS in patients with low H2T (<13.8) was 4.2 months. No significant difference in OS was observed among any groups. In the exploratory analysis using regression tree analysis (recursive partitioning), the first split of the tree was based on an H2T cutoff of 16.1, separating patients with low HER2 expression (H2T <16.1) from those with high HER2 expression (H2T ≥16.1). Patients were next segregated by intermediate HER2 expression (16.1 ≤H2T ≤68.3) and very high HER2 expression (H2T >68.3). H2T cutoffs of 16.1 and 68.3 to define low, intermediate, and high groups were found to have greater discrimination. Median PFS (15.7 months) and OS (47.6 months) were longest in the subgroup characterized as H2T-intermediate (16.1 ≤H2T ≤68.3), H2T-low (≤3.89), and p95-low (≤3.75), compared with other groups (median PFS range, 4.0-7.8 months; median OS range, 23-27 months). In the entire group of HER2-positive, trastuzumab-treated patients, low (normal) H2T (≤16.1) and very high H2T (>68.3) were correlated with shorter PFS and OS. A subsequent study (2014) that used VeraTag to quantify p95HER2 found significantly shorter PFS and OS among H2T-positive, hormone receptor-positive, trastuzumab-treated patients with high p95, using a different cutoff of 2.8.\textsuperscript{18} This association was not found among hormone receptor-negative patients.

Han et al (2012) performed a similar retrospective analysis in 52 women with locally advanced or metastatic HER2-positive (3+ on IHC or gene amplification by FISH) breast cancer that had progressed after treatment with an anthracycline, a taxane, and trastuzumab.\textsuperscript{15} Patients were treated with lapatinib and capecitabine until disease progression or intolerance. Among all patients, median TTP was longer in patients with high H2T (>13.8; 5.0 months) than in patients with low H2T (<13.8; 1.8 months; p=0.047). However, a cutoff of 14.95 had greater discrimination (lower chi-square p value). Results were similar using this cutoff; median TTP in patients with high H2T (>14.95) was 5.2 months and in those with low H2T (<14.95), 1.8 months (p=0.018). No significant association was found between H2T levels and OS using either cut point. Among subgroups defined by H3T levels, median TTP was significantly longer (5.6 months) in patients with both high H2T (>14.95) and high H3T (>0.605) than in other groups (2.2 months; p=0.002).

Duchnowska et al (2012) investigated the correlation between H2T in primary breast cancers and time-to-brain metastasis (TTBM) in HER2-positive advanced breast cancer patients treated with trastuzumab.\textsuperscript{16} The patient sample included 142 consecutive patients who were administered trastuzumab-based therapy for HER2-positive metastatic breast cancer. HER2/neu gene copy number was quantified as the HER2/CEP17 ratio by central laboratory FISH. HER2 protein was quantified as H2T by the HERmark assay in FFPE tumor samples. HER2 variables were correlated with clinical features, and TTBM was measured from the initiation of trastuzumab-containing therapy. A higher H2T level (continuous variable) correlated with shorter TTBM, whereas HER2 amplification by FISH and a continuous HER2/CEP17 ratio were not predictive (p=0.013, 0.28, and 0.25, respectively). In the subset of patients that was centrally determined by FISH to be HER2-positive, an above-the-median H2T level (>58) was significantly associated with a shorter TTBM (HR=2.4, p=0.005), whereas this was not true for the median HER2/CEP17 ratio by FISH (p=0.4). The correlation between a continuous H2T level and TTBM was confirmed on multivariate analysis (HR=3.3, p=0.024). The authors concluded that their data revealed a strong relationship between quantitative HER2 protein expression level and risk for brain relapse in HER2-positive advanced breast cancer patients and that quantitative assessment of HER2 protein expression may inform and facilitate refinements in therapeutic treatment strategies for selected subpopulations of patients in this group.

Barros et al (2014) used proximity ligation assays to characterize specific HER2 heterodimers and their association with breast cancer-specific survival (BCSS) and disease-free interval.\textsuperscript{17} Tumor samples were from patients who had primary operable, invasive breast cancer at a single center in England. Among 1858 unselected patients, high levels of all 3 HER2 heterodimers (HER2/HER1 [EGFR], HER2/HER3, HER2/HER4) showed statistically worse BCSS and disease-free interval compared with low levels (range of HRs for BCSS, 0.62-0.66 [95% CI, 0.45 to 0.92]; p≤0.014; range of HRs for disease-free interval, 0.64-0.72 [95% CI, 0.47 to 0.98]; p≤0.037). Cut points were determined using X-tile, a graphical method that has been used in breast cancer research.\textsuperscript{19} However, among the subgroup of 224 patients who were HER2-positive by IHC and FISH,
associations between HER2 heterodimers and patient outcomes were not statistically significant, regardless of trastuzumab therapy. In a follow-up study, Green et al (2014) showed that HER2/HER3 heterodimers were significantly associated with shorter BCSS among unselected estrogen receptor-positive patients, but not among estrogen receptor-negative patients.20 Among the subset of HER2-positive patients, there was no association between HER2/HER3 heterodimers and BCSS in estrogen receptor-positive or-negative patients who had or had not received trastuzumab.

Yardley et al (2015) in their retrospective multicenter study examined the correlation of results obtained by various HER2 methods with OS of breast cancer patients.9 The authors used a predefined, published HERmark clinical cutoff (13.8 RF/mm²) to define H2T levels as H2T low and H2T high in OS analysis.13 Kaplan-Meier analysis was performed in cases that had HER2 testing results and available survival data (177 cases with local IHC, 188 cases with central IHC, 65 cases with local FISH, 190 cases with local HER2 status, 190 cases with HERmark). OS analysis revealed a significant correlation between shorter OS and HER2 positivity by local IHC (HR=2.6, p=0.016), central IHC (HR=3.2, p=0.015), and HERmark (HR=5.1, p<0.001) in this cohort of patients, most of whom received no HER2-targeted therapy. The OS curve of discordant low (HER2 positive but H2T low, 10% of all cases) was aligned with concordant negative (HER2 negative and H2T low, HR=1.9, p=0.444), but showed a significantly longer OS than concordant positive (HER2 positive and H2T high, HR=0.31, p=0.024). Conversely, the OS curve of discordant high (HER2 negative but H2T high, 9% of all cases) was aligned with concordant positive (HR=0.41, p=0.105), but showed a significantly shorter OS than concordant negative (HR=41, p<0.001).

Section Summary: Clinically Valid
Retrospective studies have reported an association between H2T levels and survival outcomes. However, for these analyses, different cut points were used and results.

Clinically Useful
Data on the clinical utility of HERmark are lacking. Clinical trials are needed to understand the relation between quantitative HER2 expression and homodimer measurements with clinical outcomes in breast cancer patients stratified by the HERmark assay receiving anti-HER2 therapy in the adjuvant and metastatic settings.

Summary of Evidence
For individuals who have breast cancer and are undergoing assessment of HER2 status who receive quantitative total HER2 protein expression and HER2 homodimer measurement, the evidence includes validation studies and retrospective analysis of the association between levels and survival outcomes. Relevant outcomes are overall survival, disease-specific survival, test accuracy, and test validity. Retrospective analysis using HERmark have shown that the assay may predict a worse response to trastuzumab in certain populations. However, findings have been inconsistent, and no clear association with clinical outcomes has been shown. Additionally, cut points for defining patient groups varied across studies. Clinical utility of the HERmark assay has not been demonstrated, and clinical trials are needed to determine the impact on clinical outcomes of patients stratified by the HERmark assay. The evidence is insufficient to determine the effects of the technology on health outcomes.

Supplemental Information
Practice Guidelines and Position Statements
National Comprehensive Cancer Network guidelines on the treatment of breast cancer (v.2.2017) do not address the use of HERmark.21

U.S. Preventive Services Task Force Recommendations
Not applicable.
Medicare National Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Palmetto GBA determines coverage and reimbursement for laboratories that perform molecular diagnostic testing and submit claims to Medicare in Medicare Jurisdiction E (California, Nevada, and Hawaii). Palmetto GBA’s decisions apply for all molecular diagnostic tests for Medicare. Palmetto GBA has assessed HERmark and determined that the test meets criteria for analytic and clinical validity and clinical utility as a reasonable and necessary Medicare benefit. Effective December 9, 2011, Palmetto GBA will reimburse HERmark services for patients with breast cancer.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in October 2017 did not identify any ongoing or unpublished trials that would likely influence this review.

Appendix

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.76

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>1c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
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</tr>
<tr>
<td>2a. Diagnostic</td>
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<tr>
<td>2b. Prognostic</td>
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</tr>
<tr>
<td>2c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
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</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
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</tr>
<tr>
<td>5. Reproductive testing</td>
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<tr>
<td>5a. Carrier testing: preconception</td>
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<tr>
<td>5b. Carrier testing: prenatal</td>
<td></td>
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<tr>
<td>5c. In utero testing: aneuploidy</td>
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<tr>
<td>5d. In utero testing: familial variants</td>
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</tr>
<tr>
<td>5e. In utero testing: other</td>
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<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
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</tbody>
</table>

References

2.04.76 Quantitative Assay for Measurement of HER2 Total Protein Expression and HER2 Dimers

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**Documentation for Clinical Review**

- No records required

**Coding**

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement.

**IE**

The following services may be considered investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>CPT®</td>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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<tr>
<td></td>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
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<tr>
<td>HCPCS</td>
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<tr>
<td>ICD-10</td>
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<tr>
<td>Procedure</td>
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<tr>
<td>Diagnosis</td>
<td>All Diagnoses</td>
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</tbody>
</table>

**Policy History**

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
<th>Reason</th>
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<tbody>
<tr>
<td>03/01/2016</td>
<td>BCBSA Medical Policy adoption</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>05/01/2017</td>
<td>Policy revision without position change</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>02/01/2018</td>
<td>Policy revision without position change</td>
<td>Medical Policy Committee</td>
</tr>
</tbody>
</table>

**Definitions of DecisionDeterminations**

**Medically Necessary**: A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

**Investigational/Experimental**: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation**: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.
Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.