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

Policy # 00321
Original Effective Date: 11/16/2011
Current Effective Date: 12/18/2013

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Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

Based on review of available data, the Company considers the assessment of HER2 status by quantitative total HER2 protein expression and HER2 homodimer measurement to be **investigational.***

Background/Overview

Novel assays that quantitatively measure total HER2 protein expression and homodimers have been developed in an effort to improve the accuracy and consistency of HER2 testing.

The HER-family of receptor tyrosine kinases (EGFR/HER1, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4) plays a major role in the pathogenesis of many solid tumors. In approximately 25-30% of breast cancers, overexpression of HER2 has been linked to shorter disease-free and overall survival (OS), 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 disease-free and OS 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.

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. Immunohistochemistry provides a semiquantitative measure of protein levels (scored as 0, 1+, 2+ and 3+) and the interpretation may be subjective. Fluorescence in situ hybridization 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, it is not always representative of protein expression, and multiple studies have failed to demonstrate a relationship 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 show to experience better outcomes on trastuzumab than those scored negative by those assays, differences in the degree of expression or amplification by these methods have generally not been shown to discriminate between groups with different outcomes. Immunohistochemistry 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. 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 (CISH).

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Normally, HER2 activates signaling pathways by dimerizing with ligand-bound EGFR-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, Monogram Biosciences, South San Francisco, CA) was developed to quantify total HER2 expression and HER2 homodimers in formalin-fixed, paraffin-embedded tissue samples.

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)
The FDA does not regulate in-house or "home brew" tests for HER2, tests developed and used at unique or individual laboratory sites.

The HERmark assay has been validated according to the specifications prescribed by the Clinical Laboratory Improvement Amendments and is performed in a College of American Pathologists-certified clinical reference laboratory at Monogram Biosciences.

Rationale/Source

Introduction

Validation of biomarker assessment to improve treatment outcomes is a multistep process. In general, important steps in the validation process address the following:

- **Analytic validity**: measures technical performance, i.e., whether the test accurately and reproducibly detects the biomarker of interest.
- **Clinical validity**: measures the strength of the associations between the selected biomarkers and clinical status.
- **Clinical utility**: determines whether the use of specific biomarker assessments to guide treatment decisions improves patient outcomes such as survival or adverse event rate compared to standard treatment without genotyping.

Technical Performance of the Assay (Analytic Validity)

The HERmark assay uses a proprietary, proximity-based platform to measure H2T and H2D. Antibody binding to HER2 and other HER proteins releases fluorescent reporter tags (VeraTag™, Monogram Biosciences)‡. Proximity of a bound HER2 antibody to a different bound HER antibody indicates a HER2 protein (H2T); proximity of 2 bound HER2 antibodies indicates a HER2 homodimer (H2D). Quantification of fluorescence permits quantification of H2T and H2D.

The HERmark assay is currently commercially available only for quantification of HER2 total protein expression (H2T) and HER2 homodimers (H2D) in breast cancer. The company plans to validate use of the HERmark test to measure HER heterodimers. The company website indicates that assays currently are available for HER2:HER1 and HER2:HER3 heterodimers; these apparently are for use in drug development.

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HER2 protein quantification was normalized to tumor area and compared to 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. 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 $\log_{10}$, and HER2 homodimer levels were consistent with crosslinking and immunoprecipitation results.

Huang and colleagues compared results of the HERmark assay with those of IHC and FISH centrally performed at the Mayo Clinic in 237 archived formalin-fixed, paraffin- embedded (FFPE) breast cancers. IHC had already been performed at the time of initial diagnosis in all of the cases but was repeated for the purpose of this validation, and interpreted by one reviewer and scored as negative, equivocal, or positive according to the American Society of Clinical Oncologists/College of American Pathologists (ASCO/CAP) guidelines. Reflex FISH for *HER2* gene amplification had also been performed at the time of initial diagnosis on all 94 of the IHC 2+ cases. 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 IHC positive subgroup, 1 (2%), 3 (6%), and 48 (92%) were classified as negative, equivocal, and positive by HERmark, respectively. The overall concordance was 67%, with a weighted κ of 63% (95% confidence interval [CI]: 55-70%) calculated using the κ statistic. When equivocal cases were excluded from the HERmark and immunohistochemical results, positive and negative concordance between HERmark and central immunohistochemical testing was 98%. The overall concordance was 98%, with a κ of 95% (95% CI: 89-100%).

Reflex FISH was performed on 94 breast cancers that had been determined as 2+ immunohistochemically at the time of initial diagnosis. Variable H2T and H2D levels were correlated to corresponding results for the HER2/centromere 17 (HER2/CEP17) ratio. Of the 94 cases that were 2+ immunohistochemically, 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 x illumination buffer volume] per mm^2 of invasive tumor [RF/mm^2].) Of the 62 FISH-negative cases, 24 (39%), 21 (34%), and 17 (27%) were determined as negative, equivocal, and positive by HERmark, respectively. Of the 5 FISH-equivocal cases, 1 (20%), 2 (40%), and 2 (40%) were determined as negative, equivocal, and positive by HERmark, respectively. Of the 27 FISH-positive cases, 3 (11%), 6 (22%), and 18 (67%) were determined as negative, equivocal, and positive by HERmark, respectively.

<u>How well does HERmark predict subpopulation response to trastuzumab? (Clinical Validity)</u>
Bates and colleagues (2011) measured HER2 protein expression (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 (KM) analyses were carried out comparing the groups for time-to-progression (TTP) and OS. Cox multivariate analyses were carried out to identify correlates of clinical outcome. Bootstrapping analyses were carried out to test the robustness of the

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results. Time-to-progression 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; $p < 0.0001$) 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 require confirmation in larger controlled clinical cohorts.

Joensuu and colleagues (2011) reported the results of measurement of total HER2 content (H2T) using HERmark from formalin-fixed tumor tissue of 899 women (89%) who participated in the FinHer trial (ISRCTN76560285) to determine if very high tumor total HER2 content influences outcome in early breast cancer treated with adjuvant trastuzumab plus chemotherapy. In a CISH test, 197 (21.9%) patients had HER2-positive cancer and were randomly assigned to receive trastuzumab or control. Tumor H2T levels varied greatly, by 1,808-fold. High H2T levels strongly correlated with a positive HER2 status by CISH ($p < 0.0001$). 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: 0.33-4.62; $p = 0.75$), whereas the rest of the patients with HER2-positive disease by CISH (87%) did benefit (HR for distant recurrence: 0.52; 95% CI: 0.28-1.00; $p = 0.050$). The authors concluded that patients with HER2-positive breast cancer with very high tumor HER2 content may benefit less from adjuvant trastuzumab compared with those whose cancer has more moderate HER2 content.

Toi and colleagues (2010) investigated the relationship between quantitative measurements of HER2 expression (H2T) or HER2 homodimers (H2D) and OS in a clinic-based population of 72 patients drawn from 6 oncology clinics in Japan who had metastatic breast cancer and had been treated with at least one chemotherapy regimen prior to receiving trastuzumab. Patients were originally selected for treatment with trastuzumab by 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 the end of the follow-up period. The median duration of patient follow-up period was 18.2 months. The median duration of trastuzumab treatment was 14.6 months. As a whole, 2-year survival rate of the cohort was 60.8% (95% CI: 48.4-73.2%). In the 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 the subgroups with the 25% highest and lowest H2T values had substantially lower risk of death than the middle 2 subgroups. Dividing the cohort into high HER2-expressing (≥ the median value of H2T) and low HER2- expressing (< the median value of H2T) sub-groups and using the Cox regression analysis with the continuous H2T value in each of the 2 subgroups, patients with higher values for HER2 expression lived longer than those with lower values for H2T in the high HER2- expressing group (HR: 0.06; $p = 0.010$; 95% CI: 0.01-0.51). In contrast, in the low HER2-expressing group, the opposite trend (those with lower H2T values were favored) was observed (HR: 16.0; $p = 0.017$; 95% CI: 1.64-155.9). The authors concluded that their data suggest that there are 2 subpopulations of patients in this cohort that behave differently with respect to HER2 expression and OS and that the quantitative amount of HER2 expression measured by HERmark may be a new useful marker

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to identify a more relevant target population for trastuzumab treatment in patients with metastatic breast cancer.

Lipton and colleagues (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 IHC 3+ or 2+/FISH-positive and treated with trastuzumab- containing regimens, 98 had both central FISH and HER2 total expression values. Sixty-six of 76 central FISH-positive patients (87%) had high HER2 total expression levels (concordant positive), and 19 of 22 central FISH-negative patients (86%) were HER2 total expression low (concordant negative). Fourteen percent (3 of 22) of central FISH-negative patients were HER2 total expression high (discordant HER2 total expression high), and 13% (10 of 76) of central FISH-positive patients were HER2 total expression low (discordant HER2 total expression low). The concordant positive group had a significantly longer time to progression (TTP median: 11.3 months) compared with the concordant negative group (median TTP: 4.5 months; HR: 0.42; $p < 0.001$), and also compared with the discordant HER2 total expression low group (median TTP: 3.7 months; HR: 0.43; $p = 0.01$). The discordant HER2 total expression low group behaved similarly compared with concordant negatives (HR: 1; $p = 0.99$). In analyses restricted to central FISH-positive patients only ($n = 77$), Cox proportional hazards multivariate regression identified HER2 total expression as an independent predictor of TTP (HR: 0.29; $p = 0.0015$) 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 and colleagues (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 H3T analysis were available from 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 exploratory analysis using regression tree analysis (recursive partitioning), the first split of the tree was based on a H2T cutoff of 16.1, separating patients with low HER2 expression (H2T < 16) from those with high HER2 expression (H2T ≥ 16.1). The patients were next segregated by intermediate HER2 expression (16.1 ≤ H2T ≤ 68.3) versus 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), H3T-low (≤ 3.89), and p95 low (≤ 3.75), compared to other groups (median PFS 4.0-7.8 months; median OS 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.

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. Patients were treated with lapatinib and capecitabine until disease progression or intolerance. Among all patients, median TTP was longer in patients with high H2T (>

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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 and colleagues (2012) investigated the correlation between quantitative HER-2 expression in primary breast cancers and the time-to-brain metastasis (TTBM) in HER-2+ advanced breast cancer patients treated with trastuzumab. The study group included 142 consecutive patients who were administered trastuzumab-based therapy for HER-2+ metastatic breast cancer. HER-2/neu gene copy number was quantified as the HER-2/centromeric probe for chromosome 17 (CEP17) ratio by central laboratory FISH. HER-2 protein was quantified as total HER-2 protein expression (H2T) by the HERmark assay in formalin-fixed, paraffin-embedded tumor samples. HER-2 variables were correlated with clinical features and TTBM was measured from the initiation of trastuzumab-containing therapy. A higher H2T level (continuous variable) was correlated with shorter TTBM, whereas HER-2 amplification by FISH and a continuous HER-2/CEP17 ratio were not predictive (p = 0.013, .28, and .25, respectively). In the subset of patients that was centrally determined by FISH to be HER-2+, 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 HER-2/CEP17 ratio by FISH (p = 0.4). 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 the quantitative HER-2 protein expression level and the risk for brain relapse in HER-2+ advanced breast cancer patients and that quantitative assessment of HER-2 protein expression may inform and facilitate refinements in therapeutic treatment strategies for selected subpopulations of patients in this group.

Summary of Clinical Validity

Retrospective studies report an association between H2T levels and survival outcomes. However, for these analyses, different cut points are used and results are variable. (Table 1)

Table 1. Summary of studies of HERMark clinical validity

Reference	Cutoffs used		Result	Favored group		
	Low	High		Low	Intermediate	High
Bates 2011	< 13.8	> 68.5	The group with intermediate H2T levels experienced the longest TTP and OS.		✓	
Joensuu 2011	not applicable	≥ 125.4[a]	Patients with HER2-positive breast cancer with very high tumor HER2 content may benefit less from adjuvant trastuzumab compared with those whose cancer has more moderate HER2 content.	✓	✓	

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Toi 2010	< median H2T[b]	≥ median H2T[b]	Patients with higher H2T values (> 75%ile) lived longer than those with lower H2T values in the high HER2-expressing group. Patients with lower H2T values live longer than those with higher H2T values in the low HER2-	✓		✓
Lipton 2010	< 13.8	> 68.5	Better response to trastuzumab at higher levels of HER2 total expression was			✓
Lipton 2013	< 16.1	> 68.3	Low H2T and high H2T were correlated with shorter PFS and OS.		✓	
Han 2012	< 13.8	≥ 13.8	TTP was longer in patients with high H2T than in patients with low H2T		✓	✓
Duchnowska 2012	< 58[c]	≥ 58[c]	Correlation between a continuous H2T level and TTBM was confirmed on multivariate	✓	✓	

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

Clinical Utility

Data on the clinical utility of HERmark are lacking. Clinical trials are needed to understand the relationship 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.

Ongoing Clinical Trials

No current trials were identified at ClinicalTrials.gov using the search terms “HERMark” or “VeraTag.”

Summary

Retrospective analyses using HERmark have shown that the assay may predict a worse response to trastuzumab in certain populations. However, the findings are inconsistent, and no clear association with clinical outcomes has been shown. Additionally, cut points for defining patient groups varied across studies. The 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.

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Codes used to identify services associated with this policy may include (but may not be limited to) the following:

Code Type	Code
CPT	84999
HCPCS	No codes
ICD-9 Diagnosis	All diagnoses
ICD-9 Procedure	No codes

Policy History

Original Effective Date: 11/16/2011
Current Effective Date: 12/18/2013
11/03/2011 Medical Policy Committee review
11/16/2011 Medical Policy Implementation Committee approval. New policy.
11/01/2012 Medical Policy Committee review
11/28/2012 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
12/12/2013 Medical Policy Committee review
12/18/2013 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
Next Scheduled Review Date: 12/2014

*****Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
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