Comparison of Central HER-2 Tests with Quantitative HER-2 Expression and HER-2 Homodimer Measurements Using a Novel Proximity Based Assay

Weidong Huang, MD, PhD[1]; Monica Reinholz, PhD[3]; Jodi Weidler, PharmD[1]; Yolanda Lie, MS[1]; Yi-Chen Wu, MS[1]; Colombe Chappey, PhD[1]; Jeannette Whitcomb, PhD[2]; Wilma Lingle, PhD[3]; Robert Jenkins, MD, PhD[3]; Jeffrey S. Larson, PhD[2]; Yuping Tan, MS[2]; Michael Bates, MD[1]; Edith A. Perez, MD[4]. Departments of [1]Clinical Research and [2]Clinical Lab Operation, Monogram Biosciences, Inc., South San Francisco, Calif; [3]Department of Laboratory Medicine and Pathology, Mayo Clinic, Minn; [4]Department of Medicine, Mayo Clinic, Jacksonville, Fla.

Weidong Huang, MD, PhD, FCAP
Monogram Biosciences, Inc.
345 Oyster Point Blvd.
South San Francisco, CA 94080
weidong.huang@monogrambio.com

BACKGROUND

The accuracy and reliability of standard methods, immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), to assess HER-2 status has recently been a subject of debate(1). The best method to assess HER-2 status remains controversial. We have developed a novel assay (HERmark, Monogram Biosciences, Inc., South San Francisco, Calif.) that provides precise quantification of total HER-2 expression (H2T) and HER-2 homodimer (H2D) in formalin-fixed paraffin-embedded (FFPE) tissues(2,3). We compared H2T and H2D generated by the HERmark assay to HER-2 expression by IHC and HER-2 gene amplification by FISH performed in a central laboratory (Mayo Clinic, Minn.).

METHODS

FFPE Tissue Samples: 158 invasive breast cancer cases were selected for biomarker study at Mayo Clinic, Minn.

HERmark Assay: Novel Proximity Based Technology

H2T and H2D are detected through the release of a fluorescent tag ("VeraTag reporter") conjugated to a monoclonal antibody directed against the cytoplasmic domain of HER-2 (Ab8, LabVision). For the H2T assay, this antibody is paired with a biotinylated second antibody directed against the C-terminus of HER-2 (Ab15, Labvision), or with biotinylated Ab8 for the H2D assay. The "molecular scissors" (streptavidin-conjugated methylene blue) that is subsequently added and bound to the biotinylated antibody liberates singlet O_2 upon irradiation with red light. The release of VeraTag reporter molecules (Pro11, Fig. 1) requires proximity of the VeraTag antibody to a second HER-2 "scissors" antibody (proximity based assay). Signal quantified by capillary electrophoresis is normalized to tumor area on the FFPE tissue section. The continuous H2T results are also grouped as HERmark Negative, HERmark Equivocal, and HERmark Positive (Fig. 3.1).

HER-2 by central IHC and central FISH

IHC was performed using the HercepTest kit (DAKO, Carpinteria, Calif.) and the results were interpreted as Negative, Equivocal, or Positive according to the recent ASCO/CAP guidelines for HER-2 testing(1). FISH was performed using the PathVysion Her-2/neu probe kit (Vysis Inc, Downers Grove, IL). The HER-2 gene to chromosome 17 centromere (HER-2/cep17) ratio was calculated. Both IHC and FISH tests were performed in a central laboratory (Mayo Clinic, Minn.).

HER-2 by DASL assay

HER-2 gene expression was determined by the DASL (cDNA-mediated Annealing, Selection, extension and Ligation) assay (Illumina; San Diego, Calif.) in a central laboratory (Mayo Clinic, Minn.)(4).

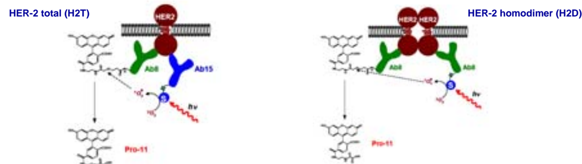

Figure 1: The principle of HERmark assay -- novel proximity based technology

A monoclonal antibody specific for a unique epitope of HER-2 is conjugated to a fluorescein VeraTag reporter (Pro11) or a molecular scissors (S) by means of a cleavable tether. The molecular scissors liberates singlet O_2 upon irradiation with red light. The free radicals cleave all thioether bonds in close proximity (within approximately 30-100 nM), releasing the "VeraTag reporter." The signal (Pro11) can then be collected and analyzed on a capillary electrophoresis (CE) array. Each VeraTag reporter is designed with a unique charge-mass ratio and can thus be identified and quantitated by comparison to assay standards. The standard unit of VeraTag measurement from tumor samples is relative peak area (RPA) x collection volume (uL)/ tumor area (mm^2).

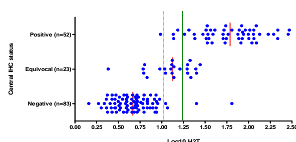

Figure 3.1: HER-2 expression by HERmark and central IHC

- HERmark Equivocal: defined by the two green vertical lines
- HERmark Negative: area to the left of the Equivocal zone
- HERmark Positive: area to the right of the Equivocal zone

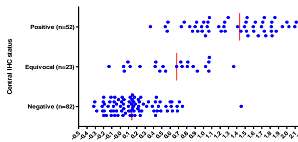

Figure 3.2: HER-2 homodimer by HERmark and HER-2 expression by central IHC

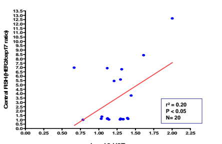

Figure 4.1: HER-2 expression by HERmark and HER-2/cep17 ratio by FISH

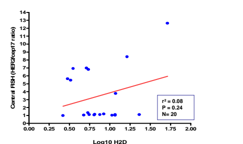

Figure 4.2: HER-2 homodimer by HERmark and HER-2/cep17 ratio by FISH

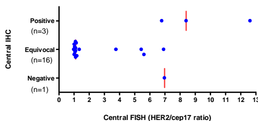

Figure 4.3: HER-2 expression by central IHC and HER-2/cep17 ratio by FISH

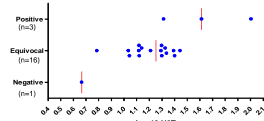

Figure 4.4: HER-2 expression by central IHC and HERmark

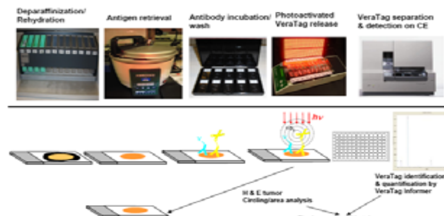

Figure 2: The workflow of HERmark assay in formalin-fixed paraffin embedded (FFPE) tissues

Table 1: Concordance of HER2 by central IHC and HERmark

	Central IHC Negative		Central IHC Equivocal		Central IHC Positive		Total
HERmark Status	N	%	N	%	N	%	
Negative	79	95%	6	26%	1	2%	86
Equivocal	2	2%	9	39%	3	6%	14
Positive	2	2%	8	35%	48	92%	58
Total	83		23		52		158

Table 2: HER2 concordance: Equivocal considered negative by both assays

	Central IHC Negative		Central IHC Positive		Total
HERmark Status	N	%	N	%	
Negative	96	91%	4	8%	100
Positive	10	9%	48	92%	58
Total	106		52		158

Table 3: HER2 concordance: Equivocal considered positive by both assays

	Central IHC Negative		Central IHC Positive		Total
HERmark Status	N	%	N	%	
Negative	79	95%	7	9%	86
Positive	4	5%	68	91%	72
Total	83		75		158

Table 4: HER2 concordance: Equivocal cases excluded by both assays

	Central IHC Negative		Central IHC Positive		Total
HERmark Status	N	%	N	%	
Negative	79	98%	1	2%	80
Positive	2	2%	48	98%	50
Total	81		49		130

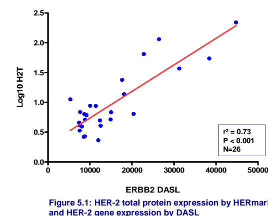

Figure 5.1: HER-2 total protein expression by HERmark and HER-2 gene expression by DASL

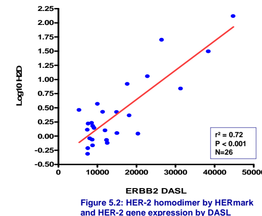

Figure 5.2: HER-2 homodimer by HERmark and HER-2 gene expression by DASL

RESULTS and DISCUSSION

- The VeraTag technology enables precise quantitation of protein expression and protein-protein complex in formalin-fixed, paraffin-embedded tissues.
- The HERmark assay accurately measures continuums of total HER-2 expression (H2T) and HER-2 homodimer (H2D) over a wide dynamic range (2-3 log).
- Total HER-2 expression by HERmark showed a high concordance with HER-2 IHC performed by a central laboratory.
- In a subset of cases (n=20, with 16 IHC Equivocal), there was a poor correlation between HERmark (H2T and H2D) and HER-2/cep17 ratio of central FISH. But, H2T by HERmark appeared to correlate better with central IHC as compared to FISH correlating with central IHC. More cases are currently being tested to further investigate this subset of cases.
- There was a good correlation between HERmark (H2T and H2D) and HER2 gene expression determined by DASL assay.
- The precise quantification of total HER-2 expression and the novel HER-2 homodimer measure may provide better predictive tests for targeted HER-2 therapy(5).

References:

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