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Quantitative measurements of HER2 expression and HER2 homodimers using a novel proximity based assay: comparison with HER2 status by immunohistochemistry and chromogenic in situ hybridization in the FinHer study

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BACKGROUND

The accuracy and reliability of current methods, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), to assess HER2 status has recently been a subject of debate (1). The best method to assess HER2 status remains controversial. We developed a novel assay (HERmark, Monogram Biosciences, Inc.) that provides precise quantification of total HER2 expression (H2T) and HER2 homodimer (H2D) in formalin-fixed paraffin-embedded (FFPE) tissues (2). We compared H2T and H2D generated by the HERmark assay to results of HER-2 tests by local IHC, central chromogenic in situ hybridization (CISH) and central IHC retesting of breast cancers from the FinHer study.

METHODS

FFPE Tissue Samples

899 invasive breast cancer cases of the FinHer study (Fig. 2) that had adequate invasive tumor tissues for the HERmark assay were included.

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 local IHC testing

HER-2 expression was initially determined by immunohistochemistry (IHC) according to the guidelines of each participating institution of the FinHer study, and recorded as HER-2 IHC negative or positive (3).

HER-2 by central CISH testing

When HER-2 IHC test was scored 2+ or 3+ (on a scale of 0, 1+, 2+, or 3+), the HER2/neu gene amplification status was determined by means of chromogenic in situ hybridization (CISH) in one of two central reference laboratories (3,4).

HER-2 by central IHC retesting

Central HER-2 IHC retesting was performed independently by the PhenoPath Laboratories (Seattle, WA), using the DAKO polyclonal A0485 and Envision + detection kit (5), and the results were interpreted as HER-2 Negative, Equivocal, or Positive according to the recent ASCO/CAP guidelines for HER-2 testing (1).

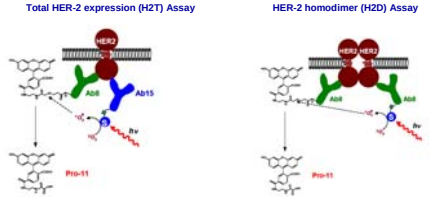

Figure 1: The principle of HERmark assay -- novel proximity based technology

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).

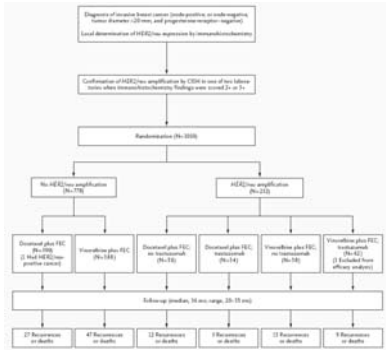

Figure 2.1: The design of the FinHer trial

CISH denotes chromogenic in situ hybridization, and FEC a regimen of fluorouracil, epirubicin, and cyclophosphamide.

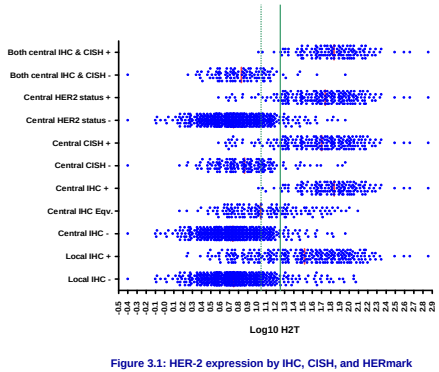

Figure 3.1: HER-2 expression by IHC, CISH, and HERmark

- 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

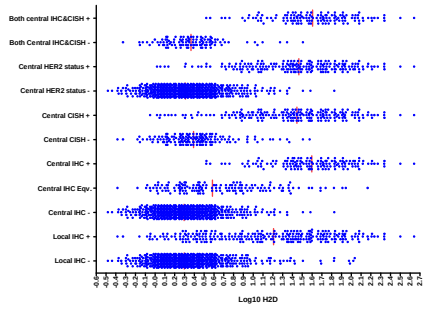

Figure 3.2: HER-2 homodimer (H2D) by HERmark

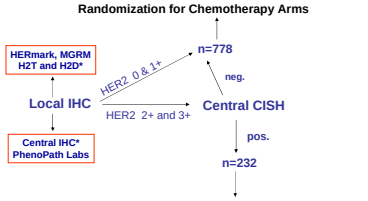

Figure 2.2: The FinHer study and the assessment of HER2 Status

*In addition to local IHC and central CISH, 899 cases that had adequate invasive tumor tissues were retrospectively tested by the HERmark assay and central HER-2 IHC.

Table 1: HER2 testing - HERmark assay comparison, 2x3

	Local IHC-	Local IHC+	Central CISH -	Central CISH +	Central HER2 Status -*	Central HER2 Status +*	Both Central IHC&CISH -	Both Central IHC&CISH +
HERmark Negative	523 (82%)	60 (23%)	165 (68%)	18 (8%)	536 (82%)	12 (6%)	82 (79%)	1 (1%)
HERmark Equivocal	74 (12%)	29 (11%)	35 (23%)	12 (6%)	86 (13%)	9 (5%)	22 (20%)	2 (2%)
HERmark Positive	41 (6%)	171 (64%)	14 (9%)	167 (85%)	31 (5%)	173 (89%)	6 (5%)	329 (98%)
Total	*638*	*260*	*154*	*197*	*653*	*194*	*110*	*332*
Reclassification**	23%		23%		16%		33%	

*Central HER2 status: Negative = Central IHC 0, 1+, or IHC equivocal+central CISH; Positive = Central IHC 3+, or IHC equivocal+central CISH+
**Reclassification: (1) IHC-/CISH negative, but HERmark Equivocal or Positive, and (2) IHC+/CISH positive, but HERmark Equivocal or Negative

Table 2: Concordance of HER2 by central IHC and HERmark, 3x3

	Central IHC Negative	Central IHC Equivocal	Central IHC Positive	Total
HERmark Negative	513 (84%)	70 (48%)	1 (1%)	584
HERmark Equivocal	73 (12%)	27 (18%)	3 (2%)	103
HERmark Positive	26 (4%)	49 (34%)	137 (97%)	212
Total	612	146	141	899
Concordance: 75%; (513+27+137)/899				

Table 3: HER2 concordance 2x2; Equivocal cases excluded by both assays

	Central IHC Negative	Central IHC Positive	Total
HERmark Negative	513 (95%)	1 (1%)	514
HERmark Positive	26 (5%)	137 (99%)	163
Total	539	138	677
Concordance: 96%; (513+137)/677			

Table 4: HER2 concordance 2x2, HERmark Equivocal excluded

	Central IHC&CISH Both Negative	Central IHC&CISH Both Positive	Total
HERmarkNegative	82 (93%)	1 (1%)	83
HERmark Positive	6 (7%)	129 (99%)	135
Total	88	130	218
Concordance: 97%; (82+129)/218			

RESULTS and DISCUSSION

- The VeraTag technology enables precise quantification of protein expression and protein-protein complexes in formalin-fixed, paraffin-embedded tissues.
- The HERmark assay accurately measures total HER-2 expression (H2T) and HER-2 homodimer (H2D) over a wide dynamic range (~3 logs).
- Total HER-2 expression by the HERmark assay correlated well with HER-2 tests by IHC and CISH performed independently by central laboratories.
- Higher concordance was observed between H2T by HERmark and HER-2 by more stringent central tests (central IHC, central CISH, and their combinations), as compared with local HER-2 IHC tests.
- In 13-23% of the cases, the HER-2 status (negative or positive, as defined by conventional IHC&CISH tests) was reclassified by the HERmark assay (HERmark negative, equivocal, or positive; Table 1). The clinical significance of such reclassification is currently under investigation (6,7).
- The precise quantification of total HER-2 expression and the novel HER-2 homodimer measurement may provide better predictive tests for targeted HER-2 therapy (6,7).

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