

Correlation of quantitative total HER2 expression and HER2 homodimers with histopathologic characteristics of breast cancers in the FinHer study

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BACKGROUND

We recently reported that the HERmark assay (Monogram Biosciences, Calif.) accurately measures the distributions of total HER2 expression (H2T) and HER2 homodimers (H2D) over a wide (~3 logs) dynamic range in formalin-fixed, paraffin-embedded (FFPE) tissues, and that a higher concordance was observed between H2T and HER2 assessed by more stringent central testing as compared with local HER2 testing by immunohistochemistry (Joensuu et al, 2008 SABCS).¹ In this follow-up analysis, H2T and H2D were correlated with histopathologic characteristics of breast cancers of the FinHer study.

METHODS

FFPE Tissue Samples
 899 invasive breast cancer cases from the FinHer study with adequate tumor content (Figure 2) were tested with HERmark. Histopathologic characteristics of the breast cancers, including HER2 status by immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH), estrogen receptor/progesterone receptor (ER/PR) status, Ki67, tumor grade, tumor size, and lymph node metastasis were provided by the FinHer study (Joensuu et al, *N Engl J Med* 2006).²

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 O₂ upon irradiation with red light. The release of VeraTag reporter molecules (Pro11, Figure 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 (Figure 4.1).

HER-2 by local IHC testing
 HER2 expression was initially determined by immunohistochemistry (IHC) according to the guidelines of each participating institution of the FinHer study, and recorded as HER2 IHC negative or positive.²

HER-2 by central CISH testing
 When HER2 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.^{4,5}

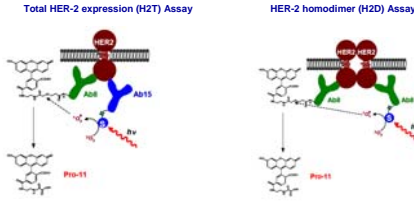


Figure 1: The principle of HERmark assay -- novel proximity based technology

A monoclonal antibody specific for a unique epitope of HER-2 is conjugated to a fluorescent VeraTag reporter (Pro11) or a molecular scissors (S) by means of a cleavable tether. The molecular scissors liberates singlet O₂ upon irradiation with red light. The free radicals cleave all the ether 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 quantified by comparison to assay standards. The standard unit of VeraTag measurement from tumor samples is relative peak area (RPA) x collection volume (μL) / tumor area (mm²).

Table 1. Characteristics of the Patients and Tumors at Baseline*

Variable	All Participants (N=1002)	Participants with HER2+ve (N=1002)	P	Participants with HER2+ve (N=1107)	ER/PR (N=1107)	ER/PR (N=1107)
Age, yr†						
Median	59.8	51.0	0.72	51.4	48.9	0.19
Range	25.65-87	26.9-86.8		25.65-86	27.54-84	
WHO performance status - no. (%)						
0	455 (91)	455 (90)	0.57	105 (91)	107 (90)	0.64
1	47 (9)	51 (10)		11 (9)	9 (8)	
No. of metastatic nodules only - no. (%)						
0	57 (11)	54 (11)	0.73	12 (10)	25 (22)	0.06
1-3	300 (60)	316 (62)		64 (55)	68 (58)	
≥4	47 (9)	51 (10)		10 (9)	10 (9)	
Distance of primary tumor - no. (%)						
≤ 9 cm	30 (6)	52 (10)		8 (7)	8 (7)	
> 9 cm	174 (35)	180 (36)		38 (33)	27 (23)	
< 2 cm	206 (41)	209 (41)		69 (59)	61 (52)	
Not available	2 (0)	1 (0)		1 (1)	0 (0)	
Histologic type - no. (%)			0.98†			0.51†
Ductal	300 (78)	308 (78)		109 (91)	109 (98)	
Lobular	99 (25)	99 (25)		10 (8)	11 (10)	
Other	13 (3)	12 (3)		0 (0)	2 (2)	
Distance of primary tumor - no. (%)			0.39†			0.51†
1	76 (15)	74 (15)		2 (2)	3 (3)	
2	186 (37)	211 (42)		39 (34)	33 (28)	
3	214 (44)	201 (40)		73 (63)	77 (66)	
Not available	26 (5)	22 (4)		2 (2)	3 (3)	
Stromal type - no. (%)			0.50			0.36
Positive	358 (71)	372 (72)		58 (50)	51 (44)	
Negative	144 (29)	136 (27)		35 (30)	35 (30)	
Progesterone receptor status - no. (%)			0.35			0.13
Positive	283 (56)	301 (59)		45 (39)	34 (29)	
Negative	219 (44)	207 (41)		71 (61)	82 (71)	
HER2/neu amplification - no. (%)			0.67			1.00
Absent	389 (77)	388 (78)		0 (0)	0 (0)	
Present	113 (23)	118 (24)		113 (97)	118 (100)	

*Whenever any test used 100, because of rounding. WHO denotes World Health Organization.
 †The P value for the discrete type as compared with binomial and other types.
 ‡The P value for the grade 1 or 2 as compared with grade 0.
 §New cases who had a tumor with HER2/neu amplification did not participate in randomization for treatment.

(Adapted from Joensuu et al, *N Engl J Med* 2006).²

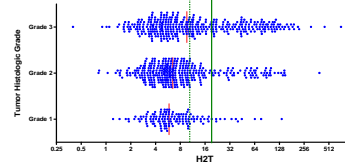


Figure 3.1: Total HER2 expression (H2T) and tumor histologic grade

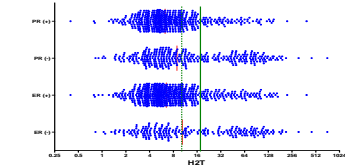


Figure 4.1: Total HER2 expression (H2T) and ER/PR status

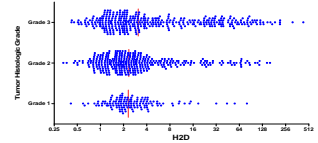


Figure 3.2: HER2 homodimers (H2D) and tumor histologic grade

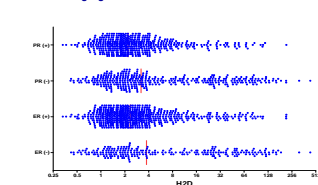


Figure 4.2: HER2 homodimers (H2D) and ER/PR status

Table 2. Distribution of Clinicopathologic Features in the Three HERmark Categories

Characteristic	HERmark Negative		P [†]	HERmark Equivocal		P [†]	HERmark Positive		P [†]
	No.	%		No.	%		No.	%	
Age, years			0.99			0.99			0.99
≤ 50	246	42.1		51	49.5		102	48.1	
> 50	338	57.9		52	50.5		110	51.9	
Histologic type			0.99			0.60			< 0.0001
Invasive ductal carcinoma	427	71.1		82	79.6		192	90.6	
Invasive lobular carcinoma	146	25.0		18	17.5		16	7.5	
Others	11	1.9		3	2.9		4	1.9	
Tumor grade			0.99			0.2			< 0.0001
1	108	19.4		15	15.3		13	6.2	
2	251	45.1		37	37.8		65	31.1	
3	198	35.5		46	46.9		131	62.7	
Tumor size, cm			0.72			0.99			0.99
≤ 2	248	43.1		56	54.9		89	42.6	
> 2	328	56.9		46	45.1		120	57.4	
Lymph node status			0.99			0.99			0.34
Negative	50	8.6		11	10.7		31	14.6	
Positive	534	91.4		92	89.3		181	85.4	
Estrogen receptor status			0.99			< 0.0001			< 0.0001
Negative	122	20.9		20	19.4		102	48.1	
Positive	462	79.1		83	80.6		110	51.9	
Progesterone receptor status			0.99			< 0.0001			< 0.0001
Negative	200	34.3		37	35.9		137	64.6	
Positive	383	65.7		66	64.1		75	35.4	
Ki 67			0.99			0.0015			< 0.0001
Negative (≤ 10% positive nuclei)	180	34.5		25	20.0		21	10.6	
Positive (> 10% positive nuclei)	342	65.5		58	60.9		177	89.4	
HER2 status			0.0024			< 0.0001			< 0.0001
Negative	566	96.9		91	88.3		45	21.2	
Positive	18	3.1		12	11.7		167	78.9	

†P values given are the results of a chi-square test, corrected for multiple testing by the Bonferroni method.
 ‡HERmark negative versus HERmark equivocal
 §HERmark equivocal versus HERmark positive
 ¶HERmark positive versus HERmark negative

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 HER2 expression (H2T) and HER2 homodimer (H2D) over a wide dynamic range (~3 logs).

• Total HER2 expression by the HERmark assay significantly correlated with HER2 status assessed by IHC and CISH in the FinHer study. However, reclassification of HER2 status by HERmark was observed in 18.5% (166/899) of the cases (reclassification defined as (1) HER2 negative, but HERmark Equivocal or Positive; and (2) HER2 positive, but HERmark Equivocal or Negative).

• HERmark positive breast cancers were significantly associated with invasive ductal carcinoma, high tumor grade, estrogen/progesterone receptor negativity, and Ki67 positivity. The quantitative HER2 measurement confirms the known correlations between HER2 expression and histopathologic characteristics of breast cancer.

• The predictive ability of precise quantification of total HER2 expression and HER-2 homodimer levels is under active investigation.⁶

References:

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Figure 2: The design of the FinHer study
 CISH denotes chromogenic in situ hybridization, and FEC a regimen of fluorouracil, epirubicin, and cyclophosphamide.

(Adapted from Joensuu et al, *N Engl J Med* 2006).²