

# Discordant HER2 total and HER2 homodimer levels by HERmark analysis in matched primary and metastatic breast cancer

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## INTRODUCTION

- HER-2/neu is over-expressed in approximately 25% of primary invasive breast cancers.
- HER2 assay of tumor tissue is a critical step for selection of patients for HER2-targeted treatment (trastuzumab, lapatinib).
- We have previously reported that quantitative measurements of HER2 total (H2T) and HER2 homodimers (H2D) protein expression using the HERmark assay identifies sub-populations of "HER2-positive" patients (by IHC and/or FISH) that have different clinical outcomes on trastuzumab (Leitzel et al, ASCO 2008; Lipton et al, SABCS 2008).
- Previous studies report up to a 20% discordance in HER2 status using conventional IHC or FISH analysis between the primary and metastatic breast tumors.
- In this study, we correlated HER2 total and homodimer levels in 27 matched primary and metastatic breast tumor tissues from the same patient.

## METHODS

### HER Family HERmark Assays

- Proximity-based assays that quantify HER2 total (H2T) and HER2 homodimer (H2D) protein expression in formalin-fixed, paraffin-embedded (FFPE) specimens.
- Each assay employs two monoclonal antibodies that minimize background and enhance specificity.
- 7-10 times more sensitive than IHC.
- Validated according to CLIA specifications.
- 27 patients had matched primary and metastatic FFPE tumor sections (5 microns) tested in the HERmark assay to quantify and compare their H2T and H2D expression levels.

### DEMOGRAPHICS

- FFPE tissues were available from 27 primary breast cancers and metachronous metastatic sites.
- Metastatic lesions included 7 skin, 5 lymph node, 3 bone, 3 pleura, 2 brain, 2 chest wall, and 5 other soft tissue lesions.
- The median elapsed time between matched primary and metastatic sites was 71 mo. (range 9-137 mo).
- During the time period between the primary specimen harvest and the metastatic biopsy, patients were treated as follows:
  - 6 were treated with chemotherapy alone
  - 10 received hormonal therapy without trastuzumab
  - 3 received trastuzumab
  - 3 received no treatment
  - 5 treatment was unknown

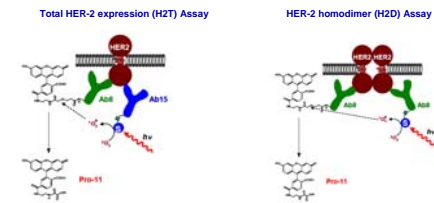


Figure 1: The principle of HERmark assay – novel proximity based technology

A monoclonal antibody specific for a unique epitope of HER-2 is conjugated to a fluorescein VeraTag reporter (Pro11) or a molecular scissor (S) by means of a cleavable tether. The molecular scissors liberates single O<sub>2</sub> 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 (μL) tumor area (mm<sup>2</sup>).

## HERmark Assay Workflow: H2T, H2D

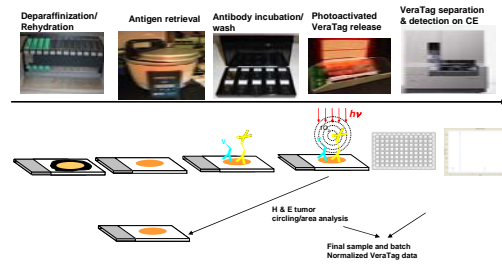


Figure 2: The workflow of the HERmark assay on FFPE tissue

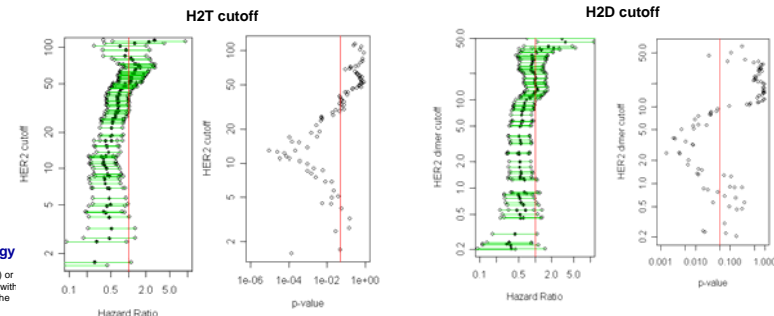


Figure 3: H2T and H2D cutoff identification by TTP positional scanning

Clinical cutoffs for H2T and H2D were chosen by positional scanning analysis and selection of the cutoff associated with the lowest p-value for TTP in a study of 101 metastatic breast cancer patients treated with 1st-line trastuzumab-containing therapy (Lipton et al, SABCS 2008). For H2T, this cutoff was 13.8. For H2D, the selected cutoff was 2.95.

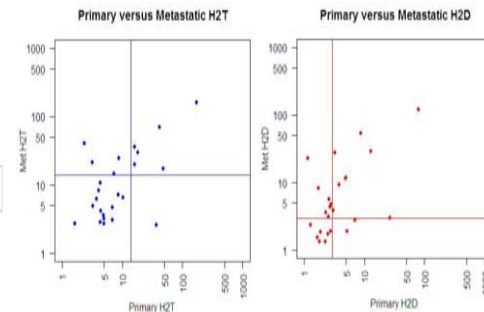


Figure 4: H2T and H2D in primary breast cancer versus metastatic site

## RESULTS and DISCUSSION

- For the whole population, there was a weak to moderate positive correlation between primary and metastatic cancers with H2T ( $r^2=0.36$ ,  $p<0.001$ ) and for H2D ( $r^2=0.27$ ,  $p<0.006$ ).
- Using the optimized time to progression (TTP) positional scanning cutoff for H2T defined previously:
  - 4/20 patients (20%) converted from low to high H2T.
  - 1/7 (14%) converted from high to low H2T.
- Using the H2D cutpoint:
  - 7/15 patients (47%) converted from low to high,
  - 3/12 (25%) converted from high to low H2D.
- Overall discordance between primary and metastatic sites was 19% for H2T, and 37% for H2D.

- The substantial HER2 total and HER2 homodimer discordance observed between matched primary and metachronous metastatic breast cancer sites needs confirmation in a larger cohort. If confirmed, patients with elevated HERmark levels in metastatic breast cancer sites should be evaluated in clinical trials for response to HER2-directed therapy.

### Acknowledgements

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### References

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Table 1: Correlation between primary and metastatic H2T measurements

	Metastatic tumor	
	Low H2T N (%)	High H2T N (%)
Primary tumor	16 (80)	4 (20)
High H2T	1 (14)	6 (86)
Total	17	10

Table 2: Correlation between primary and metastatic H2D measurements

	Metastatic tumor	
	Low H2D N (%)	High H2D N (%)
Primary tumor	8 (53)	7 (47)
High H2D	3 (25)	9 (75)
Total	11	16

Overall H2D discordance: 37%  
Overall discordance was defined as (4+1)/27