Quantitative measurements reveal elevated levels of HER2-HER3 heterodimers in brain metastases compared to matched primary breast cancers

CAP17 #138

Renata Duchnowska[1], Weidong Huang[2], Gerald Wallweber[2], Jeff Sperinde[2], Tomasz Trojanowski[3], Tomasz Mandat[4], Anna Kowalczyk[5], Bogumiła Czartoryska Arłukowicz[6], Barbara Radecka[7], Bozena Jarosz[3], Wojciech Biernat[5], Jacek Jassem[5].

[1]Military Institute of Medicine, Warsaw, Poland, [2]Monogram Biosciences, Laboratory Corporation of America® Holdings, South San Francisco, CA, [3]Medical University of Lublin, Lublin, Poland, [4]Institute of Oncology, Warsaw, Poland, [5]Medical University of Gdansk, Gdansk, Poland, [6]Białystok Oncology Center, Białystok, Poland, and [7]Opole Oncology Center, Opole, Poland.

I. Abstract

Context: The HER2-HER3 heterodimer (H23D) is recognized as the most potent initiator of HER2 mediated cell signaling. Limited data exists on H23D levels in primary or metastatic tumor tissues, primarily due to a lack of quantitative assays. Using a novel dual-antibody, proximity-binding immunoassay platform (VeraTag®, Monogram Biosciences, South San Francisco, California), we describe the first characterization of H23D levels in primary breast cancers and matched brain metastases.

Design: H23D levels were measured in 75 formalin-fixed, paraffin-embedded (FFPE) primary breast cancers and matched brain metastases using the VeraTag H23D assay. HER2 levels were measured by conventional immunohistochemistry and the HERmark® Breast Cancer Assay (Monogram Biosciences).

Results: H23D levels spanned nearly a 2-log10 dynamic range. H23D levels were significantly higher in matched brain metastases than in primary breast tumors (Wilcoxon p=0.0065), implicating H23D signaling in brain metastasis. H23D levels correlated significantly with HER2 levels as measured by HERmark in both primary breast tumors (Spearman r = 0.5841, p<0.0001) and brain metastases (Spearman r = 0.6724, p<0.0001). The correlation between H23D and HER2 based on immunohistochemistry category was significant in brain metastases (Jonckheere p<0.0001), and trended toward significance in primary breast tumors (Jonckheere p=0.1744).

Conclusions: Significantly higher levels of H23D in brain metastases implicate H23D signaling in these lesions, and suggest that H23D levels may serve as a clinically useful biomarker for HER2-targeted therapies. The evaluation of H23D in additional cohorts and its clinical significance is warranted.

II. VeraTag® Assay Workflow

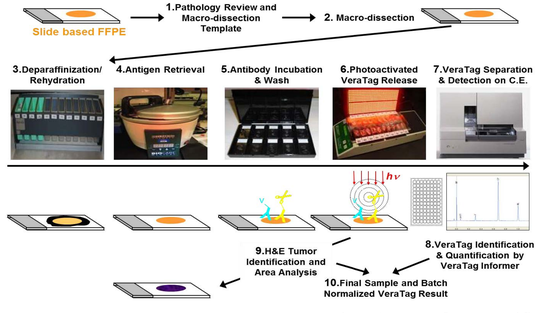


Figure 1 : VeraTag® Assay Workflow

III. Quantitative VeraTag Assays

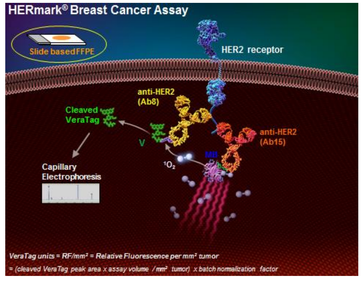


Figure 2A: HERmark® Breast Cancer Assay

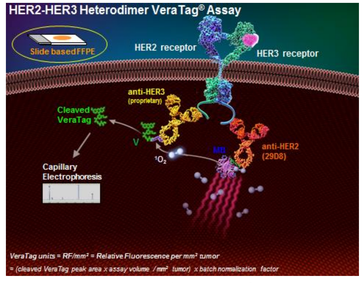
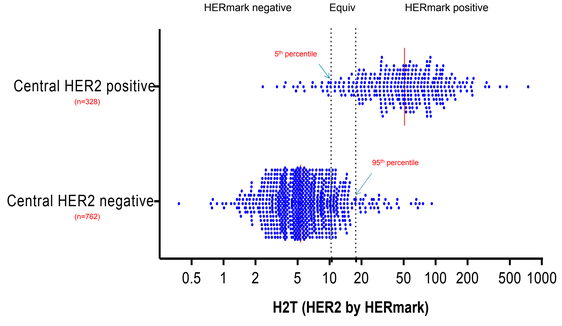


Figure 2B: HER2-HER3 Heterodimer VeraTag® Assay

Figure 2: Quantitative VeraTag Assays. Total HER2 protein expression (H2T) was measured using the HERmark assay (Fig. 2A) as previously described (Am J Clin Pathol 134:303, 2010). H2T was quantified through the release of a fluorescent tag (V) conjugated to a HER2 monoclonal antibody (Ab-8; Thermo Fisher). The antibody was paired with a biotinylated second HER2 monoclonal antibody (Ab15; Thermo Fisher). V reporters were cleaved by singlet O_2 ($^1O_2$), produced by an avidin-linked photosensitizer molecule upon illumination with red light. The cleaved VeraTag, quantified by capillary electrophoresis, was normalized to invasive tumor area on the formalin-fixed, paraffin-embedded (FFPE) tissue section. Similarly, the HER2-HER3 heterodimer (H23D, Fig. 2B) was quantified using an antibody proximity pair of VeraTag conjugated to HER3 B9A11 (proprietary, Monogram Biosciences), and HER2 monoclonal antibody (29D8; Cell Signaling) with a biotinylated goat anti-rabbit secondary antibody as previously described (Clin Cancer Res. 2015 Oct 15;21(20):4597-606).

IV. HER2 Classification by HERmark

Total HER2 expression (H2T) by the HERmark assay was measured in 1,090 breast cancers that had been previously determined centrally as negative or positive. Cutoffs for HERmark negative, equivocal and positive were taken from the 95th percentile of the central HER2 negatives and the 5th percentile of the central HER2 positives. Adapted from *Huang et. al. Am J Clin Pathol 2010: 134:303-311.*

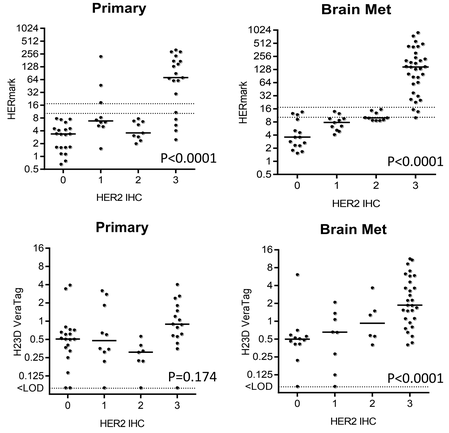


V. Patient characteristics

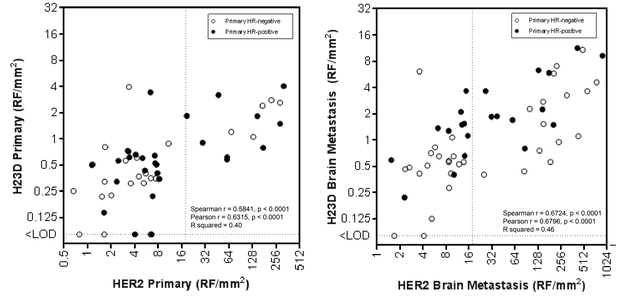
Patient Characteristic	N (%)
Age at Diagnosis	
Median	47
Range	27-76
Histologic Type	
Invasive ductal carcinoma	61 (81)
Invasive lobular carcinoma	6 (8)
Others	8 (11)
Histologic Grade	
1	4 (5)
2	29 (39)
3	38 (51)
Unknown	4 (5)
Estrogen Receptor (ER)	
ER(+)	34 (45)
ER (-)	41 (55)
Progesterone Receptor (PR)	
PR(+)	28 (37)
PR (-)	46 (61)
Unknown	1 (1)
Hormone Receptor (HR)*	
HR(+)	35 (47)
HR (-)	39 (52)
Unknown	1 (1)
Number of Brain Metastases	
1	46 (61)
2-3	17 (23)
>3	4 (5)
Unknown	8 (11)

Table 1. Seventy five (75) formalin-fixed, paraffin-embedded primary breast cancers and matched brain metastases were included in the study. Histologic type, histologic grade and hormone receptor status are of the primary tumors.
*Hormone receptor positive was defined as either ER or PR positive.

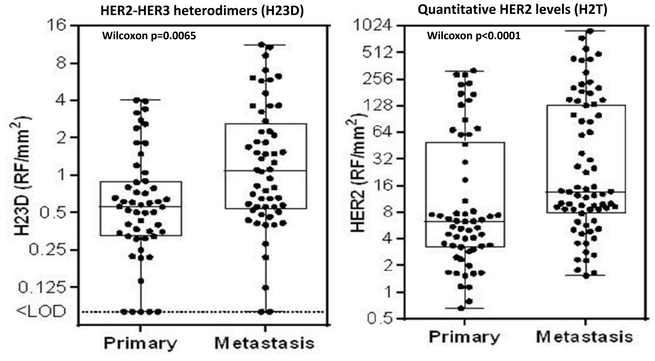
VI. VeraTag assays vs. HER2 IHC



VII. HER2-HER3 heterodimer vs. quantitative HER2



VIII. Brain metastasis vs. matched primary



IX. Conclusions

- **The H23D VeraTag assay quantified HER2-HER3 heterodimer (H23D) levels in FFPE tissues of primary breast cancer and matched brain metastasis with nearly a 2-log10 dynamic range .**
- **Significantly higher levels of H23D in brain metastases suggest that H23D signaling may play an important role in brain metastasis of breast cancer.**
- **Clinical studies on H23D expression as a clinically useful biomarker for HER2-targeted therapies are currently on going.**