

Quantitative measurement of HER3 total protein (H3T) and association with clinical outcome in HER2 positive metastatic breast cancer patients treated with trastuzumab

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Introduction

>The heterodimerization of HER3, with HER2 and the subsequent activation of the AKT pathway, has been implicated in both regulation of tumor cell growth and in resistance to HER2 targeted therapies such as trastuzumab.

>Currently available methods of measuring H3T in formalin-fixed, paraffin-embedded (FFPE) samples are insufficiently sensitive or specific; thus the impact of quantitative measurement of H3T expression on clinical response to trastuzumab has not been evaluated.

>We have developed a highly quantitative, accurate, precise, sensitive, and reproducible H3T assay in FFPE samples based on the VeraTag™ technology platform.

>Quantitative levels of HER2 total protein (H2T) and H3T expression were determined by the VeraTag technology in 81 tumors from patients with trastuzumab-treated metastatic breast cancer. A H2T cutoff derived by positional scanning analysis¹ was used to sub-divide the patients into HER2-normal (N=26, median TTP = 4.1 months) and HER2-overexpressing (N=55, median TTP = 11.1 months, HR=0.43, p=0.0002) groups

>In the HER2-overexpressing group, high H3T expression, as defined by a positional scanning cutoff analysis, correlated with shorter median time to progression (N=25, median TTP = 6.1 months, HR=2.7, p=0.0002) compared with low H3T expression (N=30, median TTP=13.1 months)

>In order to enhance the performance characteristics of the H3T assay even further, significant improvements in the assay sensitivity (down to ~1000 receptors/cell) and dynamic range (30-fold increase) are incorporated and are being evaluated in clinical samples.

1. VeraTag™ Technology

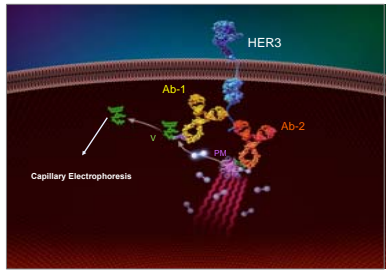


Figure 1. The H3T assay measurement comprises a dual antibody format, one VeraTag conjugated (Ab-1) and one biotin conjugated (Ab-2). Antibodies are bound to H3T. Streptavidin-methylene blue is added to FFPE slide, the slide is illuminated resulting in single O2 release and subsequent cleavage of VeraTag (V) in close proximity. VeraTag reporters are quantified on Capillary Electrophoresis (CE).

¹Leitzel K, et al. Oral presentation, ASCO 2008.

2. HER3 Total Protein Assay Workflow

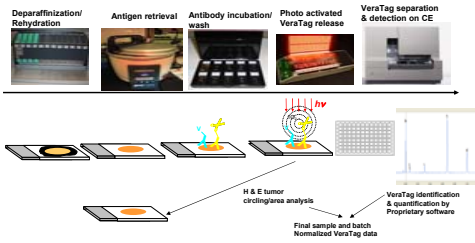


Figure 2. The HER3 assay workflow consists of epitope retrieval, antibody incubation, illumination/cleavage, CE/trans analysis of VeraTags, tumor area analysis, sample/batch normalization steps.

3. HER3 Total Assay is Accurate, Sensitive, and Reproducible

3a. Accuracy

Cell Line	IHC Score	VeraTag	ELISA (HER3) ng/ml	Flow Cytometry (pericapsule) cells
293 Clone 1	3+	18.01	592.2	188,045
MDA-MB-453	1+	1.00	46.2	26,716
MDA-MB-468	1+	0.24	2.5	5,669
SKOV3	0	0.06	0.4	500

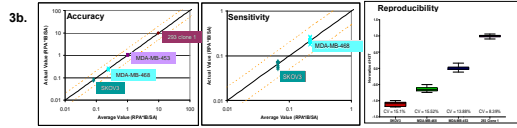
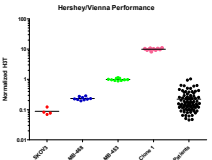


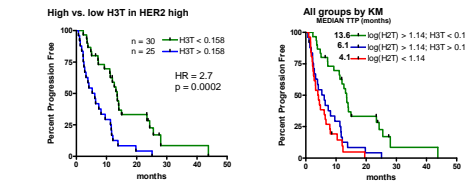
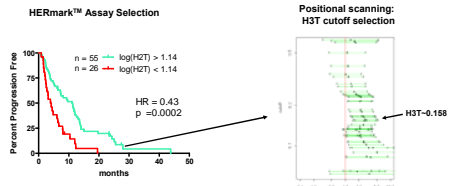
Figure 3a. Cell lines spanning a wide dynamic range of HER3 expression were grown/expanded and the following assays were performed on the same lot of cells for accuracy determination (ELISA, Flow cytometry, IHC, VeraTag). 3b. Assay is accurate such that the rank order of HER3 expression is preserved throughout dynamic range. Sensitivity is ~5000 receptors/cell and inter-assay reproducibility of assay controls is ~8-16 %CV.

4. Description of Clinical Cohort and Analytical Performance

- > HER2-positive metastatic breast cancer (IHC 3+ by Herceptest, or IHC 2+, FISH+ by Vysis) from a single institution accruing 1999-2006
- > ECOG 0-2 performance status, life expectancy ≥ 12 weeks
- > No prior trastuzumab
- > Treated with either trastuzumab + chemotherapy or single-agent trastuzumab
- > N=81



5. Clinical Significance of H3T Measurement in HER2+ Patients

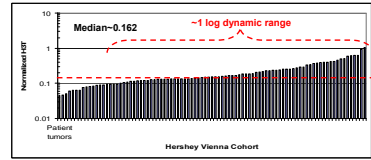


Summary of KM data

Subgroup Comparison	HR	p value
log(H2T)>1.14; H3T>0.158 (blue curve) vs log(H2T)<1.14 (red curve)	0.74	0.28
log(H2T)>1.14; H3T<0.158 (green curve) vs log(H2T)<1.14 (red curve)	0.30	<0.0001
log(H2T)>1.14; H3T>0.158 (blue curve) vs log(H2T)>1.14; H3T<0.158 (green curve)	2.7	0.0002

6. Challenges of Applying to Routine Patient Testing

>Small dynamic range around the median patient value (see figure below) resulting in clustering of samples increasing equivocal zone (potential for false positives and negatives). Reproducibility within 2-2.5 fold.



>Requirement of larger tumor areas (>25mm²) to allow for accurate patient results (to eliminate potential of false positives due to TA contribution)

7. Increased Dynamic Range and Sensitivity of H3T Assay

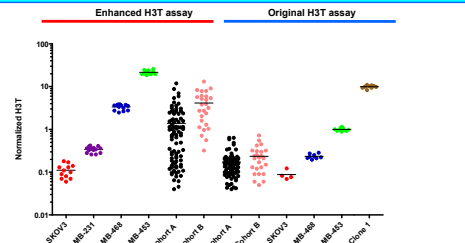


Figure 7. Assay improvements included increased efficiency of both VeraTag cleavage, and CE injection, resulting in significant improvements in sensitivity and dynamic range as measured in two independent invasive ductal carcinoma breast cancer patient cohorts (Cohort A: no selection; Cohort B: HER2+ selected).

8. Analytical Correlation Between Original and Enhanced Assay

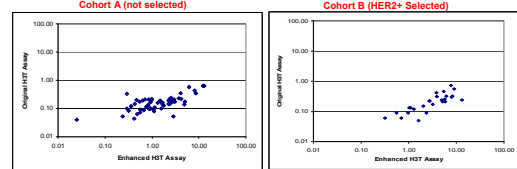


Figure 8. An unselected population of invasive ductal carcinoma vs a HER2+ selected population were run in the original and the improved version of the HER3 total assay. The resulting data indicated a good correlation between the two assays in the upper part of the dynamic range with the expected non-linearity at the lower end of the dynamic range.

Summary

>We have developed a highly quantitative, reproducible, sensitive, and accurate assay to measure H3T in FFPE tumors

>HER3 is expressed at significantly lower levels than HER2 and the biological dynamic range is relatively small in HER2+ breast tumors

>These results demonstrate that quantitative measurement of H3T, in a cohort of patients with HER2 positive metastatic breast cancer, correlates with TTP in response to trastuzumab and could indicate a population of patients with worse outcome requiring additional therapeutic intervention

>The dynamic range of the first generation H3T assay was limited (~1 log) in breast tumors. Consequently assay improvements were incorporated to increase the assay sensitivity ~5-fold (~5000 down to ~1000 H3T receptors/cell) and the dynamic range in FFPE tumors ~30-fold. This enhanced version of the H3T assay is entering clinical evaluation.

>Additional studies involving larger patient cohorts are required to confirm these preliminary results and are currently being planned.

>We anticipate that H3T measurement, along with measurements of the activated HER3 pathway (HER2/HER3 heterodimer, HER3-PI3K), using VeraTag™ technology will be valuable in stratifying patients for treatment with HER-targeted therapies.