

MILLER SCHOOL OF MEDICINE UNIVERSITY OF MIAMI

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Correlation of quantitative p95HER2, HER3, and HER2 protein expression with pathologic complete response (pCR) in HER2-positive breast cancer patients treated with neoadjuvant (NEO) trastuzumab containing therapy

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Introduction

- ➤ Prognostic and/or predictive biomarkers associated with pathologic complete response (pCR) to trastuzumab (Herceptin®, Genentech) based regimens in HER2-positive breast cancer patients in the neoadjuvant (NEO) treatment setting are lacking.
- ➤ The presence of pathologic complete response (pCR) has been shown to correlate with disease-free survival in patients treated with NEO regimens¹ and is commonly used as a surrogate marker of outcome in the NEO setting.
- ➤ **Study Aim**: In this study, we sought to investigate the prognostic value of quantitative measures of HER2 (H2T), p95HER2 (p95) and HER3 (H3T) protein expression and likelihood of pCR in a cohort of patients with locally advanced, HER2-positive breast cancer treated with NEO chemotherapy and trastuzumab.
- Biomarkers of Interest
- 1. p95HER2 [HER2-M611-CTF] (p95)
- p95 is a signaling-competent fragment of the HER2 receptor arising from alternative translation at an internal initiation codon (position 611) resulting in a truncated protein lacking the extracellular domain². In pre-clinical studies, p95 has been shown to correlate with increased activation of signaling cascades³, cell migration⁴ and tumorigenesis².
- The prognostic value of p95HER2 by VeraTag® was demonstrated in 2 independent cohorts of patients with HER2 positive metastatic breast cancer treated with trastuzumab where high tumor p95 levels were associated with shorter progression-free survival (PFS) and overall survival (OS)^{5,6}.
- Limited data exist on the use of p95HER2 as a prognostic or predictive biomarker in the neoadjuvant setting.

2. HER2 / quantitative HER2 (H2T)

- HER2, by immunohistochemistry and/or in situ hybridization (FISH/CISH), is a well-described prognostic biomarker and is predictive of clinical outcomes in HER2+ breast cancer patients in metastatic and adjuvant treatment settings.
- Higher H2T levels by a novel quantitative HER2 assay (HERmark[®], Monogram Biosciences) have also been associated with shorter PFS in 2 independent cohorts of patients with HER2 metastatic breast cancer treated with trastuzumab.^{5,7}
- We previously observed an association between higher quantitative H2T levels by HERmark® and likelihood of pCR in a small cohort of patients with locally advanced HER2 positive breast cancer treated with a NEO docetaxel (T), carboplatin (C), trastuzumab (H) (TCH) regimen.⁸

3. HER3 (H3T)

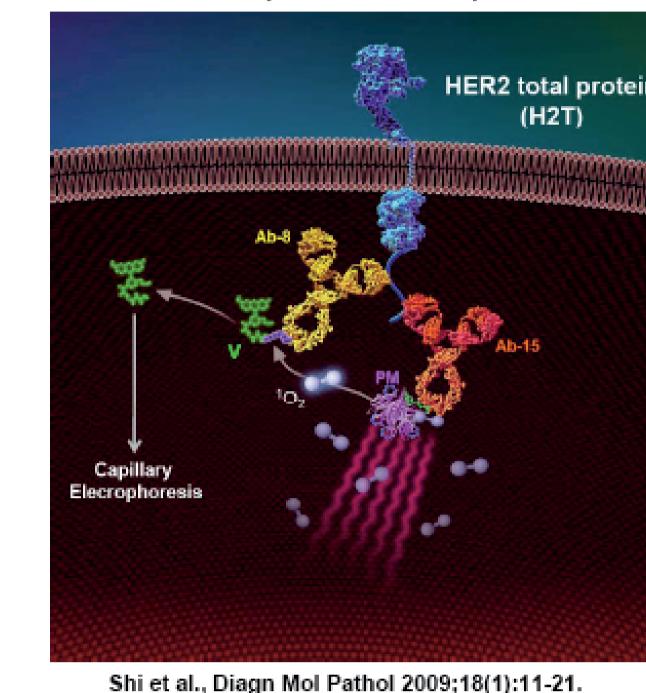
 Although data are lacking in the neoadjuvant setting, higher quantitative H3T levels by VeraTag were associated with shorter PFS in a cohort of patients with HER2 positive metastatic breast cancer treated with trastuzumab.⁹

Materials and Methods

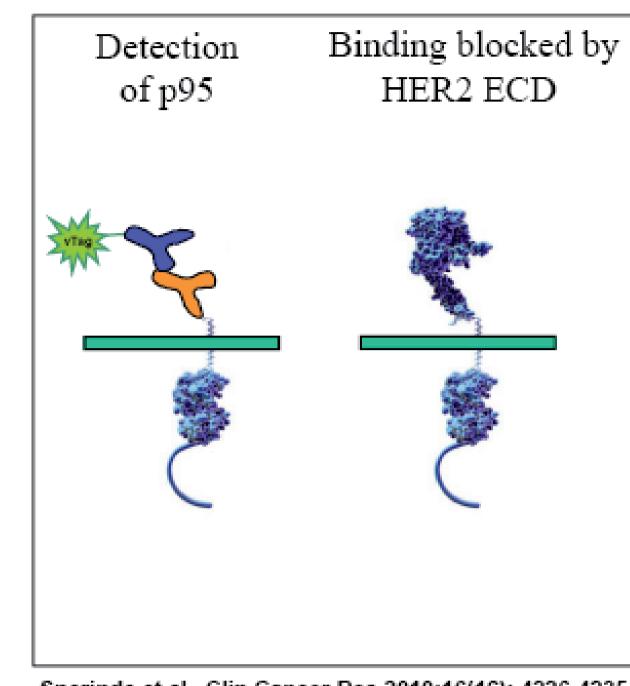
- This was a blinded, prospectively designed biomarker analysis of a retrospective cohort of patients with stage I to III breast cancer treated at a tertiary referral center in Miami.
- This study was reviewed and approved by the Ethics Committee (Institutional Review Board) at the University of Miami.
- Inclusion criteria
- Invasive Breast cancer, HER2 positive (+) (IHC 3+, or IHC 2+/FISH+)
- Pre-operative (neoadjuvant) chemotherapy regimen containing trastuzumab followed by surgery
- Available pre-therapy FFPE tumor biopsy
- Exclusion criteria
- Confirmed stage IV disease
- Refusal of surgery
- Unavailable tissue specimen
- Unavailable pCR data
- Pre-treatment FFPE breast tumor samples were assayed for quantitative H2T, p95, and H3T by HERmark® / VeraTag® assays (Monogram Biosciences). Samples with insufficient tumor area (<10mm²) or missing pCR data were excluded from analysis.
- Outcomes: pCR was defined as absence of invasive tumor in the breast at surgery;
 PFS was defined as time from start of neoadjuvant therapy to progression or censor.

Methods - VeraTag Assays

VeraTag[®] FFPE HER2 assay¹⁰ (HERmark[®])



VeraTag[®] FFPE p95 assay⁵



N = 45

Sperin

pCR Results

	N	pCR	non-pCR	p-value*
All Patients	45	21 (46.7%)	24 (53.3%)	
ER Status				
Positive	21	3 (14.3%)	18 (85.7%)	< 0.0001
Negative	24	18 (75%)	6 (25%)	
HER2 IHC				
2+ / FISH +	10	3 (30%)	7 (70%)	0.296
3+	35	18 (51.4%)	17 (48.6%)	
HERmark (H2T)				
Positive (> 17.8)	36	19 (52.8%)	17 (47.2%)	0.143
Neg /equiv (≤ 17.8)	9	2 (22.2%)	7 (77.8%)	

* Fisher Exact Test

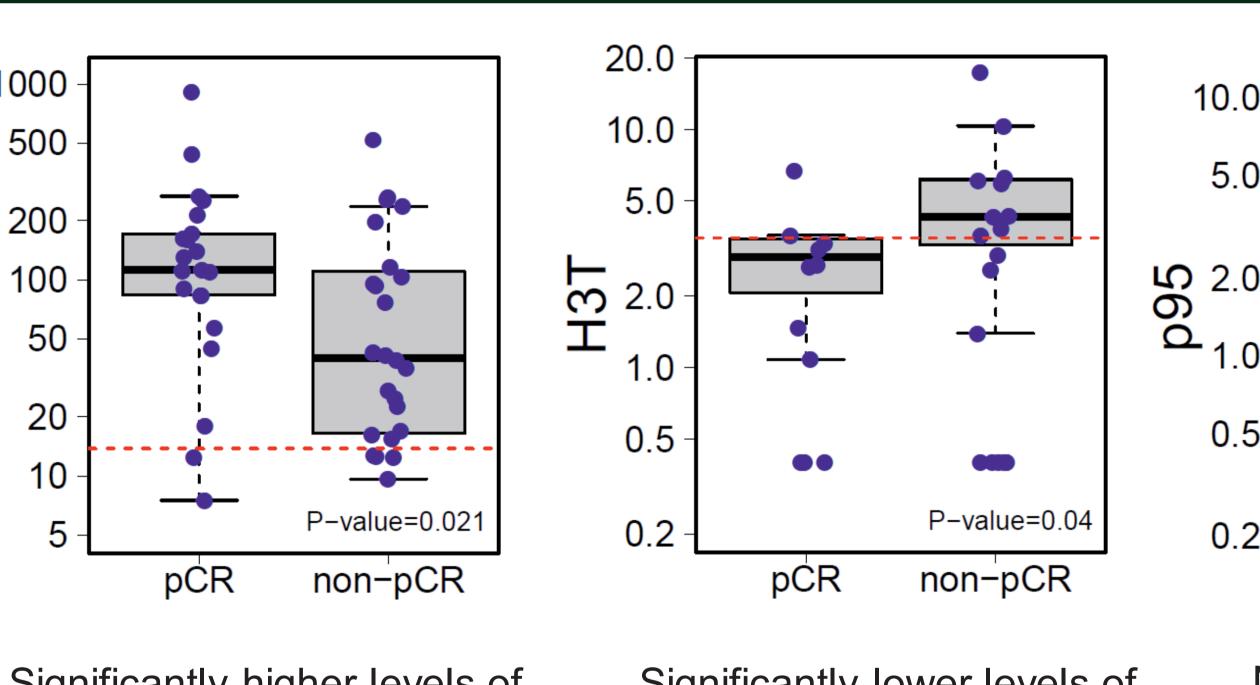
Cohort Characteristics

Patient Characteristics	N =	N = 45	
	N	%	
Age			
	median	53	
	range:	27-73	
Menopausal status			
Pre	16	36%	
Peri	1	2%	
Post	27	60%	
Unknown	1	2%	
Ethnicity			
Hispanic	30	67%	
Non-Hispanic	11	24%	
Haitian/Caribbean Islander	2	4%	
Other/Unknown	2	4%	
Race			
African descent	13	29%	
White	19	42%	
Asian	1	2%	
Other	11	24%	
Unknown	1	2%	

Clinical/Tumor Characteristics

N	%			
nor Size (baseline, longest dimension, cm)				
median	4			
range:	1.5-14			
17	38%			
27	60%			
1	2%			
14	31%			
7	16%			
23	51%			
1	2%			
10	22%			
35	78%			
39	87%			
1	2%			
5	11%			
43	96%			
1	2%			
1	2%			
	median range: 17 27 1 14 7 23 1 10 35 39 1 5 43 1			

Quantitative H2T, H3T or p95 vs. pCR



Significantly higher levels of H2T were observed in pCR vs. non-pCR cases.

Significantly lower levels of H3T were observed in pCR vs. non-pCR cases.

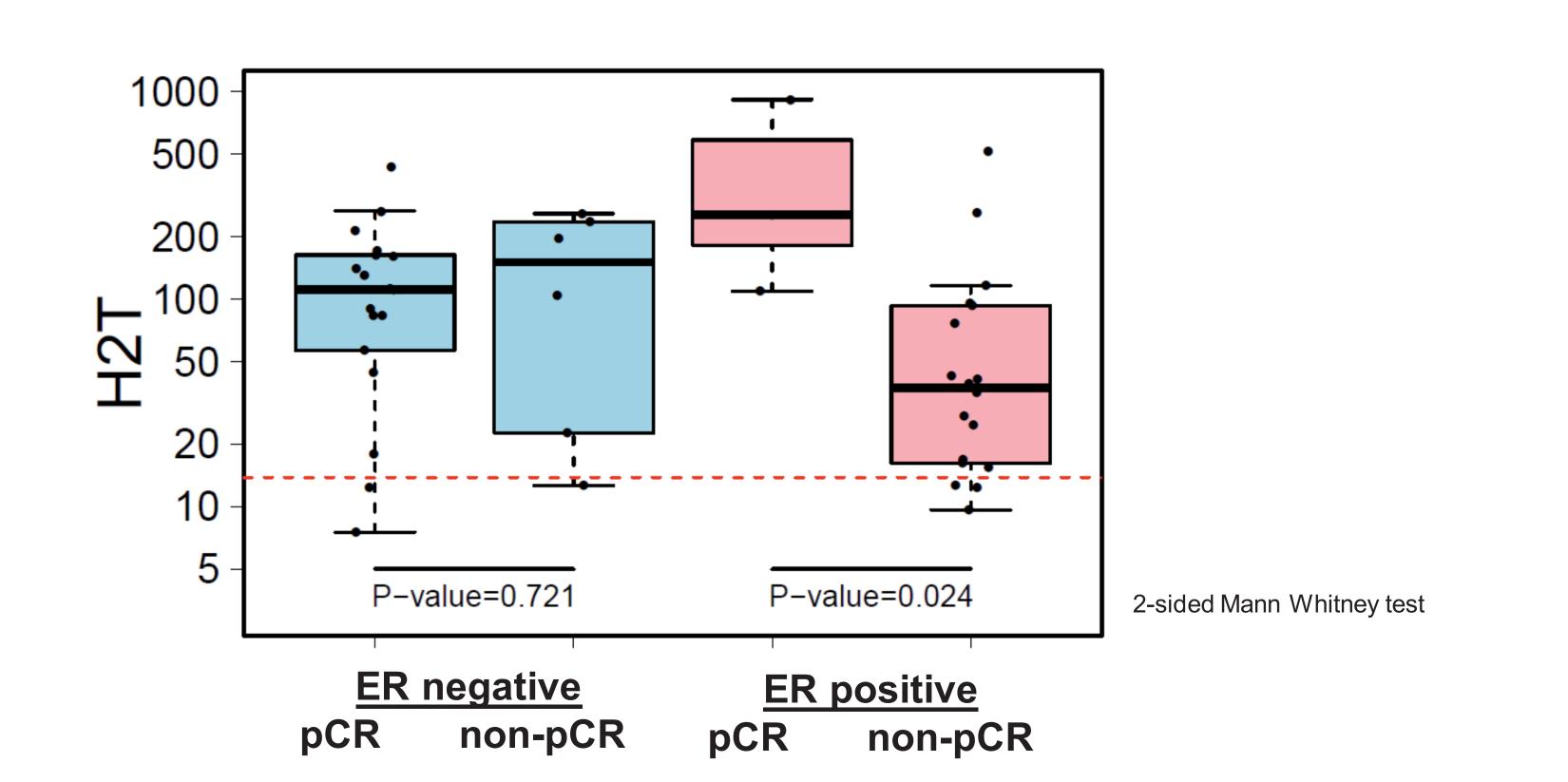
Non-significant trend in higher p95 levels in pCR vs. non-pCR cases. Influence of ER?

P-value=0.0

non-pCR

Note: The dotted red lines on each plot denote the current clinical cutoffs for each assay: (H2T=13.8; p95=2.8; H3T=3.5)^{7, 5,10} Distributions were compared using 1-sided Mann Whitney test.

Quantitative H2T vs. pCR, stratified by ER status



Prediction of pCR using logistic regression

Univariate continuous	Estimate	p-value
Log10 H2T	1.3	0.045
Log10 p95 (H2T>13.8)	0.99	0.225
Log 10 H3T (all)	-0.61	0.41
Univariate categorical	Estimate	p-value
HEDmark HOT (as = 17.9)	1 26	0.42

Jnivariate categorical	Estimate	p-value
HERmark H2T (co=17.8)	1.36	0.12
o95 low (co=2.8)	-0.59	0.46
H3T low (co=3.5)	1.61	0.076
ER negative	2.89	0.00022
HER2 IHC	0.9	0.77

In univariate analyses:

- Higher quantitative H2T was significantly associated with pCR, whereas other continuous biomarkers (H3T or p95) were not.
- ER negativity was strongly associated with likelihood of pCR.

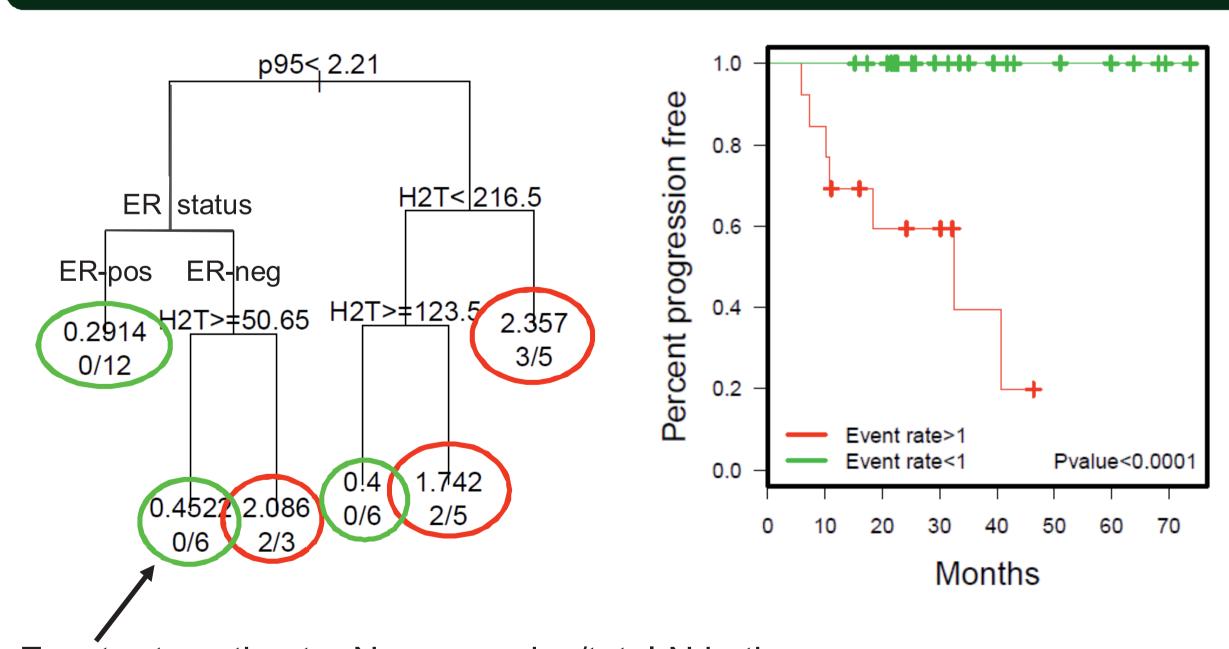
Multivariate Model for pCR

Multivariate: H2T + ER + p95	Estimate	p-value
Log10 H2T	6.1	0.012
ER negative	3.82	0.027
p95 low (co=2.8)	3.25	0.074

In multivariate analyses:

Higher H2T, ER negativity, and low p95 were independently found to correlate or trend with likelihood of pCR.

Predictive Modeling for PFS



Event rate estimate, N progression/total N in the group

- ➤ Recursive partitioning¹¹ was used to correlate continuous H2T, H3T and p95 measurements and other clinical characteristics with PFS in patients confirmed to be HER2 positive by HERmark (clinical cutoff H2T>13.8)⁷.
- Cases were classified into 2 subgroups with very different PFS outcomes, based on their quantitative H2T and p95 levels and their ER status.
- Cases with better outcomes (no progression events until censored, N=24, median follow-up=34.3 months)
- Cases that were more likely to progress (7 out of 13 patients progressed, median PFS: 10.7 months; median follow-up: 27.2 months).
- > Probabilities of progression for the subgroups derived from the optimal tree were then compared using Kaplan-Meier analysis.

Results Summary

- This study was the first analysis of the correlation between H2T, p95 and H3T and clinical outcomes in HER2+ breast cancer pts treated with trastuzumab+chemotherapy in the NEO setting. Despite the limitations of a small, retrospective dataset, interesting and significant correlations were observed:
- Higher H2T levels were observed in pCR vs. non-pCR cases (p=0.021).
- Lower H3T levels were observed in pCR vs. non-pCR cases (p= 0.038).
- Outcomes stratified by ER status:
- There was no difference in H2T levels between pCR and non-pCR in the ER-negative subset (median H2T =111.5 vs. 150.5, respectively).
- However, within the ER-positive group, patients with pCR had significantly higher H2T compared to patients with non-pCR (median H2T = 254 vs. 37.2, respectively), p=0.024.
- ➤ Multivariate models demonstrate that increasing H2T, ER-negativity, and low p95 correlate or trend with pCR (p=0.012, 0.027, and 0.074, respectively).
- ➤ An exploratory recursive partitioning analysis showed that patients who are more likely to experience disease progression could be identified using a combination of their quantitative H2T and p95 levels and their ER status.

Conclusions

- ➤ These data suggest that quantitative H2T and p95 may provide additional information on response to trastuzumab-based regimens in HER2 positive breast cancer, particularly ER+ breast cancer.
- Additional investigation into the possible relationship between quantitative levels of HER2, p95, and HER3 expression and response to HER2 targeted therapy in larger neoadjuvant cohorts is warranted.

Acknowledgements

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