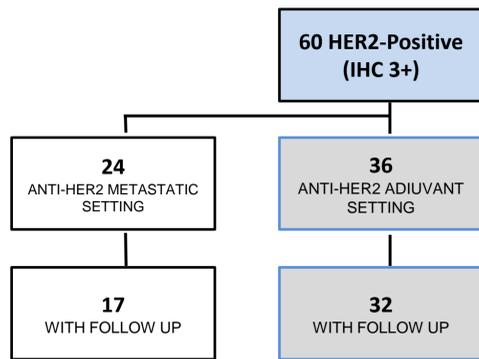


Background

There is evidence that increased absolute levels of HER2 receptor in breast cancer positively correlated with a clinical benefit from anti-HER2 therapies. Current methods of HER2 evaluation are essentially based on semiquantitative immunohistochemistry (IHC) assays that do not allow for absolute HER2 quantification in formalin fixed and paraffin embedded (FFPE) clinical samples. Thus, a significant portion of patients are classified as HER2-positive (IHC 3+) but actually express low levels of the receptor. We hypothesized that the benefit from trastuzumab or other anti-HER2 therapies is lower in this subgroup of patients compared to those with high receptor levels.

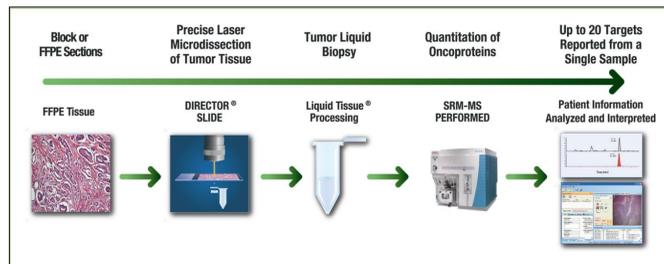
Methods



Sample population

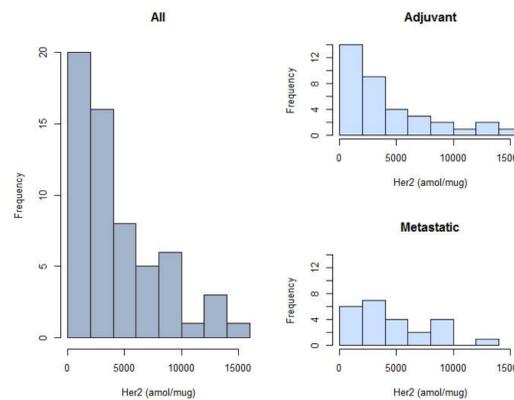
- 60 FFPE primary breast cancer HER2-positive by IHC (3+) previously treated with anti-HER2 therapy
- Patients receiving an anti-HER2 treatment in either adjuvant or metastatic settings.

Quantitative Multiplex Mass Spec Assay



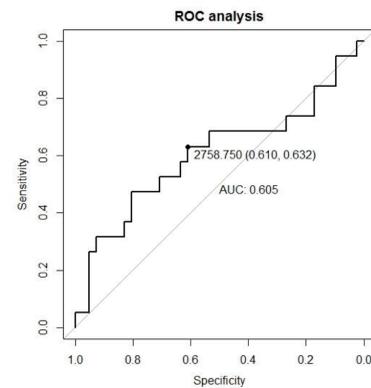
Liquid Tissue-selected reaction monitoring (LT-SRM) workflow analysis of FFPE tissue. Tumor was microdissected using laser micro-dissection and then solubilized to tryptic peptides using Liquid-Tissue[®] technology. Absolute quantitation was accomplished through the use of heavy labeled peptides identical to endogenous analytical targets (HER2, Her3, EGFR, IGF1R, cMet, etc.) and simultaneous detection of endogenous target and synthetic labeled heavy peptide using triple quadrupole mass spectrometer.

HER2 Abundance in Breast Cancer



Absolute amounts of HER2 Expression as quantitated by mass spectrometry: Absolute HER2 quantification by LT-SRM shows high variability of HER2 expression within a patient population classified homogeneously as 3+ by IHC.

HER2 (amol/μg)	All Dataset	Adjuvant Setting	Metastatic Setting
Mean	4278.8	4152.0	4468.9
Std Dev	3668.0	3882.3	3393.2
Median	2930.1	2172.7	3461.6
Min	283.3	287.8	283.3
Max	14938.3	14938.3	12840.0



ROC analysis: The curves were constructed by computing the sensitivity and specificity of increasing levels of HER2 in predicting events. The threshold obtained and chosen to stratify tumors into low and high HER2 (2758.75 amol/μg) is the point closest to the top-left part of the plot with optimal sensitivity or specificity.

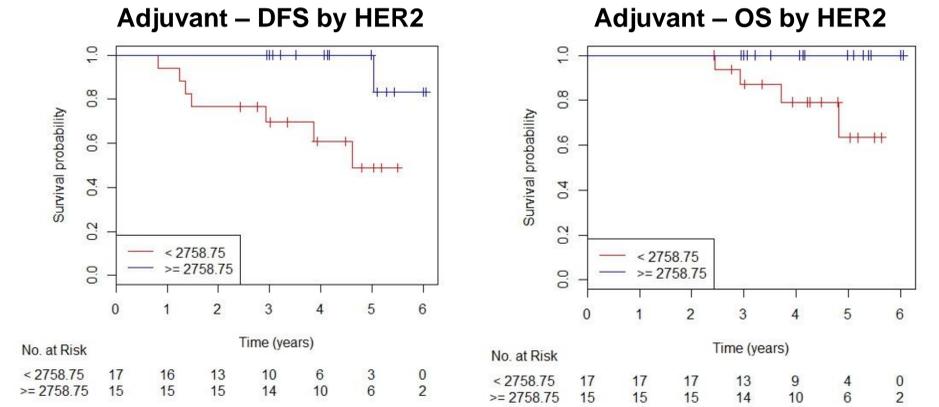
Multivariate Analysis Results

	HR	CI _{95%} (HR)	p-Value
Hazard (HR negative)	0.54	0.08 - 3.49	0.514
Hazard (HR positive)			
Hazard (Grade G2)	1.05	0.20 - 5.39	0.954
Hazard (Grade G3)			
Hazard (TX - T1)	0.53	0.09 - 3.04	0.475
Hazard (T2 -T4)			
Hazard (NX - N0)	6.96	1.25 - 38.69	0.027
Hazard (N1 - N3)			
Hazard (HER2 < 2758.75)	0.08	0.01 - 0.89	0.040
Hazard (HER2 ≥ 2758.75)			

Multivariate Cox Model of DFS including Hormone receptors (HR), tumor grade, T and N status, and HER2 in adjuvant setting.

High HER2 levels are an independent predictor of increased DFS in patients receiving an adjuvant anti-HER2 treatment.

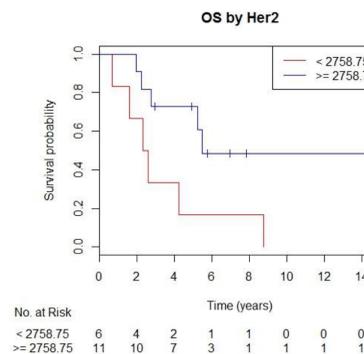
Survival Analyses Results



HER2 (amol/μg)	N	Events	Mean (SD)	HR (CI _{95%})	p-value
< 2758.75	17	7	3.38 (1.50)	0.11	0.013
≥ 2758.75	15	1	4.40 (1.09)	(0.01-0.89)	

HER2 (amol/μg)	N	Events	Mean (SD)	HR (CI _{95%})	p-value
< 2758.75	17	4	4.03 (1.06)	na	0.039
≥ 2758.75	15	0	4.43 (1.11)		

Metastatic - OS by HER2



HER2 (amol/μg)	N	Events	Mean (SD)	HR (CI _{95%})	p-value
< 2758.75	6	6	3.37 (2.89)	0.29	0.037
≥ 2758.75	11	5	5.51 (3.55)	(0.09- 0.99)	

Kaplan-Meier survival curves according to HER2 levels in patients receiving anti-HER2 therapies. High HER2 levels (≥2758.75 amol/μg) significantly correlate with increased DFS and OS in adjuvant setting and also significantly correlates with OS in first-line metastatic setting.

Conclusions

- HER2 protein levels as measured by mass spectrometry significantly vary within HER2 IHC-positive tumors.
- High HER2 protein levels is associated with longer DFS and OS in patients receiving an adjuvant anti-HER2 treatment.
- High HER2 protein levels are an independent predictive factor of response to anti-HER2 treatment in adjuvant setting.
- High HER2 protein levels is associated with longer OS in patients receiving an anti-HER2 as first-line metastatic treatment.
- This cut-off value is currently being tested in an independent set to validate this observation.