Correlation of quantitative p95HER2 and total HER2 levels with clinical outcomes in a combined analysis of two cohorts of trastuzumab-treated metastatic breast cancer patients

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Background

Expression of p95HER2 (p95), a truncated form of HER2 also known as p110 or M611-CTF, is a possible trastuzumab resistance mechanism and has been associated with poor prognosis in trastuzumab-treated HER2-positive metastatic breast cancer (MBC). Previously we reported on optimal clinical cutoffs for quantitative p95 (Clin Cancer Res, 16:4226, 2010) and quantitative HER2 protein expression (Cancer, 116:5168, 2010) that defined patient subsets with different progression-free survival (PFS). These cutoffs were confirmed in an independent trastuzumab-treated MBC cohort (ASCO 2011, #586). Here, using individual patient data, we performed an analysis on the combined data set of 243 cases from the discovery and validation cohorts to derive optimal cutoffs for quantitative p95 and H2T.

Methods

Both quantitative HER2 (H2T) and p95 assays employed the VeraTag® method (Monogram Biosciences, So. San Francisco, CA) to quantify protein expression in formalin-fixed, paraffin-embedded (FFPE) tumor samples from two cohorts of 101 and 142 cases of trastuzumab-treated MBC with 7.4 and 9.2 months median PFS, respectively. All analyses were stratified by hormone receptor status, tumor grade (3 vs. 1+2) and cohort.

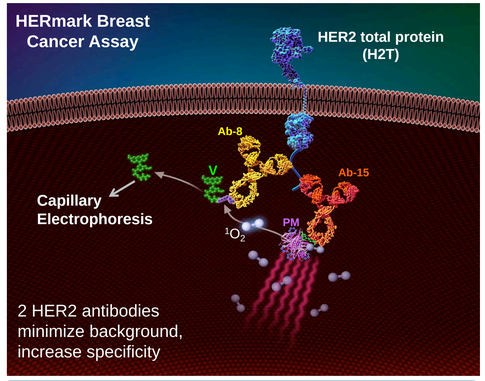
Quantitative HER2 Assay

Total HER2 protein expression (H2T) was quantified using the HERmark® assay as previously described (*Huang et al. Am J Clin Pathol 134:303, 2010*). H2T was quantified through the release of a fluorescent tag (*V* for "VeraTag® reporter", see Figure) conjugated to a HER2 monoclonal antibody (mAb). The antibody is paired with a biotinylated second HER2 mAb. An avidin-linked photosensitizer molecule (*PM*) produces singlet O_2 ($^1O_2$) upon illumination with red light. Signal (*V*) quantified by capillary electrophoresis is normalized to invasive tumor area on the FFPE tissue section. H2T measurements are compared to pre-specified cutoffs for HERmark negative (H2T≤10.5 Relative Fluorescence / mm^2 tumor [RF/mm^2]) and HERmark positive (H2T>17.8 RF/mm^2) with Equivocal defined as 10.5<H2T≤17.8, derived from the <5th percentile of centrally determined HER2-positives and the >95th percentile of centrally determined HER2-negatives, respectively, within a reference database of 1,090 breast cancer patient samples.

Quantitative p95 Assay

P95HER2 (p95) was quantified using the VeraTag platform with a proprietary mAb specific for the M611-CTF form of p95 as described in Clin Cancer Res, 16:4226, 2010.

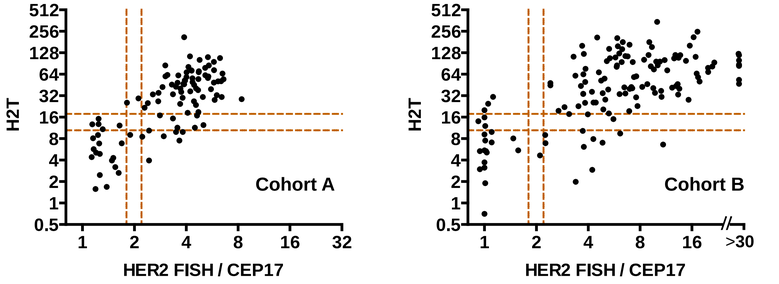
Quantitative HER2 Assay



Patient/Tumor Characteristics

Characteristic	No. (range or %)
Median age at diagnosis	54 (25–85 yr)
HERmark category for primary tumor	
Negative (H2T ≤ 10.5)	49 (20%)
Equivocal (10.5 < H2T ≤ 17.8)	20 (8.2%)
Positive (H2T > 17.8)	174 (72%)
Tumor grade	
Grade 1 (well differentiated)	5 (2.1%)
Grade 2 (moderately differentiated)	72 (30%)
Grade 3 (poorly differentiated)	166 (68%)
N/A (data not available)	*1*
Hormone receptor status	
Negative (ER and PgR negative)	139 (57%)
Positive (ER or PgR positive)	103 (43%)
N/A (data not available)	*2*

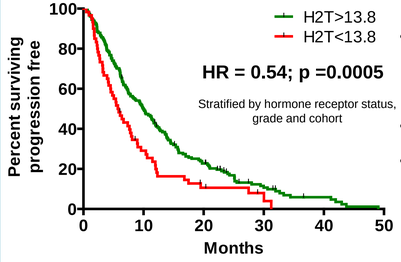
HER2 expression level in two cohorts



Cohort B had higher quantitative HER2 protein expression than **Cohort A**, but this was consistent with higher HER2 gene copy numbers.

Progression free survival vs. quantitative HER2

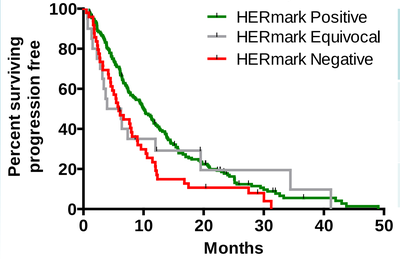
Test of HER2 cutoff (H2T=13.8) from Cancer, 116:5168 (2010)



- The cutoff derived from the training cohort and verified in the test cohort (ASCO 2011, #586) was applied to the combined data set.
- H2T<13.8: n=60. H2T>13.8: n=183.
- The optimal cutoff for the combined data set was H2T=12.75 RF/mm^2 (HR=0.48; p<0.0001, unadjusted).

Test of HERmark cutoffs pre-defined per central HER2 determination*

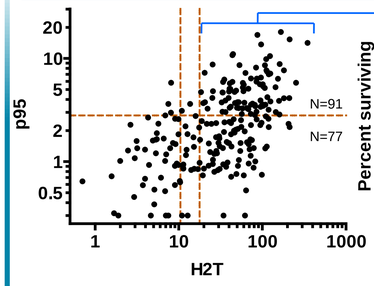
*See Methods section for derivation of analytical HERmark cutoffs from database of 1,090 tumors.

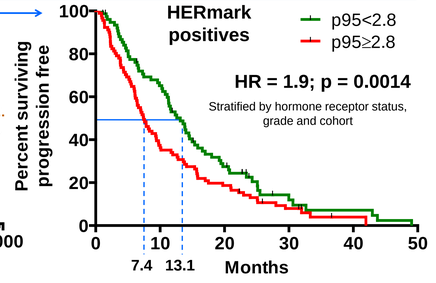


HERmark category (Stratified by hormone receptor status, grade and cohort)	N	PFS vs. HERmark negative group	
		HR	p-value
Negative	49	1	1
Equivocal	20	0.98	0.94
Positive	174	0.52	0.0006

HERmark-positive vs. HERmark-equivocal: HR=0.57; p=0.057

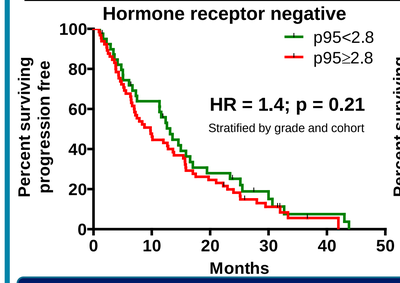
Progression free survival vs. quantitative p95

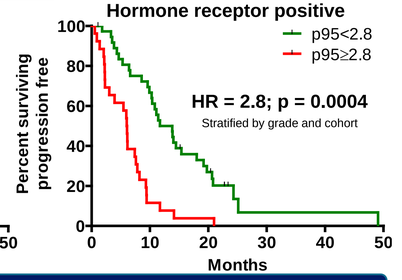




- Median PFS was 7.4 months and 13.1 months for p95≥2.8 and p95<2.8, respectively.
- Increasing continuous p95 was also correlated with shorter PFS (HR=1.9/log; p=0.022).
- The cutoff of p95=2.8 gave similar results in the HER2 FISH positive population (HR=1.7; p=0.0047).
- The optimal cutoff for the combined set was p95=2.7 (HR=2.0, p=0.0009, unadjusted).

Influence of hormone receptor status





Summary

- The H2T=13.8 cutoff derived from cohort A (Cancer, 116:5168, 2010) and tested in cohort B (ASCO 2011, #586) gave a similar difference in PFS (HR~0.5) to pre-defined HERmark cutoffs derived from concordance studies with central HER2 status.
- The p95=2.8 cutoff derived from cohort A (CCR, 16:4226, 2010) and tested in cohort B (ASCO 2011, #586) was prognostic in both the HERmark and FISH-positive populations.
- The shorter PFS observed for cases with p95≥2.8 was strongly influenced by the hormone receptor positive subgroup.
- These results are consistent with other studies in the metastatic setting. The role of p95 in the neo-adjuvant and adjuvant settings is yet to be determined.