

# Identification of a sub-population of metastatic breast cancer patients with very high HER2 expression levels and possible resistance to trastuzumab

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## Abstract

**Background:** Many HER2-positive patients with metastatic breast cancer (MBC) fail to respond to trastuzumab. We previously reported that precise quantitation of HER2 expression (H2T) by the HERmark assay identified a sub-population of IHC 3+, FISH(+) (positive) patients with low H2T levels that responded poorly to trastuzumab (Lipton, SABCS 2008, abs #32). Here we identify a sub-population of FISH(+) patients with very high H2T levels, that experience clinical outcomes that are indistinguishable from those of FISH(-) (negative) patients with low H2T levels.

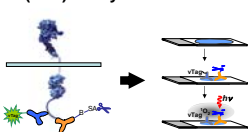
**Methods:** The HERmark assay was used to measure H2T in formalin-fixed, paraffin-embedded (FFPE) primary breast tumor specimens from 99 women treated with trastuzumab for MBC. Specimens were also tested by central FISH. A sub-population treatment effect pattern plot (STEEP) was generated to examine the progression-free survival (PFS) rate at 12 months after treatment with trastuzumab across the distribution of H2T. Kaplan-Meier (KM) analyses were performed comparing the PFS of FISH(-), H2T low ( $\log_2(\text{H2T}) < 1.25$ ) patients with those of FISH(+), H2T high ( $\log_2(\text{H2T}) \geq 1.95$ ) and FISH(+), H2T intermediate ( $1.25 < \log_2(\text{H2T}) < 1.95$ ) groups. Cutoffs were identified by lowest p-value in a positional scanning analysis.

**Results:** The PFS rate improved gradually with increasing H2T in STEEP analyses. At the highest levels of H2T, an abrupt decrease in the PFS rate was observed, consistent with a reduction in susceptibility to trastuzumab. KM analyses demonstrated that patients who were FISH(+), H2T intermediate had a significantly longer PFS than patients who were FISH(-), H2T low (median PFS 12.6 vs. 4.5 mos; HR=0.34;  $p < 0.0001$ ). Patients that were FISH(+), H2T high experienced a PFS that was no better than patients that were FISH(-), H2T low (median PFS 4.6 vs. 4.5 mos; HR=0.87;  $p=0.08$ ).

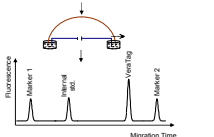
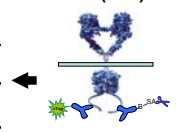
**Conclusions:** Precise quantitation of HER2 expression levels allows the identification of multiple sub-populations of HER2(+) patients that have different clinical outcomes on trastuzumab. MBC patients with very high levels of H2T could represent a sub-group with *de novo* resistance to trastuzumab.

## Methods- VeraTag assays

### VeraTag FFPE HER2 total (H2T) assay



### VeraTag FFPE HER2 homodimer (H2D) assay



1. Biotin labeled Ab and VeraTag reporter labeled Ab bind to HER2.
2. Streptavidin-functionalized sensitizer-dye binds to biotin labeled Ab.
3. Red light illumination of the section generates singlet oxygen.
4. Singlet oxygen induces cleavage and release of VeraTag reporter into solution.
5. Solution is collected and analyzed by capillary electrophoresis.
6. Integral of VeraTag signal (RPA) is normalized to tumor area (TA).

## Patient characteristics

Characteristic	Value (range, %)	Characteristic	Value (range, %)
Total patients	99	Treatment	
Mean follow-up (months)	32.0 (11.8-72.3)	trastuzumab + chemotherapy	87 (87.9%)
Mean age	55.2 (27.6-85.4)	trastuzumab only	12 (12.1%)
<b>Hormonal status</b>		<b>Line of chemotherapy</b>	
ER+ PR+	15 (15.2%)	First line	72 (72.7%)
ER+ PR-	19 (19.2%)	Second line	17 (17.2%)
ER- PR+	3 (3%)	Third line	8 (8.1%)
ER- PR-	60 (60.6%)	Unknown	2 (2.0%)
unknown	2 (2.0%)	<b>Number of metastatic sites</b>	
		< 3	57 (57.6%)
		≥ 3	42 (42.4%)

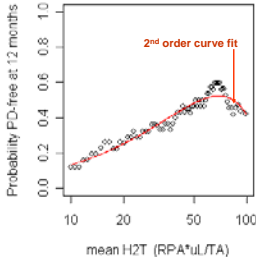
## Outcome as a function of HER2

• HER2 (H2T) was measured by VeraTag.

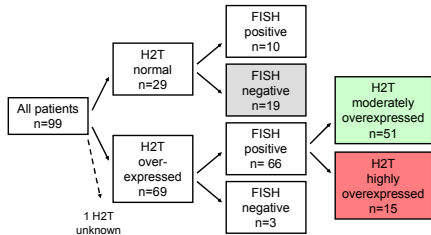
• Probability of progression at 12 months was calculated for bins of 30 patients ordered smallest to largest H2T.

• A trend of increasing probability of remaining progression-free past 12 months was observed for increasing H2T.

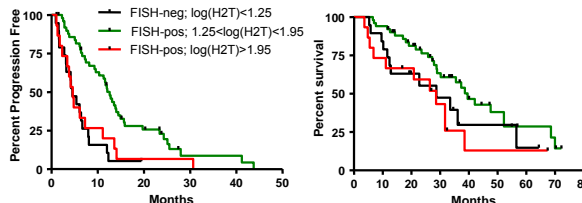
• A discontinuity in this trend was observed for bins with mean H2T of ~70.



## Subgroups for comparison



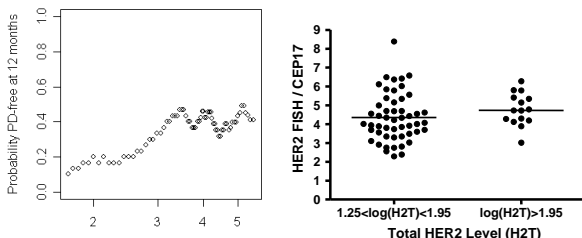
## Patients with very high HER2 outcomes similar to FISH<sup>-</sup>



FISH Status	HER2 Total Status	n	Median TTP (mo)	TTP HR (p-value) v. FISH(-)	Median OS (mo)	OS HR (p-value) v. FISH(-)
FISH-	$\log_2(\text{H2T}) < 1.25$	19	4.5	1 (1)	28.7	1 (1)
FISH+	$1.25 < \log_2(\text{H2T}) < 1.95$	51	12.6	0.34 ( <b><math>p &lt; 0.0001</math></b> )	39.6	0.56 (0.085)
FISH+	$\log_2(\text{H2T}) > 1.95$	15	4.6	0.87 (0.68)	28.6	0.79 (0.58)

- The H2T VeraTag assay identified patients with tumors having highly overexpressed HER2 and poor performance on trastuzumab.
- Results were similar when the 9 patients who received trastuzumab-only were excluded (vs. FISH-neg, TTP HR=0.36 ( $p=0.0004$ ) and 0.92 ( $p=0.8$ ) for intermediate and highest H2T, respectively).
- Results were similar when the 4 IHC 2+ patients were excluded (vs. FISH-neg, TTP HR=0.35 ( $p < 0.0001$ ) and 0.79 ( $p=0.5$ ) for intermediate and highest H2T, respectively).
- Inclusion of only p95 negatives in the OS analysis increases the distinction between the intermediate H2T group and the FISH-negative: HR=0.37 ( $p=0.013$ ).

## Poor performing subgroup not identified by FISH

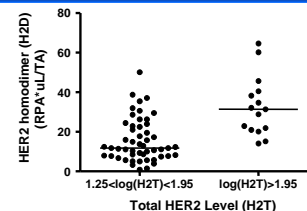


A scan of percent progression at 12 months shows only a plateau at elevated HER2 FISH/CEP17 copy numbers.

Distributions for the HER2 FISH/CEP17 copy numbers appear similar ( $p=0.2$ ) for the two groups with different HER2 levels and TTP.

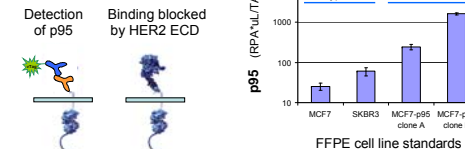
## Influence of HER2 homodimers

HER2 homodimers (H2D) measured by the VeraTag assay were also found to be elevated in the poor performing group.



## Influence of p95HER2

### p95 detection in FFPE samples



Six of 15 patients with  $\log_2(\text{H2T}) > 1.95$  were p95-positive by a VeraTag assay.

## Summary

- In this cohort a small subgroup of patients with highly overexpressed HER2 experienced a shorter TTP than those with moderate HER2 overexpression even though all were FISH positive.
- Neither the magnitude of HER2 overexpression nor the outcome for this small subgroup was predictable by FISH/CEP17 copy number.
- The TTP and OS for this subgroup were similar to that of a subgroup that was FISH negative and expressed normal levels of HER2 by the VeraTag assay.
- MBC patients with very high levels of H2T may represent a sub-group with *de novo* resistance to trastuzumab.
- Possible mechanisms that may account for the poor performance observed in the subgroup with highly overexpressed HER2:
  - Insufficient trastuzumab.
  - Increased signaling via formation of heterodimers that are not completely suppressed by trastuzumab.
  - Generation of C-terminal fragments of HER2 such as HER2p95. Six of 15 patients in the subgroup were HER2p95-positive by a VeraTag assay.
- These results require confirmation in larger independent datasets of patients with MBC.