

Total HER2 and HER2 homodimer levels predict response to trastuzumab.

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INTRODUCTION

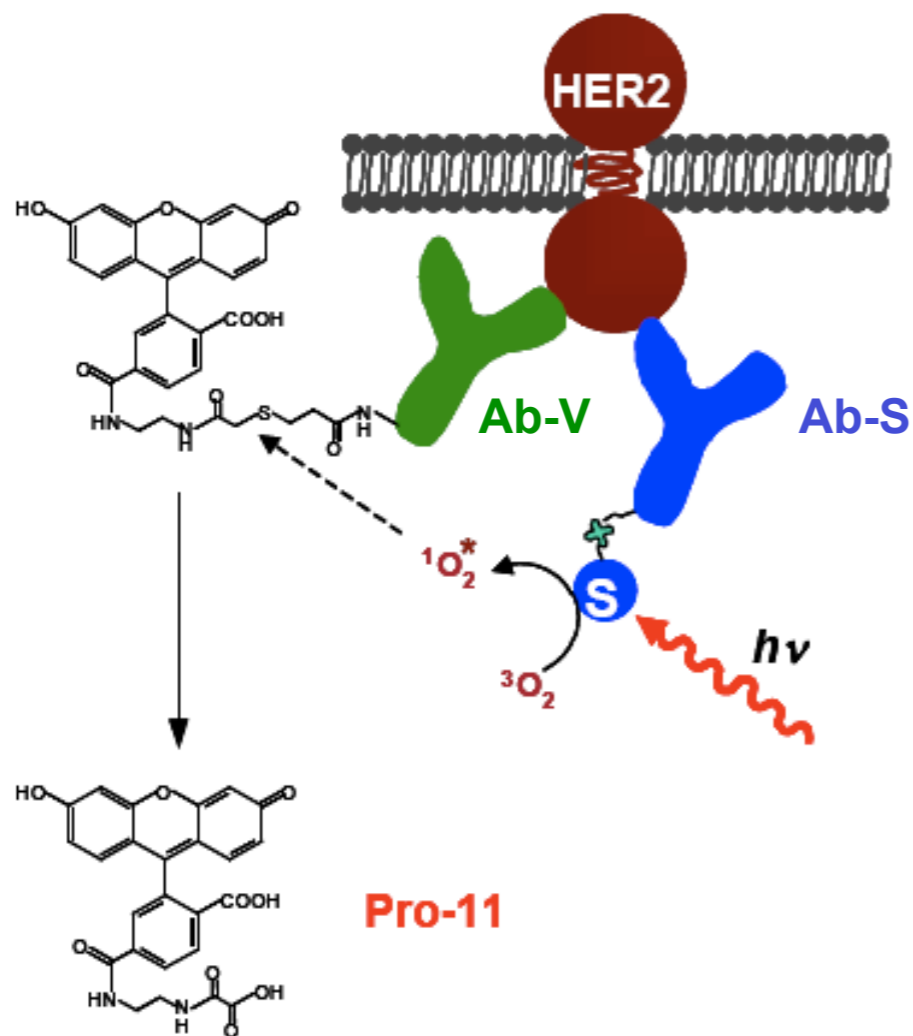
- HER2/neu is over-expressed or amplified in 20-25% of primary invasive breast cancers and is associated with poor prognosis.
- Using IHC / FISH methods to select patients for trastuzumab-based therapy, only half with HER2-positive metastatic breast cancer will respond.

The VeraTag Assay

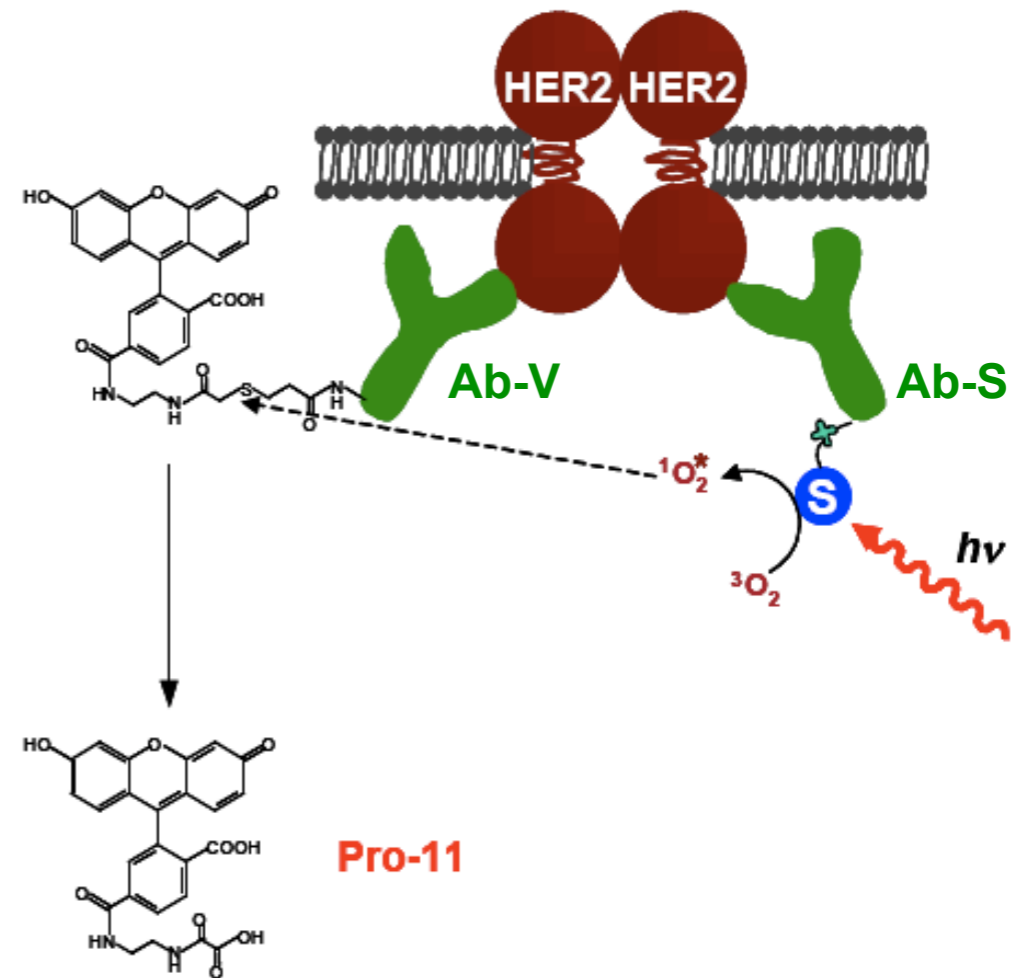
- Proximity-based method using formalin-fixed paraffin-embedded (FFPE) specimens
- Quantitates total HER2 expression (H2T) and HER2:HER2 dimers (H2D)

VeraTag Assay

Total HER2 expression (H2T)

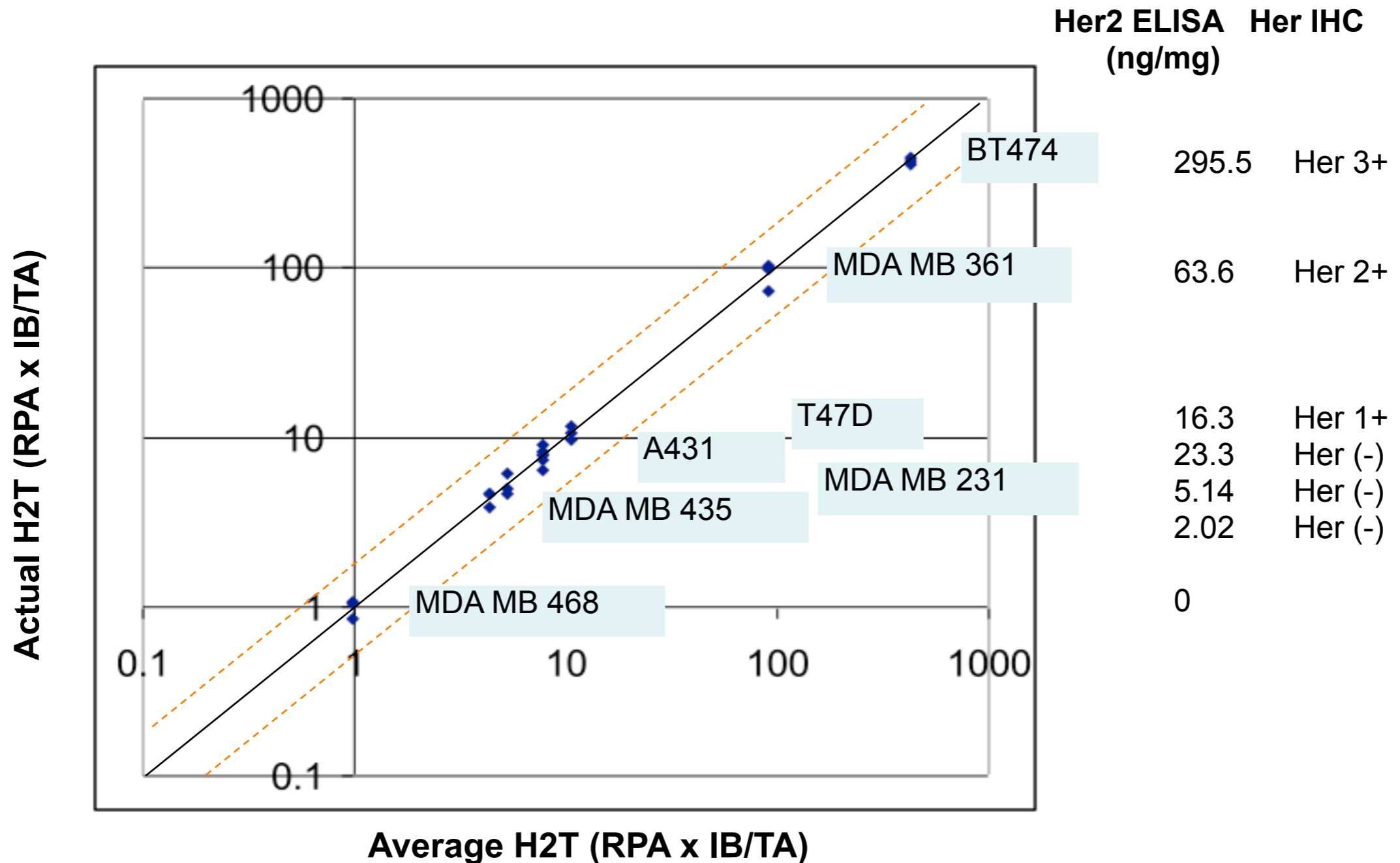


HER2 homodimer (H2D)



Monoclonal antibodies conjugated either to fluorescein (Ab-V/Pro-11) or to a molecular scissors (Ab-S) target distinct epitopes in the intracellular domain of HER2. Exposure to red light activates the molecular scissors causing the production of singlet O_2 . If the Ab-V/Pro-11 is within range (~ 80 nm) of the liberated O_2 , the thioether linkage is cleaved and the fluorescein reporter (Pro-11) is released, harvested, and quantitated by capillary electrophoresis. VeraTag's standard unit of measurement is (relative peak area x collection buffer volume) / (invasive tumor area in mm^2). (RPA x IB/TA)

VeraTag HER2 Total Assay: Breast Cancer Cell Lines



BT474 > MDA-MB-361 > T47D >~ A431 >~ MDA-MB-231 >~ MDA-MB435 >~ MDA-MB-468

The H2T and H2D assays are validated to CLIA specifications.
Similar results for H2D. RPA x IB/TA is the standard unit of measure for VeraTag assays.

Patient Inclusion Criteria

- 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 \geq 12 weeks
- No prior trastuzumab
- Treated with either trastuzumab + chemotherapy or single-agent trastuzumab

Statistics

- H2T and H2D were correlated with ORR (CR+PR), TTP, and OS.
- Methods: Test for trend, Log rank, Kaplan Meier, and Cox Proportional Hazards regression analysis.
- The optimal cutpoint for H2T and H2D was selected using positional scanning for the lowest TTP p-value (above vs. below the optimal cutpoint).

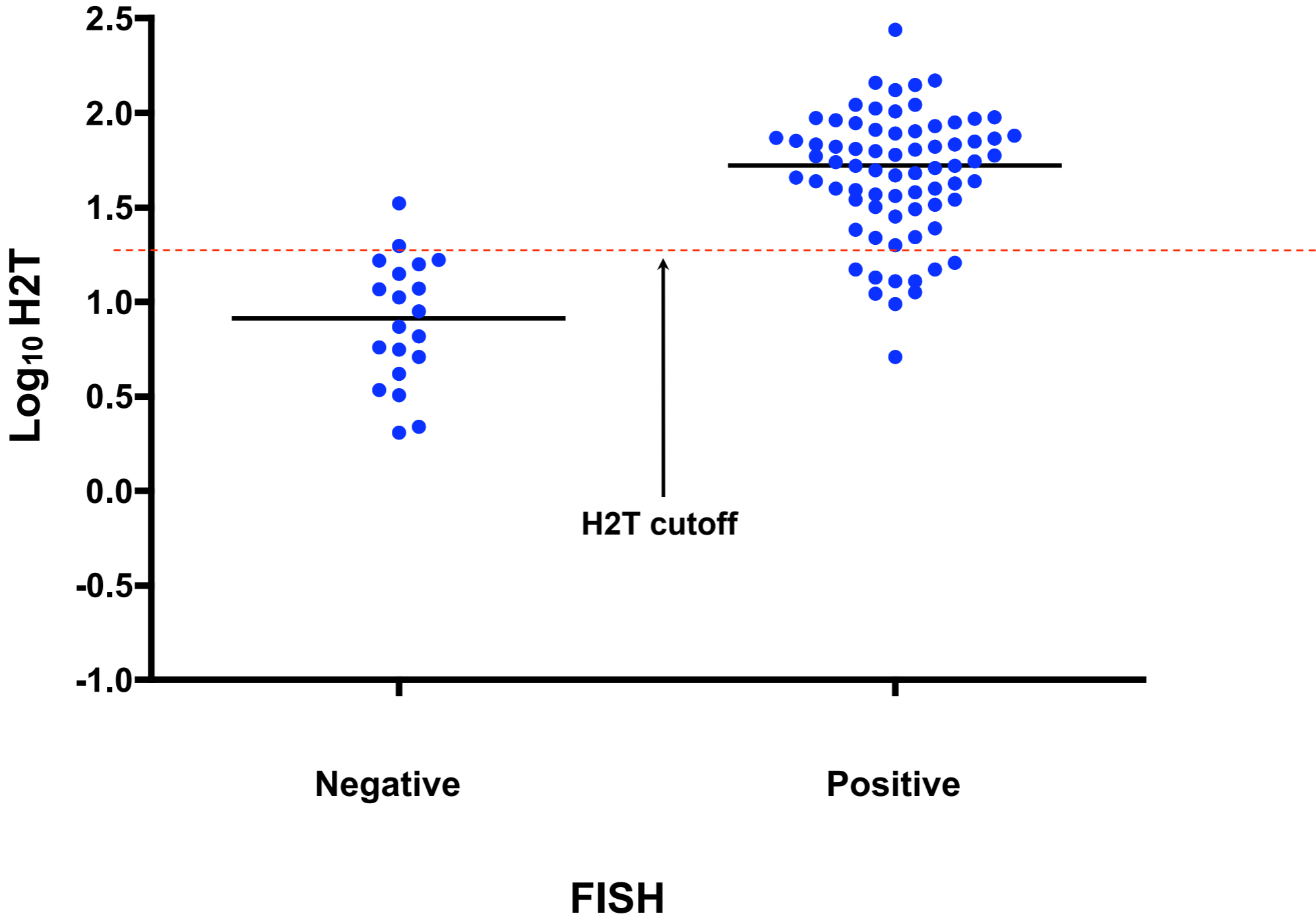
Demographics

Total number of patients	103
Mean follow-up (months)	34.3 (11.8 - 77.9)
Mean age (range)	55.3 (27.6 - 85.4)
H2T (> Cutoff)	70 (69 %)
H2D (> Cutoff)	68 (67 %)
Number of metastatic sites	
< 3	61 (59 %)
≥ 3	42 (41%)
Treatment	
Trastuzumab + chemotherapy	91 (88%)
Trastuzumab only	12 (12%)
Line of chemotherapy	
First line	76 (74%)
Second line	17 (17%)
Third line	8 (7%)
Unknown	2 (2%)

HER2 Status

<u>IHC</u>	<u>Patients (n=103)</u>
2+	5
FISH+	5
3+	96
FISH+	68
FISH-	20
FISH unknown	8
Unknown	2
FISH+	2

Distribution of H2T values by FISH



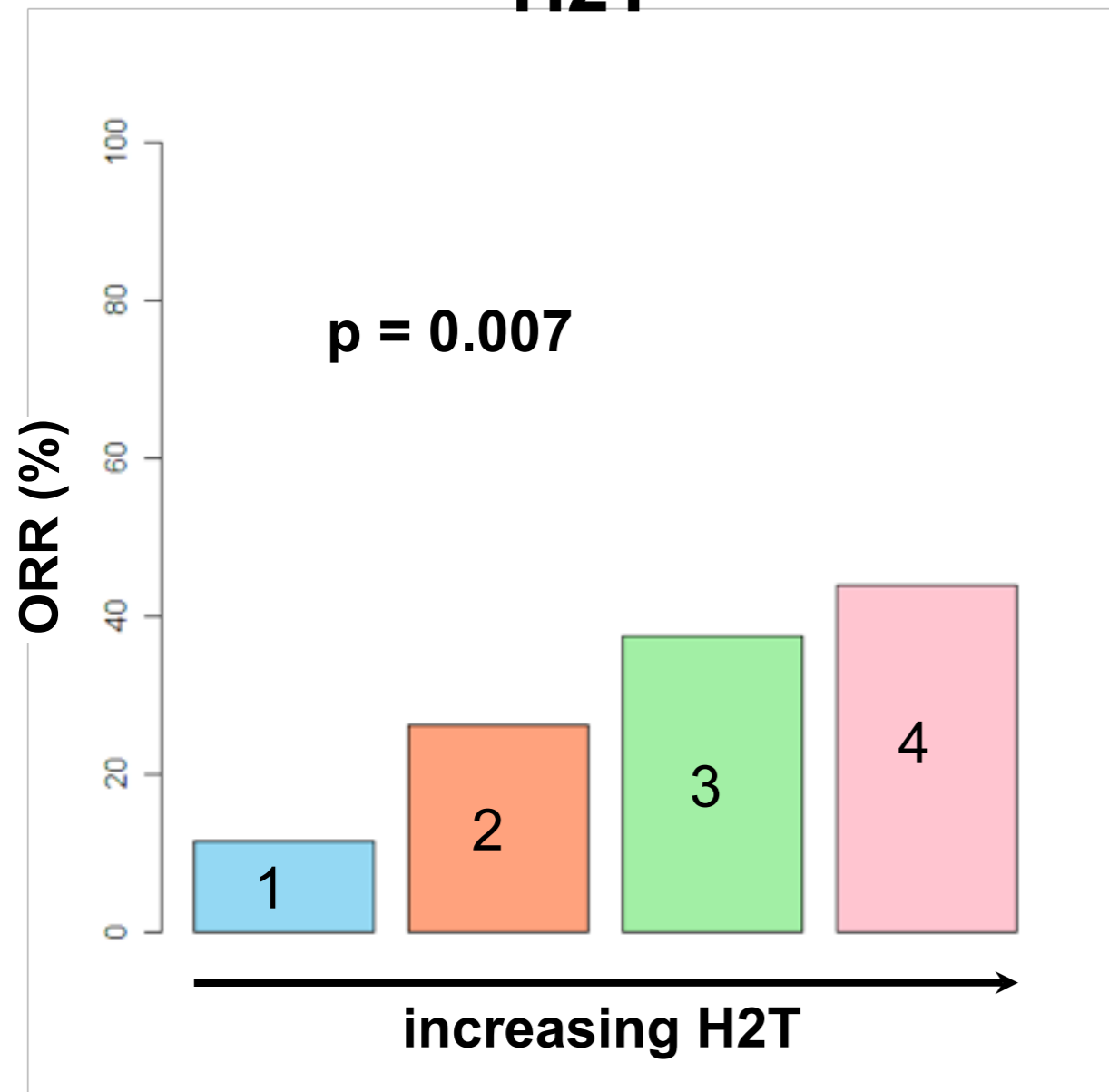
Discordance of H2T values and FISH

FISH status	H2T		Total
	not elevated	elevated	
negative	18	2	20
positive	10	64	74
Total	28	66	94

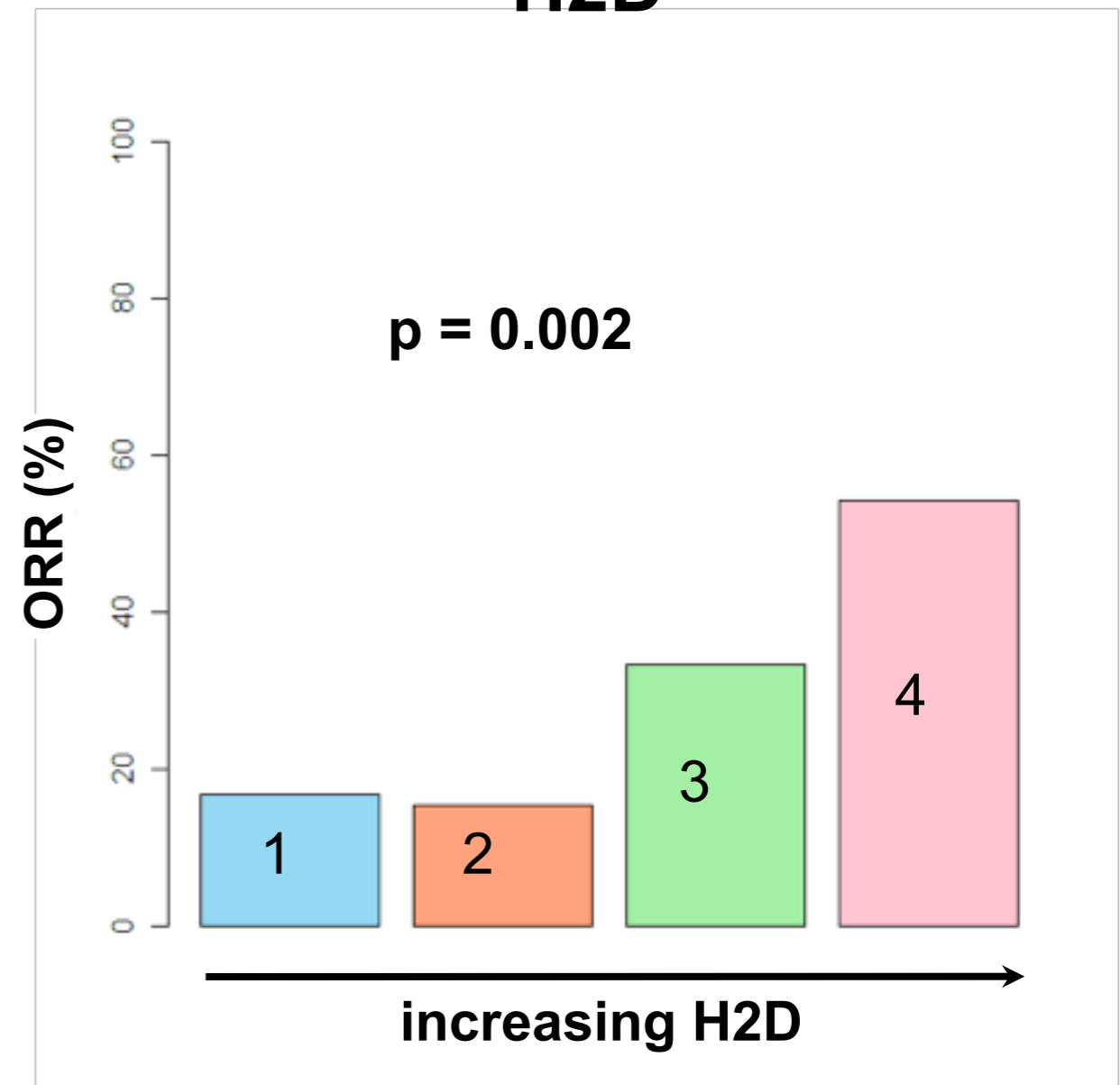
**FISH / H2T
discordance rate = 13 %**

Objective Response (CR+PR) H2T, H2D by quartiles (N=103)

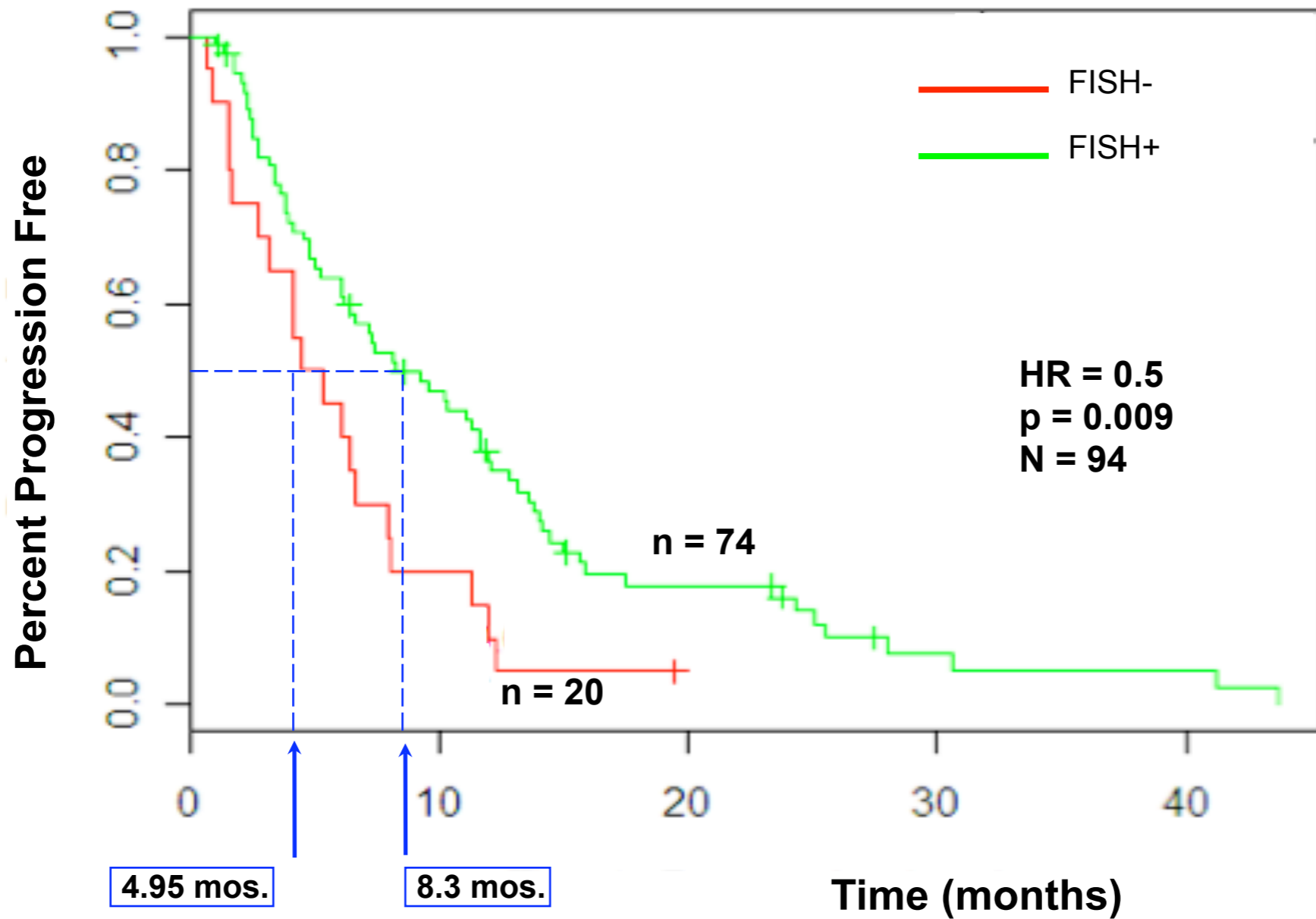
H2T



H2D

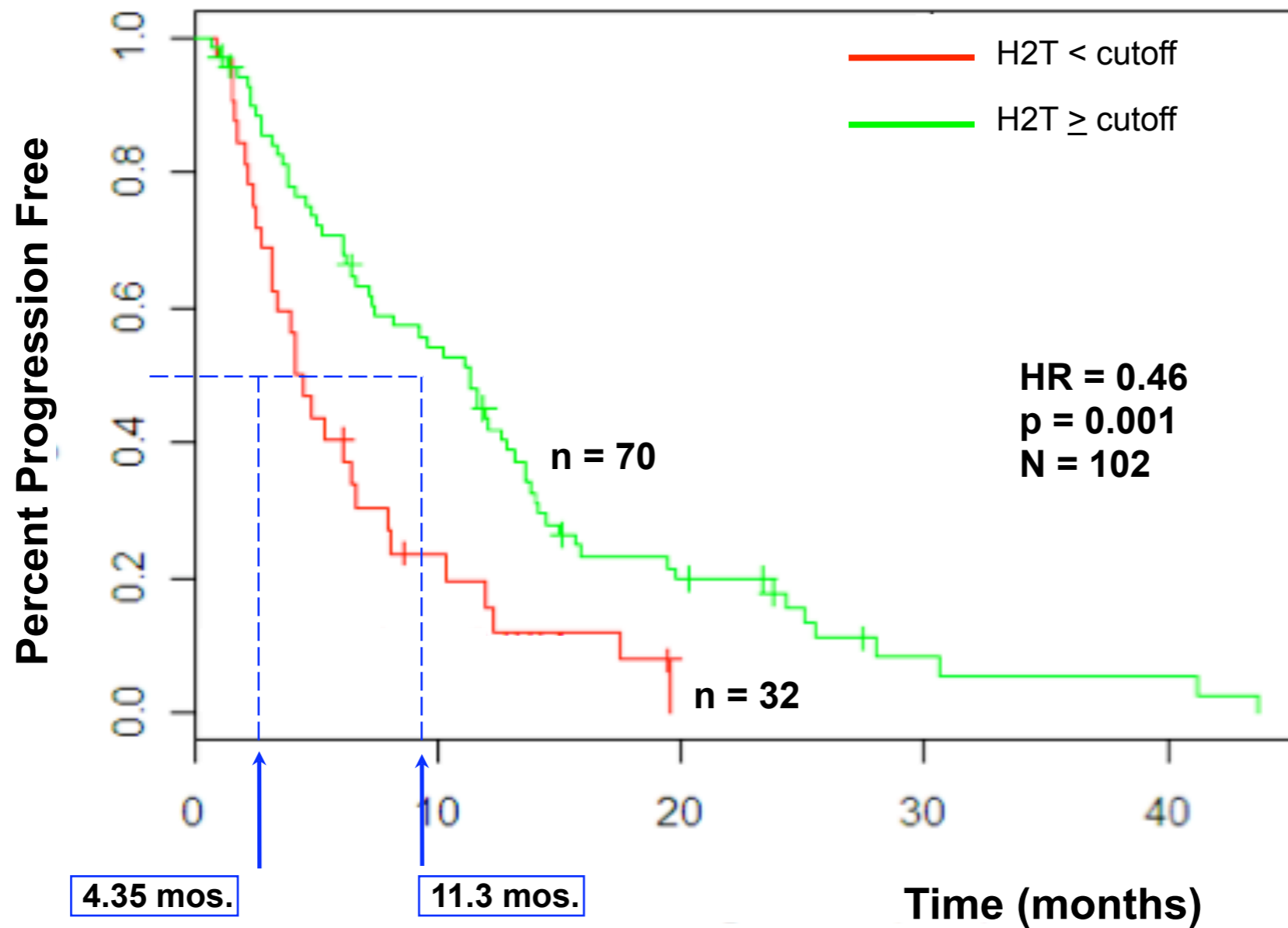


TTP : FISH



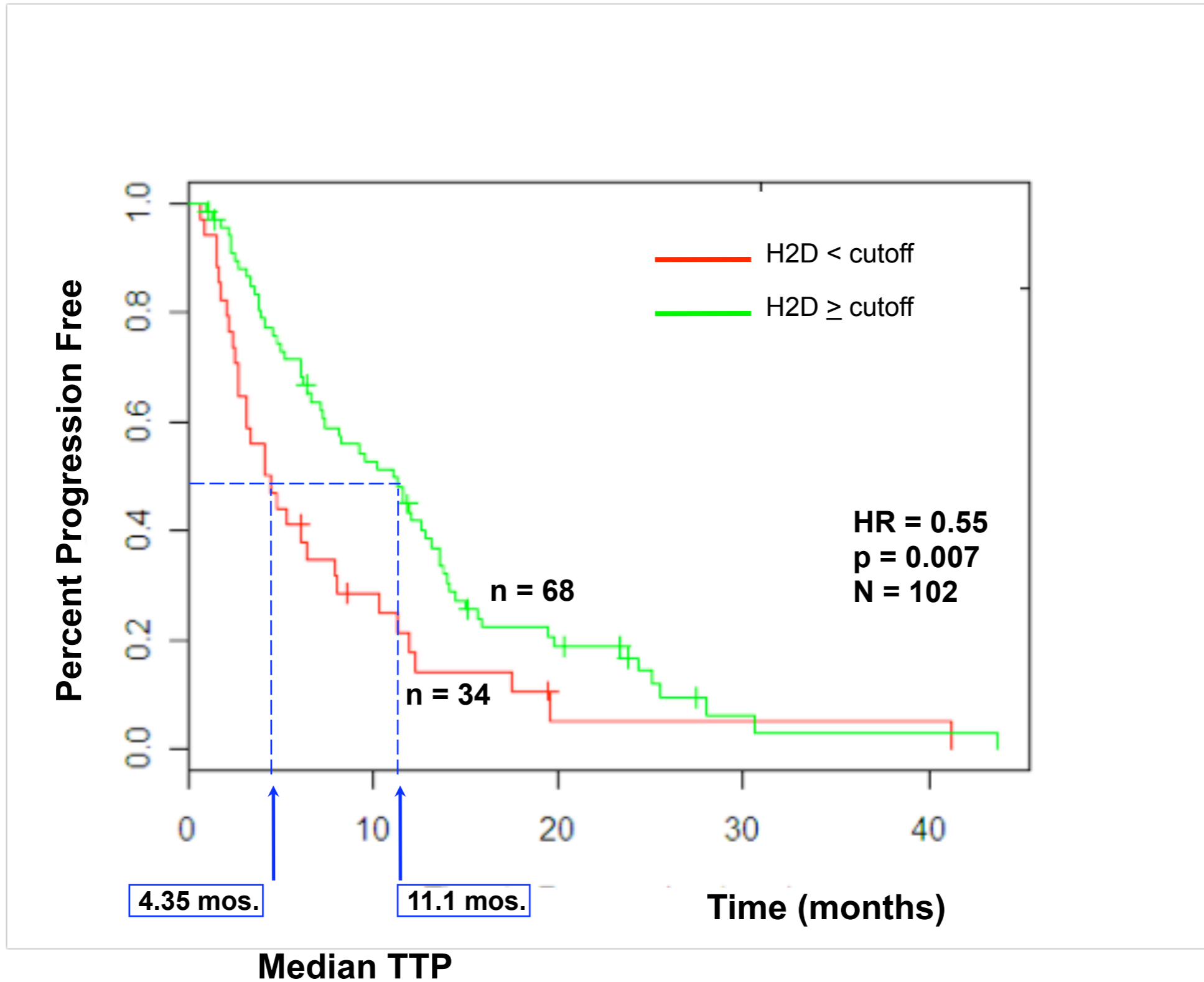
Median TTP

TTP : H2T



Median TTP

TTP : H2D



Overall Survival: by HER2 assay

	Median OS (months)		
Assay	negative / below cutoff	positive / above cutoff	p-value
FISH	28.7	31.8	0.29
H2T	28.7	37.4	0.16
H2D	26.6	37.4	0.11

Multivariate Analysis: TTP

Variable	without H2T or H2D		with H2T		with H2D	
	Hazard Ratio	P-value	Hazard Ratio	P-value	Hazard Ratio	P-value
Metastatic sites ≥ 3 vs <3	3.1	0.000004	3.4	0.000002	3.3	0.000002
Skin Metastasis Yes vs No	2.1	0.013	2	0.015	2	0.014
FISH Pos vs Neg	0.52	0.02	1.1	0.8	0.92	0.8
H2T High vs Low	-	-	0.39	0.018	-	-
H2D High vs Low	-	-	-	-	0.53	0.092

*Variables included in Cox model: Number of metastatic sites, treatment length, line of therapy, ER status, PR status, age at time of treatment, IHC status (2+ vs. 3+), IHC H-score, FISH status, CEA, p53, 15.3, time from sample to treatment, time from primary diagnosis to first metastasis, time from diagnosis of metastatic disease to treatment, H2T, H2D, H2D/H2T, mets to CNS, mets to lung, mets to liver, mets to lymph node, skin involvement/mets, mets to bone, other.

Multivariate Analysis: Overall Survival

	without H2T or H2D		with H2T		with H2D	
Variable	Hazard Ratio	P-value	Hazard Ratio	P-value	Hazard Ratio	P-value
Age	0.97	0.048	0.97	0.021	0.97	0.024
Metastatic sites ≥ 3 vs <3	1.6	0.000007	1.6	0.000003	1.6	0.000004
ER Pos vs Neg	0.49	0.03	0.48	0.03	0.51	0.039
FISH Pos vs Neg	0.65	0.23	1.4	0.57	1.6	0.36
H2T High vs Low	-	-	0.4	0.058	-	-
H2D High vs Low	-	-	-	-	0.35	0.026

*Variables included in Cox model: Number of metastatic sites, treatment length, line of therapy, ER status, PR status, age at time of treatment, IHC status (2+ vs. 3+), IHC H-score, FISH status, carcinoembryonic antigen (CEA), p53, 15.3, time from sample to treatment, time from primary diagnosis to first metastasis, time from diagnosis of metastatic disease to treatment, H2T, H2D, H2D/H2T, mets to CNS, mets to lung, mets to liver, mets to lymph node, skin involvement/mets, mets to bone, other.

Multivariate Analyses: FISH+ only (N=74)

	TTP			
	H2T		H2D	
Variable	Hazard Ratio	P-value	Hazard Ratio	P-value
Metastatic sites ≥ 3 vs <3	3.6	0.00002	3.2	0.00004
H2T High vs Low	0.3	0.002	-	-
H2D High vs Low	-	-	0.53	0.094

	Survival			
Age	0.97	0.058	0.97	0.057
Metastatic sites ≥ 3 vs <3	1.8	0.000005	1.7	0.00003
H2T High vs Low	0.25	0.006	-	-
H2D High vs Low	-	-	0.31	0.017

CONCLUSIONS

- In HER2- positive metastatic breast cancer (IHC3+ or FISH +), high HER2 total and HER2 homodimer levels selected patients more likely to respond to trastuzumab-containing therapy.
- In multivariate analysis, HER2 total and HER2 homodimer levels were significantly correlated with TTP and OS.
- These results need further confirmation in trials of adjuvant and metastatic HER2-targeted therapy.

Acknowledgements

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