Quantitative HER2 levels and steroid receptor expression in primary breast cancers and in matched brain metastases

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Abstract

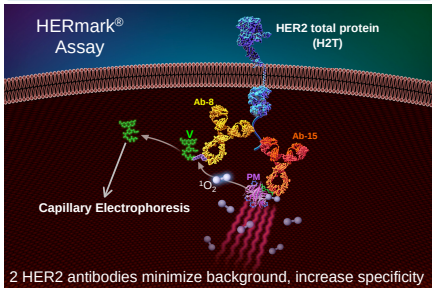
Background: Breast cancer patients with human epidermal growth factor receptor (HER2)-positive tumors are at high risk for brain metastases. In the current study we examined expression of estrogen receptor (ER), progesterone receptor (PgR) and HER2 in primary breast tumors and in matched brain metastases, as changes of their levels might reflect modes of escape from therapy.

Methods: Fifty-three pairs of matched formalin-fixed paraffin-embedded samples from primary breast cancers and brain metastases were assayed for ER and PgR status by immuno-histochemistry, whereas HER2 expression was quantified using the novel HERmark® assay. Nuclear staining of ER and PgR ≥10%, and relative fluorescence of HER2 >17.8/mm² were considered as positive results.

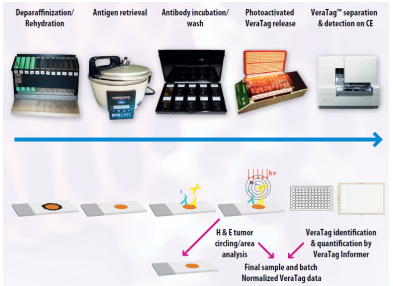
Results: HER2 levels in brain metastases were generally higher than in the primary tumors (p = 3e-6), with a median increase of 1.9-fold (range 0.08 to 199-fold). There were also substantial differences in ER and PgR status between primary tumors and brain metastases. Loss of steroid receptor positivity in brain metastases was more frequent than its gain (ER: 46% vs. 26%; p = 0.16; PgR: 57% vs. 23%; p = 0.044). These changes resulted in a net increase in the number of HER2-positive/ER-negative brain metastases, which more than doubled the proportion of primary breast tumors with this phenotype (26% vs. 11%, respectively; p = 0.08). Additionally, HER2 levels in the primary tumors significantly correlated with overall survival when stratified by ER status of the primary tumor (p = 0.011).

Conclusions: Brain metastases of breast cancer show significant changes in steroid receptor status and in quantitative HER2 levels compared to matched primary tumors. These data provide a rationale for future studies and may help in designing treatment strategies that target the most likely escape pathways of breast cancer.

Methods - H2T VeraTag® method



Methods - H2T VeraTag Workflow

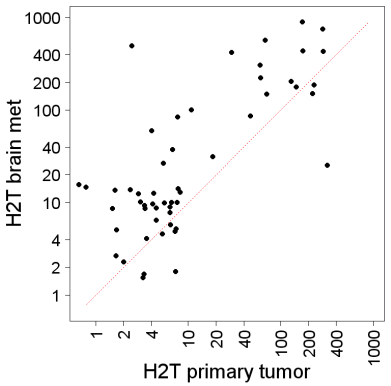


Patient Characteristics

Characteristic		Category	N	%
HER2 protein (HERmark H2T)[a]	primary	positive	16	30
		equivocal	1	2
		negative	36	68
	brain met	positive	22	42
		equivocal	8	15
		negative	23	43
ER	primary	positive	26	49
		negative	27	51
	brain met	positive	21	40
		negative	32	60
PgR	primary	positive	21	40
		negative	31	58
		unknown	1	2
	brain met	positive	17	32
		negative	36	68
Grade	primary	G3	27	51
		G1 + G2	24	45
		unknown	2	4
Pathology Type	primary	ductal	43	81
		lobular	6	11
		ductal+lobular	2	4
		other	1	2
		unknown	1	2
Dominant metastatic site		soft tissue	1	2
		bone	2	4
		viscera	46	87
		unknown	4	8
Age at progression		median	51	
		range	29 - 76	

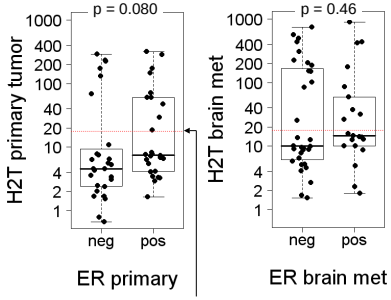
[a]HERmark positive is defined as H2T>17.8. HERmark negative is defined as H2T≤10.5. Equivocals are between these two limits. These cutoffs were previously found to coincide with central lab determined 95th % of HER2-negatives and 5th % of HER2-positives.

Changes in HER2 level by HERmark



HER2 expression measured by HERmark (H2T) was often higher in brain metastases than in the primary tumors, with a medan 1.9-fold increase (paired Wilcoxon rank, p = 3e-6).

Changes in ER status



H2T>17.8 is HERmark positive, previously found to coincide with >95th percentile of central lab determined HER2 negatives.

Changes in hormone receptor and HER2 status

ER and PgR status

Brain Metastasis	Primary Tumor ER −	Primary Tumor ER +
ER −	20	12
ER +	7	14

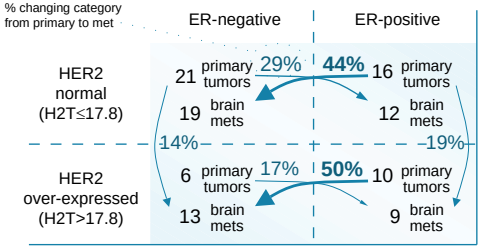
46% loss vs. 26% gain; Fisher p = 0.16

Brain Metastasis	Primary Tumor PgR −	Primary Tumor PgR +
PgR −	24	12
PgR +	7	9

57% loss vs. 23% gain; Fisher p = 0.044

Fluxes between HER2/ER subgroups

net flux to HER+/ER-



- No loss of HER2-positivity from primary to brain met
- More change in ER than HER2 status → selective pressure?
- No cases with changes in *both* ER and HER2 category

Correlation with Overall Survival*

Univariate correlations	HR	p
H2T primary (continuous)	1.6	**0.035**
H2T met (continuous)	1.2	0.29
HER2 IHC	1.1	0.55
HER2 IHC met	1.0	0.74
ER primary	0.67	0.21
ER met	0.70	0.29
Grade	1.2	0.52
trastuzumab treatment	0.98	0.96

*Overall survival measured from detection of brain metastasis to death or censor.

H2T and ER of primary tumor plus trastuzumab correlate with overall survival

3-variable model - **primary tumor**	HR	p
H2T primary (continuous)	3.0	**0.0001**
ER primary	0.43	**0.017**
trastuzumab treatment	0.29	**0.021**

3-variable model - **brain metastasis**	HR	p
H2T met (continuous)	1.4	0.14
ER met	0.66	0.24
trastuzumab treatment	0.70	0.48

Summary

- H2T showed a median increase of 1.9-fold from primary tumor to brain metastasis.
- Substantial changes in ER status was observed between primary tumor and brain metastases with change from ER-positive to ER-negative occurring more often, possibly due to selective pressure of treatment.
- H2T measured in the primary tumor tissue correlated with overall survival, although H2T measured in the brain metastasis did not.