

# Patterns of HER-family receptor dimerization in trastuzumab susceptible and trastuzumab resistant cell lines

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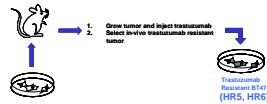
**Background:** HER2 overexpression in breast carcinoma is associated with accelerated disease progression and poor prognosis in breast cancer. Trastuzumab, a monoclonal antibody targeting the extracellular domain of HER2, is effective in the treatment of metastatic breast cancer. However, most patients treated with trastuzumab eventually develop clinical resistance. To investigate the role of HER-family receptors in trastuzumab resistance, we measured HER-family receptor expression, dimerization, and phosphorylation in trastuzumab susceptible and resistant cell lines.

**Method:** Trastuzumab resistant BT474 and SKBR3 cell lines were generated by propagation of the cells either *in vivo* (BT474) or *in vitro* (SKBR3) in the presence of trastuzumab. Lysates were made from cell cultures and Proximity-based multiplexed assays were used to detect and quantify HER1, HER2, and HER3 expression and phosphorylation levels, as well as HER1/HER2, HER1/HER3, HER2/HER2, and HER2/HER3 dimers. Samples are incubated with a mixture of HER specific antibodies conjugated either with fluorescent reporter tags (eTag), or a reporter tag releasing agent (chemical scissor). Reporter molecules are released based on proximity to the scissor in a photochemical reaction and subsequently separated by capillary gel electrophoresis.

**Results:** Total HER2 and HER3 were found to be similar in trastuzumab susceptible and resistant BT474 and SKBR3 cell lines. Strikingly, however, HER1 levels were found to be significantly elevated in both BT474 and SKBR3 resistant cells. Resistant BT474 cell lines also exhibited markedly increased levels of HER1/HER2 heterodimers. Resistant SKBR3 cells showed both an increase in HER1/HER2 and HER2/HER2 dimerization. This was also consistent with significantly higher HER2 phosphorylation in resistant SKBR3 cells.

**Conclusion:** The development of trastuzumab resistance in these cell line models correlated with HER1 expression and the appearance of HER1/HER2 heterodimers. Since signaling initiated by such heterodimers is ineffectively antagonized by trastuzumab, these data suggest that proliferative signaling by HER1/HER2 dimers may represent a mechanism of trastuzumab resistance in breast cancer.

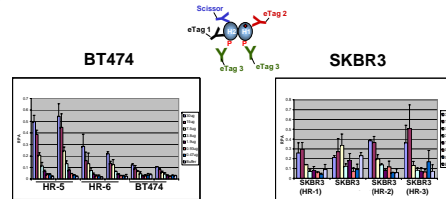
## Trastuzumab resistant BT474



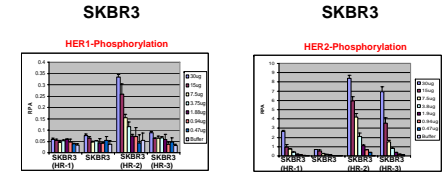
## Trastuzumab resistant SKBR3



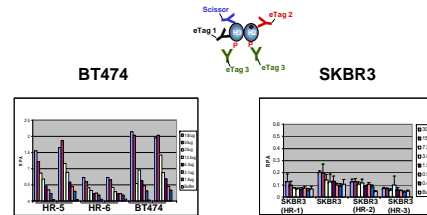
## Detection and quantification of HER1/HER2 heterodimers



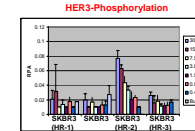
## Detection and quantification of HER1, HER2, HER3 Phosphorylation



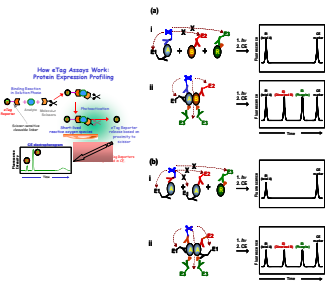
## Detection and quantification of HER2/HER3 heterodimers



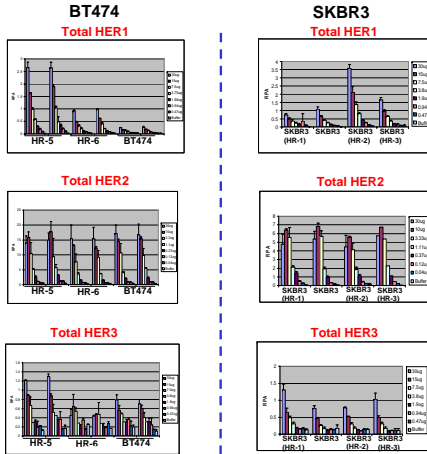
## SKBR3



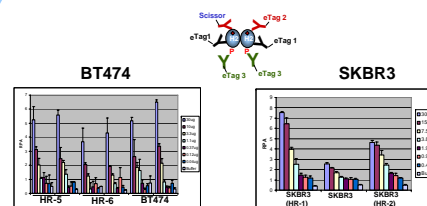
## Proximity eTag assay



## Detection and quantification of HER1, HER2, HER3



## Detection and quantification of HER2/HER2 homodimers

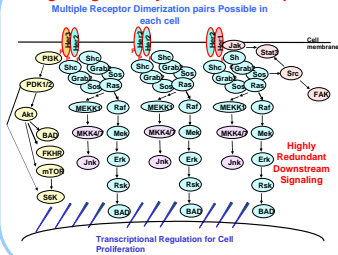


## SUMMARY

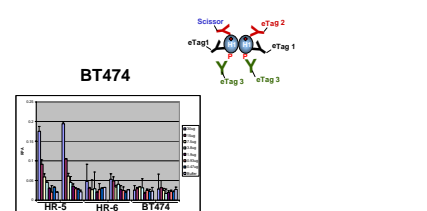
Cell Line	Monogram controls									
	HER1/HER2	HER1/HER3	HER2/HER2	HER2/HER3	HER3/HER3	HER1	HER2	HER3	HER2/HER2	HER2/HER3
BT474	***	***	***	***	***	***	***	***	***	***
SKBR3	***	***	***	***	***	***	***	***	***	***

Cell Line	Monogram controls									
	HER1/HER2	HER1/HER3	HER2/HER2	HER2/HER3	HER3/HER3	HER1	HER2	HER3	HER2/HER2	HER2/HER3
BT474	***	***	***	***	***	***	***	***	***	***
SKBR3	***	***	***	***	***	***	***	***	***	***

## Signaling Pathways from HER Receptors



## Detection and quantification of HER1/HER1 homodimers



## Conclusions

- Trastuzumab resistant BT474 and SKBR3 cells showed higher levels of HER1 as compared to trastuzumab susceptible BT474 and SKBR3 cell lines.
- Total HER2 and HER3 receptor levels were similar in trastuzumab resistant and trastuzumab susceptible BT474 and SKBR3 cell lines.
- Higher levels of HER1/HER2 heterodimers were present in trastuzumab resistant BT474 and SKBR3 cell lines.
- Resistant SKBR3 cells also showed an increase in HER2/HER2 homodimers and HER2 phosphorylation.
- HER1/HER1, HER2/HER3, receptor dimerization levels were similar in trastuzumab resistant and trastuzumab susceptible BT474 and SKBR3 cell lines.