

# HER2 (ErbB2) Receptor Homodimerization Sensitizes Cells to Trastuzumab

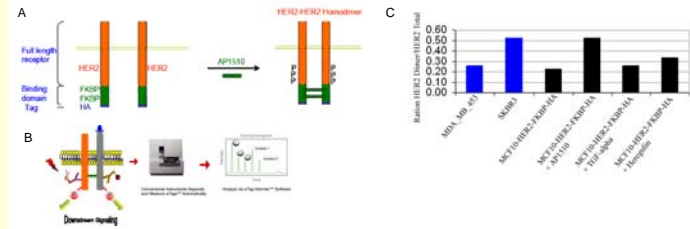
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## Introduction

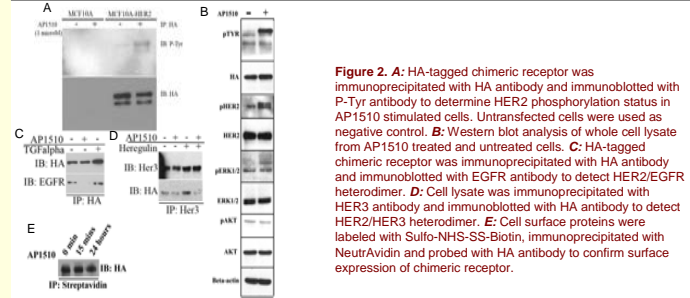
Amplification of the HER2 oncogene is present in approximately 25% of invasive breast cancers. Trastuzumab, an antibody against the ectodomain of the HER2 receptor tyrosine kinase (RTK), is approved for the treatment of HER2-overexpressing breast cancers. HER2 can be activated by homodimerization in cells with high levels of the RTK or by engagement with ligand-activated EGFR (ErbB1) or HER3 (ErbB3) co-receptors. About 50% of patients with HER2+ breast cancer either do not respond or eventually become resistant to trastuzumab. One proposed mechanism of resistance is overexpression of EGFR and HER3 ligands. Indeed, structural data using ErbB receptor ectodomains demonstrated that trastuzumab is unable to block ligand-induced EGFR/HER2 and HER2/HER3 heterodimers. Thus, we hypothesized that HER2+ breast cancers containing high levels of EGFR/HER2 and HER2/HER3 heterodimers, will be less responsive to trastuzumab compared to HER2+ tumors with undetectable or low levels of these heterodimers. To test this hypothesis, we developed a cell system where HER2 hetero- and homodimerization can be conditionally regulated. A retroviral vector encoding full-length human HER2 fused to HA-tagged ligand-binding domain of FK506 binding protein (FKBP) was transduced into MCF10A mammary epithelial cells. Treatment of MCF10A-HER2-FKBP-HA cells with the ligand AP1510 resulted in homodimerization and tyrosine phosphorylation of HER2 chimera. HA-pulldown of lysates from AP1510-stimulated cells did not precipitate EGFR or HER3, suggesting presence of only HER2 in the induced dimers. HER2 homodimerization was confirmed by fluorescent antibody-based dimer-detection VeraTag assays using formalin-fixed-paraffin-embedded (FFPE) cells. Treatment with AP1510 stimulated P-Erk and monolayer cell growth but not P-Akt. In 3-D matrigel, AP1510-treated cells formed invasive acini while untreated cells failed to grow. The EGFR ligands EGF and TGF $\alpha$  and the HER3 ligand heregulin stimulated EGFR/HER2 and HER2/HER3 heterodimerization, respectively, as well as MCF10A-HER2-FKBP-HA acini growth in 3D. Trastuzumab completely inhibited AP1510-stimulated but not EGF, TGF $\alpha$ , or heregulin-stimulated growth in monolayer and in 3-D matrigel. Moreover, trastuzumab induced apoptosis, as assessed by BrdU incorporation, in AP1510-treated cells but not on untreated cells. Lapatinib ditosylate (GW572016), an inhibitor of EGFR and HER2 tyrosine kinases, blocked EGF, heregulin and AP1510-induced growth in 3-D matrigel. Pertuzumab, an antibody that blocks ligand-induced HER2 heterodimerization, inhibited heregulin-induced but not AP1510-induced growth in 3-D matrigel.

## Development of a Cell system with controlled her2 homo-dimerization



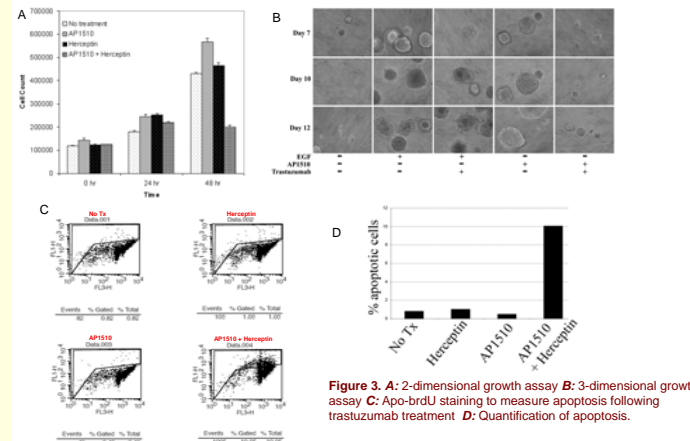
**Figure 1. A:** ErbB chimeric receptor vector. Cartoon of the chimeric receptors and the synthetic dimerizing ligand. Phosphorylated HER2 homodimers can be induced to form by the addition of AP1510 which binds to FKBP. **B:** Antibody linked proximity based VeraTag assay. **C:** Detection of HER2 homodimers in AP1510 treated cells by the VeraTag Assay.

## AP1510 induces her2 homodimerization and phosphorylation



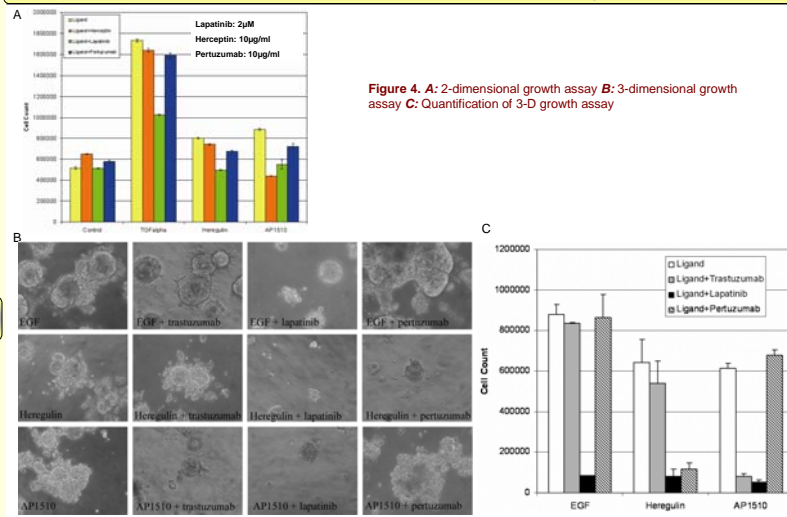
**Figure 2. A:** HA-tagged chimeric receptor was immunoprecipitated with HA antibody and immunoblotted with P-Tyr antibody to determine HER2 phosphorylation status in AP1510 stimulated cells. Untransfected cells were used as negative control. **B:** Western blot analysis of whole cell lysate from AP1510 treated and untreated cells. **C:** HA-tagged chimeric receptor was immunoprecipitated with HA antibody and immunoblotted with EGFR antibody to detect HER2/EGFR heterodimer. **D:** Cell lysate was immunoprecipitated with HER3 antibody and immunoblotted with HA antibody to detect HER2/HER3 heterodimer. **E:** Cell surface proteins were labeled with Sulfo-NHS-SS-Biotin, immunoprecipitated with NeutrAvidin and probed with HA antibody to confirm surface expression of chimeric receptor.

## Her2 homodimerization sensitizes cells to trastuzumab therapy



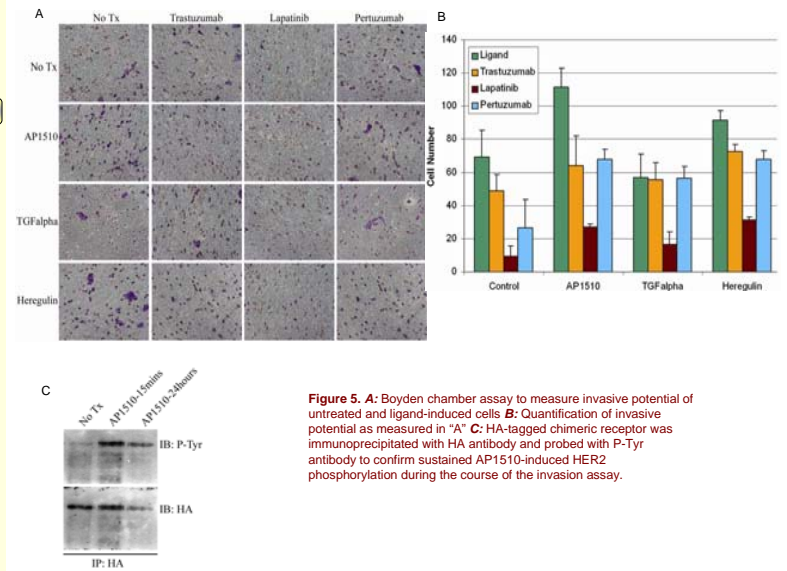
**Figure 3. A:** 2-dimensional growth assay **B:** 3-dimensional growth assay **C:** Apo-brdU staining to measure apoptosis following trastuzumab treatment **D:** Quantification of apoptosis.

## Trastuzumab does not inhibit HER2/HER3 and HER2/EGFR induced growth



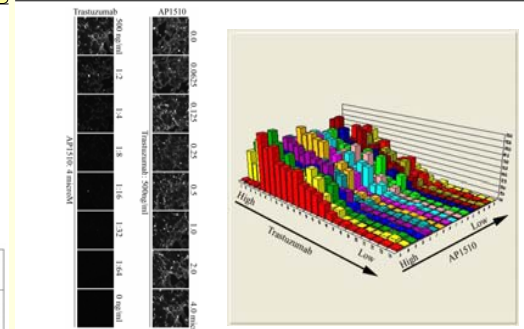
**Figure 4. A:** 2-dimensional growth assay **B:** 3-dimensional growth assay **C:** Quantification of 3-D growth assay

## Differential response of HER2 inhibitors depending on receptor dimerization status



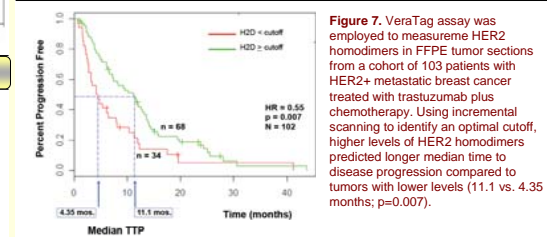
**Figure 5. A:** Boyden chamber assay to measure invasive potential of untreated and ligand-induced cells **B:** Quantification of invasive potential as measured in "A" **C:** HA-tagged chimeric receptor was immunoprecipitated with HA antibody and probed with P-Tyr antibody to confirm sustained AP1510-induced HER2 phosphorylation during the course of the invasion assay.

## AP1510 does not affect trastuzumab binding to HER2



**Figure 6.** Fluorescent microvolume assay technology (FMAT) assay to determine trastuzumab binding to HER2 in AP1510 treated and untreated cells. Fluorescently (Alexa 647) labeled trastuzumab was added to cells in presence of increasing concentration of AP1510 for 1 hour at 37° C after which fluorescence was measured using the microplate reader.

## Higher HER2 homodimer levels correlate with longer TTP



**Figure 7.** VeraTag assay was employed to measure HER2 homodimers in FFPE tumor sections from a cohort of 103 patients with HER2+ metastatic breast cancer treated with trastuzumab plus chemotherapy. Using incremental scanning to identify an optimal cutoff, higher levels of HER2 homodimers predicted longer median time to disease progression compared to tumors with lower levels (11.1 vs. 4.35 months; p=0.07).

## Conclusions

- Treatment of MCF10A stably expressing HER2-FKBP-HA with the bivalent ligand AP1510 induced HER2 homodimers but no association of HER2 with endogenous EGFR or HER3 receptors.
- Activation of HER2 homodimers with AP1510 induced receptor tyrosine phosphorylation and p-ERK1/2 but not p-AKT.
- When cultured in 3-D matrigel, cells induced with AP1510 forms invasive mammary acini while untreated cells failed to grow.
- Trastuzumab completely inhibited AP1510-stimulated but not EGF, TGF $\alpha$ , or heregulin-stimulated growth of MCF10A/HER2-FKBP-HA cells both in monolayer and in 3-D matrigel.
- AP1510 induced HER2 homodimerization did not affect trastuzumab binding to MCF10A/HER2-FKBP-HA cells.
- High levels of HER2 homodimers in patients with HER2+ metastatic breast cancer correlated with a longer time to progression following treatment with trastuzumab and chemotherapy.
- We speculate that because of their inability to potentially activate P-Akt, cells more dependent on HER2-containing homodimers, are less able to bypass trastuzumab action.