Therapeutics, Targets, and Chemical Biology

Trastuzumab Has Preferential Activity against Breast Cancers Driven by HER2 Homodimers

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Abstract

In breast cancer cells with HER2 gene amplification, HER2 receptors exist on the cell surface as monomers, homodimers, and heterodimers with EGFR/HER3. The therapeutic antibody trastuzumab, an approved therapy for HER2+ breast cancer, cannot block ligand-induced HER2 heterodimers, suggesting it cannot effectively inhibit HER2 signaling. Hence, HER2 oligomeric states may predict the odds of a clinical response to trastuzumab in HER2-driven tumors. To test this hypothesis, we generated nontransformed human MCF10A mammary epithelial cells stably expressing a chimeric HER2-FKBP molecule that could be conditionally induced to homodimerize by adding the FKBP ligand AP1510, or instead induced to heterodimerize with EGFR or HER3 by adding the heterodimer ligands EGF/TGFβ or heregulin. AP1510, EGF, and heregulin each induced growth of MCF10A cells expressing HER2-FKBP. Trastuzumab inhibited homodimer-mediated but not heterodimer-mediated cell growth. In contrast, the HER2 antibody pertuzumab, which blocks HER2 heterodimerization, inhibited growth induced by heregulin but not AP1510. Lastly, the HER2/EGFR tyrosine kinase inhibitor lapatinib blocked both homodimer- and heterodimer-induced growth. AP1510 triggered phosphorylation of Erk1/2 but not AKT, whereas trastuzumab inhibited AP1510-induced Erk1/2 phosphorylation and Shc-HER2 homodimer binding, but not TGFβ-induced AKT phosphorylation. Consistent with these observations, high levels of HER2 homodimers correlated with longer time to progression following trastuzumab therapy in a cohort of patients with HER2-overexpressing breast cancer. Together, our findings confirm the notion that HER2 oligomeric states regulate HER2 signaling, also arguing that trastuzumab sensitivity of homodimers may reflect their inability to activate the PI3K (phosphoinositide 3-kinase)/AKT pathway. A clinical implication of our results is that high levels of HER2 homodimers may predict a positive response to trastuzumab. Cancer Res; 71(5); 1871–82. ©2011 AACR.

Introduction

ErbB receptors are transmembrane tyrosine kinases comprising four members: ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4 (1). Dysregulation of ErbB expression has been associated with cancers of the lung, breast, head and neck, gastrointestinal tract, ovary, brain, and prostate (2–4). HER2 gene amplification and protein overexpression, present in about 25% of invasive breast cancers (5), are associated with poor patient prognosis. In HER2-overexpressing breast cancer cells, HER2 is present in a complex equilibrium of preassociated active and inactive homodimers, heterodimers, and monomers on the cell surface (6). Recruitment of HER2 to its coreceptors potentiates signaling by HER2-containing heterodimers (7, 8). In HER2-overexpressing cells, the kinase-impaired HER3 coreceptor is the main adaptor that directly couples to the phosphatidylinositol-3 kinase PI3K (phosphoinositide 3-kinase)/AKT pathway (9). HER2 overexpression also activates the Ras/Raf/MEK (MAP/ERK kinase)/MAPK (mitogen activated protein kinase) pathway via the recruitment of the adaptor proteins Grb2 and Shc (10).

Trastuzumab, an antibody against the ectodomain of HER2, is approved for the treatment of HER2-overexpressing breast cancer (11, 12). Overall, trastuzumab is clinically effective but a significant proportion of HER2-overexpressing breast cancer patients either do not respond or eventually become resistant to trastuzumab (13–15). Predictive assays to reliably identify...
HER2-overexpressing cancers that will respond or not to existing anti-HER2 therapy are not yet available.

Several studies have reported on possible mechanisms of resistance to trastuzumab. For example, amplification of PI3K signaling due to loss of lipid phosphatase PTEN or expression of PI3KCA-activating mutations is associated with lower response to trastuzumab (16, 17). Another pathway to resistance is overexpression of ligands of EGFR and HER3/4 (18). This is consistent with structural and cellular data using ErbB receptor ectodomains, which show that trastuzumab is unable to block ligand-induced EGF/HER2 and HER2/HER3 heterodimers (19, 20). Thus, we hypothesized that HER2-overexpressing breast cancers containing high levels of EGF/HER2 and HER2/HER3 heterodimers, surrogate markers of ErbB ligand-induced transactivation of HER2, will exhibit a lower response to trastuzumab compared with HER2* tumors with undetectable or low levels of these heterodimers. In order to identify differential signaling induced by HER2-containing homo- and heterodimers and to develop potential biomarkers of response to trastuzumab, we have developed a cell system in which HER2 dimerization can be conditionally regulated.

Materials and Methods

**Generation of MCF10A cells expressing HER2–FKBP–HA chimeric receptors**

Vector-expressing HER2–FKBP–HA chimeras was generated as described in Supplementary Methods (21). Retroviruses expressing HER2 chimeras were produced by transfecting Phoenix-Ampho cells, using published methods (22), and then utilized to transduce MCF10A human mammary epithelial cells. Stably transfected cells were selected in 1 mg/mL G418 (22).

**Cell culture, receptor ligands, and inhibitors**

MCF10A–HER2–FKBP–HA cells were maintained in DMEM/F-12 medium supplemented with epidermal growth factor (EGF; 20 ng/mL; Invitrogen/Gibco), cholera toxin (100 ng/mL; Sigma), hydrocortisone (500 ng/mL; Sigma), insulin (10 μg/mL; Invitrogen/Gibco), and 5% horse serum (HS; Hyclone). For experiments examining receptor dimerization and signaling, cells were treated with ligands ± inhibitors, as described in Supplementary Methods.

**Three-dimensional Matrigel growth assay**

About 5 × 10^3 cells/well were seeded in 8-well chamber slides in DMEM/F-12 medium supplemented with cholera toxin, hydrocortisone, insulin and 2% HS on growth factor–reduced Matrigel (BD Biosciences), as described (23). Ligands ± inhibitors were added at the time of seeding cells and replenished along with fresh media every 3 days. Acinar growth was quantified as described in Supplementary Methods.

**Cross-linking, immunoprecipitation, and immunoblotting**

Cells were cross-linked with bis(sulfosuccinimidyl)suberate (BS3), as described (24). After cross-linking, cells were lysed in Triton lysis buffer, followed by immunoprecipitation with an HER2 antibody Ab8 (Neomarkers), as described (24, 25). For immunoblot analysis, cells were lysed in 1% NP-40 buffer containing protease and phosphatase inhibitors. Samples were sonicated for 10 seconds and centrifuged at 14,000 rpm for 5 minutes at 4°C; protein concentrations were quantitated using the BCA assay (Pierce). Immunoprecipitations were done with HER3 or HA antibodies, followed by protein G (for HA) or protein A (for HER3) beads (Sigma), as described (26). Immune complexes and whole cell lysates were subjected to SDS-PAGE and transferred onto nitrocellulose membrane. Primary antibodies for immunoblotting included total AKT, S473-pAKT, T308-pAKT, HA, pErk1/2, Erk1/2 (Cell Signaling), HER3, EGFR (Santa Cruz Biotechnology), HER2 (Neomarkers), actin (Sigma), and the 4G10 phosphotyrosine antibody (Millipore).

**Cell-surface biotinylation**

The Cell Surface Protein Isolation kit (Pierce) was used for biotinylation studies according to the manufacturer’s protocol and as described in Supplementary Methods.

**Gene expression profiling**

RNA was isolated from cells grown on 100-mm dishes, using the ToTALLY RNA kit from Ambion following the manufacturer’s protocol. Synthesis of cRNA target, its hybridization to Human Gene 1.0 ST microarrays, and scanning of those arrays was done using Affymetrix GeneChip products and reagents at the Vanderbilt Microarray Shared Resource. Data were analyzed as described in Supplementary Methods (27). Raw data are available in GEO (accession # GSE22288).

**Reverse-phase protein array**

Cell lysates were prepared for reverse-phase protein array (RPPA), as described previously (28–30), RPPA was done, and data were analyzed as described in Supplementary Methods.

**Fluorescent proximity–based antibody-dependent dimer detection (VeraTag) assay**

VeraTag assays with tumor sections and cells treated with receptor ligands were carried out as described previously (25) and in Supplementary Methods.

**Study patient population**

A description of clinical characteristics of the study population is provided in Table 1. Response to treatment was documented by review of all imaging studies according to Southwest Oncology Group (SWOG) criteria, as previously described (31). Further details are provided in Supplementary Methods.

**Statistical analysis**

Clinical cutoffs for HER2 homodimer levels (HER22) were determined by positional scanning analysis and selection of the cutoff associated with the lowest P value for time to progression (TTP). Correlation of HER22 levels with clinical outcome was determined as described in Supplementary Methods.
HA cells were treated with receptor ligands–FKBP following AP1510 treatment (Fig. 1C, left). To confirm that revealed activation of pErk1/2 but not T308- or S473-pAKT was confirmed by precipitation with an HA antibody followed AP1510-induced tyrosine phosphorylation of HER2 (24 hours) synthetic FKBP ligand AP1510 (Fig. 1B, left). Sustained Immunoprecipitation of cross-linked HER2 confirmed induc-
We undertook step-down quadratic regression analysis for pattern recognition in time course studies (ref. 34; Supplementary Fig. 1B) and significance analysis using the Significance Analysis of Microarray (SAM) algorithm [false discovery rate (FDR) = 0%; Fig. 3A] on RPPA from ligand-stimulated cells. We also studied activation of signaling downstream of the PI3K/AKT (as phosphorylation of AKT, GSK3, mTOR, p70S6, 4EBP, and S6) and MAPK (as phosphorylation of MAPK, MEK, Ser118 Erk1/2, and JNK) pathways (35). RPPA assays confirmed that TGFα induced both PI3K and MAPK pathways, whereas AP1510 stimulated only the MAPK pathway as measured by signaling downstream of these two pathways (Supplementary Fig. 2). RPPA analysis also confirmed previously reported association between HER2/HER3 heterocomplex formation and activation of Src measured as phosphorylation at Y416 (Fig. 3A, iii and iv; refs. 36, 37). This is consistent with a previous report in which heregulin induced recruitment of HER3 to an HER2-Src heterodimer (36). Furthermore, 2 studies have reported on heregulin-induced activation of HER3/HER2 dimers resulting in Src kinase activation (37, 38). In contrast to HER3/HER2 heterodimers, RPPA analysis showed that HER2 homodimers and HER2/EGFR heterodimers did not induce Src.Y416 phosphorylation.

We next generated microarrays on RNA isolated from cells treated with receptor ligands. Analysis of transcriptional events induced by activation of ErbB dimers indicated that unique genes were regulated by HER2/HER2, HER2/EGFR, and HER2/HER3 dimers (Fig. 3B). SAM identified 323 genes (FDR = 0%) that are significantly different among the 4 treatment groups (Supplementary Fig. 3). Furthermore, gene expression data from triplicate RNA samples from different treatments cluster into distinct groups, indicating that HER2-containing dimers activate unique transcriptional profiles (Fig. 3C). A comparative analysis (Ingenuity Pathway Software) of the effect of activated ErbB receptors on canonical pathways involved in human malignancies revealed that HER2/HER2 and HER2/EGFR, but not HER2/HER3, can modulate the “cell cycle: G2/M DNA damage checkpoint regulation” pathway (Fig. 3D). Cyclin B1 (CCNB1), a known regulator of G2/M transition (39), is associated with aggressive pheno-
type in breast cancer (40). Interestingly, our RPPA data show that HER2 homodimers and HER2/EGFR heterodimers, but not HER2/HER3 heterodimers, enhance CCNB1 expression (Fig. 3A, i, ii, and vi), thus concurring with the Ingenuity Pathway analysis.

In a recent study, hierarchical clustering of gene expression data from 58 HER2-overexpressing patients identified a 158-gene signature that can distinguish between patients with poor and good prognoses (27). To examine if this HER2-derived prognostic predictor (HDPP) gene signature correlated with the ligand-induced RNA expression signatures, we merged our microarray data with the "good prognosis" and "poor prognosis" centroids (27). Complete hierarchical clustering was done using 139 of 158 centroid genes (due to platform representation). Consistent with broader induction of signaling, the TGFβ-induced signature clustered with the poor prognostic signature whereas AP1510-induced signature clustered with the good prognostic signature (Supplementary Fig. 4). Although heregulin-treated cells showed a trend toward increased similarity with the poor prognostic signature, they clustered with the good prognostic signature (Supplementary Fig. 4). This analysis further supports differential molecular outputs of different HER2-containing dimers and is compatible with EGFR/HER2 heterodimers being the key complex associated with a worse prognosis.

**Trastuzumab blocks HER2 homodimer- but not heterodimer-induced Erk1/2 activity**

We next sought to determine if trastuzumab selectively blocks signaling downstream of the ErbB receptor network. Immunoblot analysis of lysates from ligand-stimulated FKBP–HA cells showed that trastuzumab blocked AP1510-induced but not TGFβ- or heregulin-induced pErk1/2. Consistent with the cell growth data, trastuzumab did not inhibit TGFβ- or heregulin-induced pAKT (Fig. 4A). Cell-surface biotinylation studies indicated that trastuzumab can induce similar HER2 internalization in AP1510-, TGFβ-, and heregulin-treated cells (Fig. 4B), suggesting that the selective inhibitory effect of trastuzumab on Erk1/2 was not due to a differential effect on receptor binding and subsequent receptor downregulation from the cell surface.

The adaptor protein Shc is recruited by HER2 to induce MAPK signaling (10); thus, we examined whether trastuzumab impaired the engagement of Shc by activated HER2. Chimeric HER2 was precipitated using an HA antibody from cells treated with different ligands/C6 trastuzumab followed by

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**Figure 2.** Cell growth following HER2 homodimerization is inhibited by trastuzumab. A, cells (5 x 10^3/well) were seeded in DMEM/F-12 media with cholera toxin, hydrocortisone, insulin, and 2% HS. Cells were treated with receptor ligands AP1510 (1 μmol/L), EGF (5 ng/mL), heregulin (10 ng/mL) ± trastuzumab (20 μg/mL), and lapatinib (1 μmol) ± pertuzumab (20 μg/mL) for 12 days. Media, ligands, and inhibitors were replenished every 3 days. B, Matrigel was dissolved (see Methods) and cells were counted to quantify 3D cell growth. Each bar graph represents the mean ± SD of duplicate wells.
Shc immunoblot analysis. AP1510, TGFα, and heregulin enhanced the association of HER2–FKBP–HA with the 66-, 52-, and 46-kDa forms of Shc. Treatment with trastuzumab dissociated Shc only from AP1510- but not from TGFα- or heregulin-stimulated cells (Fig. 4A, bottom two panels). This result suggests that trastuzumab selectively blocks HER2 homodimer–induced Erk1/2 by causing a dissociation of Shc from HER2 homodimers. We next postulated that inhibition of MAPK should phenocopy the effect of trastuzumab on AP1510-stimulated FKBP–HA cells and that EGFR/HER2 heterodimer–induced cell growth should be insensitive to MAPK inhibitors because of the induction of the prosurvival AKT pathway. Thus, we treated AP1510- and EGF-stimulated cells in 3D Matrigel with the dual PI3K/mTOR inhibitor BEZ-235 (41) and MEK1/2 inhibitor CI-1040 (42). Treatment with CI-1040 completely inhibited AP1510- but not EGF-induced FKBP–HA acinus formation. BEZ-235 blocked EGF- and AP1510-stimulated cell growth (Fig. 4C and D). Consistent with these data, CI-1040

Figure 3. RPPA and RNA microarrays. Differential signaling induced by ErbB2-containing dimers. A, protein lysates were prepared from cells treated with AP1510, TGFα, or heregulin for 0, 0.25, 0.50, 1, 2, 4, and 8 hours, as indicated in Materials and Methods. Hierarchical clustering was done with proteins that underwent statistically significant change (identified using the SAM algorithm with an FDR of 0%) compared with control for each time point. Specific proteins are indicated to the right of each panel. B–D, RNA was isolated from control, AP1510-, TGFα-, or heregulin-treated cells (48 hours) and subjected to microarray analysis as indicated in Materials and Methods. B, Venn diagram was drawn with up- or downregulated genes with each treatment. Only genes that underwent at least a 1.5-fold change ($P < 0.05$) were included. C, hierarchical clustering was done with a gene list generated by the SAM algorithm (FDR 0%) to identify differential gene sets induced by HER2 homo- and heterodimers. D, Ingenuity Pathway analysis to identify distinct physiologic pathways modulated by HER2 homo- and heterodimers. The Y-axis represents Fisher’s exact test $P$ value. A Y-axis value of greater than 1.3 (yellow, line threshold) is equivalent to a value of $P < 0.05$. 

inhibited pErk1/2 in both AP1510- and EGF-induced cells whereas BEZ-235 blocked EGF-induced as well as basal pAKT (Fig. 4E). Although AP1510-induced growth is driven by Erk1/2 activity, we believe that blocking the prosurvival PI3K/AKT pathway by BEZ-235 is sufficient to suppress growth in the absence of any compensatory upregulation of pErk1/2.

Although it is possible that the inhibition of mTOR also plays a role in suppressing AP1510-induced growth by BEZ-235, we chose the drug to ensure complete inhibition of the PI3K/AKT/mTOR network. Such a strategy may minimize the possibility of PI3K pathway reactivation (41). These results suggest that the increased sensitivity of HER2 homodimer-driven growth to trastuzumab can be attributed to selective inhibition of Erk1/2 and low levels of active AKT.

**High HER2 homodimer levels correlate with response to trastuzumab**

We expanded our study to measure levels of HER2-containing homo- and heterodimers in a panel of 3 trastuzumab-sensitive and 5 trastuzumab-resistant breast cancer cell lines with HER2 gene amplification (Supplementary Table 2). We employed a fluorescent antibody–based proximity assay (VeraTag). This assay can quantify protein–protein interactions in formalin-fixed, paraffin-embedded (FFPE) cell pellet or tissue sections and involves the use of 2 HER2 monoclonal antibodies, one conjugated to a fluorescent reporter tag and the other linked to a photosensitizer molecule. Photoactivation with UV light results in the photosensitizer generating singlet oxygen, which then cleaves the fluorescent tag on the second antibody. The sphere of influence of the singlet oxygen is limited by the proximity of receptors to which the antibodies are directed (25). The cleaved fluorescent tag can then be quantified using capillary electrophoresis and used as an indicator of levels of protein–protein interaction. Using this assay, we confirmed that HER2 homodimers were induced only in AP1510-treated but not TGFα- or heregulin-treated, formalin-fixed FKBP–HA cells (Fig. 5A).
There was a trend for higher levels of HER2/HER2 homo-dimer (HER22) in trastuzumab-sensitive lines compared with the resistant lines ($P = 0.07$; Fig. 5B (left) and D (left)). We realize that this is a small sample size and as such the power from the number of cell lines studied may not be enough for any difference in dimer levels to achieve statistical significance. Interestingly, we also observed a trend for higher total HER3 ($P = 0.07$) and ligand-independent HER2/HER3 heterodimer...
levels in sensitive lines than in resistant lines (Fig. 5C and D, right). This observation points to the possibility that in these 3 sensitive lines (BT474, SKBR3, and SUM225), trastuzumab blocks the high levels of both HER2 dimers and ligand-independent HER2 dimers (43).

Finally, we employed this assay to quantify levels of HER2 dimers in FFPE sections from HER2-overexpressing primary breast cancers that had been treated with trastuzumab and for which outcome data were available (Table 1). Patients were sorted into 4 groups according to quartiles of measured HER2 homodimer (HER22) levels. ORR and CB were calculated for each subgroup of patients. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. B, univariate KM analysis examining TTP using HER22. Cutoffs were determined by positional scanning and used to differentiate high HER22 from low HER22. C, univariate KM analysis examining OS using HER22. Cutoffs determined in B were used in C.

Figure 6. High HER2 homodimer levels correlate with response to trastuzumab. A, tumor sections from paraffin blocks of HER2+ primary tumors were subjected to the VeraTag assay as indicated in Materials and Methods. Patients were divided into 4 groups on the basis of quartiles of measured HER2 homodimer (HER22) levels. ORR and CB were calculated for each subgroup of patients. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. B, univariate KM analysis examining TTP using HER22. Cutoffs were determined by positional scanning and used to differentiate high HER22 from low HER22. C, univariate KM analysis examining OS using HER22. Cutoffs determined in B were used in C.

HER2; P = 0.07) levels in sensitive lines than in resistant lines (Fig. 5C and D, right). This observation points to the possibility that in these 3 sensitive lines (BT474, SKBR3, and SUM225), trastuzumab blocks the high levels of both HER2 dimers and ligand-independent HER2 dimers (43).

Finally, we employed this assay to quantify levels of HER2 dimers in FFPE sections from HER2-overexpressing primary breast cancers that had been treated with trastuzumab and for which outcome data were available (Table 1). Patients were sorted into 4 groups according to quartiles of measured HER22. Overall response rate (ORR) and clinical benefit (CB) were calculated for each subgroup of patients as outcome measures (Fig. 6A). In both analyses, a statistically significant trend favoring better responses at higher levels of HER22 (test for trend P = 0.002 for ORR and P = 0.012 for CB) was observed. Univariate Kaplan–Meier (KM) analyses examining TTP were done. Cutoffs determined by positional scanning were used to discriminate “high” from “low” HER22 (≥HER22 vs. <HER22 = 2.5). Statistically significant relationships were observed with those patients exhibiting higher levels of HER22 (HR = 0.43; P = 0.0015) experiencing longer TTP than those patients with lower HER22 (Fig. 6B). Median TTP was 4.2 and 11.3 months for the subgroups with HER22 values below and above the cutoff, respectively. Univariate KM analyses were also done using overall survival (OS) as the outcome end point (Fig. 6C). Patients with higher levels of HER22 exhibited a median OS of 37.4 months versus 26.6 months in those with HER22 levels below the cutoff (HR = 0.54; P = 0.039).

Discussion

In tumors with HER2 gene amplification, activation of HER2 network results in downstream activation of PI3K/AKT (9) and MAPK (10). The HER2 antibody trastuzumab has changed the natural history of breast cancer overall. However, many patients with HER2 gene–amplified tumors do not respond
or eventually progress after therapy with trastuzumab (14, 44), thus suggesting the presence of de novo and acquired mechanisms of drug resistance. Coexistent mutational alterations in PI3K/AKT pathway have been associated with a lower response to trastuzumab in preclinical and retrospective clinical cohorts (16, 17). HER2+ tumors with high levels of HER2 C-terminal fragments that lack the trastuzumab-binding epitope have also been reported to be resistant to trastuzumab (45). Despite these important leads, there is no widely used biomarker(s) that can reliably predict lack of benefit from trastuzumab in patients bearing HER2-overexpressing tumors.

In this study, we developed a cell-based model in which we conditionally regulated activation of the ErbB network (Fig. 1). Using this model, we identified distinct transcriptional and signaling profiles induced by activation of HER2-containing homo- and heterodimers (Fig. 3). HER2 homodimers activated predominantly the MAPK but not the PI3K/AKT pathway (Fig. 3; Supplementary Figs. 1 and 2). Likely as a result of the high levels of EGFR in MCF10A cells (46) used to generate the FKBP–HA cells, the EGFR ligand TGFα induced a broader signaling spectrum than AP1510 (Fig. 3; Supplementary Figs. 1 and 2). Furthermore, gene expression induced by the EGFR ligand was associated with a recently reported poor prognosis signature in patients bearing HER2-overexpressing breast cancers (27).

All ligands used in this study, AP1510, TGFα (or EGF), and heregulin, stimulated FKBP–HA cell growth. Interestingly, trastuzumab was only effective at inhibiting AP1510-stimulated, HER2 homodimer–induced cell growth (Fig. 2). This action correlated with the ability of trastuzumab to induce dissociation of Shc from HER2 homodimers and subsequent inhibition of pErk1/2 (Fig. 4A). This is consistent with earlier reports supporting trastuzumab-mediated inhibition of both cell growth and Erk1/2 in cell lines (47). Furthermore, published clinical data from Mohsin and colleagues have shown that in HER2-overexpressing primary human tumors, levels of pErk1/2 but not pAKT as assessed by immunohistochemistry of tumor sections are reduced by treatment with trastuzumab given alone (48). Of note, TGFα- and heregulin-induced Erk1/2 activation was resistant to trastuzumab. Moreover, HER2 heterodimer–induced growth was resistant to the MEK inhibitor CI-1040 (Fig. 4C and D), likely due to the simultaneous activation of AKT, which was not observed with AP1510. These data also suggest that HER2 homodimer–induced growth is sensitive to trastuzumab because of the inability of these dimers to potently activate the prosurvival AKT pathway. These results imply that because of the more limited signaling output, HER2+ tumors with high levels of HER2 homodimers may exhibit a better outcome than tumors in which HER2 is predominantly in a hetero-oligomeric conformation as supported by the data shown in Supplementary Fig. 4.

An implication of these results is that clinically applicable assays that measure the oligomeric conformations of HER2 in HER2+ tumors will help predict the odds of response to trastuzumab. Indeed, in a cohort of patients bearing metastatic HER2-overexpressing breast cancer, higher HER2 homodimer levels measured with a proximity-based, antibody-dependent HER2 dimer detection assay correlated with better clinical outcome following therapy with trastuzumab and chemotherapy (Fig. 6). We realize that the results of this analysis are limited by the small size of the patient cohort, the retrospective nature of the study, and the heterogeneous chemotherapies with which patients were treated. Furthermore, the cutoff value used to distinguish between high and low HER2 homodimer levels applied to the outcome analysis was derived from the same cohort. As such, the observed relationships and derived cutoff values will require validation in an independent cohort before being regarded as widely useful. However, consistent with our observations from in vitro experiments, this data set suggests that trastuzumab has preferential activity against tumors driven predominantly by HER2 homodimer–induced signaling. Whether the converse is true with assays measuring HER2-containing heterodimers will require further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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