# PI3K Inhibition Overcomes Trastuzumab Resistance: Blockade of ErbB2/ErbB3 Is Not Always Enough

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**Trastuzumab targets ErbB2 and is used for treating ErbB2-overexpressing breast cancers. In this issue of *Cancer Cell*, Junttila et al. show that trastuzumab disrupts ligand-independent ErbB2/ErbB3/PI3K complexes and blocks AKT signaling; if PI3K is mutated, complex disruption does not inhibit AKT, which explains why trastuzumab is ineffective in some tumors.**

Approximately 20% of breast tumors possess the 17q11-24 *ERBB2*-containing amplicon and show a dramatic overexpression of ErbB2 (reviewed in Hynes and Lane, 2005). Trastuzumab is a humanized antibody targeting ErbB2 and benefits patients with ErbB2-overexpressing breast cancer, particularly when used in the adjuvant setting. However, not all patients whose tumors overexpress ErbB2 respond to trastuzumab treatment. In spite of a vast amount of research and clinical data, there are still important unresolved questions related to trastuzumab's mechanism of action and to predicting patient response.

ErbB2 is a member of the ErbB receptor tyrosine kinase (RTK) family, which also includes EGFR, ErbB3, and ErbB4. Under normal physiological conditions, activation of these receptors is controlled by spatial and temporal expression of their ligands. Ligand binding induces formation of ErbB receptor homo- and heterodimers, resulting in activation of the cytoplasmic kinase domain, which promotes phosphorylation of specific tyrosine residues (P-Y) and stimulates intracellular signaling cascades (Hynes and Lane, 2005). ErbB2 is ligandless and functions as a coreceptor with other ligand-bound ErbBs. Of the four ErbB receptors, ErbB3 is best suited to activate the PI3K/AKT pathway due to having multiple p85/p110α binding sites. ErbB3 binds heregulins (HRG) but has impaired kinase activity and only signals as a complex with another ErbB, preferably ErbB2 (Hynes and Lane, 2005). In breast cancer cells, overexpressed ErbB2 is highly phosphorylated in the absence of ligands and the PI3K/AKT pathway is constitutively active. However, ErbB2 does not function alone: it requires ErbB3 as a link to the PI3K/AKT pathway (Holbro et al., 2003) (Figure 1A, middle). Previous studies have revealed how ligand binding regulates ErbB receptor dimerization and where trastuzumab binds ErbB2 (reviewed in Leahy, 2008).

Junttila and colleagues now show that trastuzumab causes destabilization of ligand-independent constitutive ErbB2/ErbB3 complexes, uncoupling of ErbB3 from ErbB2, and blockade of downstream PI3K/AKT signaling in ErbB2-overexpressing tumor cells (Junttila et al., 2009). This effect of trastuzumab may be one important mechanism underlying its clinical activity, however, as discussed in their paper other mechanisms are also likely to be relevant in cancer patients. Furthermore, this study shows that there are differences between ligand-independent and ligand-induced ErbB2/ErbB3 complexes. Despite the fact that ErbB2-overexpressing tumor cells have high levels of P-Y ErbB3 and high PI3K/AKT activity, HRG treatment causes a further increase in both. Moreover, HRG prevents trastuzumab from disrupting ErbB2/ErbB3 complexes. However, pertuzumab, an antibody that binds ErbB2′s dimerization arm (reviewed in Leahy, 2008) does prevent HRG-induced ErbB2/ErbB3 complex formation (Figure 1B, right side). Taken together, the results show that the ligand-independent, trastuzumab-sensitive ErbB2/ErbB3 complex is structurally distinct from HRG-induced ErbB2/ErbB3 heterodimers. It will be interesting to uncover if there are differences in the proteins and intracellular pathways downstream of these two ErbB2/ErbB3 complexes.

Additional important results presented in this study are related to the PI3K/AKT pathway. Activating mutations in *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, are relatively common in breast cancer. Furthermore, decreased expression of PTEN, the phosphatase that dephosphorylates PIP3, is often found in breast cancers (Stemke-Hale et al., 2008). Previous analyses of trastuzumab-treated ErbB2 overexpressing breast cancer patients suggest that low PTEN levels or *PIK3CA* activating mutations are markers for poor response to trastuzumab (Berns et al., 2007; Nagata et al., 2004).

The work of Junttila and colleagues not only impinges on these findings, but also shows how resistance can be overcome. They demonstrate that ErbB2-overexpressing breast tumor lines with low PTEN or activating *PIK3CA* mutations do not respond to trastuzumab; AKT activity and tumor cell proliferation remain high. Interestingly, in all tumor cells, irrespective of the presence or absence of PI3K pathway mutations, trastuzumab disrupts ligand-independent ErbB2/ErbB3 complexes, leading to a loss of p85/p110α from ErbB3. However, in trastuzumab-treated cells with mutant PI3K or low PTEN, AKT activity remains high (Figure 1A, left side). These results suggest that, despite uncoupling p85/p110α from the ErbB2/ErbB3 complex, mutant PI3K remains localized at the membrane, perhaps using its Ras-binding domain, where it continues to catalyze PIP3 formation, AKT activation, and tumor cell proliferation.

Based on the importance of the PI3K pathway in human cancers, much effort is going into the development of PI3K/AKT pathway inhibitors (Garcia-Echeverria and Sellers, 2008). GDC-0941

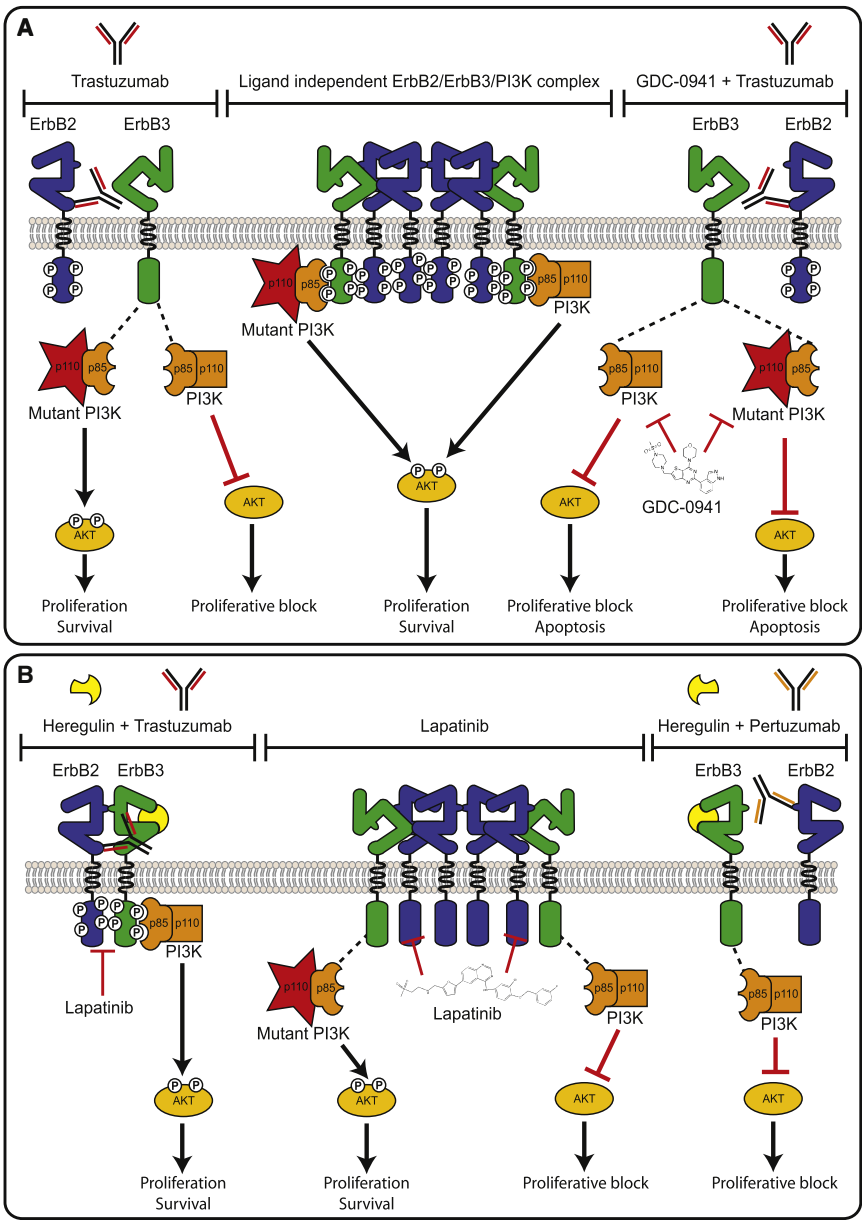


**Figure 1. Targeting ErbB2/ErbB3/PI3K**

(A) Junttila et al. (2009) provide evidence that a ligand-independent, constitutive complex of ErbB2/ErbB3/PI3K functions as an oncogenic unit in ErbB2-overexpressing breast cancer cells (middle). Trastuzumab disrupts the ErbB2/ErbB3 interaction and decreases ErbB3 phosphorylation, which in turn uncouples PI3K from ErbB3 and leads to a block of AKT phosphorylation that correlates with inhibition of proliferation of tumor cells (left side). Activating mutations of the PI3K catalytic subunit (e.g., E542K, E545K, and H1047R) result in constitutive active PI3K independent of its association with ErbB3, which maintains AKT signaling even in the presence of trastuzumab and thereby renders the cells resistant to trastuzumab (left side). GDC-0941, a selective PI3K inhibitor, synergizes with trastuzumab in inducing not only a proliferative block but also apoptotic cell death of ErbB2-overexpressing cells with wild-type PI3K (right side). Moreover, GDC-0941 inhibits the growth and survival of trastuzumab-insensitive PI3K mutant breast tumor cell lines (right side).

(B) HRG-binding induces a major conformational change in ErbB3 enabling its interaction with ErbB2 through its domain II dimerization arm (left side). Pertuzumab, an antibody that binds ErbB2's dimerization arm disrupts ligand-induced heterodimer formation and downstream signaling (right side) (reviewed in Leahy, 2008). EGFR/ErbB2 TKIs like lapatinib are able efficiently inhibit HRG-induced signaling, whereas trastuzumab binding to domain IV of ErbB2 has no effect on signaling and proliferation (left side) (reviewed in Hynes and Lane, 2005). Lapatinib also decreases downstream signaling pathway activity and blocks proliferation of ErbB2-overexpressing tumor cells (middle) (reviewed in Hynes and Lane, 2005). However, activating PI3K mutations confer resistance to lapatinib and cells maintain PI3K/AKT signaling (middle) (Eichhorn et al., 2008).

is a PI3K inhibitor with selective activity toward the class 1A isoforms p110α, p110β, and p110δ. Junttila and colleagues show that even in ErbB2-overexpressing tumor cells with no PI3K pathway mutations, the addition of GDC-0941 to trastuzumab has a synergistic effect on blockade of AKT activity. Furthermore, compared to individual treatment with GDC-0941 or trastuzumab, combination treatment has stronger in vitro and in vivo antiproliferative activity and causes an increase in cell death. Importantly, in trastuzumab-resistant tumor cells, GDC-0941 decreases AKT activity, lowers in vitro tumor cell proliferation, and blocks xenograft outgrowth (Figure 1A, right side).

Several other PI3K inhibitors are in clinical development, many of these block mTOR in addition to the PI3K class 1A isoforms (Garcia-Echeverria and Sellers, 2008). Since PI3K is crucial for correct insulin signaling, hyperglycemia as a potential serious side-effect of its inhibition is a major concern in developing PI3K inhibitors. Although there is no formal published report yet, preliminary reports on several of these compounds did not reveal any serious side effects. All PI3K inhibitors are still in early developmental phases so it is too early to say whether they will become useful for breast cancer treatment.

The results presented by Sliwkowsi and colleagues strongly suggest that it should be possible to overcome trastuzumab resistance in patients with PI3K pathway mutations. What about lapatinib, a dual EGFR/ErbB2 kinase inhibitor which was FDA approved for treatment of ErbB2-overexpressing breast cancer based on its effectiveness in patients who failed on trastuzumab (Geyer et al., 2006)? Lapatinib can block most combinations of ErbB dimers, and it is likely that these characteristics contributed to its efficacy in trastuzumab-resistant tumors (Figure 1B, left side). An important question is whether lapatinib will work in tumors with PI3K pathway mutations. There are not many clinical reports regarding this issue, the *PIK3CA* mutation status was not examined in most clinical studies. However, low PTEN levels did not preclude lapatinib response (Johnston et al., 2008). On the other hand, introduction of active PI3K mutants rendered the tumor cells resistant to lapatinib in a

breast cancer model, which was reversed by NVP-BEZ235, a dual PI3K-mTOR inhibitor, treatment (Eichhorn et al., 2008) (Figure 1B, middle). Given the frequency of mutations in this pathway, PI3K appears to be an excellent target for therapy. The oncology field eagerly awaits further information on the clinical usefulness of PI3K inhibitors.

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# Inflamed Snail Speeds Metastasis

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**Macrophage infiltration and inflammatory cytokines are powerful drivers of tumorigenesis and metastasis. Wu et al., in this issue of *Cancer Cell*, show that TNFα-dependent NFκB activation induces COP9 signalosome-mediated inhibition of GSK3β and the $SCF^{\beta\text{-}TRCP}$ ubiquitin ligase, thus leading to stabilization of the transcription factor Snail and promoting cell migration and metastasis.**

Transcription factors are prime targets for regulation by ubiquitin-dependent proteolysis. One of the best studied examples is β-catenin, a coactivator of the TCF-LEF family of DNA-binding proteins. In the absence of Wnt signaling, β-catenin is constitutively phosphorylated by glycogen synthase kinase 3β (GSK3β) on two serine residues. This phosphorylation also requires the scaffold protein axin and the adaptor protein adenomatous polyposis coli (APC), which appear to facilitate the recruitment of β-catenin to GSK3β. β-catenin phosphorylation triggers its recognition by the β-TRCP substrate receptor of a SKP1-CUL1-F-box protein (SCF) ubiquitin ligase, thus resulting in ubiquitylation and proteasomal degradation. Canonical Wnt signaling reverses β-catenin destruction by preventing its GSK3β-dependent phosphorylation.

In many colon cancers, this reversal is mimicked by mutations in APC, thus leading to increased β-catenin-TCF activity and growth promoting gene expression. Within the inflammatory tumor microenvironment, this effect can be potentiated by two additional stimuli that are provided by tumor-associated macrophages (TAMs). The first comes in the form of Wnt ligands, and the second is the proinflammatory cytokine TNFα. Even in the absence of APC mutations, TAM-derived TNFα can promote the nuclear accumulation of β-catenin and gastrointestinal tumorigenesis (Oguma et al., 2008). The cytokine does so not by activating its prominent target NF-κB, but by driving Akt1-mediated, inhibitory phosphorylation of GSK3β, which prevents β-catenin from being targeted to $SCF^{\beta\text{-}TRCP}$.

The new study by Wu et al. (2009) enacts a remarkably similar plot with partly the same cast. Once again, a transcription factor, Snail, escapes ubiquitylation by $SCF^{\beta\text{-}TRCP}$ and proteasomal degradation. Once again, this involves TAMs and their secreted TNFα. And once again, this potentiates the tumor phenotype, this time promoting metastasis. However, the newly discovered pathway has also distinctly novel aspects and brings fresh players to the stage.

Snail is a transcription factor that represses the expression of *E-cadherin* and thereby confers onto epithelial cells a fibroblast-like behavior that includes increased motility. This process, known as epithelial-mesenchymal transition (EMT), occurs at the invasive front of tumors, the same site where tumor infiltration by TAMs takes place. Wu et al. now link these events by demonstrating that TAM-derived TNFα leads to the stabilization of Snail, which is otherwise a highly unstable protein targeted for degradation by GSK3β-dependent phosphorylation and $SCF^{\beta\text{-}TRCP}$-mediated ubiquitylation (Zhou et al., 2004). As with β-catenin, TNFα-mediated Snail stabilization occurs through inhibition of GSK3β-dependent