Optimal Targeting of HER2–PI3K Signaling in Breast Cancer: Mechanistic Insights and Clinical Implications

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Abstract

The combination of a PI3K inhibitor with trastuzumab has been shown to be effective at overcoming trastuzumab resistance in models of HER2+ breast cancer by inhibiting HER2–PI3K–FOXO–survivin signaling. In this review the potential clinical implications of these findings are discussed. Cancer Res; 73(13): 1–4. ©2013 AACR.

Trastuzumab, a monoclonal antibody against the receptor encoded by the HER2 protooncogene, is the mainstay of treatment for patients with HER2 gene–amplified breast cancer. The main mechanisms of action of the antibody are antibody-dependent cell mediated cytotoxicity (ADCC) with reconstitution of adoptive immunity to HER2 (1, 2), downregulation of HER2 from the cell surface (3), and disruption of ligand-independent HER2–HER3 dimers, thus partially inhibiting postreceptor phosphoinositide 3-kinase (PI3K) signaling (4). Despite its effectiveness in both the adjuvant and metastatic settings, therapeutic resistance to trastuzumab remains an important clinical problem. A number of preclinical studies have proposed several molecular mechanisms whereby tumors can evade the action of trastuzumab. These mechanisms include engagement of alternate signaling pathways, alterations in antibody binding to HER2, loss of the apoptotic response, or evasion of the immunomodulatory effects conferred by trastuzumab; in several cases, analysis of cohorts of patients treated with trastuzumab has suggested that at least some of these mechanisms are operative in vivo (5). Signaling to PI3K–Akt as a result of HER2–HER3 dimerization is the most important survival pathway downstream of HER2 (4, 6). Several lines of evidence suggest that inhibition of PI3K–Akt is critical for the antitumor effect of HER2-directed therapies. Indeed, several of the proposed mechanisms of resistance to trastuzumab involve persistence or reactivation of PI3K signaling via alternative amplified receptor tyrosine kinases and/or mutations in PI3K pathway components, thus suggesting that direct and more sustained inhibition of PI3K might be a strategy to overcome or prevent resistance to trastuzumab or other HER2 inhibitors.

There are a number of therapeutic inhibitors of PI3K–Akt currently in clinical development. Results from several preclinical studies suggest that trastuzumab resistance is overcome by PI3K pathway inhibitors (4, 7, 8). Inhibition of nodes in the PI3K pathway downstream of AKT may also prove to be an effective and potentially less toxic strategy. For example, emerging clinical evidence suggests that blockade of mTOR downstream of HER2 with the TORC1 inhibitor everolimus, in combination with trastuzumab and a taxane, can induce significant clinical responses in patients with metastatic HER2+ breast cancer who had previously progressed on trastuzumab therapy (9, 10). Thus, the recent study by Chakrabarty and colleagues (11) is timely and of interest because it provides further insights into why maximal inhibition of PI3K is required for optimal inhibition of HER2+ breast cancer cells. This work also raises some important considerations for the future clinical development of PI3K inhibitors in this subtype of breast cancer that will be discussed herein.

In this study, the authors used the ATP-competitive, pan-PI3K (p110α) inhibitor, XL147, to inhibit PI3K in several HER2 gene–amplified trastuzumab-resistant cell lines. These included the Herceptin-resistant HR5 and HR6 cells that escape trastuzumab action by upregulation of EGFR and HER3 ligands (12), and two cell lines with somatic genetic alterations in the PI3K pathway: HCC1954 and SUM190 cells, both with "hotspot" activating mutations in PIK3CA, the gene encoding the p110α catalytic subunit of PI3K. PTEN is the lipid phosphatase that dephosphorylatesPIP3, the product of PI3K activity. Both "hotspot" mutations in PIK3CA confer PI3K signaling beyond that of the wild-type enzyme (13, 14). Thus, loss of PTEN and PIK3CA mutations amplifies PI3K signaling beyond a level conferred by HER2 overexpression alone and, as a result, counteracts the action of trastuzumab and other HER2 inhibitors. In several retrospective studies, aberrant activation of PI3K as defined by either of these alterations, that is, PTEN loss or PIK3CA mutation, was statistically correlated with decreased benefit from trastuzumab in patients with metastatic HER2+ breast cancer (15–19). Treatment with the PI3K inhibitor XL147 prevented growth and/or induced apoptosis in all trastuzumab–resistant cells, thus confirming their dependence on PI3K. Even though trastuzumab alone had no effect, combining trastuzumab with the PI3K inhibitor resulted in additive effects compared with XL147 alone.
Induction of apoptosis of primary breast tumors after neoadjuvant trastuzumab, as measured by cleaved caspase-3 immunohistochemistry, has been reported previously (20). The current study provides mechanistic insights into how HER2 function is connected to apoptosis by exploring differences between antibody-sensitive and resistant cells. The investigators first noted that survivin, a member of the inhibitor of apoptosis family of proteins, was the only apoptosis-related protein modulated upon treatment with the combination of XL147 and trastuzumab. In antibody-sensitive cells, survivin is downregulated by trastuzumab alone, whereas in resistant cells, addition of a PI3K inhibitor to trastuzumab is required to achieve such effect on survivin levels. In this case, blockade of PI3K–AKT inhibits the phosphorylation of FoxO factors, which, in turn, translocate to the nucleus, where they repress the transcription of survivin. Furthermore, modulation of FoxO function using dominant-negative or constitutively active FoxO mutants uncoupled survivin from PI3K signaling. An interesting aspect of these studies is the demonstration that downregulation of survivin was sufficient to restore sensitivity to trastuzumab in drug-resistant cells.

Another interesting finding from this work was the observation that treatment of trastuzumab-resistant cell lines with PI3K inhibitors reduced their cancer stem cell (CSC) fraction. These CSCs or tumor-initiating cells are hypothesized to be resistant to therapy and thus are able to repopulate the tumor after treatment, potentially accounting for cancer recurrences (21). Therefore, strategies that eliminate CSCs may overcome drug resistance and prevent cancer relapses. In trastuzumab-sensitive HER2 gene–amplified tumors, the antibody has been proposed to target this CSC fraction (22, 23). In the resistant cells used in this study, treatment with XL147, but not trastuzumab, reduced CSCs as measured by mammosphere formation, aldehyde dehydrogenase (ALDH) activity, and interleukin (IL)-8 expression. Again, the combination of trastuzumab with the PI3K inhibitor was more effective in some cases even though trastuzumab itself had little effect. Derepression of FoxO-mediated transcription also explained the effects of IL-8. Knockdown via siRNA of FoxO3a upregulated IL-8 mRNA levels as well as mammosphere formation. Survivin also played a role in maintenance of the CSC fraction, as downregulation of survivin with siRNA decreased mammosphere formation and ALDH activity.

Next, this study tested the combination of trastuzumab and XL147 in athymic mice with established trastuzumab-resistant human tumors. In two different resistant xenografts, treatment with trastuzumab and the PI3K inhibitor blocked PI3K signaling, reduced ALDH1 and IL-8, both markers of stem-like cells, and survivin levels. Finally, the authors explored the role of survivin in patients with HER2 þ breast cancer treated with neoadjuvant trastuzumab plus chemotherapy. Survivin mRNA levels in tumors were significantly reduced after treatment in 5 of 13 patients who exhibited a clinical response. Furthermore, in tumors of patients who did not have a response, survivin mRNA levels were statistically higher than those in patients who responded.

In summary, this work is the first to elucidate molecular mechanisms of combined inhibition of HER2 þ breast cancer cells with trastuzumab and a PI3K inhibitor. This research also shows a link between HER2, PI3K signaling, tumor cell apoptosis, and cancer stem-like cell maintenance through FoxO-mediated suppression of survivin and IL-8. We speculate that both of these cellular effects, induction of apoptosis and elimination of CSCs, may explain the ability of (1 year) adjuvant trastuzumab to completely eradicate micrometastases and potentially induce cures. During early therapy, the major effect of trastuzumab may be to elicit apoptotic and antisignaling responses, whereas longer therapy is required to eradicate the smaller, more intrinsically refractory CSC fraction.

This preclinical study also raises questions regarding the clinical development of combinations of PI3K and HER2 antagonists in HER2 þ breast cancer. Should PI3K and HER2 inhibitors be combined upfront, or should the PI3K inhibitor be added at the time of progression on trastuzumab? Would a

Figure 1. Inhibition of the HER2–PI3K–FOXO-survivin axis by trastuzumab and PI3K inhibitors. Blockade of HER2 by trastuzumab alone with PI3K inhibitors in trastuzumab-sensitive and -resistant cells results in inactivation of Akt. This results in hypophosphorylation of FOXO transcription factors which, in turn, translocate to the nucleus, where they repress transcription of IL-8 and survivin. The end result is loss of maintenance of cancer stem cells and antiapoptotic proteins such as survivin.
p110α-specific inhibitor be superior to a pan-PI3K inhibitor in this setting? Should all patients be treated with PI3K inhibitors in combination with trastuzumab? Or should this combinatorial approach be reserved for those patients with HER2+ tumors that also harbor mutations in the PI3K pathway? For what duration should the PI3K inhibitor be used in combination with trastuzumab? Perhaps the mechanistic insights provided by this study can assist in the answers to these questions.

First, the authors noted that even in antibody-sensitive cells, addition of the PI3K inhibitor resulted in a greater magnitude of response compared with trastuzumab alone. This may not be surprising, given the importance of sustained inhibition of PI3K signaling that is required for the antitumor effect of trastuzumab (4, 6). Another rationale for the simultaneous use of both inhibitors is the ability of trastuzumab to interfere with the enhanced HER2–HER3 dimerization resulting from compensatory FoxO-dependent upregulation of HER3 mRNA and protein that has been reported following the inhibition of PI3K–Akt (24, 25). In preclinical studies, trastuzumab was only a partial inhibitor of HER2–HER3 signaling to PI3K. Hence, not surprisingly, clinical studies have shown superior efficacy of “double HER2 blockade” with combinations of trastuzumab with lapatinib or trastuzumab with pertuzumab, compared with lapatinib or trastuzumab alone, respectively (26, 27). Obviously, the tolerability and efficacy of a HER2–PI3K targeting strategy will require testing in the clinic. If toxicities limit such a combination, data in this report would support a sequential strategy whereby addition of a PI3K inhibitor at the time of progression on trastuzumab could restore sensitivity to the antibody. This would be in line with controlled clinical trials showing the merits of continuing trastuzumab at the time of progression (27–29). An alternative approach that may also avoid the toxicity of a pan-PI3K inhibitor would be use of a p110α isoform–specific inhibitor. Recent studies in the HER2/neu transgenic mouse model of HER2+ breast cancer showed the requirement for p110α for cancer growth and progression and showed that selective targeting of p110α but not p110β was effective at blocking growth of the transgenic tumors (30).

Ideally, only patients who are likely to develop resistance to trastuzumab should be selected for treatment with the HER2–PI3K–targeted combination. However, at the present time, biomarkers that predict de novo or acquired resistance are unknown. The PI3K–FOXO–survivin mechanism outlined in this study suggests that measurement of pretreatment survivin levels may aid in identifying patients who are less likely to respond to trastuzumab. Survivin expression alone has been shown to correlate with poorer outcome in breast cancer (31), and survivin is one of 16 genes in the 21-gene Oncotype DX recurrence score used for risk stratification of patients with ER+ HER2-negative breast cancer (32). We speculate that survivin expression in addition to biomarkers of aberrant PI3K activation, such as PIK3CA mutations and PTEN loss, should provide a more robust predictor than any parameter alone.

Finally, the reduction in the tumor-initiating cell fraction upon treatment with XL147 and trastuzumab raises the question of whether these inhibitors should be tested in the adjuvant setting as well as the metastatic setting. We speculate that through targeting the stem cell fraction by adding a PI3K antagonist to adjuvant therapy, the duration of adjuvant trastuzumab could be reduced while maintaining or even improving its efficacy. This will be a difficult question to answer directly given the required size of a clinical trial to test such a question. One alternative might be to test whether the rate of pathologic complete response after neoadjuvant anti-HER2 therapy is improved by the addition of a PI3K inhibitor. The Neo-ALTTO study showed an improvement in the rate of pathologic complete response using the combination of trastuzumab with lapatinib versus either drug alone (33), so it would be feasible to compare the effect of dual blockade with or without a PI3K inhibitor in this setting. Indeed, the success of a therapeutic combination identified in the neoadjuvant setting may serve as a surrogate predictor of long-term clinical benefit (34).

In conclusion, the data presented in this article underscore the importance of PI3K signaling in HER2-mediated tumor progression. The results extend our understanding of how the HER2–PI3K axis modulates downstream effectors of apoptosis and maintenance of tumor-initiating cells. Important questions remain as to how to best incorporate PI3K inhibitors into the treatment of HER2+ breast cancer, but there is an increasing consensus that they should be considered as an integral part of HER2-targeted combinations.

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No potential conflicts of interest were disclosed.

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Conception and design: B.N. Rexer, C.L. Arteaga
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