"PIKing" the Winner for Phosphatidylinositol 3-Kinase Inhibitors in ErbB2-Positive Breast Cancer: Let's Not "PTENed" It's Easy!

□□*Commentary on Lu et al., p. 5883*

Matthew J. Ellis and Robert Crowder

ErbB2 targeting with the monoclonal antibody trastuzumab and, more recently, with the small-molecule inhibitor lapatinib have made significant inroads into the mortality and morbidly associated with ErbB2-positive breast cancer. However, these agents are only partially effective and resistance remains a significant and poorly understood problem. The observation that *ERBB2* gene–amplified breast cancers frequently have additional somatic mutations in genes encoding components of the ErbB2 signal transduction cascade suggests that resistant tumors evade ErbB2 targeting through incomplete pathway inactivation. To date, these potential resistance-inducing mutations have been found in genes that serve the phosphoinositol 3-kinase (PI3K) pathway and include the α-catalytic subunit of phosphoinositol 3-kinase, *PIK3CA* (1); loss of the tumor suppressor and lipid phosphatase, phosphatase and tensin homologue (*PTEN*; ref. 2); and amplification of a second chromosome 17 gene, *RPS6KB1*, which is a downstream target of mammalian target of rapamycin (mTOR; ref. 3). Other potential players include the *AKT2* gene, which may be amplified on occasion (4), as can the gene for the potent PI3K activating receptor tyrosine kinase insulin-like growth factor receptor I (5).

A determination of the clinical effect of PI3K pathway gene amplification or mutation on trastuzumab and lapatinib efficacy requires at least three approaches: (*a*) correlations between the clinical efficacy of ErbB2-targeted agents and the presence of PI3K pathway mutations, preferably in the context of randomized trials; (*b*) the development of preclinical models to dissect out the signal transduction effects of various mutation combinations and to test PI3K-directed pharmacologic interventions that could enhance the efficacy of ErbB2-targeted agents; and (*c*) carefully conducted clinical trials of the combination of ErbB2 inhibitors with PI3K pathway inhibitors with further efforts in the correlative science arena to try and determine the tumor subset(s) that derive maximum benefit from the drug combination in question. None of this is easy because each class of PI3K pathway mutation occurs at relatively low frequency (except α catalytic subunit mutation) and our collections of ErbB2-positive tumors consist mostly of paraffin blocks from adjuvant trastuzumab studies where DNA and RNA quality is compromised. High-throughput sequencing and array comparative genomic hybridization techniques still demand high-quality DNA, although technical advances are being made. A bioinformatics problem is readily evident as we are faced with multiple mutation/amplification defined subsets and multiple exploratory comparisons demanding large sample sizes for test/validation approaches. A third problem is that our collections of trastuzumab/lapatinib–resistant tumors remain very limited. This is a very significant deficiency because until we have a good quality tumor bank, we cannot address the question of acquired somatic mutations induced by the selective pressure of ErbB2-targeting agents.

The article by Lu et al. (6) illustrates some of the problems with preclinical modeling. The number of breast cancer cell lines with *ERBB2* gene amplification to use for preclinical proof of principle experiments is very limited. This group has focused on the role of PTEN loss in trastuzumab resistance, but, to date, a cell line that is both ErbB2 amplified and PTEN null has not been described. This is not that surprising given the fact that somatic mutations that result in PTEN loss occur in <10% of breast cancers. They therefore chose to model the $ErbB2^+$ $PTEN^-$ genotype with a tumorigenic derivative of the $ErbB2^+$ BT474 cell line by knocking down PTEN expression through the application of PTEN antisense oligonucleotides. Of course, this approach has a few caveats. Acute deficiency of PTEN is not the same as a loss of function somatic mutation because long-term compensatory signaling events, perhaps in association with other mutations, may not have taken place. Furthermore, the BT474 cell line carries an activating mutation in the α catalytic subunit of PI3K (1). The combination of PTEN loss and *PIK3CA* mutation is thought to be very uncommon in clinical samples, which raises a question about how well the model they are using will predict clinical outcomes. Finally, if PTEN does have a role, it seems to be specific to trastuzumab because PTEN status does not seem to affect the efficacy of lapatinib (7).

Despite these caveats, the experiments that Lu et al. present are interesting and provoke debate on several issues. First of all, their *in vitro* experiments suggest that triciribine and the rapamycin analogue RAD001 produce more robust cell death in the presence of trastuzumab and are synergistic in cell proliferation experiments. These interactions seem to be modestly enhanced by the PTEN antisense oligonucleotide, but it is tempting to speculate that the presence of the *PI3KCA* mutation in these cells already sets them up for these types of observations. All six of the agents tested in their model had quite significant activities as single agents and were often more active than trastuzumab (although we are missing full accounting of the activity of these agents in the absence of

Authors' Affiliation: Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri

Received 7/10/07; accepted 7/19/07.

Requests for reprints: Matthew J. Ellis, Medical Oncology, Washington University in St. Louis, Campus Box 8056, 660 South Euclid Street, St. Louis, MO 63110. Phone: 314-362-8866; Fax: 314-362-3091; E-mail: mellis@im.wustl.edu.

doi:10.1158/1078-0432.CCR-07-1698

PTEN knockdown). The *in vivo* xenograft data looked very promising for the combination of triciribine and trastuzumab although the PTEN NS control experiment was not presented to show that low PTEN was required for their *in vivo* observations.

So what is to be made of their claim that triciribine should be resurrected? First of all, the point should be made that triciribine and, for that matter, edelfisone are not engineered AKT inhibitors and certainly have off-target effects. For example, analogues of edelfisone also affect c-jun NH_2-terminal kinase and extracellular signal–regulated kinase in a complex cell-specific manner (8, 9). A close look at Fig. 3 raises the question of whether triciribine affects the expression of p70 S6 kinase, and it is very possible that binding to an artificial nucleoside analogue like triciribine could destabilize multiple signal transduction proteins. Rapamycin analogues were not specifically engineered to inhibit mTOR and only partially inhibit mTOR through an indirect mechanism involving an interaction with an mTOR binding protein (10). Furthermore, as shown in Lu et al.'s article, compensatory upstream activation of AKT in response to rapamycin analogues may limit the efficacy of these agents. Fortunately, better engineered agents are on the way, with direct and specific inhibitors of PI3K, mTOR, AKT, insulin-like growth factor-I receptor, and S6 kinase in clinical development. These are all likely to be tested in combinations with ErbB2-targeting agents. We therefore suspect that these newer agents will have greater momentum than less specific agents with checkered drug development histories. That being said, the proof is in the clinical trial pudding, and we strongly suspect we are in for a treat about the clinical trial results arising from the important hypothesis addressed by this article.

References

1. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005; 65:2554–9.
2. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004;6:117–27.
3. van der Hage JA, van den Broek LJ, Legrand C, et al. Overexpression of P70 S6 kinase protein is associated with increased risk of locoregional recurrence in node-negative premenopausal early breast cancer patients. Br J Cancer 2004;90:1543–50.
4. Bacus SS, Altomare DA, Lyass L, et al. AKT2 is frequently up-regulated in HER-2/neu-positive breast cancers and may contribute to tumor aggressiveness by enhancing cell survival. Oncogene 2002;21: 3532–40.
5. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst 2001; 93:1852–7.
6. Lu CH, Wyszomierski SL, Tseng LM, et al. Pre-clinical Testing of Clinically Applicable Strategies for Overcoming Trastuzumab Resistance Caused by PTEN Deficiency. Clin Cancer Res 2007;13:5883–8.
7. Xia W, Husain I, Liu L, et al. Lapatinib antitumor activity is not dependent upon phosphatase and tensin homologue deleted on chromosome 10 in ErbB2-overexpressing breast cancers. Cancer Res 2007;67: 1170–5.
8. Momota H, Nerio E, Holland EC. Perifosine inhibits multiple signaling pathways in glial progenitors and cooperates with temozolomide to arrest cell proliferation in gliomas *in vivo*. Cancer Res 2005;65: 7429–35.
9. Hideshima T, Catley L, Yasui H, et al. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and *in vivo* cytotoxicity in human multiple myeloma cells. Blood 2006;107: 4053–62.
10. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 2006;5:671–88.