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# Unfavorable Drug Interactions in Targeted Breast Cancer Therapy

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**Trastuzumab is used to treat HER2-positive breast cancer; erythropoietin (EPO) is used to alleviate chemotherapy-associated side effects. In this issue of *Cancer Cell*, Liang et al. show that many breast tumors express EPO receptor (EpoR), whose activation by EPO recovers signaling pathways downregulated by trastuzumab, thereby blunting trastuzumab's therapeutic effect.**

Chemotherapeutic agents possessing different mechanisms of action used in combination are more effective than single agents for treatment of cancer patients. Insights into molecular alterations in cancer cells have led to an enhanced understanding of cancer etiology and to the development of drugs targeting the causal components. The prediction of optimal drug combinations, however, continues to be difficult and is often based on empirical clinical evidence. Genetic and biochemical approaches have revealed the most important genes and proteins in the transformation process and how these components interact in organized pathways. Sequence analyses of cancer genomes have been correlated with epidemiologic and clinical observations to assign ''driver'' or ''passenger'' characteristics to individual mutations (Bozic et al., 2010). Activated signaling pathways and expression of particular signaling proteins have become important indicators for treatment choice and for therapeutic success.

HER2 is a certified cancer ''driver''; HER2 overexpression due to gene amplification is observed in 20%–30% of breast cancers. Patients with tumors overexpressing HER2 have a worse clinical prognosis and shorter time to relapse (reviewed in Hynes and Lane, 2005). The development of trastuzumab, a recombinant monoclonal antibody directed against HER2, is considered a milestone in targeted anticancer agents. For patients with early stage, HER2-positive breast cancer, trastuzumab treatment in combination with chemotherapy has shown significant clinical benefits (Smith et al., 2007); however, not all patients with the genetic lesion respond. In HER2-positive breast cancer, the receptor is heavily phosphorylated and many signaling pathways are constitutively active. The mechanisms underlying the clinical activity of trastuzumab are still not completely understood, and are likely to be multiple. There is an emerging consensus, however, that downregulation of the PI3K/Akt pathway contributes to trastuzumab response. Indeed, HER2-positive breast cancer cell lines with *PIK3CA* mutations are insensitive to trastuzumab treatment (Junttila et al., 2009). Moreover, patients whose tumors have low PTEN levels or activating *PIK3CA* mutations tend to have a lower response to trastuzumab (Nagata et al., 2004; Berns et al., 2007). Taken together, trastuzumab resistance might result when tumor cells are able to circumvent trastuzumab's activity on pathways downstream of HER2.

Erythropoietin (EPO) is frequently used to alleviate anemia, a complication arising from chemotherapy and/or from the release of cytokines by cancer cells. Anemia is a cause of morbidity and might also contribute to patient mortality (reviewed in Rizzo et al., 2010). EPO is a cytokine that stimulates erythropoiesis by activating EpoR, its specific cell surface receptor that has been identified on erythroid precursor cells. The Jak2 tyrosine kinase associates with EpoR and EPO binding activates Jak2, thereby stimulating PI3K/Akt and Erk pathways, as well as NF-kB and Stat5 activation (Elliott et al., 2008). As a result, there is proliferation and terminal differentiation of erythroid colony forming units (CFUe) in the bone marrow (Figure 1). Recombinant human erythropoietin (rHuEPO) has replaced blood transfusions and has had important benefits for cancer patients. However, detrimental effects elicited by rHuEPO have become a point of discussion and concern. In addition to the risks of venous thrombosis following rHuEPO treatment, there is a concern that EpoR, which is expressed on many tumors, might respond to its ligand, leading to enhanced tumor proliferation or survival (Rizzo et al., 2010).

Liang et al. (2010) have directly addressed some of these problems in the context of HER2-positive breast cancer and trastuzumab treatment. Initially, they used a specific antibody on primary breast tumors and found that EpoR is expressed at some level in approximately 80% of the tumors; there was no correlation with HER2 positivity or negativity. Thus, the majority of HER2-positive cancers also express EpoR. Next, in vitro studies carried out with HER2-positive, EpoR-expressing breast cancer cell lines showed that the receptor is functional and that rHuEPO treatment stimulates PI3K/Akt and Erk pathways, and in some cases, Stat5. As mentioned previously, treatment of HER2-positive tumor cells with trastuzumab blocks PI3K/Akt and Erk pathway activity. However, the concurrent exposure of HER2-positive, EpoR-positive models to rHuEPO and trastuzumab counteracted the inhibitory effects of trastuzumab on these pathways. Moreover, long-term exposure of these cells to rHuEPO overcame the negative effects of trastuzumab and enhanced the survival and proliferation of HER2-positive breast cancer cells. Strikingly, the effects were confirmed in

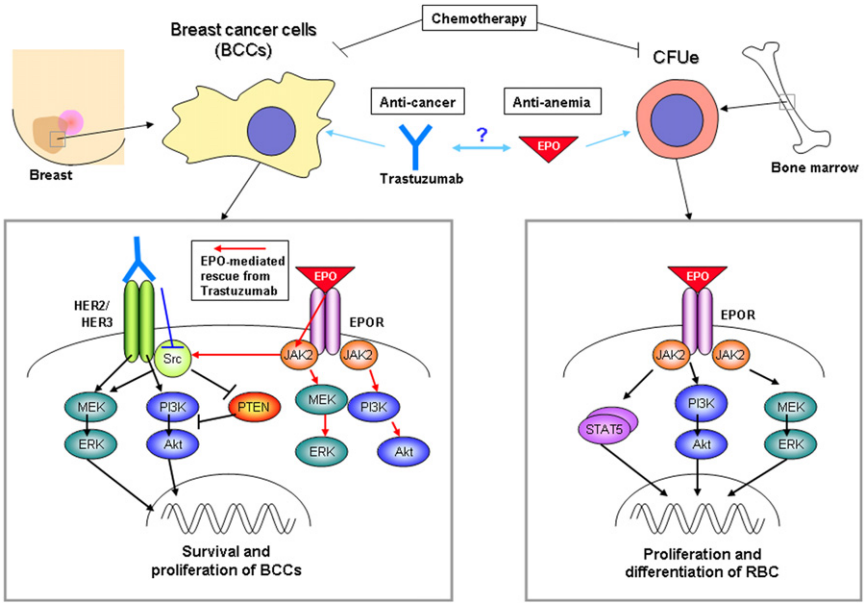


**Figure 1. Trastuzumab and EPO Interact Unfavorably in HER2-Positive Breast Cancer**
Trastuzumab binds the extracellular domain of HER2, thereby inhibiting HER2/HER3 complexes and downstream signaling cascades (Src, PI3K and MEK) that are constitutively active in tumor cells overexpressing HER2. EPO is a hematopoietic cytokine that supports survival, proliferation, and differentiation of erythroid progenitors (erythroid colony forming units CFUe) in the bone marrow. The EpoR is expressed not only in the hematopoietic compartment but also on cells of some cancer tissues. EPO binding to EpoR stimulates numerous pathways (Stat5, PI3K, and MEK) via activation of the receptor-associated JAK2 kinase.

xenograft transplantation experiments in which the growth of HER2-positive tumors was totally suppressed by treatment with trastuzumab but rescued by the simultaneous administration of rHuEPO to the mice.

The mechanism underlying the antagonism between trastuzumab and rHuEPO on tumor cell proliferation and survival is likely due to the overlap in signal transduction components activated downstream of HER2 and EpoR. Although the ligand receptor systems are clearly distinct, a small number of molecular pathways are commonly activated by both receptors. The authors focused on the PI3K/Akt and Erk pathways as well as Src kinase, each of which is affected by both receptor systems. Trastuzumab disrupts the HER2/HER3/PI3K complex (Junttila et al., 2009), which has an important role in its anticancer activity. Furthermore, it has been observed that at least in some models, trastuzumab reduces the HER2/Src association and induces PTEN activation (Nagata et al., 2004). Treatment of HER2-positive tumor cells with rHuEPO stimulated the association between HER2 and Src and increased activity of PI3K/Akt and Erk, thereby counteracting the effects of trastuzumab (Figure 1).

To test the clinical relevance, Liang and colleagues retrospectively examined HER2-positive metastatic breast cancer patients treated with trastuzumab, with or without chemotherapy, for progression- free survival and overall-survival intervals; one group of patients had been treated concurrently with rHuEPO and trastuzumab. Their analysis suggested that in this small cohort of patients, concurrent rHuEPO might have contributed to trastuzumab resistance. Importantly, they analyzed PTEN levels and *PIK3CA* mutation status in eight samples and found that four of the trastuzumab-resistant tumors were PTEN positive and *PIK3CA* wild type. Thus, not all trastuzumab-resistant patients have mutations that have been predicted to confer insensitivity to the antibody, suggesting that rHuEPO might have influenced trastuzumab response via its ability to activate specific pathways like Src or PI3K/Akt.

ErbB-targeted drugs have had important clinical activity (Baselga and Swain, 2009); however, further advances are necessary, since many patients who are initially responsive to ErbB receptor-targeted therapies become resistant. Although multiple mechanisms responsible for drug resistance have been suggested, unfavorable interactions between targeted drugs might be contributors that, up to now, have been underestimated. In addition to monitoring breast tumors for HER2 expression, testing for EpoR expression could help in clinical decisions and facilitate the choice of optimal drug combinations. The improved prediction of combined drug effects will be beneficial not only for the patients, but will also prevent the use of costly anticancer agents that have unfavorable interactions.

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