Unraveling Resistance to Trastuzumab (Herceptin): Insulin-Like Growth Factor-I Receptor, a New Suspect

Joan Albanell, Jose Baselga

Trastuzumab (Herceptin) is a humanized antibody directed against the extracellular domain of the tyrosine kinase receptor HER2 that has shown clinical activity against HER2-overexpressing breast tumors *(1–4).* HER2, the targeted receptor, is a member of the epidermal growth factor (EGF) receptor family of receptors, also known as the type I receptor tyrosine kinase family [for review, *see (5)*]. HER2 is overexpressed in 25%–30% of breast cancers, and its overexpression is associated with a high risk of relapse and death *(6).* In this group of tumors with unfavorable prognosis, trastuzumab has been a valuable addition to standard therapy, with the pivotal studies demonstrating a clear survival benefit *(2,3).* However, even in the selected group of patients with very high levels of HER2 overexpression who derive the greatest benefit from trastuzumab therapy, the response rate from this highly specific, targeted agent is limited in magnitude and duration *(7).*

This observation leads to the fundamental questions about the mechanism of action of trastuzumab in HER2-positive breast cancer cells and how these cells escape from its antitumor effects. The answer to these questions is particularly challenging because the mechanisms of action of trastuzumab have not been characterized fully and appear to be complex [for review, *see (8,9)*]. To date, the known mechanisms of trastuzumab's action include decreased expression of HER2 from the tumor cell surface *(10),* initiation of G_1 arrest and induction of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ *(8),* prevention of HER2 cleavage *(11),* inhibition of angiogenesis *(12),* and induction of immune mechanisms *(13).* Alterations in the HER2 receptor or in downstream signaling pathways that mediate any of these effects may be responsible for some cases of primary or acquired resistance to trastuzumab. Possible mechanisms could be expression of truncated HER2 receptors that cannot bind antibodies, mutations of downstream molecules (i.e., ras activation or PTEN deletion), a low level of $p27^{Kip1}$, and a decreased immune function in patients with advanced breast cancer.

Resistance to trastuzumab, however, may not only depend ultimately on its efficacy (or lack of efficacy) in inhibiting HER2 but also on whether HER2 activation is responsible, single-handedly, for the sustained growth, proliferation, and survival of a well-established tumor. In this regard, it is highly unlikely that a single, active tyrosine kinase receptor or intracellular tyrosine kinase may be solely responsible for a malignant phenotype *(14),* although a notable exception to this rule may be the critical role that the BCR-ABL tyrosine kinase plays in chronic myeloid leukemia and the high response rate of this disease to STI-571, a specific inhibitor of the BCR-ABL kinase *(15).* The emerging single-agent efficacy data with trastuzumab and other anti-growth factor receptor agents in epithelial tumors are demonstrating modest response rates, further supporting the theory that targeting just one receptor may not be enough to optimize responses *(16).* Furthermore, HER2 is a receptor without a cognate ligand, and transactivation of HER2 by other members of the EGF receptor family is important for the growth of HER2-overexpressing breast cancer cells. As a result, combined therapies with trastuzumab and a specific inhibitor of the EGF receptor tyrosine kinase result in enhanced growth inhibition and apoptosis *(17–20).*

The report by Lu et al. *(21)* in this issue of the Journal represents a further step ahead to unravel resistance to trastuzumab because it provides evidence for a critical role of insulin-like growth factor-I receptor (IGF-IR) signaling in the response to trastuzumab. The IGF-IR is a transmembrane tyrosine kinase receptor that is activated by binding of the IGF ligands. The hypothesis that IGF-IR signaling could influence the response to trastuzumab was raised from an extensive body of data indicating that this receptor plays an important role in breast cancer [reviewed in *(22)*], as follows: IGFs exert proliferative and antiapoptotic effects in many breast cancer cell lines *(23,24),* targeted disruption of the IGF-IR with anti-receptor antibodies or antisense RNAs to this receptor limits breast cancer proliferation, the IGF-IR and its ligands are expressed in many human breast tumors, and high levels of circulating IGF-I predict an increased risk of breast cancer in premenopausal women *(25).*

Lu et al. *(21)* have demonstrated that an increased level of IGF-IR signaling adversely interferes with trastuzumab's action on cell growth. They used two human breast cancer cell line models complementary in terms of IGF-IR expression—MCF-7/HER2-18 cells, which overexpress HER2 by transfection and endogenously express activated IGF-IRs, and SKBR3 cells, which endogenously overexpress HER2 but express few IGF-IRs (about 10% the number in MCF-7/HER2–18 cells). In MCF-7/HER2-18 cells, trastuzumab inhibited growth only when IGF-IR signaling was blocked by cotreatment with the anti-IGF-IR antibody α-IR3 or the IGF-binding protein-3 (IGFBP-3). In contrast to the basal resistance of MCF-7/HER2-18 cells to trastuzumab, SKBR3 cells, which have a low level of IGF-IR expression, were sensitive to trastuzumab. Additional compelling evidence to link the IGF-IR to the trastuzumab response was provided by the observation that SKBR3 cells became resistant to trastuzumab when cells were genetically altered to overexpress IGF-IRs (SKBR3/IGF-IR). Addition of IGFBP-3, which decreased IGF-IR signaling, restored the ability of trastuzumab to suppress growth. On the basis of these studies,

Affiliations of authors: J. Albanell, Medical Oncology Service, Vall d'Hebron University Hospital, Barcelona, Spain; J. Baselga, Medical Oncology Service, Vall d'Hebron University Hospital, and Universitat Autonoma de Barcelona.

Correspondence to: Jose Baselga, M.D., Medical Oncology Service,Vall d'Hebron University Hospital, Paseo Vall d'Hebron 119–129, 08035 Barcelona, Spain (e-mail: baselga@hg.vhebron.es).

Lu et al. *(21)* appropriately propose that strategies that target IGF-IR signaling may prevent or delay development of resistance to trastuzumab. A link between IGF-IR signaling in the modulation of response to monoclonal antibodies has been shown also for the EGF receptor, which is closely related to HER2. In this regard, a recent study *(26)* has shown that IGF-I signaling temporarily prevented the apoptosis mediated by the anti-EGF receptor monoclonal antibody C225 in the EGF receptor auxotroph cell line DiFi. It is interesting that such inhibition was sensitive to an inhibitor of the phosphatidylinositol 3-kinase (PI-3K)/Akt pathway *(26).* Because the PI-3K/Akt pathway is poorly suppressed by trastuzumab in breast cancer cells *(17),* it is tempting to speculate that this pathway may play an important role in the observations by Lu et al *(21).*

What are the molecular signaling events underlying the interference of IGF-IR signaling in trastuzumab response? Lu et al. *(21)* characterized opposing effects of trastuzumab and IGF-IR on $p27^{Kip1}$. The induction of $p27^{Kip1}$ by anti-HER2 antibodies contributes to their effects on growth inhibition *(8).* However, in SKBR3/IGF-IR cells, the baseline levels of $p27^{Kip1}$ were very low compared with those in SKBR3/neo control cells, and trastuzumab could not induce $p27^{Kip1}$ expression. This effect might be mediated by interference between the IGF-IR and HER2 in signaling pathways that regulate $p27^{Kip1}$. Such downstream pathways include ras/raf/mitogen-activated protein kinase or, as mentioned above, PI3-K/Akt signaling, and it would be of practical interest to characterize this possibility because we have inhibitors of these pathways in clinical development. In addition, because IGF-IR signaling has been linked to antiapoptotic effects and resistance to several anticancer treatments *(22,26,27),* it would be relevant to study whether IGF-IR targeting when combined with trastuzumab results in an enhanced apoptosis. Searching for interactions between both receptor systems will not be an easy task, because diverse study models have revealed different hierarchical cross-regulations between HER2 and the IGF-IR *(28,29).*

It is evident that trastuzumab development has been a model of a rationally designed targeted treatment, where laboratory predictions have been followed by remarkably successful clinical studies. There are, however, many unanswered biologic questions regarding the mechanisms of action of trastuzumab and causes of resistance to this antibody that warrant further investigation. In the meantime, the study by Lu et al. *(21)* sheds light on a potential strategy—the inhibition of IGF-IR signaling—to prevent or reverse resistance to trastuzumab. Based on the results obtained by Lu et al., it would be important to characterize the coexpression of HER2 and members of the IGF-IR signaling pathway in breast tumors. In particular, analysis of baseline breast tumor biopsy specimens from patients treated with trastuzumab would provide critical insights into the possible role of IGF-IR in primary clinical responsiveness to the antibody. If an association between IGF-IR activation and resistance to trastuzumab were established in the clinic, it would be a strong signal to combine anti-IGF-IR and anti-HER2 therapies in patients with breast cancer.

REFERENCES

(1) Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, et al. Phase II study of weekly intravenous recombinant humanized anti-$p185^{HER2}$ monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 1996;14:737–44.

(2) Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17:2639–48.

(3) Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783–92.

(4) Vogel C, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. First-line, single-agent Herceptin® (trastuzumab) in metastatic breast cancer: a preliminary report. Eur J Cancer 2001;37 Suppl 1: 25–9.

(5) Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.

(6) Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.

(7) Mass RD, Sanders C, Charlene K, Johnson L, Everett T, Anderson S. The concordance between the clinical trials assay (CTA) and fluorescence *in situ* hybridization (FISH) in the Herceptin pivotal trials [abstract]. Proc ASCO 2000;19:291.

(8) Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin Oncol 1999;26(4 Suppl 12):60–70.

(9) Baselga J, Albanell J. Mechanism of action of anti-HER2 monoclonal antibodies. Ann Oncol 2001;12 Suppl 1:S35–41.

(10) Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol 1989;9:1165–72.

(11) Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res 2001;61:4744–9.

(12) Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B, et al. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo:* angiogenic implications for signal transduction therapy of solid tumors. Am J Pathol 1997;151: 1523–30.

(13) Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. Nat Med 2000;6: 443–6.

(14) Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100: 57–70.

(15) Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031–7.

(16) Baselga J. Targeting the epidermal growth factor receptor: a clinical reality. J Clin Oncol 2001;19(18 Suppl):41S–44S.

(17) Anido J, Albanell J, Rojo F, Codony-Servat J, Arribas J, Baselga J. Inhibition by ZD1839 (Iressa) of epidermal growth factor (EGF) and heregulin induced signaling pathways in human breast cancer cells [abstract]. Proc ASCO 2001;20:1712.

(18) Moasser MM, Basso A, Averbuch SD, Rosen N. The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res 2001;61: 7184–8.

(19) Moulder SL, Yakes M, Bianco R, Arteaga C. Small molecule EGF receptor tyrosine kinase Inhibitor ZD1839 (IRESSA) inhibits HER2/neu (erb-2) overexpressing breast tumor cells [abstract]. Proc ASCO 2001;20:8.

(20) Normanno N, Campiglio M, De Luca A, Somenzi G, Maiello M, Ciardiello F, et al. Cooperative inhibitory effect of ZD1839 ("Iressa") in combination with trastuzumab ("Herceptin") on human breast cancer cell growth. Ann Oncol. In press 2002.

(21) 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.

(22) Zhang X, Yee D. Tyrosine kinase signalling in breast cancer: insulin-like

growth factors and their receptors in breast cancer. Breast Cancer Res 2000;22:170–5.

(23) Chan TW, Pollak M, Huynh H. Inhibition of insulin-like growth factor signaling pathways in mammary gland by pure antiestrogen ICI 182,780. Clin Cancer Res 2001;7:2545–54.

(24) Bartucci M, Morelli C, Mauro L, Ando' S, Surmacz E. Differential insulin-like growth factor I receptor signaling and function in estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells. Cancer Res 2001;61:6747–54.

(25) Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393–6.

(26) Liu B, Fang M, Lu Y, Mendelsohn J, Fan Z. Fibroblast growth factor and insulin-like growth factor differentially modulate the apoptosis and G1 arrest induced by anti-epidermal growth factor receptor monoclonal antibody. Oncogene 2001;20:1913–22.

(27) Peruzzi F, Prisco M, Dews M, Salomoni P, Grassilli E, Romano G, et al. Multiple signaling pathways of the insulin-like growth factor 1 receptor in protection from apoptosis. Mol Cell Biol 1999;19:7203–15.

(28) Ram TG, Dilts CA, Dziubinski ML, Pierce LJ, Ethier SP. Insulin-like growth factor and epidermal growth factor independence in human mammary carcinoma cells with c-erbB-2 gene amplification and progressively elevated levels of tyrosine-phosphorylated p185erbB-2. Mol Carcinog 1996;15:227–38.

(29) Balana ME, Labriola L, Salatino M, Movsichoff F, Peters G, Charreau EH, et al. Activation of ErbB-2 via a hierarchical interaction between ErbB-2 and type I insulin-like growth factor receptor in mammary tumor cells. Oncogene 2001;19:34–47.