Commentary

Trastuzumab, an appropriate first-line single-agent therapy for HER2-overexpressing metastatic breast cancer

Carlos L Arteaga

Departments of Medicine and Cancer Biology, Vanderbilt University School of Medicine; and Breast Cancer Research Program, Vanderbilt-Ingram Comprehensive Cancer Center, Nashville, Tennessee, USA

Corresponding author: Carlos L Arteaga (e-mail: carlos.arteaga@vanderbilt.edu)

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Abstract

Overexpression of the HER2/Neu (ErbB2) proto-oncogene is associated with breast cancer progression and poor patient prognosis. Herceptin (trastuzumab) is a humanized IgG1 against the ectodomain of the HER2 receptor. In combination with chemotherapy, it induces regression of HER2-overexpressing metastatic breast tumors and prolongs patient survival. Single-agent Herceptin in patients with HER2-amplified breast tumors also induces a definite objective response and clinical benefit rates, and is well tolerated. These data suggest that Herceptin is an effective first-line single-agent therapy for a predictable cohort of metastatic breast cancers and can therefore be used as a platform for therapeutic discovery against tumors that overexpress HER2.

Keywords: breast cancer, clinical trials, erbB2, HER2/Neu, Herceptin

Introduction

The HER2/Neu proto-oncogene product is a member of the erbB family of transmembrane receptor tyrosine kinases, which also includes the epidermal growth factor receptor (EGFR, HER1, ErbB1), HER3 (ErbB3), and HER4 (ErbB4). Except for HER2, binding of receptor-specific ligands to the extracellular domain of EGFR, HER3, and HER4 results in the formation of homodimeric and heterodimeric phosphorylated, kinase-active complexes into which HER2 is recruited as a preferred partner [1–3]. Even though HER2 is unable to interact directly with HER-activating ligands, it can potentiate signaling by its co-receptors and/or increase the binding affinity of ligands to EGFR and HER3 (reviewed in [1]). Studies with HER2-overexpressing breast cancer cell lines and human tumors have shown constitutive phosphorylation of HER2 [4,5]. The biochemical basis for this activation is not clear but it is consistent with the ability of wild-type Neu, the rat homolog of human HER2, to multimerize and become activated in a ligand-independent fashion when present in cells at high density [6]. Overexpression of HER2 is associated with transformation of mammary epithelial cells [7,8] as well as shorter survival in patients with breast carcinoma [9,10]. The association of HER2 with poor patient prognosis, the ability to measure HER2 levels prospectively in diagnostic tumor tissue, and the lack of an apparent physiological role of HER2 in the adult, initially suggested this receptor as a rational therapeutic target in human breast cancer.

Herceptin: mechanisms of action and clinical activity

One therapeutic approach against HER2-overexpressing breast cancers is the generation of Herceptin (trastuzumab), a humanized IgG1 that binds to residues 529–626 in the HER2 ectodomain [11]. The mouse hybridoma counterpart of Herceptin, 4D5, partly removes HER2 from the plasma membrane [12,13] and/or induces HER2 homodimerization (CL Arteaga, unpublished data), potentially preventing HER2 molecules from interacting with other HER co-receptors and thus impairing the growth of HER2-dependent tumor cells. A recent study demonstrated that Herceptin blocks the metalloprotease-induced cleavage of HER2 [14]. Proteolytic

ADCC = antibody-dependent, cell-mediated cytotoxicity; DCIS = ductal carcinoma *in situ*; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridization; PI3K = phosphoinositide 3-kinase.

cleavage of HER2 results in the generation of a 95 kDa amino-terminal membrane-associated fragment and shedding of the receptor's ectodomain [15]. The constitutive kinase activity of the 95 kDa cytoplasmic fragment and the association of high levels of the shed HER2 ectodomain with poor patient outcome [16,17] suggest that cleavage of HER2, potentially blocked by Herceptin, might be of clinical significance. Clynes and colleagues [18] reported that the inhibitory effect of the antibody against HER2-dependent xenografts is markedly abrogated in mice lacking the receptor FcγRIII, strongly implying that antibody-dependent, cell-mediated cytotoxicity (ADCC) is the predominant mechanism of action of the humanized IgG1. More recently, Yakes and colleagues [19] reported that, in HER2-overexpressing tumor cells, treatment with Herceptin results in inhibition of phosphoinositide 3-kinase (PI3K) and the serine/threonine kinase Akt. In that study, the antitumor effect of Herceptin was reversed by forced expression of activated Akt, suggesting that, in addition to immune mechanisms, the antibody might exert its antitumor effect by a blockade of PI3K/Akt signaling.

Although the mechanisms of action of Herceptin against patients' tumors remain to be elucidated, it has been shown to induce tumor regressions in up to 20% of heavily pretreated metastatic breast cancers [20,21]. Vogel and colleagues [22] recently reported the results of a clinical trial of single-agent Herceptin used as first-line therapy in 111 patients with metastatic breast tumors with high levels of HER2. Clinical responses were limited to tumors exhibiting HER2 overexpression or HER2 gene amplification as determined by 3+ immunohistochemistry or excess copies of HER2 by fluorescence *in situ* hybridization (FISH), respectively. In this cohort, the objective response rate and the clinical benefit rate, as defined by stable disease lasting longer than 6 months, were a robust 35% and 48%, respectively [22]. Although an accurate assessment of the median duration of clinical response was not possible because of patient censoring, 57% of the responding patients were free of disease progression at more than 12 months of follow-up, underscoring the durability of the responses. Remarkably, the median duration of survival for all enrolled patients was 24.4 months, almost identical to the 25.1 months median survival of patients with HER2-overexpressing metastatic breast tumors treated with chemotherapy and Herceptin in a previous study [23]. Taken together, these clinical data confirm a role for HER2 in the progression of human breast carcinomas. They also suggest that a cohort of breast cancers remain HER2-dependent until advanced metastatic phases of the disease. In addition, high levels of HER2 protein overexpression and/or HER2 gene amplification accurately predict reasonable odds of response to Herceptin and can therefore be used for rational patient selection with this molecule-directed therapy.

Rationale for future clinical directions

The rate of HER2 gene amplification in ductal carcinoma *in situ* (DCIS) of the breast is the same as or higher than that in metastatic breast cancer [24–26]. Because DCIS is a well-established precursor of invasive breast cancer [27], this suggests that HER2 overexpression occurs years before the onset of advanced invasive carcinoma. The progression of DCIS to invasive and late metastatic cancer is inexorably associated with the accumulation of additional genetic alterations. On the basis of these data, one could speculate on two possibilities. First, the results of the study by Vogel and colleagues [22] might be better than could have been expected as they suggest that a cohort of metastatic breast cancers, namely the Herceptin responders, remain predominantly dependent on HER2 function until late phases in the natural history of the disease. Such a magnitude of clinical response might not necessarily apply to the inactivation of other cancer targets with single molecular therapies. Second, it would be expected that the efficacy and curative potential of Herceptin would increase if used in earlier phases of the disease and/or in patients with lower tumor burden, that is, for the prevention of progression of preinvasive cancers or for the treatment of subclinical micrometastases in the adjuvant setting. Several clinical trials with Herceptin in the adjuvant setting in HER2-overexpressing breast cancers are under way worldwide. It is envisaged that enrollment into these large adjuvant trials and the potential general use of Herceptin in the adjuvant setting will change the natural history of HER2-amplified breast carcinoma. As these trials continue to enroll patients, one could expect a decrease in the overall percentage of metastatic breast tumors diagnosed *de novo* that express high levels of the proto-oncogene.

Slamon and colleagues [23] reported the ability of Herceptin to increase the clinical benefit from first-line chemotherapy against metastatic breast cancers that overexpress HER2. These ground-breaking studies documented a 25% improvement in survival in patients that received concurrent Herceptin and chemotherapy compared with chemotherapy alone. Interestingly, however, the median survival (25.1 months) of patients in this trial receiving the combination was almost identical to the 24.4-month median survival of patients enrolled in the single-agent Herceptin trial performed by Vogel and colleagues [22]. In both of these studies, the eligibility was similar whereas Herceptin was much better tolerated in the single-agent trial than was the combination of chemotherapy plus antibody in the study by Slamon and colleagues. Although one cannot exclude referral biases that might have resulted in different patient populations in these two trials, the published demographics for the subjects enrolled in these studies indicate that they were similar when compared by age, performance status, percentage of steroid receptor-positive tumors, number of

metastatic sites, and prior adjuvant chemotherapy. If these HER2-overexpressing patient cohorts were similar, as the patients' published characteristics would support, this might suggest, first, that in the combination therapy study the antitumor effect was mainly due to Herceptin, and, second, that Herceptin is a reasonable first-line single agent in oligosymptomatic patients that fit the eligibility of the study by Vogel and colleagues and can be used as a platform onto which other molecular therapies, not necessarily chemotherapy, can be added. Those added therapies would be those that target molecules or mechanisms of *de novo* or acquired Herceptin resistance.

Therapeutic resistance

Most metastatic breast tumors with HER2 gene amplification and/or very high levels of HER2 protein do not respond to Herceptin, suggesting the possibility that in most late breast cancers, HER2 has become dispensable for tumor viability and progression. This fact, together with the frequent overexpression of HER2 in non-invasive disease and the eventual escape from Herceptin action in patients with HER2-amplified metastatic breast cancers, suggests *de novo* and acquired mechanisms of therapeutic resistance. The antitumor effect of HER2 inhibitors such as Herceptin would require the subversion of key post-receptor signaling pathways and cell cycle/anti-apoptosis regulatory molecules that mediate the transforming effects of HER2. These post-receptor pathways are shared with heterologous receptor networks [28,29] and/or with heterodimers of the same HER (ErbB) network that are not affected by Herceptin. These data therefore imply that tumor cells are intrinsically endowed with signaling mechanisms of compensation that can counteract the blockade of HER2 function by single-agent Herceptin.

The mechanisms of *de novo* or acquired resistance to Herceptin are not yet known. However, recent reports suggest some possibilities. Indeed, overexpression of the IGF-I receptor abrogates the effect of Herceptin against HER2-overexpressing SKBR-3 cells. Addition of the IGF-I receptor antibody αIR3 or IGF-BP3 reverses Herceptin resistance [30]. Herceptin potently inhibits PI3K and Akt in HER2-dependent, antibody-sensitive cells [19,31]. Conversely, Herceptin neither decreases PI3K activity nor inhibits the growth of HER2-overexpressing MKN gastric cancer cells [32]. Transduction of vectors encoding activated Akt prevents the Herceptin-induced inhibition of proliferation of BT-474 cells and apoptosis in SKBR-3 cells [19]. These data suggest that inhibition of PI3K/Akt signaling is required for the antitumor effect of HER2 inhibitors such as Herceptin. Many breast cancers harbor genetic alterations resulting in aberrant PI3K/Akt signaling or overexpress heterologous receptor networks, such as IGF-IR, that potently activate PI3K/Akt. We speculate that these coexisting alterations in HER2-overexpressing breast cancers dampen or abrogate Herceptin action. On the basis of these data, a combined therapeutic approach with HER2 and PI3K/Akt inhibitors would be worth investigating in these tumors.

High levels of activated EGFR abrogate the efficacy of Herceptin against HER2-gene-amplified cancer cells [32] and this resistance is reversed by ATP-competitive inhibitors of the EGFR tyrosine kinase [33]. In addition, the EGFR antibody C225 synergizes with 4D5 against HER2-overexpressing ovarian cancer cells [34]. Moreover, the EGFR kinase inhibitor ZD1839 inhibits HER2 phosphorylation by itself and potentiates the antitumor effect of Herceptin against breast cancer xenografts [35–37]. Taken together these results suggest, first, that activated EGFR can potentially mediate escape from Herceptin action, and second, that combinations of Herceptin with EGFR inhibitors are synergistic against HER2-overexpressing tumors that also express EGFR. This hypothesis is currently being tested in phase II studies of Herceptin in combination with the EGFR kinase inhibitors ZD1839 or OSI-774.

Another approach that might shed light on mechanisms of Herceptin resistance is the sequencing of the HER2 gene from tumors known to be progressing on Herceptin therapy. It should be emphasized that the studies documenting a lack of somatic mutations of HER2 in primary breast cancers [38] predate the development and use of Herceptin. Thus, the possibility of acquired HER2 mutations as a result of the selective pressure of HER2 blockade has never been explored and is worth revisiting in breast cancers 'selected' for Herceptin resistance *in vivo*.

Finally, one possible exploratory trial design to address resistance mechanisms would be the administration of neoadjuvant Herceptin followed by chemotherapy to newly diagnosed HER2 gene-amplified tumors. Herceptin can be started as a single agent for the first 4 weeks before the addition of chemotherapy, while tumor core biopsies are obtained weekly. This approach has been used effectively with antiestrogens. These studies have shown that as little as 14 days of therapy with tamoxifen results in a marked reduction of breast cancer cell proliferation as measured by Ki67 immunohistochemistry of tumor sections but this reduction was limited to estrogen-receptor-positive tumors [39–41]. Any excess tissue from the initial and follow-up core biopsies can be used to detect novel RNAs or proteins and their changes as a function of inhibition of cell proliferation and/or inhibition of cell survival after treatment with Herceptin. These two endpoints will be measured by Ki67 immunohistochemistry and TUNEL (terminal transferase deoxytidyl uridine end labeling), respectively. By focusing on differences in dynamic changes on RNA and/or protein expression profiles between responders and non-responders, this approach should detect markers of resistance to Herceptin and/or mechanisms causally associated with the resistant phenotype.

Summary

The data summarized above clearly imply that Herceptin is an effective first-line single-agent therapy for HER2-overexpressing metastatic breast cancers. Herceptin can be used as a platform onto which other molecular therapies, perhaps those targeting molecules that mediate *de novo* or acquired escape from the HER2 inhibitor, can be added. Because Herceptin is well tolerated, scientifically rational combinations of the antibody with other molecule-targeted drugs are medically justifiable as first-line therapy in metastatic disease. Whether giving Herceptin alone as initial therapy jeopardizes subsequent survival in response to treatment with Herceptin and chemotherapy requires further investigation. Elucidation of the preferential molecular mechanisms of escape from Herceptin and/or HER2 dependence will define new rational targets against which drugs either are available or are to be developed. Drugs against these targets can be combined with Herceptin to prevent *de novo* or acquired resistance and to enhance therapeutic efficacy. If tumor-specific and well tolerated, combinations of anti-signaling drugs that include Herceptin should become a robust therapeutic alternative to non-specific cytotoxic chemotherapy against HER2-overexpressing breast carcinoma.

Competing interests

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Correspondence

Carlos L Arteaga MD, Division of Oncology, Vanderbilt University Medical Center, 2220 Pierce Avenue, 777 PRB, Nashville, TN 37232-6307, USA. Tel: +1 615 936 3524; Fax: +1 615 936 1790; e-mail: carlos.arteaga@vanderbilt.edu