Lapatinib Enhances Herceptin-mediated Antibody-dependent Cellular Cytotoxicity by Up-regulation of Cell Surface HER2 Expression

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Abstract. *Background: Although it was previously reported that lapatinib combined with Herceptin improved the progression-free survival rate compared with lapatinib alone for patients with Herceptin-refractory HER2-positive metastatic breast cancer, the mechanism is purported to be an antiproliferative effect relating to the synergism of these two agents. Materials and Methods: We evaluated how lapatinib interacts with Herceptin in HER2-positive breast cancer, with a particular focus on Herceptin-mediated antibody-dependent cellular cytotoxicity (ADCC). Results: In an in vitro assay, lapatinib induced HER2 expression at the cell surface of HER2-positive breast cancer cell lines, leading to the enhancement of Herceptin-mediated ADCC. Furthermore, we present a case report in which a second Herceptin treatment following lapatinib resulted in the marked shrinkage of multiple metastatic tumors in HER2-positive breast cancer. Conclusion: Lapatinib may have the potential to convert Herceptin-refractory to Herceptin-sensitive tumors in HER2-positive breast cancer by up-regulation of the cell surface expression of HER2.*

Human epidermal growth factor receptor (*EGFR*) 2 (HER2) is one of four members of the ErbB receptor tyrosine kinase protein family, and this proto-oncogene encodes a 185-kDa transmembrane glycoprotein (1). The overexpression and gene amplification of HER2 is seen in a variety of human tumors, including 25-30% of breast and ovarian, renal cell, esophageal squamous cell, and gastric and colorectal carcinomas (2-5). In particular, the overexpression and gene amplification of HER2 in breast cancer correlates with a poorer prognosis and more aggressive tumor growth (6).

Trastuzumab (Herceptin), a fully humanized monoclonal antibody targeting the extracellular domain of HER2 protein, exhibits potent growth-inhibitory activity against HER2-overexpressing tumors (7) and improves overall survival in patients with HER2-overexpressing metastatic breast cancer (8). The diverse mechanisms of Herceptin include the down-regulation of HER2 receptors (7), blockade of signaling pathways (7), inhibition of angiogenesis (9), activation of apoptotic signals of tumor cells (10), and enhancement of the immune system, such as via antibody-dependent cellular cytotoxicity (ADCC) (7, 11-13).

Lapatinib is a dual tyrosine kinase inhibitor (TKI) targeting both EGFR and HER2 tyrosine kinase domains (14), and has recently been approved by the U.S. Food and Drug Administration for the treatment of HER2-expressing advanced breast cancer. Lapatinib competes with the adenosine triphosphate binding site located within the intracellular kinase domain of EGFR and HER2, and has mechanisms of action distinct from those of Herceptin (15-17). Furthermore, Scaltriti *et al*. and our group also showed that lapatinib induces the accumulation of HER2 at the cell surface and enhances the effects of Herceptin-mediated ADCC (18, 19). In several clinical trials, lapatinib prolonged progression-free survival in patients with HER2-overexpressing advanced or metastatic breast cancer, including those with Herceptin-refractory disease, and was established as effective treatment for them (15, 20-23).

In this study, we evaluate whether lapatinib induces the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines, resulting in the enhancement of Herceptin-mediated ADCC. Furthermore, we present a case report in which lapatinib showed the potential to convert Herceptin-refractory to Herceptin-sensitive tumors in HER2-positive breast cancer.

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Materials and Methods

Reagents. Penicillin streptomycin and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phycoerythrin (PE)-conjugated mouse anti-HER2/neu antibody, PE-conjugated mouse IgG1 k isotype control immunoglobulin, and fluorescein isothiocyanate (FITC)-conjugated Annexin V, 7-amino-actinomycin D (7-AAD) (Becton Dickinson, Franklin Lakes, NJ, USA) were used for flow cytometric analysis. Lapatinib (Tykerb) was purchased from GlaxoSmithKline (Brentford, UK).

Cell lines. SK-BR-3 and BT474, which are HER2-overexpressing breast cancer cell lines, were obtained from the American Type Culture Collection (Rockville, MD, USA). All cell lines were maintained in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) with 5% fetal calf serum (Invitrogen), 50 U/ml penicillin, and 2 mM L-glutamine.

Western blotting. Tumor cell lines were treated with 100 nM of Lapatinib or DMSO for 1 h. After incubation, cell pellets were solubilized in electrophoresis sample buffer and boiled for 10 min, and then the same amount of protein (30 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to a polyvinylidene fluoride microporous membrane (Millipore, Billerica, MA, USA) blocked in phosphate-buffered saline (PBS) with 5% milk powder. After blocking, the membrane was probed with primary antibodies to HER2/ErbB2, phosphorylated HER2/ErbB2 (Tyr1221/1222), or β-actin (Cell Signalling Technology, Danvers, MA, USA), and then probed with HRP-linked goat anti-rabbit antibody (Cell Signalling Technology). Blots were visualized by enhanced chemiluminescence with ECL Plus according to the manufacturer's protocol (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Antibody-dependent cell-mediated cytotoxicity (ADCC) assay. Tumor cell lines were exposed to DMSO or 100 nM of lapatinib for 48 h, following which they were used as targets, and treated targets cells were labelled with 50 μ Ci of ^{51}Cr for 60 min. Peripheral blood mononuclear cells (PBMCs) from healthy donors obtained by Ficoll–Paque separation (GE Health Care, Uppsala, Sweden) were used as effector cells. The assays were performed in triplicate in a 96-well U-bottom plate with Herceptin (Roche, Basel, Switzerland) or Rituxan (Roche) as a negative control. As an isotype-matched control mAb for Herceptin having an ADCC capacity, we used Rituxan as a negative control for Herceptin in ADCC assay. Targets (5×10^3 well) and various ratios of effector cells were co-cultured for 8 h in 200 μl of X-VIVO medium supplemented with 50 IU/ml of IL-2 (Shionogi & Co., Ltd., Osaka, Japan). After 8-h incubation, the ^{51}Cr released into the supernatant (100 μl) was measured with a gamma counter, and the rate of specific lysis was calculated according to the formula: % specific lysis=100 × (experimental cpm–spontaneous cpm) (maximum cpm–spontaneous cpm).

Flow cytometric analysis. Tumor cell lines were exposed to DMSO or 100 nM of lapatinib for 48 h. After incubation, treated cells were immunofluorescently labeled according to a previously established immunofluorescent staining protocol (24), and then cells were analyzed using FACS Calibur (Becton Dickinson) and Cellquest™ software (Becton Dickinson).

Statistics. Student's unpaired *t*-test was performed to determine significance, and *p*-values <0.05 were considered significant.

Results

Lapatinib induced the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines. The effect of lapatinib on the inhibition of HER2 phosphorylation was examined by Western blotting, and the accumulation of HER2 at the cell surface was examined by flow cytometric analysis following treatment with lapatinib. As shown in Figure 1A, lapatinib inhibited the phosphorylation of HER2 in HER2-overexpressing breast cancer, in line with previous reports (18, 19). Furthermore, we revealed that lapatinib induced the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines BT474 and SK-BR-3 (Figure 1B), confirming the findings of our previous report for esophageal squamous cell carcinoma (19).

Lapatinib enhanced Herceptin-mediated ADCC in HER2-positive breast cancer cell lines. We and Scaltriti *et al*. recently reported that Lapatinib enhances the effects of Herceptin-mediated ADCC, with the accumulation of HER2 at the cell surface caused by lapatinib pre-treatment (18, 19). In the present study on breast cancer cells, pre-treatment of SK-BR-3 and BT474 with lapatinib resulted in almost 2-fold increment of Herceptin-mediated ADCC compared with the DMSO control and there were significant differences in the Herceptin-mediated ADCC between pre-treated targets with DMSO and with lapatinib (Figure 1C). These observations indicated that lapatinib induced the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines, leading to the enhancement of Herceptin-mediated ADCC.

Case Report

Herceptin treatment following Lapatinib was effective for metastatic HER2-positive breast cancer. We present the case of a 57-year-old woman who had HER2-positive left breast cancer at stage IIA according to the Tumor-Node-Metastases Classification of the International Union against Cancer (the 6th edition). After neoadjuvant chemotherapy including three courses of docetaxel (75 mg/m^2, every three weeks) following four courses of doxorubicin and cyclophosphamide (60 mg/m^2 of doxorubicin and 60 mg/m^2 of cyclophosphamide, every three weeks), wide excision and axillary lymph node dissection were performed at the Yamanashi Hospital of Social Insurance in March 2005. Pathological findings revealed an invasive papillotubular carcinoma with lymph node metastasis that was estrogen receptor-positive and progesterone receptor-negative, with a HER2 score of 3+ (immunohistochemistry). Although 1 mg/day of anastrozole for five years and 50 Gy of irradiation for the left residual mammary gland were administered after the operation, left parasternal lymph node metastasis appeared in March 2008 (Figure 2A). Therapy of Herceptin (4 mg/kg initially followed by 2 mg/kg, weekly)

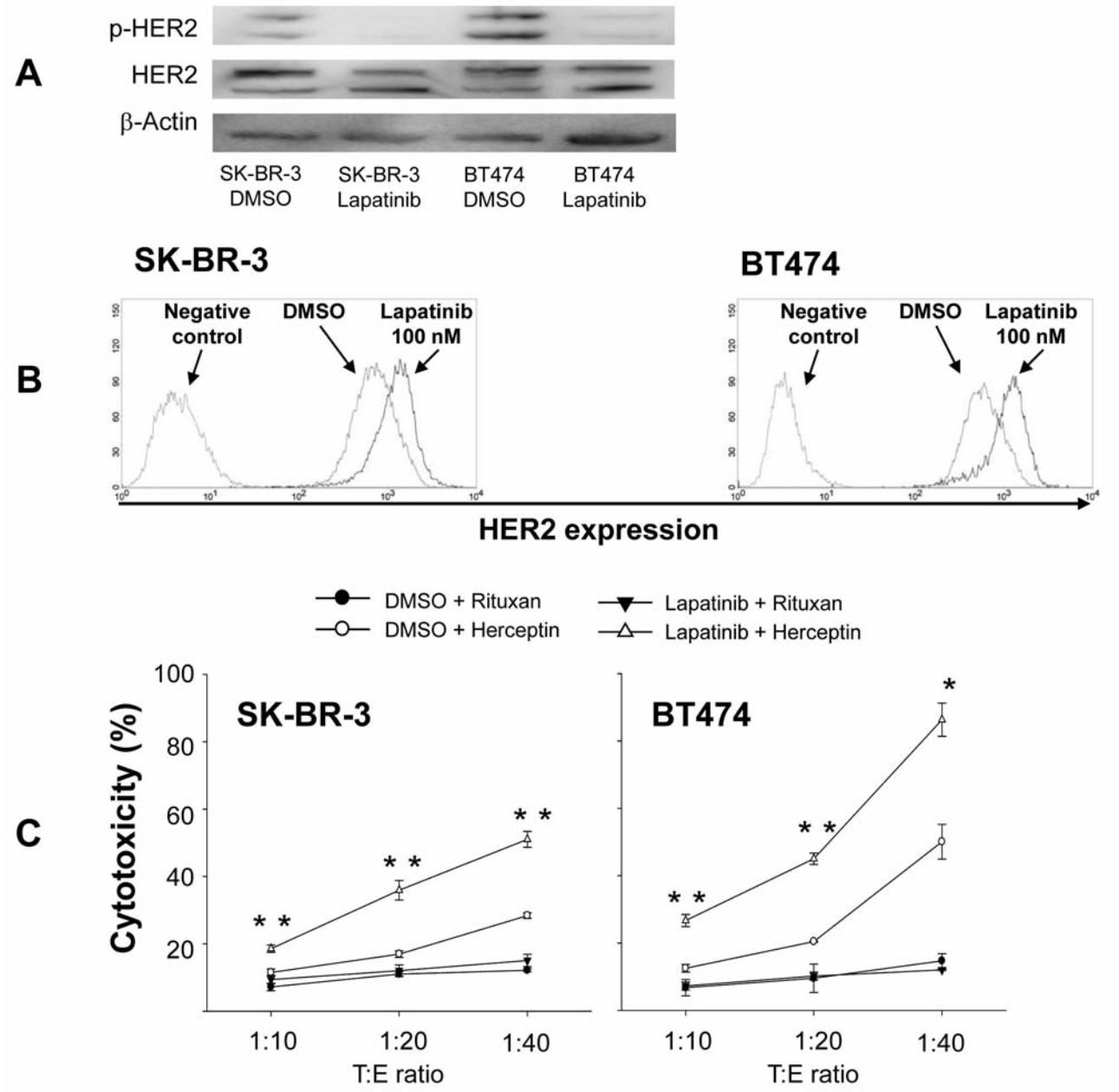

Figure 1. *Lapatinib led to the accumulation of HER2 at the cell surface and enhanced Herceptin-mediated Antibody-dependent cell-mediated cytotoxicity (ADCC) in HER2-over-expressing breast cancer cell lines. A: HER2 phosphorylation in SK-BR-3 and BT474, which are well known as HER2-overexpressing cell lines, was inhibited by lapatinib. Both cell lines were treated with lapatinib (100 nM) or DMSO control for 1 h. Western blotting to detect HER2 and phosphorylated HER2 (p-HER2) was conducted as described in the Materials and Methods. B: HER2 expression on SK-BR-3 and BT474 cells after treatment with lapatinib (100 nM) or DMSO control for 48 h was assessed by flow cytometry. Dead and/or apoptotic cells were excluded with Annexin V and 7-amino-actinomycin D staining. C: After SK-BR-3 and BT474 cells were treated with DMSO or 100 nM of lapatinib for 48 h, assay of ADCC was performed as described in the Materials and Methods. Representative data are shown from three independent experiments. We compared the activity of Herceptin-mediated ADCC between pre-treated targets with DMSO and with lapatinib. Data were analyzed using a non-paired Student's t-test. Significant difference at p-values **<0.01 and *<0.05.*

and capecitabine (2,400 mg/day, 3 weeks on, 1 week off) was started in August 2008. Treatment with Herceptin and capecitabine was effective for the metastasis of the left parasternal lymph node, defined as a complete response (CR), and the CR lasted for eight months with this treatment (Figure 2B). However, the parasternal lymph node metastasis re-appeared (Figure 2C, left panel), along with bilateral infraclavicular and right hilar lymph node metastases (Figure

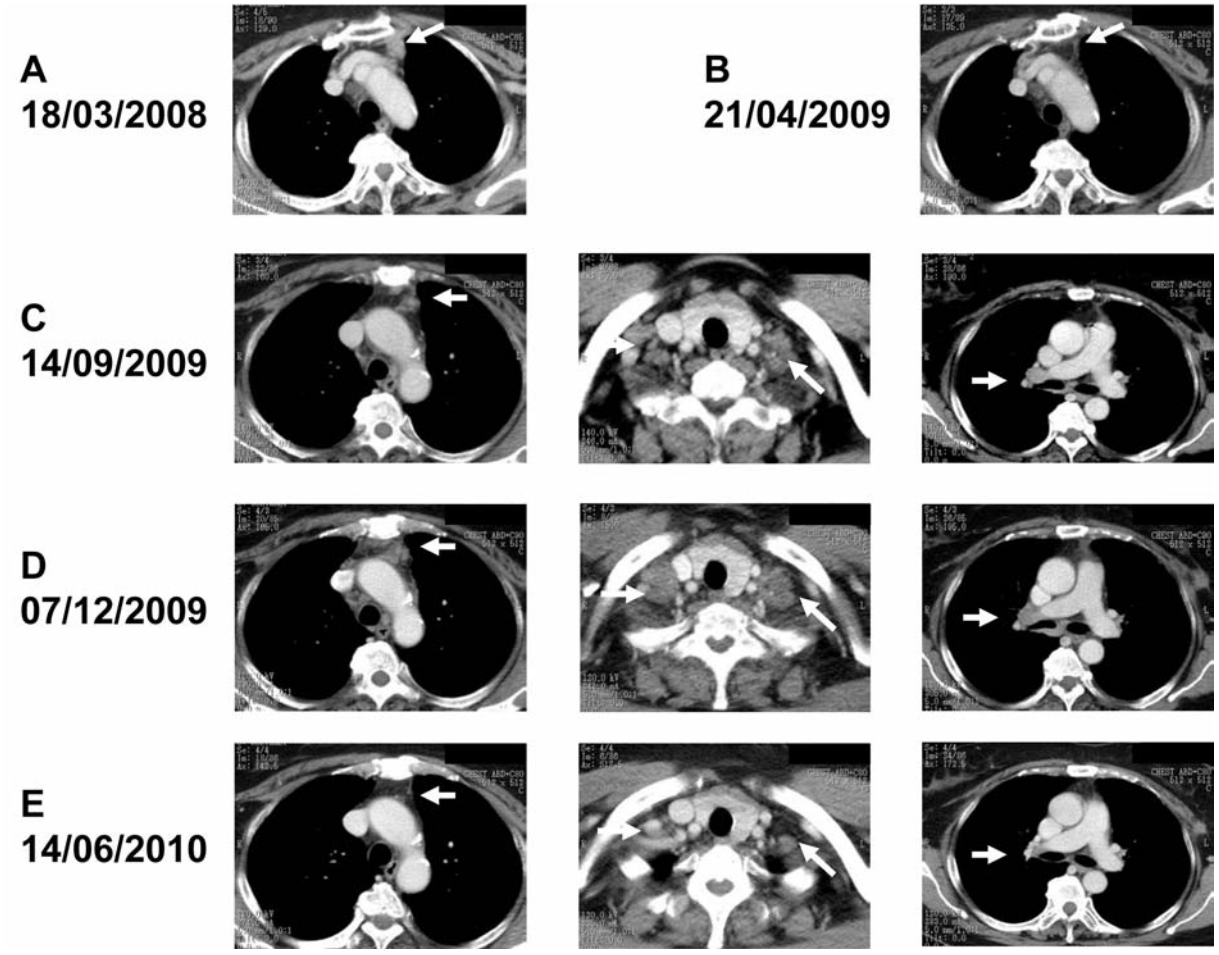

Figure 2. *Chest computed tomography findings for metastatic tumors of breast cancer. A: The metastasis of the left parasternal lymph node (arrow). B: A complete response at eight months after the initial treatment with Herceptin and capecitabine (arrow). C: Re-growth of the left parasternal lymph node metastasis (left panel, arrow) along with bilateral infraclavicular and right hilar lymph node metastases (middle and right panels, arrows). D: Progressive disease showing multiple lymph node metastases after treatment with lapatinib and capecitabine (arrows). E: Marked shrinkage of multiple lymph node metastases induced by the second treatment with Herceptin and capecitabine following treatment with lapatinib and capecitabine (arrows).*

2C, middle and right panels) in September 2009, in parallel with the elevation of tumor markers (carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), and national cancer center-ST439 (NCC-ST439)) (Figure 3). As the patient was diagnosed as a Herceptin-failure case, lapatinib and capecitabine treatment was administered from September 2009. Following this, although the metastases in the right hilar lymph node and parasternal lymph node were defined as stable disease (SD), metastasis in the bilateral infraclavicular lymph node was defined as progressive disease (PD) in December 2009 (Figure 2D). Thereafter, a second round of Herceptin and capecitabine treatment was administered from December 2009. Of interest, 6 months after this second treatment, all of the lymph node metastases showed marked shrinkage (Figure 2E), along with negative tumor markers (CEA, CA15-3, and NCC-ST439)(Figure 3). Thus, the clinical course of this case strongly suggests that lapatinib has the potential to convert HER2-positive Herceptin-refractory breast tumor to Herceptin-sensitive tumors.

Discussion

The present report provides novel and clinically important findings relevant to Herceptin and lapatinib treatments for HER2-positive breast cancer. Firstly, lapatinib induced the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines, resulting in the enhancement of Herceptin-mediated ADCC, as evaluated by an *in vitro* assay. Secondly, the case report strongly suggests that lapatinib has the potential to convert HER2-positive Herceptin-refractory

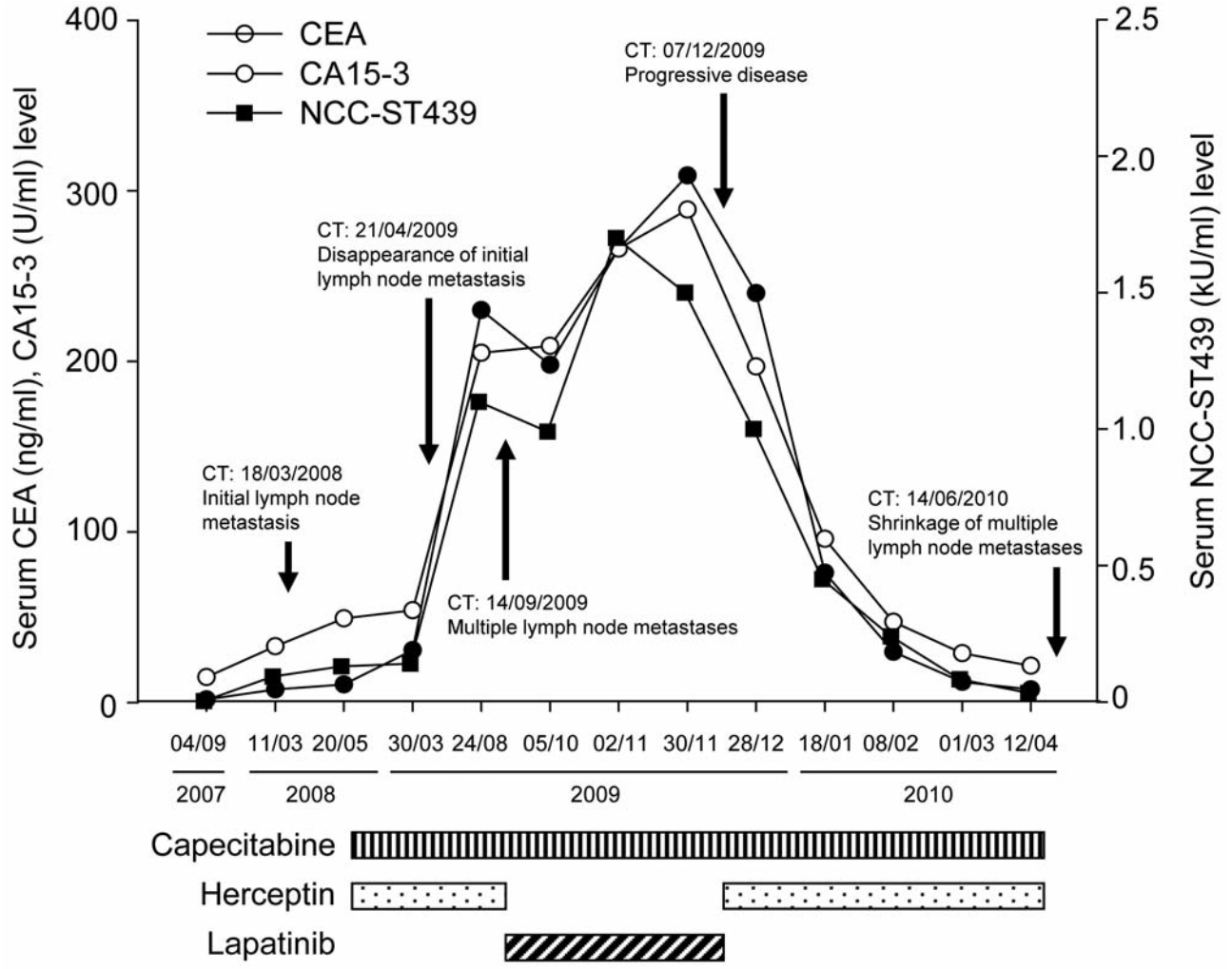

Figure 3. *Clinical course of a breast cancer patient with lymph node metastases. Serum carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), and national cancer center-ST439 (NCC-ST439) levels are shown according to the clinical course on treatment with Herceptin (4 mg/kg initially followed by 2 mg/kg, weekly), lapatinib (1,250 mg/day, every day), and capecitabine (2,400 mg/day, 3 weeks on, 1 week off).*

breast tumors to Herceptin-sensitive tumors. Taken together, these findings show lapatinib might be able to enhance the Herceptin-sensitivity of HER2-positive breast cancer by up-regulation of the cell surface expression of HER2.

In pre-clinical models, lapatinib combined with Herceptin was superior to single-drug treatment, and enhanced apoptosis as a result of the synergistic inhibitory effect of HER2 signaling was proposed as a mechanism in HER2-positive breast cancer cells (16, 25). Furthermore, in a clinical model, it was recently reported that lapatinib combined with Herceptin significantly improved the progression-free survival rate and clinical benefit compared to lapatinib alone in patients with HER2-positive metastatic Herceptin-refractory breast cancer (15). These data provide a rationale to use lapatinib in combination with Herceptin for patients with HER2-positive breast cancer. In addition, we put forward a novel mechanism in the present study that lapatinib induced the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines, resulting in the enhancement of Herceptin-mediated ADCC. There is very limited information describing the cell surface expression of HER2 induced by lapatinib, whereby the combination of the two agents triggered complete tumor regression in a HER2-positive xenograft model through enhanced Herceptin-mediated ADCC with HER2 accumulation at the cell surface due to lapatinib (18).

In the clinical course of the case report described here, since we did not use lapatinib and Herceptin in the same period and Herceptin-refractory tumors were converted to Herceptin-sensitive tumors following lapatinib treatment

alone, there would appear to be no synergistic effects, such as enhanced apoptosis, induced by the two agents. Rather lapatinib might alter tumor sensitivity to Herceptin, by inducing the accumulation of HER2 at the cell surface, resulting in the enhancement of Herceptin-mediated ADCC. In addition to the present study in breast cancer, we recently reported that lapatinib had a similar effect on HER2 receptors and Herceptin-mediated ADCC in esophageal squamous cell carcinoma (19). Thus, the enhanced cell surface expression of HER2 induced by lapatinib might be a general phenomenon among different types of HER2-positive tumor.

Most of the recent reports on pre-clinical and clinical models of HER2-positive breast cancer showed the clinical benefit of the simultaneous treatment of lapatinib with Herceptin, mainly aiming at the synergistic action of the antiproliferative or proapoptotic effects induced by the two drugs (15, 16, 18, 25). In the present study, we propose a novel mechanism in which lapatinib induces the accumulation of HER2 at the cell surface of HER2-positive breast cancer cell lines, resulting in the enhancement of Herceptin-mediated ADCC.

References

1 Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U, Levinson A and Ullrich A: Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with *neu* oncogene. Science *230*: 1132-1139, 1985.

2 Seliger B, Rongcun Y, Atkins D, Hammers S, Huber C, Storkel S and Kiessling R: HER-2/neu is expressed in human renal cell carcinoma at heterogeneous levels independently of tumor grading and staging and can be recognized by HLA-A2.1-restricted cytotoxic T lymphocytes. Int J Cancer *87*: 349-359, 2000.

3 Mimura K, Kono K, Hanawa M, Mitsui F, Sugai H, Miyagawa N, Ooi A and Fujii H: Frequencies of HER-2/neu expression and gene amplification in patients with oesophageal squamous cell carcinoma. Br J Cancer *92*: 1253-1260, 2005.

4 Al-Kuraya K, Novotny H, Bavi P, Siraj AK, Uddin S, Ezzat A, Sanea NA, Al-Dayel F, Al-Mana H, Sheikh SS, Mirlacher M, Tapia C, Simon R, Sauter G, Terracciano L and Tornillo L: *HER2, TOP2A, CCND1, EGFR* and *C-MYC* oncogene amplification in colorectal cancer. J Clin Pathol *60*: 768-772, 2007.

5 Takehana T, Kunitomo K, Kono K, Kitahara F, Iizuka H, Matsumoto Y, Fujino MA and Ooi A: Status of c-erbB-2 in gastric adenocarcinoma: a comparative study of immunohistochemistry, fluorescence *in situ* hybridization and enzyme-linked immunosorbent assay. Int J Cancer *98*: 833-837, 2002.

6 Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A and Press MF: Studies of the *HER-2/neu* proto-oncogene in human breast and ovarian cancer. Science *244*: 707-712, 1989.

7 Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM and Fox JA: Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin Oncol *26*: 60-70, 1999.

8 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J and Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med *344*: 783-792, 2001.

9 Izumi Y, Xu L, di Tomaso E, Fukumura D and Jain RK: Tumour biology: herceptin acts as an anti-angiogenic cocktail. Nature *416*: 279-280, 2002.

10 Cuello M, Ettenberg SA, Clark AS, Keane MM, Posner RH, Nau MM, Dennis PA and Lipkowitz S: Down-regulation of the erbB-2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. Cancer Res *61*: 4892-4900, 2001.

11 Clynes RA, Towers TL, Presta LG and Ravetch JV: Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. Nat Med *6*: 443-446, 2000.

12 Kono K, Takahashi A, Ichihara F, Sugai H, Fujii H and Matsumoto Y: Impaired antibody-dependent cellular cytotoxicity mediated by Herceptin in patients with gastric cancer. Cancer Res *62*: 5813-5817, 2002.

13 Mimura K, Kono K, Hanawa M, Kanzaki M, Nakao A, Ooi A and Fujii H: Trastuzumab-mediated antibody-dependent cellular cytotoxicity against esophageal squamous cell carcinoma. Clin Cancer Res *11*: 4898-4904, 2005.

14 Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH, Ellis B, Pennisi C, Horne E, Lackey K, Alligood KJ, Rusnak DW, Gilmer TM and Shewchuk L: A unique structure for epidermal growth factor receptor bound to GW572016 (lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res *64*: 6652-6659, 2004.

15 Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J and O'Shaughnessy J: Randomized study of lapatinib alone or in combination with trastuzumab in women with ERBB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol *28*: 1124-1130.

16 Konecny GE, Pegram MD, Venkatesan N, Finn R, Yang G, Rahmeh M, Untch M, Rusnak DW, Spehar G, Mullin RJ, Keith BR, Gilmer TM, Berger M, Podratz KC and Slamon DJ: Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res *66*: 1630-1639, 2006.

17 Zhou Y, Li S, Hu YP, Wang J, Hauser J, Conway AN, Vinci MA, Humphrey L, Zborowska E, Willson JK and Brattain MG: Blockade of EGFR and ERBB2 by the novel dual EGFR and ErbB2 tyrosine kinase inhibitor GW572016 sensitizes human colon carcinoma GEO cells to apoptosis. Cancer Res *66*: 404-411, 2006.

18 Scaltriti M, Verma C, Guzman M, Jimenez J, Parra JL, Pedersen K, Smith DJ, Landolfi S, Ramon y Cajal S, Arribas J and Baselga J: Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. Oncogene *28*: 803-814, 2009.

19 Mimura K, Kono K, Maruyama T, Watanabe M, Izawa S, Shiba S, Mizukami Y, Kawaguchi Y, Inoue M, Kono T, Choudhury A, Kiessling R and Fujii H: Lapatinib inhibits receptor phosphorylation and cell growth and enhances antibody-dependent cellular cytoxicity (ADCC) of EGFR and HER2 overexpressing esophageal cancer cell lines. Int J Cancer: 8 APR 2011, DOI: 10.1002/ijc.25896.

20 Johnston S, Trudeau M, Kaufman B, Boussen H, Blackwell K, LoRusso P, Lombardi DP, Ben Ahmed S, Citrin DL, DeSilvio ML, Harris J, Westlund RE, Salazar V, Zaks TZ and Spector NL: Phase II study of predictive biomarker profiles for response targeting human epidermal growth factor receptor 2 (HER-2) in advanced inflammatory breast cancer with lapatinib monotherapy. J Clin Oncol *26*: 1066-1072, 2008.

21 Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S and Cameron D: Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med *355*: 2733-2743, 2006.

22 Gomez HL, Doval DC, Chavez MA, Ang PC, Aziz Z, Nag S, Ng C, Franco SX, Chow LW, Arbushites MC, Casey MA, Berger MS, Stein SH and Sledge GW: Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol *26*: 2999-3005, 2008.

23 Di Leo A, Gomez HL, Aziz Z, Zvirbule Z, Bines J, Arbushites MC, Guerrera SF, Koehler M, Oliva C, Stein SH, Williams LS, Dering J, Finn RS and Press MF: Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. J Clin Oncol *26*: 5544-5552, 2008.

24 Ando T, Mimura K, Johansson CC, Hanson MG, Mougiakakos D, Larsson C, Martins da Palma T, Sakurai D, Norell H, Li M, Nishimura MI and Kiessling R: Transduction with the antioxidant enzyme catalase protects human T-cells against oxidative stress. J Immunol *181*: 8382-8390, 2008.

25 Xia W, Gerard CM, Liu L, Baudson NM, Ory TL and Spector NL: Combining lapatinib (GW572016), a small molecule inhibitor of ERBB1 and ERBB2 tyrosine kinases, with therapeutic anti-ERBB2 antibodies enhances apoptosis of ERBB2-overexpressing breast cancer cells. Oncogene *24*: 6213-6221, 2005.

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