—Original—

Paclitaxel Enhances Antibody-dependent Cell-mediated Cytotoxicity of Trastuzumab by Rapid Recruitment of Natural Killer Cells in HER2-positive Breast Cancer

Daishu Miura[1,2], Kimiyasu Yoneyama[3], Yoshiaki Furuhata[4] and Kazuo Shimizu[1]

[1]Department of Endocrine Surgery, Nippon Medical School Hospital
[2]Department of Breast and Endocrine Surgery, Toranomon Hospital
[3]Department of Breast Surgery, National Cancer Center East
[4]Department of Surgery, Japanese Red Cross Medical Center

Abstract

Introduction: An important mechanism by which trastuzumab inhibits the growth of human epidermal growth factor receptor 2 (HER2)-positive breast cancer cells is the activation of a host tumor response via antibody-dependent cell-mediated cytotoxicity (ADCC). Although paclitaxel has a synergistic effect in combination with trastuzumab, whether ADCC is enhanced by paclitaxel is not known. In the present study we examined whether adding paclitaxel to trastuzumab enhances ADCC and also investigated the kinetics of effector cells in ADCC.

Materials and Methods: The subjects were 20 patients with HER2-positive breast cancer: 9 received the combination of trastuzumab (4 mg/kg as a loading dose and 2 mg/kg weekly) and paclitaxel (80 mg/m^2 weekly) and 19 received monotherapy with trastuzumab. In blood samples (mononuclear cells) obtained before and 10 minutes after administration of chemotherapy, ADCC and the number of effector cells, including natural killer (NK) cells, monocytes, and CD64+ cells, were compared in each case. The ADCC was analyzed with a ^{51}Cr releasing assay using the SK-BR-3 cell line, and the fractions of NK cells (both CD16+ [FcγRIII] and CD56+) and CD64+ (FcγRI) cells were analyzed with flow cytometry.

Results: The mean ADCC level increased 20% after trastuzumab monotherapy and 126% (p<0.05) after combination therapy with trastuzumab and paclitaxel. All 9 patients receiving combination therapy had increased ADCC levels. The number of NK cells increased 51% after trastuzumab monotherapy and 112% (p<0.05) after combination therapy. No significant changes were found in monocytes (39% increase) or CD64+ cells (53% increase) after trastuzumab monotherapy, but monocytes decreased 40% (p<0.05) and CD64+ cells decreased 24% after combination therapy.

Conclusions: Adding paclitaxel to trastuzumab significantly enhances ADCC, with levels twice as great as with trastuzumab monotherapy, through a rapid recruitment of NK cells. This finding suggests that the combination of trastuzumab and paclitaxel has a stronger-than-expected synergistic effect in HER2-positive breast cancer.
(J Nippon Med Sch 2014; 81: 211–220)

Key words: antibody-dependent cell-mediated cytotoxicity, human epidermal growth factor receptor 2, natural killer cells, Fcγ receptors, trastuzumab

Correspondence to Daishu Miura, MD, Department of Breast and Endocrine Surgery, Toranomon Hospital, 2–2–2 Toranomon, Minato-ku, Tokyo 105–8470, Japan
E-mail: daishu@marine.email.ne.jp
Journal Website (http://www.nms.ac.jp/jnms/)

Introduction

The humanized human epidermal growth factor receptor 2 (HER2)/*neu* immunoglobulin G (IgG)-1 monoclonal antibody trastuzumab is an effective treatment for HER2/*neu*-positive breast cancer. Trastuzumab is also effective as a single agent and when combined with cytotoxic agents[1,2]. Moreover, several randomized clinical studies have shown that patients who are treated concurrently with trastuzumab and cytotoxic agents tend to have longer disease-free survival (DFS) than do patients treated sequentially[1–3].

The major mechanism of action of trastuzumab is believed to be the abrogation of intracellular HER2 signaling through pathways, including PI3K/Akt and Ras/mitogen-activated protein kinase, leading to cell-cycle arrest, reduction in angiogenesis, inhibition of extracellular domain cleavage, and antibody-dependent cellular cytotoxicity (ADCC)[4–7]. The mechanism of action of trastuzumab demonstrated *in vitro* in HER2-overexpressing cells has not been consistently confirmed in *in vivo* studies[8]. ADCC is effectively triggered upon ligation of Fc receptors, either via IgG or Fcγ-receptor antibodies. Three classes of Fc receptors for IgG have been identified: FcγRI CD64, FcγRII CD32, and FcγRIII CD16. Because the Fc portion of trastuzumab is a human IgG1 type, trastuzumab binds both to HER2-expressing cells via the Fab portion and to cells that express Fc receptors, such as human peripheral blood mononuclear cells (PBMCs). The ADCC is mediated by natural killer (NK) cells via cell-surface FcγRIII, initiating a sequence of cellular events culminating in the release of cytotoxic granules containing perforin and granzymes[5]. Important ADCC-mediating effector cells that express receptors for the Fc portion of IgG include monocytes that primarily express FcγRI and II, granulocytes expressing FcγRI and II, and NK cells expressing FcγRIII. Of the various effector cells against HER2-overexpressing cell lines, NK cells display the highest cytotoxicity in trastuzumab-mediated ADCC[9,10].

Several studies have suggested that ADCC is an important mechanism for trastuzumab-induced responses *in vivo* and that ADCC through FcγRIII, as is present on NK cells, is a main cytotoxic effect for trastuzumab-coated HER2-overexpressing cells[4,6,11–14]. Two *in vivo* pilot studies have evaluated the potential role of ADCC in the mechanism(s) of action of trastuzumab. Repka et al.[13] have demonstrated that treatment with a combination of trastuzumab and interleukin 2 leads to NK-cell expansion and NK cell-mediated ADCC against HER2-overexpressing cells. Additionally, they have shown that serum from treated patients retains residual ADCC 2 to 8 weeks after the last trastuzumab injection. Gennari et al.[12] have shown that PBMCs of trastuzumab-treated patients have cytopathic activity against HER2-overexpressing cells *in vitro*, with ADCC more pronounced in tumors demonstrating a good response to treatment than in those exhibiting a poor response. Tsavaris et al.[14] have reported that treatment with taxanes, especially docetaxel, leads to increased serum concentrations of several cytokines and enhancement of NK-cell activity 4 weeks after treatment.

We examined the mechanism of ADCC associated with effector cells in trastuzumab monotherapy and in combination therapy with trastuzumab and paclitaxel. The study had 2 primary aims. The first was to determine whether the combination of trastuzumab and paclitaxel enhances ADCC with PBMCs. If so, when does the synergistic effect occur? The second aim was to determine the rapid kinetics of PBMCs following trastuzumab monotherapy and trastuzumab-plus-paclitaxel combination therapy.

Materials and Methods

Patients

From 2004 through 2006, 20 consecutive patients with HER2-positive breast cancer who received the following treatments and signed an informed consent for research form were enrolled in the study, which was approved by the institutional review board. Seven patients were receiving neoadjuvant treatment, and 13 had metastatic

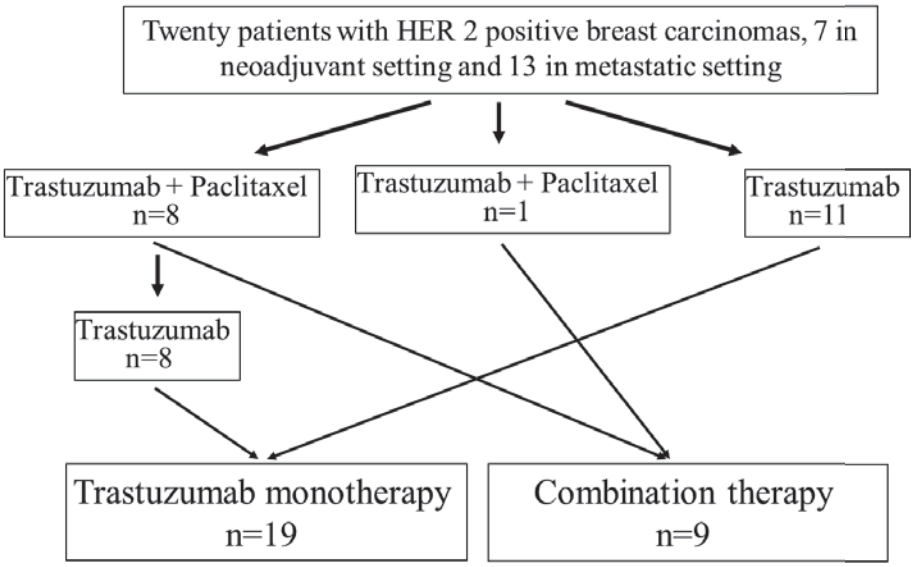

Fig. 1 Patient flow chart. Of 20 patients with HER2-positive breast cancer, 19 patients received trastuzumab monotherapy and 9 patients received combination therapy.

disease. Eight patients received trastuzumab plus paclitaxel for 3 to 4 months followed by trastuzumab alone, 1 patient received combination treatment with trastuzumab plus paclitaxel, and 11 patients received trastuzumab alone. We thus examined 19 patients who received trastuzumab and 9 patients who received trastuzumab plus paclitaxel **(Fig. 1)**.

Immunohistochemistry and Fluorescence In-situ Hybridization

The following inclusion criterion was used: HER2-positive breast cancer confirmed with 3+ immunohistochemical staining or with fluorescence in-situ hybridization (FISH). Patients meeting this criterion were treated with trastuzumab monotherapy or with combination therapy with trastuzumab and paclitaxel. Positivity for HER2 was determined according to the American Society of Clinical Oncology/College of American Pathologists guidelines. Immunohistochemical studies were performed with a polyclonal antibody kit (Herceptest, Dako, Glostrup, Denmark) by the Department of Pathology, Toranomon Hospital, according to the manufacturer's instructions. Standard scoring of 3+ staining was considered positive for HER2 overexpression. FISH was performed with the PathVysion HER2 DNA FISH kit (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions. For each case, at least 20 nonoverlapping invasive cancer nuclei were scored for centromere enumeration probe (CEP) 17 and HER2 signals. A *HER*2 to CEP17 ratio >2.2 was also considered to indicate HER2 overexpression.

Treatment

Trastuzumab was administered at a loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg infused over 90 minutes thereafter. Paclitaxel was administered at 80 mg/m^2 for 1 hour with 6 mg of dexamethasone every week. Peripheral blood samples were collected before and 10 minutes after administration.

ADCC Assay

From samples of whole blood collected before and 10 minutes after the completion of the chemotherapy infusion, PBMCs were isolated by centrifugation over a discontinuous Ficoll gradient and then adjusted to a density of 5×10^6 cells/mL. A set of blood samples (pretreatment and posttreatment) was collected from a patient once for each regimen. Target cells (SK-BR-3; a HER2-overexpressing breast cancer cell line) were labeled with 50 μCi of ^{51}Cr for 2 hours. After extensive washing with RF10+ medium, the target cells were

adjusted to a density of 1×10^5 cells/mL. Mononuclear cells (effector cell/target cell [E/T] ratio=25 and 50), 1.0 μg/mL of trastuzumab (10 μL), and target cells were then added to the wells of microtiter plates. After the preliminary experiment of a trastuzumab-mediated ADCC assay with E/T ratios of 6.25, 12.5, 25, 50, and 100, we decided that ratios of 25 and 50 were appropriate for 1.0 μg/mL of trastuzumab. After incubation at 37℃ for 4 hours, ^{51}Cr release was measured in 150 μL of supernatant from triplicate samples. Release of ^{51}Cr was measured as counts per minute (cpm). Cytotoxicity is expressed as a percentage calculated according to the following formula: specific lysis (%)=experimental cpm (trastuzumab+) − spontaneous release cpm (trastuzumab−)/maximum release cpm (whole target cell lyses)−spontaneous cpm (trastuzumab−). We compared the cytotoxicity of samples obtained before and 10 minutes after administration in each patient.

Effector Cells

Effector cells were obtained from patients at times different from those when cells were obtained for the ADCC study. Blood samples were collected before and 10 minutes after drug administration in the same manner as for the ADCC assay. Monocytes and CD64+ cells were measured with forward-scatter/side-scatter gated flow cytometry. The NK cells were measured with CD16/CD56 gated flow cytometry. Because CD64 is found on both monocytes and neutrophils, there was some overlap with respect to the population of CD64+ cells. We analyzed both cell types because monocytes bearing the high-affinity CD64 antigen also contribute to ADCC. We compared the number of effector cells obtained before and 10 minutes after drug administration in each patient.

Statistical Analysis

All statistical analyses were performed with the SPSS statistical software package (SPSS Inc., Chicago, IL, USA). Comparisons between pretreatment and posttreatment and the mean percent changes in ADCC and effector cell numbers were analyzed by means of the paired *t*-test and the *t*-test for 1 sample (two-sided), respectively. The correlation between percent ADCC and number of NK cells was assessed with Pearson's correlation. A *p*-value of <0.05 was considered to indicate statistical significance.

Results

Figure 2 shows the changes in ADCC in the 19 patients who received trastuzumab monotherapy. Some degree of ADCC enhancement was observed in 14 of the 19 patients after trastuzumab monotherapy. We found significant increases in posttreatment ADCC at an E/T cell ratio of 50 (p=0.015), but found no significant change at an E/T ratio of 25 (p=0.186). Although the degree of significance at E/T ratios of 25 and 50 was discordant, the results were consistent for the 14 patients with enhanced ADCC responses. The mean percentage change in ADCC after trastuzumab monotherapy was not significant at an E/T ratio of 25 (20%; 95% confidence interval [CI], −0.8% to 41.0%; p=0.059) or at an E/T ratio of 50 (23%).

Figure 3 shows the change in ADCC in the 9 patients who received combination therapy with trastuzumab and paclitaxel. All patients showed enhanced cytotoxicity after administration. The ADCC increased significantly at E/T ratios of both 25 (p=0.005) and 50 (p=0.002). The mean increase in ADCC after combination therapy with trastuzumab and paclitaxel was 126% (95% CI, 2.1%−243.1%) and represents a significant increase (p=0.049). This percentage was 1.9 times as high as that after trastuzumab monotherapy, when ADCC was 20% higher than that before treatment. At an E/T ratio of 50, the mean increase in posttreatment ADCC was 52% (p=0.002).

Figure 4 shows the changes in the number of NK cells with treatment. After trastuzumab monotherapy, the number of NK cells increased in 14 of 19 patients. The number of cells increased significantly after treatment (p=0.012), with the mean percentage increase in the number of NK cells after treatment being 51% (p=0.002, 95% CI, 21.2%−79.9%). All 9 patients who received trastuzumab-plus-paclitaxel combination therapy had pronounced and

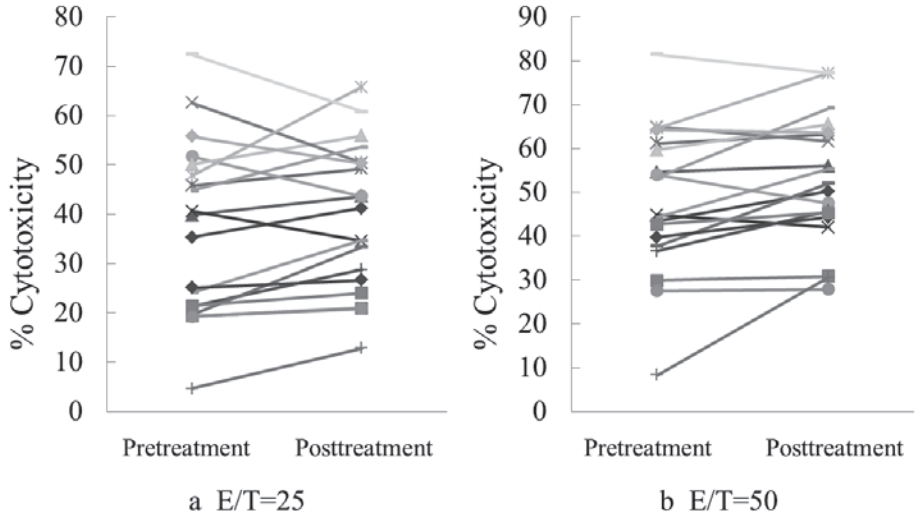

Fig. 2 Changes in ADCC after trastuzumab monotherapy. The change in ADCC after treatment was not significant at (a) an E/T ratio of 25 (p=0.186) but was significant at (b) an E/T ratio of 50 (p=0.015).

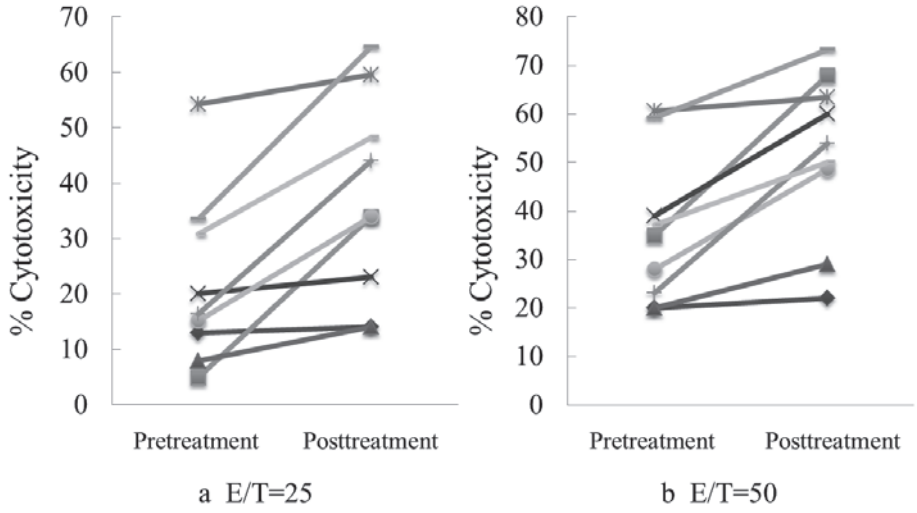

Fig. 3 Changes in ADCC after combination therapy with trastuzumab and paclitaxel. Changes in ADCC after treatment were significant (a) at an E/T ratio of 25 (p=0.005) and (b) at an E/T ratio of 50 (p=0.002).

statistically significant increases in the number of NK cells after administration (p=0.004); the mean percentage increase in number of cells after treatment was 112% (p=0.007; 95% CI, 40.9%–182.7%).

Figure 5 shows the changes in the number of monocytes with treatment. Trastuzumab monotherapy did not significantly affect the number of monocytes (p=0.256), with the mean percentage increase being 39% (p=0.131; 95% CI, −12.8% to 91.2%). However, the number of monocytes decreased significantly after combination therapy with trastuzumab and paclitaxel (p=0.015); the mean percentage change in the number of monocytes after treatment was −40% (p=0.026; 95% CI, −74.4% to −6.2%).

Figure 6 shows the changes in the number of CD 64+ cells with treatment. The number of CD64+ cells did not change significantly (p=0.325) after trastuzumab monotherapy (mean percentage change, 53%; 95% CI, −7.9% to 114.4%; p=0.084). Furthermore, combination therapy with trastuzumab and paclitaxel also had no significant effect (p=0.080) on the number of CD64+ cells (mean

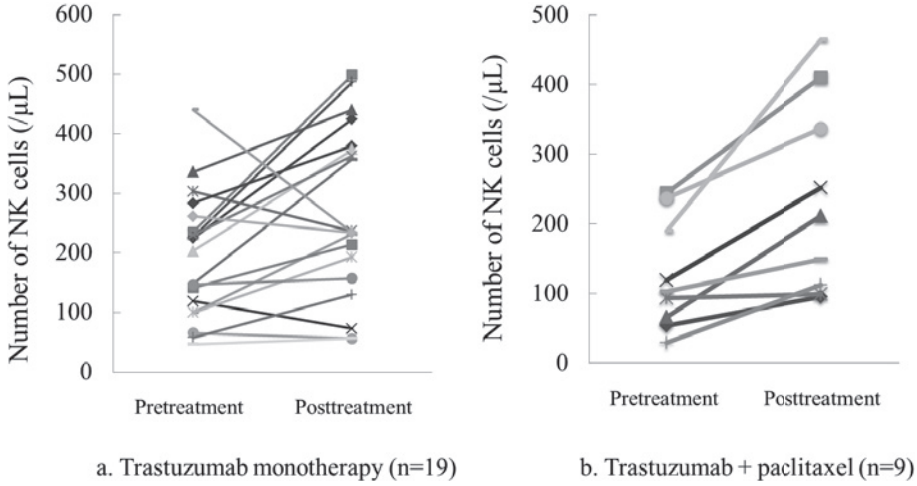

Fig. 4 Changes in the number of natural killer (NK) cells in each patient. The number of NK cells increased significantly after (a) trastuzumab monotherapy (p=0.012) and (b) combination therapy with trastuzumab and paclitaxel (p=0.004).

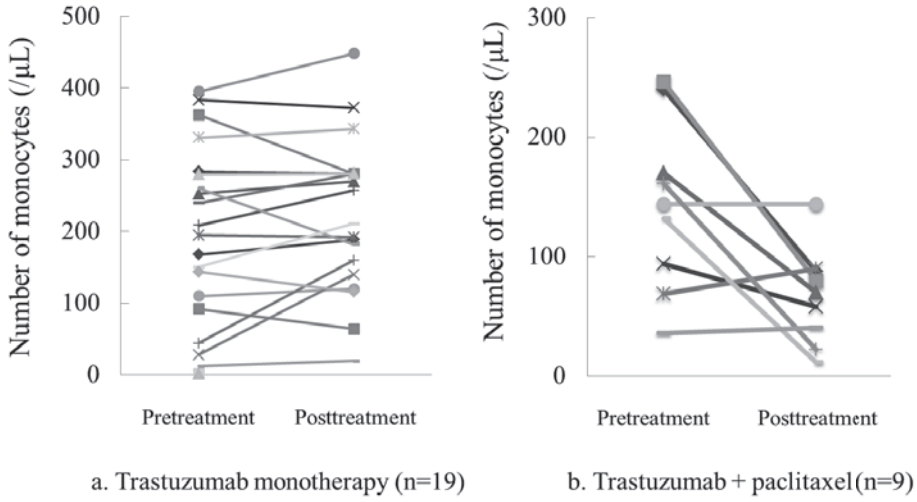

Fig. 5 Changes in the number of monocytes in each patient. The number of monocytes did not change significantly after (a) trastuzumab monotherapy (p=0.256) but decreased significantly after (b) trastuzumab and paclitaxel combination therapy (p=0.015).

percentage change, −24%; 95% CI, −50.3% to 1.5%; p=0.061).

We found marked changes in *in vitro* ADCC and the number of effector cells after combination therapy with trastuzumab and paclitaxel. The number of NK cells was rapidly increased both by trastuzumab alone and by trastuzumab plus paclitaxel. Both the ADCC associated with PBMCs and the number of NK cells detected after combination therapy with trastuzumab and paclitaxel more than doubled within a few hours. These results are summarized in **Table 1**.

We also investigated the relationship between ADCC and the number of NK cells (**Fig. 7**). A positive correlation between them was found (p= 0.002; Pearson's correlation coefficient=0.41).

Discussion

Trastuzumab is a humanized monoclonal antibody against HER2, which is amplified or overexpressed or both in 15% to 20% of invasive breast cancers[15–18].

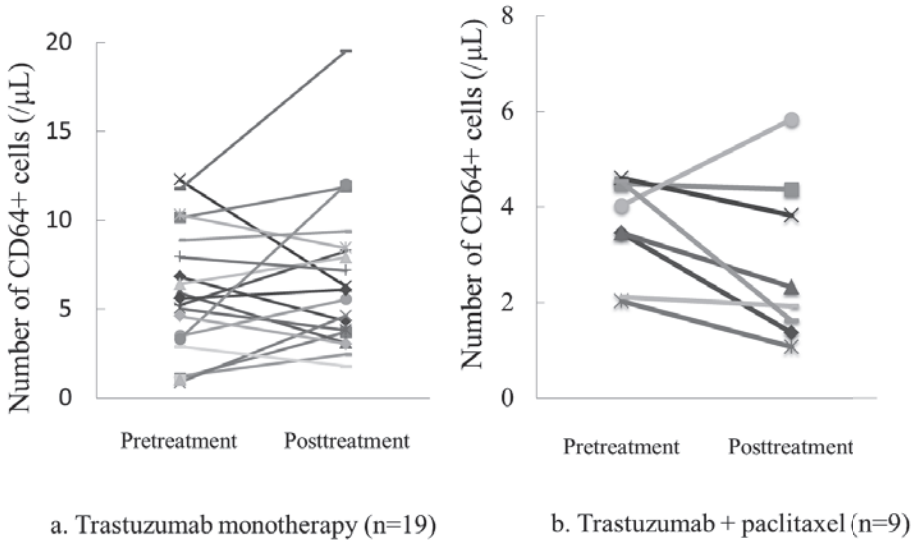

Fig. 6 Change in the number of CD64+ cells in each patient. The number of CD64+ cells did not change significantly after (a) trastuzumab monotherapy (p=0.325) or after (b) trastuzumab and paclitaxel combination therapy (p=0.080).

Table 1 Changes in ADCC and the number of NK cells, monocytes, and CD64+ cells after drug administration

	ADCC change (p, paired t test)	NK cell change (p, paired t test)	Monocyte change (p, paired t test)	CD64+ change (p, paired t test)
Trastuzumab monotherapy	↑ +20% (p=0.186 E/T25) (p=0.015 E/T50)	↑ +51% (p=0.012)	→ (p=0.256)	→ (p=0.325)
Combination of trastuzumab + paclitaxel	↑↑ +126% (p=0.005)	↑↑ +112% (p=0.004)	↓ −40% (p=0.015)	→ (p=0.080)

Breast tumors positive for HER2 are more aggressive and more susceptible to recurrence than are HER2-negative tumors[17,19]. Our results show that concomitant use of trastuzumab and paclitaxel significantly enhances ADCC relative to the pretreatment level via a rapid increase in the number of NK cells. Both ADCC (226% of baseline) and the number of NK cells (212% of baseline) doubled in our study. A positive correlation was observed between ADCC and the number of NK cells, which also increased after combination therapy. These rapid phenomena were observed within just 3 hours after the start of the chemotherapy infusion. We also found that combination therapy led to significantly greater increases in ADCC and the number of NK cells than with trastuzumab monotherapy.

Our findings are supported by the results of the North Central Cancer Treatment Group N9831[1,20] phase III randomized study of doxorubicin plus cyclophosphamide followed by paclitaxel with or without trastuzumab in women with HER2-overexpressing node-positive or high-risk node-negative breast cancer. A study comparing sequential trastuzumab (n=954) and concurrent trastuzumab (n=949) with a 6-year median follow-up and 313 events reported 5-year DFS rates of 80.1% and 84.4%, respectively. The DFS rate with concurrent trastuzumab and paclitaxel was higher than that with sequential administration (hazard ratio, 0.77), but the p value (.02) did not cross the prespecified O'Brien-Fleming boundary (.00116) for the interim analysis of the N9831 trial[1]. The DFS curves of sequential trastuzumab and of concurrent

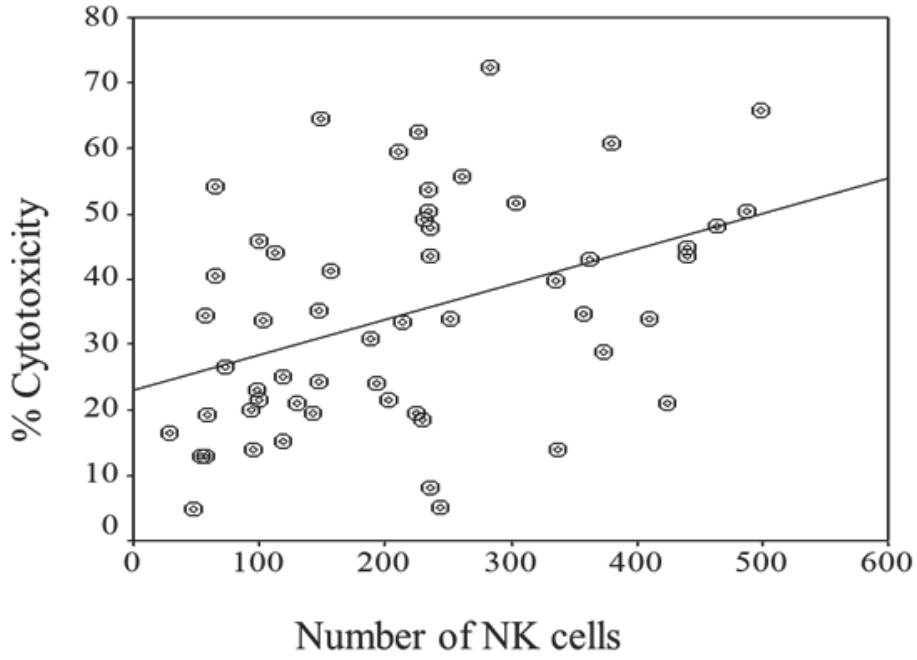

Fig. 7 Correlation between ADCC and the number of NK cells. Percent cytotoxicity was significantly correlated the number of NK cells (p=0.002; Pearson's correlation coefficient=0.41).

trastuzumab diverged gradually until 4 years after random assignment and then were nearly parallel after 4 years, suggesting that the efficacy of concurrent trastuzumab does not persist for an extended time.

An interesting assessment of clinical and immunological responses in 26 patients receiving trastuzumab monotherapy as maintenance therapy after chemotherapy was performed by Alessandra et al.[21] Both NK cell activity and ADCC (p<0.05) in 17 responders were significantly higher than those in 9 nonresponders but did not differ from those in 11 healthy control subjects. The NK cell activity of nonresponders was significantly (p<0.05) lower than that of the healthy control subjects. Although progression-free survival and NK cell activity were correlated, time to tumor progression was not correlated with the ADCC. The authors concluded that NK cell-mediated ADCC-induced lysis is correlated with the short-term response to treatment, whereas longer protection against tumor expansion seems to be mediated solely by NK activity.

In the present study, ADCC increased significantly after treatment, irrespective of the significant decrease in the number of monocytes. Our findings suggest that the number of NK cells is positively correlated with ADCC, as previous study described[6]. Clynes et al.[5] inoculated knockout mice lacking activating FcγRIII receptors with trastuzumab-sensitive BT-474 cells. In this model system, the antitumor activity of trastuzumab was reduced by about 75% but was not abolished. We believe that the pronounced increase in the number of NK cells may complement ADCC rather than decrease other effector cells. Little is known about the relationship between paclitaxel injection and the number of CD64+ cells; however, Kaur et al.[22] have reported that paclitaxel is associated with several immunosuppressive effects, such as decreased numbers and activity of dendritic cells, NK cells, and monocytes in patients with lung cancer in whom colonic polyps/colon cancer developed either during or immediately after chemotherapy.

Although we did not asses the correlation between clinical outcome and ADCC in this study, previous studies have provided evidence of a strong immune effector cell response before chemotherapy in the adjuvant setting[12,23]. Murine studies have shown that ADCC contributes to the antitumor

effects of trastuzumab in HER2-positive tumors *in vivo* [24,25]. In 2 neoadjuvant clinical studies, trastuzumab treatment was associated with increased tumor infiltration by NK cells[4,12]. Gennari et al.[12] have reported that patients with an objective response to trastuzumab-based treatment had higher numbers of infiltrating leukocytes and higher levels of ADCC. In addition to CD16+ CD56+ NK cells, several other potential effector immune cells might be involved in trastuzumab-enhanced ADCC[26].

We conclude that trastuzumab administration leads to enhanced ADCC in conjunction with a significant and rapid increase in the number of peripheral NK cells. The number of peripheral NK cells increased significantly (doubling relative to the pretreatment value) after combination therapy with trastuzumab and paclitaxel. The addition of paclitaxel to trastuzumab significantly enhanced ADCC. Compared with the pretreatment level and that following treatment with trastuzumab alone, the ADCC level doubled owing to combination therapy, and kinetic analysis showed a rapid increase in the number of effector cells. A significant positive correlation between trastuzumab-mediated ADCC and the number of NK cells was observed. In fact, the coefficients of these correlations were modest, and counting the number of NK cells might reflect the level of ADCC and its effectiveness in a regimen including trastuzumab. The conclusions of our study are limited by the small number of cases. However, to our knowledge this is the first report providing evidence that adding paclitaxel to trastuzumab significantly enhances ADCC through a rapid recruitment of NK cells. Our findings suggest that the combination of trastuzumab and paclitaxel has a stronger synergistic effect than might be expected in HER2-positive breast cancer.

Acknowledgement: The authors thank Yasunori Ohta, Naoko Inoshita, and Kenichi Ohhashi for pathological assessment. This study was presented at the Clinical Cancer Symposium during American Society of Clinical Oncology (ASCO) 2007 Annual Meeting.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Perez EA, Suman VJ, Davidson NE, et al.: Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. J Clin Oncol 2011; 29: 4491–4497.
2. Romond EH, Perez EA, Bryant J, et al.: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353: 1673–1684.
3. Slamon D, Eiermann W, Robert N, et al.: Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011; 365: 1273–1283.
4. Arnould L, Gelly M, Penault-Llorca F, et al.: Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer 2006; 94: 259–267.
5. Clynes RA, Towers TL, Presta LG, Ravetch JV: Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 2000; 6: 443–446.
6. Cooley S, Burns LJ, Repka T, Miller JS: Natural killer cell cytotoxicity of breast cancer targets is enhanced by two distinct mechanisms of antibody-dependent cellular cytotoxicity against LFA-3 and HER2/neu. Exp Hematol 1999; 27: 1533–1541.
7. Pohlmann PR, Mayer IA, Mernaugh R: Resistance to Trastuzumab in Breast Cancer. Clin Cancer Res 2009; 15: 7479–7491.
8. Mohsin SK, Weiss HL, Gutierrez MC, et al.: Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. J Clin Oncol 2005; 23: 2460–2468.
9. Kute T, Stehle Jr JR, Ornelles D, Walker N, Delbono O, Vaughn JP: Understanding key assay parameters that affect measurements of trastuzumab-mediated ADCC against Her2 positive breast cancer cells. Oncoimmunology 2012; 1: 810–821.
10. Stockmeyer B, Elsasser D, Dechant M, et al.: Mechanisms of G-CSF- or GM-CSF-stimulated tumor cell killing by Fc receptor-directed bispecific antibodies. J Immunol Methods 2001; 248: 103–111.
11. Collins DM, O'Donovan N, McGowan PM, O'Sullivan F, Duffy MJ, Crown J: Trastuzumab induces antibody-dependent cell-mediated cytotoxicity (ADCC) in HER-2-non-amplified breast cancer cell lines. Ann Oncol 2012; 23: 1788–1795.
12. Gennari R, Menard S, Fagnoni F, et al.: Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res 2004; 10: 5650–5655.
13. Repka T, Chiorean EG, Gay J, et al.: Trastuzumab and interleukin-2 in HER2-positive metastatic breast cancer: a pilot study. Clin Cancer Res 2003; 9: 2440–2446.
14. Tsavaris N, Kosmas C, Vadiaka M, Kanelopoulos P, Boulamatsis D: Immune changes in patients with advanced breast cancer undergoing chemotherapy with taxanes. Br J Cancer 2002; 87: 21–27.
15. Owens MA, Horten BC, Da Silva MM: HER2

amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. Clin Breast Cancer 2004; 5: 63–69.
16. Sjogren S, Inganas M, Lindgren A, Holmberg L, Bergh J: Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. J Clin Oncol 1998; 16: 462–469.
17. 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: 177–182.
18. Mieda J, Ohaki Y, Oguro T, et al.: Breast cancer with neuroendocrine differentiation detected by unique staining pattern of neoplastic cells in hercep test. J Nippon Med Sch 2004; 71: 203–208.
19. Slamon DJ, Godolphin W, Jones LA, et al.: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707–712.
20. Perez EA, Romond EH, Suman VJ, et al.: Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 2011; 29: 3366–3373.
21. Beano A, Signorino E, Evangelista A, et al.: Correlation between NK function and response to trastuzumab in metastatic breast cancer patients. J Transl Med 2008; 6: 25.
22. Kaur A, Dasanu CA: Rapidly progressive colonic dysplasia/neoplasia in a series of treated lung cancer patients: Is paclitaxel involved? J Oncol Pharm Pract 2013; 19: 82–85.
23. Mozaffari F, Lindemalm C, Choudhury A, et al.: NK-cell and T-cell functions in patients with breast cancer: effects of surgery and adjuvant chemo- and radiotherapy. Br J Cancer 2007; 97: 105–111.
24. 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: 60–70.
25. Spiridon CI, Guinn S, Vitetta ES: A comparison of the in vitro and in vivo activities of IgG and F(ab')2 fragments of a mixture of three monoclonal anti-Her-2 antibodies. Clin Cancer Res 2004; 10: 3542–3551.
26. Varchetta S, Gibelli N, Oliviero B, et al.: Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer overexpressing Her2. Cancer Res 2007; 67: 11991–11999.

(Received, November 5, 2013)
(Accepted, December 26, 2013)