HER-2/neu Status of Primary Breast Cancer and Corresponding Metastatic Sites in Patients with Advanced Breast Cancer Treated with Trastuzumab-based Therapy

D. PECTASIDES[1], A. GAGLIA[2], P. ARAPANTONI-DADIOTI[3], A. BOBOTA[4], C. VALAVANIS[3], V. KOSTOPOULOU[2], N. MYLONAKIS[2], A. KARABELIS[2], M. PECTASIDES[1] and T. ECONOMOPOULOS[1]

[1]Second Department of Internal Medicine-Propaedeutic, Oncology Section, Attikon University Hospital, Athens; [2]Second Department of Medical Oncology, [3]Department of Histopathology and [4]Department of Immunology, Metaxas Memorial Cancer Hospital, Piraeus, Greece

Abstract. *Background: The aim of this prospective study was to investigate whether there were changes in HER-2/neu status in newly-developed metastatic lesions following treatment with trastuzumab in advanced breast cancer patients overexpressing HER-2/neu. The utility of serological assays for HER-2/neu in such patients was also studied. Patients and Methods: Sixteen patients with HER-2/neu-overexpressing tumors (15 were 3+ by immunohistochemistry (IHC) and one 2+ by IHC and positive by the chromogenic in situ hybridization (CISH) test) were included in the study. Fourteen patients underwent biopsy and 2 patients fine-needle aspiration (FNA) of newly-developed metastatic lesions following trastuzumab treatment. All samples were assayed for HER-2 by IHC and by the CISH test. Serial serum HER-2/neu (S-HER-2) levels were measured prior to (baseline values) and during trastuzumab-based treatment by enzyme-linked immunosorbent assay (ELISA) (cut-off point: 10 ng/ml) in all patients. The patients were divided into 2 groups: those with "altered HER-2/neu status" and those with "conserved HER-2/neu status" in the metastatic region. Results: Six out of the 16 (37%) ("altered HER-2/neu status") newly-developed metastatic lesions lost their HER-2/neu overexpression and scored 0 or +1 by IHC or negative on the CISH test, while in the remaining cases (10/16, 62.5%) ("conserved HER-2/neu status"), the HER-2/neu status was unchanged (+3 by IHC or a positive CISH test). Baseline S-HER-2 levels were elevated in 5 out of 16 patients (3 of "altered HER-2/neu status", 2 of "conserved HER-2/neu status"). The serum HER-2 (S-HER-2) levels declined and returned within the normal ranges in all these 5 patients as a response to trastuzumab treatment. Following the disease progression, the S-HER-2 levels of the 3 patients with "altered HER-2/neu status" remained normal, while those of 2 with "conserved HER-2/neu status" increased. There was no statistically significant difference in the number of chemotherapeutic treatments or the median time of treatment with trastuzumab or chemotherapy between the 2 groups. Time to tumor progression (TTP) was significantly shorter in the "altered HER-2/neu status" patients (median TTP for "altered HER-2/neu status": 9.5 months, and for "conserved HER-2/neu status": 12 months; $p<0.001$). Conclusion: These data suggest that, for most patients with metastatic breast cancer treated with trastuzumab, the HER-2/neu expression as measured by IHC and/or CISH in newly-developed metastatic lesions was unchanged. However, a remarkable percentage of cases lost HER-2/neu overexpression. It is not clear whether this finding implies resistance or sensitivity to trastuzumab.*

Systemic therapy has been widely used in the treatment of metastatic breast cancer (MBC). Clinical trials of combination chemotherapy have demonstrated 50% to 70% rates of clinical response. Twenty to 30% of invasive breast cancers show HER-2/neu overexpression as a result of gene amplification which is usually associated with aggressive biological behavior (1). The HER-2 oncoprotein is a transmembrane receptor, belonging to the epidermal growth factor receptor (EGFR) family with tyrosine kinase activity. Several studies have shown a correlation between HER-2/neu overexpression and poor outcome (short disease-free survival (DFS) and overall survival (OS) in breast cancer patients) (2, 3).

Trastuzumab (Herceptin), a high-affinity humanized monoclonal antibody that recognizes HER-2, is a novel targeted-therapy for breast cancer overexpressing this

Correspondence to: D. Pectasides, Second Department of Internal Medicine-Propaedeutic, Oncology Section, Attikon University Hospital, Rimini 1, Haidari, Athens, Greece. Tel: 210-5831691, 210-6008610, Fax: 210-5831690, 210-6008610, e-mail: pectasid@otenet.gr

Key Words: HER-2/neu status, breast carcinoma, trastuzumab therapy, serum HER-2/neu.

receptor. In recent years, the efficacy of trastuzumab has been proven. Monotherapy with trastuzumab achieves an overall response rate (RR) of 35% when given as first-line treatment and 18% in patients previously treated with chemotherapy for MBC (4-6). A combination with chemotherapy as first-line treatment achieves an overall RR of 45% and survival benefit of approximately 5 months (7). It has also been proven that the efficacy of trastuzumab is highly dependent on the HER-2 status of the tumor (4, 7, 8).

There is evidence that the HER-2 status of a tumor remains fairly constant during metastatic spread, but most studies have focused on the comparison between primary tumors, lymph node metastases and distant metastases (9-14). It is reasonable to assume that oncogenes involved in the early stages of tumorigenesis remain stable during tumor progression. Knowledge of the HER-2/neu status of metastases could be of potential value for therapeutic decisions, particularly if it is different from the primary tumors following treatment with trastuzumab.

The prevalence of increased extracellular domain (ECD) in patients with primary breast cancer varies between 0% and 38%, whereas in metastatic disease the range is from 23% to 80% (15). Some women with HER-2/neu-negative tumors by tissue testing develop increased ECD concentrations in metastatic disease. Increased ECD has been correlated with poor outcome (OS and DFS).

To answer the question as to whether HER-2/neu is different in MBC, the HER-2/neu status of a set of primary breast cancer or metastatic lesions was compared to their corresponding distant metastases following treatment with trastuzumab. A serum assay for HER-2/neu was also studied for its ability to predict response to trastuzumab treatment during longitudinal follow-up.

Patients and Methods

Patients were selected among women with MBC, who developed accessible for biopsy or fine-needle aspirate (FNA), new recurrences ("second" biopsy) to assay for HER-2/neu status while receiving trastuzumab. Sixteen patients with MBC, having an "initial" tumor (any tissue sample obtained either from the primary breast tumor or from any metastatic site) with a HER-2/neu status either +3 by IHC (Herceptest™) or a positive CISH test, treated with trastuzumab, were included in the study. The patients were divided into 2 groups; those patients whose "second" biopsy reflected an altered HER-2/neu status ("altered HER-2/neu status") and those patients whose second biopsy retained the HER-2/neu overexpression ("conserved HER-2/neu status").

The number of chemotherapy regimens prior to trastuzumab therapy was not an exclusion criterion. The HER-2/neu status of the second biopsy was examined by IHC and the CISH test. All slides were reviewed by a second, independent pathologist. Multidirectional FNAs were obtained from 2 patients instead of second biopsy due to rapid deterioration of their performance status (PS). Serum HER-2 (S-HER-2) samples were also collected from the patients prior to (baseline levels) and during the trastuzumab treatment.

Immunohistochemistry. IHC was performed on formalin-fixed, paraffin-embedded breast cancer tissue samples. Immunostaining for HER-2/neu was performed with Herceptest™ (DAKO, Glostrup, Denmark) using an anti-human HER-2/neu polyclonal antibody (AO485) with a dilution of 1:100. A biotin-streptavidin-amplified method was then applied using 3,3'-diaminobenzidine (DAB) as the chromogen and hematoxylin as the counterstain. The prepared slides from the paraffin blocks prior to incubation with primary antibody were heat-pretreated in a 10 mM citrated buffer, pH 8.0, at 95˚C, 5 min x2, in a microwave oven. Scoring was performed, according to the manufacturer's guidelines (DAKO), from score 0 to score +3. The FNA specimens were also tested with the above-mentioned method.

Chromogenic in situ hybridization. The paraffin-embedded sections were deparaffinized in xylene and then in ethanol 3 times. This was followed by incubation of the slides in pretreatment buffer in a temperature-controlled microwave oven at 92˚C for 10 min, using a spot light FFPE reagent Kit. Then the slides were washed in phosphate-buffered saline (PBS) before incubating in Digest-All 3 (Zymed Inc., South San Francisco, CA, USA) for enzyme digestion for 15 min at 37˚C. The slides were washed with PBS and dehydrated with graded ethanol. The digoxigenin-labelled HER-2/neu probe (Zymed) was applied to each slide. The slides were coverslipped and sealed with rubber cement. The slides were denatured for 3 min at 93˚C and were then incubated at 37˚C for overnight hybridization in a moist slide box. After removing the coverslips, the slides were washed in 0.5 x standard saline citrate at 72˚C for 5 min and then rinsed in PBS and Tween 20. Zymed's CISH detection Kit was used, with DAB as the chromogen. The tissue sections were counterstained with hematoxylin, and mounted in DPX mountant (BDH). The CISH results were evaluated by counting red (HER-2/neu) signals in nuclei of tumor cells under a light microscope. One to 5 gene copies per nucleus in >50% of the cells were defined as no amplification. Low level amplification was defined as 6 to 10 signals per nucleus in >50% of cancer cells. Amplification was also defined when a large gene copy cluster in >50% of carcinoma cells or numerous (>10) gene copies were seen.

Serum HER-2 assay. Serum samples were collected before and during trastuzumab treatment (every 3 cycles and at disease progression) and assayed for S-HER-2. The blood samples were centrifuged at 4000xg for 15 min at room temperature and the serum was stored at –80˚C. The concentrations of S-HER-2 were measured by enzyme-linked immunosorbent assay (ELISA by Bender MedSystems). Serum samples were also collected from 50 healthy blood donors and the cut-off was calculated at 10 ng/ml (S-HER-2-negative <10 ng/ml, S-HER-2-positive >10 ng/ml).

An anti-S-HER-2 human monoclonal coating antibody was used, which is absorbed onto microwells. S-HER-2 present in the sample binds to antibodies absorbed to the microwells; an HRP-conjugated monoclonal anti-S-HER-2 antibody then binds to S-HER2 captured by the first antibody. Following incubation, the unbound enzyme-conjugated anti-S-HER-2 is removed during a wash step and the substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of soluble S-HER-2 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard

Table I. *The "altered HER-2/neu status" group: patients with a decrease in HER-2 score following disease progression after treatment with trastuzumab. All patients had a (–) CISH test.*

Case	"Initial" biopsy	"Second" biopsy	HER-2 score prior to trastuzumab	CISH test prior to trastuzumab	Post-trastuzumab HER-2 score	Post-trastuzumab CISH test
1	neck skin metastasis	neck skin metastasis	+3	positive	+1	negative
2	breast (biopsy from inflammatory inoperable breast)	chest wall	+3	positive	+1	negative
3	chest wall	chest wall	+3	positive	0	negative
4	breast (operable primary breast cancer)	chest wall	+2	positive	0	negative
5	chest wall	chest wall	+3	positive	0	negative
6	cervical lymph node	breast	+3	positive	+1	negative

curve was prepared from 7 S-HER-2 standard dilutions and the S-HER-2 sample concentration was determined. The sensitivity of the assay was 0.1 ng/ml. The overall intra-assay coefficient of variation was 7.2% (precision of the assay) calculated for mean S-HER-2 concentrations ranging from 7.0 to 167.0 ng/ml.

Statistical analysis. "Initial" and "second" tumors were correlated using the Wilcoxon signed ranks test. The number of chemotherapeutic regimens, the median time of chemotherapy and trastuzumab treatment administered between the two biopsies, were correlated using the Mann-Whitney test. All statistical analysis were performed using the Stata software version 8.0 (Stata Corp.,College Station, TX, USA). The time to progression (TTP) was calculated from the beginning of the trastuzumab therapy until disease progression.

Results

HER-2/neu status. All the patients were aged over 18 years. The HER-2/neu status prior to trastuzumab treatment ("initial" tumor) was examined from the primary breast cancer in 12 out of 16 patients and from metastatic sites in 4 patients (Tables I, II). All patients' "initial" tumor scored +3 for HER2/neu, apart from 1 that scored +2. This patient had a positive CISH test. In 6 patients (37.5%) ("altered HER-2/neu status") the HER-2/neu from the "second" biopsies scored 0 or +1. The patient who was CISH test-positive was included in this group (Table I). The CISH test was also performed in this group of patients and no amplification was detected. The remaining 10 out of 16 cases (62.5%) ("conserved HER-2/neu status"), including the 2 cases that had the FNA sampling, retained the HER-2 overexpression in the metastatic site (HER-2/neu +3, CISH test-positive) (Table II). The decrease in HER-2 score in "second" biopsies compared to that of the "initial" samples was statistically significant ($p=0.014$; Wilcoxon test). The patients' clinical details are shown in Tables III, IV. There was no statistical difference between the 2 groups in the number of chemotherapeutic regimens, the median time of chemotherapy or the trastuzumab treatment administered between the 2 biopsies (Table V). The median TTP for the whole group of patients was 11 months (range 4-36 months), 9.5 months (range: 2-14 months) for the "altered HER-2/neu status" group and 12 months (range: 7-36 months) for the "conserved HER-2/neu status" group. This difference was statistically significant ($p<0.001$). The corresponding Kaplan-Meier curve is shown in Figure 1.

Table II. *The "conserved HER-2/neu status" group: patients who retained +3 HER-2 score after disease progression. All patients had a (+) CISH test.*

Case	Initial biopsy	Second biopsy	HER-2 score prior to trastuzumab	Post-trastuzumab HER-2 score
1	breast	chest wall	3	3
2	breast	liver	3	3
3	breast	chest wall	3	3
4	breast	chest wall	3	3
5	breast	chest wall	3	3
6	breast	breast	3	3
7	breast	cervical lymph node	3	3
8	breast	cervical lymph node	3	3
9	breast	soft palate	3	3
10	breast	ovary	3	3

Serum HER-2. At baseline, only 5 out of the 16 patients (31.25%) (3 in the "altered HER-2/neu status" group and 2 in the "conserved HER-2/neu status" group) had elevated S-HER-2 levels (median: 42 ng/ml, range: 29, 2-188 ng/ml). In the remaining 11 patients, the serum concentrations were within the normal limits in all measurements, regardless of the response to trastuzumab. The fluctuations of S-HER-2 levels in patients of the "conserved HER-2/neu status" group followed the clinical course of disease (response and progression). In contrast, the S-HER-2 levels of the "altered HER-2/neu status" group were reduced and returned to within the normal limits in response to treatment, but remained normal at

Table III. *Clinical details of patients in the "altered HER-2/neu status" group.*

Case	Time between the 2 biopsies (years)	Duration of trastuzumab treatment until disease progression (years)	Duration of chemotherapy between the 2 biopsies (months)	Number of chemotherapeutic regimens between the 2 biopsies	Time to progression (months)	Best response to trastuzumab therapy
1	1.2	1.2	14	2	11	PR
2	3.7	1.6	14	3	8	PR
3	3.3	0.8	17	4	11	PR
4	10	2	26	4	8	PR
5	0.4	0.3	6	2	2	PD
6	0.8	0.6	7	1	14	PR

PR, partial remission; PD, progression of disease.

Table IV. *Clinical details of patients in the "conserved HER-2/neu status" group.*

Case	Time between the 2 biopsies (years)	Duration of trastuzumab treatment until disease progression (years)	Duration of chemotherapeutic treatment between the 2 biopsies (months)	Number of chemotherapeutic regimens between the 2 biopsies	Time to progression (months)	Best response to trastuzumab therapy
1	2.5	1.1	28	4	12	PR
2	5.5	1.5	30	4	14	PR
3	5	1.5	16	3	9	PR
4	10	1	12	3	7	PR
5	6.3	2	18	4	23	PR
6	0.9	0.6	11	2	7	PR
7	5.3	2	27	3	12	PR
8	4	1	13	3	9	PR
9	5.3	1	12	3	19	PR
10	8	3.2	5	1	36	PR

PR, partial remission.

disease progression. The fluctuations of S-HER-2 values in the 5 patients in response to treatment are illustrated in Table VI and Figure 2.

Discussion

The present study is, to our knowledge, the first to compare the HER-2/neu status of primary breast tumors or metastatic lesions with their corresponding distant metastasis in MBC patients treated with trastuzumab, using both IHC and CISH techniques.

Currently, HER-2 status is routinely assessed in tissues from primary breast carcinoma with the assumption that the result is also representative of metastatic lesions. In this study, 16 MBC patients treated with trastuzumab, with corresponding locoregional or distant metastases, were investigated by IHC and CISH. The majority (62.5%) of our cases showed identical HER-2/neu expression between

Table V. *Comparison of the clinical details between the two patient groups.*

	"Altered HER-2/neu status" group	"Conserved HER-2/neu status" group
Median duration of trastuzumab treatment until disease progression (years)	1.2	1.5
Median duration of chemotherapy between the 2 biopsies (months)	16	14
Median number of chemotherapeutic regimens between the 2 biopsies (months)	3	3

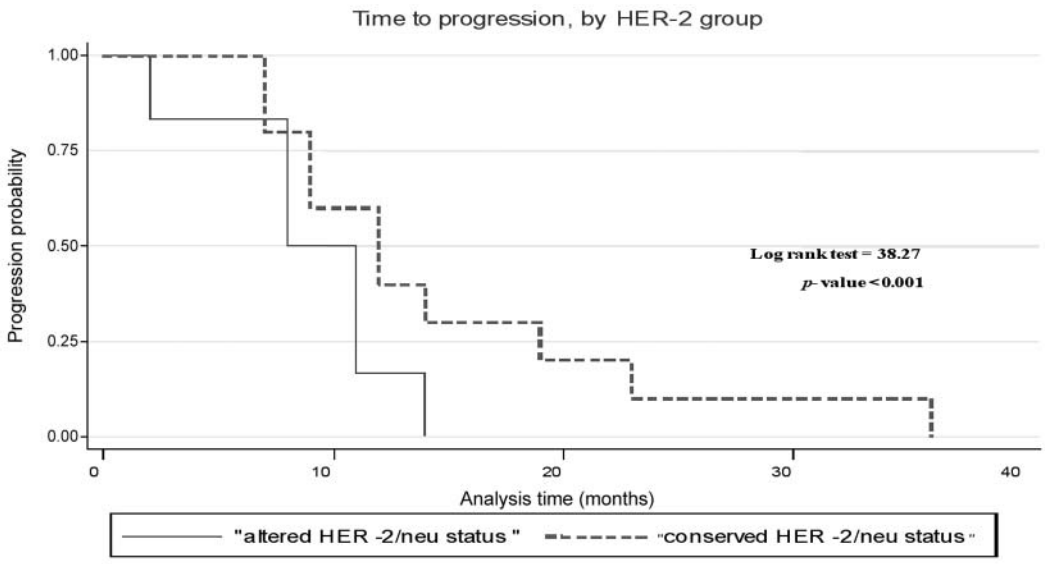

Figure 1. *Kaplan-Meier curve for time to tumor progression.*

primary breast carcinoma or metastatic lesions and their corresponding distant metastases before and after treatment with trastuzumab. Overexpression or gene amplification from the initial tumors was retained in the distant metastases following therapy with trastuzumab. This suggests that HER-2 amplification and overexpression are quite stable alterations once they have occurred. However, one could argue that the majority of biopsies (all except one) in this group of patients was obtained from the primary tumor and not from new metastatic sites. It is unknown whether the results would have been different if the samples had been obtained from the metastases. However, a subset of cases revealed loss of HER-2/neu overexpression in the distant metastases after treatment with trastuzumab in comparison with breast tumors prior to trastuzumab treatment. In this group of patients, the biopsies obtained were from metastatic sites (4 out of 6). The clinical significance of this finding is unknown. It may represent down-regulation of HER-2/neu expression following anti-HER-2 antibody exposure, as reported in preclinical models (16). It may also represent an intrinsic heterogeneity of HER-2/neu expression and tumor response or tumor sampling or testing. It is not clear whether this finding implies resistance or sensitivity to trastuzumab. This is in contrast to the current view that the HER-2/neu status of primary and metastatic breast carcinoma is identical (17-19), even in patients treated with chemotherapy (20-22).

This issue has become important in recent years due to the development and approval of trastuzumab for the treatment of HER-2/neu-overexpressing tumors. Most published studies have compared the HER-2/neu status in primary tumors with their locoregional, concurrent lymph node and distant metastases (13, 17, 18, 23). None of these studies investigated the HER-2/neu status after treatment with trastuzumab. The difficulty in obtaining biopsies from metastatic sites before and after trastuzumab therapy should be emphasized. However, it should be assumed that both types of metastases are biologically equivalent. Distant metastases may often represent clonal outgrowth with genetic alterations not detected in the primary tumors. Niehans *et al.* retrospectively assessed the HER-2/neu expression in primary breast carcinoma and different metastatic sites in 30 patients who had died from the disease (18). Eight (27%) cases were HER-2/neu-positive and, among those, there was only a single case

Table VI. *Fluctuations in S-HER-2 values during the course of disease in five patients.*

Patient	Group	Baseline S-HER-2 levels (ng/ml)	Response S-HER-2 levels (ng/ml)	Progression S-HER-2 levels (ng/ml)
1	"Altered HER-2/neu status"	188	3	2
2	"Altered HER-2/neu status"	42	5	2
3	"Altered HER-2/neu status"	36.2	-	3
4	"Conserved HER-2/neu status"	60	10	192
5	"Conserved HER-2/neu status"	29.2	0.5	26

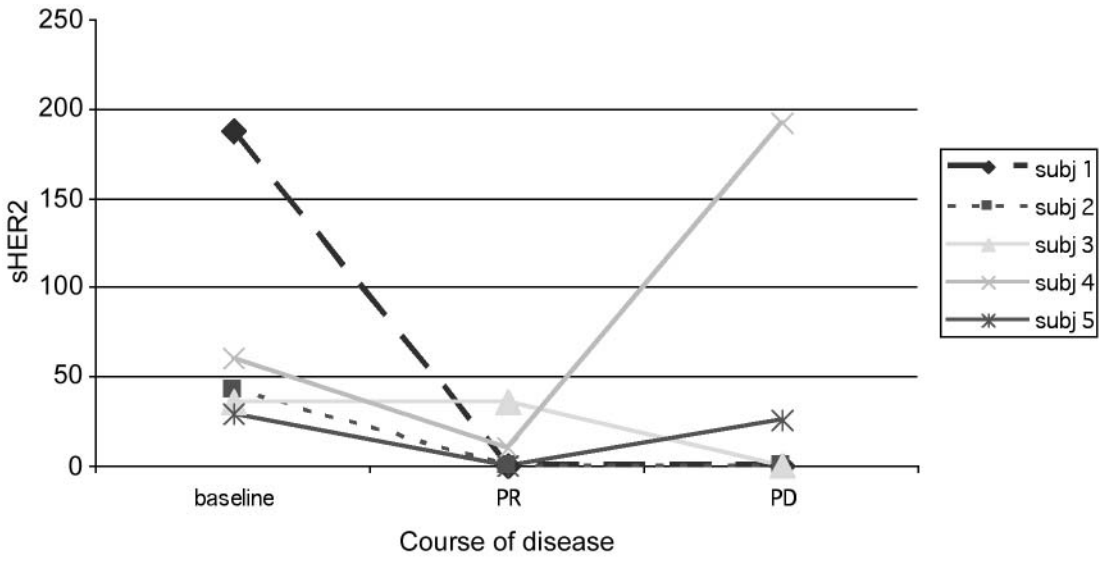

Figure 2. *Fluctuations in S-HER-2 values in response to treatment.*

of discordant staining (3%) between the primary site and 2 of its metastatic lesions. Shimizu *et al.* evaluated HER-2/neu protein levels by IHC in primary and metastatic breast cancer samples from 21 patients (13). Only 7 of the metastatic samples were from distant metastases, with the remaining 14 corresponding to locoregional relapses. The authors found no significant differences in HER-2/neu expression between the primary tumors and the corresponding metastases. In addition, other investigators found a high proportion of concordance between primary breast tumors and locoregional or distant metastases (17, 24). Gancberg *et al.* found a high level of concordance in HER-2/neu status in the primary and corresponding metastatic tumors (94% and 93% when analyzed by IHC and FISH, respectively). Most of the discordant cases demonstrated increased HER-2/neu expression in the corresponding metastatic sites in comparison to their primary breast tumors (14). Recently, Regitnig *et al.* showed increased HER-2/neu immunoreactivity by IHC in 48.4% of distant metastases (25).

Few studies have compared the HER-2 status in the primary core needle biopsy prior to the surgical specimen and following neoadjuvant chemotherapy (21, 22, 26). Varga *et al.* reported that the HER-2 status was modified in 35% of cases using IHC and in 13% using FISH (21). The initially negative (+) IHC was thus mofified as positive (+2 and +3, respectively) in the surgical specimen of 2 patients. In contrast, 6 positive cases (+2 and +3) on core biopsies were negative on the mastectomy specimens. Interestingly, 5 out of 6 +2 positive cases on IHC were negative after chemotherapy. Among +3 positive tumors, there was 1 case with loss of protein expression; however, this case showed gene amplification by FISH. Vincent-Salomon *et al.* found a concordance of 95.5% between the core biopsy and the post-treatment specimen. Only loss of HER-2 expression was observed (22). In a similar study by Burstein *et al.*, the pre-operative HER-2 status determined using IHC was reconfirmed using enzyme-linked immunosorbent assay post-operatively. In this patient selection, all initially negative cases remained negative. However, 17% of the originally strong-positive cases were found to be negative (20).

In our study, the increased concentrations of ECD were lower than those reported in the literature (15, 27, 28). S-HER-2 levels in previous studies have shown a 69-74% correlation with the clinical course of the disease (29, 30). Although the number of patients with positive baseline levels was small, there was a concordance between the S-HER-2 levels and response to trastuzumab. In all 5 patients who responded to trastuzumab, the S-HER-2 levels declined and returned within the normal range. This was probably due to the reduction of the bulk of disease or possibly trastuzumab blocks the shedding of HER-2 ECD (31). At disease progression, the S-HER-2 levels of the "conserved HER-2/neu status" patients increased. On the contrary, in patients of the "altered HER-2/neu status", having lost HER-2 overexpression, the S-HER-2 concentrations remained undetectable.

In conclusion, these data suggest that, for most patients with MBC treated with trastuzumab, the HER2/neu expression as measured by IHC in newly-developed metastatic lesions was unchanged. However, a subset of distant metastases from breast carcinomas treated with trastuzumab lost the HER-2 overexpression or amplification. The role of the HER-2 ECD in monitoring response to treatment with trastuzumab remains unclear.

The number of cases in this study was too small for final conclusions to be drawn, particularly with respect to daily clinical practice. A larger number of cases need to be investigated prospectively for final verification and recommendation for clinical practice.

References

1 Ross JS and Fletcher JA: HER-2/neu (c-erb-B2) gene and protein in breast cancer. Am J Clin Pathol *112*: S53-S67, 1999.
2 Slamon DJ, Clark GM, Wong SG *et al*: Human breast cancer: correlation of relapse and survival with amplification of the Her-2/neu oncogene. Science *235*: 177-182, 1987.
3 Andrulis IL, Bull SB, Blackstein ME *et al*: Neu/erb-2 amplification identifies a poor-prognosis group of women with node-negative breast cancer. JCO *16*: 1340-1349, 1998.
4 Cobleigh MA, Vogel CL, Tripathy D *et al*: Multinational study of the efficacy and safety of humanized anti-HER-2 monoclonal antibody in women who have HER-2-overexpessing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol *17*: 2639-2648, 1999.
5 Baselga J, Tripathy D, Mendelsohn J *et al*: Phase II study of weekly intravenous recombinant humanized anti-p185HER-2 monoclonal antibody in patients with HER-2/neu overexpressing metastatic breast cancer. J Clin Oncol *14*: 737-744, 1996.
6 Vogel C, Colbeigh M, Tripathy D *et al*: First-line single agent Herceptin (H) therapy for women with HER-2 overexpressing metastatic breast cancer (MBC). Eur J Cancer *36(Suppl 5)*: S53 (abstr 40), 2000.
7 Slamon DJ, Leyland Jones B, Shak S *et al*: Use of chemotherapy plus a monoclonal antibody against HER-2 for metastatic breast cancer that overexpresses HER-2. N Engl J Med *344*: 783-792, 2001.
8 Vogel CL, Cobleigh MA, Tripathy D *et al*: Superior outcomes with Herceptin (trastuzumab) (H) in fluorescence *in situ* hybridization (FISH)-selected patients. Proc ASCO *20*: 23a (abstr 86), 2001.
9 Barnes DM, Lammie GA, Millis RR *et al*: An immunohistochemical evaluation of c-erbB-2 expression in human breast carcinoma. Br J Cancer *58*: 448-452, 1988.
10 Xu R, Perle MA, Inghirami G *et al*: Amplification of HER-2/neu gene in Her-2/neu-overexpressing and nonexpressing breast carcinomas and their synchronous benign premalignant, and metastatic lesions detected by FISH in archival material. Mod Pathol *15*: 116-124, 2002.
11 Krogerus LA and Leivonen M: Her-2/neu oncogene expression in advanced breast cancer. Cancer Detect Prev *25*: 1-7, 2001.
12 Simon R, Nocito A, Hübscher T *et al*: Patterns of Her-2/neu amplification and overexpression in primary and metastatic breast cancer. J Natl Cancer Inst *93*: 1141-1146, 2001.
13 Shimizu C, Fukutomi T, Tsuda H *et al*: c-erbB-2 protein overexpression and p53 immunoreaction in primary and recurrent breast cancer tissues. J Surg Oncol *73*: 17-20, 2000.
14 Gancberg D, Di Leo A, Rouas G *et al*: Reliability of the tissue microarray-based FISH for evaluation of the HER-2 oncogene in breast carcinoma. J Clin Pathol *55*: 315-317, 2002.
15 Carney WP, Neumann R, Lipton A *et al*: Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patients with breast cancer. Clin Chem *49*: 1579-1598, 2003.
16 Drebin JA, Link VC, Stern DF *et al*: Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. Cell *41*: 697-706, 1985.
17 Tanner M, Jarvinen P and Isola J: Amplification of HER-2/neu and topoisomerase II alpha in primary and metastatic breast cancer. Cancer Res *61*: 5345-5348, 2001.
18 Niehans GA, Singleton TP, Dykoski D *et al*: Stability of HER-2/neu expression over time and at multiple metastatic sites. J Natl Cancer Inst *85*: 1230-1235, 1993.
19 Gancberg D, Di Leo A, Cardoso F *et al*: Comparison of Her-2 status between primary breast cancer and corresponding distant metastatic sites. Ann Oncol *13*: 1036-1043, 2002.
20 Burstein HJ, Harris LN, Gelman R *et al*: Preoperative therapy with trastuzumab and paclitaxel followed by sequential adjuvant doxorubicin/cyclophosphamide for HER-2 overexpressing stage II or III breast cancer: a pilot study. J Clin Oncol *21*: 46-53, 2003.
21 Varga Z, Caduff R and Pestalozzi B: Stability of the HER-2 gene after primary chemotherapy in advanced breast cancer. Virchows Arch *446*: 136-141, 2005.
22 Vincent-Salomon A, Jouve M, Genin P *et al*: HER-2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process. Cancer *94*: 2169-2173, 2002.
23 Edgerton SE, Merkel D, Moore DH *et al*: HER-2/neu/erbB-2 status by immunohistochemistry and FISH: clonality and progression with recurrence and metastases. Breast Cancer Res Treat *64*: 55 (abstr 180), 2000.
24 Masood S and Bui MM: Assessment of HER-2/neu overexpression in breast cancers and their metastatic lesions: an immunohistochemical study. Ann Clin Lab Sci *30*: 259-265, 2000.
25 Regitnig P, Schippinger W, Lindbauer M *et al*: Change of HER-2/neu status in a subset of distant metastases from breast carcinomas. J Pathol *203*: 918-926, 2004.
26 Taucher S, Rudas M, Mader RM *et al*: Influence of neoadjuvant therapy with epirubicin and doxetaxel on the expression of HER-2/neu in patients with breast cancer. Breast Cancer Res Treat *82*: 207-213, 2003
27 Andersen TI, Paus E, Nesland JM *et al*: Detection of c-erbB-2 related protein in sera from breast cancer patients. Relationship to *ERBB2* gene amplification and c-erbB-2 protein overexpression in tumour. Acta Oncol *34*: 499-504, 1995.
28 Watanabe N, Miyamoto M, Tokuda Y *et al*: Serum c-erbB-2 in breast cancer patients. Acta Oncol *33*: 901-904, 1994.
29 Schöndorf T, Hoopmann M, Warm W *et al*: Serologic concentrations of HER-2/neu in breast cancer patients with visceral metastases receiving trastuzumab therapy predicts the clinical course. Clin Chem *48*: 1360-1362, 2002.
30 Gaglia A, Bobota A, Kostopoulou V *et al*: Serial measurement of serum HER-2/neu levels in trastuzumab-treated metastatic breast cancer patients. Sixteenth International Congress on Anti-Cancer Treatment, abstracts pp. 156, 2005.
31 Molina MA, Codony-Servat J, Albanell J *et al*: Trastuzumab (Herceptin), a humanized anti-HER-2/neu receptor monoclonal antibody inhibits basal and activated HER2 ectodomain cleavage in breast cancer cells. Cancer Res *61*: 4744-4749, 2001.

Received September 1, 2005
Revised November 11, 2005
Accepted November 23, 2005