to prevent or delay the action of trastuzumab and was associated with the development of resistance to trastuzumab[53]. It also has been suggested that the heterodimerization between IGF-1R and HER-2 contributes to trastuzumab resistance to breast cancer cells[54]. Furthermore, an anti-IGF-1R monoclonal antibody can partially restore the sensitivity of the cells to trastuzumab[51]. These studies suggest that, to predict responsiveness to trastuzumab, assays for HER-2 and other molecules such as IGF-1R might be necessary.

Other challenges are hormone receptor-negative and HER-2-negative high-grade breast cancers. Because estrogen receptor, progesterone receptor, and HER-2 are all negative in these cases, they are called "triple negatives." Although it is assumed that only chemotherapy is applicable in such cases, a relatively high ratio of patients with these cancers are resistant to chemotherapy, which often have a rapid clinical course. The diagnostic, epidemiological, histopathological, and molecular characterization of this tumor group is now under investigation.

Although trastuzumab therapy is now of profound clinical utility, the prognosis of patients with HER-2-overexpressing breast cancers is still poor, especially those with lymph node metastasis and/or in metastatic or recurrent disease. Better and more effective regimens are anticipated.

Acknowledgments

The author thanks Dr. Kansei Komaki, Department of Surgery, Tokushima University Hospital, for encouraging the preparation of manuscript. The contents of this paper were presented at the 6th Annual Meeting of the Japanese Breast Cancer Trialist's Group held in Tokushima on March 5, 2005. This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labor, and Welfare, Japan.

References

1) Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2- overexpressing metastatic breast cancer. *J Clin Oncol* 20:719-726, 2002.
2) Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, 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.
3) Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, Pusztai L, Green MC, Arun BK, Giordano SH, Cristofanilli M, Frye DK, Smith TL, Hunt KK, Singletary SE, Sahin AA, Ewer MS, Buchholz TA, Berry D, Hortobagyi GN: Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: Results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J Clin Oncol* 23:3676-3685, 2005.
4) Romond EH, Perez EA, Bryant J, Suman V, Geyer CE Jr, Davidson N, Tan-Chiu E, Martino S, Paik S, Kaufman P, Swain SM, Pisansky T, Fehrenbacher L, Kutteh LA, Vogel V, Visscher DW, Yothers G, Jenkins R, Brown AM, Dakhil SR, Mamounas E, Lingle WL, Klein PM, Ingle JN, Wolmark N: Doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab as adjuvant therapy for patients with HER-2 positive operable breast cancer. *N Engl J Med* 353:1673-1684, 2005.
5) Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinister M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolch MS, Gelber RD; Herceptin Adjuvant (HERA) Trial Study Team, Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 353:1659-1672, 2005.
6) Tsuda H: Prognostic and predictive value of c-erbB-2 (HER-2/neu) gene amplification in human breast cancer. *Breast Cancer* 8:38-44, 2001.
7) Goldhirsch A, Glick JH, Gelber RD, Coats AS, Thurlimann B, Senn HJ; Panel members. Meeting highlights: international expert consensus on the primary therapy of early breast cancer. *Ann Oncol* 16:1569-1583, 2005.
8) Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, Symmans WF, Pusztai L, Bloom KJ: The HER-2/neu gene and protein in breast cancer 2003: Biomarker and target of therapy. *Oncologist* 8:307-325, 2003.
9) Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG, Somerfield MR: American Society of Clinical Oncology Tumor Markers Expert Panel. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: Clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 19:1865-1878, 2001; Erratum *J Clin Oncol* 19:4185-4188, 2001.
10) 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* 235:177-182, 1987.
11) Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Yamamoto H, Miyajima N, Toyoshima K, Yamamoto T, Yokota J, Sakamoto H, Yoshida T, Terada M, Sugimura T: Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: hst-1/int-2 and c-erbB-2/ear-1. *Cancer Res* 49:3104-3108, 1989.

12) Wright C, Angus B, Nicholson S, Sainsbury JR, Cairns J, Gullick WJ, Kelly P, Harris AL, Horne CH: Expression of c-erbB-2 oncoprotein: A prognostic indicator in human breast cancer. *Cancer Res* 49:2087-2090, 1989.
13) Clark GM, McGuire WL: Follow-up study of HER-2/neu amplification in primary breast cancer. *Cancer Res* 51:944-948, 1991.
14) Zhang G-J, Tsuda H, Adachi I, Fukutomi T, Yamamoto H, Hirohashi S: Prognostic indicators of breast cancer patients with one to three regional lymph-node metastases with special reference to alterations in expression levels of bcl-2, p53, and c-erbB-2 proteins. *Jpn J Clin Oncol* 27:371-377, 1997.
15) Tsuda H, Sakamaki C, Tsugane S, Fukutomi T, Hirohashi S: A prospective study on the significance of gene and chromosome alterations as prognostic indicators of breast cancer patients with lymph node metastases. *Breast Cancer Res Treat* 48:21-32, 1998.
16) Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR, Flom KJ: HER-2/neu gene amplification characterized by fluorescence in situ hybridization: Poor prognosis in node-negative breast carcinomas. *J Clin Oncol* 15:2894-2904, 1997.
17) Tsutsui S, Ohno S, Murakami S, Hachitanda Y, Oda S: Prognostic value of c-erbB2 expression in breast cancer. *J Surg Oncol* 79:216-223, 2002.
18) van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, Nusse R: Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med* 319:1239-1245, 1988.
19) Allred DC, Clark GM, Tandon AK, Molina R, Tormey DC, Osborne CK, Gilchrist KW, Mansour EG, Abeloff M, Eudey L: HER-2/neu in node-negative breast cancer: Prognostic significance of overexpression influenced by the presence of in situ carcinoma. *J Clin Oncol* 10:599-605, 1992.
20) Muss HB, Thor AD, Berry DA, Kute T, Liu ET, Koerner F, Cirrincione CT, Budman DR, Wood WC, Barcos M, Henderson IC: c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med* 330:1260-1266, 1994.
21) Paik S, Bryant J, Tan-Chiu E, Yothers G, Park C, Wickerham DL, Wolmark N: HER2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-15. *J Natl Cancer Inst* 92:1991-1998, 2000.
22) Di Leo A, Gancberg D, Larsimont D, Tanner M, Jarvinen T, Rouas G, Dolci S, Leroy JY, Paesmans M, Isola J, Piccart MJ: HER-2 amplification and topoisomerase IIalpha gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. *Clin Cancer Res* 8:1107-1116, 2002.
23) Wang J, Buchholz TA, Middleton LP, Allred DC, Tucker SL, Kuerer HM, Esteva FJ, Hortobagyi GN, Sahin AA: Assessment of histologic features and expression of biomarkers in predicting pathologic response to anthracycline-based neoadjuvant chemotherapy in patients with breast carcinoma. *Cancer* 94:3107-3114, 2002.
24) Hamilton A, Larsimont D, Paridaens R, Drijkoningen M, van de Vijver M, Bruning P, Hanby A, Houston S, Treilleux I, Gusatalla JP, Van Vreckem A, Sylvester R, Piccart M: A study of the value of p53, HER2, and Bcl-2 in the prediction of response to doxorubicin and paclitaxel as single agents in metastatic breast cancer; a companion study to EORTC 10923. *Clin Breast Cancer* 1:233-242, 2000.
25) Tetu B, Brisson J, Plante V, Bernard P: p53 and c-erbB-2 as markers of resistance to adjuvant chemotherapy in breast cancer. *Mod Pathol* 11:823-830, 1998.
26) Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M: Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: Evidence from a phase III randomized trial. *J Clin Oncol* 19:3808-3816, 2001.
27) Ellis IO, Bartlett J, Dowsett M, Humphreys S, Jasani B, Miller K, Pinder SE, Rhodes A, Walker R: Best Practice No 176: Updated recommendations for HER2 testing in the UK. *J Clin Pathol* 57:233-237, 2004.
28) Bilous M, Dowsett M, Hanna W, Isola J, Lebeau A, Moreno A, Penault-Llorca F, Ruschoff J, Tomasic G, van de Vijver M: Current perspectives on HER2 testing: A review of national testing guidelines. *Mod Pathol* 16:173-182, 2003.
29) Trastuzumab Pathology Committee. Guide for HER-2 test. Chugai Pharmaceutical Co., Tokyo, 2003 (in Japanese).
30) Simon R, Nocito A, Hubscher T, Bucher C, Torhorst J, Schraml P, Bubendorf L, Mihatsch MM, Moch H, Wilber K, Schotzau A, Kononen J, Sauter G: Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. *J Natl Cancer Inst* 93:1141-1146, 2001.
31) Gancberg D, Di Leo A, Cardoso F, Rouas G, Pedrocchi M, Paesmans M, Verhest A, Bernard-Marty C, Piccart MJ, Larsimont D: Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. *Ann Oncol* 13:1036-1043, 2002.
32) Shimizu C, Fukutomi T, Tsuda H, Akashi-Tanaka S, Watanabe T, Nanasawa T, Sugihara K: c-erbB-2 protein overexpression and p53 immunoreaction in primary and recurrent breast cancer tissues. *J Surg Oncol* 73:17-20, 2000.
33) Cardoso F, Di Leo A, Larsimont D, Gancberg D, Rouas G, Dolci S, Ferreira F, Paesmans M, Piccart M: Evaluation of HER2, p53, bcl-2, topoisomerase II-alpha, heat shock proteins 27 and 70 in primary breast cancer and metastatic ipsilateral axillary lymph nodes. *Ann Oncol* 12:615-620, 2001.
34) Meng S, Tripathy D, Shete S, Ashfaq R, Haley B, Perkins S, Beitsch P, Khan A, Euhus D, Osborne C, Frenkel E, Hoover S, Leitch M, Clifford E, Vitetta E, Morrison L, Herlyn D, Terstappen LWMM, Fleming T, Fehm T, Tucker T, Lane N, Wang J, Uhr J: HER-2 gene amplification can be acquired as breast cancer progresses. *Proc Natl Acad Sci USA* 101:9393-9398, 2004.
35) Tsuda H, Hirohashi S: Multiple developmental pathways of highly aggressive breast cancers disclosed by

comparison of histological grades and c-erbB-2 expression patterns in both the non-invasive and invasive portions. *Pathol Int* 48:518-525, 1998.
36) Tsuda H, Akiyama F, Terasaki H, Hasegawa T, Kurosumi M, Shimadzu M, Yamamori S, Sakamoto G: Detection of HER-2/neu (c-erb B-2) DNA amplification in primary breast carcinoma. Interobserver reproducibility and correlation with immunohistochemical HER-2 overexpression. *Cancer* 92:2965-2974, 2001.
37) Mass R, Sanders C, Kasian C, Everett T, Johnson L, Anderson S: The concordance between the clinical trials (CTA) and fluorescence in situ hybridization (FISH) in the herceptin pivotal trials. *Proc Am Soc Clin Oncol* 19:75a, 2000.
38) 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* 5:63-69, 2004.
39) Dowsett M, Bartlett J, Ellis IO, Salter J, Hills M, Mallon E, Watters AD, Cooke T, Paish C, Wencyk PM, Pinder SE: Correlation between immunohistochemistry (HercepTest) and fluorescence in situ hybridization (FISH) for HER-2 in 426 breast carcinomas from 37 centers. *J Pathol* 199:418-423, 2003.
40) Tsuda H, Sasano H, Akiyama F, Kurosumi M, Hasegawa T, Osamura RY, Sakamoto G: Evaluation of interobserver agreement in scoring immunohistochemical results of HER-2/neu (c-erbB-2) expression detected by the HercepTest, Nichirei polyclonal antibody, CB11, and TAB250 in breast carcinoma. *Pathol Int* 52:126-134, 2002.
41) Press MF, Bernstein L, Zhou J-Y, Villalobos IE, Anderson S, Cobleigh MA, Vogel CL, Mass R: Low-level HER-2/neu gene amplification by fluorescence in situ hybridization and responsiveness to single agent herceptin immunotherapy among women with metastatic breast cancer. *Proc Am Soc Clin Oncol* 22:182, 2004.
42) Nunes RA, Harris LN: The HER2 extracellular domain as a prognostic and predictive factor in breast cancer. *Clin Breast Cancer* 3:125-135, 2002.
43) Sugano K, Ushiama M, Fukutomi T, Tsuda H, Kitoh T, Ohkura H: Combined measurement of the c-erbB-2 protein in breast carcinoma tissues and sera is useful as a sensitive tumor marker for monitoring tumor relapse. *Int J Cancer* 89:329-336, 2000.
44) Esteva FJ, Valero V, Booster D, Guerra LT, Murray JL, Pusztai L, Cristofanilli M, Arun B, Esmaeli B, Fritsche HA, Sneige N, Smith TL, Hortobagyi GN: Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpresing metastatic breast cancer. *J Clin Oncol* 20:1800-1808, 2002.
45) Burstein HJ, Harris LN, Marcom PK, Lambert-Falls R, Havlin K, Overmoyer B, Friedlander RJ Jr, Gargiulo J, Strenger R, Vogel CL, Ryan PD, Ellis MJ, Nunes RA, Bunnell CA, Campos SM, Hallor M, Gelman R, Winer EP: Trastuzumab and vinorelbine as first-line therapy for HER2-overexpressing metastatic breast cancer: Multicenter phase II trial with clinical outcomes, analysis of serum tumor markers as predictive factors, and cardiac surveillance algorithm. *J Clin Oncol* 21:2889-2895, 2003.
46) Bethune-Volters A, Labroquere M, Guepratte S, Hacene K, Neumann R, Carney W, Pichon MF: Longitudinal changes in serum HER-2/neu oncoprotein levels in trastuzumab-treated metastatic breast cancer patients. *Anticancer Res* 24:1083-1089, 2004.
47) Fornier MN, Seidman AD, Schwartz MK, Ghani F, Thiel R, Norton L, Hudis C: Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: Association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate. *Ann Oncol* 16:234-239, 2005.
48) Elkin EB, Weinstein MC, Winer EP, Kuntz KM, Schnitt SJ, Weeks JC: HER-2 testing and trastuzumab therapy for metastatic breast cancer: A cost-effectiveness analysis. *J Clin Oncol* 22:854-863, 2004.
49) Yaziji H, Goldstein LC, Barry TS, Werling R, Hwang H, Ellis GK, Gralow JR, Livingstone RB, Gown AM: HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA* 291:1972-1977, 2004.
50) Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, Brown A, Yothers G, Anderson S, Smith R, Wickerham DL, Wolmark N: Real-world performance of HER2 testing-National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 94:852-854, 2002.
51) Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, Addo FK, Murphy B, Ingle JN, Perez EA: Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 94:855-857, 2002.
52) Blackwell KL, Burstein H, Pegram M, Storniolo AM, Salazar VM, Maleski JE, Lin X, Spector N, Stein SH, Berger MS: Determining relevant biomarkers from tissue and serum that may predict the response to single agent lapatinib in trastuzumab refractory metastatic breast cancer. *Proc Am Soc Clin Oncol* 23:193s, 2005.
53) Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M: Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (herceptin). *J Natl Cancer Inst* 93:1852-1857, 2001.
54) Robinson A, Ellard S, Speers C, Turbin D, Yorida E, Rajput A, Thomson T, Huntsman D, Jelmon K: Clinical and molecular predictors of sustained response to trastuzumab in metastatic breast cancer. *Proc Am Soc Clin Oncol* 23:4s, 2005.