Review Article

Biological and Clinical Significance of *HER2* Overexpression in Breast Cancer

Junichi Kurebayashi

The product of the *HER2/neu* proto-oncogene, *HER2*, is the second member of the human epidermal growth factor receptor (*HER*) family of tyrosine kinase receptors and has been suggested to be a ligand orphan receptor. Ligand-dependent heterodimerization between *HER2* and another *HER* family member, *HER1*, *HER3* or *HER4*, activates the *HER2* signaling pathway. The intracellular signaling pathway of *HER2* is thought to involve ras-MAPK, MAPK-independent S6 kinase and phospholipase C-γ signaling pathways. However, the biological consequences of the activation of these pathways are not yet completely known.

Amplification of the *HER2* gene and overexpression of the *HER2* protein induces cell transformation and has been demonstrated in 10% to 40% of human breast cancer. *HER2* overexpression has been suggested to associate with tumor aggressiveness, prognosis and responsiveness to hormonal and cytotoxic agents in breast cancer patients. These findings indicate that *HER2* is an appropriate target for tumor-specific therapies. A number of approaches have been investigated: (1) a humanized monoclonal antibody against *HER2*, rhuMAb*HER2* (trastuzumab), which is already approved for clinical use in the treatment of patients with metastatic breast cancer; (2) tyrosine kinase inhibitors, such as emodin, which block *HER2* phosphorylation and its intracellullar signaling; (3) active immunotherapy, such as vaccination; and (4) heat shock protein (Hsp) 90-associated signal inhibitors, such as radicicol derivatives, which induce degradation of tyrosine kinase receptors, such as *HER2*.

Breast Cancer 8:45-51, 2001.

Key words: *HER2*, Overexpression, Breast cancer, Biologic therapy, Antibody, Hsp90

HER2 Signaling Pathway (Table 1)

The product of the *HER2* gene (also known as c-*erb*B-2 and *neu*), *HER2*, encodes a 185 kDa transmembrane glycoprotein and belongs to the human epidermal growth factor receptor (*HER*) family of receptor tyrosine kinases (RTKs)[1]. Four members of this family, *HER1* (also known as epidermal growth factor receptor)[2], *HER2*, *HER3*[3] and *HER4*[4], have been identified so far. In general, RTKs have an extracellular domain (ECD) which interacts with polypeptide ligands. Ligand binding to the ECD results in the formation of receptor homo- or heterodimers and stimulation of intrinsic kinase activity, which leads to the phosphorylation of tyrosine residues in the intracellular domain[5]. These serve as docking sites for a number of src-homologous 2-domain proteins including the adaptor proteins, SHC[6], Grbs[7] and the p85 subunit of phosphatidylinositol 3-kinase (PI3K)[8]. These adaptor molecules link RTKs to intracellular signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway[9], MAPK-independent p70/p85 S6 kinase cascade[10] or the phospholipase C (PLC)-γ signaling pathway[11]. However, the biological consequences of the activation of these pathways are not yet completely known.

Although a number of ligands for the *HER* family members have been identified, such as epidermal growth factor (EGF)[12], heparin binding EGF-like growth factor (HB-EGF)[13], betacellulin[14] and heregulins[15], no ligand has been reported to directly bind to *HER2*. Therefore, *HER2* is recognized as a ligand orphan receptor. However, recent studies have revealed that all ligands for the *HER* family members induce *HER2* tyrosine

Department of Breast and Thyroid Surgery, Kawasaki Medical School, Japan.
Reprint requests to Junichi Kurebayashi, Department of Breast & Thyroid Surgery, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan

Abbreviations:
HER, Human epidermal growth factor receptor; Hsp, Heat shock protein, RTK, Receptor tyrosine kinase, ECD, Extracellular domain; PI3K, Phosphatidylinositol 3-kinase, MAPK, Mitogen-activated protein kinase; PLC, Phospholipase C; EGF, Epidermal growth factor; HB-EGF, Heparin binding EGF-like growth factor; PCR, Polymerase chain reaction, FISH, Fluoroscene *in situ* hybridization; MAb, Monoclonal antibody, ER, Estrogen receptor

Received June 8, 2000, accepted July 25, 2000

Table 1. *HER2* Signaling Pathway

EGF-related ligands	Receptors	*HER2* heterodimers	Intracellular signaling
EGF and HB-EGF	*HER1*	*HER1-HER2*	ras-MAPK and PLC-γ
betacellulin	*HER1*	*HER1-HER2*	ras-MAPK and PLC-γ
	HER4	*HER2-HER4*	ras-MAPK
heregulins	*HER3*	*HER2-HER3*	ras-MAPK and PI3K/p70/p85^{S6K}
	HER4	*HER2-HER4*	ras-MAPK

phosphorylation by triggering heterodimerization between *HER2* and another *HER* family receptor, *HER1*, *HER3* or *HER4*. In other words, *HER2* is the preferred heterodimerization partner of all the *HER* family receptors and mediates lateral signaling in target cells[16, 17].

Oncogenic Potential of HER2 Overexpression

The *HER2* oncogene was originally identified as a result of DNA transfection and focus formation assays using DNA isolated from a rat neuroblastoma[1]. This oncogene becomes activated in the rat by a point mutation within the transmembrane region. However, no point mutation has been identified in human tumors. Amplification and/or overexpression of the *HER2* oncogene is related to malignancy. *In vitro* assay systems, such as focus transformation and anchorage-independent growth, have showed that *HER2* overexpression mediates transformation of normal cells including mammary epithelial cells[18, 19]. In addition, an elegant study of transgenic mice carrying an activated *HER2* gene driven by a mouse mammary tumor virus promoter demonstrated that *HER2* activation leads to a stepwise progression of carcinogenesis in mammary epithelium[20]. A recent study also showed that the expression of activated *HER2* under normal transcriptional control was not sufficient for the initiation of mammary carcinogenesis but that amplification of the *HER2* oncogene resulted in the appearance of mammary tumors[21]. These findings obtained from both *in vitro* and *in vivo* studies suggest that *HER2* overexpression plays a critical role in the development of mammary tumors.

Evaluation of HER2 Overexpression in Breast Cancer (Table 2)

A number of methodologies have been applied to evaluate amplification and overexpression of the *HER2* gene in breast cancer[22]. Gene amplification has been measured by Southern blot analysis or slot-blot analysis of tumor tissue-derived DNA. Recently, polymerase chain reaction (PCR) methods and *in situ* hybridization have also been used. Amplification of the *HER2* gene has been identified in 10% to 40% of breast cancers. The lower frequencies probably reflect contamination of surrounding normal tissues and degradation of tumor DNA. Notably, the fluorescence *in situ* hybridization (FISH) technique has been used to measure the number of *HER2* gene copies in formalin-fixed archival specimens[23] as well as fine-needle aspiration samples[24]. FISH has been reported as a rapid, reproducible and reliable method of detecting *HER2* gene amplification. *HER2* mRNA expression has been evaluated by Northern blot analysis, *in situ* hybridization or PCR methods using frozen tumor tissues. However, instability of mRNA in tumor samples may interfere with the quantification of *HER2* mRNA. Quantitative and qualitative evaluation of *HER2* protein has been performed using immunohistochemistry on frozen and archival tumor tissues, and using Western blot analysis or enzyme immunoassays on solubilized tumor extracts. It is suggested that contamination of tumor samples with normal stromal cells and connective tissues dilutes *HER2* protein levels.

Table 2. Evaluation of Amplification and Overexpression of the *HER2* Gene

Gene amplification
Southern blot/slot blot analysis
Fluoroscene *in situ* hybridization (FISH)*
PCR analysis
Protein expression
Immunohistochemistry*
Western blot analysis
Enzyme immunoassay
mRNA expression
Northern blot analysis
PCR analysis
In situ hybridization

*practical and reliable

Immunohistochemistry with anti-*HER2* antibodies and archival tissues is a practical and reliable method used to evaluate *HER2* protein expression in breast cancer. However, comparisons among different antibodies have demonstrated different levels of sensitivity and specificity on archival tissue samples[25]. Indeed, the procedures of fixation, embedding and storage of tumor samples substantially influence *HER2* protein conservation. Additionally, lack of a uniform scoring system to interpret *HER2* staining is another important issue affecting the evaluation of *HER2* expression. Recently, the U.S. Food and Drug Administration has approved an immunohistochemical test kit to determine tumor *HER2* status[26]. In the near future, a recombinant humanized anti-*HER2* monoclonal antibody (MAb) (rhuMAb*HER2*, trastuzumab, Genentech Inc., South San Francisco, CA, USA)[27-31] will be used for the treatment of breast cancer in Japan. *HER2* overexpression in tumor tissues is an essential indicator for the use of this MAb. Therefore, a clear guideline for evaluating *HER2* expression in tumor samples should be made in Japan as soon as possible.

A very recent report has indicated that the low interobserver reproducibility in evaluating *HER2* status by immunohistochemistry necessitates further confirmation by FISH before treatment decisions are made. It is also suggested that negative staining for *HER2* always correlates with a lack of gene amplification but positive membranous staining does not always predict gene amplification, and that immunohistochemical staining may be considered as a useful screening test[32]. These findings prompt us to prospectively compare immunohistochemistry and FISH for the evaluation of *HER2* status as a predictor of response to trastuzumab in breast cancer.

HER2 Overexpression as a Prognostic Marker

Because of the above problems in determining tumor *HER2* status, the results of breast cancer outcome studies and of studies to investigate the association between *HER2* overexpression and conventional prognostic factors have not been uniform. However, most studies have suggested a correlation between *HER2* overexpression and other parameters indicative of tumor progression, such as tumor size, lymph node metastasis, high histological grade, absence of hormone receptor expression, aneuploidy and high proliferation index[22]. Although it is well established that *HER2* overexpression is indicative of poor prognosis in node-positive breast cancer patients, there is no consensus on its predictive value in node-negative patients[33-35]. These findings suggest an important biological role of *HER2* overexpression in the progression of breast cancer. Recently, *HER2* activity can be measured using antibodies that recognize only the tyrosine-phosphorylated form of *HER2*[36,37]. However, no data has been reported so far on the relevance of *HER2* activation as a more reliable prognostic factor.

Table 3. *HER2* Overexpression as a Predictive Factor of Response to Therapy

	HER2 overexpressing tumors
Response to endocrine therapy	
Adjuvant setting	poor/detrimental
Therapy for metastatic diseases	poor
Response to anthracycline-based regimens	
Adjuvant setting	controversial
Therapy for metastatic diseases	unknown
Response to CMF	
Adjuvant setting	controversial
Therapy for metastatic diseases	unknown
Response to taxanes	
Adjuvant setting	unknown
Therapy for metastatic diseases	favorable?
Response to trastuzumab	
Adjuvant setting	unknown
Therapy for metastatic diseases	favorable

HER2 Overexpression as a Predictive Factor of Response to Endocrine Therapy (Table 3)

A strong correlation between *HER2* overexpression and estrogen receptor (ER) negativity in breast cancer has been demonstrated. Some experimental work has suggested the action mechanisms of this phenomenon. First, it is suggested that estrogen down-regulates the transcription of the *HER2* gene[38,39]. Thus, *HER2* gene expression may be partially suppressed by estrogen in tumors expressing ER. Second, it has been reported that transfection of *HER2* gene, which causes *HER2* overexpression, into hormone-dependent breast cancer cells reduces the hormone dependency of these cells[40]. Additionally, transfection of heregulin, which causes activation of *HER2* signaling, into these cells also reduces their hormone-dependency as well as the function of ER[41]. These findings may explain the negative relationship between *HER2* and ER expression.

In contrast, approximately half of breast cancers overexpressing *HER2* are ER-positive[42]. Moreover, recent retrospective studies have revealed that *HER2* overexpression in breast cancer predicts unresponsiveness to hormonal therapy in the adjuvant setting[43] as well as in the treatment of recurrent disease[44]. It should be noted that tamoxifen treatment in the adjuvant setting was suggested to be detrimental in patients with tumor overexpressing *HER2*[43]. Another report indicates that the serum level of the soluble *HER2* ECD is more predictive of response to hormone therapy than the ER status[45]. These findings suggest that hormone therapy would not be indicated in patients with tumors expressing both ER and *HER2*. Otherwise, our experimental study suggested that a combined treatment with antiestrogen and anti-*HER2* MAb, rhuMAb*HER2*, synergistically inhibited the growth of human breast cancer cells expressing both ER and *HER2*[46]. This new strategy may be useful and should be tested in the treatment of patients with breast cancer.

HER2 Overexpression as a Predictive Factor of Response to Chemotherapy (Table 3)

Recently, some retrospective studies have pointed to the importance of *HER2* overexpression in the response to doxorubicin, to CMF therapy or to paclitaxel. A clinical study indicated that patients receiving a dose-intensive doxorubicin-based chemotherapy had significantly longer disease-free survival and overall survival if their tumors overexpressed *HER2*[47]. However, this is not the case in patients receiving a low-dose doxorubicin-based chemotherapy[47]. Two subsequent studies supported the hypothesis that a dose-intensive doxorubicin-based chemotherapy is more active in patients with tumors overexpressing *HER2*. However, both studies emphasized the complexity of the interactions, including an important role for p53 in predicting the response to therapy[48, 49]. Further validation of these parameters is needed before clinical implementation. In addition, the action mechanisms of these interactions remain to be elucidated. Very recently, it has been reported that coamplification of *HER2* and topoisomerase II α genes was detected in primary breast cancers[50]. Doxorubicin is a topoisomerase II α inhibitor and the expression levels of topoisomerase II α protein in breast cancer cells correlated with sensitivity to doxorubicin. These findings may explain the altered chemosensitivity to doxorubicin reported in *HER2*-amplified breast cancers.

Table 4. *HER2* Overexpression as a Target for Therapy

Monoclonal antibodies against *HER2*
humanized monoclonal antibody (trastuzumab)
bispecific monoclonal antibodies
conjugates of monoclonal antibodies with chemotherapeutic agents
Tyrosine kinase inhibitors
emodin
Active immunotherapy
vaccination
Hsp90-associated signal inhibitors
geldanamycin derivatives
radicicol derivatives

The potential role of *HER2* as a predictor of adjuvant CMF therapy is still controversial. Two retrospective studies suggested that patients with *HER2*-negative tumors seemed to benefit from this therapy, whereas patients with *HER2*-positive tumors did not[51, 52]. Recently, a retrospective study using the samples collected from the "CMF versus no treatment" clinical trial has indicated that *HER2*-positive patients strongly benefit from CMF adjuvant therapy, both in terms of disease-free and overall survival[53].

A retrospective study suggested that recurrent breast cancer patients with *HER2*-positive tumors responded significantly better to monotherapy with taxanes than those with *HER2*-negative tumors[54]. Further studies are clearly needed to clarify this hypothesis.

HER2 Overexpression as a Target for Therapy (Table 4)

1) MAb against HER2

Although there are several therapeutic strategies using MAb against *HER2*-positive breast cancer, such as bispecific MAb with specificities for both H*ER2* and the molecules that trigger cytotoxicity[55] and as a conjugate with chemotherapeutic drugs[56], the development and introduction of a humanized MAb against *HER2*, rhuMAb*HER2*, are discussed here because this MAb is already approved for clinical use in the treatment of breast cancer in Western countries and will be approved for clinical use in Japan in the near future.

The murine MAb 4D5 was produced against the ECD domain of *HER2* and proved to cytostati-

cally inhibit the growth of human tumor cells that overexpress *HER2*[57]. However, because of occurrence of human anti-mouse antibodies, murine antibodies are inadequate for clinical use. Thus, MAb 4D5 was humanized by inserting its complementary-determining regions into the human immunoglobulin G1 framework[27]. This humanized MAb is called rhuMAb*HER2* or trastuzumab (Genentech Inc.). In a phase II study including heavily pretreated patients with *HER2*-overexpressing metastatic breast cancer, intravenous administration of this MAb as a single agent was found to be safe, and 11.6% and 37% of treated patients obtained objective response and stable disease, respectively[28]. This is the first evidence that MAb against growth factor receptors can achieve objective response in patients with solid tumors. Recently, this MAb in combination with first-line chemotherapy (doxorubicin plus cyclophosphamide or paclitaxel) was found to be more effective than chemotherapy alone in phase II and III trials of patients with *HER2*-overexpressing metastatic breast cancer[31]. Combination therapies of this MAb with other chemotherapeutic agents, such as cisplatinum[30] and navelbine[58], are also suggested to be effective. Additionally, in our laboratory, combined treatments with this MAb and hormonal agents[46] as well as radiation therapy are under investigation. However, the precise action mechanisms of this MAb on regulating tumor growth both *in vitro* and *in vivo* are not yet fully understood. Finally, unexpected cardiotoxicity has been reported in the use of this MAb alone or in combination with cytotoxic agents. Cardiac function should be monitored in patients receiving this MAb.

2) Tyrosine Kinase Inhibitors

One of the tyrosine kinase inhibitors, emodin, was reported to inhibit *HER2* tyrosine kinase activity and to repress the transformation ability and growth rate of *HER2*-overexpressing breast cancer cells preferentially[59]. Emodin was also shown to sensitize *HER2*-overexpressing tumor cells to chemotherapeutic agents both *in vitro* and *in vivo*[60]. These findings indicate that combined treatment with tyrosine kinase inhibitors and chemotherapeutic agents may be a promising strategy against *HER2*-overexpressing tumors.

3) Active Immunotherapy

A series of studies has revealed that a portion of breast cancer patients develop antibody and cellular immune responses specific for *HER2*[61]. In addition, the detection of *HER2*-specific antibodies in breast cancer patients is reported to correlate with *HER2* overexpression by the primary tumors[62]. It is not yet known whether immunity to *HER2* predicts improved survival, but existent immunity predicts that vaccines will be able to promote immunity to *HER2*. Effective immunization regimens of active immunotherapy have been explored[61].

4) Heat Shock Protein (Hsp) 90-associated Signal Inhibitors

Hsp90 is a molecular chaperone and plays a key role in the stability and function of multiple signaling molecules, such as *HER2*, Raf-1 and mutant p53. Benzoquinone ansamycins[62], such as geldanamycin, and radicicol[64] have been known to specifically interact with an Hsp90 amino-terminal nucleotide-binding domain, disrupting the chaperone's association with its client-signaling proteins and leading to their subsequent destabilization and proteolysis. Both have a potent antitumor effect and are considered to be novel anticancer agents. Indeed, a geldanamycin derivative is under a phase I clinical trial[65].

Recently, a novel oxime derivative of radicicol, KF25706, was synthesized at the Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. (Sunto-gun, Shizuoka, Japan)[66]. Preclinical study revealed that this agent could exhibit both *in vitro* and *in vivo* antitumor activity through inhibition of the Hsp90 family chaperone function. Notably, this agent drastically depleted Hsp90-associated molecules, such as *HER2*. No liver or renal toxicity of this agent was reported. Recently, we have been involved in studies of the antitumor effect of KF25706 and a more potent new derivative, KF58333, on human breast cancer cells both *in vitro* and *in vivo*. Both agents exhibited a potent growth inhibitory effect *in vitro* and a long-lasting antitumor effect on xenografts in nude mice associated with a significant antiangiogenic effect (manuscript in preparation). These agents may be good candidates for further clinical development.

Acknowledgments

The author is grateful to Drs. Hiroshi Sonoo, Shigeru Yamamoto, Hironori Kunisue, Masafumi Kurosumi and Takemi Otsuki for their continuous support. This work was supported by Research Project Grants from Kawasaki Medical School.

References

1) Yamamoto T, Ikawa S, Akiyama T, *et al*: Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. *Nature* 319:230-234, 1986.
2) Ullrich A, Coussens L, Hayflick JS, *et al*: Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309:418-425, 1984.
3) Kraus MH, Issing W, Miki T, *et al*: Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc Natl Acad Sci USA* 86:9193-9197, 1989.
4) Plowman GD, Green JM, Culouscou JM, *et al*: Heregulin induces tyrosine phosphorylation of *HER*4/P180erbB4. *Nature* 366:473-475, 1993.
5) van der Geer P, Hunter T, Lindberg RA: Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol* 10:251-337, 1994.
6) Pelicci G, Lanfrancone L, Grignani F, *et al*: A novel transforming protein (SHC) with an SH2 domain is implicated in mitogenic signal transduction. *Cell* 70:93-104, 1992.
7) Lowenstein EJ, Daly RJ, Batzer AG, *et al*: The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 70:431-442, 1992.
8) Fedi P, Pierce JH, di Fiore PP, *et al*: Efficient coupling with phosphatidylinositol 3-kinase, but not phospholipase C gamma or GTPase-activating protein, distinguishes ErbB-3 signaling from that of other ErbB/EGFR family members. *Mol Cell Biol* 14:492-500, 1994.
9) Egan SE, Weinberg RA: The pathway to signal achievement. *Nature* 365:781-783, 1993.
10) Ming XF, Burgering BM, Wennstrom S, *et al*: Activation of p70/p85 S6 kinase by a pathway independent of p21ras. *Nature* 371:426-429, 1994.
11) Peles E, Levy RB, Or E, *et al*: Oncogenic forms of the neu/*HER*2 tyrosine kinase are permanently coupled to phospholipase C gamma. *EMBO J* 10:2077-2086, 1991.
12) Savage CR Jr, Inagami T, Cohen S: The primary structure of epidermal growth factor. *J Biol Chem* 247:7612-7621, 1972.
13) Higashiyama S, Abraham JA, Miller J, *et al*: A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* 251:936-939, 1991.
14) Shing Y, Christofori G, Hanahan D, *et al*: Betacellulin: a mitogen from pancreatic beta cell tumors. *Science* 259:1604-1607, 1993.
15) Peles E, Bacus SS, Koski RA, *et al*: Isolation of the neu/*HER*-2 stimulatory ligand: a 44 kd glycoprotein that induces differentiation of mammary tumor cells. *Cell* 69:205-216, 1992.
16) Goldman R, Levy RB, Peles E, *et al*: Heterodimerization of the erbB-1 and erbB-2 receptors in human breast carcinoma cells: a mechanism for receptor transregulation. *Biochemistry* 29:11024-11028, 1990.
17) Graus-Porta D, Beerli RR, Daly JM, *et al*: ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J* 16:1647-1655, 1997.
18) Di Fiore PP, Pierce JH, Kraus MH, *et al*: erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells. *Science* 237:178-182, 1987.
19) Zhai YF, Beittenmiller H, Wang B, *et al*: Increased expression of specific protein tyrosine phosphatases in human breast epithelial cells neoplastically transformed by the neu oncogene. *Cancer Res* 53:2272-2278, 1993.
20) Muller WJ, Sinn E, Pattengale PK, *et al*: Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell* 54:105-115, 1988.
21) Andrechek ER, Hardy WR, Siegel PM, *et al*: Amplification of the neu/erbB-2 oncogene in a mouse model of mammary tumorigenesis. *Proc Natl Acad Sci USA* 97:3444-3449, 2000.
22) Menard S, Tagliabue E, Campiglio M, *et al*: Role of *HER2* gene overexpression in breast carcinoma. *J Cell Physiol* 182:150-162, 2000.
23) Pauletti G, Godolphin W, Press MF, *et al*: Detection and quantitation of *HER*-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene* 13:63-72, 1996.
24) Sauter G, Feichter G, Torhorst J, *et al*: Fluorescence in situ hybridization for detecting erbB-2 amplification in breast tumor fine needle aspiration biopsies. *Acta Cytol* 40:164-173, 1996.
25) Busmanis I, Feleppa F, Jones A, *et al*: Analysis of cerbB2 expression using a panel of 6 commercially available antibodies. *Pathology* 26:261-267, 1994.
26) Roche PC, Ingle JN: Increased *HER*2 with U.S. Food and Drug Administration-approved antibody. *J Clin Oncol* 17:434, 1999.
27) Carter P, Presta L, Gorman CM, *et al*: Humanization of an anti-p185*HER*2 antibody for human cancer therapy. *Proc Natl Acad Sci USA* 89:4285-4289, 1992.
28) Baselga J, Tripathy D, Mendelsohn J, *et al*: Phase II study of weekly intravenous recombinant humanized anti-p185*HER*2 monoclonal antibody in patients with *HER*2/neu-overexpressing metastatic breast cancer. *J Clin Oncol* 14:737-744, 1996.
29) J, Norton L, Albanell J, *et al*: Recombinant humanized anti-*HER*2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against *HER*2/neu overexpressing human breast cancer xenografts. *Cancer Res* 58:2825-2831, 1998.
30) Pegram MD, Lipton A, Hayes DF, *et al*: Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185*HER*2/neu monoclonal antibody plus cisplatin in patients with *HER*2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 16:2659-2671, 1998.
31) Slamon D, Leyland-Jones B, Shank S: Addition of Herceptin (humanized anti-*HER*2 antibody) to first line chemotherapy for *HER*2 overexpressing metastatic breast cancer (*HER*2+/MBC) markedly increases anticancer activity: A randomized, multinational controlled phase III trial. *Proc Am Soc Clin Oncol* 17:98a, 1998.
32) Hoang MP, Sahin AA, Ordonez NG, *et al*: *HER*-2/neu gene amplification compared with *HER*-2/neu protein overexpression and interobserver reproducibility in invasive breast carcinoma. *Am J Clin Pathol* 113:852-859, 2000.
33) Paik S, Hazan R, Fisher ER, *et al*: Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: Prognostic significance of erbB-2 protein overexpression in primary breast cancer. *J Clin Oncol* 8:103-112, 1990.
34) Wright C, Angus B, Nicholson S, *et al*: Expression of c-erbB-2 oncoprotein: a prognostic indicator in human

breast cancer. *Cancer Res* 49:2087-2090, 1989.
35) Rosen PP, Lesser ML, Arroyo CD, *et al*: Immunohistochemical detection of *HER*2/neu in patients with axillary lymph node negative breast carcinoma. A study of epidemiologic risk factors, histologic features, and prognosis. *Cancer* 75:1320-1326, 1995.
36) Bangalore L, Tanner AJ, Laudano AP, *et al*: Antiserum raised against a synthetic phosphotyrosine-containing peptide selectively recognizes p185neu/ erbB-2 and the epidermal growth factor receptor. *Proc Natl Acad Sci USA* 89:11637-11641, 1992.
37) Bacus SS, Chin D, Yarden Y, *et al*: Type 1 receptor tyrosine kinases are differentially phosphorylated in mammary carcinoma and differentially associated with steroid receptors. *Am J Pathol* 148:549-558, 1996.
38) Read LD, Keith D Jr, Slamon DJ, *et al*: Hormonal modulation of *HER*-2/neu protooncogene messenger ribonucleic acid and p185 protein expression in human breast cancer cell lines. *Cancer Res* 50:3947-3951, 1990.
39) Newman SP, Bates NP, Vernimmen D, *et al*: Cofactor competition between the ligand-bound oestrogen receptor and an intron 1 enhancer leads to oestrogen repression of ERBB2 expression in breast cancer. *Oncogene* 19:490-497, 2000.
40) Benz CC, Scott GK, Sarup JC, *et al*: Estrogen-dependent, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with *HER*2/neu. *Breast Cancer Res Treat* 24:85-95, 1993.
41) Tang CK, Perez C, Grunt T, *et al*: Involvement of heregulin-beta2 in the acquisition of the hormone-independent phenotype of breast cancer cells. *Cancer Res* 56:3350-3358, 1996.
42) McCann AH, Dervan PA, O'Regan M, *et al*: Prognostic significance of c-erbB-2 and estrogen receptor status in human breast cancer. *Cancer Res* 51:3296-3303, 1991.
43) Carlomagno C, Perrone F, Gallo C, *et al*: c-erb B2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. *J Clin Oncol* 14:2702-2708, 1996.
44) Wright C, Nicholson S, Angus B, *et al*: Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer. *Br J Cancer* 65:118-121, 1992.
45) Leitzel K, Teramoto Y, Konrad K, *et al*: Elevated serum c-erbB-2 antigen levels and decreased response to hormone therapy of breast cancer. *J Clin Oncol* 13:1129-1135, 1995.
46) Kunisue H, Kurebayashi J, Otsuki T, *et al*: Anti-*HER*2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and *HER*2. *Br J Cancer* 82:46-51, 2000.
47) Muss HB, Thor AD, Berry DA, *et al*: 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.
48) Thor AD, Berry DA, Budman DR, *et al*: erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *J Natl Cancer Inst* 90:1346-1360, 1998.
49) Paik S, Bryant J, Park C, *et al*: erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. *J Natl Cancer Inst* 90:1361-1370, 1998.
50) Jarvinen TA, Tanner M, Rantanen V, *et al*: Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. *Am J Pathol* 156:839-847, 2000.
51) Allred DC, Clark GM, Tandon AK, *et al*: *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.
52) Gusterson BA, Gelber RD, Goldhirsch A, *et al*: Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. *J Clin Oncol* 10:1049-1056, 1992.
53) Menard S, Valagussa P, Pilotti S, *et al*: *HER*2 overexpression and response to CMF in lymph node-positive breat cancer. *Proc Am Soc Clin Oncol* 18:69a, 1999.
54) Baselga J, Seidman AD, Rosen PP, *et al*: *HER*2 overexpression and paclitaxel sensitivity in breast cancer: therapeutic implications. *Oncology* (Huntingt) 11 (Suppl 2):43-48, 1997.
55) Disis ML, Cheever MA: *HER*-2/neu protein: a target for antigen-specific immunotherapy of human cancer. *Adv Cancer Res* 71:343-371, 1997.
56) Jinno H, Ueda M, Enomoto K, *et al*: Effectiveness of an adriamycin immunoconjugate that recognizes the C-erbB-2 product on breast cancer cell lines. *Surg Today* 26:501-507, 1996.
57) Lewis GD, Figari I, Fendly B, *et al*: Differential responses of human tumor cell lines to anti-p185*HER*2 monoclonal antibodies. *Cancer Immunol Immunother* 37:255-263, 1993.
58) Burstein HJ, Kuter I, Richardson PG, *et al*: Herceptin and vinorelbine for *HER*2-positive metastatic breast cancer: a phase II study. *Proc Am Soc Clin Oncol* 19:102a, 2000.
59) Zhang L, Chang CJ, Bacus SS, *et al*: Suppressed transformation and induced differentiation of *HER*-2/neu-overexpressing breast cancer cells by emodin. *Cancer Res* 55:3890-3896, 1995.
60) Zhang L, Lau YK, Xia W, *et al*: Tyrosine kinase inhibitor emodin suppresses growth of *HER*-2/neu-overexpressing breast cancer cells in athymic mice and sensitizes these cells to the inhibitory effect of paclitaxel. *Clin Cancer Res* 5:343-353, 1999.
61) Disis ML, Calenoff E, McLaughlin G, *et al*: Existent T-cell and antibody immunity to *HER*-2/neu protein in patients with breast cancer. *Cancer Res* 54:16-20, 1994.
62) Disis ML, Pupa SM, Gralow JR, *et al*: High-titer *HER*-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. *J Clin Oncol* 15:3363-3367, 1997.
63) Whitesell L, Mimnaugh EG, De Costa B, *et al*: Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc Natl Acad Sci USA* 91:8324-8328, 1994.
64) Sharma SV, Agatsuma T, Nakano H: Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene* 16:2639-2645, 1998.
65) Kelland LR, Sharp SY, Rogers PM, *et al*: DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J Natl Cancer Inst* 91:1940-1949, 1999.
66) Soga S, Neckers LM, Schulte TW, *et al*: KF25706, a novel oxime derivative of radicicol, exhibits in vivo antitumor activity via selective depletion of Hsp90 binding signaling molecules. *Cancer Res* 59:2931-2938, 1999.