Perspectives

HER-2-Targeted Therapy: Lessons Learned and Future Directions

Rita Nahta and Francisco J. Esteva[1]

Departments of Breast Medical Oncology [R. N., F. J. E.] and Molecular and Cellular Oncology [F. J. E.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

HER-2 is overexpressed in 20–25% of invasive breast cancers and is associated with an aggressive tumor phenotype and reduced survival rates. The HER-2 status of a tumor is the critical determinant of response to the HER-2-targeted antibody trastuzumab. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from HER-2-targeted therapy. Trastuzumab combined with chemotherapy increases response rates, time to progression, and survival. However, the majority of cancers that initially respond to trastuzumab begin to progress again within 1 year. This minireview describes HER-2 targeting strategies currently in use or in stages of development for the treatment of breast cancer.

Introduction

The primary goal of novel anticancer drug design is to directly target specific molecular lesions found in tumor cells in the hopes of improving cancer cure rates and reducing cytotoxicity in normal cells. Advances in molecular biology have facilitated the identification of tumor markers that not only predict prognosis and therapeutic response but may also function as potential therapeutic targets (1, 2).

HER-2 (*erb*B2/neu) is an EGFR[2]-related tyrosine kinase receptor that is overexpressed in 20–25% of invasive breast cancers (3, 4). The oncogenic potential of HER-2 was demonstrated in part by its ability to transform normal fibroblasts (5) and to produce breast cancer in transgenic mice when overexpressed under the control of the mouse mammary tumor virus promoter (6–8). Overexpression of HER-2 occurs primarily through amplification of the wild-type *her-2* gene and is associated with poor disease-free survival (3, 9–13) and may be associated with resistance to certain types of chemotherapy (14–16).

HER-2 has become an important therapeutic target in breast cancer for several reasons. (*a*) HER-2 levels correlate strongly with the pathogenesis and prognosis of breast cancer. (*b*) The level of HER-2 in human cancer cells with gene amplification is much higher than that in normal adult tissues, potentially reducing the toxicity of HER-2-targeting drugs. (*c*) HER-2 is present in a very high proportion of tumor cells (17), and tumors with high expression (*i.e.*, an IHC score of 3+) often show uniform, intense immunohistochemical staining (18), suggesting that anti-HER-2 therapy would target most cancer cells in a given patient. (*d*) HER-2 overexpression is found in both the primary tumor and metastatic sites (19), indicating that anti-HER-2 therapy may be effective in all disease sites.

Assessment of HER-2 Status

The American Society of Clinical Oncology recommends evaluation of HER-2 status in all primary breast tumors, either at the time of diagnosis or upon recurrence (20). The HER-2 status of a tumor provides prognostic information and is the critical determinant of response to the HER-2-targeted Ab trastuzumab. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from trastuzumab.

Several methods for assessing the HER-2 status of tumors are listed in Table 1. Currently, the two most common methods of measuring HER-2 levels in the clinical setting are IHC and FISH (11–13, 21–24). IHC is the most widely used method and entails staining paraffin-embedded tissue with a HER-2-specific Ab. When using commercially available kits such as HercepTest (Dako, Carpinteria, CA) and Pathway HER2 (Ventana, Tucson, AZ), staining is graded semiquantitatively on a scale from 0 (no detectable HER-2) to 3+ (high HER-2 expression) on the basis of comparison with cell lines of known HER-2 receptor density. Tumors with a staining score of 3+ are the most responsive to trastuzumab (12, 25–27). The disadvantages of IHC include the subjective interpretation and semiquantitative nature of results. Currently available IHC kits provide control slides against which samples are compared. Such standardization is essential to assuring accurate assessment of HER-2 status (12).

FISH detects *her-2* gene amplification and is more specific and sensitive than IHC (11, 28). Importantly, FISH offers quantitative results, possibly eliminating subjectivity and variability among different laboratories. Furthermore, FISH more accurately predicts prognosis and response to trastuzumab than does IHC, because the subset of patients whose tumors overexpress HER-2 in the absence of gene amplification are less likely to respond to trastuzumab-based therapy (12, 27, 29). In general, IHC and FISH demonstrate a concordance rate of approximately 80% (30–32). The FDA has approved the use of IHC and FISH for selecting patients for trastuzumab-based therapy. Although IHC is the more widely used method, FISH should be performed

Received 4/24/03; revised 7/10/03; accepted 7/10/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

F. J. E. is a recipient of a Mentored Patient-Oriented Research Career Development Award from the National Cancer Institute (K23 CA82119).

[1] To whom requests for reprints should be addressed, at Department of Breast Medical Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 424, Houston, TX 77030-4009. Phone: (713) 792-2817; Fax: (713) 745-5768; E-mail: festeva@mdanderson.org.

[2] The abbreviations used are: EGFR, epidermal growth factor receptor; MAb, monoclonal antibody; FDA, United States Food and Drug Administration; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; ECD, extracellular domain; MBC, metastatic breast cancer; cdk, cyclin-dependent kinase; AC, doxorubicin plus cyclophosphamide; TCH, taxanes, platinum salts, and trastuzumab; FTI, farnesyl transferase inhibitor; TKI, tyrosine kinase inhibitor; Ab, antibody.

Table 1 Methods of assessing HER-2 status

Method	Advantages	Disadvantages	Clinical use
Western blot	Widely available; relatively inexpensive	Semiquantitative; Ab variability; tumor extract is required	Not in clinical use
PCR	Rapid; specific; sensitive; small amount of starting material	Semiquantitative	Not in clinical use
IHC	Widely available; relatively inexpensive	Semiquantitative; Ab variability; subjective interpretation	FDA-approved; most frequently used clinically
FISH	Specific; quantitative; strong correlation with response to trastuzumab	Expensive; requires specialized equipment not widely available	FDA-approved; valuable for confirmation of HER-2 status if IHC score is 2+
ECD ELISA	Serum easily obtained	ECD levels do not always correlate with tumor load	FDA-approved to monitor response to chemotherapy; multicenter prospective study ongoing in patients on trastuzumab

Table 2 Proposed mechanisms of action of trastuzumab

Mechanism	Ref. no.
Internalization and degradation of HER-2: disrupts receptor dimerization; disrupts downstream signaling pathways	89
G_1 arrest and reduced proliferation: induces $p27^{kip1}$-cdk2 complex formation; induces $p27^{kip1}$ levels	37 and 38
Apoptosis: inhibits Akt activity	36 and 90
Suppresses angiogenesis: reduces tumor vasculature *in vivo*; reduces expression of proangiogenic VEGF,[a] TGF-α, Ang-1, PAI-1; induces antiangiogenic TSP-1	39 and 91
Immune-mediated responses: ADCC; stimulates natural killer cells	92
Inhibits HER-2 ECD proteolysis	43

[a] VEGF, vascular endothelial growth factor; TGF, transforming growth factor; Ang-1, angiopoietin 1; PAI-1, plasminogen-activator inhibitor 1; TSP-1, thrombospondin 1; ADCC, antibody-dependent cellular cytotoxicity.

on tumors scoring 2+ by IHC (HercepTest scoring system) because FISH status improves the prediction of likelihood of response to trastuzumab (23).

Another method under investigation for predicting response to trastuzumab is the quantification of serum levels of the HER-2 ECD. The HER-2 ECD is shed into blood and is readily measured using ELISA as a circulating tumor antigen in the serum of 20–40% of patients with MBC (31, 33). The advantage of this method is that blood is relatively easy to collect, allowing routine monitoring of changes in HER-2 status in response to HER-2-targeted therapies. Our group recently showed that the rate of response to docetaxel and trastuzumab therapy was higher for patients whose levels of HER-2 ECD were high at baseline than for patients who had low HER-2 ECD levels before initiation of treatment (27). However, there is no established clinical role for monitoring changes in HER-2 ECD over time, and this approach remains investigational. A prospective multicenter study is ongoing to evaluate the role of the HER-2 ECD assay for patients with MBC who are undergoing trastuzumab-based therapy.

Mechanisms of Action of Trastuzumab

Trastuzumab (Herceptin; Genentech, South San Francisco, CA), a recombinant humanized MAb directed against the ECD of the HER-2 protein, is the only HER-2-targeted therapy approved by the FDA for the treatment of MBC. Although the mechanisms by which trastuzumab induces regression of HER-2-overexpressing tumors are incompletely defined, several molecular and cellular effects have been observed *in vitro* (Table 2). Trastuzumab and the murine MAb 4D5, from which trastuzumab is derived, induce HER-2 receptor internalization and degradation in a dose-dependent manner in the BT474 and SKBR3 HER-2-overexpressing breast cancer cell lines (34, 35). Down-regulation of HER-2 disrupts receptor dimerization and signaling through the downstream phosphatidylinositol 3′-kinase cascade (36). Cells treated with trastuzumab undergo arrest during the G_1 phase of the cell cycle, with a concomitant reduction in proliferation (35). Cell cycle arrest is accompanied by induction of the cdk inhibitor $p27^{kip1}$ and increased formation of $p27^{kip1}$-cdk2 complexes (35, 37, 38). Additional mechanisms of trastuzumab that have been demonstrated *in vivo* include suppression of angiogenesis via induction of antiangiogenic factors and repression of proangiogenic factors (39), activation of Ab-dependent cellular cytotoxicity (40–42), and inhibition of proteolytic cleavage of the HER-2 ECD (34, 43). *In vitro* studies showed that trastuzumab is synergistic with a variety of chemotherapies (44), and Pietras *et al.* (45) showed that treatment with trastuzumab prevented DNA repair following the impact of DNA-damaging drugs. However, the mechanism of synergies observed with other chemotherapy agents *in vitro* is unknown.

Clinical Trials with Trastuzumab

Initial Phase I trials of trastuzumab showed that the Ab was safe and that its pharmacokinetics were reliable (46). Response rates to trastuzumab given as a single agent ranged from 12% to 34%, depending in part on the method used to determine HER-2 status and the prior treatment received by the patients (26, 47, 48). In a pivotal randomized clinical trial, Slamon *et al.* (25) showed that combining trastuzumab with either AC or single-

agent paclitaxel produced longer time to progression, higher response rates, and improved survival rates compared with chemotherapy alone. However, the administration of AC plus trastuzumab caused severe cardiac dysfunction (25, 49, 50). Although HER-2 is not overexpressed in cardiomyocytes, HER-2, together with its coreceptor, HER-4, and the ligand heregulin, is essential for normal development of the heart ventricle. Conditional knockout mice lacking HER-2 gene expression in ventricular cardiomyocytes developed severe dilated cardiomyopathy (51). Clinical trials are under way to evaluate the safety of epirubicin and liposomal anthracyclines in combination with trastuzumab (52). Non-anthracycline-containing trastuzumab-based regimens that have shown promising results include cisplatin (53), paclitaxel administered weekly (32), docetaxel (27), vinorelbine (54), and gemcitabine (55). Combinations of TCH are highly synergistic *in vitro* (56, 57). Preliminary data from Phase II studies of TCH have shown a high response rate and an extended time to progression (58). A Phase III, randomized trial showed an improvement in median time to progression for patients treated with trastuzumab, paclitaxel, and carboplatin (13 months) compared with patients receiving trastuzumab and paclitaxel [7 months (59)]. Slamon *et al.* (60) recently reported a time to progression of 17 months for patients with HER-2-amplified MBC treated with docetaxel, carboplatin, and trastuzumab. A randomized trial of docetaxel and trastuzumab with and without carboplatin is ongoing.

Perhaps the most promising application of trastuzumab therapy will be in the adjuvant setting. Cooperative groups are conducting large randomized trials. The National Surgical Adjuvant Breast and Bowel Project-B31 protocol is randomizing node-positive, HER-2-positive breast cancer patients to four cycles of AC followed by four cycles of paclitaxel with or without trastuzumab. The Intergroup Protocol N9831 is testing a similar approach using weekly paclitaxel. In addition, trastuzumab is being administered either concomitantly with paclitaxel or after completion of AC and paclitaxel therapy. Both studies allowed HER-2 testing at local hospitals initially. However, a significant number of false-positive results were noted, and a more centralized testing approach was implemented to assure proper patient selection (61, 62). The Breast Cancer International Research Group (BCIRG Protocol 006) is evaluating the role of docetaxel with and without trastuzumab after AC chemotherapy. A third experimental arm incorporates the TCH regimen. This protocol includes node-positive and high-risk node-negative patients; HER-2 status is determined using FISH at a central laboratory. The Herceptin Adjuvant Trial is a large-scale international clinical trial led by the Breast International Group in which patients are randomized to trastuzumab *versus* no further treatment after completion of adjuvant/neoadjuvant chemotherapy. Patients receiving trastuzumab will be randomly assigned to 1 year or 2 years of trastuzumab therapy.

Future Directions

In most patients who initially respond to trastuzumab, disease progression is noted within 1 year. Combining trastuzumab with novel agents and novel strategies for targeting HER-2 may increase the magnitude and duration of response. Many new agents are currently in the preclinical or early clinical stages of development.

Trastuzumab plus the anti-EGFR TKI ZD1839 (Iressa; AstraZeneca, Wilmington, DE) produced complete remission of BT474 breast tumor xenografts (63). Because HER-2 and EGFR coexpression occurs in 10–36% of mammary carcinomas and defines one of the most aggressive tumor phenotypes, blockade of both receptors is an important therapeutic strategy. The Eastern Cooperative Oncology Group is conducting a Phase II trial in which patients with HER-2-overexpressing, trastuzumab-naive MBC will be treated with combined ZD1839 and trastuzumab (64). Blockade of EGFR may prevent transactivation of HER-2, improving response rates to trastuzumab. Such a combination may also be considered for trastuzumab-resistant tumors, in which compensatory signaling by EGFR may inhibit the response to trastuzumab.

In preclinical studies, the FTI R115777 (tipifarnib, Zarnestra; Janssen Pharmaceutica, Titusville, NJ) has demonstrated activity in breast cancer cells (65) and is being studied in combination with trastuzumab. Although breast cancers rarely demonstrate Ras mutations, aberrant Ras signaling via activated growth factor receptors such as HER-2 and EGFR may be a target for FTIs and may be inhibited to a greater degree when FTIs are combined with trastuzumab. Another novel combination being tested in patients with MBC is trastuzumab plus the cdk inhibitor flavopiridol, which together have been shown to synergistically inhibit the survival of HER-2-overexpressing breast cancer cells (66, 67). Inhibitors of the Akt cell survival pathway are also being explored as therapies in HER-2-overexpressing breast cancer. Constitutive Akt signaling is often observed in growth factor receptor-positive tumors and may contribute to trastuzumab resistance. One of the AKT inhibitors undergoing clinical testing is CCI-779 (Wyeth-Ayerst, Madison, NJ), a water-soluble ester analogue of rapamycin that inhibits the kinase mTOR downstream from Akt (68). Clinical trials of CCI-779 documented objective responses in patients with refractory breast cancer (69). Ongoing biomarker studies are evaluating the molecular mechanisms of CCI-779 in patients with early-stage breast cancer.

Novel HER-2-targeting agents, including MAbs, TKIs, and vaccines, are being developed and tested in patients with MBC (Table 3). The recombinant humanized HER-2 MAb 2C4 (Genentech) sterically blocks dimerization of HER-2 with other HER receptors (70). Thus, 2C4 should block signaling from HER-2/HER-3 and HER-2/EGFR heterodimers. Cho *et al.* (71) recently described the crystal structure of HER-2 complexed with trastuzumab. The HER-2 conformation confirms its ability to interact with other HER receptors in the absence of ligand. Altering HER-2 heterodimers has the potential to block compensatory signaling in HER-2-overexpressing tumor cells treated with trastuzumab and inhibit signaling in cells that express normal levels of HER-2. Phase I clinical trials of 2C4 in breast cancer are currently being conducted and include patients whose tumors express normal HER-2 levels.

To increase the potency of Ab-directed therapy, the specificity of the antigen-binding site has been combined with a wide variety of effector agents, including toxins (72). Using this approach, trastuzumab has been linked with the toxin DM-1 in ongoing preclinical studies. Additionally, recombinant mole-

Table 3 Novel HER-2-targeting agents

Agent	Class of compound	Phase of development in MBC	Source
Trastuzumab-DM1	MAb-toxin conjugate	Preclinical	Genentech
2C4	MAb	I	Genentech
CI-1033	TKI	II	Pfizer
GW572016	TKI	II	Glaxo Smithkline
E1A	Transcriptional inhibitor	I	Targeted Genetics
2B1	Bispecific Ab against HER-2 and F_c RIII	II	Chiron
AutoVac	DNA vaccine	II	Pharmexa

cules in which the Ab-combining site is fused directly to the toxin have been developed and show strong selectivity for HER-2 binding (72, 73). Recombinant toxins show promise in that they can be safely delivered to experimental animals at effective doses and may penetrate tumors more effectively than trastuzumab alone (74, 75). However, one limitation facing the development of toxin targeting is the potential for immune response to the protein.

In addition to Abs targeting the HER-2 ECD, TKIs that directly inhibit the cytoplasmic tyrosine kinase domain of growth factor receptors are being developed. Several of these agents inhibit more than one HER/erbB receptor. CI-1033 (PD183805; Pfizer, New York, NY) is an orally available pan-HER TKI that irreversibly inhibits all HER receptors. Homologous kinase domains shared by the HER receptors can be targeted by small molecule pan-HER inhibitors to simultaneously block signaling from all active receptors (76). Phase I trials of single-agent CI-1033 in which pre- and posttreatment tumor biopsy specimens were studied for biomarkers revealed a 40–50% reduction in EGFR and HER-2 phosphorylation, which correlated with decreased proliferation. Although partial remissions and stable disease occurred primarily in patients with squamous cell skin cancer and advanced-stage non-small cell lung cancer, respectively, one heavily pretreated patient with breast cancer has remained in a CI-1033 Phase I trial for more than 6 months without disease progression (77). Current clinical trials include testing of CI-1033 in patients with MBC whose disease did not respond to trastuzumab therapy. GW572016 (GlaxoSmithKline, Research Triangle Park, NC) is another novel inhibitor of the EGFR and HER-2 tyrosine kinases undergoing clinical testing in breast cancer patients. This agent has shown remarkable *in vitro* and *in vivo* activity, leading to growth arrest and/or apoptosis in EGFR- and HER-2-dependent tumor cell lines. GW572016 markedly reduced tyrosine phosphorylation of EGFR and erbB2 and inhibited activation of extracellular signal-regulated kinase 1/2 and AKT, downstream effectors of proliferation and cell survival, respectively (78). Ongoing studies are evaluating the safety and efficacy of GW572016 as a single agent and in combination with other biological agents. A multicenter, Phase II study is evaluating the efficacy of GW572016 as monotherapy for patients who develop progressive disease while on trastuzumab-based therapy. Because trastuzumab resistance is a considerable clinical problem that may be due to compensatory signaling by other HER receptors, pan-HER inhibitors such as CI-1033 and GW572016 may offer a new therapeutic strategy in this patient population.

In addition to the previously discussed strategies that target the HER-2 protein, strategies that prevent the synthesis of HER-2 mRNA are also being developed. One such strategy is derived from the finding that the HER-2 gene can be repressed by the introduction of the adenovirus E1A gene (79). Delivery of E1A expression constructs into human tumor cell lines using liposomes has resulted in inhibition of HER-2 expression and loss of tumorigenicity (80). A Phase I clinical trial of E1A therapy showed that intracavitary injection of the EIA gene complexed with DC-Chol cationic liposome (DCC-E1A; Targeted Genetics) is feasible in patients with breast cancer (81).

Two approaches to immunotherapy that rely on targeting by anti-HER-2 Abs have been developed; both are designed to deliver immune effector cells to the tumor. The first approach is to use a single chimeric protein molecule that features two Ab-binding specificities: (*a*) one that binds HER-2; and (*b*) one that binds an immune cell via CD16, Fc receptor III (82), or CD3 (83). The toxicity of this therapy has been assessed in Phase I clinical studies, and there is evidence that a biologically relevant concentration of the experimental therapeutic can be achieved (84, 85).

DNA and peptide-based vaccine strategies designed to specifically boost HER-2 immunity are being tested in patients with MBC. Initial results demonstrated that significant levels of HER-2 immunity can be generated with active immunization and that the T cells generated against HER-2 do not produce an autoimmune response against cells with normal HER-2 levels (86). However, initial strategies using single HLA binding epitopes to induce cytotoxic $CD8^+$ T cells produced transient responses (86, 87). More recent approaches generating active immunization against HER-2 with $CD4^+$ T-helper epitopes resulted in the development of T-cell immunity in 92% of patients with MBC, ovarian cancer, and non-small cell lung cancer, with responses persisting in 38% of these patients at a follow-up time of 1 year (88). The clinical role of cancer vaccines remains to be defined. HER-2 vaccines may be useful as adjuvant therapies to prevent relapse by establishing an effective memory response or as treatments for patients whose disease has progressed during treatment with HER-2 MAb (85, 87).

Conclusions

Currently, the optimal duration of HER-2-targeted treatment is unknown. In most patients who initially respond to trastuzumab, disease progression begins again within 1 year. A clearer understanding of the mechanisms that contribute to trastuzumab resistance is needed to increase the magnitude and duration of response. Elucidating the molecular changes that

occur as tumors progress on trastuzumab therapy will allow the design of targeted therapies to be used in combination with or after trastuzumab. Additionally, new HER-2 targeting strategies are in preclinical and clinical development stages.

Acknowledgments

We thank Kate O'Suilleabhain for editorial assistance.

References

1. Esteva, F. J., and Hayes, D. F. Monoclonal antibody-based therapy of breast cancer. *In:* M. L. Grossbard (ed.), Monoclonal Antibody-Based Therapy of Cancer, pp. 309–338. New York: Marcel Decker, 1998.

2. Atkins, J. H., and Gershell, L. J. Selective anticancer drugs. Nat. Rev. Drug Discov., *1:* 491–492, 2002.

3. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science (Wash. DC), *235:* 177–182, 1987.

4. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A., and Press, M. F. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science (Wash. DC), *244:* 707–712, 1989.

5. Hudziak, R. M., Schlessinger, J., and Ullrich, A. Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. Proc. Natl. Acad. Sci. USA, *84:* 7159–7163, 1987.

6. Bouchard, L., Lamarre, L., Tremblay, P. J., and Jolicoeur, P. Stochastic appearance of mammary tumors in transgenic mice carrying the mmtv/c-neu oncogene. Cell, *57:* 931–936, 1989.

7. Guy, C. T., Webster, M. A., Schaller, M., Parsons, T. J., Cardiff, R. D., and Muller, W. J. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc. Natl. Acad. Sci. USA, *89:* 10578–10582, 1992.

8. Guy, C. T., Cardiff, R. D., and Muller, W. J. Activated neu induces rapid tumor progression. J. Biol. Chem., *271:* 7673–7678, 1996.

9. Press, M. F., Bernstein, L., Thomas, P. A., Meisner, L. F., Zhou, J. Y., Ma, Y., Hung, G., Robinson, R. A., Harris, C., el-Naggar, A., Slamon, D. J., Phillips, R. N., Ross, J. S., Wolman, S. R., and Flom, K. J. 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.

10. Depowski, P., Mulford, D., Minot, D., Bourne, P., McCabe, C., and McHugh, L. Comparative analysis of Her-2/neu protein overexpression in breast cancer using paraffin-embedded tissue and cytologic specimens. Mod. Pathol., *15:* 70A, 2002.

11. Press, M. F., Slamon, D. J., Flom, K. J., Park, J., Zhou, J. Y., and Bernstein, L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J. Clin. Oncol., *20:* 3095–3105, 2002.

12. Leonard, D. S., Hill, A. D., Kelly, L., Dijkstra, B., McDermott, E., and O'Higgins, N. J. Anti-human epidermal growth factor receptor 2 monoclonal antibody therapy for breast cancer. Br. J. Surg., *89:* 262–271, 2002.

13. Joensuu, H., Isola, J., Lundin, M., Salminen, T., Holli, K., Kataja, V., Pylkkanen, L., Turpeenniemi-Hujanen, T., von Smitten, K., and Lundin, J. Amplification of erbB2 and erbB2 expression are superior to estrogen receptor status as risk factors for distant recurrence in $pT_1N_0M_0$ breast cancer: a nationwide population-based study. Clin. Cancer Res., *9:* 923–930, 2003.

14. Gusterson, B. A., Gelber, R. D., Goldhirsch, A., Price, K. N., Save-Soderborgh, J., Anbazhagan, R., Styles, J., Rudenstam, C. M., Golouh, R., Reed, R., Martinez-Tello, F., Tiltman, A., Torhorst, J., Grigolato, P., Bettelheim, R., Neville, A. M., Burki, K., Castiglione, M., Collins, J., Lindtner, J., and Senn, H. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. J. Clin. Oncol., *10:* 1049–1056, 1992.

15. Paik, S., Bryant, J., Park, C., Fisher, B., Tan-Chiu, E., Hyams, D., Fisher, E. R., Lippman, M. E., Wickerham, D. L., and Wolmark, N. erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. J. Natl. Cancer Inst. (Bethesda), *90:* 1361–1370, 1998.

16. Paik, S., Bryant, J., Tan-Chiu, E., Yothers, G., Park, C., Wickerham, D. L., and 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. (Bethesda), *92:* 1991–1998, 2000.

17. Eccles, S. A. The role of c-erbB-2/HER2/neu in breast cancer progression and metastasis. J. Mammary Gland Biol. Neoplasia, *6:* 393–406, 2001.

18. Paik, S., Hazan, R., Fisher, E. R., Sass, R. E., Fisher, B., Redmond, C., Schlessinger, J., Lippman, M. E., and King, C. R. 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.

19. Niehans, G. A., Singleton, T. P., Dykoski, D., and Kiang, D. T. Stability of HER-2/neu expression over time and at multiple metastatic sites. J. Natl. Cancer Inst. (Bethesda), *85:* 1230–1235, 1993.

20. Bast, R. C., Jr., Ravdin, P., Hayes, D. F., Bates, S., Fritsche, H., Jr., Jessup, J. M., Kemeny, N., Locker, G. Y., Mennel, R. G., Somerfield, M. R., and 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.

21. Pauletti, G., Dandekar, S., Rong, H., Ramos, L., Peng, H., Seshadri, R., and Slamon, D. J. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence *in situ* hybridization and immunohistochemistry. J. Clin. Oncol., *18:* 3651–3664, 2000.

22. Bilous, M. Predicting HER2 status of breast cancer from basic pathology features: HER2 status of 1500 breast cancers determined by immunohistochemistry and fluorescence *in situ* hybridisation with pathology correlation. Eur. J. Cancer, *38* (Suppl.)*:* S52, 2002.

23. Fornier, M., Risio, M., Van Poznak, C., and Seidman, A. HER2 testing and correlation with efficacy of trastuzumab therapy. Oncology (Huntingt.), *16:* 1340–1342, 2002.

24. Schaller, G., Evers, K., Papadopoulos, S., Ebert, A., and Buhler, H. Current use of HER2 tests. Ann. Oncol., *12* (Suppl. 1)*:* S97–S100, 2001.

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

26. Vogel, C. L., Cobleigh, M. A., Tripathy, D., Gutheil, J. C., Harris, L. N., Fehrenbacher, L., Slamon, D. J., Murphy, M., Novotny, W. F., Burchmore, M., Shak, S., Stewart, S. J., and 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.

27. Esteva, F. J., Valero, V., Booser, D., Guerra, L. T., Murray, J. L., Pusztai, L., Cristofanilli, M., Arun, B., Esmaeli, B., Fritsche, H. A., Sneige, N., Smith, T. L., and Hortobagyi, G. N. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J. Clin. Oncol., *20:* 1800–1808, 2002.

28. Persons, D. L., Bui, M. M., Lowery, M. C., Mark, H. F., Yung, J. F., Birkmeier, J. M., Wong, E. Y., Yang, S. J., and Masood, S. Fluorescence *in situ* hybridization (FISH) for detection of HER-2/neu amplification in breast cancer: a multicenter portability study. Ann. Clin. Lab. Sci., *30:* 41–48, 2000.

29. Mass, R. D., Press, M., Anderson, S., Murphy, M., and Slamon, D. Improved survival benefit from Herceptin (trastuzumab) in patients selected by fluorescence *in situ* hybridization (FISH). Proc. Am. Soc. Clin. Oncol., *20:* 85, 2001.

30. Jacobs, T. W., Gown, A. M., Yaziji, H., Barnes, M. J., and Schnitt, S. J. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. J. Clin. Oncol., *17:* 1983–1987, 1999.

31. Harris, L. N., Liotcheva, V., Broadwater, G., Ramirez, M. J., Maimonis, P., Anderson, S., Everett, T., Harpole, D., Moore, M. B., Berry, D. A., Rizzeri, D., Vredenburgh, J. J., and Bentley, R. C. Comparison of methods of measuring HER-2 in metastatic breast cancer patients treated with high-dose chemotherapy. J. Clin. Oncol., *19:* 1698–1706, 2001.

32. Seidman, A. D., Fornier, M., Esteva, F. J., Tan, L., Kaptain, S., Bach, A., Panageas, K. S., Arroyo, C., Valero, V., Currie, V., Gilewski, T., Theodoulou, M., Moynahan, M. E., Moasser, M., Sklarin, N., Dickler, M., D'Andrea, G., Cristofanilli, M., Rivera, E., Hortobagyi, G. N., Norton, L., and Hudis, C. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. J. Clin. Oncol., *19:* 2587–2595, 2001.

33. Baselga, J. Is circulating HER-2 more than just a tumor marker? Clin. Cancer Res., *7:* 2605–2607, 2001.

34. Baselga, J., Albanell, J., Molina, M. A., and Arribas, J. Mechanism of action of trastuzumab and scientific update. Semin. Oncol., *28* (Suppl.)*:* 4–11, 2001.

35. Sliwkowski, M. X., Lofgren, J. A., Lewis, G. D., Hotaling, T. E., Fendly, B. M., and Fox, J. A. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin. Oncol., *26* (Suppl. 12)*:* 60–70, 1999.

36. Yakes, F. M., Chinratanalab, W., Ritter, C. A., King, W., Seelig, S., and Arteaga, C. L. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res., *62:* 4132–4141, 2002.

37. Lane, H. A., Beuvink, I., Motoyama, A. B., Daly, J. M., Neve, R. M., and Hynes, N. E. ErbB2 potentiates breast tumor proliferation through modulation of $p27^{Kip1}$-Cdk2 complex formation: receptor overexpression does not determine growth dependency. Mol. Cell. Biol., *20:* 3210–3223, 2000.

38. Lane, H. A., Motoyama, A. B., Beuvink, I., and Hynes, N. E. Modulation of p27/Cdk2 complex formation through 4D5-mediated inhibition of HER2 receptor signaling. Ann. Oncol., *12* (Suppl.)*:* 21–22, 2001.

39. Izumi, Y., Xu, L., di Tomaso, E., Fukumura, D., and Jain, R. K. Tumour biology: Herceptin acts as an anti-angiogenic cocktail. Nature (Lond.), *416:* 279–280, 2002.

40. Carter, P., Presta, L., Gorman, C. M., Ridgway, J. B., Henner, D., Wong, W. L., Rowland, A. M., Kotts, C., Carver, M. E., and Shepard, H. M. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc. Natl. Acad. Sci. USA, *89:* 4285–4289, 1992.

41. Clynes, R. A., Towers, T. L., Presta, L. G., and Ravetch, J. V. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. Nat. Med., *6:* 443–446, 2000.

42. Lewis, G. D., Figari, I., Fendly, B., Wong, W. L., Carter, P., Gorman, C., and Shepard, H. M. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol. Immunother., *37:* 255–263, 1993.

43. Molina, M. A., Codony-Servat, J., Albanell, J., Rojo, F., Arribas, J., and Baselga, J. Trastuzumab (Herceptin), a humanized anti-HER2 receptor monoclonal antibody, inhibits basal and activated HER2 ectodomain cleavage in breast cancer cells. Cancer Res., *61:* 4744–4749, 2001.

44. Pegram, M., Hsu, S., Lewis, G., Pietras, R., Beryt, M., Sliwkowski, M., Coombs, D., Baly, D., Kabbinavar, F., and Slamon, D. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene, *18:* 2241–2251, 1999.

45. Pietras, R. J., Fendly, B. M., Chazin, V. R., Pegram, M. D., Howell, S. B., and Slamon, D. J. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene, *9:* 1829–1838, 1994.

46. Baselga, J. Phase I and II clinical trials of trastuzumab. Ann. Oncol., *12* (Suppl.)*:* 49–55, 2001.

47. Baselga, J., Tripathy, D., Mendelsohn, J., Baughman, S., Benz, C. C., Dantis, L., Sklarin, N. T., Seidman, A. D., Hudis, C. A., Moore, J., Rosen, P. P., Twaddell, T., Henderson, I. C., and Norton, L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J. Clin. Oncol., *14:* 737–744, 1996.

48. Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G., and Slamon, D. J. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER-2 overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J. Clin. Oncol., *17:* 2639–2648, 1999.

49. Keefe, D. L. Trastuzumab-associated cardiotoxicity. Cancer (Phila.), *95:* 1592–1600, 2002.

50. Seidman, A., Hudis, C., Pierri, M. K., Shak, S., Paton, V., Ashby, M., Murphy, M., Stewart, S. J., and Keefe, D. Cardiac dysfunction in the trastuzumab clinical trials experience. J. Clin. Oncol., *20:* 1215–1221, 2002.

51. Ozcelik, C., Erdmann, B., Pilz, B., Wettschureck, N., Britsch, S., Hubner, N., Chien, K. R., Birchmeier, C., and Garratt, A. N. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. Proc. Natl. Acad. Sci. USA, *99:* 8880–8885, 2002.

52. Winer, E. P., and Burstein, H. J. New combinations with Herceptin® in metastatic breast cancer. Oncology (Basel), *61* (Suppl.)*:* 50–57, 2001.

53. Pegram, M. D., Lipton, A., Hayes, D. F., Weber, B. L., Baselga, J. M., Tripathy, D., Baly, D., Baughman, S. A., Twaddell, T., Glaspy, J. A., and Slamon, D. J. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J. Clin. Oncol., *16:* 2659–2671, 1998.

54. Burstein, H. J., Kuter, I., Campos, S. M., Gelman, R. S., Tribou, L., Parker, L. M., Manola, J., Younger, J., Matulonis, U., Bunnell, C. A., Partridge, A. H., Richardson, P. G., Clarke, K., Shulman, L. N., and Winer, E. P. Clinical activity of trastuzumab and vinorelbine in women with HER-2 overexpressing metastatic breast cancer. J. Clin. Oncol., *19:* 2722–2730, 2001.

55. O'Shaughnessy, J. A., Vukelja, S., Marsland, T., Kimmel, G., Ratnam, S., and Pippen, J. Gemcitabine and trastuzumab for HER-2 positive metastatic breast cancer: preliminary results of a Phase II study. Breast Cancer Res. Treat., *69:* 302, 2001.

56. Konecny, G., Fritz, M., Untch, M., Lebeau, A., Felber, M., Lude, S., Beryt, M., Hepp, H., Slamon, D., and Pegram, M. HER-2/neu overexpression and *in vitro* chemosensitivity to CMF and FEC in primary breast cancer. Breast Cancer Res. Treat., *69:* 53–63, 2001.

57. Pegram, M. D., Lopez, A., Konecny, G., and Slamon, D. J. Trastuzumab and chemotherapeutics: drug interactions and synergies. Semin. Oncol., *27* (Suppl. 11): 21–25, 2000.

58. Nabholtz, J. M., Crown, J., Yonemoto, L., Tannenbaum, S., Klimo, P., Patel, R., Fumoleau, P., Sanchez, J., Prady, C., Villa, D., *et al.* Results of two open-label multicenter pilot Phase II trials with Herceptin in combination with docetaxel and platinum salts (cis- or carboplatin) (TCH) as therapy for advanced breast cancer in women overexpressing HER2. Breast Cancer Res. Treat., *64:* 327, 2000.

59. Robert, N., Leyland-Jones, B., Asmar, L., Belt, R., Ilegbodu, D., Loesch, D., Raju, R., Valentine, E., Sayre, R., Albain, K., Cobleigh, M., McCullough, C., Fuchs, L., and Slamon, D. Phase III comparative study of trastuzumab and paclitaxel with and without carboplatin in patients with HER2/Neu positive advanced breast cancer. Breast Cancer Res. Treat., *76* (Suppl. 1)*:* 35, 2002.

60. Slamon, D. J., Patel, R., Northfelt, R., Pegram, M., *et al.* Phase II pilot study of Herceptin combined with Taxotere and carboplatin in metastatic breast cancer patients overexpressing the HER2-Neu protooncogene: a pilot study of the UCLA Network. Proc. Am. Soc. Clin. Oncol., *20:* 193, 2001.

61. Paik, S., Bryant, J., Tan-Chiu, E., Romond, E., Hiller, W., Park, K., Brown, A., Yothers, G., Anderson, S., Smith, R., Wickerham, D. L., and Wolmark, N. Real-world performance of HER2 testing: National Sur-

gical Adjuvant Breast and Bowel Project experience. J. Natl. Cancer Inst. (Bethesda), *94:* 852–854, 2002.

62. Roche, P. C., Suman, V. J., Jenkins, R. B., Davidson, N. E., Martino, S., Kaufman, P. A., Addo, F. K., Murphy, B., Ingle, J. N., and Perez, E. A. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. J. Natl. Cancer Inst. (Bethesda), *94:* 855–857, 2002.

63. Moulder, S. L., Yakes, F. M., Muthuswamy, S. K., Bianco, R., Simpson, J. F., and Arteaga, C. L. Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells *in vitro* and *in vivo*. Cancer Res., *61:* 8887–8895, 2001.

64. Arteaga, C. L., Moulder, S. L., and Yakes, F. M. HER (erbB) tyrosine kinase inhibitors in the treatment of breast cancer. Semin. Oncol., *29:* 4–10, 2002.

65. Kelland, L. R., Smith, V., Valenti, M., Patterson, L., Clarke, P. A., Detre, S., End, D., Howes, A. J., Dowsett, M., Workman, P., and Johnston, S. R. D. Preclinical antitumor activity and pharmacodynamic studies with the farnesylprotein transferase inhibitor R115777 in human breast cancer. Clin. Cancer Res., *7:* 3544–3550, 2001.

66. Nahta, R., Iglehart, J. D., Kempkes, B., and Schmidt, E. V. Rate-limiting effects of cyclin D1 in transformation by ErbB2 predicts synergy between Herceptin and flavopiridol. Cancer Res., *62:* 2267–2271, 2002.

67. Nahta, R., Trent, S., Yang, C., and Schmidt, E. V. Epidermal growth factor receptor expression is a candidate target of the synergistic combination of trastuzumab and flavopiridol in breast cancer. Cancer Res., *63:* 3626–3631, 2003.

68. Hidalgo, M., and Rowinsky, E. K. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene, *19:* 6680–6686, 2000.

69. Chan, S., Johnson, S., and Scheulen, M. E. First report: a Phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. Proc. Am. Soc. Clin. Oncol., *21:* 175, 2002.

70. Agus, D. B., Akita, R. W., Fox, W. D., Lewis, G. D., Higgins, B., Pisacane, P. I., Lofgren, J. A., Tindell, C., Evans, D. P., Maiese, K., Scher, H. I., and Sliwkowski, M. X. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell, *2:* 127–137, 2002.

71. Cho, H. S., Mason, K., Ramyar, K. X., Stanley, A. M., Gabelli, S. B., Denney, D. W., Jr., and Leahy, D. J. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature (Lond.), *421:* 756–760, 2003.

72. Batra, J. K., Kasprzyk, P. G., Bird, R. E., Pastan, I., and King, C. R. Recombinant anti-erbB-2 immunotoxins containing *Pseudomonas* exotoxin. Proc. Natl. Acad. Sci. USA, *89:* 5867–5871, 1992.

73. Wels, W., Harwerth, I. M., Mueller, M., Groner, B., and Hynes, N. E. Selective inhibition of tumor cell growth by a recombinant single-chain antibody-toxin specific for the erbB-2 receptor. Cancer Res., *52:* 6310–6317, 1992.

74. King, C. R., Fischer, P. H., Rando, R. F., and Pastan, I. The performance of e23(Fv)PE, recombinant toxins targeting the erbB-2 protein. Semin. Cancer Biol., *7:* 79–86, 1996.

75. Reiter, Y., Pai, L. H., Brinkmann, U., Wang, Q. C., and Pastan, I. Antitumor activity and pharmacokinetics in mice of a recombinant immunotoxin containing a disulfide-stabilized Fv fragment. Cancer Res., *54:* 2714–2718, 1994.

76. Rusnak, D. W., Affleck, K., Cockerill, S. G., Stubberfield, C., Harris, R., Page, M., Smith, K. J., Guntrip, S. B., Carter, M. C., Shaw, R. J., Jowett, A., Stables, J., Topley, P., Wood, E. R., Brignola, P. S., Kadwell, S. H., Reep, B. R., Mullin, R. J., Alligood, K. J., Keith, B. R., Crosby, R. M., Murray, D. M., Knight, W. B., Gilmer, T. M., and Lackey, K. The characterization of novel, dual ErbB-2/EGFR, tyrosine kinase inhibitors: potential therapy for cancer. Cancer Res., *61:* 7196–7203, 2001.

77. Allen, L. F., Lenehan, P. F., Eiseman, I. A., Elliott, W. L., and Fry, D. W. Potential benefits of the irreversible pan-erbB inhibitor, CI-1033, in the treatment of breast cancer. Semin. Oncol., *29:* 11–21, 2002.

78. Xia, W., Mullin, R. J., Keith, B. R., Liu, L. H., Ma, H., Rusnak, D. W., Owens, G., Alligood, K. J., and Spector, N. L. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. Oncogene, *21:* 6255–6263, 2002.

79. Yu, D., Suen, T-C., Yan, D-H., Chang, L-S., and Hung, M-C. Transcriptional repression of the neu protooncogene by the adenovirus 5 E1A gene products. Proc. Natl. Acad. Sci. USA, *87:* 4499–4503, 1990.

80. Yu, D., Matin, A., Xia, W., Sorgi, F., Huang, L., and Hung, M. C. Liposome-mediated *in vivo* E1A gene transfer suppressed dissemination of ovarian cancer cells that overexpress HER-2/neu. Oncogene, *11:* 1383–1388, 1995.

81. Hortobagyi, G. N., Ueno, N. T., Xia, W. Y., Zhang, S., Wolf, J. K., Putnam, J. B., Weiden, P. L., Willey, J. S., Carey, M., Branham, D. L., Payne, J. Y., Tucker, S. D., Bartholomeusz, C., Kilbourn, R. G., de Jager, R. L., Sneige, N., Katz, R. L., Anklesaria, P., Ibrahim, N. K., Murray, J. L., Theriault, R. L., Valero, V., Gershenson, D. M., Bevers, M. W., Huang, L., Lopez-Berestein, G., and Hung, M. C. Cationic liposome-mediated E1A gene transfer to human breast and ovarian cancer cells and its biologic effects: a Phase I clinical trial. J. Clin. Oncol., *19:* 3422–3433, 2001.

82. Weiner, L. M., Holmes, M., Adams, G. P., LaCreta, F., Watts, P., and Garcia de Palazzo, I. A human tumor xenograft model of therapy with a bispecific monoclonal antibody targeting c-erbB-2 and CD16. Cancer Res., *53:* 94–100, 1993.

83. Shalaby, M. R., Carter, P., Maneval, D., Giltinan, D., and Kotts, C. Bispecific HER2 × CD3 antibodies enhance T-cell cytotoxicity *in vitro* and localize to HER2-overexpressing xenografts in nude mice. Clin. Immunol. Immunopathol., *74:* 185–192, 1995.

84. Weiner, L. M., Clark, J. I., Davey, M., Li, W. S., Garcia de Palazzo, I., Ring, D. B., and Alpaugh, R. K. Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc γ RIII. Cancer Res., *55:* 4586–4593, 1995.

85. Weiner, L. M., Clark, J. I., Ring, D. B., and Alpaugh, R. K. Clinical development of 2B1, a bispecific murine monoclonal antibody targeting c-erbB-2 and Fc γ RIII. J. Hematother., *4:* 453–456, 1995.

86. Knutson, K. L., Schiffman, K., Cheever, M. A., and Disis, M. L. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369–377, results in short-lived peptide-specific immunity. Clin. Cancer Res., *8:* 1014–1018, 2002.

87. Bernhard, H., Salazar, L., Schiffman, K., Smorlesi, A., Schmidt, B., Knutson, K. L., and Disis, M. L. Vaccination against HER-2/neu oncogenic protein. Endocr. Relat. Cancer, *9:* 33–44, 2002.

88. Disis, M. L., Gooley, T. A., Rinn, K., Davis, D., Piepkorn, M., Cheever, M. A., Knutson, K. L., and Schiffman, K. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. J. Clin. Oncol., *20:* 2624–2632, 2002.

89. Sarup, J. C., Johnson, R. M., King, K. L., Fendly, B. M., Lipari, M. T., Napier, M. A., Ullrich, A., and Shepard, H. M. Characterization of an anti-p185her2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. Growth Regul., *1:* 72–82, 1991.

90. Cuello, M., Ettenberg, S. A., Clark, A. S., Keane, M. M., Posner, R. H., Nau, M. M., Dennis, P. A., and Lipkowitz, S. Down-regulation of the erbB-2 receptor by trastuzumab (Herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. Cancer Res., *61:* 4892–4900, 2001.

91. Petit, A. M., Rak, J., Hung, M. C., Rockwell, P., Goldstein, N., Fendly, B., and Kerbel, R. S. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: angiogenic implications for signal transduction therapy of solid tumors. Am. J. Pathol., *151:* 1523–1530, 1997.

92. Cooley, S., Burns, L. J., Repka, T., and Miller, J. S. 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., *27:* 1533–1541, 1999.