Management of HER2-Positive Breast Cancer

By Eric P. Winer, MD, Martine J. Piccart-Gebhart, MD, PhD, Hope S. Rugo, MD, and George W. Sledge Jr, MD

Overview **HER2-positive breast cancer accounts for approximately 20% of all cases of breast cancer. These tumors have a distinct natural history that, in the absence of systemic therapy, is associated with a short disease-free interval and an aggressive course in the metastatic setting. Once HER2 is activated, signaling through the HER2 pathway plays a major role in cell growth and survival, as well as the potential to metastasize. For the past 7 years, trastuzumab, generally combined with chemotherapy, has been a standard treatment in women with HER2-positive metastatic disease. Results from large adjuvant trials have now established the role of trastuzumab in the adjuvant setting as well. Until recently, preoperative treatment with trastuzumab-based regimens was administered within the context of clinical trials for several years. With the findings of the adjuvant trials, preoperative therapy with trastuzumab will be used increasingly. There are many unanswered questions about the optimal use of trastuzumab, largely because of an insufficient understanding of the underlying biology. One of the most critical questions concerns the mechanism(s) of resistance to trastuzumab. Greater knowledge in this area will ultimately lead to therapeutic approaches that minimize the chance of resistance.**

HER2-POSITIVE TUMORS account for approximately 20% of all breast cancers. In the absence of systemic treatment, these tumors are associated with a particularly aggressive clinical course. HER2-positive tumors tend to be larger than HER2-negative tumors, present more commonly with lymph node involvement, and are more likely to be associated with a short disease-free survival. HER2-positive disease may be resistant to cyclophosphamide, methotrexate, and fluorouracil-type chemotherapy,[1,2] although reports in the literature are mixed. With greater consistency, HER2-positive disease appears to be particularly sensitive to anthracyclines.[3-5] Approximately 50% of HER2-positive cancers are hormone receptor negative, and the remainder are positive for estrogen and/or progesterone receptor.[6,7] It is unclear, however, whether patients with HER2-positive disease derive as much benefit from hormone therapy as those who have HER2-negative tumors.

In the setting of *HER2* gene amplification or high levels of HER2 expression, the HER family of receptors and their associated signal-transduction pathways play a dominant role in cell growth and survival. Inhibition of HER2 with trastuzumab, the only molecularly targeted treatment approved for breast cancer apart from endocrine agents, appears to have a profound effect on the natural history of the disease. The rational development of trastuzumab started in 1984 with the identification of the HER2/*neu* oncogene,[8] which was soon followed by its cloning[9,10] and the demonstration that an anti-HER2 monoclonal antibody inhibits *neu*-transformed cells.[11] The bench-to-bedside process was initiated in 1987, when *HER2* amplification was found to be correlated with a poor prognosis in breast cancer,[12] and attracted considerable attention with the report in 2001 that trastuzumab combined with chemotherapy improved survival among women with metastatic HER2-positive disease.[13] More recently, five randomized clinical trials have shown that adjuvant trastuzumab reduces the risk of recurrent HER2-positive disease by roughly 50%,[6,7,14,15] a magnitude rarely observed in breast cancer trials. This article addresses the use of trastuzumab in the adjuvant, preoperative, and metastatic settings, and discusses the potential mechanisms underlying trastuzumab resistance.

ADJUVANT THERAPY WITH TRASTUZUMAB

Five recently reported trials have established the efficacy of adjuvant treatment with trastuzumab in women with HER2-positive breast cancer: the Herceptin Adjuvant (HERA) trial,[6] the combined North American National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 and North Central Cancer Treatment Group (NCCTG) N9831 trials,[7] the Breast Cancer International Research Group (BCIRG) 006 trial,[14] and the Finnish trial.[15] This section describes the trial designs and patient populations, analyzes the safety and efficacy findings, and notes questions that remain to be answered.

Trial Designs

Table 1 summarizes the designs of the five adjuvant trials. Although a total of 13,353 patients were enrolled on these trials, a substantial number of patients are not included in the current analyses. A total of 1,694 patients in the 2-year trastuzumab group in the HERA trial have been excluded because the current results for this group are protected by the independent data monitoring committee; 842 patients in the group that received chemotherapy followed by trastuzumab in the N9831 trial have been excluded because this group is unsuitable for combined analysis with the NSABP B31 trial, and 334 patients (325 in B31 and N9831 combined and nine in HERA) have been excluded because follow-up data are pending. Finally, 152 patients in N9831 have been excluded because they were enrolled in the control group during temporary closure of

From the Dana-Farber Cancer Institute, Boston, MA; Jules Bordet Institute, Brussels, Belgium; University of California, San Francisco, CA; Indiana University Medical Center, Indianapolis, IN.

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Address reprint requests to Eric Winer, MD, Dana-Farber Cancer Institute, 44 Binney St, Room D1210, Boston, MA 02115; e-mail: ewiner@partners.org.

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Table 1. Trial Designs

Trial	Accrual	No. of Patients Included in Analysis	Treatment Regimens	Trastuzumab Schedule	Primary Endpoint	
HERA	5,090	3,387	Any accepted CT alone T given 1 yr after completion of CT T given 2 yr after completion of CT (not included in analysis)	Every 3 wk	OS	DFS for combined analysis
NSABP B31	2,043	1,736	AC × 4→P × 4 AC × 4→P × 4 + T P given every 3 wk	Weekly	DFS	
Intergroup N9831	2,766	1,615	AC × 4→P × 4 AC × 4→P × 4 + T starting concurrently with P AC × 4→P × 4 + T starting after P (not included in analysis) P given every 3 wk	Weekly	DFS	
BCIRG 006	3,222	3,222	AC × 4 → D × 4 AC × 4 → D × 4 + T starting concurrently with D DCb × 6 + T D given every 3 wk	Weekly with CT, then every 3 wk	DFS	
FinHer	232	232	V or D × 3, with or without 9 wk of T→FEC × 3	Weekly	RFS	

Abbreviations: HERA, Herceptin Adjuvant; CT, chemotherapy; T, trastuzumab; yr, year; wk, week; OS, overall survival; NSABP, National Surgical Adjuvant Breast and Bowel Project; AC, doxorubicin and cyclophosphamide; →, followed by; P, paclitaxel; DFS, disease-free survival; BCIRG, Breast Cancer International Research Group; D, docetaxel; DCb, docetaxel and carboplatin; FinHer, Finnish trial; V, vinorelbine; FEC, fluorouracil, epirubicin, and cyclophosphamide; RFS, recurrence-free survival.

the concomitant trastuzumab chemotherapy group because of safety concerns.

The administration of trastuzumab after chemotherapy in the HERA trial permits the application of its findings to the wide variety of chemotherapy regimens used throughout the world. The HERA design, however, delayed the start of trastuzumab until a median of 8 months after surgery. In contrast, the delay was approximately 4 months in B31 and N9831, and 1 month in the Finnish trial and in the platinum and taxane group in BCIRG 006. The important question of the optimal timing of adjuvant trastuzumab remains unanswered at this time.[16,17] Unfortunately, there may never be a clear and absolute answer to this question because time to initiation of trastuzumab is confounded by other variables (e.g., sequential compared with concurrent administration with chemotherapy).

The effect of differences among the trials in trastuzumab and taxane scheduling on the outcome is unlikely to be important for several reasons. First, the large Eastern Cooperative Oncology Group (ECOG) 1199 trial showed similarities in disease-free survival whether the taxane (docetaxel or paclitaxel) was given every 3 weeks or weekly[18] (although these are early results, and a separate analysis for hormone-receptor–negative disease has not yet been performed). In addition, trastuzumab has a long half-life, and it has similar antitumor activity in advanced breast cancer whether given weekly or every 3 weeks (although a head-to-head comparison of these two schedules has never been performed).[19-22] The long half-life of trastuzumab suggests that blood levels of the drug persisted in the Finnish patients while they were receiving fluorouracil, epirubicin, and cyclophosphamide (FEC) — an unusual feature of this small trial, which may have taken advantage of anthracycline and trastuzumab synergy.

Some similarities among patient characteristics across the five trials include young age (median, approximately 50), a large proportion of high-grade tumors (60%–70%), and planned endocrine therapy because of hormone-receptor–positive tumors in roughly 50% of the patients.

Differences include the proportion of women with node-negative disease (low in B31 and N9831 combined and in the Finnish trial, but substantial in HERA and BCIRG 006), the use of taxanes (in all patients in B31–N9831 and BCIRG 006 but in ≤ 50% in the European trials), and the geographic distribution (wide only in HERA and BCIRG 006). By design, women with highly significant cardiac risk factors were excluded from all trials because of the documented adverse interaction between trastuzumab and anthracyclines in advanced disease.[13] The same was true for women with cardiac dysfunction (measured by echocardiography or multiple gated acquisition scanning). However, the time at which cardiac function was assessed varied substantially among the trials: after surgery, after 3 months of doxorubicin-based chemotherapy, or at completion of locoregional therapy and chemotherapy. The most favorable selection, in this regard, occurred in the HERA trial.

Safety and Compliance

With the exception of hypersensitivity, which has been seen only occasionally and mainly with the first infusion, cardiotoxicity (principally congestive heart failure) is the most important adverse effect of trastuzumab. Its incidence is around 1.4% in women receiving the drug as a single agent.[20,22] Symptomatic or asymptomatic cardiac dysfunction occurs in 13% of patients given trastuzumab concomitantly with paclitaxel and in 27% of those given trastuzumab with an anthracycline.[13] For this reason, all investigations of trastuzumab in the adjuvant setting

Table 2. Cardiotoxicity

	Trial Regimen																			
	HERA				NSABP B31				NCCTG N9831						BCIRG 006					
	Observation		T × 1 yr		AC→P		AC→P + T		AC→P		AC→P + T		AC→P→T		AC→D		DCb + T		AC → D + T	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Women at risk	1,693		1,694		872		864		807		808		842		1,050		1,056		1,068	
Cardiac deaths	1		0		1		0		0		1		NA		0		0		0	
Class 3–4 CHF NYHA*	0	0	9	0.5	3	0.3	30	3.4	0	0	20	2.4	12	1.4	3	0.2	4	0.3	17	1.5

Abbreviations: HERA, Herceptin Adjuvant; NSABP, National Surgical Adjuvant Breast and Bowel Project; NCCTG, North Central Cancer Treatment Group; BCIRG; Breast Cancer International Research Group; T, trastuzumab; AC, doxorubicin and cyclophosphamide; →, followed by; P, paclitaxel; D, docetaxel; DCb, docetaxel and carboplatin; NA, not available; CHF, congestive heart failure; NYHA, New York Heart Association.

*No CHF class 3–4 reported in the Finnish trial.

have required careful cardiac monitoring and stopping rules specified for cardiotoxicity.

Table 2 summarizes the most important cardiotoxicity results reported with adjuvant trastuzumab use so far. Although cross-trial comparisons are problematic and must be interpreted with caution, this table suggests three things. First, concomitant administration of trastuzumab with a nonanthracycline-based regimen such as docetaxel and carboplatin carries a risk of severe congestive heart failure that is low and similar to the risk associated with nontrastuzumab regimens (0%–0.3%). Second, administration of anthracycline and taxane chemotherapy followed by trastuzumab was associated with a slight increase in the risk of cardiac dysfunction (1.4%) in the sequential group in N9831, although the risk was lower (0.5%) in the HERA trial. Third, concomitant administration of trastuzumab and a taxane after four cycles of doxorubicin and cyclophosphamide was associated with a risk of severe congestive heart failure ranging from 1.5% with docetaxel (in BCIRG 006) to 2.4% with weekly paclitaxel (in N9831) and 3.4% with paclitaxel given every 3 weeks (in NSABP B31). It is unclear whether these figures reflect actual regimen-specific differences in the risk of severe congestive heart failure, differences related to pre-existing cardiac risk factors (e.g., diabetes, smoking, and hypertension) in the study populations, or variability across the studies because of chance.

Adverse effects that are unexpected or difficult to manage have not been seen in any of the trials, with the exception of nine cases of interstitial pneumonitis, possibly related to trastuzumab, in B31 and N9831, two of which were fatal, and two fatal "pneumonias" in the docetaxel and carboplatin and trastuzumab group in BCIRG 006. In the HERA trial, no such events were reported, but the rate of infections in general was slightly higher in the trastuzumab group than in the comparison group (1.7% vs. 0.6%).

Compliance has been excellent. The rate of patient-initiated discontinuation of trastuzumab (for reasons other than toxicity or disease progression) was 2.5% in the HERA trial and 6% in B31–N9831. In all the trials, the follow-up has been too short to analyze the rate of secondary leukemias.

Efficacy

The highly reproducible and striking therapeutic benefit of adjuvant trastuzumab is shown in Table 3: 39% to 52% reductions in the rates of recurrence — all highly

Table 3. Trial Results

	Trial Regimen								
	HERA		NSABP B31 + NCCTG N9831		BCIRG 006			Finnish Trial	
	Observation (1693 patients)	T × 1 yr (1694 patients)	Control (1679 patients)	T × 1 yr (1672 patients)	AC→D	AC→D + T	DCb + T	Control (115 patients)	T × 9 wk (116 patients)
No. of Events for DFS*									
All events	220	127	261	133	147	77	98	26	11
Distant events	154	85	193	96	113	52	67	NA	NA
HR for DFS	0.54		0.48		0.49				
						0.61		0.46	
95% CI	0.43 to 0.57		0.39 to 0.59		0.37 to 0.65	0.47 to 0.79			
p	< 0.0001		< 0.0001		< 0.0001	0.0002		0.0078	
Events for OS	37	29	92	62	36	20	28	6	14
HR for OS	0.74		0.67			NA		0.43	
95% CI	0.47 to 1.23		0.48 to 0.93			NA			
p	0.26		0.015			NA		0.08	
Median follow-up	1 year		2 years			≈ 2 years		38 months	

Abbreviations: HERA, Herceptin Adjuvant; NSABP, National Surgical Adjuvant Breast and Bowel Project; NCCTG, North Central Cancer Treatment Group; BCIRG, Breast Cancer International Research Group; yr, year; T, trastuzumab; AC, doxorubicin and cyclophosphamide; D, docetaxel; DCb, docetaxel and carboplatin; wk, week; NA, not available; DFS, disease-free survival; HR, hazard ratio; OS overall survival.

* Defined in all trials as breast cancer relapses, second malignancies, and deaths. In contrast, the Finnish trial used recurrence-free survival.

statistically significant — were observed in the trials at median follow-up times ranging from 1 to 3 years. These early recurrences were at distant sites in roughly two-thirds of the cases. A statistically significant overall survival benefit is currently seen only in B31–N9831. Because it must be presumed that virtually all of the patients in the control group received trastuzumab when they developed metastatic disease, the survival benefit strongly suggests that trastuzumab is more effective when used earlier in the course of the disease.

Subgroups With the Greatest Benefit From Trastuzumab

The benefits seen with trastuzumab have been similar across virtually all subgroups defined by clinical parameters. The reduction in the annual risk of recurrence is similar across the trials in women with hormone receptor–positive and hormone-receptor–negative disease. Trastuzumab does not alter the natural history of all HER2-overexpressing breast cancers. Experience with the drug in advanced disease shows that resistance can occur, and there are clearly relapses among women who have received trastuzumab in the adjuvant setting. Current knowledge of resistance mechanisms is still limited.[23]

Collection of tumor blocks and serum is ongoing in the adjuvant trials, in the hope that molecular signatures will be identified that can be used to predict the success or failure of trastuzumab. At the 2005 San Antonio Breast Cancer Symposium (San Antonio, TX), Paik presented preliminary, but provocative results, obtained from translational research related to NSABP–B31. Patients with tumors characterized by coamplification of *cMyc* and *HER2* seem to have the greatest benefit from adjuvant trastuzumab, a phenomenon explained by the fact that trastuzumab can turn on the proapoptotic function of deregulated cMyc.[24]

Optimizing Adjuvant Chemotherapy for HER2-positive Disease

The magnitude of the treatment effect, which is substantial in all adjuvant trastuzumab trials, raises the question of whether less aggressive and safer chemotherapy regimens could be developed in the future. An interesting, although retrospective, analysis carried out by the BCIRG 006 investigators suggests that coamplification of the *topoisomerase II-alpha* gene occurs in one-third of patients with HER2-positive tumors and that such patients may have a benefit from anthracycline-based regimens, whereas patients who have HER2-positive tumors without coamplification of *topoisomerase II-alpha* do not appear to have this same benefit and may be good candidates for efficacious, nonanthracycline-based regimens.[14] If independently and prospectively confirmed, this observation would have important implications for clinical practice. There is also growing interest in the investigation of liposomal anthracyclines in this population because these agents might decrease the risk of cardiac toxicity.

Unanswered Questions

Data emerging from the control groups in the trastuzumab trials are likely to enrich the understanding of the natural history of adjuvant treatment with chemotherapy alone in women with HER2-overexpressing breast cancer, at least in the cases in which no cross-over to trastuzumab will be performed. A first look at this group in the HERA trial showed substantially different risks of relapse at 2 years according to hormone receptor status and node status. The risk of relapse was 33% for women with four or more positive nodes (independent of hormone receptor status), 25% for women with hormone-receptor–negative disease and one to three positive nodes, 18% for those with hormone-receptor–negative and node-negative disease, and approximately 10% for women with hormone-receptor–positive disease and either node-negative disease or one to three positive nodes.[25] These figures should by no means be used as the basis for withholding trastuzumab in women who would have met the eligibility criteria for the trials, but they may assist the clinician and the patient in deciding whether to initiate adjuvant trastuzumab earlier or later, especially if anthracycline therapy is planned, since the risk of cardiotoxicity is probably lower with later initiation of trastuzumab (as observed in the HERA trial). Caution is required for the use of adjuvant trastuzumab in women with small, node-negative tumors who would not have been eligible for the trials. On the basis of the data reviewed, caution is particularly warranted in the case of women with small, node-negative, hormone-receptor–positive tumors.

Implications for Clinical Practice

There is level one evidence that adjuvant trastuzumab is an effective therapy and that its benefit exceeds its risks in most patients. Nevertheless, a careful risk-benefit assessment needs to be done for each woman and each of the strategies studied so far — upfront administration of trastuzumab with carboplatin and docetaxel, four cycles of an anthracycline regimen followed by trastuzumab in combination with a taxane, and administration of trastuzumab at the completion of chemotherapy.

Finally, clinicians should remember that HER2 overexpression and/or amplification was assessed under stringent conditions in the five trials. In clinical practice, the worst scenarios would be offering trastuzumab to a patient with a false-positive result and withholding trastuzumab from a patient with a false-negative result. To minimize the chance of false-positive or false-negative results, it is essential that clinicians work with a highly experienced laboratory.

PREOPERATIVE TRASTUZUMAB FOR HER2-POSITIVE BREAST CANCER

During the past decade, there has been increased use of preoperative therapy for women with operable breast cancer. Both preoperative chemotherapy and endocrine therapy have been shown to increase the proportion of women who can undergo breast-conserving surgery as a result of tumor shrinkage.[26,27] Disease-free survival and

overall survival appear to be identical whether treatment is administered before or after surgery. From a research standpoint, there are two major advantages of evaluating new therapies in the preoperative setting. First, because tumor tissue can be easily accessed before, during, and after the completion of therapy, there is great potential for correlative studies. Second, a preoperative response in the breast, particularly a pathologic complete response, appears to be a reliable indicator of long-term outcomes. This correlation allows investigators to draw preliminary, hypothesis-generating conclusions based on early results. The following discussion reviews selected preoperative trials that have been performed with trastuzumab, considers the implications of the large adjuvant trials for the preoperative treatment of HER2-positive disease, and highlights approaches for future clinical trials.

Single-arm Preoperative Trials

Burstein et al conducted a phase II trial of preoperative treatment with paclitaxel (175 mg/m^2 every 3 weeks for four cycles) and trastuzumab (a loading dose of 4 mg/kg followed by 2 mg/kg weekly for 11 weeks) in women with stage II or III HER2-positive breast cancer.[28] A total of 40 women, including six with inflammatory breast cancer, were enrolled. Of the 40, eight patients had 2 or more HER2 overexpression and 32 had 3 or more overexpression. After the 12-week treatment program, definitive surgery was performed, followed by four cycles of standard doxorubicin and cyclophosphamide chemotherapy (doxorubicin, 60 mg/m^2, and cyclophosphamide, 600 mg/m^2, administered every 3 weeks). The overall clinical response rate was 75% and the pathologic complete response rate was 18% with this preoperative 12-week regimen. The study also demonstrated that the majority of patients (18 of 24) who had tumors with 3 or more HER2 overexpression at diagnosis and residual disease at the time of surgery continued to have 3 or more overexpression.

Harris et al completed a trial using preoperative vinorelbine and trastuzumab, also administered for a total of 12 weeks.[29] In this study, patients received a weekly regimen of vinorelbine administered concurrently with trastuzumab at a dose of 25 mg/m^2. In keeping with the results of the paclitaxel and trastuzumab trial, the clinical response rate was 88%, and the pathologic response rate among the first 40 patients enrolled in the study was also 18%. Approximately half of the patients had tumors that were positive for estrogen and/or progesterone receptor, and while the ability to look for subset differences is extremely limited in a study of this size, there was no apparent correlation between hormone receptor status and response to trastuzumab. A preliminary microarray analysis showed that the nonresponding tumors tended to cluster together, suggesting that specific and potentially identifiable genes are responsible for the lack of response to a trastuzumab-based regimen (L. Harris. personal communication).

Other studies have evaluated other regimens in the preoperative setting. Lee et al administered docetaxel at a dose of 70 mg/m^2 and cisplatin also at 70 mg/m^2 every 3 weeks in combination with trastuzumab.[30] Investigators have also used docetaxel and epirubicin in combination with trastuzumab[31] and doxorubicin/cyclophosphamide followed by paclitaxel and trastuzumab,[32] all administered in the preoperative setting. With each of these regimens, the pathologic complete response rate has been in the range of 15% to 25%.

Randomized M. D. Anderson Trial

Buzdar et al from M. D. Anderson Cancer Center (Houston, TX) have reported the results of a small randomized trial comparing the sequential use of four cycles of paclitaxel (225 mg/m^2) followed by four cycles of FEC (fluorouracil, 500 mg/m^2; epirubicin, 75 mg/m^2; and cyclophosphamide, 500 mg/m^2), all administered every 3 weeks either with or without concurrent trastuzumab.[33] The study was designed to accrue 164 patients, but was stopped prematurely because of a marked difference in the rate of pathologic complete response between the group that received trastuzumab and the group that did not (control group). Whereas the 19 patients in the control group had a 25% pathologic complete response rate (95% CI, 7%-52%), the 23 patients treated with the regimen containing trastuzumab had a pathologic complete response rate of 65% (95% CI, 43%-84%). Despite the use of concurrent epirubicin and trastuzumab, none of the patients developed clinical congestive heart failure. The investigators subsequently reported the findings in a second cohort treated with the same trastuzumab-containing regimen as part of an extended phase II study.[34] In 22 additional patients, the pathologic complete response rate was 54%.

Although one has to be careful in making comparisons across trials, the regimen evaluated by Buzdar et al appears to result in a substantially higher pathologic complete response rate than any previous trastuzumab-containing preoperative regimen. Possible explanations for this finding include a more favorable study population (unlike other studies, the trial conducted by Buzdar et al did not include any patients with tumors larger than 5 cm), the use of both an anthracycline and a taxane preoperatively, the concurrent administration of the anthracycline with trastuzumab, and/or the administration of a 6-month course of treatment, including 6 months of trastuzumab, preoperatively. As noted previously, the investigators did not see significant cardiac toxicity, and the regimen was otherwise well tolerated. Nevertheless, concerns about the concurrent use of an anthracycline and trastuzumab remain, and these data, therefore, need to be viewed with some measure of caution until there are more extensive safety data. A trial within the American College of Surgeons Cooperative Group is evaluating this regimen further.

Trastuzumab Monotherapy and Preliminary Biologic Insights

Other investigators have evaluated the use of trastuzumab monotherapy before either a combination of trastuzumab and chemotherapy or surgery. Mohsin et al treated 35 patients who had locally advanced, HER2-positive

disease with a 3-week course of trastuzumab monotherapy.[35] They reported an objective clinical response rate of 22% after the 3-week course of treatment. Core biopsies for correlative studies were performed at baseline, after 1 week of treatment, and after the completion of 3 weeks of therapy. There were no changes in markers of proliferation; in particular, both Ki67 and p27 remained largely unchanged. However, apoptosis was significantly increased after the first week of therapy, suggesting that apoptosis rather than a change in cell-cycle kinetics accounts for trastuzumab's clinical effect.

Gennari at al treated 11 patients with a 4-week course of preoperative trastuzumab monotherapy.[36] Five of 11 patients had an objective response to therapy. As in the previous study, no change in proliferation was seen, nor was there evidence of downmodulation of HER2. Gennari et al suggested that antibody-dependent cellular cytotoxicity may be important on the basis of their observation that there was marked infiltration of lymphoid cells in all tumors and even more extensive infiltration of leukocytes in those tumors that responded to therapy.

Although these studies should be viewed as preliminary, they provide an important avenue for exploring mechanisms of sensitivity and resistance to trastuzumab and/or other HER2-directed therapy in the future. The hope is that the preoperative setting can be used to help identify new treatment targets and to determine which patients are unlikely to do well with current therapeutic approaches. The North American Intergroup and the Breast International Group are each considering trials in the preoperative setting that would provide important insights for the design of the next generation of adjuvant trials.

Clinical Implications

The results of the large adjuvant trials, summarized previously, have clearly changed the approach to preoperative treatment of women with HER2-positive disease.[6,7,14] Given the impressive disease-free survival advantage seen with the preoperative use of trastuzumab, this agent has rapidly been incorporated into standard clinical practice. Outside of a clinical trial, it is reasonable for a woman with HER2-positive disease to receive a trastuzumab-containing regimen preoperatively in the hope of reducing the size of the tumor and increasing the chance of breast conservation. In this setting, treatment with four cycles of doxorubicin/cyclophosphamide followed by either paclitaxel or docetaxel administered concurrently with trastuzumab represents a reasonable standard. With the use of this approach, surgery would typically be performed at the completion of the taxane therapy and then followed by trastuzumab monotherapy for a total of 1 year. If there is a desire to avoid an anthracycline, treatment with docetaxel, carboplatin and trastuzumab as administered in the BCIRG trial is a reasonable alternative. The administration of chemotherapy alone followed by surgery and then trastuzumab (that is, the approach used in the HERA trial) is not unreasonable, but because the goal of preoperative therapy is to maximize the response in the hope of decreasing the extent of surgery, the use of trastuzumab preoperatively is an inherently more appealing approach. It should be reiterated, however, that there is no compelling reason to administer preoperative treatment outside of a clinical trial unless a patient has inoperable disease or requires a mastectomy but would prefer to undergo breast conservation.

A variety of preoperative clinical trials are either underway or planned for women with HER2-positive disease. Within the North American Intergroup and the Breast International Group, there is interest in using the preoperative setting to identify regimens that can be taken forward into large adjuvant trials. Trastuzumab has dramatically reduced the risk of recurrence in the adjuvant setting, but a minority of patients still have recurrent disease. Newer biologically based approaches, administered either in addition to or in lieu of trastuzumab, will be tested in the preoperative setting in the hope that these therapies will lead to further improvements in outcome.

TREATMENT OF METASTATIC HER2-POSITIVE BREAST CANCER

Despite remarkable advances in the treatment of breast cancer, more than 40,000 women will die of metastatic disease this year in the United States alone. The treatment of patients with metastatic breast cancer is a substantial part of any general oncology practice. When trastuzumab became commercially available in the late 1990s, the treatment of patients with metastatic HER2-positive disease changed dramatically. While there have been substantial improvements in the treatment of HER2-positive metastatic breast cancer, many unanswered questions and challenges remain. Ultimately, many of these challenges may be seen in the adjuvant setting as well.

Initial Studies

Trastuzumab was first tested as a single agent[37] or in combination with cisplatin[38] in patients with HER2/*neu*-amplified, multiply pretreated metastatic breast cancer. Therapy was administered on a weekly basis, and initial pharmacokinetic analysis defined the half-life as approximately 8 days (subsequent data confirmed a half-life close to 21 days) and the optimal dosing level as an initial loading dose of 4 mg/kg, followed by 2 mg/kg weekly. No dose-limiting toxicities were found in either study, and response rates ranged from 12% for single-agent therapy to 24% for trastuzumab combined with cisplatin.

Preclinical evaluation of the effect of various chemotherapeutic agents in combination with trastuzumab in HER2-positive breast cancer cell lines demonstrated significant interactions, including enhanced cytotoxicity (synergistic) and additive effects, and what appeared to be reduced cytocidal effectiveness (antagonism).[17] It is important to note that these data are from cell lines, and the difference between additive and synergistic effects has not been confirmed clinically. In addition, the antagonistic effect noted in vitro has unclear clinical implications. Nonetheless, these data were used to design multiple clinical trials combining trastuzumab with chemotherapy.

Table 4. Pivotal Combination and Single-Agent Trials of Trastuzumab in Metastatic Breast Cancer

Study	Regimen	No. of Patients	HER2 Staining	Overall Response Rate		Median TTP (months)		Overall Survival (months)	
				%	p	duration	p	duration	p
Slamon et al[13]									
phase III, first-line	AC or P + T vs. AC or P alone	469	2 or 3+	50 vs. 32	< 0.0001	7.4 vs. 4.6	< 0.0001	25.1 vs. 20.3	0.046
Marty et al[39]									
phase III, first-line	D + T vs. D alone	186	3+	61 vs. 34	0.002	11.7 vs. 6.1	0.0001	31.2 vs. 22.7	0.033
Cobleigh et al[19]									
phase II, in pretreated patients	T	222	2 or 3+	15		9.1 (median duration of response)		13	
Vogel et al[15]									
phase II, first-line	T	114	2 or 3+	26; FISH+, 35		3.8		24.4	

Abbreviations: TTP, time to progression; AC, doxorubicin and cyclophosphamide; P, paclitaxel; T, trastuzumab; D, docetaxel; FISH, fluorescence in situ hybridization.

Pivotal Trials

Following the studies demonstrating the use of trastuzumab as a single agent, a number of pivotal trials were performed in patients with known HER2-positive disease (Table 4). The trial that led to approval by the Food and Drug Administration,[13] as well as a subsequent confirmatory study,[39] evaluated trastuzumab combined with doxorubicin/cyclophosphamide, paclitaxel, or docetaxel. The most remarkable finding in both trials was that the addition of trastuzumab to first-line chemotherapy for metastatic breast cancer significantly improved survival, along with a marked improvement in response rates and time to disease progression. This was true despite the fact that a majority of patients with disease progression crossed over from single-agent chemotherapy to trastuzumab. Two other studies evaluated the antibody as single-agent therapy in patients with untreated disease[20] or moderately pretreated disease.[19] Response rates ranged from 15% by independent evaluation in patients receiving one to two prior chemotherapy regimens to 35% in untreated patients with fluorescence in situ hybridization (FISH) -positive metastatic disease.

The trial by Slamon et al[13] and the supporting single-agent study[19] defined the unique cardiac toxicity of trastuzumab as well as the best laboratory methods for identifying HER2-positive disease. The most important adverse event was cardiac dysfunction of New York Heart Association class III or IV. Cardiac dysfunction occurred in 27% of patients receiving doxorubicin/cyclophosphamide plus trastuzumab as compared with 8% of those receiving doxorubicin/cyclophosphamide alone, and in 13% of patients receiving paclitaxel plus trastuzumab as compared with 1% of those receiving paclitaxel alone.[40] Cardiac function generally improved with medical therapy and withdrawal of trastuzumab therapy; restarting treatment is feasible in specific cases.[41] Cardiac dysfunction occurred in only 4.7% of patients treated in the single-agent study.[19] Thus, it became clear that exposure to doxorubicin, and probably all anthracyclines, potentiates the cardiac toxicity of trastuzumab, and this combination was largely abandoned. Additional investigations have evaluated less cardiotoxic anthracyclines in combination with trastuzumab to see whether efficacy can be maintained with minimal cardiac risk. Trials combining trastuzumab with liposomal anthracyline preparations[42] and with epirubicin[43] have been reported, with variable cardiac data. Additional information would be required to use these combinations in clinical practice.

The assay used to determine HER2/*neu* status for the pivotal trial was an immunohistochemical (IHC) test with a combination of two antibodies against the HER2/*neu* receptor, 4D5 and CB11 (the clinical trials assay). Women were eligible for the trial if their tumors stained at a 2 or more or 3 or more level by IHC. Subsequent testing of tumor blocks from patients on this study revealed that only approximately 30% of patients with 2 or higher staining by IHC had gene amplification as measured by FISH, compared with 89% of those with 3 or more staining.[44] In addition, FISH positivity, defined as a ratio of the gene copy number to centromere 17 of 2 or greater, was strongly correlated with an improved response to trastuzumab. The response rate in patients with FISH-negative tumors was identical between the trastuzumab and no-trastuzumab groups. Testing can be performed with an approved IHC test; however, all patients with 2 or more staining must have testing confirmed by FISH.[45] The alternative is to perform FISH testing alone on all samples.

Other Chemotherapy–Trastuzumab Combinations

Many other combinations of chemotherapy and trastuzumab have been tested in phase II trials.[46] Weekly paclitaxel or docetaxel combined with trastuzumab is safe and effective. HER2-positive breast cancer cell lines treated with trastuzumab have decreased ability to repair cisplatin-induced DNA damage,[47] suggesting that cisplatin combined with trastuzumab could be an effective regimen. Indeed, the addition of either carboplatin or cisplatin to docetaxel and trastuzumab has resulted in encouraging response rates and time to progression.[48] A small randomized trial compared trastuzumab and paclitaxel with the same regimen combined with carboplatin (TPC) every 3 weeks.[49] Response rates and time to progression were significantly improved in the patients receiving carboplatin. Overall survival was similar in the

two groups. Weekly dosing of TPC is associated with less myelosuppression but a similar response rate.[50]

One of the most active nontaxane chemotherapeutic agents combined with trastuzumab is vinorelbine, with response rates in the range of 68% to 78% in the first-line setting.[51] An advantage of this regimen is the absence of associated alopecia. Other combinations with demonstrated safety and efficacy in phase II studies include trastuzumab and gemcitabine,[52] with or without a platinum salt, and trastuzumab with capecitabine. A study evaluating trastuzumab combined with the novel taxane nab-paclitaxel is ongoing.

The optimal sequence of chemotherapy or hormone therapy and trastuzumab for patients with HER2/*neu*-positive metastatic breast cancer has not been established. The choice of treatment depends on the extent of disease, performance status, coexisting conditions, and patients' preferences regarding expected toxicities. Certainly, the improved survival seen with taxanes and the high response rate seen in phase II studies with vinorelbine suggest that one of these two regimens would be the preferred first approach to treatment in the metastatic setting. However, in patients with limited or asymptomatic disease, trastuzumab alone may be appropriate as first-line therapy, with minimal impact on quality of life. Disease that is both hormone receptor-positive and HER2/*neu*-positive may respond to hormone therapy.

Trastuzumab can be given safely and with apparent efficacy every 3 weeks at three times the weekly dose (a loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks). One recent trial showed lower mean trough but higher peak trastuzumab concentrations with similar average exposure.[21] The long half-life of monoclonal antibodies is convenient in terms of allowing less frequent dosing but has complicated attempts to study the benefits of continuing trastuzumab after progression of disease with initial single-agent or combination therapy. In addition, patients and physicians have been reluctant to stop using the antibody after progression. One trial that randomly assigned patients to either trastuzumab with vinorelbine or vinorelbine alone was closed because of poor accrual. At this time, the benefit of continuing trastuzumab with second-line chemotherapy after progression is unclear, although safety has been demonstrated in patients who continued on trastuzumab in the initial pivotal trial[53] as well as in retrospective reviews.[54,55]

Trastuzumab Combined With Hormone Therapy

The combination of trastuzumab with hormone therapy is an attractive, minimally toxic regimen that could be effective in the 50% of cancers that overexpress both HER2/*neu* and the estrogen or progesterone receptor. Signaling through the HER2/*neu* receptor has been implicated in resistance to hormone therapy, particularly to the selective estrogen receptor modulator, tamoxifen.[56] A clinical trial comparing the aromatase inhibitor anastrozole with the combination of trastuzumab and anastrozole in more than 200 patients has been completed but not yet reported. The data from this study will help direct a trial evaluating the use of a completely nonchemotherapy regimen in early-stage, hormone-receptor–positive, HER2-positive breast cancer. Combinations with the pure estrogen receptor antagonist fulvestrant are also being evaluated.

New Directions

Both acquired and new resistance to trastuzumab is a continuing challenge for the treatment of HER2-positive disease. Combinations of trastuzumab with novel biologic therapy as well as newer agents designed to target HER2/*neu* are under investigation. Ongoing trials are evaluating trastuzumab combined with bevacizumab and other targeted therapies. The brain is a common site of metastasis in HER2-positive disease specifically.[57] Multiple investigators have reported an incidence of central nervous system disease in the range of 25% to 35% in women with HER2-positive breast cancer.[58] Trastuzumab does not cross the blood–brain barrier and is, therefore, ineffective therapy for brain metastases. It is not uncommon for the brain to be the only site of recurrent and uncontrollable disease in patients whose other sites of visceral disease continue to be well controlled with the use of trastuzumab alone.[59] Oral tyrosine kinase inhibitors are small molecules that offer the advantage of oral dosing and target the internal tyrosine kinase portion of the receptor, but also may cross the blood–brain barrier. Lapatanib is a novel tyrosine kinase inhibitor of the HER1 and HER2 receptors. Activity has been reported in trastuzumab-refractory disease,[60] and preliminary efficacy has been seen in the setting of brain metastases. A small study of lapatinib in patients with brain metastases, sponsored by the National Cancer Institute (Bethesda, MD), has recently completed accrual, and a larger, multinational study has recently opened. In addition, a study of patients with untreated metastatic HER2-positive disease is randomly assigning 130 women to receive either 1,500 or 500 mg per day of lapatanib.[61] An interim analysis of the first 60 patients showed a partial response rate of 28% and a stable disease rate of 40%, with minimal toxicity that included rash, pruritis, and diarrhea. Lapatanib is also being studied in combination with trastuzumab and paclitaxel as first-line chemotherapy for metastastic disease, either alone or in combination with trastuzumab in women with progression on trastuzumab, and in combination with other novel agents, including bevacizumab.

Although in vitro data suggested that the combination of trastuzumab and gefitinib would be effective, a phase I/II study demonstrated a response rate of only 19%, with a time to progression of only 3 months.[62] An ongoing study is evaluating the combination of erlotinib with trastuzumab.

Pertuzumab is a novel antibody that prevents ligand-induced dimerization and homodimerization of the HER2/*neu* receptor so that the receptor is unable to bind to any of its HER partners. This might be a more effective way of inhibiting signaling through the receptor. A recently completed phase I trial showed activity in solid tumors.[63] Another targeted approach to HER2-positive disease is the use of chemotherapy-filled liposomes with antibodies against HER2 attached to the cell surface — a smart bomb

designed to attack the tumor site with potentially less systemic toxicity. One anti-HER2 immunoliposome has been developed and should be in clinical trials within the next year.[64] There has been long-standing interest in the use of vaccines to redirect the immune response in the treatment of cancer; however, clearly defined unique targets are required. HER2 is a possible target for immunotherapy with vaccines. A number of studies are ongoing.

In summary, the identification of a unique prognostic factor for metastatic breast cancer in 1987 led to the development of a targeted antibody that revolutionized the treatment of this aggressive disease, resulting in remarkable improvements in response rates, progression-free survival, and overall survival. The primary toxicity involves the myocardium. However, with appropriate treatment and, if necessary, withdrawal of the drug, cardiac muscle function appears to recover in the majority of patients. Brain metastases remain a difficult problem, but there is a glimmer of hope on the horizon. Given the enormous effect of trastuzumab on early-stage HER2-positive breast cancer, it is highly likely that in the future, all women with recurrent HER2-positive disease will have been exposed to the antibody. Initial exposure to trastuzumab will fortunately reduce the number of women with metastatic disease, but will also pose a greater treatment challenge in this group because of trastuzumab resistance. Current and planned trials should help evaluate the effect of novel targeted therapies in treating or perhaps preventing metastatic disease in this setting.

TRASTUZUMAB RESISTANCE

Efficacy and resistance are two sides of the same coin. Just as a complete understanding of how trastuzumab eliminates HER2-positive breast cancer is lacking, so also is a lack of good understanding of why some HER2-positive tumors fail to respond to trastuzumab. Yet, trastuzumab resistance is a clinical reality. In carefully selected populations of patients with HER2-positive metastatic breast cancer, no more than one-third of patients respond to initial trastuzumab monotherapy. Similarly, in the setting of microscopic metastatic disease, adjuvant trastuzumab reduces the annual hazard ratio by only 50%, suggesting that a substantial proportion of these tumors are resistant to trastuzumab.

Trastuzumab resistance has already attracted research in both the laboratory and the clinic. As Vidal et al suggest, failure to respond to trastuzumab can be examined in the context of a small number of common resistance mechanisms[65]: suboptimal drug delivery, altered target expression, an altered target, modified target-regulating proteins, and signaling by alternative pathways. Each of these mechanisms has been explored in the laboratory. All remain to be fully examined in the clinic.

Suboptimal Drug Delivery

Incomplete blockade of the HER2 extracellular domain because of inadequate trastuzumab delivery has several potential explanations. First, rapid clearance of trastuzumab from the body could result in inadequate circulating levels. Population-based data from trials of trastuzumab monotherapy in women with metastatic breast cancer suggest that rapid antibody clearance of trastuzumab reduces overall survival, although it does not impair treatment response.[66] Clearance of trastuzumab is a function of the number of metastatic sites, shed extracellular domain levels, and body weight.[67] Circulating extracellular domain, in theory, could act as a serum sink for trastuzumab. In reality, however, circulating extracellular domain represents only a small fraction of the total load of HER2 antigen in patients with advanced breast cancer.[67] In clinical trials of trastuzumab monotherapy, there has been no clear correlation between shed extracellular domain levels and response.[20]

Another form of suboptimal delivery could involve organ-specific sanctuary sites. The occurrence of central nervous system metastases in patients with responding peripheral metastases suggests that the central nervous system may represent one such sanctuary site for HER2-positive breast cancer, where inadequate antibody penetration may result in therapeutic failure (reviewed by Duchnowska and Szczylik[68]).

Altered Target and Target Expression

If the presence of HER2 at the gene and protein level is associated with a response to trastuzumab, is resistance associated with the loss of HER2 amplification and overexpression? Current evidence, derived primarily from preclinical studies, suggests that HER2-positive cell lines in which resistance to trastuzumab has been induced continue to overexpress HER2 on the cell surface.[69] Similarly, a cell line derived from a patient with clinical trastuzumab resistance continued to overexpress HER2 mRNA and protein.[70] These preclinical and cell-line data notwithstanding, adequate clinical data to suggest whether loss of HER2 expression is associated with the development of resistance is not currently available.

Because trastuzumab recognizes a specific epitope in the extracellular portion of the HER2 protein, alterations in or masking of that target could result in loss of activity. Two such potential alterations have been identified. Nagy et al, working with a cell line established from a patient with a trastuzumab-resistant breast cancer, demonstrated that the membrane-associated mucin MUC4 masked HER2 and inhibited trastuzumab binding.[71] Other preclinical work has focused on the truncated HER2 receptor ($p95^{ErbB2}$), which lacks the extracellular domain and is, therefore, insensitive to trastuzumab, although not to small-molecule receptor tyrosine kinase inhibitors.[72] The clinical importance of these resistance mechanisms is unknown.

Modified Target-Regulating Proteins

Several lines of evidence suggest that downstream regulators of HER2 function may be altered in trastuzumab-resistant breast cancer cells. Nahta et al, examining trastuzumab-resistant SKBR3 HER2-overexpressing cell lines, demonstrated downregulation of the cyclin-dependent kinase inhibitor $p27^{kip1}$, with an associated increase in cyclin-dependent kinase-2 activity.[69] The PI3K/AKT pathway has also been implicated in trastuzumab resistance.

Trastuzumab activates and stabilizes PTEN (which normally opposes activation of the PI3K/AKT pathway). Conversely, loss or inactivation of PTEN is associated with trastuzumab resistance in vitro.[73] An initial clinical study in a small patient cohort receiving trastuzumab for advanced breast cancer suggests that this in vitro phenomenon may have clinical relevance.[73] Although larger data sets remain to be examined, the above work points to the possibility that agents targeting the PI3K/AKT pathway have the potential to reverse resistance to trastuzumab.

The presence of pathways not directly interacting with the HER2 pathway has also been implicated in relative resistance to trastuzumab-based therapy. The *cMyc* oncogene is coamplified with *HER2* in approximately 30% of cases.[74] NSABP investigators hypothesized that patients with *cMyc*-amplified tumors, by virtue of independent signaling through the cMyc pathway, would derive less benefit from trastuzumab-based therapy. An analysis of patients treated on the NSABP B31 adjuvant trastuzumab trial, however, demonstrated that those with *cMyc*-amplified tumors had statistically superior recurrence-free survival. Kim et al suggest that the proapoptotic function of dysregulated cMyc needs to be counterbalanced by an antiapoptotic signal for such cells to develop cancer. Once this antiapoptotic signal is reversed (e.g., with trastuzumab), apoptosis is initiated.[74] This analysis does not argue against the use of trastuzumab in patients without *cMyc* amplification, but does demonstrate a smaller benefit in such patients than in those with *cMyc* amplification.

Signaling By Alternative Pathways

Just as HER2 overexpression can represent an escape mechanism for estrogen receptor blockade, invocation of alternative growth factor receptor pathways might allow HER2-positive breast cancers to escape trastuzumab. Preclinical investigations have suggested that overexpression of insulin-like growth factor 1 receptor (IGF1R), whether natural or induced, results in relative resistance to trastuzumab and that blockade of IGF1R signaling restores sensitivity to trastuzumab.[75] Overexpression of IGF1R in HER2-positive breast cancer cells in vitro has also been shown to antagonize the trastuzumab-induced increase in the level of the Cdk inhibitor $p27^{Kip1}$, resulting in restoration of Cdk2 activity and attenuation of cell cycle arrest in G_1 phase.[76] Clinical data on IGF1R as a potential resistance mechanism are limited. Kostler et al have recently reported that IGF1R protein expression does not correlate with trastuzumab resistance in metastatic breast cancer.[77]

In conclusion, several potential mechanisms of trastuzumab resistance have been identified, but compelling clinical evidence for most of these mechanisms is unavailable. Trastuzumab resistance, like resistance to hormone therapies, is probably multifactorial and will therefore be difficult to reverse in the clinic. The search for resistance mechanisms will ultimately have value to the extent that such mechanisms are reversible.

CONCLUSION

There has been dramatic progress in the treatment of HER2-positive breast cancer in the past decade. Initial studies with trastuzumab in the metastatic setting led to large adjuvant trials, which have produced impressive results. There remain many unanswered questions and clinical challenges for oncologists treating women with HER2-positive disease. Collaborations among laboratory scientists, clinical scientists, and women with HER2-positive disease have tremendous promise. The goal should be the elimination of mortality for HER2-positive disease in the next 5 to 10 years.

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Author	Employment or Leadership Positions (Commercial Firms)	Consultant or Advisory Role	Stock Ownership	Honoraria	Research Funding	Expert Testimony	Other Remuneration
Martine J. Piccart-Gebhart		Johnson & Johnson; Bristol-Myers Squibb; sanofi-aventis US; GlaxoSmithKline; Pfizer Oncology; Shering-Plough; Novartis Oncology; AstraZeneca		Pfizer Oncology; Roche; sanofi-aventis US; Shering-Plough; Novartis Oncology; Lilly Oncology; AstraZeneca; Amgen, Inc.	Bristol-Myers Squibb		
Hope S. Rugo		Genentech BioOncology; GlaxoSmithKline			GlaxoSmithKline; Genentech BioOncology		
George W. Sledge, Jr.*							
Eric P. Winer		GlaxoSmithKline; Genentech BioOncology		Genentech BioOncology; GlaxoSmithKline	Genentech BioOncology; GlaxoSmithKline		

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