Current Challenges in HER2-positive Breast Cancer

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Overview: **There has been substantial progress in the management of human epidermal growth factor receptor 2 (HER2)–positive breast cancer during the last 15 years. Trastuzumab chemotherapy combinations now form the cornerstone of therapy in both adjuvant and metastatic disease and are increasingly used in the preoperative setting. As a result, the natural history of these tumors has been dramatically altered and their prognosis with anti-HER2 therapy has improved relative to HER2-negative tumors in general. There are several clinical scenarios involving HER2-positive breast cancer in which management is less clear cut. These include the optimal management of subcentimeter tumors, optimal first-line chemotherapy combinations with trastuzumab, and the management of HER2-positive brain metastases. Changes in receptor status over the course of disease progression have been described in multiple settings, and it is unclear how this should influence clinical decision making. This review will focus on these controversies and highlight relevant clinical trials that have contributed to optimizing the approach to treatment, and better understanding the underlying biology of HER2-positive disease.**

HER2 BELONGS TO A family of transmembrane receptors that are potent mediators of tumor proliferation, and is overexpressed in 15% to 20% of invasive breast cancer. Trastuzumab is a humanized monoclonal antibody directed against the extracellular domain of the HER2 receptor. The U.S. Food and Drug Administration (FDA) has approved it for use in early and metastatic HER2-positive amplified breast cancer in combination with chemotherapy. This represents the single most important advancement in breast cancer care since the targeting of the estrogen receptor (ER). Lapatinib, a small-molecule tyrosine kinase inhibitor that inhibits the kinase activity of HER2 and HER1, has demonstrated benefit in combination with chemotherapy as second-line therapy in metastatic HER2-positive disease, and is also approved by the FDA.[1] Although anti-HER2 therapies have been shown to reduce the risk of recurrence and improve survival in women with stage I to III HER2-positive breast cancer,[2-5] and improve survival in the metastatic setting,[1,6] a substantial proportion of patients experience relapse after adjuvant trastuzumab therapy, and resistance is inevitable in the metastatic setting. Here, we review several ongoing clinical controversies in HER2-positive breast cancer, specifically focusing on areas in which there is no current standard of care. These include the management of small HER2-positive tumors, optimal first-line chemotherapy for advanced disease, management of HER2-positive brain metastases, and management of discordant HER2 status. Because of its breadth and because several reviews covering the topic have been published recently, this paper does not review novel therapeutics.

Management of Small, Node-negative HER2-positive Breast Cancers

Small, node-negative, HER2-positive breast cancers are not uncommon. Although there is some evidence that point to poorer outcomes without treatment, the management of this clinical scenario remains controversial. There remain limited data on outcomes for patients with lower-risk, stage I HER2-positive breast cancers because the seminal adjuvant trastuzumab trials generally excluded patients with small tumors, although some accrued a moderate number of patients with node-negative disease (Table 1).[2-5,7,8] There are currently no prospective phase III trial data to support the routine use of adjuvant trastuzumab and chemotherapy in women with subcentimeter tumors. Current guidelines from St. Gallen and the European Society for Medical Oncology (ESMO) do not recommend adjuvant trastuzumab and chemotherapy for T1N0 HER2-positive tumors.[8,9] The current U.S. National Comprehensive Cancer Network (NCCN) guidelines has however factored in the indirect evidence obtained from retrospective and subset analysis, and recommends consideration of trastuzumab-based therapy in T1bN0 tumors ($>$ 0.5 cm), and in particular, the hormone receptor-negative subset.[10] There is wide variation in clinical practice; some patients with T1N0 tumors do not receive any adjuvant therapy, whereas others receive a variety of chemotherapy combinations with or without trastuzumab.

Prognostic outcomes of T1N0 breast cancers obtained from the Surveillance, Epidemiology, and End Results database demonstrate an excellent overall prognosis and a 10-year breast cancer-specific overall mortality rate of approximately 5%.[11] Independent poor prognostic features identified in this subgroup include high tumor grade and the presence of lymphovascular invasion.[12,13] Small HER2-positive tumors have substantially poorer outcomes than do small HER2-negative tumors, with recurrence rates in the magnitude of 10% to 30%, and hazard estimates for recurrence risk of two- to five-fold compared with similarly sized HER2-negative tumors.[14-16] Studies in small HER2-positive tumors are, however, limited by their retrospective nature, heterogeneity of adjuvant treatments received, small cohort sizes, and low event rates.

In light of the generally poorer prognosis of small HER2-positive compared with HER2-negative tumors, would they derive the same relative degree of benefit with trastuzumab and chemotherapy as do larger HER2-positive tumors? Important insights can be gained from retrospective and subset analyses of the adjuvant trastuzumab trials. In the Herceptin Adjuvant (HERA) study, 1 year of adjuvant trastuzumab resulted in an overall hazard ratio (HR) for disease-free survival (DFS) of 0.64 in a small subset of patients with 1- to

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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1092-9118/10/1-10

Table 1. Overview of Seminal Adjuvant Trastuzumab Trials

Trial	Eligibility Requirements	Treatment	HR for DFS	Citation
NSABP B-31	Node-positive disease	AC→T vs. AC→TH	0.48	Romond et al[3]
NCCTG N9831	Node-positive disease or high-risk node-negative disease	AC→T vs. AC→TH (concurrent)	0.48	Romond et al[3]
	Tumor ≥ 2 cm and ER+/PR+, or tumor ≥ 1 cm and ER− and PR−	AC→T→H (sequential)	0.87	Perez et al[18]
HERA	Node-positive disease or node-negative disease with tumor ≥ 1 cm	Chemotherapy vs. chemotherapy→H	0.64	Piccart-Gebhart et al[2]
BCIRG 006	Node-positive disease or node-negative disease and one of the following risk factors: tumor > 2 cm, ER− and PR−, grade 2–3, or age < 35	AC→D vs. AC→DH vs. DCb + H	0.61 0.67	Slamon et al[4]
FinHER	Node-positive disease or Node-negative disease with tumor ≥ 2 cm and PR−	D or V + FEC vs. D or V + FEC + T (9 weeks)		Joensuu et al[5]
FNCLCC-PACS 04	Node-positive disease	FEC or ED vs. FEC or ED + T	0.86	Spielmann et al[7]

Abbreviations: HR, hazard ratio; DFS, disease-free survival; AC, doxorubicin plus cyclophosphamide; Cb, carboplatin; D, docetaxel; FEC, flourouracil, epirubicin, and cyclophosphamide; ED, epirubicin plus docetaxel; H, trastuzumab; T, paclitaxel; V, vinorelbine; ER, estrogen receptor; PR, progesterone receptor.

2-cm, node-negative tumors (95% CI, 0.54 to 0.76; median follow-up, 23.5 months).[2,17] An equivalent relative benefit to the overall cohort was also found in the node-negative subgroup (HR = 0.59; 95% CI, 0.39 to 0.91) and in patients with 1- to 2-cm, node-negative tumors (HR = 0.53; 95% CI, 0.26 to 1.07). Similarly in the Breast Cancer International Research Group (BCIRG) 006 trial, patients with node-negative tumors derived the same relative benefit from trastuzumab as did the overall cohort.[4]

The clinical dilemma we are therefore faced with in tailoring treatment to small, node-negative HER2-positive tumors is the balance between a smaller absolute benefit with trastuzumab plus chemotherapy and its potential toxicity. Although anthracyclines has been shown to be beneficial in HER2-positive tumors, anthracycline-trastuzumab combinations are associated with a small but meaningful risk of cardiac toxicity in 1.9% to 3.8% of patients, potentially outweighing the benefits in this lower-risk population.[18] In contrast, the anthracycline-sparing combination of trastuzumab, docetaxel and carboplatin resulted in a considerably lower cardiac event rate of 0.4% in the BCIRG 006 study, and a shorter 9-week treatment with trastuzumab plus either docetaxel or vinorelbine in the FinHER study reported a symptomatic cardiac failure rate of 0.9%.[5,18] Both these regimens were however associated with a moderate degree of noncardiac toxicity.

One strategy is to use less intensive and therefore potentially less toxic treatment compared with the regimens explored in the randomized adjuvant trials. In a phase II study in women with HER2-positive metastatic breast cancer, weekly paclitaxel and trastuzumab resulted in a 67% to 81% response rate, and a 6% incidence of grade 3 or 4 neutropenia.[19] Our group instituted a phase II, multicenter, nonrandomized study of weekly paclitaxel plus trastuzumab for 12 weeks, followed by maintenance trastuzumab for 9 months in patients with node-negative, HER2-positive tumors that are smaller than 3 cm (information available at ClinicalTrials.gov; identifier NCT00542451). This trial has completed accrual of 410 patients, of whom approximately 50% had tumors smaller than 1 cm. Outcomes that will be reported include recurrence rates and, importantly, the tolerability and toxicity of the regimen. Estimates of DFS will be analyzed for the study as a whole and for subgroups of patients determined by tumor size (< 1 cm vs. ≥ 1 cm) and hormone receptor status.

Another strategy aimed at improving the benefit-to-toxicity ratio is combined HER2 blockade with trastuzumab and lapatinib without chemotherapy. In the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO) preoperative trial, a 6-week biologic window of anti-HER2 therapy without chemotherapy demonstrated a superior objective clinical response rate of trastuzumab plus lapatinib compared with either drug alone (67.1%, 30.2%, and 52.6%, respectively).[20] After the biologic window, paclitaxel was added to the anti-HER2 treatment for a further 12 weeks. The pathologic complete response rate was higher with paclitaxel plus dual HER2 inhibition compared with the trastuzumab or lapatinib combinations (51.3%, 29.5%, and 24.7%, respectively). Lastly, the FinHER study demonstrated that trastuzumab plus chemotherapy for 9 weeks resulted in a better distant DFS compared with chemotherapy alone (HR = 0.65; 95% CI, 0.38 to 1.12; p = 0.12; median

KEY POINTS

- Small, node-negative HER2-positive tumors have better outcomes than do larger HER2-positive tumors, and consideration of potential toxicities should strongly influence any recommendations for adjuvant therapy.
- There is efficacy data of trastuzumab in combination with paclitaxel, docetaxel, and vinorelbine in the first-line metastatic setting, suggesting that trastuzumab is a therapeutic equalizer that renders the choice of partnering chemotherapy secondary, allowing for the selection of a treatment regimen that will be best tolerated.
- The most promising systemic therapies for HER2-positive central nervous system (CNS) metastases following cranial irradiation are lapatinib and capecitabine, a combination approved by the U.S. Food and Drug Administration for HER2-positive breast cancer after progression during treatment with trastuzumab. There is an urgent need to increase therapeutic options in this subgroup of patients with novel agents and multimodality treatment through trials with specific CNS end points.
- Discordances in HER2 assessment can influence clinical decision making with anti-HER2 therapies. Reassessment of tumor phenotype at relapse is recommended, particularly after a substantial interval period has passed since the last pathologic assessment.

follow-up, 62 months), suggesting that a shorter duration of treatment may also be a valid strategy for this subgroup.[5]

In summary, small, node-negative HER2-positive tumors have better outcomes than do larger HER2-positive tumors, and as such, consideration of potential toxicities should strongly influence any recommendations on adjuvant therapy. Within the realm of subcentimeter HER2-positive tumors, there is a relative lack of data with T1a (< 0.5 cm) tumors and microinvasive tumors. The optimal chemotherapy regimen in this subgroup is debatable, and it is unlikely that there would be a definite answer derived from prospective phase III trials as a result of its low prevalence and event rates that would necessitate a large cohort. Overall, our threshold for treatment mirrors that put forth by the NCCN, that consideration of trastuzumab and chemotherapy should be limited to tumors 0.5 cm in size and larger. The toxicity trade-off in smaller tumors may not be justifiable with current adjuvant regimens used in larger or node-positive HER2-positive tumors. An abbreviated course of treatment with a better toxicity profile such as the combination of paclitaxel and trastuzumab may suffice for small tumors, and additional data will inform this decision in the years ahead. The ultimate goal is still to identify biomarkers that can predict which patients actually require therapy and which do not. The incorporation of this subset of patients and correlative biomarker studies in trials of newer HER2-targeted agents is essential for this field to ultimately move forward.

First-line Chemotherapy for Metastatic HER2-positive Breast Cancer

Trastuzumab has substantially altered the natural progression of HER2-positive metastatic breast cancer. The overall survival rate with one line of trastuzumab-containing therapy in initial studies was 25 months.[6] HER2 remains a viable target in patients whose disease has progressed during treatment with trastuzumab, and ongoing efficacy is demonstrated with trastuzumab and other anti-HER2 therapies even after progression during treatment with a trastuzumab-containing regimen.[1,21] In our institution, analysis of 113 patients with metastatic HER2-positive breast cancer indicated that patients received a median of four trastuzumab-containing regimens and a median survival of 3.5 years (95% CI, 3.0 to 4.4 years; E. Olsen, personal communication, January 2011), a substantial improvement on the survival rates reported with one anti-HER2 regimen.[6]

Although there is clinical rationale for the ongoing targeting of HER2 beyond progression during trastuzumab treatment, the optimal chemotherapy partner, particularly in first-line treatment, is less straightforward. The combination of paclitaxel and trastuzumab is currently the only combination approved by the FDA on the basis of the improvement in overall survival in the initial registration trial.[6] Trastuzumab plus docetaxel has also demonstrated a survival advantage over docetaxel alone in a randomized phase II trial.[21] More recently, the randomized phase III Herceptin plus Navelbine or Taxotere (HERNATA) trial comparing first-line treatment of metastatic HER2-positive disease with trastuzumab plus docetaxel or vinorelbine reported equivalent time to progression (TTP; median, 15.3 vs. 12.4 months; HR = 0.94; p = 0.67) and overall survival (median, 35.7 vs. 38.8 months; HR = 1.01; p = 0.98) in both treatment cohorts.[23] Importantly, the rates of grade 3 or 4 toxicity requiring dose reductions and treatment discontinuation were lower with the vinorelbine combination. These results mirror those of the earlier Trastuzumab and Vinorelbine or Taxane (TRAVIOTA) trial, which was terminated as a result of poor accrual. In this trial, we reported that trastuzumab plus vinorelbine did not demonstrate a significant difference in TTP (median, 8.5 months vs. 6.0 months; p = 0.09) and objective response rates (51% vs. 40%; p = 0.37) compared with trastuzumab plus a taxane.[24]

The results of these second-generation trials have substantial implications on first-line treatment options for HER2-positive tumors, and more importantly, on the direction of future clinical trials in this breast cancer subtype. There is efficacy data for trastuzumab in combination with paclitaxel, docetaxel, and vinorelbine in the first-line metastatic setting, suggesting that trastuzumab is a therapeutic equalizer that renders the choice of partnering chemotherapy secondary. Because the majority of women with HER2-positive tumors would have received trastuzumab plus taxane in the adjuvant setting, trastuzumab plus vinorelbine represents an attractive option in the first-line treatment in the metastatic setting. Importantly, oncologists and their patients can choose among varieties of trastuzumab-chemotherapy combinations and select the treatment program that will be best tolerated. There is little evidence that chemotherapy dose intensification plus trastuzumab will improve outcomes.[25,26] The focus of clinical trials should therefore be shifted away from optimizing the existing chemotherapy partners of trastuzumab, and focus on the development of other novel and HER2-targeting agents.

Management of HER2-positive Central Nervous System Metastases

The central nervous system (CNS) has emerged as an important sanctuary site for metastases and is substantial source of morbidity and mortality in patients with HER2-positive breast cancer. More effective therapies are extending the survival of these patients who have historically died as a result of extra-CNS disease before developing CNS metastasis. This paradox is thought to result from the confluence of factors including the biologic behavior or HER2-positive tumors, a treatment (trastuzumab) that is relatively more effective in controlling visceral compared with CNS metastases, and improved imaging techniques resulting in earlier detection of CNS metastases. Despite the use of whole-brain radiotherapy (WBRT) and radiosurgery, a substantial proportion of patients with HER2-positive CNS metastases die as a result of neurologic causes rather than progressive extra-CNS disease. As the survival of patients with HER2-positive tumors, even after the diagnosis of CNS progression, continues to improve, the need for effective salvage therapies after WBRT will only increase. Treatment options for CNS metastases remain limited, and further complicating this issue are the exclusion of patients with CNS metastases from many clinical trials and the challenges involved with the evaluation of CNS disease, particularly after WBRT. In this setting, differentiating tumor progression from radiation necrosis can be challenging.

HER2-positive breast tumors are biologically predisposed to CNS relapse. In multivariate analyses of risk factors for CNS metastases before trastuzumab therapy, HER2 overex-

pression, tumor size of 2 cm or larger, and hormone-receptor negativity were independent prognostic factors for the development of CNS metastases in newly diagnosed breast cancer. HER2-positive tumors associated with approximately a two- to fourfold increase in risk compared with HER2-negative tumors.[27,28] Although the clinical course of patients with HER2-positive breast tumors has changed substantially with the widespread use of trastuzumab, its effect on the incidence of CNS metastases is not clear. There were limited differences reported between the trastuzumab and nontrastuzumab treatment cohorts in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31, North Central Cancer Treatment Group (NCCTG) N9831, and HERA adjuvant trials.[2,3,29] A substantial effect on the time to CNS metastases was seen in a large retrospective study of metastatic HER2-positive patients with breast cancer with first-line trastuzumab-containing therapy compared with patients who did not receive trastuzumab (13.1 vs. 2.1 months, respectively).[30] Several studies have demonstrated a dichotomy in the progression of CNS and visceral metastases with trastuzumab-containing therapy, supporting the hypothesis that improvements in the systemic control of advanced HER2-positive disease with trastuzumab have led to an "unmasking" of CNS metastases that might have otherwise have remained clinically silent.[31]

The majority of studies in CNS metastases with systemic chemotherapy are retrospective and limited by small patient numbers. CNS response rates with temozolomide, liposomal doxorubicin, topotecan, and cisplatin have generally been disappointing.[32] The most promising CNS antitumor activity has been seen with capecitabine in both untreated and pretreated patients, and when administered in combination with temozolomide and lapatinib.[33,34] Few patients developed CNS progression in the registration trial of lapatinib plus capecitabine compared with capecitabine alone.[1] The event rate was small, and therefore this finding did not reach statistical significance. Our group reported a pilot study in patients with progressive HER2-positive CNS disease, which, despite not meeting its primary end point, demonstrated a modest response with single-agent lapatinib.[35] In light of these results, our group designed a larger, multicenter trial, whereby 242 patients with progressive HER2-positive metastases, prior trastuzumab treatment, and WBRT received treatment with lapatinib.[34] The protocol was later amended to permit patients who had experienced progression during treatment with lapatinib to have the option of adding capecitabine. Although the CNS response rate to lapatinib alone was modest (6% objective response), 21% of patients had a 20% or greater volumetric reduction in CNS lesions, and this was associated with a clinical improvement in neurologic symptoms and an improvement in progression-free survival (PFS). In patients who subsequently received lapatinib plus capecitabine, additional responses were observed with 20% of patients obtaining an objective CNS response and 40% or greater volumetric reduction in CNS lesions. Finally, on the basis of the preclinical finding that lapatinib may sensitize HER2-positive cells to radiation, our group has also explored the combination of lapatinib and WBRT in a phase I study.[36] Although the study did not meet the predefined criteria for feasibility, the combination of WBRT and a lower dose of lapatinib may represent a multimodality therapy for further exploration.

In summary, HER2-positive CNS metastases are increasing in prevalence and importance as the natural history of HER2-positive breast cancer evolves with better therapies. The current standard of care for patients with HER2-positive CNS metastases is still WBRT, stereotactic radiosurgery, and consideration of surgery for oligometastases. The most promising systemic therapies after WBRT are lapatinib plus capecitabine, a regimen currently approved by the FDA for HER2-positive breast cancer after progression during treatment with trastuzumab. As the survival of patients with metastatic HER2-positive breast cancer improves, there is an urgent need to increase therapeutic options in this area, and the best hope lies with novel agents and multimodality treatment through trials with specific CNS end points.

Discordant HER2 Status and Utility of HER2 As a Predictive Therapeutic Marker

Accurate assessment of HER2 is critical for the management of breast cancer. HER2 testing has improved with newer assays and increased standardization as a result of published algorithms and guidelines that include stringent laboratory accreditation standards, proficiency testing, and competency assessment.[37] Despite technical and interpretative improvements, changes in receptor status over the course of disease progression have been described in multiple settings: between diagnostic biopsy and surgical resection; between primary diagnosis and at surgical resection after neoadjuvant therapy; and between primary tumor, metastatic tumor, and circulating tumor cells (CTCs). Furthermore, there is also substantial discordance between HER2-positivity by standard pathology assays and molecularly defined, HER2-enriched intrinsic subtypes. What accounts for these discordances, and what are their therapeutic implications?

Biologic explanations for these discrepancies include intratumoral heterogeneity, as demonstrated by the occasional finding of focal clusters of HER2-positive cells within a predominantly HER2-negative tumor. Tumor evolution may also result in HER2 conversion occurring in the context of disease progression or therapy. One hypothesis for HER2 conversion is that tumors comprising heterogeneous cell populations, and in some tumors, anti-HER2 therapy may eliminate HER2-positive clones, leaving behind only the HER2-negative clones. Another hypothesis is that the loss of HER2 expression/amplification represents a resistance mechanism to anti-HER2 therapy. It is likely that the underlying mechanisms for HER2 conversion are more complex and varied. Although studies comparing HER expression after systemic therapy are limited by small numbers, up to one-third of previously HER2-positive tumors have been shown to lose HER expression/amplification.[38] This discordance predicted a shorter TTP compared with that of patients whose HER2 status was concordant. Current treatment approaches presume not only concordance between primary tumor and residual micrometastatic disease, but also homogeneity within a tumor. These findings highlight the importance of reassessing the tumor marker status after neoadjuvant therapy, and ideally at progression with metastatic disease. The optimal management and efficacy of anti-HER2 therapy in this setting is not clear however.

The converse situation of HER2 gain has been noted in several studies. In a minority of patients with HER2-

negative primary breast cancers, metastatic biopsies show new evidence of HER2 overexpression.[39] In this setting, the management will usually be altered to include anti-HER2 therapy. Despite the potential impact of revised therapy, repeat tumor biopsies are not routinely performed, primarily because of the invasive nature of the test. CTCs represent a potential noninvasive method to assess the metastatic tumor phenotype. By using antibody, size-based enrichment techniques and/or polymerase chain reaction (PCR)-based strategies, it is possible to detect rare CTCs (1 in 1 million to 1 in 100 million) in the blood of patients with advanced breast cancer. There is increasing evidence correlating CTC number with PFS and overall survival in these patients.[40] In addition to enumeration, CTCs can be molecularly characterized by using immunofluorescence, RNA, or genomic methods to identify characteristics of CTCs that correlate with response or resistance to therapy. It has been shown that approximately 30% of patients with primary HER2-negative breast cancer demonstrate evidence of HER2 expression or amplification in CTCs at tumor progression.[41] In our attempt to better understand whether these patients would benefit from anti-HER2 therapy, our group has initiated a single-arm phase II clinical trial with trastuzumab-based therapy in patients with metastatic breast cancer, a history of HER2-negative primary tumors and HER2-positive CTCs (information available at ClinicalTrials.gov; identifier NCT01185509).

Finally, as gene profiling and RNA profiling platforms are increasingly used in correlative and investigational studies, such as intrinsic subtyping by using either microarray or PAM50 reverse-transcriptase PCR technology, discordances in HER2 classification by using these platforms compared with standard pathologic assessment are emerging.[42] Classic pathologic markers used for breast cancer classification do not fully recapitulate the molecularly defined intrinsic breast cancer subtypes. The HER2-enriched intrinsic subtype is characterized by a predominance of clinical HER2-positive and highly proliferative tumors that lack expression of the basal cluster genes, and show low expression of the luminal cluster genes relative to the luminal A and B intrinsic subtypes.[43] In a combined data set of 400 patients, 106 (26.5%) were found to belong to the HER2-enriched intrinsic subtype, and comprised a heterogeneous mix of clinicopathologic categories including 51% ER-negative/HER2-positive, 15% ER-positive/HER2-positive, 16% ER-positive/HER2-negative, and 18% ER-negative/HER2-negative tumors. Interestingly, 34% of tumors in the HER2-enriched intrinsic subtype were HER2-negative by standard pathology, suggesting that these tumors may be driven by a similar functional event to that of HER2 amplification. Conversely, within the hormone receptor-negative/HER2-positive tumors, 50% to 88% comprise the HER2-enriched intrinsic subtype, and the hormone receptor-positive/HER2-positive group of tumors primarily comprising luminal B and HER2-enriched intrinsic subtypes. These data suggest that the information provided by the intrinsic subtypes may complement and expand on those provided by clinicopathologic markers. The anti-HER2 responses in HER2-positive tumors stratified according to their HER2-enriched intrinsic subtype remain to be seen, as does whether HER2-negative tumors (by standard pathology critera) that belong to the HER2-enriched intrinsic subtype would respond to anti-HER2 therapy.

Despite the success of HER2 as a predictive biomarker for anti-HER2 therapy, many patients with HER2-positive tumors do not benefit from trastuzumab. Recently, the robustness of HER2 as a predictive biomarker for trastuzumab was called into question in a study reporting outcomes from the NSABP B-31 adjuvant trial. Patients with HER2 2+ and 3+ tumors on immunohistochemistry (IHC) were randomly assigned to receive chemotherapy with or without trastuzumab. In this study there was no significant association found between HER2 copy number and therapeutic benefit ($p = 0.60$) in 174 patients with tumors that were deemed HER2-negative on central testing (fluorescent in situ hybridization negative and IHC $< 3+$). There was a surprising benefit with the addition of trastuzumab.[44] This thought-provoking report suggests that the benefit of trastuzumab may extend beyond tumors with HER2 amplification. Of course, all of these patients did have tumors that were deemed HER2-positive at the local laboratory. We must, however, be reminded that the accurate determination of HER2 and benefit from anti-HER2 therapy may be two separate, although inter-related, issues. In another recent report, the 70-gene Mammaprint signature identified a subgroup of patients with HER2-positive breast cancer with favorable outcomes in the absence of adjuvant trastuzumab and chemotherapy.[45] The 10-year distant DFS was 84% in patients with the good-prognosis gene signature compared with 55% in patients with the poor-prognosis gene signature. After adjusting for known prognostic variables and hormone therapy, the HR for distant DFS in the good prognosis compared with the poor prognosis gene signature groups was 5.8 (95% CI, 1.3 to 26.7; $p = 0.03$). These two studies bring to light some of the limitations of HER2 as a predictive therapeutic marker for trastuzumab.

The heterogeneity in trastuzumab response of HER2-positive tumors is most likely explained by the presence or absence of other modifying factors that affect the HER2 signaling pathway, and therefore trastuzumab response. The basis for the heterogeneity in trastuzumab response may lie in the possibility that a biologically active HER2 signaling pathway rather than the presence of HER2 amplification predicts for response to anti-HER2 therapy. It is perhaps not surprising that the ability of a single biomarker to predict therapeutic response is unlikely to account for all of the heterogeneity within HER2-positive tumors, just as with the case of the heterogeneity of endocrine therapy with ER as a therapeutic biomarker. The challenge is therefore to define these additional defects and its therapeutic implications.

In summary, discordance in HER2 status is not uncommon and can influence clinical decision making. We recommend reassessment of tumor phenotype at relapse when possible, particularly after a substantial interval has passed since the last pathologic assessment. There are no prospective data to guide what one should do in the setting of HER2 discordance, and one should consider providing treatment according to the most recent tumor assessment. Outside of a clinical trial, patients whose tumors fail to demonstrate HER2 amplification with standard criteria should not receive treatment with HER-directed therapy. Novel approaches such as HER2 assessment of CTCs may provide an alternative means of phenotyping metastatic disease. The therapeutic utility of trastuzumab in the intrinsic subtype of HER2-enriched tumors is not known, but this type of

analysis may potentially provide importantly insights into therapeutic heterogeneity of trastuzumab in HER2-positive tumors.

Conclusion

There has been dramatic progress in the clinical outcomes of early and advanced HER2-positive breast cancer, for which trastuzumab-chemotherapy combinations are now the cornerstone of therapy. Much of this success has resulted from the concurrent improvements made in the development of HER2 as a reliable biomarker for prognosis and more importantly, for anti-HER2 therapy. Despite improved survival outcomes with anti-HER2 therapies, a substantial proportion of patients with HER2-positive disease still ultimately experience relapse after adjuvant trastuzumab therapy, and trastuzumab resistance is inevitable in the metastatic setting. As we focus our attention on less common subgroups of patients with HER2-positive disease, it is unlikely that it would be possible to derive definite answers from traditional prospective phase III trials as a result of several factors. These include the low prevalence and event rates with small HER2-positive tumors, which would necessitate a financially prohibitive trial with a large patient cohort; challenges unique to the assessment of disease and treatment response in brain metastases; and the relative rarity of HER2 discordance. However, early-phase trials and single-arm studies may reveal vital insights. Importantly, well-designed prospective correlative experiments will aid our understanding of some of these complex and challenging clinical scenarios.

Authors' Disclosures of Potential Conflicts of Interest

Author	Employment or Leadership Positions	Consultant or Advisory Role	Stock Ownership	Honoraria	Research Funding	Expert Testimony	Other Remuneration
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