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Maximizing the response to Herceptin® therapy through optimal use and patient selection

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The aggressiveness of human epidermal growth factor receptor-2 (HER2)-positive breast cancer and the poor prognosis of women with this disease demand the availability of accurate and reliable tests for HER2 status and the optimization of HER2-targeted therapy. The distinctive clinical pattern of HER2-positive breast cancer underlines the importance of testing for HER2 status and efforts are ongoing to validate the two major methods in use—immunohistochemistry (IHC), which measures cell membrane HER2 expression, and fluorescence *in situ* hybridization (FISH), which measures gene copy number. Clinical trial results demonstrate that there is an association between strong HER2 overexpression (IHC 3+) and optimal response to therapy with the novel recombinant HER2 antibody Herceptin®. High levels of concordance between IHC 3+ and FISH-positive status have been observed, and response to treatment with Herceptin® is similar for patients whose breast cancers are IHC 3+ and those who are FISH-positive. Observations to date have led to the formulation of an algorithm for HER2 status determination and Herceptin® use which recommends that: (i) the HER2 status of all women with breast cancer be determined at presentation, (ii) all IHC 3+ and FISH-positive patients with metastatic disease should receive Herceptin®, (iii) Herceptin® should be used early in the course of metastatic breast cancer and preferably first line, and (iv) Herceptin® therapy should be continued until disease progression. [© 2001 Lippincott Williams & Wilkins.]

Key words: Breast cancer, fluorescence *in situ* hybridization, HER2, Herceptin®, immunohistochemistry.

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Introduction: why test for human epidermal growth factor receptor-2 (HER2)?

Breast tumors often express high levels of growth factors and their receptors. Of these, the ErbB tyrosine kinase receptor system, which consists of four homologous receptors [the human epidermal growth factor receptor (c-erbB-1/EGFr/HER1), c-erbB-2 (HER2 or HER2/*neu*), c-erbB-3 (HER3) and c-erbB-4 (HER4)], has been studied most extensively.[1] These receptors are transmembrane tyrosine kinase receptors with growth-stimulating activity. HER cell surface receptors appear to exist as monomers that form active dimers that are stabilized by ligand binding.[2,3] HER2, because it is the preferred dimerization partner for the other members of the HER family[4] and forms heterodimers with particularly high signaling potency compared with homodimers or heterodimers not containing HER2,[5–7] has a central role in signal transduction.

It is known that amplification of the HER2 gene plays a pivotal role in oncogenic transformation and tumorigenesis.[8–10] Amplification of the HER2 gene and/or overexpression of the HER2 protein (HER2 positivity) has been identified in 20–30% of breast cancers and such HER2 positivity is an independent predictor of prognosis.[11] Slamon *et al.*[12] first showed a link between a HER2-positive status and decreased overall and disease-free survival; since that time, a significant independent correlation between HER2 status and prognosis has been demonstrated in the majority of large studies.[11,13,14]

Numerous studies have also been carried out to investigate the relationship between HER2 status and response to therapy. HER2 positivity is linked to estrogen-independent cell growth and relative insensitivity to the anti-estrogen tamoxifen. De Laurentiis *et al.*[15] performed a meta-analysis of seven trials involving 1110 breast cancer patients and concluded that HER2-positive patients treated with endocrine therapy are less likely to respond to treatment than HER2-negative patients (overall odds ratio for disease progression 2.46). Furthermore, data from the 20-year update of the Naples GUN trial have shown positive HER2 status to be a strong predictor of tamoxifen failure

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Table 1. HER2 status as a predictor of response to anthracycline-based chemotherapy (immunohistochemical staining of paraffin-embedded specimens was used in all studies)

Study	No. of cases	Relationship of HER2-positive status to treatment outcome
Paik *et al.*[35]	638	predicted better response to anthracyclines
Ravdin *et al.*[36]	595	predicted better response to anthracyclines + tamoxifen compared with tamoxifen alone
Thor *et al.*[37]	992	predicted better response to standard versus suboptimal anthracycline dosages
Clahsen *et al.*[18]	440	not predictive of response to anthracycline-based chemotherapy
Rozan *et al.*[19]	323	not predictive of response to anthracycline-based chemotherapy

independently of estrogen receptor status and other major prognostic factors.[16]

In contrast, available data suggest a link between HER2 overexpression and chemosensitivity to anthracyclines. Table 1 summarizes findings from trials in which immunohistochemistry (IHC) was used to determine HER2 status. Although a number of studies have indicated that HER2 status has no value in the prediction of response to anthracycline therapy,[17–20] it should be noted that most of these involved smaller numbers of patients (which may have precluded the showing of statistical significance) than those in which an association was found. Data also indicate that there may be an association between HER2-positive status and relative resistance to CMF (cyclophosphamide, methotrexate and 5-fluorouracil) therapy.[21–24]

These findings, particularly those relating to the importance of knowledge of HER2 status in clinical decision making, indicate that HER2 testing should be routine for any women diagnosed with breast cancer. This article reviews how and when to test, the use of test results to select patients for therapy using the anti-HER2 monoclonal antibody Herceptin®, and the optimal use of Herceptin®.

How and when to test for HER2 status

Techniques used to determine HER2 status

Any test used to detect biological markers in cancer must be technically and clinically validated, i.e. the specificity, sensitivity and accuracy of the test and relationship of results to disease outcome need to be established.[25] In the case of HER2, immunohistochemistry (IHC), which measures HER2 expression at the cell membrane level, and fluorescence *in situ* hybridization (FISH), which measures gene copy number within the cell, are most widely available and are useful and powerful techniques for the determination of HER2 status in tumor tissue.[26,27] However, the IHC and FISH tests used to determine HER2 status vary between laboratories, and no one HER2 assay technique or specific test can be shown to determine HER2 status most accurately and reliably.[28] In this situation it is important to ensure that any test used is accurate and reliable, and test validation should be a primary goal because it produces good interlaboratory and interobserver concordance.[25–27,29]

Some of the problems with IHC and FISH that can be addressed by test validation and the application of strict testing protocols include: (i) variability in tissue fixation, method of processing and duration of sample storage (IHC), (ii) the antibody and staining procedure used (IHC), (iii) the need for antigen retrieval, which introduces variability (IHC), (iv) scoring the test result, with subjectivity and setting cut-off levels being problems for IHC and FISH, respectively, (v) length of time required to produce results due to the number of cells that have to be scored (FISH), (vi) loss of tissue architecture and thus the ability to relate signal to tumor cells (FISH), and (vii) the need for specialized equipment to visualize signal (FISH) that is not currently commonly available in clinical laboratories. Despite these concerns, validated IHC is currently the most widely available assay for use in routine HER2 testing and is most familiar to pathologists. FISH is recognized to be more reproducible than IHC[27,29] and its use is increasing, particularly as an adjunct to IHC in situations where IHC results are inconclusive. This use is based partly on studies showing that tissues that show HER2 gene amplification are generally strongly positive by IHC.[30–32] It is thus possible that the quantitative scoring system used for FISH may overcome any issues with the qualitative scoring of IHC.

When to test for HER2 status

HER2 amplification/overexpression is known to be an early event in the development of breast cancer, occurring prior to, or with the development of, atypical hyperplasia.[33] Furthermore, the HER2 status of primary tumors and their metastases has been shown to be similar.[34] As discussed above, tumors that are HER2 positive are particularly aggressive,[11–14] and HER2 status influences both prognosis and the response to standard therapies for breast cancer: Slamon *et al.* showed that women with HER2-positive breast cancer survive 3 years, less than half the survival duration (6–7 years) of women with HER2-negative

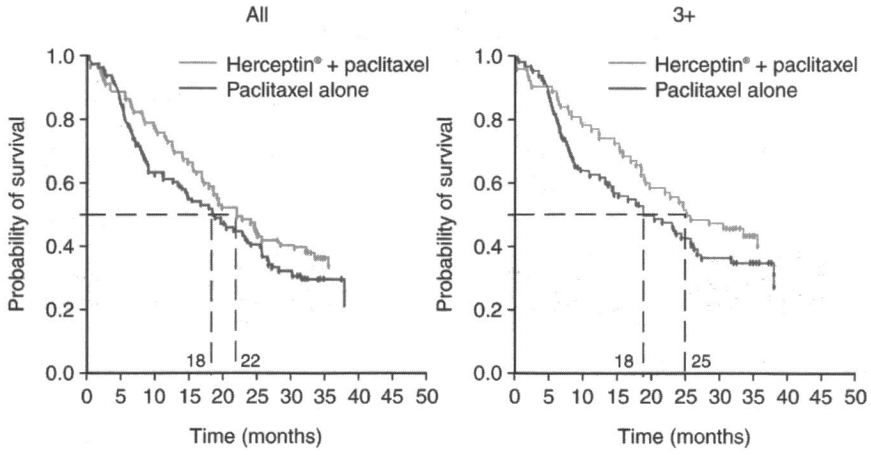

Figure 1. Benefit of treatment in terms of median survival in patients receiving paclitaxel with ($n = 92$) or without ($n = 96$) Herceptin® in a pivotal phase III clinical study. Of the total 469 participants in this study, 349 were classified as HER2 3+ by IHC. Note the increase in median survival from 22 in all patients to 25 months in HER2 3+ patients when Herceptin® was added to therapy. Study H0648g cut-off October 1999.

disease;[12] moreover, HER2 positivity has been linked to a poor response to hormonal therapy,[15] sensitivity to optimal anthracycline doses[35–37] and relative resistance to CMF.[21–24] Finally, HER2 testing of stored tissue specimens can add to the variability of results obtained. Testing fresh tissue specimens would overcome this difficulty. HER2 testing of biopsies from all women at diagnosis would avoid the necessity of taking new biopsies from all women who experience a recurrence of primary breast cancer.

The above considerations indicate that routinely establishing the HER2 status of breast cancer patients at presentation will provide useful information for clinical decision making both in the primary and metastatic setting. Furthermore, this will help to ensure the accuracy of test results.

Selecting patients for treatment with Herceptin®

Preclinical data indicate that cells showing higher levels of HER2 overexpression are inhibited by anti-HER2 monoclonal antibodies to a greater extent than those with lower levels (Genentech, data on file). This suggests that patients who are more strongly HER2-positive should respond better to Herceptin® therapy. As discussed elsewhere,[38] the novel anti-HER2 monoclonal antibody Herceptin® has been shown to produce durable objective tumor responses and to improve survival in patients with metastatic breast cancer in two pivotal clinical trials.[39,40] These effects are particularly marked in patients with high levels of HER2 expression (HER2 3+) as determined using the IHC testing protocol stipulated for both trials.[39,40] In the pivotal phase II trial of second/third-line Herceptin® monotherapy (4 mg/kg initial i.v. dose followed by weekly infusion of 2 mg/kg in 222 patients), the overall response rate was 15%. However, the response rate was 18% in the 172 patients in this trial who were HER2 3+.[39] In addition, median survival was extended from 13 months in the overall study population to 16.4 months in HER2 3+ patients.[38] These findings are supported by data from a study of 114 patients treated with first-line Herceptin® monotherapy.[41] In this population, the overall response rate was 26% and increased to 35% in HER2 3+ patients.

In the combination therapy study in 469 patients, the addition of Herceptin® to chemotherapy (anthracycline or paclitaxel based) produced an increase in median survival of 45% (29 versus 20 months) in HER2 3+ patients compared with a 25% increase (25 versus 20 months) in the overall population (Roche, data on file).[40] Similarly, other measures of efficacy, including time to progression (TTP) and response rate, also improved more in HER2 3+ patients than in the overall population (TTP 7.8 versus 7.4 months, response rate 56 versus 50%). The increased benefit of adding Herceptin® to chemotherapy was also seen in the HER2 3+ patients in the subgroups (anthracycline plus Herceptin® subgroup, HER2 3+ versus all: response rate 60 versus 56%, TTP 8.1 versus 7.8 months, survival 31 versus 27 months; paclitaxel plus Herceptin® subgroup, HER2 3+ versus all: response rate 49% versus 41%, TTP 7.1 versus 6.9 months, survival 25 versus 22 months) (Figure 1) (Roche, data on file).[40]

These results imply that the main target population for Herceptin® therapy should be HER2 3+ patients. However, it is known that a proportion of HER2 2+ patients also respond to Herceptin®. For example, in the pivotal phase II trial of second/third-line monotherapy, 6% of HER2 2+ patients responded to therapy.[39] The question of how to identify these patients in order to ensure that all

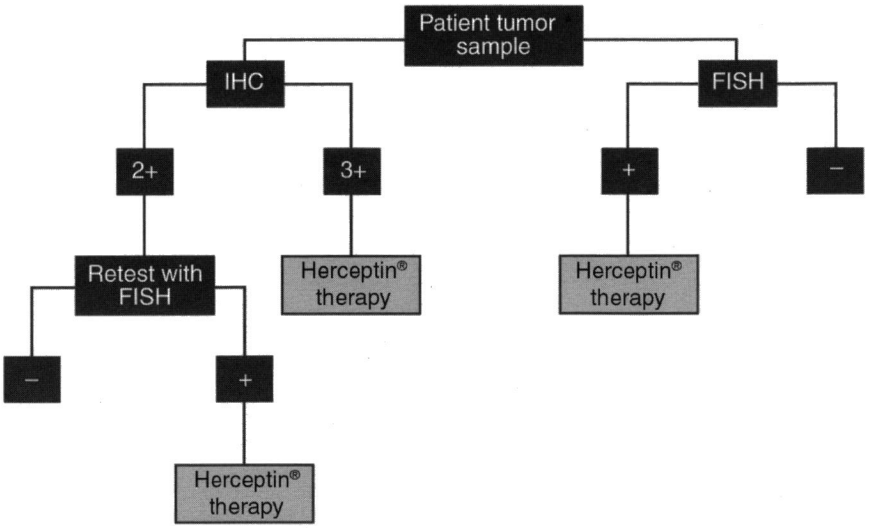

Figure 2. HER2 testing algorithm.

patients who are most likely to benefit from Herceptin® receive this therapy is addressed below.

Concordance between IHC and FISH and clinical outcomes

To examine the association between HER2 status on IHC and FISH, Mass *et al.* [42] compared the status determined using the clinical trial assay (IHC) with that measured using the PathVysion™ FISH assay. A random sample of 623 specimens (1:1 ratio of HER2-positive:HER2-negative) was taken from the 5998 samples screened for the pivotal clinical trials. The overall concordance between the assays was 81.3%. However, concordance increased with increasing levels of HER2 expression: rates of gene amplification in the HER2 0, 1+, 2+ and 3+ groups were 4.2, 6.7, 23.9 and 89.3%, respectively. Thus, substantial HER2 overexpression, as determined by IHC, is highly concordant with FISH-positive status. However, a significant minority of IHC 2+ patients are FISH positive.

The FISH status of patients in these trials has also been linked to treatment outcome. Similar clinical benefit was obtained with Herceptin® monotherapy in FISH-positive and IHC 3+ patients in the pivotal phase II trial.[42] Median TTP was identical at 3.2 months in both subgroups, and response rates in FISH-positive and IHC 3+ patients were 21 and 18%, respectively. In patients receiving paclitaxel plus Herceptin® in the pivotal phase III trial, median survival and response rates were identical (25 months and 49%) and median TTP were similar (7.0 and 7.1 months, respectively) in FISH-positive and IHC 3+ patients. Thus, FISH analysis identifies HER2-positive patients who are likely to respond to Herceptin®-containing therapy as well as IHC 3+ patients. Because the FISH-positive patient group includes a number of IHC 2+ patients, it appears that FISH may be able to identify IHC 2+ patients who are likely to respond to Herceptin®. Thus, retesting of HER2 2+ patients with FISH may be warranted.[42]

Recommendations for testing for Herceptin® eligibility

These observations allow a HER2 testing algorithm for use in selecting patients for Herceptin® therapy to be proposed (Figure 2). This algorithm considers IHC and FISH to be viable alternative testing techniques. Using these tests, IHC 3+ and FISH-positive patients can be considered definitively HER2 positive and thus eligible for Herceptin® therapy. Patients who are shown to be IHC 2+ status should be retested with FISH to confirm their eligibility for Herceptin® therapy.[26,31,32,42]

Recommendations for use of Herceptin®

When to treat with Herceptin®

Optimizing how Herceptin® is used can provide patient benefits. Current evidence supports the early use of Herceptin®, either as monotherapy or in combination with chemotherapy. Analysis of data from the pivotal trials[39,40] and the first-line monotherapy trial[41] shows marked increases in overall objective tumor response rates and median survival when Herceptin® is used as first-line compared to second/third-line monotherapy and when Herceptin® is used in combination with paclitaxel up front (Table 2). In considering these results, it is essential to remember that 65% of patients who were initially treated with paclitaxel alone in the pivotal phase III trial later

Table 2. Clinical benefit of Herceptin® as first-line monotherapy or in combination with paclitaxel

Treatment	Tumor response rate (%)	Median TTP (months)	Median survival (months)
Paclitaxel alone (phase III combination study)	17	3.0	18.0
Herceptin® + paclitaxel (phase III combination study)	49	7.1	25.0
Second/third-line Herceptin® monotherapy (phase II monotherapy study)	18	3.3	16.4
First-line Herceptin® monotherapy (phase II first-line therapy study)	35	3.5	24.4

Subgroup analyses of patients with HER2 3+ status participating in pivotal phase II monotherapy and phase III combination therapy studies,[40,42] and a phase II study of first-line monotherapy with Herceptin® in patients with metastatic breast cancer (Roche, data on file).

received Herceptin® at disease progression.[40] If adding Herceptin® to paclitaxel at this stage had similar effects to using these agents in combination as first-line therapy, it would be expected that outcomes would be similar. It is also interesting to note that patients treated with first-line Herceptin® monotherapy survived for a similar length of time as those treated with Herceptin® plus paclitaxel.

These results indicate that for optimal efficacy Herceptin® should be used as first-line therapy either in combination with paclitaxel to exploit the synergy between these agents observed in both preclinical and clinical studies, or as monotherapy in women who are not eligible for standard chemotherapy. Current data regarding the use of Herceptin® in combination with chemotherapy in patients who have received prior chemotherapy for metastatic disease are preliminary, but indicate that this approach is active and well tolerated.[43,44] Furthermore, second/third-line monotherapy has also been shown to be effective, producing durable tumor responses,[39] and should be considered for all HER2-positive metastatic breast cancer patients who have received first-line chemotherapy.

Recommended Herceptin® dose

Preclinical and early clinical studies of Herceptin® demonstrated that the half-life of Herceptin® increases with increasing Herceptin® dose.[45] Based on this, a fixed dose schedule of 250 mg followed by 100 mg i.v. weekly was selected and shown to have antitumor efficacy and to be well tolerated.[46,47] Finally, a dose based on body weight was introduced to optimize efficacy and safety: a 4 mg/kg initial dose given over 90 min by i.v. infusion, followed by 2 mg/kg i.v. weekly. This is the dose schedule that was used in the pivotal trials[39,40] and proved to be effective and well tolerated.

Several trials have examined different Herceptin® dose schedules. The first-line monotherapy trial compared the standard dose with a schedule involving an 8 mg/kg initial dose followed by 4 mg/kg weekly.[41] The efficacy and tolerability of the two regimens proved to be similar, with response rates in the 4 → 2 and 8 → 4 mg/kg groups of 25 and 27%, respectively, TTP of 3.5 and 3.8 months, respectively, and survival of 22.9 and 25.8 months, respectively. Moreover, the study suggested a higher rate of certain adverse events in the 8 → 4 mg/kg group. Thus, it is currently recommended that the standard regimen of 4 mg/kg i.v. followed by 2 mg/kg i.v. weekly be used in all patients treated with Herceptin®.

It is possible that a dose schedule involving 3-weekly Herceptin® may be introduced. Preliminary data for patients treated with an initial dose of 8 mg/kg i.v. followed by 6 mg/kg i.v. every 3 weeks in combination with paclitaxel suggest that this regimen is well tolerated and has pharmacokinetics that compare favorably to those observed for weekly Herceptin® plus paclitaxel in the pivotal trial.[48] Such a schedule would be more convenient for patients, particularly in the adjuvant setting, but further research is required before it can be recommended outside clinical trials.

Duration of Herceptin® therapy

In clinical trials performed to date, Herceptin® therapy has been continued until disease progression. The rationale for this is partly data showing that the withdrawal of Herceptin® results in tumor regrowth *in vitro*[49] and considerations of the mechanism of action of Herceptin®,[50,51] which is likely to have a tumor suppressing as well as a cytotoxic effect. Treatment with Herceptin® until progression is the approach that has produced the clinical benefits, including the survival advantage, observed using this novel agent.[39–41,43,44] Furthermore, this approach has proved to be well tolerated.[39,40] Therefore, it is recommended that Herceptin® should be continued until disease progression.

Treatment with Herceptin® beyond disease progression might also be warranted. Preclinical data indicate that Herceptin® has antitumor efficacy while cells are exposed to the drug, but that tumors regrow rapidly when Herceptin® is withdrawn.[49] Furthermore, different interactions between the mechanisms of action of Herceptin® and those of agents with which it is combined are partly responsible for the synergistic and additive effects of Herceptin® combination therapy.[49,52] Therefore, the addition of chemotherapy to Herceptin® monotherapy or changing chemotherapeutic agent at disease progression may produce greater clinical benefit than withdrawing Herceptin® and substituting chemotherapy. Data from a

recent analysis of patients who continued to receive Herceptin® after disease progression in the pivotal phase III trial support this. Of 235 patients initially treated with Herceptin® plus chemotherapy, 93 received Herceptin® either alone or in combination with chemotherapy at disease progression.[53] The response rate among these patients was 11%, response duration was 6.7 months and no new side effects of Herceptin® were observed with up to 12 months of therapy. This indicates that patients who progress on Herceptin® plus chemotherapy may respond to a further course of Herceptin®-containing therapy. Therefore, this approach requires further investigation and clinical confirmation.

Conclusions

HER2-positive breast tumors are aggressive and require effective and specific therapy. The HER2 receptor is an important prognostic marker in breast cancer and is a specific target for the humanized monoclonal antibody Herceptin®. Thus, routine determination of the HER2 status of all women diagnosed with breast cancer is warranted in order to optimize patient care. Both IHC and FISH can be used to accurately determine HER2 status.

In terms of selecting patients for Herceptin® therapy, current evidence indicates that those who are HER2 3+ on IHC or FISH positive obtain greatest benefit from Herceptin® therapy and constitute the main target population. However, some HER2 2+ patients also respond; it appears that retesting using FISH can identify these patients.

Patients with metastatic breast cancer who have been identified as being eligible should ideally receive Herceptin® as first-line therapy, whether it is administered in combination with chemotherapy or as monotherapy. Second/third-line Herceptin® also provides clinical benefit. Herceptin® should be administered according to the standard dose regimen (4 mg/kg i.v. followed by 2 mg/kg i.v. weekly) until disease progression.

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