Prediction of Response to Neoadjuvant Chemoendocrine Therapy in Primary Breast Carcinomas


ABSTRACT

Our aim was to determine whether biological molecular markers can predict response to neoadjuvant chemoendocrine therapy in patients with early breast cancer. Ninety patients (median age 56 years; range, 28–69 years) with primary operable breast carcinoma were studied. They were treated with four 3-weekly cycles of chemotherapy with mitozantrone, methotrexate (± mitomycin C), and tamoxifen prior to surgery. Fine-needle aspiration was used to obtain samples from patients prior to therapy, and the following parameters were assessed: estrogen receptor (ER), progesterone receptor (PgR), p53, Ki67, Bcl-2, and c-erbB-2 measured by immunocytochemistry, and ploidy and S-phase fraction (SPF) by flow cytometry. The tumors of 78% of the subjects responded (complete response, 9%; partial response, 69%) and 22% did not (no change, 20%; progressive disease, 2%). Response rates according to disease stage and patient age were as follows: T1, 74%; T2, 79%; T3/T4, 78%; age ≤50 years, 76%; >50, 79% (P = not significant). Response rates for other parameters were as follows: ER-positive, 82%, and −negative, 70%; PgR-positive, 86%, and −negative, 71%; p53-positive, 74%, and −negative, 81%; Bcl-2-positive, 85%, and −negative 61%; c-erbB-2-positive, 57%, and −negative, 93%; Ki67 high, 77%, and low, 81%; SPF high, 77%, and low, 77%; aneuploid, 71%; and diploid, 85%. Only the difference for c-erbB-2 was statistically significant (P = 0.007).

INTRODUCTION

Neoadjuvant therapy with chemotherapy and/or tamoxifen is increasingly being used in the treatment of patients with primary breast carcinomas. Previous studies have clearly established that neoadjuvant therapy causes significant tumor regression of primary breast carcinomas, thus allowing more conservative surgery (in particular, fewer mastectomies) to be performed (1–3). A benefit in terms of prolongation of survival remains to be established.

During neoadjuvant therapy, the tumor response can be directly measured and thus be used as a surrogate marker of sensitivity to therapy. In three studies of neoadjuvant therapy, it has been shown that patients who achieve CR or PR have a better outcome than patients who have stable or progressive disease (4–6). The ability to predict the response to neoadjuvant therapy may allow for the early identification of nonresponders. Nonresponders could be offered alternative treatment strategies, such as high-dose therapy. Because therapy is instituted prior to surgical resection, prediction of tumor response must be made on biopsy material obtained from the primary tumor. This can be achieved by FNA, which is a reliable, relatively atraumatic, cost-effective, and relatively painless procedure (7) that can be repeated during therapy. FNA can be used in conjunction with immunocytochemistry and flow cytometry to measure multiple molecular markers from primary breast carcinomas (8). These markers include the hormone receptors, ER, and PgR, the tumor suppressor gene p53, the apoptosis inhibitor Bcl-2, the oncogene c-erbB-2 and the proliferative markers of SPF and Ki67. Previous studies have shown that hormone receptor status predicts for response to endocrine therapy (9, 10). SPF and Ki67 have been shown to predict for response to cytotoxic chemotherapy (11, 12). Although experimental evidence suggests that p53 mutation results in increased resistance to chemotherapy (13), early clinical data appear to contradict this (14). The role of Bcl-2 in predicting response has not been adequately addressed in clinical studies, but experimental studies suggest that Bcl-2 expression is able to confer multidrug resistance to cells.
Prediction of Response to Chemoendocrine Therapy

PATIENTS AND METHODS

Ninety consecutive patients presenting with operable primary breast carcinoma during the study period of January 1, 1991, through February 28, 1995, were studied. Inclusion criteria were: age less than 70 years, informed consent, nonmetastatic operable breast carcinoma, suitable for treatment by surgery, radiotherapy, cytotoxic chemotherapy, and tamoxifen. All patients had a positive FNA cytology for malignancy. Carcinoma in situ could not be excluded by cytology. The median age was 56 years (range, 28-69 years); 33 patients were ≤50 years old and 57 were >50 years old. The distribution of cases according to tumor size, clinical node status, and menopausal status is shown in Table 1.

Seventy-three of these patients were participants in the Royal Marsden Hospital neoadjuvant versus adjuvant chemoendocrine therapy trial (3) for operable primary breast carcinomas. Only those randomized to the neoadjuvant arm of the trial were included in this study. The first 25 patients received 7 mg/m² Mitomycin C, 7 mg/m² Mitozantrone, and 35 mg/m² methotrexate i.v. every 3 weeks for a total of four cycles prior to surgical excision and 20 mg of tamoxifen daily (3MT). Following surgery, patients received a further four cycles of chemotherapy, and tamoxifen was continued for 5 years. Mitomycin C was omitted from the next 65 patients after the occurrence of a case of hemolytic-uremic syndrome believed to be due to an interaction of mitomycin C and tamoxifen (20). In the patients who did not receive mitomycin C, the dose of mitozantrone was increased to 11 mg/m², whereas the other drugs remained unchanged (2MT). Response rates to 2MT and 3MT had previously been shown to be similar (3).

A further 17 patients received similar treatment with chemoendocrine therapy and surgery with or without radiotherapy after four cycles but had not been randomized to the neoadjuvant trial, although they fulfilled the entry criteria for this trial. These patients were not entered in the neoadjuvant trial because they would have required a mastectomy and did not want the option of surgery first. Because they were all from the latter period of the study, they all received 2MT. For the purposes of this study, no distinction was made in the analysis between those patients who were in the randomized trial and those who were not.

Assessment of tumor response was undertaken by clinical bidimensional measurements prior to and at 3-week intervals during chemotherapy until surgery. Response was recorded according to the UICC criteria (21): CR, representing disappearance of the primary tumor; PR, indicating a reduction of ≥50%; or NC, defined as a reduction of <50% or increase in size of <25%, representing stable disease. An increase in size of ≥25% was considered to be progressive disease, and patients went on to immediate surgery. The residual palpable abnormality after good response was frequently an irregularity in the breast with no clinical evidence of measurable residual tumor and was defined as MRD. Clinical assessment of response to neoadjuvant therapy was used because this is the only evaluation of response that has been previously shown to be related to survival (4-6). Assessment of response by mammography was evaluated at our center. Although mammograms in most patients showed some response to chemotherapy, prediction of pathological outcome was not possible (22).

FNAs were taken from the tumor of all patients prior to treatment using a 23 gauge needle and a 10-ml syringe. A 2-ml cell suspension was made with MEM with phenol red and 25 mM HEPES buffer. One hundred μl of the cell suspension were placed in each of 12 Shandon cytospin chambers and centrifuged at 500 rpm for 5 min onto 3-aminopropyltriethoxsilane-coated slides. Seven slides were air-dried for 5 min prior to being stored at −80°C, while the remaining five slides were fixed in 50:50 methanol and acetone. Four of these were stored at −80°C, and the remaining fixed slide was stained with May-Grunwald-Giemsa for cytodiagnosis. The slide stained with Giemsa was scored in terms of malignancy and cellularity. ICA was subsequently performed on the stored slides that were unequivocally malignant and were of adequate cellularity.

The remainder of the cell suspension was snap frozen in liquid nitrogen and subsequently used for DNA analysis by flow cytometry.

### Table 1 Distribution of patients according to tumor size, clinical node status, and menopausal status

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of patients (total, 90)</th>
<th>%</th>
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<tbody>
<tr>
<td>T1</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>T2</td>
<td>58</td>
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<td>6</td>
</tr>
<tr>
<td>T4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>N0</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>N1</td>
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<td>14</td>
</tr>
<tr>
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<td>2</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>48</td>
<td>53</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>15</td>
<td>17</td>
</tr>
</tbody>
</table>

(15). c-erbB-2 is an oncogene that is overexpressed in approximately 30% of breast cancers and is associated with a poor prognosis (16). Additionally, there is evidence that overexpression may result in resistance to both chemotherapy and endocrine therapy (17-19).

The aim of this prospective study was to assess whether response to neoadjuvant chemoendocrine therapy could be predicted by measuring a panel of biological molecular markers in the primary tumor and correlating these with the tumor response after four cycles of treatment.
Table 2  Monoclonal antibody used for ICA and biological significance

<table>
<thead>
<tr>
<th>Marker</th>
<th>Antibody used and source</th>
<th>Biological significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>H222 (Abbott)</td>
<td>Hormone sensitivity</td>
</tr>
<tr>
<td>PgR</td>
<td>KD68 (Abbott)</td>
<td>Hormone sensitivity</td>
</tr>
<tr>
<td>p53</td>
<td>Pab240 and Pab1801 (Novocastra)</td>
<td>Tumor suppressor gene</td>
</tr>
<tr>
<td>Ki67</td>
<td>Mib1 (Binding Site)</td>
<td>Proliferation</td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>ICR12 (ICR, Sutton)</td>
<td>Oncogene</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>bcl-2 (DAKO Corp.)</td>
<td>Inhibitor of apoptosis</td>
</tr>
</tbody>
</table>

(1:10 dilution) and PAB1801 (1:20 dilution; Novocastra Laboratories Ltd., Newcastle, United Kingdom), and a streptavidin-HRP detection system. Immunostaining for Bcl-2 was performed using the Bcl-2 monoclonal antibody (as supplied by DAKO Corp.) at a dilution of 1:200 and a streptavidin-HRP detection system. Ki67 immunostaining was performed using the Mib1 antibody (as supplied by The Binding Site Ltd., Birmingham, United Kingdom) at a 1:200 dilution, and staining was detected using the avidin-biotin complex-HRP method. c-erbB-2 was detected using the ICR12 rat monoclonal antibody (supplied to us by Dr. C. Dean, Institute of Cancer Research, Sutton, Surrey, United Kingdom) and used at a dilution of 1:200.

Scoring of Immunocytochemical Staining. The immunostaining signals for ER, PgR, and p53 were evaluated by light microscopy using a scoring method representing the sum of scores of the proportion and intensity of nuclear staining (24). The proportion score represented the estimated fraction of positive staining tumor cell nuclei (0, none; 1, <10%; 2, 10%–50%; 3, 50%–75%; 4, >75%). The intensity score represented the estimated average staining intensity of positive tumor cell nuclei (0, none; 1, weak; 2, intermediate; 3, strong). A score of ≥3 was recorded as positive for ER and PgR, and ≥2 was positive for p53. The cutoff points used for ER, PgR, and p53 were those recorded as positive for ER and PgR, and 2 was positive for Ki67.

Flow Cytometry for Ploidy and SPF. The suspension fluid that had been stored in liquid nitrogen was thawed at 37°C and centrifuged at 1000 rpm for 10 min, and the pellet was resuspended in 200 μl of a stain-detergent solution consisting of PBS, 0.5 mM EDTA, 20 μg/ml propidium iodide, and 0.5% NP40 (all of the reagents were purchased from Sigma (Poole, Dorset, United Kingdom)). To this suspension, 20 μl of a 1 mg/ml −1 solution of RNase was added, and the suspension was kept on ice for 30 min before analysis.

In the early phase of the study, nuclei were analyzed on an Ortho Cytofluorograf 50H equipped with an Ortho 2150 computer system. The histograms were transferred to an IBM-compatible personal computer; further analysis and production of diagrams was performed using our own software. In the later part of the study, nuclei were analyzed on a Coulter Elite ESP linked to an IBM-compatible personal computer. Cell cycle analysis was performed using the Multicycle program (Phoenix Flow Systems, Inc). Both flow cytometers were equipped with an argon-ion laser producing 200 mW at 488 nm. On both flow cytometers, the peak and the intergrated area of the DNA signal were recorded and displayed. A gate was set to exclude clumped nuclei from the analysis (25). All the samples contained some normal (diploid) cells. The position of the G1 peak from the DNA histogram of the normal cells was compared with that of the G1 peak from the tumor and used to compute the DNA index (tumor-G1-channel/normal-G1-channel). The sample was recorded as aneuploid only if a clearly separate peak could be distinguished. Any sample with a coefficient of variation across the G1 peak >10% was excluded from the analysis.

Statistical Analysis. The significance of the effect of variables on tumor response was assessed by the Mann-Whitney test for trend (ordered categorical variables) and the two-tailed Fisher’s exact test (dichotomous variables). A multivariate logistic regression analysis was done to determine which variables had an independent effect on the likelihood of response. However, missing data on some patients weakened the power of the analysis and as a result did not reveal significant interactions between factors.

RESULTS

Results were available for analysis from 90 patients with primary breast carcinomas. The number of markers adequately scored from FNAs from these patients, together with the proportion that were positive, is shown in Table 3.

For Ki67, the median value was used to divide patients into high and low groups rather than positive/negative. Flow cytometry results were obtained in 71 cases. There were 45 (64%) aneuploid tumors and 26 (36%) diploid tumors. SPF could be determined in 35 of 45 (78%) aneuploid and all 25 (100%) of the diploid tumors. In 10 of 45 (22%) aneuploid tumors, SPF could not be determined because of overlapping peaks of the aneuploid populations. For SPF, the median value for aneuploid tumors was 12.6% and for diploid tumors, 3.7%. For the assessment of response, the high-SPF aneuploids were grouped together with the high diploids. Low aneuploids and diploids were similarly treated.

Not all parameters were measured for all patients for the following reasons: some samples were of inadequate cellularity to measure all markers from a single aspirate; there was poor

Table 3 Parameters measured by immunocytochemistry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of samples</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>77</td>
<td>57</td>
<td>74</td>
</tr>
<tr>
<td>PgR</td>
<td>78</td>
<td>49</td>
<td>63</td>
</tr>
<tr>
<td>p53</td>
<td>80</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>51</td>
<td>33</td>
<td>65</td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>45</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>Ki67</td>
<td>52</td>
<td>26</td>
<td>50*</td>
</tr>
</tbody>
</table>

*Proportion greater than the median score (>6).
preservation of some markers during storage; and samples at the beginning of the study were not adequately stored for subsequent analysis by flow cytometry.

The clinical response could be evaluated in all 90 patients after 4 cycles of chemotherapy and tamoxifen and is shown in Table 4. Eight of 90 (9%) had CR; 23 of 90 (26%) had MRD; 39 of 90 (43%) had PR; 18 of 90 (20%) had NC; and 2 of 90 (2%) had PD. For further analysis, patients were divided into two categories according to the response to treatment: responders (CR, MRD, and PR) and nonresponders (NC and PD). There were 70 responders (78%) and 20 nonresponders (22%).

The clinical response in relation to T stage, menopausal status, and age is shown in Table 5. When clinical tumor response was related to initial T stage, patients with T1 and T2 tumors had 74 and 79% response rates, respectively, whereas the response rate for T3/T4 tumors was 77%. For menopausal status, the objective response rate for pre- and perimenopausal women was 74%; and it was 75% for postmenopausal women. In relation to age, the response rate for women ≤50 years was 76%, and for women >50 years, 79%. These differences are not significant, suggesting that clinical response, as assessed by UICC criteria, was not related to pretreatment tumor size, age of patient, or menopausal status.

Table 6 shows the relationship between clinical response (CR + MRD + PR) and ER, PgR, p53, Bcl-2, and c-erbB-2. Of 57 patients with ER-positive tumors, 47 (82%) were responders, whereas of 20 patients with ER-negative tumors, there were 14 (70%) responders (P = 0.3). Of 49 patients with PgR-positive tumors, 42 (86%) were responders, and of 29 patients with PgR-negative tumors, there were 20 (69%) responders. For the tumor suppressor gene product p53, 23 (74%) of 31 patients with positive tumors and 40 (81%) of 49 patients with negative tumors were responders (P = 0.6). For Bcl-2, 28 (85%) of 33 patients with positive tumors and only 11 (61%) of 18 patients with negative tumors were responders (P = 0.08). For c-erB-2, 8 (57%) of 14 patients with positive tumors and 29 (93%) of 31 patients with negative tumors were responders; this difference was statistically significant (P = 0.007).

Table 7 shows the relationship between clinical response (CR + MRD + PR) and Ki67, SPF, and ploidy. For the proliferation marker Ki67, 20 (77%) of 26 patients with tumors with above-median values (high) and 21 (86%) of 26 patients with below-median values (low) responded (P = 1.0). For SPF, 24 of the 31 (77%) patients in the high group and 23 of the 30 (77%) in the low group responded (P = 1.0). Among patients with aneuploid tumors there were 32 of 45 (71%) responders and for patients with diploid tumors 22 of 26 (85%; P = 0.3).

### DISCUSSION

This study was undertaken to determine whether response to neoadjuvant chemotherapy and tamoxifen in patients with primary breast cancer could be predicted from the measurement prior to treatment of biological molecular markers. The ability to predict this response may allow for the optimization of systemic therapy for individual patients. Multiple molecular markers from samples taken by FNA from primary breast tumors were measured prospectively prior to systemic neoadjuvant chemoendocrine therapy. The molecular markers that were measured were chosen on the basis that they represented different biological properties of the tumors, and previous studies had established that they can be adequately measured from material obtained by FNA (8, 26).

The mitoxantrone, methotrexate ± mitomycin C combination of cytotoxic agents was used because it had been shown to be as effective, but better tolerated and with less alopecia, than an anthracycline-based regimen (vincristine, adriamycin, and cyclophosphamide) in patients with advanced breast cancer (27). The addition of tamoxifen to maximize the tumor response was based on indirect evidence from the 1992 Early Breast Cancer Triallists’ Collaborative Group report (28), which suggested that combination of tamoxifen with chemotherapy achieved greater benefits when used as adjuvant therapy than either treatment alone (29).

The clinical tumor response rates according to tumor size were as follows: 74% for T1 tumors; 79% for T2 tumors; and 78% for T3/T4 tumors. This suggests that tumor response is unrelated to initial tumor size. This result is consistent with predictions made by Norton and Simon (30) in 1977 on the influence of tumor size on chemosensitivity. However, it is at variance with a previous neoadjuvant study by Bonadonna et al. (5) in which response was inversely related to initial tumor size. This latter result may be due to response being defined as reduction in tumor size to less than 3 cm after neoadjuvant treatment rather than the conventionally used UICC criteria.

A trend toward higher responses to chemoendocrine therapy among patients with tumors that were hormone receptor positive (82% versus 70% for ER and 86% versus 69% for PgR) was observed. Previous studies have established that patients with ER- and/or PgR-positive tumors benefit more from adjuvant endocrine therapy than do those with ER- and PgR-negative tumors (28). In patients with metastatic disease, both ER and PgR predict response to endocrine therapy (31–33). ER
Therapy has been less intensely studied. Lippman et al. patients with primary breast carcinomas (9, 34–37). The rela-
positivity also predicts for response to endocrine therapy in chemotherapy but only 3 of 25 patients with high ER (>10 fmol/mg cytosol protein) responded (P < 0.0001). In contrast, Kiang et al. (59), in a retrospective series of 140 patients with advanced breast cancer, found that responses to chemotherapy were significantly higher in “ER-rich” tumors (86%) than in “ER-poor” tumors (36%; P < 0.001). These contradictory results may reflect different assays for ER, different cutoff points, different chemotherapy agents, small series numbers, and the retrospective nature of the studies. In an adjuvant trial of chemotherpay versus ovarian ablation in premenopausal women, those with ER-negative tumors had a better outcome after chemotherpay, whereas those with ER-positive tumors did better with ovarian ablation (39). Our results suggest that ER and PgR are only weak predictors of response to chemoendocrine therapy. This may be due to the regression of ER/PgR-positive tumors in response to tamoxifen, whereas ER/PgR-negative tumors may respond to the chemotherapy. Thus, when chemoendocrine therapy is used, both ER and PgR may lose part of their predictive capacity for response but still predict for better outcome. This can only be established with more patients and longer follow-up. The role of combined chemotherapy and endocrine therapy in the adjuvant setting is currently being tested in several large trials of patients with early breast cancer.

In this study, no correlation was found between SPF and Ki67, indicators of proliferative capacity, and tumor response to chemoendocrine therapy. For ploidy there were more responders (84%) among diploid tumors than among aneuploid tumors (71%), but this difference was not statistically significant. Previous studies have reported contradictory results with regard to ploidy and prediction of response to chemotherapy. Although no difference between response rates to chemotherapy and ploidy were seen in some studies (40, 41), others have shown higher response rates among aneuploid tumors (11, 42). For SPF, two previous studies of preoperative chemotherapy in primary breast carcinomas have shown significantly higher response rates among those with high SPF (11, 40). Ki67 and its relationship to chemotherapy response has only been studied in small numbers of patients, making interpretation difficult. Both of these found a nonsignificant trend toward higher response in patients with high Ki67 values (43, 44). The use of proliferation indices to predict response to endocrine therapy has been less well studied. In a series of 45 patients with advanced breast cancer and ER-positive tumors, slowly proliferating tumors (indicated by low tritiated thymidine labeling index) responded more frequently to tamoxifen than faster proliferating tumors (86% versus 60%; P < 0.05) (45). The results in our study would be consistent with a hypothesis that rapidly proliferating tumors (high SPF and Ki67, aneuploid) respond better to chemotherapy, whereas slowly proliferating tumors (low SPF and Ki67, diploid) respond better to endocrine therapy. When a combination of chemoendocrine therapy is used, these proliferation markers lose their predictive capacity.

No relationship was observed between p53 and response to treatment. Mutations of the p53 gene are common and result in a conformationally altered and nonfunctional but apparently more stable nuclear protein that is detected by standard immunocytochemistry (46). There are theoretical arguments for both increased and decreased chemosensitivity in tumors with mu-
tated p53 (47). Using a rodent model, Lowe et al. (13) found that tumors expressing the p53 gene contained a higher proportion of apoptotic cells and typically regressed after treatment with radiation or Adriamycin. In contrast, p53-deficient tumors continued to enlarge and contained few apoptotic cells after receiving the same treatments. These results suggest that defects in apoptosis, caused by inactivation of p53, can produce treatment-resistant tumors and suggest that the p53 status may be an important determinant of tumor response to therapy. Other experimental studies have supported this view (48–50). However,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
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<th>%</th>
<th>No.</th>
<th>%</th>
<th>P</th>
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<td>ER</td>
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<td>85</td>
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<td>57</td>
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<table>
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<td>77</td>
<td>21/26</td>
<td>81</td>
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<tr>
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<td>32/45a</td>
<td>71a</td>
<td>22/26b</td>
<td>85b</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Aneuploid.
* Diploid.

Table 6 Clinical response (CR + MRD + PR) in relation to ER, PgR, p53, Bcl-2, and c-erbB-2

Table 7 Clinical response (CR + MRD + PR) in relation to Ki67, SPF, and ploidy
clinical results do not appear to support this. In a retrospective study of node-positive breast cancer patients, p53 protein overexpression was found to be a marker for responsiveness to adjuvant endocrine therapy and chemotherapy (14), whereas in a series of 86 patients with primary breast carcinomas, no relationship was found between p53 levels and response to chemotherapy (51). For tumors with Bcl-2 overexpression, there was an 85% response rate, whereas in negative tumors, the response rate was 61% (this difference approached statistical significance; \( P = 0.08 \)). Bcl-2 is known to inhibit programmed cell death (apoptosis) and may be expected to have a central role in the response of tumor cells to chemotherapy, as many commonly used chemotherapeutic agents have been shown to be potent inducers of apoptosis (52). Experimental studies have shown that expression of Bcl-2 results in resistance to cell death following treatment with a variety of cytotoxic agents, including doxorubicin and methotrexate (53, 54). These results suggest that Bcl-2 should confer resistance to chemotherapy via inhibition of apoptosis. In the present study, the opposite was observed, with a trend toward more response in the Bcl-2-positive patients. However, it is consistent with a recent report showing that high Bcl-2 expression was associated with a better response to tamoxifen in metastatic breast cancer (55).

Overexpression of the protein product of the c-erbB-2 oncogene was found to predict for poor response to chemoendocrine therapy in this study. Whereas 93% of c-erbB-2-negative tumors responded, only 57% of those tumors with high expression responded \( (P = 0.007) \). Some caution needs to be exercised in the interpretation of this result, because the cutoff point for the dichotomy of tumors into positive and negative was chosen after optimized cutoff point analysis and also because c-erbB-2 analysis was performed on a smaller number of tumors than the other markers. However, only 2 of 31 c-erbB-2-negative patients did not respond, and this result is consistent with previous reported results that indicate that c-erbB-2 overexpression predicts for poor response to both chemotherapy (16, 17, 56) and endocrine therapy (18, 19, 57, 58). Because a combination of chemotherapy and tamoxifen was used, it is to be expected that those tumors overexpressing c-erbB-2 were less responsive to treatment. The poor response to systemic therapy of c-erbB-2 positive tumors may in part explain the poor prognosis of these patients.

In conclusion, in this study of primary chemoendocrine therapy in early breast cancer, a trend toward higher response rates to neoadjuvant chemoendocrine therapy for tumors that were positive for ER, PgR, and Bcl-2 was observed, but this did not reach statistical significance. In contrast, Ki67, SPF, and ploidy failed to predict for response. This may be partly due to the combination of chemotherapy and tamoxifen that was used as the neoadjuvant therapy, whereby slowly proliferating tumors may have responded better to endocrine therapy, whereas the faster-proliferating tumors responded better to chemotherapy. The lack of prediction of response by the p53 ICA status and the trend in favor of expression of Bcl-2 and tumor response that approached statistical significance are important in that they are contrary to what was predicted by in vitro and animal studies. This suggests that results from in vitro chemosensitivity studies cannot be readily applied to human tumors in vivo. The demonstration that tumors overexpressing c-erbB-2 have a much lower response rate to neoadjuvant chemoendocrine therapy is consistent with previous reports that it predicts for poor response to both chemotherapy and tamoxifen. For those patients who overexpress c-erbB-2, a prospective study should be considered for this predictive factor as a means of selecting patients for high-dose anthracyclines.

REFERENCES


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