A Human Epidermal Growth Factor Receptor 2 Expression-based Approach to Neoadjuvant Chemotherapy for Operable Breast Cancer

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Objective: We investigated the pathological effects of neoadjuvant chemotherapy based on the human epidermal growth factor receptor 2 in operable breast cancer.

Methods: This prospective clinical study was a pilot involving 63 female patients. Before surgery, patients with tumors overexpressing human epidermal growth factor receptor 2 received four cycles of 60 mg/m² anthracycline and 600 mg/m² cyclophosphamide every 3 weeks, whereas those whose tumors did not overexpress human epidermal growth factor receptor 2 received four cycles of 75 mg/m² docetaxel and 600 mg/m² cyclophosphamide every 3 weeks. A quasi-pathological complete response (i.e. absence of invasive tumor or only focal residual tumor cells) was the primary endpoint, with compliance and predictors for each regimen as secondary endpoints. If a quasi-pathological complete response was not achieved, then crossover to the alternative treatment was recommended.

Results: The quasi-pathological complete response rate was 36.5% (23 of 63) overall, 27.8% (5 of 18) for the anthracycline and cyclophosphamide regimen and 40.0% (18 of 45) for the docetaxel and cyclophosphamide regimen. Docetaxel and cyclophosphamide treatment induced a quasi-pathological complete response in most patients with triple-negative tumors (15 of 19). The relative dose intensity was 97.3% for the anthracycline and cyclophosphamide regimen and 96.6% for the docetaxel and cyclophosphamide regimen. Quasi-pathological complete response to the docetaxel and cyclophosphamide regimen was associated with low estrogen receptor and progesterone receptor expression and high MIB-1 and topoisomerase IIα expression, in univariate analyses, but only with low estrogen receptor expression in multi-variate analysis.

Conclusions: Selecting neoadjuvant chemotherapy regimens on the basis of individual human epidermal growth factor receptor 2 status improved efficacy, with docetaxel and cyclophosphamide treatment showing particular promise in tumors with the potential to be highly malignant.

Key words: breast cancer – neoadjuvant chemotherapy – HER2 – docetaxel – anthracycline – pathological complete response

INTRODUCTION

Adjuvant regimens using docetaxel and cyclophosphamide (TC) have been associated with superior overall survival rates compared with anthracycline and cyclophosphamide (AC) regimens in the treatment of breast cancer (1,2). Since these data were reported, regimens using non-anthracycline
Several genes have been reported to be associated with sensitivity to anthracyclines and taxanes. For anthracyclines, these include human epidermal growth factor receptor 2 (HER2), estrogen receptor-α (ERα), p53, topoisomerase IIα (TopoIIα) and P-glycoprotein (3–12), and for taxanes, they include β-tubulin, cytochrome P450 3A4, Tau and thiorphan (13–17). However, their roles in predicting the clinical outcome of chemotherapy for breast cancer patients remain controversial.

Among these genes, HER2 is the most promising predictor of the chemosensitivity of breast cancers, as recent meta-analyses have revealed that the benefits of anthracycline-containing regimens are still the standard therapy. It has therefore become important to consider sensitivities to these two drugs in individual patients.

PATIENTS AND METHODS

PATIENT ELIGIBILITY

Patients eligible for this study were females with Stage II or III breast cancer (T ≥2 cm, N0–3 and M0), including those with clinical stage T1N1M0 cancer. Patients staged as T1N0 were not eligible for inclusion. The other eligibility criteria were an Eastern Cooperative Oncology Group performance status of 0 and an age of ≥18 but <75 years. Patients were ineligible if they were pregnant or nursing. All patients were diagnosed as having invasive breast cancer by a core-needle biopsy before treatment. The Institutional Review Board at Yokohama City University Medical Centre, Yokohama, Japan, approved the protocol on 15 January 2007, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent was obtained from all patients prior to the study.

TREATMENT PLANS

On the basis of their HER2 status established from core-needle biopsy specimens, patients received four cycles, at standard doses, of 60 mg/m² anthracycline and 600 mg/m² cyclophosphamide (AC) or 75 mg/m² doxorubicin and 600 mg/m² cyclophosphamide (TC), every 3 weeks. Patients whose tumors overexpressed HER2, as determined by a HercepTest™ (Dako, Glostrup, Denmark) score of ≥3+, received AC, whereas patients with tumors with HercepTest™ scores of <2+ received TC. Dose reductions of 60 to 48 mg/m² doxorubicin and 75 to 60 mg/m² doxorubicin were allowed in patients with febrile neutropenia and Grade 3 or 4 non-hematological toxicities, apart from nausea, vomiting and fatigue. Patients who showed Grade 4 non-hematological toxicity that progressed during chemotherapy, or whose treatment was delayed by more than 3 weeks for any toxicity-related reason were withdrawn from the study.

When a quasi-pathological complete response (QpCR) (as described below) was not achieved following the initial treatment, crossover to the alternative treatment was recommended. For non-QpCR cases after AC treatment, weekly paclitaxel was also added to the TC regimen. The study design is illustrated in Fig. 1. All patients who underwent breast-conserving surgery were given standard radiotherapy. Hormonal therapy was given at the discretion of the treating physician.

HISTOPATHOLOGICAL AND IMMUNOHISTOLOGICAL STUDIES OF PRE-TREATMENT SPECIMENS

The core-needle biopsy specimens from patients were fixed in 20% (w/v) formalin in phosphate-buffered saline and embedded in paraffin. Hematoxylin and eosin (H&E) sections of each block were prepared for histological diagnosis and used to determine the histological type and grade.

Figure 1. Treatment plan: AC was given to patients with breast tumors overexpressing HER2 (3+), whereas other patients were given TC. In patients in whom a QpCR was not achieved, crossover treatment was recommended. AC, anthracycline and cyclophosphamide; HER2, human epidermal growth factor receptor 2; TC, doxorubicin and cyclophosphamide; QpCR, quasi-pathological complete response.
Serial 3–4 μm sections were mounted on silane-coated slides (Muto-glass, Tokyo, Japan) and dried at 37°C overnight. Immunohistochemical staining was then performed using an automatic staining machine (Dako-autostainer; Dako, Kyoto, Japan). Slides were incubated at 95°C for 40 min and de-paraffinized by four 5 min incubations in xylene, followed by 5 min each in 95%, 90% and 70% ethanol, and two 1 min washes in distilled water. Antigen retrieval was achieved by microwaving in citrate buffer (pH 6.0) for 40 min at 95°C. The primary antibodies used in this study are listed in Table 1. After treatment with endogenous peroxidase-blocking reagent (Dako), tissue sections were incubated with primary antibodies for 30 min and with secondary reagents (either Dako REAL™ EnVision™ Detection System, Dako or Ventana iVIEW DAB Universal Kit, Roche Diagnostics Inc., Tokyo Japan) for 30 min. Antibody labeling was visualized using 3,3’-diaminobenzidine (Dako) and slides were lightly counterstained with Mayer’s hematoxylin.

The ER and progesterone receptor (PgR) status was evaluated using the Allred score (18). Briefly, the proportion of positively stained tumor cells was estimated and assigned a score from 0 to 5 (0, negative; 1, 0 to <1%; 2, 1–10%; 3, 10–33%; 4, 33–66%; and 5, >66%) and an average intensity score was assigned for the positive tumor cells (0, none; 1, weak; 2, intermediate; and 3, strong). The final score in the range 0–8 was the sum of the proportion and the intensity scores. The HER2 status was scored from 0 to 3+ using the scale outlined for the HercepTest (Dako) with only HER2 3+ tumors regarded as showing overexpression. Only unambiguous membrane staining was evaluated. For MIB-1, TopoIIα and p53, only unequivocal nuclear staining was considered as a positive result. Slides were scanned at low magnification to locate an area with the most evenly labeled tissue, where a minimum of 1000 tumor cells were scored at high power (×350) and the percentage of labeled cells was calculated. Immunostaining for class III β-tubulin was graded semi-quantitatively from the proportion of tumor cells with positive nuclear staining (0, 0%; 1, 0–10%; and 2, >10%).

Statistical Methods

The primary endpoint used in this study was the overall QpCR rate. The projected QpCR rate was estimated to be ~18%, based on the results reported in previous neoadjuvant trials which included anthracycline (20,21). On this basis, the required sample size for this study was estimated to be 51 with 5% type I and 20% type II errors, and assuming overall QpCR rates of 10% and 35% for the null and alternative hypotheses, respectively. The rate of QpCR was compared between luminal B and HER2 subtypes in the AC group, and luminal A and triple-negative subtypes in the TC group, using Fisher’s exact test. The secondary endpoints included compliance with the study protocol and the predictors of a QpCR (i.e. ER, PgR, p53, MIB-1, TopoIIα and class III β-tubulin expression) for each regimen. The Mann–Whitney U-test was used to compare gene expression between the two groups. For each marker, tumors were divided into high- and low-expression groups; for ER and PgR, tumors with Allred scores of 0–3 were considered low, and 4–8 high. For p53, MIB-1, TopoIIα and class III β-tubulin, the mean values were used as the cut-off points to divide the high- and low-expression groups. Individual analyses were carried out for each predictor and multivariate analysis by logistic regression was also performed, using the Statistical Package for Social Science (SPSS) software.

RESULTS

In total, 63 patients were enrolled in this trial between February 2007 and March 2009. On the basis of their HER2 status, 18 patients were assigned to AC treatment and 45 were assigned to TC treatment. Table 2 shows the baseline characteristics of the patients. In total, 26 (41.3%) of the patients had Stage III disease. The patient characteristics were well balanced, with the exception of HER2 expression. None of the patients dropped out during neoadjuvant chemotherapy, and so the pathological responses were evaluated in all of the patients.

Pathological Tumor Response

The overall pathological breast tumor responses and the responses for each treatment regimen are shown in Fig. 2. In total, 23 of 63 (36.5%) patients showed a QpCR. QpCRs were attained by 5 of 18 (27.8%) patients who received AC.
chemotherapy and 18 of 45 (40.0%) patients who received TC treatment. The QpCR rates for each tumor subtype are shown in Fig. 3. In the TC group, the QpCR rate was significantly higher ($P < 0.001$) for the triple-negative tumors (15 of 19; 78.9%) than for the luminal A tumors (3 of 26; 11.5%). In the AC group, a QpCR was obtained in 25.0% (2 of 8) of patients with a luminal B subtype and in 33.3% (3 of 10) of patients with an HER2 subtype.

COMPLIANCE WITH TREATMENT

The completion rates were 100% for both the AC and TC groups and there were no treatment-related fatalities during the trial. The actual and relative dose intensities of epirubicin in the AC group were 29.2 mg/m²/week and 97.3%, respectively (where the target dose was 30 mg/m²/week); the corresponding values for docetaxel in the TC group were 24.2% mg/m²/week and 96.8%, respectively (where the target dose was 25 mg/m²/week). The commonest reason for reducing the dose was febrile neutropenia, which occurred in 1 of the 18 AC patients (5.6%) and in 10 of the 45 TC patients (22.2%). However, all courses of treatment were completed without delays.

PREDICTIVE FACTORS FOR AC OR TC

Table 3 shows the expression profiles of five genes in the biopsies before treatment from the two groups treated with AC or TC. There were no statistical differences in the expression of these five genes between the two groups. Individual analysis of these genes showed a statistically significant association between a QpCR to the TC regimen and ER, PgR, Topo IIα and MIB-1 expression, but no associations were found with the AC regimen. Multivariate analysis for these five predictors showed that only ER expression remained statistically significant as a predictor of QpCR to the TC regimen (Table 4).

DISCUSSION

This study has shown that planning neoadjuvant chemotherapy on the basis of HER2 status can improve the pathological responses to either anthracycline- or docetaxel-containing regimens, providing a paradigm for individualized treatment for breast cancer patients. This study has also shown that TC
was a particularly effective treatment for tumors of potentially high malignancy, such as the triple-negative subtype.

The overall QpCR rate in this study was 36.5%. Previous studies have reported QpCR rates for combined therapies with anthracyclines and taxanes of between 23.0% and 25.9% (22–24). It is generally accepted that combined therapy with anthracycline and taxane provides better results than using either drug alone (25). However, both these drugs are not always required to treat all patients, as the sensitivity to each drug varies in different individuals. It could be of benefit to use one or other of these drugs, provided that a sufficient pathological response can be achieved.

Neoadjuvant therapy allows us to choose which drugs a tumor is likely to be most sensitive to, with the possibility of following up with a different regimen if the initial therapy does not achieve an adequate response.

In the AC-treated group, the rate of QpCR (27.8%) was better than the rate reported for anthracycline-containing regimen (20,21) and comparable to the rates seen with combined treatments with taxane (22–24). This probably results from selectively administering AC to cases showing HER2 overexpression. This suggests that HER2 overexpression is at present the most promising predictor for the efficacy of anthracyclines. Other genes examined, including TopoIIα, were not found to be associated with the efficacy of the AC regimen.

The TC regimen produced a higher QpCR rate (40.0%) than the AC regimen (27.8%). However, comparing the efficacy of the two regimens directly is not very useful, because the subtypes of breast cancers treated were different in the two groups. AC treatment was given to patients with luminal B and HER2 tumors, whereas TC was administered to those with luminal A and triple-negative tumors. TC therapy was unexpectedly effective in patients with triple-negative breast

![Figure 3. Relationship between QpCR and breast cancer histological subtypes in patients given AC (A) and TC (B) regimens.](image-url)
tumors, which therefore showed an improved QpCR rate compared with luminal A tumors. The three luminal A tumors that achieved a QpCR were not highly hormone-dependent (ER+ but PgR−; data not shown). Analysis of the marker genes also showed that lower ER Allred scores were significantly associated with a QpCR to the TC regimen (P = 0.018). These results suggest that estrogen-dependent cancers are not good targets for TC treatment.

TC was reported to be effective irrespective of the hormone receptor and HER2 status of breast tumors in the US oncology research trial 9735 (2). However, our current results do not necessarily contradict these data, as a subanalysis of the 9735 trial revealed that the TC regimen was superior to the AC regimen for ER− and HER2− tumors compared with ER+ and HER2+ tumors. This supports our results, showing that the triple-negative subtype might be the best target for TC treatment. The positive correlation seen with MIB-1 and TopoIIα expression also suggested that the TC regimen might be effective in breast cancer patients with a relatively high malignancy risk.

Triple-negative breast cancer subtypes are generally more sensitive to anti-cancer agents than non-triple-negative subtypes (26). However, chemotherapy using taxane alone seems to be less promising than combined regimens that include anthracycline for treating the triple-negative subtype (27). This is probably because agents that damage DNA are theoretically more effective for treating triple-negative breast cancers than other types of drug (28). In this study, TC was unexpectedly effective against triple-negative tumors, showing a high QpCR rate, compared with combined regimens such as 5-fluorouracil, epirubicin and cyclophosphamide (FEC100) followed by 75 mg/m² docetaxel (QpCR: 25%) (24). We speculated that a combination of cyclophosphamide and docetaxel might offer improved efficacy, as synergy between these drugs had been observed in preclinical tests (29).

Although the TC regimen produced more frequent cases of febrile neutropenia than had been reported in the US oncology research trial 9735 (1), the treatment was feasible and effective, especially for ER− subtypes. We have therefore launched a randomized Phase II study to compare six cycles of TC treatment with the standard FEC100 regime followed by 100 mg/m² of docetaxel for hormone receptor-negative breast cancers at the neoadjuvant setting.

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Conflict of interest statement

None declared.

References


