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Review Article

Current Trends and Controversies over Pre-operative Chemotherapy for Women with Operable Breast Cancer

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The multi-disciplinary approach, including surgery, chemotherapy, endocrine therapy and radiation therapy, has become the standard treatment for primary breast cancer patients. The indication of pre-operative chemotherapy has been extended to women with potentially operable breast cancer based on the results of large randomized studies and has become an attractive option that extends the chance of breast conservation. The clinical and pathological responses to pre-operative chemotherapy correlates with long-term outcome. The anthracycline-containing regimen is now considered the standard. Sequential administration of non-cross-resistant drugs, namely taxanes, improves local tumor response but its long-term benefit has been controversial. Prediction of response to pre-operative chemotherapy still remains a challenge. Identification of useful predictive markers and development of molecular-targeted drugs is the key to individualized therapy in the future.

Key words: pre-operative chemotherapy – breast cancer – advantage – response – long-term outcome – prediction

INTRODUCTION

The multi-disciplinary approach, including surgery, chemotherapy, endocrine therapy and radiation therapy, has become the standard treatment for primary breast cancer patients with a high risk of recurrence. Although mortality from breast cancer is decreasing in western countries thanks mainly to early detection of the disease by mammography screening and wide usage of post-operative adjuvant systemic therapy (1), its incidence and mortality are steadily increasing in the rest of the world, including Japan (2).

When it first emerged in late 1970s, the use of pre-operative (primary) chemotherapy had been primarily limited to women with inoperable locally advanced breast cancer to enable optimal local therapy (3–5). Later on, large randomized trials proved that pre-operative chemotherapy has at least the same survival benefit as the post-operative chemotherapy (6), and its indication has been extended to women with potentially operable breast cancer.

However, with long-term survivors increasing by systemic therapy in early breast cancer, the 'survivorship' or importance of quality of life after primary therapy has recently come into the limelight. Whether an attempt at breast conservation can be made at the time of definitive surgery is one of the important issues discussed among patients and physicians. Pre-operative chemotherapy is an attractive option for those who have large tumors but a strong interest in breast conserving surgery.

In this review, we describe available evidence and discuss current controversies and future prospects of pre-operative chemotherapy, taking account of its two major clinical roles; eradication of micrometastatis and increased chance of breast conservation.

RATIONALE OF PRE-OPERATIVE CHEMOTHERAPY

Biologic rationale for pre-operative adjuvant chemotherapy was derived from the pre-clinical studies in animal models. It had been known that growth kinetics of metastatic tumors change after surgical removal of the primary lesion (7). The greatest effect of chemotherapy was observed when it was administered prior to operation (8, 9). These observations led to a hypothesis that early systemic chemotherapy prior to surgery might further reduce the risk of metastasis.

The landmark trial in a clinical setting was the National Surgical Adjuvant Breast and Bowel Project (NSABP)

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B-18 trial, which showed pre-operative chemotherapy for operable breast cancer by doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (AC) was at least as effective as post-operative adjuvant chemotherapy with the same regimen in terms of disease-free and overall survival (10). The results were consistent over a longer follow-up period (6) and the result of another large randomized trial conducted in Europe was also confirmatory (11). A recent meta-analysis of pre-operative and post-operative chemotherapy (partly including T4 disease) indicated that pre-operative chemotherapy was equivalent to post-operative therapy in terms of survival and disease progression (12).

Thus the available clinical data has not demonstrated a convincing difference in long-term outcome as hypothesized in pre-clinical studies. However, a higher proportion of women were able to undergo breast conservation surgery. In addition, because the extent of clinical and pathological responses to pre-operative chemotherapy correlates with survival (10), improved tumor response in this setting is expected to improve the overall outcome.

ADVANTAGE OF PRE-OPERATIVE CHEMOTHERAPY

The advantage of pre-operative therapy is that one can subjectively evaluate the response to systemic therapy *in vivo*. Both clinical and pathological responses have been associated with prolonged disease-free and overall survival (6, 8) and they are used as the primary endpoint in clinical trials. Unlike post-operative adjuvant chemotherapy, one can avoid or minimize the unnecessary toxicities from cytotoxic agents by changing treatment strategy when the tumor is not responding to a certain regimen.

Pre-operative chemotherapy is an attractive option for women who wish to reduce the extent of local surgery. Clinical trials provide evidences that 28–89% of women can undergo breast conserving surgery when they might not be otherwise qualified (12).

Because breasts are located on the body surface, one can easily obtain the tumor cells or tissue by either fine needle aspiration or core needle biopsy with minimal invasions. As one can also evaluate the response to systemic therapy in a subjective manner and because patients are usually chemotherapy naïve, a pre-operative setting can be an ideal *in vivo* laboratory for biomarker studies using tumor specimens.

DISADVANTAGE OF PRE-OPERATIVE CHEMOTHERAPY

The overall response rate of pre-operative chemotherapy is 75% on average (range 49–100%), whereas fewer than 5% of the patients with operable breast cancer progress during pre-operative chemotherapy and some more do not even show major responses (13). For such patients with progression, the delay of local treatment may be of disadvantage at least in terms of local control. Pre-operative chemotherapy is also associated with significantly increased risk of loco-regional disease recurrence (12).

Another potential disadvantage of pre-operative chemotherapy is the loss of initial histological information such as tumor size, nodal status and biologic markers. According to the current guidelines, application of post-operative chemotherapy is to be decided by weighing the baseline risk, endocrine responsiveness and estimated risk reduction and harm of the treatment (14). Risk of recurrence is estimated based on the clinical and pathological information obtained from surgical specimens. In a pre-operative setting the information on tumor size and nodal status will inevitably be imprecise and intra-tumor heterogeneity of histologic type, histologic grade and biomarker expression cannot be taken into account. It may potentially put patients into danger of over- or under-treatment. Currently, core-needle biopsy is mandatory prior to pre-operative chemotherapy to obtain as much pre-treatment histopathological information as possible.

TREATMENT REGIMENS

Using clinical or pathological responses as surrogate endpoints of overall survival, optimal systemic therapies have been investigated in pre-operative settings in patients with early breast cancer. The general consensus reached is that an anthracycline-containing doublet (doxorubicin or epirubicin with cyclophosphamide) or triplet (doxorubicin or epirubicin with cyclophosphamide and 5-fluorouracil) should be used as the initial chemotherapy strategy for pre-operative chemotherapy (15, 16).

The sequential use of non-cross-resistant agents is likely to augment the response of pre-operative chemotherapy (17, 18), among which taxanes are the most investigated drug. Overall, results of randomized trials indicate that the incorporation of taxane increases the rate of pathological complete response (pCR) by 6–16% compared to anthracycline/cyclophosphamide-based regimens (19, 20). Smith et al. randomized patients who achieved clinical response to the initial four cycles of cyclophosphamide/vincristin/doxorubicin/predonisone (CVAP) therapy to receive further four cycles of CVAP or four cycles of docetaxel (Aberdeen trial) (21). The sequential use of docetaxel resulted in enhanced clinical and pathological responses even in anthracycline-sensitive tumors. In NSABP-B27 trial, the addition of four cycles of docetaxel after pre-operative AC increased the clinical complete response rate (40% versus 63%), clinical overall response rate (86% versus 91%) and the pCR rate (14% versus 26%) compared with pre-operative AC therapy alone (20). However, the addition of taxane in pre-operative or post-operative setting after AC did not improve the long-term outcome in this trial (22).

Treatments incorporating molecular-targeting drugs are of interest. Trastuzumab is effective for patients with advanced

breast cancer over expressing HER2 (23). In adjuvant settings, at least one year of trastuzumab given sequentially or concomitantly with chemotherapy significantly improves disease-free and overall survival (24, 25). Moreover a short course (9 weeks) of trastuzumab administered concomitantly with docetaxel or vinorelbine seems to be effective in HER2-positive subset of patients in adjuvant settings (26).

For pre-operative settings, there are a limited number of phase II studies reporting the use of trastuzumab (25, 27, 28). The only randomized trial reported was by Buzdar et al., who compared neoadjuvant chemotherapy for HER2-positive, operable breast cancer with or without administration of trastuzumab (29). This study was closed by the recommendation of Data and Safety Monitoring Board of the institution according to early-stopping rule, because pCR rate, the primary endpoint, was strikingly superior in the chemotherapy plus trastuzumab arm (given simultaneously for 24 weeks) compared with the chemotherapy-alone arm (65% versus 26%, $p = 0.016$). We still need to confirm if this significant difference in pathological response will be translated into prolonged overall survival by long-term follow-up and also the cardiac safety of trastuzumab in combination with chemotherapy should be assessed.

CONTROVERSIES OVER PRE-OPERATIVE CHEMOTHERAPY

Evaluation of Residual Tumor for Optimal Surgery

Optimal imaging modality has not been established to definitely localize the remaining tumor. Usually, serial imaging studies are performed before and after pre-operative chemotherapy. Magnetic resonance imaging or computerized-tomography scanning may supplement conventional breast imaging studies by mammography and ultrasonography (30–33).

The use of functional imaging techniques such as fluorine-18 fluorodexyglucose positron emission tomography ([^{18}F]-FDG PET) is of interest for the evaluation of therapeutic response to systemic therapy in breast cancer. The change in [^{18}F]-FDG uptake reflects the alteration in cellular glycolysis. Some relatively small studies reported that [^{18}F]-FDG PET after a single pulse of chemotherapy predicted pCR or minimal residual disease with a sensitivity of 85–100% and a specificity of 74–85% (34–36). FDG-PET is promising for clinical application in future to detect non-responding tumor to avoid unnecessary toxicities from cytotoxic therapy.

Feasibility of Sentinel Lymph-Node Biopsy (SNB) in Patients Treated with Pre-operative Chemotherapy

Axillary staging by SNB may allow omission of axillary dissection in sentinel-node negative patients without compromising the long-term outcome (37). However the optimal timing and feasibility of SNB in the setting of pre-operative chemotherapy have not been established.

Identification rate of SNB following pre-operative chemotherapy are reported to be 84–93% and 78–93%, in single-institution series and multi-center studies (38), respectively. High false-negative rates up to 25–33% have been reported for several small single institution studies (39, 40), but in multi-institutional studies using radiocolloid with or without blue dye, false-negative rates range between 5 and 13% (38), which are similar to those observed when it was carried out before systemic chemotherapy.

There still remain concerns about the use of SNB following chemotherapy in patients with clinically positive axilla (41), SNB after chemotherapy possesses a potential to maximize the benefit of axillary downstaging by pre-operative systemic treatment, in other words, avoidance of complications related to axillary dissection and decision-making of adding further chemotherapy.

Alteration of Biological Markers

The changes in the expression of hormone receptors and HER2 protein during pre-operative chemotherapy may influence the clinical decision of adjuvant hormonal and trastuzumab therapy. In studies using immunohistochemistry, the administration of pre-operative chemotherapy did not alter the expression patterns of HER2 and hormone receptors (42–45).

However, a study was conducted to compare gene expression profile of pre-treatment biopsy specimens with those in tumors remaining after doxorubicin-containing pre-operative chemotherapy using DNA array. There were differences in the gene expression profile in tumors that showed a response, but not in tumors that did not respond to therapy (46). Biological and clinical implications of the change of gene expression profile in responding tumors need further elucidation.

Definition of Pathological Response

Primary systemic treatment is increasingly recognized as the best model for the quick development of new treatment strategies in early breast cancer. pCR after pre-operative chemotherapy has been chosen as the primary endpoint of clinical trials, because it is validated as the surrogate marker of improved outcome (47, 48). However, diverse definitions of pathological response are used by different investigators (10, 47, 49–53). Some of these grading systems allow inclusion of residual ductal caricinoma *in situ* (DCIS) without invasive component in the definition of pCR. However, there is no confirmatory data to justify the concept that there is no difference in prognosis between patients with no invasive or *in situ* disease and those with residual DCIS. Jones et al. investigated whether the prognosis for patients with residual DCIS is the same as that for patients with no residual tumor cells, but could not demonstrate significant

prognostic difference (54). However, this study was statistically underpowered to draw any conclusions.

Ideally, response to chemotherapy should be measured as a continuous variable. No system satisfies the need of accurate pathologic evaluation for the majority of patients who achieve partial or minor response to pre-operative chemotherapy. Rajan et al. proposed that the product of residual tumor size and cellularity might be a more clinically relevant indicator of tumor response than assessing tumor size alone (55). Though it is an interesting proposal, the method needs to be validated in correlation with long-term outcome.

Outcome after Pre-operative Chemotherapy and Surgery

Several studies have attempted to find more accurate predictors for survival after pre-operative chemotherapy than pCR in the primary tumor. This is because substantial risk of systemic recurrence still remains even if pCR is achieved, whereas substantial patients have excellent prognosis even if pCR is not achieved. If the long-term risk is high, they will be the candidates for clinical trials to determine whether additional aggressive therapy will be of benefit. If a good prognosis is expected even without good response to pre-operative therapy, aggressive chemotherapy might be over-treatment in pre-operative setting.

In the report of retrospective studies from Royal Marsden Hospital and M. D. Anderson Cancer Center, pathologically negative axillary lymph nodes after pre-operative chemotherapy, not pCR in the primary tumor, remained the independent prognostic factor for disease-free survival and overall survival in multivariate analysis adjusted for other prognostic factors (56–58).

It was revealed by a retrospective multivariate analysis of the clinicopathological factors of the 226 patients who had pCR after pre-operative chemotherapy that pre-operative clinical stage IIIB, IIIC, and inflammatory breast cancer, axillary lymph nodes more than 10, and pre-menopausal status were the independent prognostic factors of distant metastasis (59). In another study, only histological grading had an independent prognostic impact on disease-free and overall survival after adjustment for pCR to pre-operative chemotherapy containing doxorubicin (60). Carey et al. found that American Joint Committee on Cancer Tumor-Node-Metastasis staging after pre-operative chemotherapy was useful in prediction of distant disease-free survival and overall survival (61).

Rouzier et al. constructed nomograms combining clinical variables associated with pCR that might accurately predict pCR and distant disease-free survival (62). This was confirmed in an independent dataset within the study. The nomogram included size of residual tumor and the number of metastatic nodes at the time of surgery, histologic grade, estrogen receptor (ER) status and histologic type. On the other hand, biologic markers such as expression of HER2 (63), EGFR (64), p53 (65) or MDR1 gene (66) in tumor specimen before pre-operative chemotherapy, reduction of expression in topoisomerase II-α (70) or MLH1 (71) after pre-operative chemotherapy are suggested to predict long-term outcome. Although it is not known whether these markers would add to or replace the nomogram, development of more accurate and comprehensive tools for prediction of prognosis is awaited.

Prediction of Response to Pre-operative Chemotherapy

The pre-operative setting is ideal to explore molecular predictors of response to therapy. Various clinical and pathologic variables have been studied. Among them, ER status, histologic grade and smaller tumor size seem to be associated with the response to pre-operative chemotherapy (47, 69).

In previous retrospective studies, clinical and pathological responses to pre-operative chemotherapy appear to be lower in invasive lobular carcinoma (ILC) as compared to invasive ductal carcinoma (IDC), and patients with ILC were more likely to receive mastectomy after initial attempt for breast conservation (70–73). However, low pCR rates in ILC have not been translated into survival disadvantage (70–72). These data suggest that different approach should be taken in the clinical management of patients with ILC.

In a biomarker study, ER expression, absence of HER2 and a decrease in Ki67 correlated with good clinical responses subsequent to a pre-operative chemoendocrine therapy (74). Among other biomarkers, bcl-2 and p53 have been studied. bcl-2 has been shown to protect cells from apoptosis induced by chemotherapeutic drugs (75). Although high expression of bcl-2 has been hypothesized to play a role in resistance to chemotherapy, it is still controversial. In one study, higher bcl-2 expression at diagnosis was predictive of pCR in univariate analysis but it did not retain its impact in multivariate analysis (76), while other studies did not find any correlation between bcl-2 expression and the response (77, 78).

p53 is also a potential predictive marker. Active p53 promotes apoptosis in growth-arrested cells whereas loss of p53 function has been reported to enhance cellular resistance to various chemotherapeutics (79). In a clinical setting, in patients treated with single agent epirubicin, mutant p53 was a significant predictor for poor clinical response, but the association was weaker in patients treated with cyclophospmide/methotrexate/5FU with or without tamoxifen (65). Another study demonstrated that a tumor expressing wild-type p53 was related to resistance to single agent doxorubicin therapy in multivariate analysis (80). *TP53* gene mutation and over expression of p53 were related to epirubicin-containing chemotherapy, but response to paclitaxel seemed to be related to p53-negative tumors (81).

Tumor response and toxicities are different among individual patients. Pharmacogenomic studies aim to elucidate the genetic bases for inter-individual differences and to enable individualization of care. DNA microarray is one of the modern high-throughput biotechnologies that allow

researchers to analyze expression of multiple genes in concert and relate the findings to clinical parameters. In breast cancer, several groups have reported preliminary results suggesting that the gene expression profile of the primary tumor may predict the tumor's response to pre-operative chemotherapy (82–86). One major limitation of microarray studies is overfitting of the predictior: the number of mRNA transcripts far exceed the number of samples (87, 88). The accuracy of the predictive model is low in independent data set (89). More rigorous and critical evidence is necessary before multi-gene predictors can be accepted as a useful and reliable tool in clinical practice.

Pre-operative Endocrine Therapy

The relative benefit of chemotherapy is less in endocrine-responsive disease as compared with endocrine non-responsive disease (1) and recent consensus of the clinical community lays emphasis on the endocrine responsiveness in decision-making of adjuvant systemic therapy (14). Pre-operative endocrine therapy is an attractive alternative for endocrine-responsive disease, because it is easy to perform and can also avoid acute and late side effects caused by cytotoxic chemotherapy, but pre-operative endocrine therapy has not been accepted as the standard therapy because of the slow rate of response (90). We need more accurate measures to select the patients who are most likely to respond to endocrine therapy without compromising the potential benefit of chemotherapy.

Application to Molecular-Targeted Therapy

Molecular-targeted drugs are anticipated to individualize the therapeutic strategy based on the biology of the tumor. To date, the presence of a target still does not satisfactorily guarantee a response to therapy, but efforts are being made to elucidate the key components of the molecular pathways targeted by a specific agent.

Moshin et al. reported a pre-operative study of trastuzumab as a single agent in HER2-positive locally advanced breast cancer (91). They administered trastuzumab as a single agent for the first 3 weeks, followed by a combination of trastuzumab and docetaxel. Of note, partial response was observed in eight among 35 patients after only 3 weeks of trastuzumab. The accompanying biomarker study suggested that the main mechanism of action of trastuzumab is inhibition of the PI3K/Akt pathway, which results in an increase of apoptosis (79). The clinical role of single-agent trastuzumab in HER2-positive tumors has not been determined, but it is attractive if we can select the responders to trastuzumab as this is usually less toxic than cytotoxic chemotherapy.

A report by Polychronis et al. is unique in respect of testing the efficacy of combination of targeted therapy based on biology-derived hypothesis (92). It was a double-blind placebo controlled phase II randomize trial of pre-operative gefitinib versus gefitinib versus anastrozole in post-menopausal patients with ER- and EGFR-positive primary breast cancer. The tumors of patients assigned to combination therapy had a greater reduction of Ki67 labeling index than those assigned to gefinitib alone. Although the number of patients in this study was so small that we do not yet know whether reduction in proliferation will be translated into clinical benefit, we foresee a future of individualized therapy.

FUTURE DIRECTIONS

Pre-operative chemotherapy has become the standard of care in management of primary breast cancer. However, we should be aware that a substantial portion of patients may be over-treated by pre-operative chemotherapy because of inaccurate pre-treatment staging. In NSABP-B27 study, addition of docetaxel was beneficial in terms of disease-free survival not in complete responders or non-responders but only in partial responders in a subset analysis according to clinical response after AC. Who needs additional systemic therapy? Who can avoid systemic therapy?

Development of endocrine therapy and trastuzumab has opened the door to important therapeutic advance of 'molecular-targeted therapy'. Transcriptional profiling has revealed that expression levels of these targets, i.e. ER and HER2, are the major genetic determinants of the biology of the disease (93). Thus, we can foresee the future of systemic therapy individualized with endocrine responsiveness and involvement of HER2 signaling pathway. However, to date, the predictive value of screening test for molecular targets remains unsatisfactory.

Identification of clinically useful, prognostic and predictive molecular markers is highly anticipated to optimize therapeutic regimens. The current probability-based therapeutic strategy, 'empiric treatment' so to speak, might give way to biology-based, individualized strategy, 'marker-based treatment', when additional biologic markers are identified that make 'targeted therapy' more targeted and effective. Pharmacogenomic researches that accompany pre-operative therapy might help better understand the biology of breast cancer and thus promote the development of new therapeutic strategies.

Conflict of interest statement
None declared.

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CLINICAL TRIAL

Prediction of response to repeat utilization of anthracycline in recurrent breast cancer patients previously administered anthracycline-containing chemotherapeutic regimens as neoadjuvant or adjuvant chemotherapy

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Summary
Purpose: The aim of this study was to identify the predictors of the response to doxorubicin plus cyclophosphamide in patients with recurrent breast cancer (RBC) previously treated with anthracycline-containing regimens in a neoadjuvant or adjuvant setting.
Method: Between December 1993 and October 2005, 664 patients had received combined doxorubicin plus cyclophosphamide chemotherapy (doxorubicin, 40 mg/m^2, iv on day 1; cyclophosphamide, 500 mg/m^2, iv on day 1, every 21 days) for RBC at our institution. In this study, we retrospectively analyzed the efficacy of doxorubicin plus cyclophosphamide in 99 of these 664 RBC patients who had also previously been administered an anthracycline-based chemotherapy in a neoadjuvant or adjuvant setting.
Results: The median cumulative dose of the previously administered anthracycline was 156 mg/m^2. The median disease-free interval (DFI) and median anthracycline-free interval were 33.8 and 43.7 months, respectively. The overall response rate to doxorubicin plus cyclophosphamide therapy was 38.4% (95% CI; range, 28.8–48.0%). The median time to progression and overall survival were 6.2 and 17.5 months, respectively. The results of a multivariate logistic regression analysis revealed a significant association of the response to doxorubicin plus cyclophosphamide therapy with the DFI ($P = 0.02$); human epidermal receptor type 2 (HER2) status also tended to affect the response rate, however the association was not statistically significant ($P = 0.06$).
Conclusion: DFI and HER2 status may be associated with the response to repeat utilization of anthracycline-containing regimens in RBC patients also treated previously with anthracycline-containing chemotherapeutic regimens in a neoadjuvant or adjuvant setting.

Keywords Anthracycline · Anthracycline-free interval · Disease-free interval · Doxorubicin · HER2 status · Prediction reutilization · Recurrent breast cancer

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Introduction

Breast cancer is known as one of the chemotherapy-sensitive cancers. Neoadjuvant or adjuvant chemotherapy, for eradicating micrometastatic disease, has been shown to improve the survival of patients with early-stage breast cancer [1, 2]. Results of randomized controlled trials and meta-analyses have demonstrated the clinical benefit of anthracyclines in early breast cancer, including in node-positive and node-negative breast cancer, in both pre- and post-menopausal women. Doxorubicin and epirubicin, the two most commonly used anthracyclines, are among the most effective anticancer drugs in breast cancer chemotherapy.

The majority of early breast cancer patients currently receive anthracycline-containing chemotherapy.

However, despite the adjuvant or neoadjuvant chemotherapy, a significant proportion of these patients develop recurrence within the first 5 years. Treatment of patients with recurrent breast cancer (RBC), often poses a difficult therapeutic problem; these patients have already received the most effective therapy as their primary treatment, therefore an alternative agent that might be less effective often needs to be used.

It has been reported that in some chemosensitive tumors, reutilization of potentially active chemotherapeutic agents may be an effective treatment option [3–5]. In the case of ovarian cancer, planning of chemotherapy for recurrent disease after adjuvant chemotherapy is mainly dependent on the platinum-free interval [3]. To the best of our knowledge, there is no information available until date about the optimum anthracycline-free interval (AFI) or predictors of response to repeated utilization of anthracycline-containing regimens in cases of RBC. Similar to their efficacy in the neoadjuvant or adjuvant setting, anthracyclines have also been shown to exhibit clinical efficacy against metastatic breast cancer [6]. Therefore, it may be useful to identify patients of RBC who are likely to show response to repeat utilization of anthracycline-containing regimens.

The objective of this study was to evaluate the efficacy, in terms of the response rate, time to progression and overall survival, of combined doxorubicin plus cyclophosphamide (AC) therapy in RBC patients previously treated with anthracycline-containing chemotherapeutic regimens and to identify patients who are likely to benefit from repeat utilization of anthracycline-based chemotherapy.

Patients and methods

A total of 664 patients with RBC were treated with AC therapy between December 1993 and October 2005 at the National Cancer Center Hospital. We retrospectively selected patients who fulfilled the following selection criteria as the subjects of the present study: (1) previously administered anthracycline-containing chemotherapeutic regimens as neoadjuvant or adjuvant chemotherapy; (2) adequate bone marrow and organ function (neutrophils $\geq$1,500 μ^{-1}, platelets $\geq$100,000 μ^{-1}, AST $\leq 2.5 \times$ upper limit of normal range (ULN), ALT $\leq 2.5 \times$ ULN, serum creatinine $\leq 1.5 \times$ ULN); (3) availability of written informed consent prior to the start of treatment.

Patients were administered 3 mg of granisetron hydrochloride and 8 mg of dexamethasone intravenously (iv) 30 min prior to the doxorubicin infusion. The dosages of the chemotherapeutic drugs were as follows: doxorubicin, 40 mg/m^2, iv on day 1; cyclophosphamide, 500 mg/m^2, iv on day 1 of each 21-day cycle. Treatment with the AC therapy was continued until evidence of disease progression or of unacceptable toxicity was observed.

Patients with no bidimensionally measurable lesions were considered ineligible for the objective response evaluation. The objective responses were evaluated according to WHO criteria [7]. Patients without measurable lesions were classified as not assessable (NA). Toxicity was re-evaluated according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) ver 2.0.

Statistical analysis

Logistic regression analyses were performed to assess the response to the AC therapy of the RBC patients previously treated with anthracycline-containing chemotherapeutic regimens in a neoadjuvant or adjuvant setting and other factors. Factors with a P-value of less than 0.2 in the univariate logistic regression were examined simultaneously with multivariate logistic regression models. A stratified analysis was also performed to assess the effect of the disease-free interval (DFI) and human epidermal receptor type 2 (HER2) status, believed to be factors associated with the response to AC therapy. In regard to the DFI, its validity as a predictor was examined via an ROC analysis, and the cutoff value that yielded 75% sensitivity was selected in order to classify the patients into two categories.

DFI was measured from the date of mastectomy until observation of evidence of the first local, regional, or distant recurrence of the tumor, contralateral breast cancer, or a second primary tumor in addition to the breast tumor. AFI was measured from the last date of administration of anthracycline-containing chemotherapy until the date of re-start of AC therapy. Time to progression was measured from the first day of treatment until disease progression or the final day of follow-up without disease progression, and the overall survival time was measured from the first day of treatment until death or the final day of follow-up. Median time to progression and median overall survival were estimated by the Kaplan–Meier method.

The statistical analysis was performed with SAS, version 9.1.3 (SAS Institute, Cary, NC, USA), and the significance level was set at $P = 0.05$ (two-sided).

Results

Patient characteristics

Of the 664 patients treated with AC therapy for RBC, 99 had also previously received anthracycline-based

chemotherapy in the neoadjuvant or adjuvant setting. The patient characteristics are summarized in Table 1. The median age was 54 years (range, 31–76 years); the median performance status was 0 (range, 0–3). Median number of organs involved was 2 (range, 1–6). Most of patients (91%) had received anthracycline-based chemotherapy in the adjuvant setting while the remaining had received it in a neoadjuvant setting. The median dose of the previously administered anthracycline was 156 mg/m^2 (range, 15–360 mg/m^2). Six patients had received regimens containing anthracycline and taxanes agents as neoadjuvant or adjuvant chemotherapy. Ninety-seven patients had undergone mastectomy and remaining had undergone breast-conserving therapy. Twelve patients had also received adjuvant radiation therapy and 79 patients, adjuvant hormone therapy. The median DFI was 33.8 months (range, 3.8–191.7 months) and the median AFI was 43.7 months (range, 4.7–192.8 months). The majority of these patients (N = 66, 66.6%) had received AC therapy as first-line chemotherapy for RBC. Before AC therapy, remaining patients (N = 33) had received other chemotherapy for RBC, as follows: 21 patients, docetaxel; 13 patients, paclitaxel; 2 patients, CMF; 1 patient, capecitabine; 1 patient, vinorelbine; 1 patient, irinotecan; 1 patient each, vinorelbine and capecitabine.

Table 1 Characteristics of the 99 patients

Characteristics	Value
Median age (range)	54 (31–76)
Side (right/left)	57/42
Median ECOG performance status (range)	0 (0–3)
Median metastatic site (range)	2 (1–6)
Metastatic sites	
Lung	37
Liver	32
Bone	47
Pleural effusion	20
Lymph node	47
Soft tissue	32
No. of previous chemotherapy regimens before treatment with AC	
0	66
1	27
>1	6
Hormone status (ER or PgR)[a]	
Positive	69
Negative	30
HER2 status[a]	
Positive	9
Negative	80
Unknown	10

ECOG Eastern Cooperative Oncology Group, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal receptor type 2

[a] Hormone status and HER2 status were evaluated by immunohistochemical examination

Treatment efficacy and toxicity

A total of 482 courses of AC therapy were administered, and the median number of courses was 6 (range, 1–6). The response rate in the 99 patients was 38.4% (95% CI; range, 28.8–48.0%, 2 CR, 36 PR, 32 SD, 8 NA, and 21 PD). The objective response rates stratified according to the DFI and HER2 status are shown in Table 2. The difference in the response rate between patients with a long DFI (≥2.5 years) and those with a short DFI (<2.5 years) was statistically significant in patients with an HER2-negative status (Chi-Square test— P = 0.014). Although the response rate tended to be higher in the patients with an HER2-positive status, statistical analysis to determine the significance was not performed due to the small sample size. Age and DFI were significantly associated with the response to AC therapy in according to the results of univariate analysis (P = 0.03 and 0.03, respectively). The results of the multivariate logistic regression analysis indicated that DFI as continuous variable significantly affected the response rate to AC therapy (Odds ratio, 1.23; 95% CI: 1.03–1.48, P = 0.02), even after adjusting for the effect of the HER2 status. The HER2 status tended to affect the response rate, however, the association was not statistically significant (Odds ratio, 4.1; 95% CI: 0.94–17.8, P = 0.06). The statistical analysis revealed no significant correlation of other factors, including AFI, with the response to AC therapy. The median time to progression and overall survival were 6.2 months (Fig. 1; 95% CI: 5.6–7.6 months) and 17.5 months (Fig. 1; 95% CI: 14.6–22.2 months), respectively.

Table 2 Objective response rate to doxorubicin plus cyclophosphamide therapy according to the HER2 status and DFI

Profile	No. of patients	Response rate (95% CI)
HER2 positive[a]	9	66.7% (29.9–92.5%)
HER2 negative	90	35.6% (25.7–46.4%)
DFI ≥2.5 years[b]	52	46.2% (31.6–60.7%)
DFI <2.5 years	38	21.1% (6.8–35.3%)
Total	99	38.4% (28.8–48.0%)

[a] The number of patients with HER2 positivity was small. Therefore, Chi-Square test was not performed for the HER2-positive patients according to the DFI

[b] Long DFI was associated with a higher response rate than a short DFI in HER2-negative patients (Chi-Square test— P = 0.014)

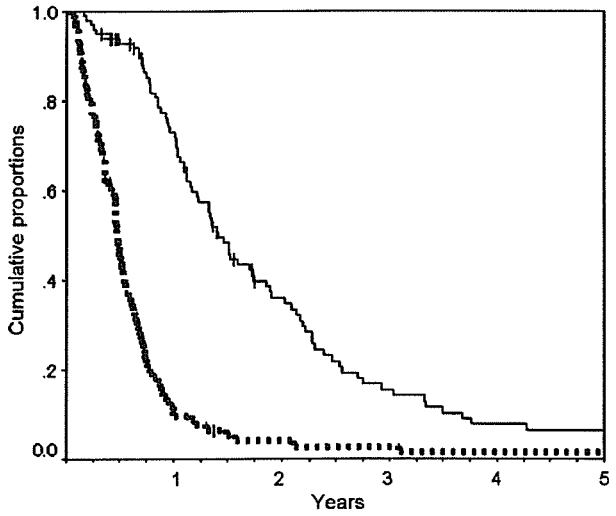

Fig. 1 Kaplan–Meier analysis of time to progression (*dotted line*) and overall survival (*solid line*). *Vertical bars* indicate censored cases

A total of 482 courses in the 99 patients were assessable for toxicity. The median cumulative dose of doxorubicin for RBC was 240 mg/m^2 (range, 40–240 mg/m^2), and the median total cumulative dose of anthracycline (after conversion to doxorubicin) was 343 mg/m^2 (range, 102–600 mg/m^2). The toxicity profile is listed in Table 3. The AC therapy was generally well tolerated and could be managed from an outpatient setting. Grade 3 or 4 neutropenia occurred in 14 patients (14.1%) and 4 patients of these developed febrile neutropenia. No cardiotoxicity was observed. No grade 4 non-hematological toxicity was reported either, and there were no unexpected adverse reactions or treatment-related deaths.

Table 3 Maximum grade (NCI-CTC ver 2.0) toxicity (% of patients)

	Maximum grade % of patients			
	1	2	3	4
Leukopenia	27	24	5	2
Neutropenia	15	21	7	7
Anemia	22	8	1	2
Thrombocytopenia	2	1	0	1
Fatigue	31	2	0	0
Appetite loss	82	7	0	0
Nausea	74	17	0	0
Vomiting	15	9	0	0
Stomatitis	21	1	0	0
Diarrhea	6	0	0	0
Constipation	8	0	0	0
Neurosensory	9	1	0	0

Discussion

This study demonstrated the activity of AC therapy even in RBC patients who had previously received anthracycline-containing chemotherapy in the neoadjuvant or adjuvant setting.

There are few reports of the efficacy of repeat use anthracycline-containing chemotherapy in metastatic or RBC patients. Although repeat use of anthracycline-containing regimens has been reported to yield objective response rates of 30–46%, there were no clear predictive factors of the response to such anthracycline agent-containing chemotherapy in case with RBC [8–12].

The results of the present study demonstrated that patients with a long DFI show favorable response to repeat use of AC as compared with patients with a short DFI. DFI, which reflect the degree of aggressiveness of the disease, had been known as one of the most important prognostic factors in cases of RBC [13]. Anthracyclines are topoisomerase inhibitors, and the topoisomerase II alpha gene has been reported to be associated with anthracycline sensitivity. Tinari et al. reported that anthracycline-sensitive breast cancer had the decreasing changes in topoisomerase II expression after anthracycline-based neoadjuvant chemotherapy and that it is an independent predictor of a long DFI [14]. Therefore, a long DFI may actually indicate inherent sensitivity to anthracycline, and a favorable response.

The role of the HER2 status in predicting the sensitivity to anthracyclines is still under debate. The topoisomerase II alpha gene is closely linked to the HER2 gene on chromosome 17 [15]. Recent studies have suggested that patients with an HER2-positive status might derive greater benefit from adjuvant chemotherapy using anthracycline-containing regimens as compared to that using non-anthracycline-containing regimens [16, 17]. While the HER2 status tended to influence the response rate to AC therapy in this study, the association was not found to be statistically significant. HER2 is generally overexpressed in 20–25% of breast cancers [18]. The relatively low frequency of patients with an HER2-positive status in the present study may also confound the result.

In the present study, all the patients had previously been treated with anthacycline-containing chemotherapeutic regimens, therefore, leukopenia or neutropenia (of any grade) was frequently observed, however, the incidence of febrile neutropenia was approximately equivalent to that reported by a previous randomized multicenter study of doxorubicin plus cyclophosphamide combination chemotherapy as

first-line treatment in cases with metastatic or RBC [19–21]. The retrospective nature of this study did not allow reliable or accurate estimation of the potential doxorubicin-related cardiotoxicity. Even though the incidence of cardiotoxicity may have been underestimated in this study, there were no cases with symptomatic cardiotoxicity or cardiotoxicity requiring treatment. Both the hematological and non-hematological toxicity profiles were similar to those reported by the Japanese randomized multicenter trial using the same standard dose of AC [21].

The use of anthracyclines in clinical practice is limited in most cases by the important drug-associated toxicity, namely, cardiotoxicity. Therefore, extensive research has been directed at the identification of methods capable of ameliorating the threat of anthracycline-related cardiotoxicity. Prevention or reduction of doxorubicin-induced cardiotoxicity would enable continuation of treatment in doxorubicin-responsive patients beyond the limit imposed by potential cardiotoxicity. The risk of anthracycline-related cardiotoxicity can be reduced by setting a cumulative dose limitation, generally in the region of 450–500 mg/m^2. However, it is recognized that injury to the myocardium begins with the first administration of anthracyclines and that cardiotoxicity begins to appear at a total cumulative dose <300 mg/m^2 [22]. In addition, several studies have reported factors that might increase the risk of doxorubicin-induced cardiotoxicity, namely, preexisting heart disease, mediastinal radiation, pediatric age group, and older patients (age >65 years) [23–25]. Also, the weekly schedule of drug administration was less cardiotoxic than the once-in-three weeks schedule [12]. Administration of doxorubicin as a prolonged infusion has been reported to be less cardiotoxic than that by bolus infusion [22, 26]. However, neither the schedule or administration rate recommended above widely accepted, because of the associated inconvenience. Encapsulated doxorubicin by pegylated liposomes is a unique formulation of doxorubicin, which reduces the maximum peak serum level of free doxorubicin and increases the half-life of the drug [27]. In addition, a randomized clinical trial demonstrated that liposomal doxorubicin was associated with a significantly reduced cumulative cardiac toxicity, while providing comparable antitumor efficacy [19]. Dexrazoxane, an iron-chelating agent, has shown the ability to reduce the cardiac toxicity induced by doxorubicin. Recent advances in safety-improvement techniques of may be expected to increase the therapeutic index of anthracyclines and enhance its overall clinical benefit.

Several studies have reported the efficacy of combined anthracycline plus other agent (e.g., taxanes, gemcitabine, vinorelbine) chemotherapy in cases with metastastic or RBC, including those previously administered anthracycline-based adjuvant chemotherapy [10, 12]. For RBC patients, the present study results suggesting the beneficial effects of doxorubicin with dexrazoxane, pegylated liposomal doxorubicin and the aforementioned combination chemotherapies may be significant.

The validity of measurement of the left ventricular ejection fraction by multiple-gated acquisition scan to predict the risk of cardiotoxicity remains controversial [28, 29]. However, at the present time, when considering the reutilization of anthracycline-containing chemotherapeutic regimens, care must be exercised to avoid patients with a high risk of anthracycline-related cardiotoxicity and careful monitoring should be conducted to detect any evidence of cardiotoxicity.

In conclusion, the results of the present study suggest that the treating physician of RBC cases may consider repeat utilization of anthracycline drug in patients likely to show a favorable response based on a long DFI and/or HER2 status. Anthracycline-containing chemotherapy may be administered as a useful chemotherapeutic option in these patients with careful monitoring for any evidence of the onset of cardiotoxicity.

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ORIGINAL ARTICLE

Correlation of p53 and MIB-1 expression with both the systemic recurrence and survival in cases of phyllodes tumors of the breast

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Abstract

Phyllodes tumors are rare primary tumors of the breast. The study aimed at evaluating the immunohistochemical features of phyllodes tumors of the breast that may be useful for predicting the clinical outcome. We examined the immunohistochemical expression of the epidermal growth factor receptor (EGFR), HER2/neu, CD117/c-kit, p53, and MIB-1, and analyzed correlations between the immunohistochemical findings and the clinical outcome. The study included 41 patients with phyllodes tumor (20 benign, 5 borderline, and 16 malignant). Systemic recurrence occurred in 9 patients. The 2-year survival rate was 84%, and the 2-year recurrence-free survival rate was 77%. Six patients developed systemic recurrence within the first year after surgery. None of the phyllodes tumors was positive for HER2/neu or CD117/c-kit. Positive staining for p53 was seen in 10 phyllodes tumors (24%), and the median MIB-1 index was 10%. Both p53 expression and the MIB-1 index, but not the expression status of EGFR, were significantly correlated with the recurrence-free and overall survival. p53 expression status and MIB-1 index may be significant prognostic factors in patients with phyllodes tumors, and careful postoperative follow-up may be important in those cases showing positive expression of p53 and/or MIB-1 index.

Keywords: Phyllodes tumor; p53; MIB-1; Systemic recurrence; Survival

Introduction

Phyllodes tumors of the breast are rare, accounting for less than 1% of all breast tumors. [16]. Phyllodes tumors occur predominantly in middle-aged women, and the average tumor size is 4–5 cm. Histopathologically, these tumors are distinguished from true sarcomas by the presence of epithelial elements within the cellular connective tissue stroma. At present, phyllodes tumors are classified into benign, borderline, and malignant subtypes based on a combination of histological features, stromal cellular atypia, mitotic activity, stromal overgrowth, and tumor margins [15]. Approximately 50% of phyllodes tumors are benign, while the

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incidence of the malignant subtype has been reported to range from 26% to 35% [16,17]. While local recurrence can occur in all phyllodes tumors, systemic recurrence may also develop in cases of borderline or malignant phyllodes tumors [8].

Several studies have been carried out in which different pathologists have evaluated the same histological slides, resulting in a discordance of up to 25% in the final histopathological typing [5,9]. It remains difficult to predict the clinical outcome of the patients based solely on the histological features, and no effective treatment strategies have been developed for systemic involvement in these cases. Previous studies have been conducted to investigate the usefulness of immunohistochemical analyses of the tumors for various tumor markers to predict the clinical outcome. Immunohistochemical detection of p53 expression, commonly used as an identification for tumor-suppressor gene mutation, has been correlated with tumor grade [6,13,19]. Several studies on MIB-1 immunostaining, cell proliferation, have also shown a correlation between MIB-1 positivity and the histological grade [6,11,12,24]. Furthermore, several studies have also investigated the expression of other tumor markers in phyllodes tumors, including actin, epidermal growth factor receptor (EGFR), HER2/neu, BM28/cdc, CD34, CD117/c-kit, platelet-derived growth factor, and vascular endothelial growth factor [4,7,18,19,21–23], but these previously conducted studies did not add substantially to the information already provided by standard histopathological analysis.

The aim of the present study was to conduct an immunohistochemical analysis to determine the expression status of EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 in phyllodes tumors. We assessed the correlation between the results of the immunohistochemical analysis and the clinical outcome in an attempt to identify factors predictive of the prognosis in cases of phyllodes tumors of the breast.

Patients and methods

The study group consisted of all patients with phyllodes tumor of the breast diagnosed at the National Cancer Center Hospital, Tokyo, between 1994 and 2004. The histological sections were re-reviewed by a single pathologist (T.H.) for diagnosis. Patient history and follow-up data were obtained by a review of the medical records. Recurrence-free survival (RFS) time was measured from the time of surgery until the appearance of systemic recurrence or until the last day of follow-up without evidence of systemic recurrence, and the overall survival time (OS) was measured from the time of surgery until the last day of follow-up or death, whichever came earlier.

Immunohistochemical analysis of tissue samples

Immunohistochemical staining of the tissue sections obtained from formalin-fixed, paraffin-embedded blocks was performed for EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 using the labeled streptavidin–biotin method. The antibodies used for the immunohistochemical staining were as follows: EGFR (EGFR pharmDx Kit, 2-18C9, DakoCytomation, Glostrup, Denmark), HER2/neu (CB11, BioGenex, San Ramon, USA), CD117/c-kit (A4502, DakoCytomation, Glostrup, Denmark), p53 (DO7, DakoCytomation, Glostrup, Denmark), and MIB-1 (Immunotech, Marseille, France). The anti-EGFR monoclonal antibody, clone 2-18C9, which binds to an epitope located near the ligand-binding domain on the extracellular domain of EGFR, has been shown to be specific for EGFR and not to cross-react with HER2 or other receptors of the HER family [20].

The immunohistochemical analysis of the primary tumor in all patients was conducted by the same investigator (T.H.), blinded to the clinical status of the patients. The intensity of the immunochistochemical staining for p53, EGFR, HER2/neu, and CD117/c-kit was also similarly scored as 0, negative; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. Negative controls, in which the primary antibody was omitted, were also included in each run. As for positive controls, invasive breast cancers showing strong staining (3+) were used as the positive controls for EGFR and HER2/neu staining, and tissue mast cells showing strong staining (3+) were used as the internal positive controls for CD117/c-kit staining. The proportion of positive cells was categorized as sporadic (positive cells $<10\%$); focal ($11\% <$ positive cells $<50\%$); and diffuse (positive cells $\geq 50\%$). The immunohistochemical scores of 2+ and 3+ with focal to diffuse distribution were considered to represent positive expression of the respective markers.

The MIB-1 index was defined as the percentage of nuclei showing positive staining calculated after counting 1,000 neoplastic cells per slide. The cut-off point for the value of the MIB-1 index ($>11.2\%$ vs. $\leq 11.2\%$) was defined based on the results of a previous study [13].

Statistical analysis

The Kaplan–Meier method was used to describe the distribution of the RFS and the median OS. The prognostic factors of primary tumor size (≤ 10 cm vs >10 cm) and histological types (benign vs. borderline vs. malignant) were analyzed statistically. The relationships between the expression of the biomarkers (EGFR, p53, and MIB-1 index) and the clinical outcomes of the patients were compared with the log-rank test, and the