Table II. Relationship between clinico-pathological parameters and the status of M30 and MIB-1, and therapeutic response.

Factors	Clinical response NR vs R	Pathological response grade 0-1 vs grade 2-3	Pre M30 ≥35 vs <35	Pre MIB-1 ≥240 vs <240	Post M30 ≥35 vs <35	Post MIB-1 ≥240 vs <240
Menopausal status						
Negative vs positive	NS	NS	NS	NS	NS	NS
Pre-tumor size						
T0-T3 vs T4	NS	NS	NS	NS	NS	NS
Pre-nodal status						
N0-1 vs N2-3	NS	NS	NS	NS	NS	NS
Estrogen receptor						
Negative vs positive	NS	NS	NS	0.0018	NS	0.0021
Progesterone receptor						
Negative vs positive	0.0405	NS	NS	0.0069	NS	NS

NR, non-responder (NC + PD); R, responder (CR + PR); NS, not significant.

patients underwent operations after 2-4 cycles of FAC treatment and continued the same FAC treatment post-operatively until the full dose of antharacycline (500 mg/m^2). No patients received radiation therapy or endocrine therapy. Patients were followed-up at least at 3-month intervals, and recurrence was confirmed by histological examination or image examination. Twenty-five patients developed recurrence and 19 patients died of breast cancer within the follow-up period (median follow-up: 2.7 years when censored at relapse).

Evaluation of tumor response. Response of the primary tumors by chemotherapy was evaluated according to criteria established by the Japanese Breast Cancer Society, which are essentially the same as those of the World Health Organization. CR (complete response), is defined as the disappearance of tumor; PR (partial response), refers to a decrease in tumor size of ≥50%; NC (no change), indicates a decrease in tumor size of ≤50% or an increase of tumor size by <25%; PD (progressive disease), indicates an increase in tumor size of ≥25%.

The grading of the pathological efficiency by chemotherapy, which is evaluated by microscopic evaluation by a skilled pathologist, was also categorized according to the criteria established by the Japanese Breast Cancer Society. Grade 3, is defined as the complete disappearance of variable cancer cells on the examined specimens; grade 2, refers to apparent degeneration of 2/3 or more of the population of observed cancer cells; grade 1, indicates observation of degenerative cells in less than 2/3 of examined tumor cells; grade 0, indicates lack of findings of degenerative cancer cells on specimens.

Determination of conventional biological markers. All specimens, fixed in 10% formaldehyde solution and embedded in paraffin, were examined by conventional procedures to obtain a histological diagnosis. Estrogen receptor (ER) and progesterone receptor (PgR) levels were determined with ELISA systems from Otsuka Assay Institute (Tokushima, Japan) as reported previously, or by immunohistochemistry (12,13). The cut-off values for ER and PgR in biochemical assay were 13 fmol/mg protein and 10 fmol/mg protein, respectively. For immunohistochemical determination of ER and PgR, tumors containing 10% or more receptor-positive cells were regarded as receptor-positive status. Her-2 and p53 immunostainings were performed as previously described (12).

Tumor growth kinetics assessed by CK18NE and Ki-67 antigen. The 4 µm-thick sections, mounted on the silane-coated glass, were dewaxed in xylene, rehydrated through descending concentrations of alcohol, and treated with 0.3% hydrogen peroxide in methanol for 15 min to inhibit the endogenous peroxidase activity. The sections for MIB-1 staining were autoclave pre-treated for 15 min at 121°C in a solution of 10 mM citrate buffer (pH 6.0) and the section for M30 staining were pre-treated with pepsin solution (1 mg/ml in phosphate buffer) for 15 min at 37°C. The mouse monoclonal antibody M30 CytoDeath (Roche, Basel, Switzerland) against caspase-cleaved CK18NE was incubated with tissue sections at a 1:200 dilution for 30 min at room temperature. The mouse monoclonal antibody against Ki-67 (clone MIB-1; Dako, Glostrup, Denmark) was incubated with tissue sections at a 1:50 dilution for 1 h at room temperature. All of the slides were processed using the commercial Elite ABC kit (Vectastain, Vector Laboratories, Burlingame, CA) directed against mouse IgG. Diaminobenzidine was used as the final chromogen and Meyer's hematoxylin was used for counterstain.

At least 1000 neoplastic cells were analyzed by at least three different microscopic views in each case. The ratio of positively stained cells, cytoplasmic staining for M30 and nuclear staining for MIB-1, was counted and the M30 index and MIB-1 index were calculated as the average of these counts, respectively. All immunohistochemical stainings were evaluated without knowledge of clinical outcome. Two experienced pathologists independently confirmed the histological diagnosis of all specimens. Positive and negative controls were run in parallel.

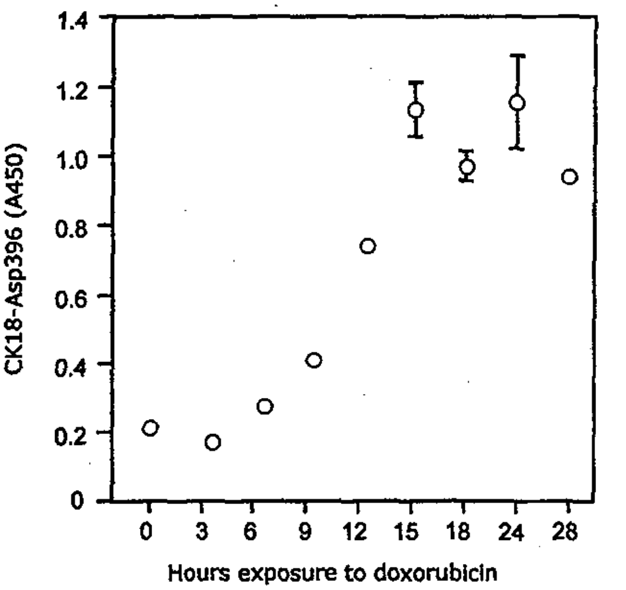

Figure 1. Increase of M30 by doxorubicin *in vitro*. A time-course experiment showing the increase of M30 by 1 μg/ml doxorubicin treatment in MDA-MB-231 cells. Each plot indicates the value of mean ± standard deviation (SD). Values which have minimum SD did not show the area of SD on the graph. A significant difference was observed between 0 and 15 h (p>0.0001, *t*-test).

Statistical analysis. The relationship between examined biomarkers and the clinical variables, including the clinicopathological factors and treatment characteristics listed in Table I, were analyzed using the Chi-square test or Student's t-test. The relationship between M30 index and MIB-1 index was analyzed with the correlation test. The survival curves were plotted according to the Kaplan-Meier method, and the log-rank test was applied to compare the survival curves. Multivariate analyses were performed using the Cox proportional hazards model. All statistical tests were performed using the StatView software package (Abacus Concepts Inc.) and the findings were considered significant when the p-value was <0.05.

Results

Increase of M30 by doxorubicin in vitro. A time-course experiment showed that the M30 levels increased immediately after the exposure to doxorubicin in MDA-MB-231 cells. The increase from 0 to 15 h was statistically significant at the level of p<0.0001 by t-test (Fig. 1). This was also true for 18, 24 and 28 h.

M30 index and MIB-1 index. Pre-treatment M30 index ranged from 1 to 574 counts (median: 2.5 count/1000 neoplastic cells) and pre-treatment MIB-1 index ranged from 34 to 825 counts

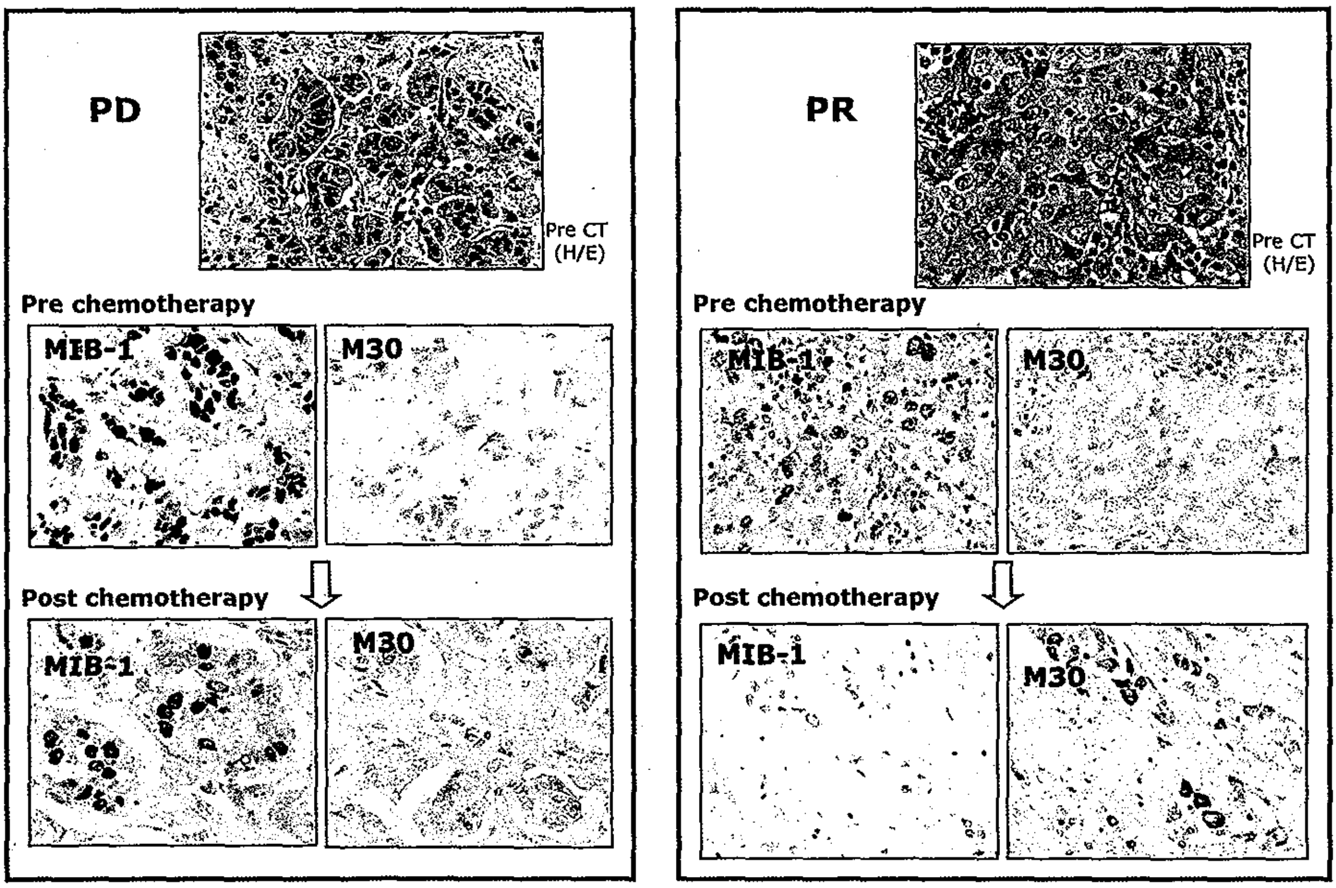

Figure 2. Representative staining of M30 and MIB-1 and the changes of growth kinetics by chemotherapy. Positive cytoplasmic staining was assessed as positive for M30, and positive nuclear staining was assessed as positive for MIB-1. M30 index and MIB-1 index were calculated as the positive cell rate of 1000 tumor cells. In non-responders (PD, clinically progressive disease), no decrease in MIB-1 index and no increase in M30 index were observed. In responders (PR, clinically partial response), MIB-1 index decreased significantly, and M30 index increased remarkably.

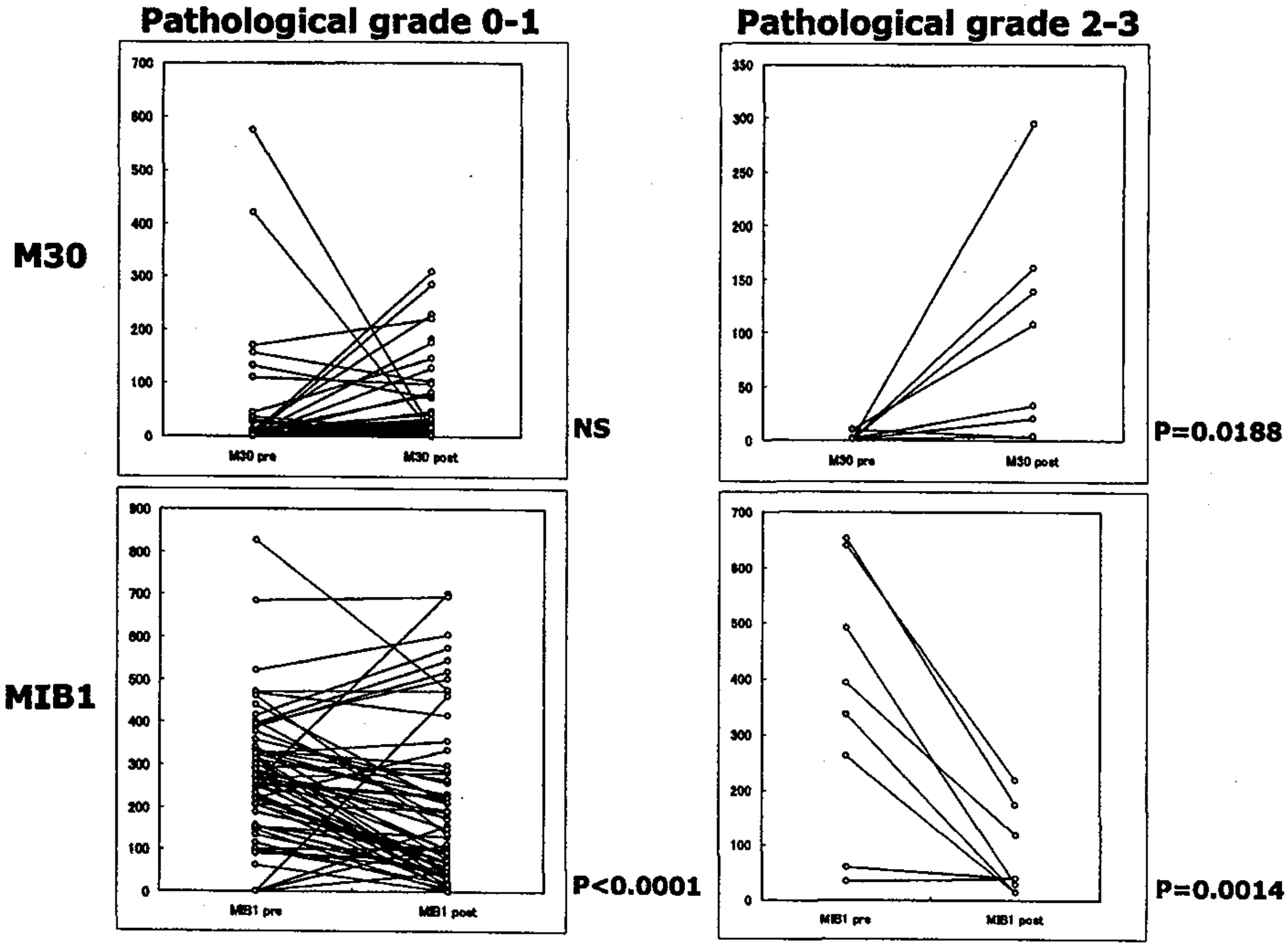

Figure 3. The relationship between the changes in M30 index and MIB-1 index and pathological response grade. The rules of Japanese Breast Cancer Society were used for assessing pathological tumor response (see Materials and methods). In pathological grade 2 patients, a significant increase in M30 index and a significant decrease in MIB-1 index were observed.

(median: 274 count/1000 neoplastic cells). Post-treatment M30 index ranged from 1 to 308 counts (median: 13 count/1000 neoplastic cells) and post-treatment MIB-1 index ranged from 1 to 701 counts (median: 124.5 count/1000 neoplastic cells). Representative cases of M30 and MIB-1 immunostainings and the changes by the treatment are shown in Fig. 2.

Relationship with clinical and pathological response to pre-CT. There was a positive correlation between clinical objective response and negativity of PgR status (p=0.0405, Chi-square test, Table II). Pre-treatment MIB-1 index and post-treatment MIB-1 index were associated with ER status significantly as shown in Table II (p=0.0018 and p=0.0021, respectively by Chi-square test). Neither pathological response nor M30 index showed a significant correlation with menopausal status, tumor size, nodal status and hormone receptor status. No significant correlation between clinical tumor response by pre-CT and M30 index, or MIB-1 index, was detected (data not shown). Any parameter, pre- and post-treatment index of M30 or MIB-1, or increase of M30 index during pre-CT, showed no significant association with clinical tumor response, but decrease of MIB-1 index during pre-CT showed significant association with clinical tumor response (data not shown). On the other hand, post-treatment M30 index increased significantly after pre-CT in histological response grade 2-3 cases (p=0.0188, t-test), whereas no significant increase was observed in histological response grade <2 patients (Fig. 3). Only one case that showed complete disappearance of tumor cells (pathological response grade 3) by pre-CT was included in this analysis.

Survival analysis. Survival analysis was conducted in a subgroup of 42 patients who underwent FAC therapy alone pre- and post-operatively, because this subgroup was a pure subset to assess the relationship of MIB-1/M30 status and therapeutic outcome of chemotherapy. No clinical CR was included in this analysis. Univariate analyses were conducted for post-treatment M30 index, post-treatment MIB-1 index and MIB-1/M30 ratio at every cut-off point by step-wise method, and it was found that >35, >140 and >5 showed the highest prognostic values for M30 index (p=0.0009), MIB-1 index (p=0.0014) and MIB-1/M30 ratio (p=0.0003) by log-rank test in disease-free survival (Table III). The tumors having <35 M30 index, having >140 MIB-1 index and >5 MIB-1/M30 ratio showed a

Table III. Univariate and multivariate analysis for survival in breast cancer patients (n=42).

Factors	DFS				OS				DFS	OS	DFS	OS
	Univariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value	Univariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value	Multivariate p-value
Pathological nodal metastasis 0-3 vs 4~	NS	0.0423	0.0016	0.0259	NS	NS	0.0311	0.0340	0.0459	NS	0.0016	0.0311
Estrogen receptor Negative vs positive	0.0185	NS	NS	NS	0.0268	NS	NS	NS	NS	NS	NS	NS
HER2 Negative vs positive	0.0163	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Post NAC tumor size T1, 2 vs T3, 4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Post M30 ≤35 vs >35	0.0009	0.0035	-	-	0.0023	0.0177	-	-	-	-	-	-
Post MIB-1 ≤140 vs >140	0.0014	NS	-	-	0.0090	NS	-	-	-	-	-	-
Post MIB-1/M30 ratio ≤5 vs >5	0.0003	-	-	0.0005	0.0025	-	-	0.0100	-	-	-	-
Pre-post M30 increase[a] ≤35 vs >35	0.0110	-	NS	-	0.0333	-	NS	-	-	-	-	-
Pre-post MIB-1 decrease[a] ≤20 vs >20	0.0001	-	<0.0001	-	0.0112	-	0.0171	-	-	-	-	-
Pre-post M30 increase/ MIB decrease[b] High/high vs others	0.0092	-	-	-	0.0025	-	-	-	0.0212	NS	-	-
Pre-post M30 increase/ MIB decrease[b] Low/low vs others	<0.0001	-	-	-	0.0112	-	-	-	-	-	<0.0001	0.0171

[a]Pre-post M30 increase and pre-post M30 increase were the differences between before and after pre-operative chemotherapy. [b]M30 increase and MIB-1 decrease were divided into low and high categories at 35, 20 respectively. DFS, disease-free survival; OS, overall survival; NS, not significant.

worse prognosis as compared with those with opposite values. In addition, increase of M30 index by the treatment and decrease of MIB-1 index by the treatment also showed potent prognostic significance (p=0.0110, p=0.0001, respectively, log-rank test). The details of univariate analyses are summarized in Table III. Survival curves by Kaplan-Meier method are shown in Fig. 4. A significant difference was demonstrated between post-treatment M30 index high, MIB-1 index low group and others (p=0.0004, log-rank test). Tumors having <5 post-treatment MIB-1/M30 ratio showed favorable prognosis (p=0.0003, log-rank test). Pre-post increase in M30 and pre-post decrease in MIB-1 index were divided into low and high categories. M30 high and MIB-1 low group showed a significantly favorable prognosis as compared with M30 low and MIB-1 high group (p<0.0001, log-rank test). Neither clinical tumor response nor pathological tumor response grade (<2 vs 2) showed any significant prognostic value (data not shown). No pathological tumor response grade 3 was seen in this 42-patient subgroup.

Multivariate analyses also confirmed that post-treatment M30 index, post-treatment MIB-1/M30 ratio and pre-post MIB-1 decrease, as well as post-treatment histological nodal status had an independent prognostic value (Table III). Similar results were obtained between disease-free survival (DFS) and overall-survival (OS).

Growth kinetics and relapse. Scatter diagrams of pre- and post-treatment M30 index and MIB-1 index are shown in Fig. 5. Eleven patients (Fig. 5, colored symbols) showed a growth kinetics with >35 M30 index and <140 MIB-1 index after pre-CT, although one case showed such growth kinetics before pre-CT (Fig. 5, red circle). A dramatic change in tumor growth kinetics revealed by M30 index and MIB-1 index was observed.

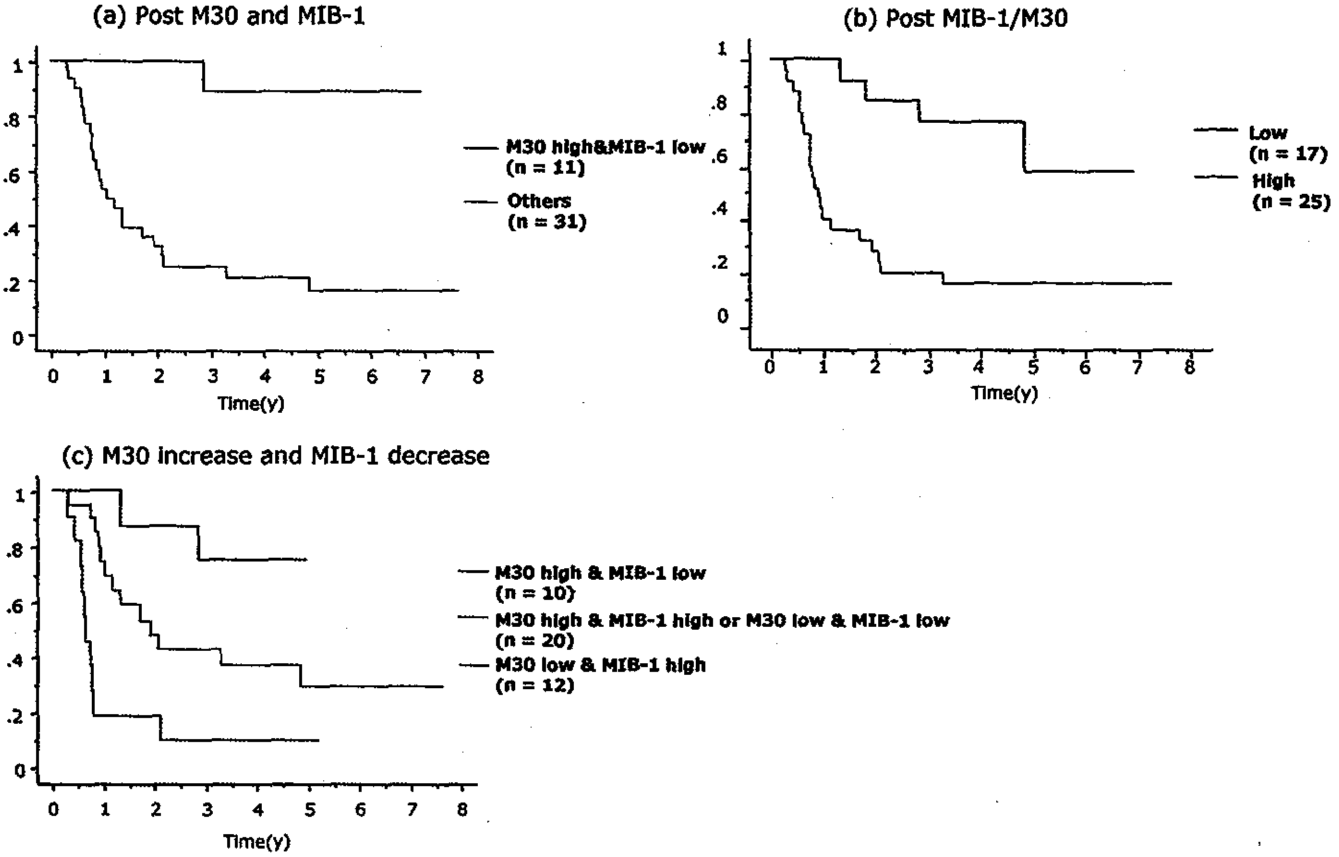

Figure 4. Disease-free survival curves stratified by M30 index and MIB-1 index. (a), Post-treatment M30 index and post-treatment MIB-1index were divided into low and high categories at 35 and 140, respectively. A significant difference was demonstrated between M30 index high, MIB-1 index low and others (p=0.0004, log-rank test). (b), Post-treatment MIB-1/M30 ratio was divided into low and high categories at 5 (p=0.0003, log-rank test). (c), Pre-post increase in M30 index and pre-post decrease in MIB-1 index were divided into low and high categories at 35 and 20, respectively. The survival analyses were conducted in the 3 groups. M30 high and MIB-1 low showed a significantly more favorable prognosis as compared with M30 low and MIB-1 high group (p<0.0001, log-rank test).

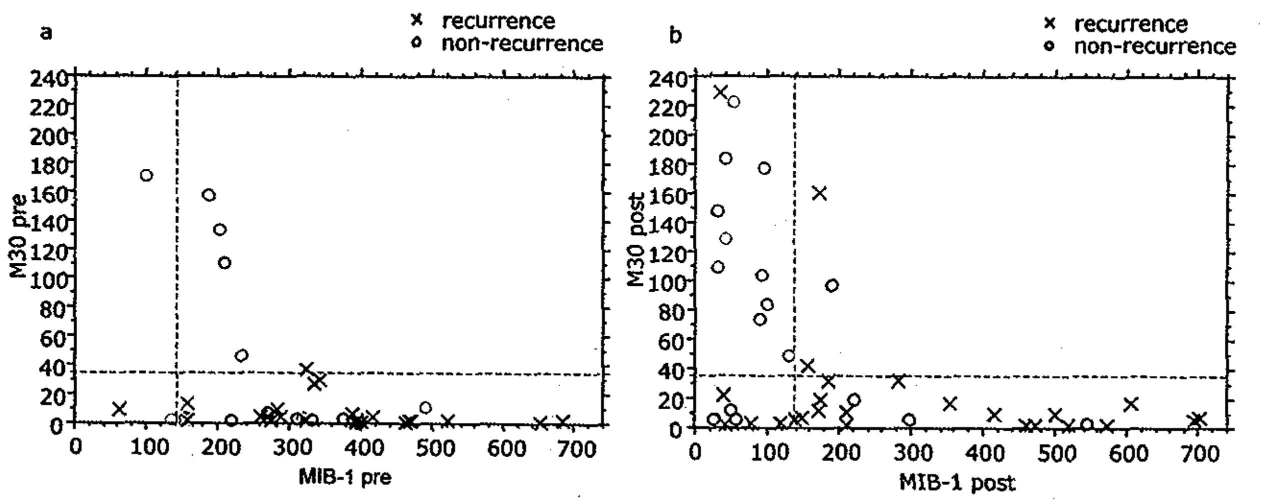

Figure 5. Scatter diagrams of M30 and MIB-1 index. Scatter diagrams of M30 index and MIB-1 index at pre-treatment and post-treatment are shown. Relapse and non-relapse are plotted with different types of marks. Eleven patients (colored legends) showed favorable growth kinetics with >35 M30 index and ≤140 MIB-1 index after pre-CT, although one case showed such growth kinetics before pre-CT (red circle). Difference in colored symbols (red, blue, green and purple) indicates the categories of M30 or MIB-1 index before pre-CT.

Discussion

Several biological markers such as p53 mutation and Her-2 overexpression have been proposed for predicting the efficacy of chemotherapy and for determining the dosages of chemotherapeutic agents, particularly anthracyclines, in adjuvant treatment of primary breast cancer (2,3,14-17) However, controversies still remain for clinical application because of the cost or for other reasons (18). There is a requirement to develop a novel practical predictive marker for adjuvant chemotherapy of primary breast cancer, especially a surrogate marker for predicting survival benefit from chemotherapy.

Among many apoptosis-related markers, M30 is characterized to detect the relatively early-phase of apoptosis in tumor cells (7-11). According to preliminary analysis comparing M30 immunostainings and the apoptosis assessed by TdT-mediated dUtp-x nick end labeling (TUNEL) method, a heterogeneous concordance in M30 positively stained tumor cells and TUNEL-positive apoptotic cells was found (data not shown). It is suggested that M30 recognizes the different phase of apoptosis from the phase detected by TUNEL analysis. Leers *et al* reported that the generation of CK18NE detected by M30 antibody occurred early in the apoptotic cascade before annexin V reactivity or positive DNA nick end labeling (7). A flow cytometric analysis proved that the majority of M30-positive cells appeared in the sub G1 peak in the cell-cycle. According to these data, an enzyme-linked immunosorbent assay of M30 is utilized for screening of pro-apoptotic drugs against cancer (9). Thereby, it was considered that tumor growth kinetics study using CK18NE, presented as the M30 index, would provide different clinical implication from others (19,20).

Pre-CT with an anthracycline-based regimen changed the M30 index or MIB-1 index dramatically in more than half of the examined cases. In particular, in pathological response grade 2 cases, M30 index increased remarkably and MIB-1 index decreased significantly. Interestingly, there was no significant correlation between the changes in these indexes and clinical tumor response. Since there is an agreement that clinical tumor response to pre-CT does not necessarily correlate with prognosis, but histological response does (5), the growth kinetics displayed by M30 and MIB-1 should be useful for predicting survival.

In the present study, survival analyses were performed in a subgroup of 42 patients who underwent FAC chemotherapy alone. Although the number of examined cases was small, the subgroup was thought to be appropriate to assess the pure effect of chemotherapy on survival, because full-dose of FAC therapy was given for all the patients pre- and post-operatively. Surprisingly, post-treatment M30 index and post-treatment M30/MIB-1 ratio (<5) showed a potent prognostic significance. Very few patient recurred in the group of patients who had a high post-treatment M30 index (>35). The changes in M30 index and MIB-1 index by the treatment also exhibited a significant prognostic value. The patients whose M30 index did not increase and MIB-1 index did not decrease significantly showed an extremely poor prognosis.

These data indicate several important points to consider. First of all, the analysis on the alteration of tumor growth kinetics using M30 index might be useful for identifying the particular subgroup that benefits from chemotherapy among non-pCR patients. There is a consensus that pCR patients after pre-CT showed a significantly favorable prognosis as compared with non-pCR patients. However, regarding literature-based research, few reports have focused on the identification of susceptible patients for chemotherapy among non-pCR patients. The method and criteria for assessment used in the present study might be useful for this purpose, although this strategy should be tested and confirmed in a large-scale clinical trial. In particular, there might be a criticism that the subgroup that showed favorable tumor growth kinetics after pre-CT, such as >35 M30 index and <140 MIB-1 index, might be potential candidates of pCR patients, because the dosages of anthracycline we used in this study were lower than the dosages commonly used in the US and Europe, and pre-operative treatment cycles might be insufficient to achieve pCR. However, even so, it is true that the changes in growth kinetics after the exposure of 2 or 4 cycles of chemotherapy result in extremely favorable prognosis. The shift of pattern in the scattered diagram of M30 index and MIB-1 index between pre- and post-treatment strongly suggests that this change is elicited by pre-CT. Secondly, these data raise an issue regarding the switch of the treatment. According to our findings, if the patients do not show favorable growth kinetics after the first pre-CT, the therapy should be switched to other non-cross resistant regimens or different types of treatments, because the prognosis is extremely poor in such cases. This is speculation from the result of this study, however, a recent report has demonstrated clearly that a sequential combination of 4 cycles of anthracycline-based regimen and 4 cycles of docetaxel increased pCR rate in about double and improved the survival as compared with the 8-cycle anthracycline-based regimen alone, even for the responders to the initial treatment of anthracycline (21). Growth kinetics should be taken into consideration in deciding treatment-switch in pre-CT. Thirdly, in common practice, we obtain the tumor sample prior to starting the pre-CT by core-needle biopsy (CNB) rather than open biopsy. In order to assess the pre-treatment tumor growth kinetics precisely, it may remain a problem in cases of CNB because of the heterogeneity issue. We need more sophisticated methods or techniques. Nevertheless, for the purpose of predicting survival, to determine the post-treatment status of tumor growth kinetics might be adequate. Presumably, post-treatment MIB-1 status would represent a combined value of 'original prognostic value' and 'therapeutic prognostic value', however post-treatment M30 status would represent more 'therapeutic prognostic value' (22-27).

There are a number of approaches to analyze and monitor chemotherapy-induced apoptosis. Expressions of Bcl-2 family and those changes in the expression or phosphorylation by chemotherapy have been examined (20,28-31). These approaches have also been utilized to identify the susceptible subgroup to endocrine therapy as well (32). For instance, Parton *et al* (20) focused upon DNA fragmentation factor (DFF)-40 cleaved by caspases and documented that the level of DFF40 that emerges after 24-h exposure to chemotherapy correlated significantly with that of active caspase 3 and caspase 6, indicating that DFF40 is a promising molecule for assessing chemotherapy-induced apoptosis. This direction of research, as well as the research on chemo-resistance and on the selection of chemo-unnecessary cases would be crucial to advance pre-CT (33).

A variety of pre-operative combination chemotherapies are being tested currently in order to achieve further survival advantages. To maximize the efforts for demonstrating the efficacy of new treatment modalities, adjuvant trials are shifted from post-operative to pre-operative, because pCR can be a surrogate marker of long-term survival outcome in case of pre-CT. This study indicated that the analysis of tumor growth kinetics with CK18NE/M30 index, in conjunction with MIB-1 index, might be useful as a surrogate marker for predicting survival benefits from chemotherapy in non-pCR patients. It is warranted to confirm its clinical usefulness in the larger collaboration studies.

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News and views

Novel insights in clinical trials with preoperative systemic therapy for primary breast cancer

Masakazu Toi[a,*], Hiroko Bando[a], Louis W.C. Chow[b]

[a]Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, 113-8677 Tokyo, Japan
[b]Department of Surgery, University of Hong Kong Medical Centre, Queen Mary Hospital, Pokfulam, Hong Kong, China

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Abstract

Preoperative systemic therapy has provided novel insights into the treatment of primary breast cancer. The knowledge of the response to the treatment helps to predict long-term survival outcome, which indicated that the patients who benefit from the treatment are identifiable at individual level. The subgroup with pathological complete response (pCR) to the preoperative chemotherapy (Pre-CT) shows a favorable prognosis, whereas the subgroup with non-pCR shows an unfavorable prognosis. These identifications enable to classify the patients into multiple categories and to consider novel therapeutic implication more efficiently and more individually. Theoretically in the preoperative treatment system it is possible to distinguish the patients who require novel therapy beyond the conventional therapy from those require no further systemic chemotherapy except the conventional therapy or those who need no systemic chemotherapy. Novel therapy development specific for non-pCR patients should be considered in the future clinical trials. In this review, we will focus on these insights elicited by Pre-CT in primary breast cancer treatment.

Keywords: Breast cancer; Individualized treatment; Pathological response; Preoperative chemotherapy

1. Introduction

The number of studies and clinical trials on preoperative systemic therapy has increased sharply in recent years. According to the literature research by *PubMed*, chemotherapy took off in early 90s and hormone therapy took off around 2000, respectively. Publications on translational researches have also increased in parallel. Why the vigorous attentions have been paid to preoperative systemic therapy? Two major reasons could be raised. One is the increment of the chances for breast-conserving therapy (BCT) and another is the knowledge of the sensitivity or resistance to the treatment at individual levels [1–6]. According to National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 trial result, preoperative 4-cycle adriamycin plus cyclophosphamide (AC) therapy drove an additional 8% chance of BCT as compared with surgical treatment at front for primary breast cancer patients [1]. Many other investigations have also confirmed a remarkable increase in BCT rate by preoperative chemotherapy (Pre-CT) [4]. It is clear that the shift in the timing of the treatment beyond the surgery brings a significant advance to the treatment of primary breast cancer.

In NSABP B-18 trial it was also revealed that tumor response, especially pathological complete response (pCR), promises a favorable prognosis whereas non-pCR predicts a poor prognosis at 8-year follow-up time [4] In addition, it is also speculated that node-negative conversion, from histologically node-positive to node-negative in the axilla, could be caused by preoperative AC chemotherapy in about 30% of the cases. Recent 8-cycle sequential regimens such as 4-cycle anthracycline followed by 4-cycle docetaxel are noted to have achieved even higher pCR rates from 25% to 34% [7]. The estimated node-negative conversion rate seems to have also

* Auteur correspondant.
Adresse e-mail : maktoi77@wa2.so-net.ne.jp (M. Toi)

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increased proportionally. For these backgrounds, a novel consensus has been formed that most of primary breast cancer patients who require postoperative adjuvant chemotherapy (Post-CT) are the potential candidates for Pre-CT [8].

2. Clinical phenotypes emerged by Pre-CT

Recurrence or death is the ultimate marker for the assessment of adjuvant treatments, in either postoperative or preoperative setting. In the postoperative setting, the patients are categorized into two subgroups consisted of a group that failed to the treatment and another group that did not fail, however, in the preoperative setting, particularly in the Pre-CT, the patients can be classified into further multiple categories due to the sensitivity to the treatment like Figs. 1 and 2. These classifications raise novel therapeutic implication that conventional chemotherapy is recommended to the patients categorized as therapy-sensitive and curable property (Category A: therapy-relevant favorable prognosis group), however, new therapeutic approaches should be considered for the patients categorized as therapy-resistant and incurable property (Category D: therapy-irrelevant unfavorable prognosis group) (Table 1). The patients categorized as therapy-resistant but curable (Category C: therapy-irrelevant favorable prognosis group) might not require any type of chemotherapy. A controversy may exist in the interpretation of the patients categorized as therapy-sensitive but incurable. It might be possible to raise two types of explanation. Firstly, Pre-CT might have killed all invasive tumor cells in the breast but may not have killed all the metastatic tumor cells in the distant organs. Secondly, the killing of invasive tumor cells in primary site might, inversely, facilitate the growth of metastatic tumor cells in the secondary site that has been already indicated in animal experimental models [9]. From therapeutic point of view, the former hypothesis may suggest that new approaches based upon the conventional therapy is considerable for the future, whereas the latter hypothesis indicates that novel therapeutic approaches based upon totally new therapeutic concept are required to obtain the better outcome.

Table 1
Tumor properties relating to the treatment and therapeutic implication

Subgroup	Phenotype	Properties of treatment	Implication	Therapeutic approach
A	Responder non-recurrence	Sensitive/curable	Therapy-relevant favorable prognosis	Conventional therapy
B	Responder recurrence	Sensitive/curable	Therapy-irrelevant unfavorable prognosis	Novel approach or modification of conventional therapy
C	Non-responder non-recurrence	Resistant/curable	Therapy-irrelevant favorable prognosis	No need for therapy
D	Non-responder recurrence	Resistant/incurable	Therapy-irrelevant unfavorable prognosis	Novel approach with totally novel mechanisms

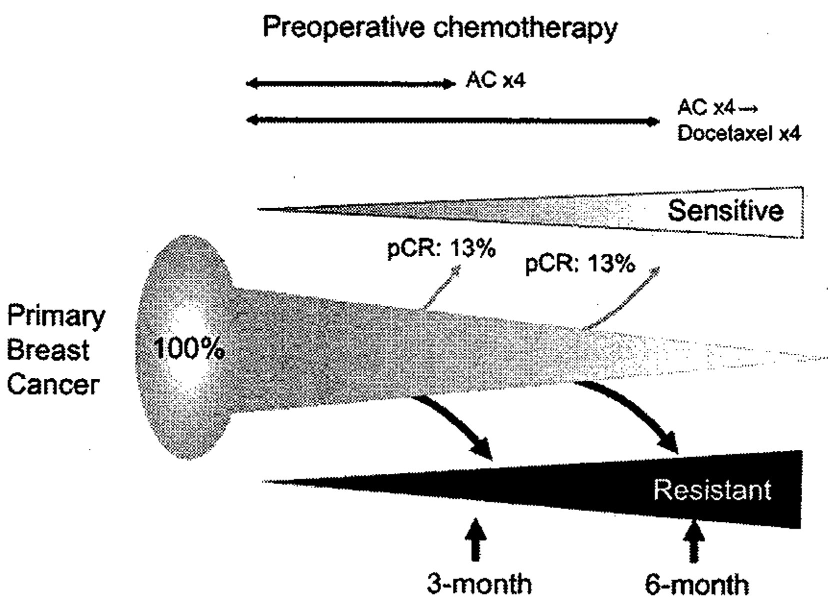

Fig. 1. A preoperative chemotherapy model simulated from NSABP B-18 and NSABP B-27 trial data. Pathological complete response (pCR) was achievable in 13% of operable primary breast cancer patients by preoperative AC (adriamycin + cyclophosphamide) 4-cycle in NSABP B-18 trial. The remaining 87% non-pCR patients were basically resistant to the AC 4-cycle treatment. According to NSABP B-27 data, pCR was achievable in further 13% of primary breast cancer patients by the additional 4-cycle treatment of docetaxel.

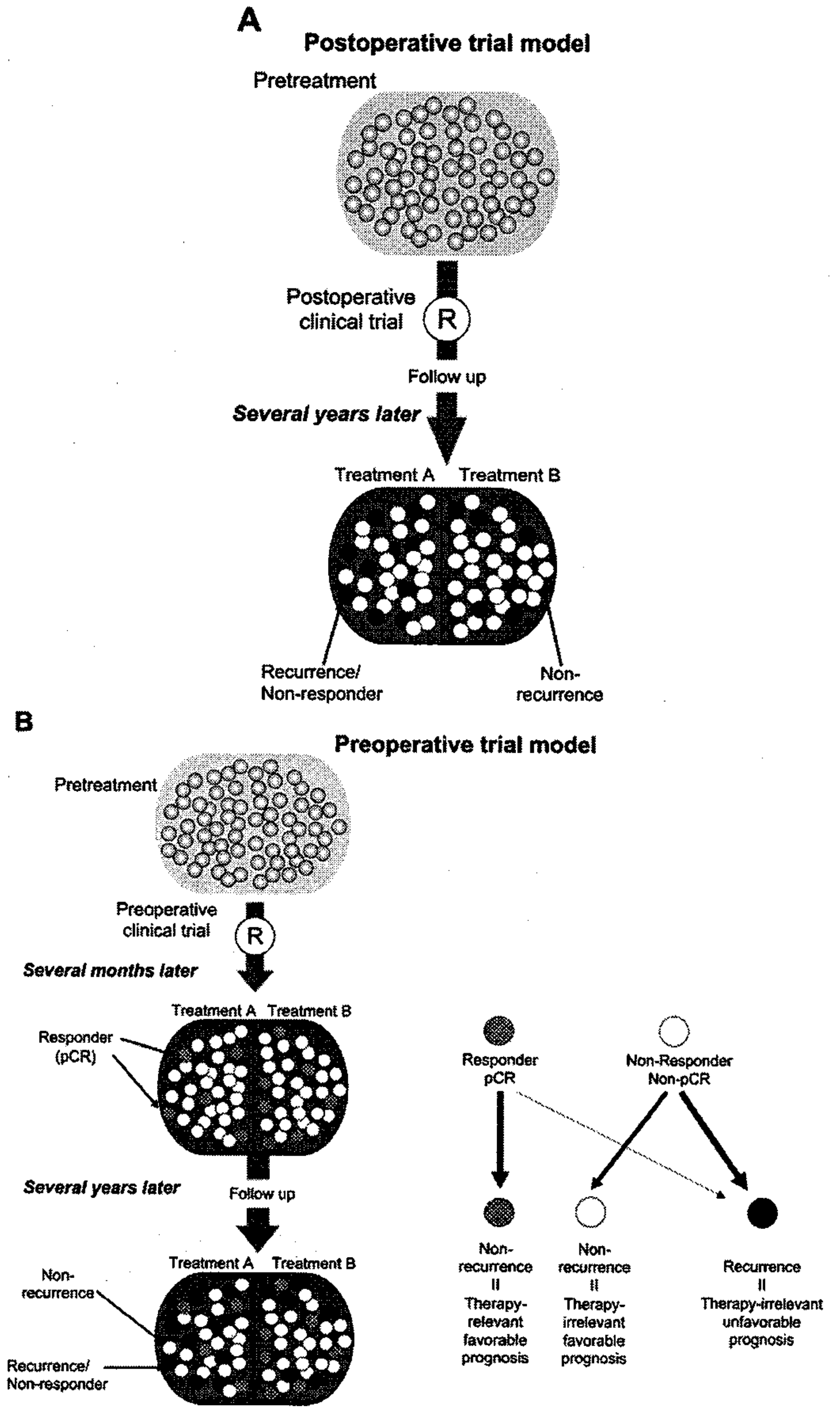

Fig. 2. A postoperative trial model and a preoperative trial model. The data sets of short-term therapy-sensitivity and long-term survival provide novel insights to clinical trials for primary breast cancer patients.

3. Simulation of the proportion of patients classified into the categories

As simulated the proportion of patients for each category according to an 8-year follow-up data of NSABP B-18, the Category A should be roughly 10%, the Category B should be several percent, the Category C should be about 50% and the Category D should be about 35% [1,2]. Therefore, we can take the conventional chemotherapy, AC 4-cycle, for about 60% of primary breast cancer patients, unless we care the toxicity. Nevertheless, we need novel therapy for the remaining 40%. As applied the tumor response data of NSABP B-27, where pCR rate was approximately 26%, a simulation exhibits that the Category A should be about 20%, the Category C should be about 50%, and the Category B and the Category D should be about 30% in total. Approximately, 30% of the patients would require novel therapeutic approaches. Only preoperative treatment fashion enables to provide such information as who do not require any type of chemotherapy, who do not require additional chemotherapy and who do require novel therapy. In the future clinical trials with new therapeutic tools, the shift of the patients from Category B or Category D to Category A would occur.

4. Translational research

These classifications according to tumor response and survival outcome are also useful for conducting efficient translational researches. As illustrated by Hayes et al., it is important to distinguish predictive markers form prognostic markers [10]. In the postoperative situation, no treatment control (local therapy ± hormone therapy alone) is indispensable for analyzing pure prognostic value or pure predictive value of the 'so-called' prognostic markers. Nevertheless, in the situation of preoperative therapy, the study for Category A could reveal the factors relating pure predictive value of the treatment and the study for Category C could reveal the factors relating pure prognostic value even in one-arm study. Studies of subgroups of Category B and Category D would be useful for exploring new therapeutic targets. In a setting of clinical trials comparing new treatment arm with conventional treatment arm, the pure prognostic value emerged from the study of Category C could be validated during the analysis in a clinical trial, because the therapy-irrelevant favorable prognosis group would be same between the two arms.

5. Response-based individualized treatment system

It has been demonstrated that postoperative hormone therapy drives a significant survival benefit to hormone-receptor (HR) positive patients but not to HR negative patients [11–13]. Therefore, there is no doubt about that tumor phenotyping on HR ought to be done before starting any type of therapy [8]. Her-2 status should be also determined before starting the therapy because of a tight association with the effect of treatment trastuzumab that targets Her-2 molecule specifically [14].

After the exclusion of minimal risk patients those would not require any type of systemic chemotherapy, most of high-risk primary breast cancer patients could be treated by Pre-CT [15]. After confirming the pathological tumor response, the choice of postoperative systemic therapy might be divers. For pCR patients, cytostatic systemic therapy such as hormone therapy or no systemic therapy might be considered, however for non-pCR patients, additional treatments including other types of chemotherapy may have to be taken into consideration (Fig. 3).

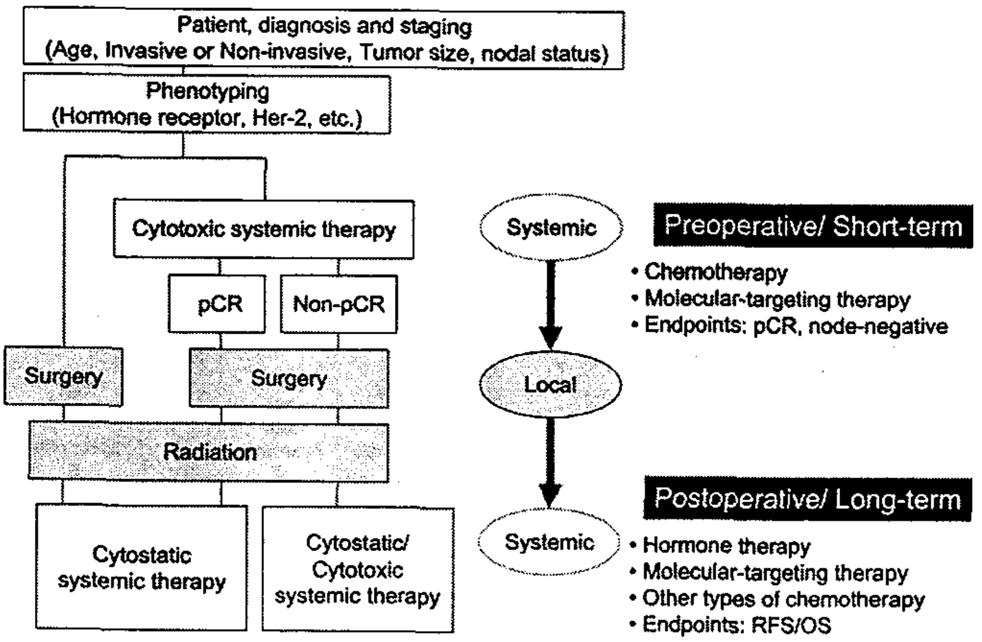

Fig. 3. A strategy with preoperative systemic treatment.

Tests of new therapeutic approaches will be targeted for non-pCR patients in order to avoid over-treatment. Translational researches will help to clarify the subpopulation that requires the new types of therapy more precisely and to explore new therapeutic target [15]. These response-based treatment strategy seems to be promising to improve the survival outcome of primary breast cancer patients efficiently and individually.

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Original Article

An Evaluation of Predictive Factors Involved in Clinical or Pathological Response to Primary Chemotherapy in Advanced Breast Cancer

Reiki Nishimura*[1], Kazuharu Nagao*[1], Haruhiko Miyayama*[2], Masakazu Matsuda*[1], Ken-ichirou Baba*[1], Yukio Matsuoka*[1], Hiroya Yamashita*[1], and Makoto Fukuda*[1]

***Background*:** The usefulness of primary chemotherapy has been widely recognized and applied to routine clinical practice to improve prognosis by downstaging. Nevertheless, none of many trials has been able to show a positive effect of primary chemotherapy in terms of prognosis, and predictive factors of outcome have not been defined and are still under investigation.

***Methods*:** Primary chemotherapy was given to 50 patients with advanced breast cancer. Predictive factors involved in clinical or pathological response to primary chemotherapy (3 cycles of CE(F) therapy) were investigated.

***Results*:** The response rate in all patients was 56.0% (CR: 3 patients PR: 25 patients) and 64.1% in patients without distant metastases. MIB-1 was related to the clinical response and EIC (extensive intraductal component) was related to the pathological response; the response was high in patients with EIC negative tumors. Responders had tumors with higher proliferative activity, which decreased significantly after chemotherapy. Patients with a decrease of more than 30% in proliferative activity after chemotherapy had significantly higher disease-free survival rates.

***Conclusion*:** The proliferative activity and EIC status were useful predictors of clinical or pathological response to primary chemotherapy. A decrease in proliferative activity by chemotherapy significantly correlated with clinical response and reflected a favorable prognosis. The number of patients benefiting from primary chemotherapy might steadily increase by detecting these predictive factors.

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Key words: Primary chemotherapy, Breast cancer, Proliferative activity, Extensive intraductal component

The spread of medical examination for breast cancer and educational campaigns has increased the number of cases of early breast cancer, but cases of large tumor or primary cases with distant metastasis are still occasionally observed. Hence both early diagnosis and treatment of advanced cases are important issues. Further, the selection of the treatment suitable for each case is also important, since not only endocrine therapy but also chemotherapy is often effective for breast cancer. With regard to surgery, breast-conserving surgery has frequently been used in Japan since the latter half of the 1980's and is now recognized as routine surgery. The indications for breast-conserving surgery have recently been extended, downstaging by chemotherapy followed by limited surgery is performed on large tumors. The NSABP B-18 trial[1] demonstrated that preoperative chemotherapy increased the ratio of patients who could undergo breast-conserving surgery without worsening prognosis.

The authors have reported[2,3] that the evaluation of proliferative activity is useful as a predictive factor of the response to chemotherapy in advanced or recurrent breast cancer. It has been also reported[4] that c-erbB-2 positive patients are responsive to regimens containing doxorubicin. Although one of the most important factors correlating with surgical margin in breast-conserving surgery is positive EIC (extensive intraductal component)[5], it is not clear whether or not intraductal lesions respond to chemotherapy. Selection of suitable treatment in each case is thus required.

In this study, cases were either accumulated

*[1]Department of Surgery and *[2]Clinical Pathology, Kumamoto City Hospital, Japan.
Reprint requests to Reiki Nishimura, Department of Surgery, Kumamoto City Hospital, 1-1-60 Kotoh, Kumamoto City, Kumamoto 862-8505, Japan.

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prospectively and the relationship between clinicopathological factors and the response to chemotherapy assessed clinically or pathologically, or outcomes after operation were investigated to evaluate predictive factors of the response to primary chemotherapy and outcome in advanced breast cancer.

Patients and Methods

Patients

Primary chemotherapy was given to 50 patients with primary advanced breast cancer who gave informed consent to the Department of Surgery, Kumamoto City Hospital between July 1996 and December 2000. As shown in Table 1, the mean tumor diameter was 7.8 cm and the mean patient age was 49.8 years. The rate of estrogen receptor (ER) positivity was 44% (22/50). Out of 50 patients, 11 cases had distant metastases without symptoms. Operations were performed on 38 cases after 3 cycles of chemotherapy, and they received 3 cycles of the same chemotherapy postoperatively. Six cases responding to therapy refused surgery, and the chemotherapy regimen was changed in 5 nonresponders after 3 cycles of chemotherapy. Needle biopsies were performed to confirm the diagnosis before initial chemotherapy. Factors involved in the response to therapy were investigated in these cases after the 3 cycles of primary chemotherapy. The local ethics committee approved this study, and informed consent was obtained by signing and dating a consent form prior to entering this study.

Table 1. Biological and Clinical Characteristics of Breast Cancer Patients

Age: median (range)	49.8 years (28-76)
Tumor size: median (range)	7.8 cm (3.1-15.0)
Menopausal Status	
pre-	26 (52%)
post-	24
ER (estrogen receptor)	
(+)	22 (44%)
(−)	28
p53	
(−)	19 (40%)
(+)	29
c-erbB-2	
(−)(+)	34 (72%)
(++)(+++)	13
Extensive intraductal component (EIC)	
(−)	25 (52%)
(+)	23
Proliferative activity (MIB-1)	
~19%	10 (21%)
20%~	37
Distant metastasis	
with	11 (22%)
without	39
Surgery	
BCS	11 (22%)
Mastectomy	27 (M0: 21)
(−)	12

BCS: breast-conserving surgery

Treatment and Criteria for Evaluation of Response

Therapies were divided into 2 regimens containing epirubicin: CEF therapy (Cyclophosphamide: 100 mg/day p.o. for 14 days, Epirubicin 40 mg/m^2 every 21 days, 5-FU 500 mg/m^2 every 21 days) and CE therapy (Cyclophosphamide: 600 mg/m^2 every 21 days, Epirubicin 60 mg/m^2 every 21 days). The response of the primary breast tumors to therapy was evaluated after 3 cycles and surgery was performed in the 3rd week after the last cycle. The number of patients receiving each regimen was 36 and 14 respectively. The clinical response was grouped into 4 grades[6]: complete response (CR), the disappearance of breast cancer; partial response (PR), a decrease of 50% or more in tumor size (bidimensionally measured); no change (NC), a decrease of less than 50% or an increase of less than 25%; progressive disease (PD), an increase of 25% or more. Pathological response was grouped into 5 grades[7]: Grade 3, the complete disappearance of cancer cells; Grade 2, changes such as degeneration or necrosis in 2/3 or more of the whole lesion; Grade 1b, changes in more than 1/3 and less than 2/3 of the whole lesion; Grade 1a, changes in 1/3 or less of the whole lesion; Grade 0, almost no response. Patients with grade 3 or 2 response were considered as pathological responders. The evaluation by pathologists was conducted without knowledge of clinical response.

Evaluation Items

Histological grade and extensive intraductal component (EIC) were investigated in 50 patients by preoperative drill needle biopsy and HE staining. Histological grade and EIC as well as lymph node metastasis were evaluated in isolated specimens from 38 patients after surgery. The histological grade was classified into 3 categories according to the method of Bloom and Richardson[8]. EIC was judged positive when intraductal lesions were detected in 25% or more of the primary tumor[9]. Proliferative activity was judged by immunostaining for MIB-1 (Ki-67 antibody, Immunotech, Marseille, France). The fraction of proliferating cells was based on a count of at least 500 tumor cells and classified into 2 degrees (≦ 19%, 20% +)[10]. Decrease in proliferative activity after chemotherapy was calculated as a ratio (%): the difference in value between post- and pre-chemotherapy over the pre-chemotherapy value. The expression of p53 and c-erbB-2 were evaluated by immunostaining (LSAB method) with mouse monoclonal anti-p53 antibody (Japan Tanner Corporation, Tokyo, Japan) and rabbit polyclonal c-erbB-2 antibody (Dako, Glostrup, Denmark). The staining pattern of p53 was divided into three groups: ++ (homogenous and diffuse staining), + (heterogeneous or focal staining > 5% of cancer cells), and negative (focal staining < 5% of cancer cells). The staining pattern of c-erbB-2 was divided into 4 groups: +++ (strong and diffuse staining), ++ (moderate and diffuse staining), + (focal staining > 10% of cancer cells), and negative. These factors were examined before and after chemotherapy. Estrogen receptor (ER) and progesterone receptor (PgR) were determined by the EIA (enzyme immunoassay) method and judged positive when greater than 14 fmol/mg protein and 13 fmol/mg protein, respectively.

Statistical Methods

The relationships between the clinical and pathological response to chemotherapy and clinicopathological factors (Table 2 and 3), between the clinical response and the pathological response (Table 4), and changes in expression of p53 and c-erbB-2 pre- and post-primary chemotherapy were evaluated by the χ^2 test and Fisher's exact test. Logistic regression analysis (SPSS) was used for univariate analysis of factors to predict a response to chemotherapy. The statistical significance in the change in MIB-1 between pre- and post-therapy and the relationship between mean MIB-1 and the clinical response was investigated by the Student's t-test. The cumulative disease-free survival after surgery for breast cancer was calculated by the Kaplan-Meier method and evaluated by the logrank test. The median observation period after surgery was 21 months. Patients with grade 3 response (pathological CR) were included with patients showing a decrease in tumor size of 30% or more.

Results

Relationship between Clinical Response and Clinicopathological Factors of Primary Chemotherapy

Of the 50 patients, 56.0% (CR: 3 patients, PR: 25 patients) clinically responded to the primary chemotherapy. Patients without distant metastases had a response rate of 60.5% (Table 1). There was no significant difference in response rate between the 2 regimens: CEF 19/36 (52.8%), CE 9/14 (64.3%) ($p = 0.49$). Hence differences in regimen were not taken into consideration. No marked relationship was observed between the clinical response and age, menopausal status, ER, PgR, histological grade, expression of p53, or EIC status. However, a relationship was detected between clinical response, proliferative activity (MIB-1) and expression of c-erbB-2 ($p = 0.037$ and $p = 0.07$, respectively) using the χ^2 test (Table 2). Logistic regression analysis (univariate) revealed that proliferative activity was a significant factor contributing to clinical response. The mean MIB-1 was 43.8% in patients showing CR or PR, 31.0% in patients with PD or NC; the difference was significant ($p = 0.028$).

Relationship between Pathological Response and Clinicopathological Factors of Primary Chemotherapy

Table 3 shows that there was no apparent relationship between pathological response and age, menopausal status, PgR, histological grade, expression of p53 or MIB-1, while a marginal or significant relationship was noted between the pathological response and ER, expression of c-erbB-2 and EIC status ($p = 0.09$, $p = 0.08$ and $p = 0.012$, respectively). By logistic regression analysis (univariate) patients with negative EIC were significantly more likely to have pathological response.

Relationship between Clinical Response and Pathological Response to Primary Chemotherapy

Table 4 shows the relationship between the clinical response and the pathological response. There was a significant relationship between the two; of 3 patients with CR, 2 patients undergoing surgery were pathologically graded as 2 or 3, but most NC and PD patients did not have pathological response. However, about a third of PR patients did not show any pathological response.

Table 2. Relationship between Clinical Response to Primary Chemotherapy and Clinicopathological Factors in Advanced Breast Cancer

Variables	Clinical Response			*p* value	Logistic Regression Analysis		
	CR + PR	NC	PD		Category	Relative Risk	*p* value
Age							
~35 y.o	5 (71.4)	2	0		51~/~35	0.36	
~50 y.o	12 (60.0)	6	2	0.60	51~/36~50	0.54	0.47
51 y.o ~	11 (47.4)	11	1				
Menopausal Status							
pre-	17 (65.4)	9	0		post-/pre-	0.38	0.13
post-	9 (45.8)	10	3	0.12			
ER (Estrogen receptor)							
+	13 (59.1)	9	0		+/−	1.06	0.93
−	15 (53.6)	10	3	0.28			
PgR (Progesterone receptor)							
+	12 (62.5)	7	0		+/−	1.69	0.41
−	16 (53.8)	12	3	0.34			
p53							
(−)	9 (50)	9	1				
(+)	3 (42.9)	5	0	0.32	+/−	1.75	0.67
(++)	15 (73.3)	5	1				
c-erbB-2							
(−)	19 (63.3)	14	0				
(+)	1	0	0	0.06	+/−	0.46	0.42
(++)(+++)	7 (37.5)	4	2				
EIC (Extensive intraductal component)							
(−)	15 (57.7)	9	2		+/−	0.68	0.53
(+)	13 (54.2)	10	1	0.80			
Histological Grade							
I	2 (100)	0	0				
II	16 (53.3)	13	1	0.55	III/I + II	0.98	0.98
III	10 (55.6)	6	2				
MIB-1							
~19%	3 (20.0)	6	2	0.037	20~/~19	8.80	0.006
20%~	25 (68.8)	13	1				
mean ± SD	43.8 ± 21.2	31.0 ± 21.0		0.028			

Change in Expression of p53, c-erbB-2 and MIB-1 Status between Pre- and Post-Primary Chemotherapy

When expression of p53 and c-erbB-2 before chemotherapy was compared with expression after chemotherapy, the concordance rates were as high as 86.7% and 93.3% respectively in 30 patients in which both could be determined, which suggests that the expression of these markers was not affected by chemotherapy (Table 5). The mean of MIB-1 positive cells was evaluated in 36 patients excepting 2 patients histologically judged to have no cancer. The MIB-1 value in CR and PR patients was 46.8% before chemotherapy and significantly decreased to 34.7% after chemotherapy, while that in NC and PD patients was 29.0% before chemotherapy and increased to 32.3% after chemotherapy, although this was not a significant change. Altogether, there was no change in MIB-1 between pre- and post-chemotherapy, but the proliferative activity in CR and PR patients was high before chemotherapy and was markedly decreased after chemotherapy. When a decrease in proliferative activity was classified to 3 groups ≧30%, 29-0.1%, and 0%, there was a significant relationship between the decrease and clinical response (Table 6).

Table 3. Relationship between Pathological Response to Primary Chemotherapy and Clinicopathological Factors in Advanced Breast Cancer

Variables	Pathological Response			*p* value	Logistic Regression Analysis		
	Grade 3, 2	1b	1a, 0		Category	Relative Risk	*p* value
Age							
~35 y.o	3 (50)	0	3		51~/~35	0.46	
~50 y.o	2 (14.3)	2	10	0.21	51~/36~50	2.05	0.42
51 y.o ~	5 (27.8)	0	13				
Menopausal Status							
pre-	5 (25.0)	1	14				
post-	5 (27.8)	1	12	0.98	post-/pre-	1.09	0.91
ER (Estrogen receptor)							
+	2 (12.5)	0	14				
−	8 (36.4)	2	12	0.09	+/−	0.23	0.10
PgR (Progesterone receptor)							
+	3 (20.0)	0	12				
−	7 (30.4)	2	14	0.34	+/−	0.56	0.47
p53							
(−)	4 (30.8)	0	9				
(+)	1 (12.5)	0	7	0.44	+/−	0.67	0.61
(++)	4 (25.0)	2	10				
c-erbB-2							
(−)	8 (28.6)	1	19				
(+)	0	0	2	0.08	+/−	0.35	0.37
(++)(+++)	1 (14.3)	2	4				
EIC (Extensive intraductal component)							
(−)	9 (47.4)	1	9				
(+)	1 (5.3)	1	17	0.012	−/+	4.86	0.008
Histological Grade							
I	0 (0.00)	0	1				
II	6 (27.3)	1	15	0.97	III/I + II	0.96	0.96
III	4 (26.7)	1	10				
MIB-1							
~19%	2 (22.2)	1	6	0.65	20~/~19	1.75	0.54
20%~	8 (27.6)	1	20				

Postoperative Disease-Free Survival as a Function of Clinical or Pathological Response and the Decrease in Proliferative Activity in Patients with Primary Chemotherapy

Thirty-eight patients with advanced breast cancer underwent surgery after primary chemotherapy. Breast-conserving surgery was performed for 11 of 32 (34.4%) patients without distant metastases (Table 1). The cumulative disease-free survival was investigated in 32 patients without distant metastases to evaluate the relationship between the clinical or pathological response and prognosis after surgery (Fig 1A, 1B). The disease-free survival was evaluated according to a decrease in proliferative activity pre- and post-chemotherapy (Fig 1C). The disease-free survival of clinical responders, pathological responders, and patients with a decrease of 30% or more in proliferative activity was higher than that of clinical non-responders, pathological

Table 4. Comparison of Clinical Response with Pathological Response after Primary Chemotherapy

Pathological Response	Clinical Response				Total (*p* value)	
	CR	PR	NC	PD		
0 + 1a	0	12	14	0	26	
1b	0	1	0	1	2	$p = 0.0002$
2 + 3	2	6	2	0	10	

Table 5. Changes in Expression of p53 and c-erbB-2 between Pre- and Post-Primary Chemotherapy in Breast Cancer

Degree of expression	p53 (post-chemotherapy)			c-erbB-2 (post-chemotherapy)		
	−	+	++	−	+	++/+++
Pre-chemotherapy						
−	9	2	0	20	2	0
+	1	3	1	0	2	0
++	0	0	14	0	0	6
p value		< 0.0001			< 0.0001	
Correspondence rate		86.7% (26/30)			93.3% (28/30)	

Table 6. Relationship between Changes in MIB-1 Values and Clinical Response after Primary Chemotherapy in Breast Cancer

Clinical Response	No. of cases	MIB-1 (mean ± SD: %)		p value	Decrease in MIB-1			p value
		Pre-	Post-chemotherapy		~30%	29~0.1%	0%~	
CR + PR	19	46.8 ± 21.4	34.7 ± 23.6	0.005	12	2	7	
NC + PD	17	29.0 ± 21.0	32.3 ± 19.4	0.60	1	8	8	0.002
Total	36	38.4 ± 22.8	33.6 ± 21.4	0.08	13	10	15	

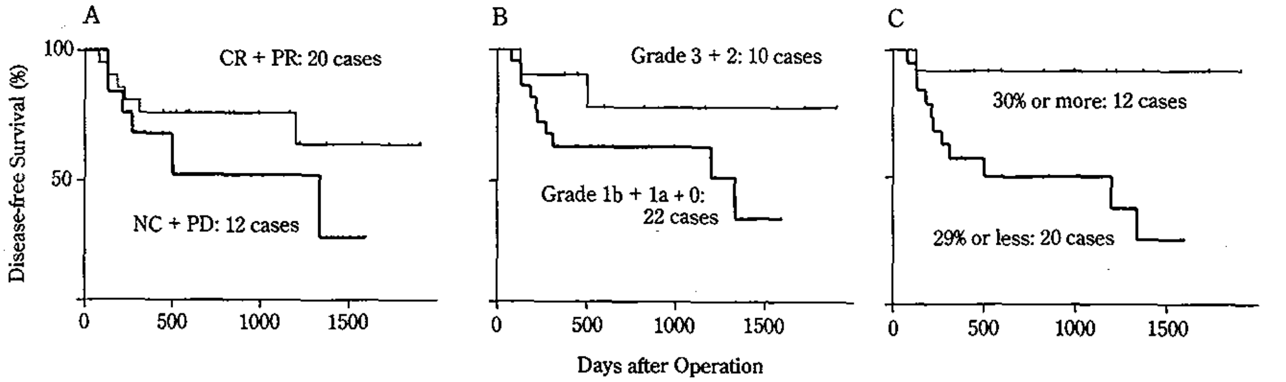

Fig 1. Postoperative disease-free survival as a function of clinical or pathological response and decrease in proliferative activity in advanced breast cancer with primary chemotherapy. (A) Postoperative disease-free survival according to clinical response. (B) Postoperative disease-free survival according to pathological response. (C) Postoperative disease-free survival according to decrease in proliferative activity after chemotherapy.

The cumulative disease-free survival was investigated in 32 patients without distant metastases to evaluate the relationship between the clinical or pathological response and the prognosis after surgery (A, B). The disease-free survival was evaluated according to the decrease in proliferative activity pre- and post-chemotherapy (C). The disease-free survival of clinical responders, pathological responders, and patients with a decrease of 30% or more in proliferative activity was higher than that of clinical non-responders, pathological non-responders, and patients with a decrease of 29% or less, respectively ($p = 0.24$, $p = 0.14$, and $p = 0.011$, respectively).

non-responders, and patients with a decrease of 29% or less, respectively ($p = 0.24$, $p = 0.14$, and $p = 0.011$, respectively). Accordingly, the decrease in proliferation was a useful predictor of outcome after primary chemotherapy in advanced breast cancer.

Discussion

Primary or neoadjuvant chemotherapy in breast cancer is very useful in that it enables us to observe the response to chemotherapy *in vivo*, achieve downstaging, and avoid mastectomy. It is also expected that the therapy is effective for micrometastasis when it is effective and improves the prognosis. The response rate to primary chemotherapy has been reported to be 50-89%[1, 11-14]. However, predictive factors of response have not been specified so far. Factors which predict patients who will respond to primary chemotherapy for downstaging and breast-conserving surgery have not been defined either, although elucidating such factors would be indispensable for these patients. In this study, chemotherapy including epirubicin was performed on patients with primary advanced breast cancer and the response was clinically and pathologically evaluated to investigate predictive factors.

It has frequently been reported[1, 15, 16] that the prognosis of patients who respond to chemotherapy is favorable. In this study, the postoperative disease-free survival of clinical or pathological responders was higher than that of non-responders, which means that chemotherapy is advantageous for responsive patients but disadvantageous for unresponsive patients. As patients who do not respond to one regimen may respond to another regimen, it is possible to avoid choosing an ineffective chemotherapy if predictive factors of the response are defined. It has been reported so far that c-erbB-2 negativity[14, 16, 17], ER positivity[16] or negativity[15] and p53 negativity[18, 19] are effective predictive factors of CR and PR, but it has been also reported[4] that c-erbB-2 positive patients respond to adjuvant chemotherapy. It was reported[13] that a decrease of more than 25% in the score of proliferative activity assessed by MIB-1 after 1 cycle was a significant predictive factor. The present study showed that patients with higher proliferative activity before chemotherapy tended to have higher response rate, and the activity declined in CR and PR patients but increased in NC and PD patients. However, no apparent relationship between proliferative activity and the pathological response was noted. This means that the therapy clinically achieved PR by reducing the tumor size but was not pathologically effective for the remaining tumor, suggesting that the tumor was not homogenous. The authors have reported[2, 3] that patients with ER negative or highly proliferative tumors responded to chemotherapy in cases of recurrent breast cancer, and the same result was obtained in the present study. That is, patients with high proliferative activity also responded to primary chemotherapy. The expression of c-erbB-2 or p53 was neither strongly related to the pathological response nor affected by chemotherapy. Regarding postoperative disease-free survival, a decrease in proliferation after chemotherapy was a more useful predictor than clinical or pathological response.

Another fruitful result of the present study was that the presence or absence of EIC was related to pathological response, that is, primary chemotherapy was less effective for EIC positive patients. One of the factors correlated with positive surgical margin in breast-conserving surgery is EIC positivity[5]. The present study indicates that primary chemotherapy was not likely to be pathologically effective for patients with positive EIC, suggesting that breast-conserving surgery is likely not feasible in these patients. There has been a report[12] showing that breast-conserving surgery is not feasible even when the tumor size is reduced after preoperative chemotherapy if *in situ* lesions remain. Tumors with EIC had higher ER-positivity rates than those without EIC[20], and the rate of pS2 expression, which is associated with ER and response to endocrine therapy, in intraductal lesions was significantly higher than in invasive lesions[21, 22]. These findings suggest that EIC positive tumors more frequently respond to endocrine therapy rather than chemotherapy. Preoperative chemotherapy with an anthracycline-containing regimen had little effect on intraductal lesions, suggesting that the combined use of radiation therapy and endocrine therapy may be required when breast-conserving surgery is considered for patients with EIC. However, the effectiveness of these therapies should be further investigated because it was reported[23] that the time until recurrence was simply extended by radiation therapy in patients with positive surgical margins.

These results demonstrate that the proliferative activity and presence or absence of EIC are useful predictors of the response to primary chemotherapy, and that the primary chemotherapy was of benefit to responders since breast-conserving surgery was feasible and achieved satisfactory postoperative results in these patients. Micrometastasis was believed to be controlled in these patients with satisfactory postoperative results. The detection of predictive factors of therapy to control distant micrometastasis will be needed in the future since individualized treatment is required. However, for preoperative treatment preceding breast-conserving surgery, not only control of distant micrometastasis but also the effect on intramammary spread needs to be studied further. It is expected that the number of patients benefiting from detection of these predictive factors will steadily increase.

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