Neoadjuvant Chemotherapy with Combined infusional 5-Fluorouracil, Adriamycin and Cyclophosphamide in Locally Advanced Breast Cancer

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Neoadjuvant Chemotherapy with Combined infusional 5-Fluorouracil, Adriamycin and Cyclophosphamide in Locally Advanced Breast Cancer

Directed by Professor Hyun Cheol Chung

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Purpose: The goal of neoadjuvant chemotherapy is the downstaging of locally advanced breast cancer (LABC). The author evaluated the efficacy and safety of neoadjuvant chemotherapy with an infusional 5-FU, adriamycin and cyclophosphamide (iFAC) regimen in LABC patients.

Methods: 82 LABC patients were treated with iFAC chemotherapy, which was composed of infusional 5-FU ($1000mg/m^2$, continuous intravenous infusion, day 1-3), adriamycin ($40mg/m^2$, intravenous bolus, day 1), and cyclophosphamide ($600mg/m^2$, intravenous bolus, day 1) every 3 weeks. Surgery was performed when tumor response was at a maximum. Adjuvant chemotherapy with iFAC and radiotherapy

were performed after surgery. Patients with positive hormonal receptor status or in a post-menopausal state were also treated with hormonal therapy. c-erbB-2 status was determined by both immunohistochemisty (IHC) and fluorescence *in situ* hybridization (FISH) in surgical specimens of 29 patients.

Results: Of the 82 patients, downstaging occurred in 71 patients (86.6%). However, four of them were still unresectable because of increased axillary node size in 1 patient, newly developed breast lesion in 2, and unchanged fixed axillary node in 1, respectively. Five of 11 patients without downstaging became resectable due to decreased breast tumor size. As a result, 72 patients (67 patients with downstaging plus 5 patients without downstaging) were resectable (resectability rate, 87.8%). The clinical response rate was 84.2% (CR, 17.1%; PR, 67.1%) and the pathologic complete response rate was 7.8%. Of the clinical responders (69 patients), 51 (73.9%) were early responders who showed a maximum clinical response at $\leq$ 3 cycles of iFAC, while 18 (26.1%) were late responders who showed a maximum response at > 3 cycles of iFAC. During 891 cycles of chemotherapy, grade 3/4 hematological toxicities were leukopenia (36.0%), anemia (0.8%), and thrombocytopenia (0.5%). One patient experienced septic shock resulting from pneumonia, and 3 patients showed congestive heart failure. However, there were no treatment-related deaths. The median follow-up period of the 82 patients was 51 months and the median overall survival

duration was 66 months. The median disease-free and overall survival durations for 64 resected patients were 45 and 89 months, respectively. c-erbB-2 positivity was 31.0% by FISH and 37.9% by IHC with the concordance rate of 93.1% (27/29). A trend was noted that disease-free and overall survivals were prolonged in patients without expression of c-erbB-2 by IHC. An early response to chemotherapy was identified as a favorable prognostic factor of locoregional recurrence-free, distant recurrence-free, disease-free, and overall survivals. A smaller breast tumor size (<10cm) was a favorable predictor of an early response (hazard ratio=0.1, p=0.003).

Conclusion: Neoadjuvant chemotherapy with iFAC was found to have a comparable response rate with that of bolus FAC and acceptable toxicity in LABC. Moreover, an early response to neoadjuvant iFAC chemotherapy was a favorable prognostic factor, and initial tumor size was the only significant predictor of the early response.

Neoadjuvant chemotherapy with combined infusional 5-fluorouracil, adriamycin and cyclophosphamide in locally advanced breast cancer

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I. Introduction

Locally advanced breast cancer (LABC), despite its reducing frequency, remains a challenge in terms of achieving local and distant disease control. In mammographically screened populations, stage III breast cancer seldom amounts to 5% of those diagnosed. However, in medically underserved areas of the United States and in many other countries, LABC represents 30% to 50% of newly diagnosed malignant breast neoplasm[1-2]. Some controversy exists in terms of the definition of LABC. Most investigators include inoperable stage IIIB, whereas some investigators include both operable stage III and stage IIIC by virtue of a positive supraclavicular node[3]. As at the beginning of this study, the author administered neoadjuvant chemotherapy to patients with operable stage II cancers as well as to those with inoperable locally advanced cancers, the study population was

too heterogeneous. The author restaged patients who received neoadjuvant iFAC chemotherapy in accordance with the American Joint Cancer Committee (AJCC) staging system revised in 2002[4] and defined LABC to comprise stage IIIA, IIIB, and IIIC.

The rationales for neoadjuvant chemotherapy in LABC are to make unresectable tumors into resectable status, and to treat micrometastasis at the earliest possible time. Neoadjuvant chemotherapy followed by locoregional therapy is becoming an accepted strategy in LABC and the 5-year overall survival rates have improved from 10-20% with local therapy alone to 30-60% with the multidisciplinary approach[5]. The most effective regimens, judged by objective response criteria, usually contain adriamycin. Generally three to four cycles of treatment results in the clinical response of 50~90% and the pathologic complete response (pCR) rate of less than 20% [6, 7]. Since a combined bolus 5-FU, adriamycin and cyclophosphamide (FAC) regimen produced a good tumor response in metastatic breast cancers [8, 9] and has been investigated by the M. D. Anderson group in a neoadjuvant setting (Hortogagyi et al. 1983)[10], the bolus FAC regimen has been widely used as a neoadjuvant chemotherapy in LABC with a clinical response of 73~88% and a pCR of 8~23%[11-13].

Protracted infusion of 5-FU is attractive because the duration of exposure is an important determinant of cytotoxicity[14]. This was based on an observation of improved *in vitro* sensitivity to

prolonged, low-dose 5-FU exposure than to short, high dose exposure[15]. However, infusional FAC has rarely been evaluated in a neoadjuvant setting of LABC.

As individual variation and tumor heterogeneity are major obstacles in cancer treatment, many investigators have tried to identify predictive or prognostic molecular markers after chemotherapy in order to predict the efficacy of treatment or survival. Tumors overexpressing c-erbB-2 are known to be related to poor prognosis, but are more likely to respond to adriamycin-containing regimens[17-19]. In addition, several molecular markers including Ki67 and p53 have also been evaluated as prognostic or predictive markers. However, relevance of these markers in clinical practice remains contoversial[17-19]. Hence, our study was designed to evaluate the efficacy and safety of an infusional FAC (iFAC) regimen as a neoadjuvant chemotherapy in LABC, and to identify predictive and prognostic markers for this regimen.

II. STUDY AIMS

The aims of this study were to evaluate the efficacy and safety of an iFAC regimen as a neoadjuvant chemotherapy in LABC, and to identify the prognostic factors of survival and predictive factors of response. The primary endpoint was the response rate and the secondary endpoints were the downstaging rate, disease-free survival (DFS), overall survival (OS), toxicity, and dose intensity (DI).

III. PATIENTS AND METHODS

1. Eligibility criteria

Patients with locally advanced breast cancer of stage IIIA, IIIB, or IIIC including inflammatory breast cancer were eligible for this study. Other eligibility criteria were: i) age ≤70 years, ii) histologically proven infiltrative ductal or lobular carcinoma, iii) Eastern Oncology Cooperative Group (ECOG) performance status of ≤ 2, and iv) adequate bone marrow (neutrophils ≥2x10^3/μl, platelets ≥100x10^3/μ l, Hb ≥10.0 g/dl), renal (serum creatinine ≤1.5x upper normal limit) and liver function (serum bilirubin ≤1.5x upper normal limit, aspartate aminotransferase[AST], alanine aminotransferase[ALT] ≤1.5x upper normal limit) and v) no previous chemo-, radio- or hormone therapy. Patients with other malignancies or bilateral breast cancers were excluded.

2. Treatment scheme

The iFAC regimen was administered according to the following schedule: 5-FU 1000mg/m^2 continuous intravenous infusion on days 1 to 3, adriamycin 40mg/m^2 intravenous (i.v.) bolus injection on day 1, and cyclophosphamide 600mg/m^2 i.v. bolus on day 1. Treatment was repeated every 3 weeks. When the tumor response reached a maximum, as determined by no change in tumor size for 2 consecutive treatment cycles, the resectability

was assessed by an oncologic surgeon. When the tumor became resectable, curative surgical resection was undertaken. After surgery, adjuvant chemotherapy with iFAC and radiotherapy were performed based on the pathologic response evaluation. The planned total cycles of iFAC were 12 in maximum including neoadjuvant chemotherapy with a total cumulative adriamycin dose of $480mg/m^2$. If the tumor was unresectable by evaluation, chemotherapy with an alternate regimen and/or radiation was followed. Hormonal treatment was combined in patients who were hormonal receptor positive or in a post-menopausal state (Fig. 2).

3. Response and toxicity evaluation

Tumor measurements were determined by physical examination and by mammography and/or ultrasonography. Tumor measurements were performed at baseline and every third cycle, and when needed. Clinical response was defined according to the World Health Organization (WHO) criteria[20]. A complete response (CR) required the complete disappearance of all evidence of disease in any known site and the absence of a new lesion for at least 4 weeks. Partial response (PR) was defined as a reduction of at least 50% in the sum of the products of the two largest perpendicular dimensions of a measurable lesion for at least 4 weeks, with no progression of evaluable disease and the absence of a new lesion. Progressive disease

(PD) was defined as an increase $\geq 25\%$ in the sum of the products of the two largest perpendicular dimensions of measurable lesions or evidence of new areas of malignant disease, or the definite worsening of evaluable disease. Stable disease (SD) was defined as changes in the target lesion failed to meet the criteria for PR or PD, or in the absence of regression and new areas of malignant disease for at least 4 weeks. An *early response* was defined as a maximum clinical response at $\leq$ 3 cycles of iFAC chemotherapy, whereas a *late response* was defined as a maximum clinical response occurred at > 3 cycles. The author assigned patients with a late or no response to a 'late/no response group'. Assigning pathologic response categories other than pCR after neoadjuvant chemotherapy is arbitrary, because the baseline pathologic tumor size is unknown. Considering that the larger the residual disease, the worse the prognosis, the author dichotomized residual disease into microscopic residual disease (microRD, breast tumor $\leq$ 1cm and negative axillary node) and macroscopic residual disease (macroRD, breast tumor >1cm or positive axillary node).

The level of toxicity was evaluated and graded using WHO criteria[20]. Granulocyte colony-stimulating factor (G-CSF) was injected if grade 3/4 neutropenia occurred. The subsequent treatment cycle was delayed until complete recovery from toxicity was achieved. The administered dose was reduced by 25% if grade 3/4 non-hematological toxicity occurred or if grade

3/4 hematological toxicity was sustained for more than 2 weeks.

4. Follow-up evaluation after the completion of anti-cancer treatment

Patients were evaluated every 6 months after the completion of neoadjuvant chemotherapy, surgery, adjuvant chemotherapy, and adjuvant radiation. Cardiac function testing was performed by either radioventriculography or echocardiography if patients showed dyspnea, dyspnea on exertion, or cardiomegaly on plain chest X-ray. During the follow-up period, any suspected recurrence was confirmed by biopsy where possible. Typical nodules in liver or lung, indicated by imaging studies, or lytic areas on bone indicated by radioisotope bone scan and plain X-ray were accepted as recurrence without a histological confirmation. Locoregional recurrence was defined as recurrence in the chest walls, breasts, axillary nodes, or ipsilateral supraclavicular node, whereas distant recurrence was defined as systemic recurrence, not in locoregional area. Locoregional recurrence-free survival (LRFS) and distant recurrence-free survival (DRFS) were defined as the time from curative surgery to locoregional recurrence and distant recurrence, respectively. Disease-free survival (DFS) was defined as the time from curative surgery to either cancer recurrence, the occurrence of a secondary primary cancer, or death without evidence of recurrence. Overall survival (OS) was

defined as the time from chemotherapy to death from all causes.

5. Actual dose intensity and relative dose intensity

The actual dose intensity (ADI) represents the amount of an individual agent administered per week, and was calculated as follows:

$$\text{ADI (mg/m}^2\text{/week)} = \frac{\text{total delivered dose of each drug (mg/m}^2)}{\text{chemotherapy duration (week)}}$$

The relative dose intensity (RDI) was defined as the actual dose intensity divided by the planned dose intensity.

6. Evaluation scheme

The evaluation of cinical response, downstaging, and toxicity was performed in 82 patients who received neoadjuvant chemotherapy, while the evaluation of pathologic response was performed only in 64 resected patients. Moreover, the evaluation of recurrence and the estimation of disease-free survival was performed in the 64 resectd patients followed by adjuvant therapy (Fig. 1).

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┌─────────────┐     ┌─────────────┐     ┌─────────────┐
│ Neoadjuvant │ →   │  Surgery    │ →   │  Adjuvant   │ →
│chemotherapy │     │   (n=64)    │     │   therapy   │
│   (n=82)    │     │             │     │   (n=64)    │
└─────────────┘     └─────────────┘     └─────────────┘
        ↑                   ↑                   ↑
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•Clinical   •Pathologic   •Recurrence
 response   response   evaluation
 evaluation   evaluation   •Disease-free
•Downstaging   survival
 evaluation
•Toxicity
 evaluation
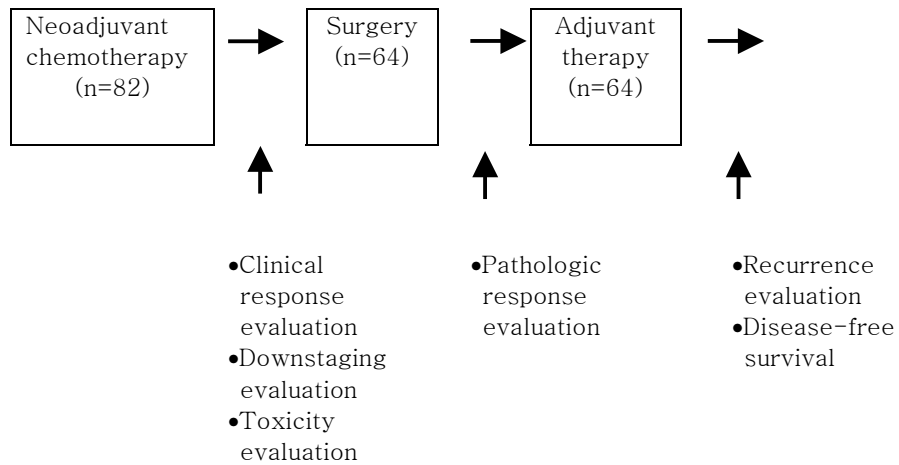
Figure 1. Evaluation scheme.

7. Immunohistochemical staining for c-erbB-2 determination

Immunohistochemical staining for c-erbB-2 was performed using polyclonal c-erbB-2 oncoprotein antibody (A0485, 1:200 dilution, Dako Cooperation, Carpinteria, CA, USA). Tissue sections (4 μm) were deparaffinized and hydrated in the normal manner, and antigen retrieval was performed by heating a slide-amounted section in a microwave oven at 800W in 0.01M sodium citrate buffer (pH 6.0). The slides were then cooled to room temperature and washed with PBS buffer. Non-specific endogenous peroxidase activity was blocked by treating with 3% hydrogen peroxidase for 10 minutes. After rewashing, sections were incubated overnight with the primary antibodies in a refrigerator. Following the application of biotinlylated link antibody and streptavidin/peroxidase for 10 minutes, a solution

of diaminobenzidine (DAB; Dako Corporation, Carpinteria, CA, USA) was added as substrate-chromogen. Finally the sections were lightly counterstained with hematoxylin. The results of c-erbB-2 immunostaining were categorized as follows: Tumors with a complete absence of staining or membrane staining in less than 10% of the tumor cells were scored as 0. Tumors with faint or barely perceptible membrane staining in less than 10% of the tumor cells with only part of the cell membrane stained were scored as 1+. Tumors with moderate complete membrane staining in more than 10% of the tumor cells were scored as 2+. Tumors with strong complete membrane staining in more than 10% of the tumor cells were scored as 3+.

8. Fluorescence *in situ* hybridization for c-erbB-2 determination

 c-erbB-2 gene status was also determined by fluorescence *in situ* hybridization (FISH). Formalin-fixed, paraffin-embedded human breast cancer tissue specimens were sectioned at 5㎛, and placed on the silane-coated slides. After deparaffinization in Histo-ClearⅡ (National Diagnostics, Manville, NJ), the slides were dehydrated through 100% ethanol and allowed to be air-dried, and then they were immersed in a pretreatment solution (Paraffin Pretreatment Reagent Kit, Vysis) at 80℃ for 30 minutes, washed in purified water, and then in 2X SSC. The slides were then incubated in protease solution (Paraffin Pretreatment Reagent Kit, Vysis) at 37℃ for 10 minutes, washed

in 2X SSC for 5 minutes at room temperature, and dried. To tightly fix the residual DNA, the slides were treated in 10% buffered formalin solution (4% formaldehyde in PBS) at room temperature for 10 minutes, and incubated in a denaturing solution (70% formamide / 2X SSC, pH 7.0-8.0) at 72℃ for 5 minutes, dehydrated through a 70, 85, and 100% ethanol gradient, and air-dried. The HER-2/*neu* probe mix consisted of a mixture of a Spectrum Green fluorophore-labeled-α-satellite DNA probe for chromosome 17 (17p11.1-q11.1) and a Spectrum Orange fluorophore-labeled DNA probe for the c-erbB-2 gene locus (17q11.2-q12) (Vysis PathVysion® HER-2 DNA Probe Kit; Vysis Inc, Downers Grove; IL, USA). 10$\mu\ell$ of probe mixture was applied directly to the target area of the slide and covered with a glass coverslip. The slides were then incubated in a pre-warmed humidified hybridization chamber at 37℃ for 14-18hr. Following hybridization, the unbound probe was removed by incubation with post-hybridization wash buffer (2X SSC / 0.3% NP-40) at 72℃ for 2 minutes. Nuclei were counterstained with 10 $\mu\ell$ of DAPI (4, 6-diamidino-2-phenylindole; Vysis Inc, Downers Grove; IL, USA), a DNA-specific nuclear stain. Probe hybridization was viewed under an Olympus BX51 Fluorescence Microscope (Olympus America, Inc.) equipped with appropriate excitation and emission filters to allow visualization of the intense orange and green fluorescent signals. c-erbB-2 and chromosome 17 nuclear signals were counted under a

microscope, which yielded the ratio of the copy numbers of the c-erbB-2 gene to chromosome 17.

9. Statistical analysis

All statistical calculations were carried out using SPSS Windows version 11.0 (SPSS Inc., USA). All p values are two-sided and α was set at 0.05. Survival curves were estimated using the Kaplan-Meier method, the log rank test was used to compare survival in the subgroups. The significant variables were submitted to multivariate analysis using the Cox's proportional hazard regression model.

IV. RESULTS

1. Characteristics of eligible patients

Eighty-two patients were enrolled between June 1991 and June 2001. The median follow-up period was 51 months (range, 7~122 months) ending on December 31, 2003. The median age of the 82 patients was 47 years (range, 29~70 years) and all tumors except 2 infiltrative lobular carcinomas fell into the category of infiltrative ductal carcinoma. ECOG performance status was 0 to 1 in all patients. Twenty patients (24.4%) had inflammatory breast cancer, and the numbers of patients with clinical stage IIIA, B, and C were 41, 30, and 10, respectively. Detailed patient characteristics are listed in table 1.

Table 1. Characteristics of patients

	Number of patients	%
Number of total patients	82	
Median age (years)	47 (range, 29~70)	
Menopause state		
premenopause	44	53.7
postmenopause	38	46.3
Initial clinical stage		
IIIA	42	51.3
T1N2M0	1	
T2N2M0	11	
T3N1M0	21	
T3N2M0	9	
IIIB	30	36.5
T4N0M0	13	
T4N1M0	11	
T4N2M0	6	
IIIC	10	12.2
T2N3M0	5	
T2N3M0	5	
Inflammatory breast cancer	20	24.4
Initial tumor size (cm)	7 (range, 1.5~18)	
Pathology		
infiltrative ductal carcinoma	80	97.5
infiltrative lobular carcinoma	2	2.5
Estrogen/progesterone receptor		
positive/ positive	17	52.4%
positive / negative	3	7.1%
negative/ positive	0	0%
negative/ negative	22	40.5%
unknown	40	–

2. Treatment results

As shown in gray-colored area of table 2, downstaging was observed in 86.6% (71/82). Downstaging was not found to correlate with clinical stage (IIIA, 85.7%; IIIB, 83.3%; IIIC, 100%) but the resectability rate inversely correlated with the clinical stage (IIIA, 83.3%; IIIB, 73.3%; IIIC, 70.0%).

Of 71 downstaged patients, 4 were still unresectable because of increased axillary node size in 1 patient, newly developed breast lesions in 2, and unchanged fixed axillary node in 1, respectively, whereas five of 11 patients without downstaging became resectable due to decreased breast tumor size. As a result, 72 patients (67 patients with downstaging plus 5 without downstaging) were resectable (resectability rate, 87.8%).

Of the 72 resectable patients, 8 refused surgery. All resectable patients underwent a modified radical mastectomy with axillary lymph node dissection, and received radiotherapy at a median dosage of 5040 cGy (range, 4860~7560 cGy).

Ten unresectable patients recieved salvage chemotherapy with platinum- or taxane-based regimen and/or radiation. Of the 10 unresectable patients, 5 underwent surgery after further treatment. (Fig. 2).

Table 2. Downstaging

Initial stage	Total number	Clinical stage after neoadjuvant chemotherapy					
		Stage 0	Stage I	Stage IIA	Stage IIB	Stage IIIA	Stage IIIB
IIIA	42	8	3	18	7	6	0
IIIB	30	3	1	14	7	0	5
IIIC	10	3	1	4	0	2	0

The numbers in gray-colored area represent downstaged patients.

Four downstaged patients were unresectable. The reasons were as follows:

   IIIB(T4N1M0)->IIB(T2N1M0): increased axillary node size

   IIIC(T3N3M0)->IIA(T2N0M0): new breast lesion

   IIIC(T2N3M0)->IIA(T1N1M0): new breast lesion

   IIIC(T2N3M0)->IIIA(T2N2M0):unchanged fixed axillary node
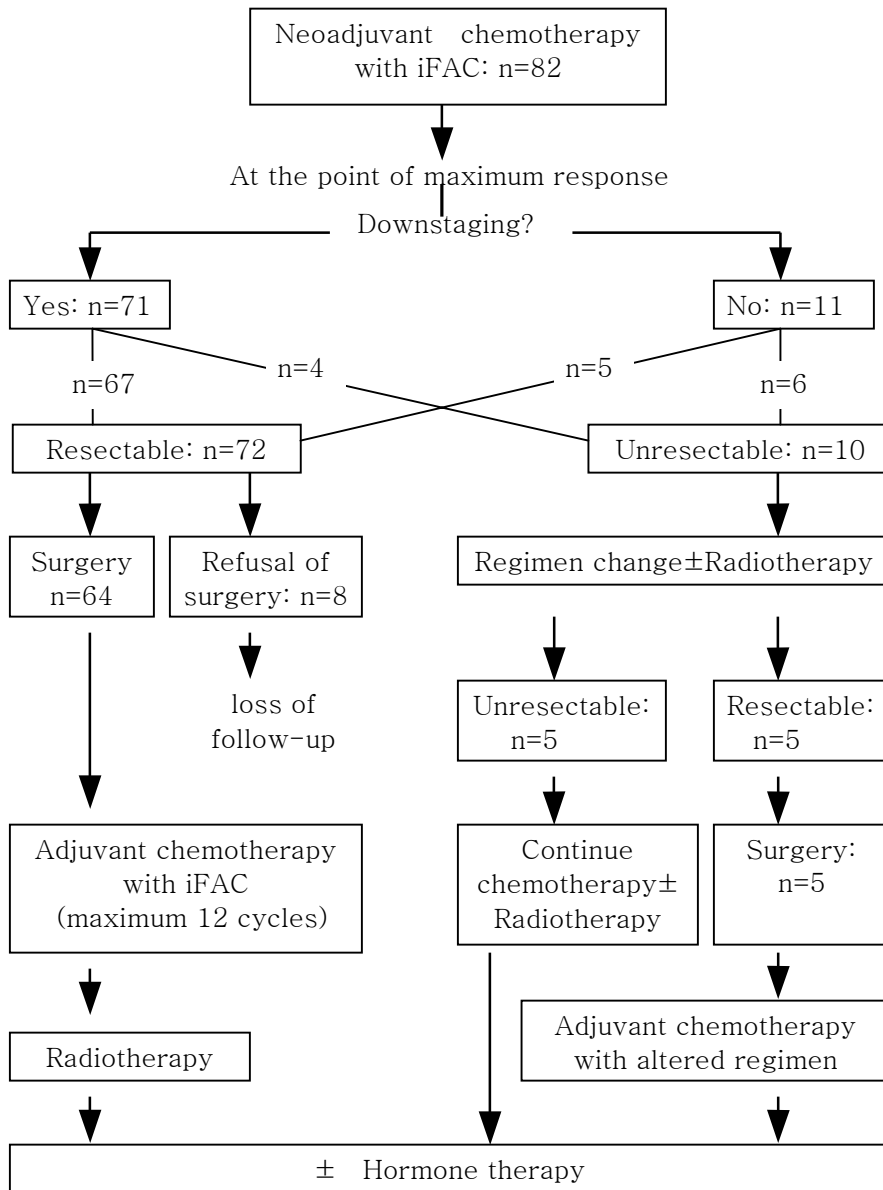
Figure 2. Treatment scheme and results.

3. Response to iFAC chemotherapy

Clinical response evaluation in breast tumor was performed for all the patients, while evaluation in axillary node and supraclavicular node was possible only for the 69 patients with palpable axillary nodes and 10 with palpable supraclavicular nodes, respectively. Clinical response rate in breast tumor was 84.4% (cCR, 25.6%; cPR, 58.8%) and clinical response rate in axillary nodes was 82.8% (cCR, 55.7%; cPR, 27.1%), and overall clinical response rate (ORR) in both breast tumor and nodes was 84.2% (cCR, 17.1%; cPR, 67.1%). A higher ORR was observed in lower clinical stages (ORR : IIIA, 87.2%; IIIB, 81.8%; IIIC, 80.0%). The pCR rate in breast tumor was 10.9% and the pCR rate in axillary nodes was 26.6%. Both cCR (59.4% versus 25.6%) and pCR (26.6% versus 10.9%) rates were higher in axillary nodes than in breast tumor. Five patients (7.8%, 5/64) achieved pCR in both in breast and axillary node, and 7 (10.9%, 7/64) achieved good pathologic response, i.e., pCR and microRD (Table 3). Discrepancy was noted between cCR and pCR as follows: Only 4 of the 14 cCR patients had pCR. Of the remaining 10 patients, 3 had a residual breast tumor without axillary lymph node involvement, 2 had axillary lymph node involvement without residual cancer in the breast, and 5 had both residual breast tumor and axillary lymph node involvement. In contrast, 4 of 5 patients with pCR had been clinically assessed as having CR and

the remaining 1 patient was assessed as having PR.

The median number of cycles required to achieve maximum response was 3 (range, 2~6). An early response was observed in 73.9%, while a late response in 26.1% of the 69 responders.

Table 3. Response rate to neoadjuvant iFAC chemotherapy

	Clinical response[1] (N=82)		Pathologic response[2] (N=64)	
primary tumor	(n=82)		(n=64)	
	CR[3]	21(25.6%)	pCR[10]	7(10.9%)
	PR[4]	49(58.8%)	no pCR	57(89.1%)
	SD[5]	6(7.3%)		–
	PD[6]	6(7.3%)		–
AXLN[7]	(n=69)		(n=64)	
	CR	41(59.4%)	pCR	17(26.6%)
	PR	18(26.1%)	no pCR	47(73.4%)
	SD	8(11.6%)		–
	PD	2(2.9%)		–
SCL[8]	(n=10)			
	CR	10(100%)		–
total response[9]	(n=82)		(n=64)	
	CR	14(17.1%)	pCR	5(7.8%)
	PR	55(67.1%)	microRD[11]	2(3.1%)
	SD	6(7.3%)	macroRD[12]	57(89.1%)
	PD	7(8.5%)		–

[1] Clinical response and [2] pathologic response were evaluated in 82 enrolled patients and in 64 resected patients with iFAC chemotherapy, respectively.
[3] CR, complete response; [4] PR, partial response; [5] SD, stable disease; [6] PD, progressive disease.
[7] AXLN, axillary lymph node; Only 69 patients had palpable axillary nodes.
[8] SCL, supraclavicular node; Only 10 patients had palpable supraclavicular nodes.
[9] Total response was assessed by the summation of responses in primary tumor, AXLN and SCL based on the WHO response criteria.
[10] pCR, pathologc complete response.
[11] microRD, microscopic residual disease; [12] macroRD, macroscopic residual disease.

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4. Recurrence pattern

The most common locoregional and distant recurrence sites were chest wall and bone, respectively. An isolated locoregional recurrence occurred in 10 patients (15.6%) and combined recurrence with a distant metastasis occurred in 8 (12.5%). Seventeen patients (26.6%) showed an exclusively distant recurrences (Table 4). Of 10 locoregionally recurred patients, 6 patients showed a systemic recurrence later, whereas a delayed locoregional recurrence was observed in 1 of 17 patients that initially recurred systemically. Five-year LRFS (5Y-LRFS) and 5-year DRFS (5Y-DRFS) rates were 68.5% and 51.3%, respectively (Fig. 3).

Table 4. Site of first recurrence

Site	Number of patients	Number of recurred patients / Number of resected patients
Locoregional		10/64(15.6%)
chest wall	3	
SCL[1]	2	
contralateral breast	2	
AXLN[2]	1	
chest wall + SCL	1	
chest wall+ contralateral breast	1	
Distant		17/64(26.6%)
bone	5	
liver	3	
neck node	3	
lung	2	
brain	1	
pericardium	1	
liver+ lung	1	
pleura+ lung+ bone	1	
Combined locoregional and distant		8/64(12.5%)
chest wall+ bone	2	
chest wall+ pleura	1	
chest wall+ lung+ brain	1	
SCL+ pleura	1	
SCL+ lung	1	
SCL+ neck node	1	
SCL+ neck node+ lung	1	

[1] SCL, supraclavicular lymph node; [2] AXLN, axillary lymph node
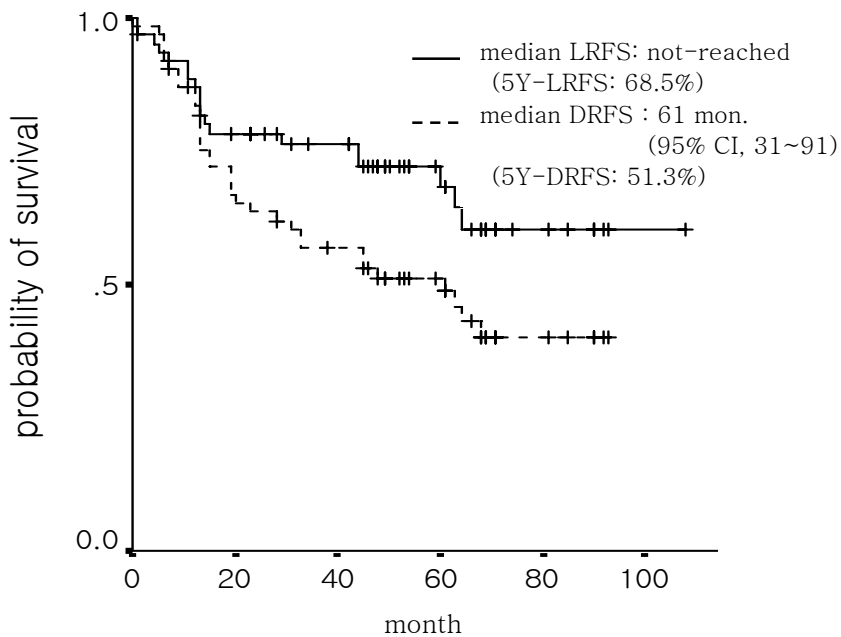
Figure 3. Local recurrence-free and distant recurrence-free survivals of resected patients (N=64). LRFS, locoregional recurrence-free survival; DRFS, distant recurrence-free survival; CI, confidence interval.

5. Survival analysis

Five of the 8 patients who refused surgery died as a result of disease-progression. Of the remaining 74 patients, one patient died from an acute myocardial infarction and 35 patients died from disease-progression. Table 5 summarizes the survival analysis.

Table 5. Summary of survival analysis

Subgroup	N	median DFS (month)	5Y-DFS[1] (%)	median OS (month)	5Y-OS[2] (%)
Total patients	82	–	–	66	50.9%
Response					
responders[3]	61	–	–	89	59.8%
no-responders	13	–	–	44	23.8%
Resection					
resected	64	45	44.7%	89	55.8%
unresected[4]	10	–	–	51	30.0%

[1]5Y-DFS, 5-year disease-free survival; [2]5Y-OS, 5-year overall survival.
[3]Responders or [4]unresected group excluded the 8 patients who refused surgery.

The median OS duration of 82 patients was 66 months with 95% confidence interval (CI) ranging from 43 to 89 months, and their 5-year OS (5Y-OS) rate was 50.9% (Fig. 4). Median OS duration was 89 months in the 61 responders (excluding the 8 patients who refused surgery) and 44 months (95% CI, 19~69 months) in the 13 no-responders (p=0.006). As shown in figure 5, median DFS duration for the 64 resected patients after iFAC chemotherapy was 45 months (95% CI, 17~73 months) and their median OS duration was 89 months (95% CI, 43~129 months). The 5-year DFS (5Y-DFS) and the 5Y-OS rates of the 64 resected patients after iFAC chemotherapy were 44.7% and 55.8%, respectively. Median OS duration and 5Y-OS were 51 months (95% CI, 45~57 months) and 30.0% for the 10 unresected patients, excluding the 8 patients who refused surgery despite showing clinical response.
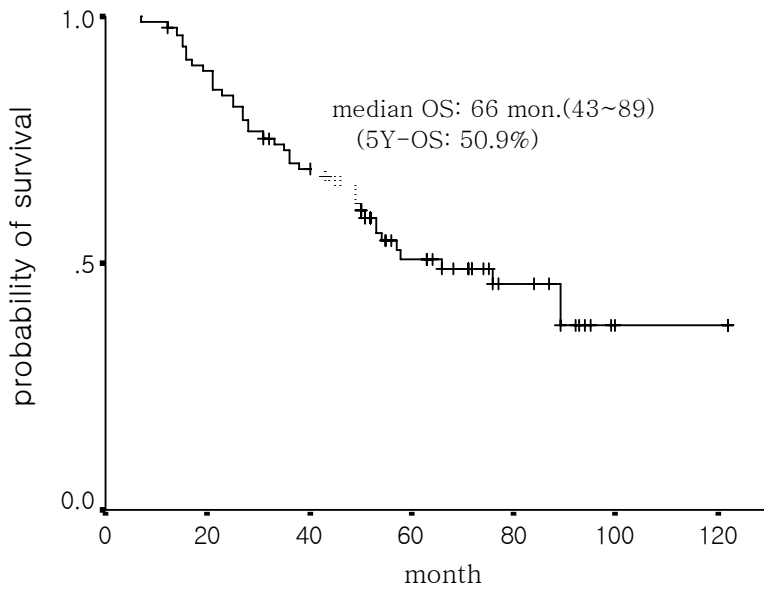
Figure 4. Overall survival of total enrolled patients (N=82).
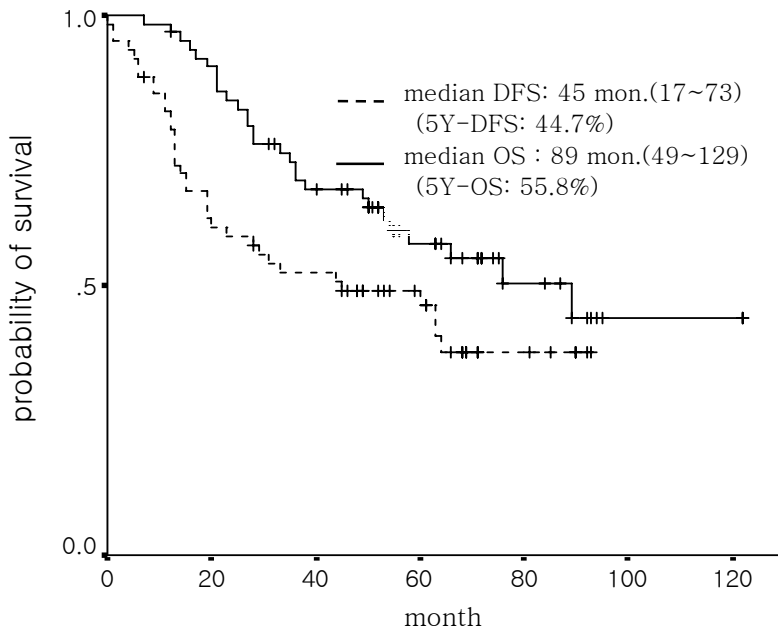
Figure 5. Disease-free and overall survivals of resected patients (N=64).

6. Toxicity

 The main toxicity resulting from iFAC chemotherapy was myelosuppression. Of a total of 891 cycles, grade 3/4 leukopenia occurred in 36.0%, anemia in 0.8%, and thrombocytopenia in 0.5% (Table 6).

Table 6. Hematologic toxicities of iFAC chemotherapy (total 891 cycles)

WHO grade	Leukopenia (%)	Anemia (%)	Thrombocytopenia (%)
1	103(11.6)	400(44.9)	40(4.5)
2	269(30.2)	106(11.9)	13(1.5)
3	292(32.8)	6(0.7)	4(0.5)
4	28(3.2)	1(0.1)	0

 Other serious toxicities were one episode of pneumonia with septic shock and 3 congestive heart failures. However, there were no treatment-related deaths. Congestive heart failure (CHF) occurred in 3 patients (3.7%) after the completion of iFAC chemotherapy. All three had received cumulative doxorubicin doses of 480 mg/m^2 and developed heart failure at 2 months, 2 months, and 46 months, respectively, after the completion of iFAC. Oral mucositis, diarrhea, and skin toxicities were mild.

7. Dose intensity

 The median duration of neoadjuvant chemotherapy was 10 weeks (range, 7~23 weeks) and the median number of iFAC

cycles in a neoadjuvant setting was 3 (range, 2~6). Dose intensity in the neoadjuvant setting was as follows: The median ADIs of 5-fluorouracil, adriamycin, and cyclophosphamide were 1000.0 mg/m^2/wk, 13.3 mg/m^2/wk, and 200.0 mg/m^2/wk, respectively. The median RDIs of 5-fluorouracil, adriamycin, and cyclophosphamide were 1.0 in all drugs, and the RDI of the combined iFAC regimen was 0.98. The dose intensity in the adjuvant setting was as follows: The median ADIs of 5-fluorouracil, adriamycin, and cyclophosphamide were 909.1 mg/m^2/wk, 12.1 mg/m^2/wk, and 181.8 mg/m^2/wk, respectively. The median RDIs of 5-fluorouracil, adriamycin and cyclophosphamide were 0.9 in all. The RDI of the combined iFAC regimen was 0.91 (Table 7).

Table 7. Dose intensity of iFAC chemotherapy

	Median ADI[1] (mg/m^2/wk)	Median RDI[2]
Neoadjuvant		
5-fluorouracil	1000.0(511.6~1000.0)	1.0(0.5~1.0)
adriamycin	13.3(7.7~13.3)	1.0(0.6~1.0)
cyclophosphamide	200.0(98.2~200.0)	1.0(0.5~1.0)
iFAC	−	0.98(0.58~1.00)
Adjuvant		
5-fluorouracil	909(353.8~1000.0)	0.9(0.6~1.0)
adriamycin	12.1(4.7~13.3)	0.9(0.4~1.0)
cyclophosphamide	181.8(70.8~200.0)	0.9(0.4~1.0)
iFAC	−	0.91(0.49~1.00)

[1] ADI: actual dose intensity; [2] RDI: relative dose intensity.

8. Determination of c-erbB-2 status

c-erbB-2 status was determined by both IHC and FISH in surgical specimens of 29 patients who had received iFAC neoadjuvant chemotherapy. Their clinical response was as follows: CR, 2; PR, 23; SD, 4. All patients received resection after iFAC chemotherapy.

Tumors with a gene copy ratio of c-erbB-2 to chromosome 17 centromere > 2.0 or with an IHC staining intensity of ≥ 2+ were considered as c-erbB-2-positive. Positivity of c-erbB-2 was 31.0% by FISH and 37.9% by IHC with the concordance rate of 93.1% (27/29). According to the IHC staining intensity, all tumors expressing 3+ by IHC were FISH-positive. Meanwhile, all FISH-positive patients expressed IHC-positive, and all FISH-negative patients but 2 expressed IHC-negative (Table 8). When gene copy number of c-erbB-2 was graded as shown in table 1, results by FISH had positive correlation with those by IHC (adjusted R^2=0.718, p<0.001, Fig. 6).

Table 8. Comparison of c-erbB-2 status in fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC)

FISH[2]	IHC[1]				Total patients
	0	1+	2+	3+	
0 (<2)	16	2	2[3]	0	20
1 (2~<10)	0	0	1	0	1
2 (10~<15)	0	0	1	1	2
3 (≥ 15)	0	0	2	4	6

[1]staing intensity by IHC; [2]grade of gene copy number by FISH.
[3]FISH/IHC discordance.
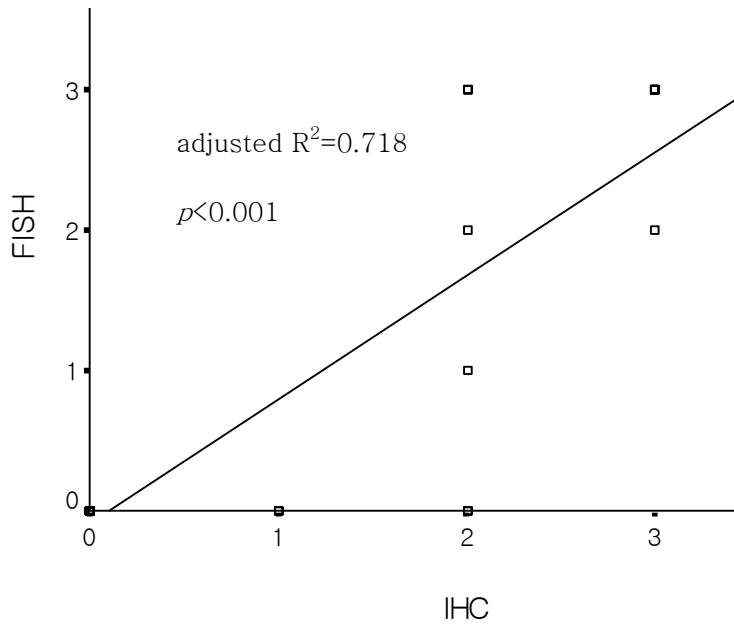
Figure 6. Correlation of c-erbB-2 determination by IHC and by FISH based on the grading system.

9. Association of clinical and pathological characteristics with c-erbB-2 status

c-erbB-2 status was recategorized as being 'positive' when synchronously positive by both methods and as 'negative' otherwise. None of the clinical and pathologic variables including stage, tumor size, and axillary node status were significantly associated with c-erbB-2 status (Table 9).

Table 9. Association of clinical and pathological characteristics with c-erbB-2 status

Variables	FISH/IHC		
	negative (n=20)	positive (n=9)	P value
Age[1] (year)	45 (33~70)	45 (34~56)	NS[4]*
Initial clinical stage			
IIIA	15	7	NS[†]
IIIB	5	2	
Initial tumor size[2] (cm)	7.4±3.4	7.0±2.2	NS[‡]
Initial axillary node status			
negative	2	1	NS[†]
positive	18	8	
Pathologic stage			
I	1	0	NS[†]
II	11	4	
III	8	5	
Pathologic tumor size[3] (cm)	3.6±2.4	4.4±2.5	NS[‡]
No. of positive axillary nodes			
0	3	0	NS[†]
1~3	7	3	
>4	10	6	

* was calculated with Mann-Whitney test.
† was calculated with Fisher's exact test..
‡ was calculated with Student's t-test.
Valuses of [1]age are median (range).
Valuses of [2]initial tumor size and [3]pathologic tumor size are mean±standard deviation.
[4]NS, not significant (p >0.05).

10. Correlation of c-erbB-2 status and clinical response

Axillary nodes were less responsive to iFAC chemotherapy in c-erbB-2-positive status than negative (p=0.02), whereas breast tumor response, total clinical response, and early responsiveness did not correlate with c-erbB-2 status (Table 10).

Table 10. Correlation of c-erbB-2 status and clinical response

Response	No. of patients	FISH[1]/IHC[2] negative (n=20)	positive (n=9)	p^* value
Breast tumor response				
$\quad$CR[3]+PR[4]	25	18	7	0.59
$\quad$SD[5]+PD[6]	4	2	2	
Axillary node response				
$\quad$CR+PR	21	17	4	**0.02**
$\quad$SD+PD	5	1	4	
Total clinical response				
$\quad$CR+PR	25	18	7	0.59
$\quad$SD+PD	4	2	2	
Early responsiveness				
$\quad$early response	16	12	4	0.69
$\quad$late/no response	13	8	5	

$^*P$ value was calculated with Fisher's exact test.
[1]FISH, fluorescence *in situ* hybridization; [2]IHC, immunohistochemistry.
[3]CR, complete response; [4]PR, partial response; [5]SD, stable disease; [6]PD, progressive disease.

11. Correlation of c-erbB-2 status and survival

Differences were not observed in DFS and OS according to the c-erbB-2 status by FISH/IHC (Fig 7), while a trend was noted that these survivals were prolonged in patients without expression of c-erbB-2 by IHC (Fig. 8).
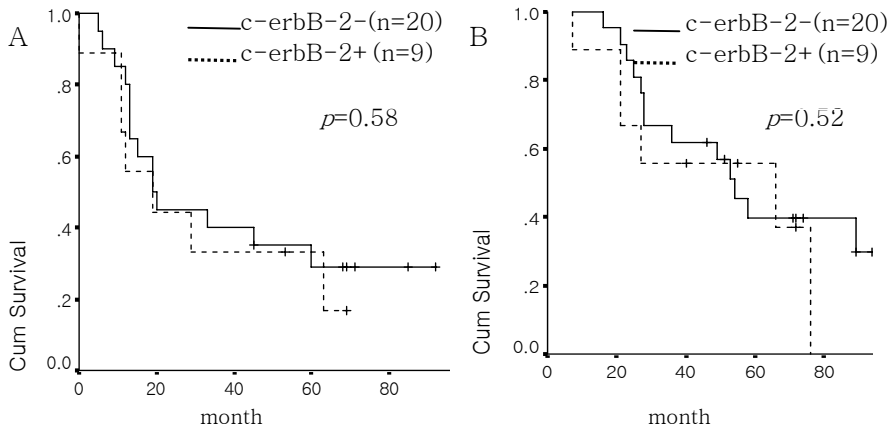
Figure 7. DFS (A) and OS (B) according to the c-erbB-2 status by FISH/IHC.
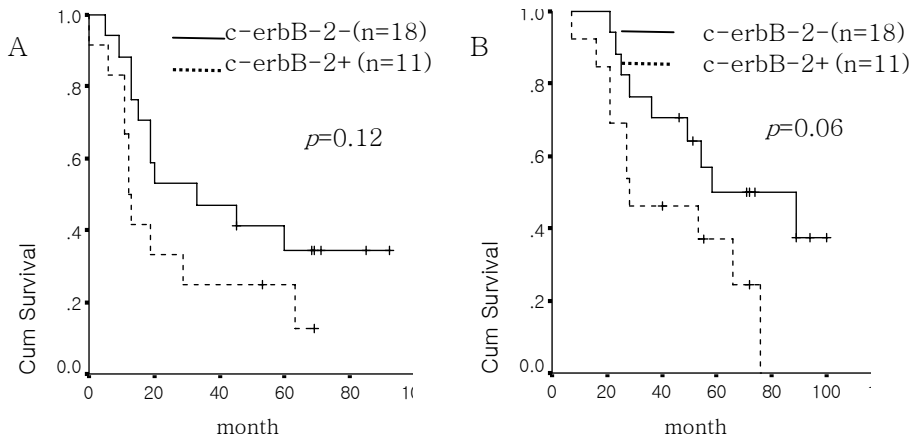
Figure 8. DFS (A) and OS (B) according to the c-erbB-2 status by IHC.

12. Prognostic factors of survival

The prognostic significance of the clinical and the pathologic variables for 5Y-LRFS, 5Y-DRFS and 5Y-DFS were evaluated by Cox's regression analysis.

Clinical T stage was not included in multivariate analysis of LRFS using the Cox's regression model due to divergency of coefficients in the Cox's model despite a significant variable in univariate analysis. The reason was that no patients with clinical T1-T2 stage experienced locaregional recurrence.

A late/no response were identified as an adverse prognostic factor of 5Y-LRFS, 5Y-DRFS, and 5Y-DFS (hazard ratio=4.1, $p<0.05$; hazard ratio=3.6, $p<0.01$; hazard ratio=4.6, $p<0.01$, respectively, Table 11).

Table 11. Univariate and multivariate analysis of prognostic factors of survival

	5Y-LRFS[1]				5Y-DRFS[2]				5Y-DFS[3]			
	univariate		multivariate		univariate		multivariate		univariate		multivariate	
	$\%^4$	p	HR[5] (95% CI[6])	p	$\%^4$	p	HR (95% CI)	p	$\%^4$	p	HR (95% CI)	p
Preoperative factors												
Size of breast tumor												
≤10cm	78.4	***	1		51.5	NS	–		52.7	**	1	
>10cm	21.8		2.3 (0.6~8.3)	NS[7]	53.0		–		13.6		2.2 (0.8~6.0)	NS
Clinical T stage												
T1-T2	100	*	–[8]		40.4	NS	–		40.0	NS	–	
T3-T4	62.5		–[8]		53.6		–		45.2		–	
Clinical LN status												
negative	60.0	NS	–		62.5	NS	–		50.0	NS	–	
positive	69.7		–		49.6		–		43.7		–	
Clinical stage												
IIIA	78.6	NS	–		48.4	NS	–		47.1	NS	–	
IIIB	56.9		–		57.0		–		39.7		–	
IIIC	66.7		–		45.7		–		50.0		–	
Clinical response												
CR+PR	70.5	*	1		54.9	***	1		47.3	***	1	
SD+PD	33.3		2.7 (0.5~16.4)	NS	0		3.1 (0.9~10.8)	NS	0		2.7 (0.7~9.9)	NS

| | 5Y-LRFS | | | | 5Y-DRFS | | | | 5Y-DFS | | | |
| | univariate | | multivariate | | univariate | | multivariate | | univariate | | multivariate | |
	%	p	HR (95% CI)	p	%	p	HR (95% CI)	p	%	p	HR (95% CI)	p
Early responsiveness												
early response	84.6	***	1		63.8	***	1		61.4	***	1	
late/no response	21.1		**4.1** (1.2~14.8)	*	23.2		**3.6** (1.5~8.6)	**	8.1		**4.6** (1.6~12.9)	**
Postoperative factors												
Number of AXLN[9]												
0	87.5	NS	–		68.8	*	1		68.8	*	1	
1~3	67.0		–		60.6		1.2 (0.2~5.9)	NS	52.8		1.8 (0.3~9.3)	NS
≥ 4	52.3		–		33.6		2.6 (0.6~11.7)	NS	23.7		2.6 (0.5~13.9)	NS
Pathologic T stage												
T0-T2	72.6	*	1	NS	54.6	NS	–		46.5	*	1	
T3-T4	54.5		1.8 (0.6~5.3)		40.8		–		35.7		0.7 (0.2~2.5)	NS
Pathologic N stage												
N0	87.8	NS	–		69.3	NS	–		69.3	NS	–	
N1-3	60.3		–		44.6		–		36.1		–	

	5Y-LRFS				5Y-DRFS				5Y-DFS			
	univariate		multivariate		univariate		multivariate		univariate		multivariate	
	%	p	HR (95% CI)	p	%	p	HR (95% CI)	p	%	p	HR (95% CI)	p
Pathologic stage												
0	71.4	NS	–		57.1	*	1		57.1	*	1	
I–II	71.2		–		60.1		0.2 (0.0~1.4)	NS	48.0		0.2 (0.0~1.2)	NS
III	60.6		–		27.8		0.9 (0.1~6.1)	NS	28.1		1.2 (0.1~12.7)	NS
Pathologic response												
CR+ microRD	71.4	NS	–		77.1	NS	–		57.1	NS	–	
macroRD	67.7		–		50.4		–		43.2		–	

[1] LRFS, local recurrence-free survival; [2] DRFS, distant recurrence-free survival; [3] DFS, disease-free survival.

[4] 5Y-LRFS (%), 5Y-DRFS (%), and 5Y-DFS (%) were estimated using the Kaplan-Meier method in univariate analysis.

[5] HR, hazard ratio; [6] CI, confidence interval.

[7] NS, not significant (p <0.05).

[8] Cofficients did not converge since no patients with clinical T1-T2 stage experienced locaregional recurrence, and thus clinical T stage was not included in multivariate analysis of LRFS using the Cox's regression model.

[9] AXLN, axillary lymph node.

∗ represents p value<0.05; ∗∗, p<0.01; ∗∗∗, p<0.001.

13. Predictive factors of an early response

As an early response was a favorable prognostic factor of 5Y-LRFS, 5Y-DRFS, and 5Y-DFS, and thus the author searched for clinical predictive factors of an early response. As shown in table 12, tumor size ($\geq$10cm versus <10cm) was the only predictive factor of an early response (hazard ratio=0.1, p=0.003).

Table 12. Univariate and multivariate analysis of predictive factors of an early response

	Univariate			Multivariate	
	Early response (%) (N=50)	Late/no response (%) (N=32)	p^* value	HR[1] (95% CI[2])	p value
Size of breast tumor					
<10cm	69.1	30.9	0.002	1	
≥ 10cm	21.4	78.6		0.1 (0.0~0.5)	0.003
IBC					
non-IBC	66.1	33.9	NS[3]	–	
IBC	45.0	55.0		–	
Clinical T stage					
T1-T2	58.8	41.2	NS	–	
T3-T4	61.5	38.5		–	
Clinical N stage					
N0	35.7	64.3	0.033	1	
N1-N3	66.2	33.8		1.0 (0.5~2.0)	NS
Clinical stage					
IIIA	66.7	33.3	NS	–	
IIIB	54.5	45.5		–	
IIIC	60.0	40.0		–	

* was calculated with χ^2 test or Fisher's exact test.
[1] HR, hazard ratio. HR was calculated in logistic regression model.
[2] CI, confidence interval.
[3]NS, not significant (p <0.05).

14. Comparison of pathologic characteristics between early and late/no responders

The pCR rate was higher for early responders than for late/no responders (9.3% versus 4.7%). And the proportion of positive axillary nodes ≤ 3 was higher (65.1% versus 42.9%, p=0.02) and the mean tumor size was smaller (2.7 cm versus 4.4 cm, p=0.09)

in early responders than in late/no responders (Table 13).

Table 13. Comparison of pathologic characteristics between early and late/no responders[1]

Variables	Early responders N=43	Late/no responders N=21	p value
Pathologic response			
pCR	4/43(9.3%)	1/21(4.7%)	−
microRD+macroRD	39/43(90.7%)	20/21(95.3%)	−
Tumor size (cm)	2.7±1.8	4.4±2.8	0.09[*]
Number of axillary nodes			
0~3	28/43(65.1%)	9/21(42.9%)	**0.02[†]**
≥4	15/43(34.9%)	12/21(57.1%)	

∗ was calculated with Student's t-test.
† was calculated with χ^2 test.
This comparison was performed in 64 resected patients.

V. DISCUSSION

LABC was considered inoperable until the emergence of neoadjuvant chemotherapy in the mid-1970's. Various active neoadjuvant chemotherapy regimens induced a high resectability rate due to effective downstaging, and an improved distant disease-control rate, achieved by treating occult micrometastasis in LABC. Because pCR correlates with patients' better outcome[21, 22], improving the pCR rate is a rational primary objective when searching for more effective regimens. This study was carried out in order to identify a more effective regimen in LABC, and thus the author report outcomes in a cohort of 82 LABC patients who were treated with a combined regimen of infusional FAC with a median follow-up duration of 51 months.

An initial investigation of continuous infusion of 5-FU in colorectal cancer demonstrated that the continuous infusion of 5-FU is more active[23] and less toxic to bone marrow than a bolus 5-FU, due to its different mode of action. Thus the author selected an iFAC regimen for the neoadjuvant chemotherapy in LABC, because of belief in the efficacy and safety of the bolus FAC regimen based on experience. Most investigators used a fixed number of neoadjuvant chemotherapy cycles, usually 3-4[10, 24]. However, in the present study, to reduce the tumor bulk prior to definitive local therapy, patients received treatment until they

reached maximum clinical response regardless of the number of cycles. The former method has the advantage that definitive local therapy is not delayed unnecessarily, though it has the disadvantage of missing an opportunity for optimal resection due to insufficient response, while the latter method is considered better with improved cCR, though it increases the risk of developing resistant clones. Our results with iFAC in LABC (ORR, 84.2%; pCR, 7.8%) were similar to the results of trials of a bolus FAC regimen conducted by the M. D. Anderson group (ORR, 73~88%; pCR, 8~23%)[11-13]. Lower pCR achieved by the current study could be explained by the fact that the M. D. Anderson group trial enrolled more stage II patients in the proportion of 28%~83%, whereas the current study enrolled only stage III patients.

The limitation of our study is that the duration of enrollment was too long, i.e., from 1991 to 2001. Tumor measurements had been made by classical methods, i.e., physical examination and mammography and/or ultrasonography. Discrepancy was noted between cCR and pCR, which was due to overestimation of the residual tumor in terms of chemotherapy−induced fibrosis, or difficulty in detecting microscopic residual tumor by classical evaluation methods. Now CT and MRI can be used to assess tumor response more precisely[25]. CT and MRI data were not included here to evaluate response because these methods were introduced for response evaluation in the late 1990's. In

addition, estrogen and progesterone hormone receptor statuses were not determined at the beginning of the study, radioactive enzyme-immunoassays were introduced to determine these in 1992, and these were replaced by immunohistochemical staining methods in 1998. However, the author had no difficulties in classifying hormone receptor status as positive or negative in the present study.

The clinical benefits of neoadjuvant chemotherapy are downstaging, resectability, and breast conservation. Downstaging and resectability rates were similar to those reported by Western studies, but breast conservation was not performed in any case in the present study. Hortobagyi reported that 23% of patients with stage IIB or III were potential breast-conservation candidates[26]. In the NSABP B-27 study, 22% of the patients with a T3 tumor received breast conservation surgery[27]. Most of the breasts of our patients were too small to conserve compared with the breast mass, and most patients did not want to conserve the breast. This may amount to a national trait, which results in the limited use of breast conservation surgery.

Higher pCR as well as higher cCR were observed in the axillary nodes than in breast tumors. Suggested reasons are i) the different sizes of primary breast tumors and nodes, as a lower tumor burden is generally responds more easily to drugs, or ii) the different predetermined sensitivities of breast tumors

and nodes to chemotherapeutic agents.

The author observed that patients detected first with locoregional recurrence also showed a higher risk of subsequent systemic recurrence. Systemic recurrence following locoregional recurrence (60.0%, 6/10) occurred more frequently than systemic recurrence with/without synchronous locoregional recurrence (44.4%, 24/54). Hence, patients who develop locoregional recurrence need to be followed more closely, because they probably have already developed systemic micrometastasis.

The toxicities of iFAC chemotherapy were mild and acceptable in general. The major toxicity was bone marrow suppression, which was manageable with G-CSF. Changing to the continuous infusion of 5-FU in the current iFAC regimen did not cause more mucositis or GI toxicity. Considering the cumulative dose of adriamycin and the fact that 1 of 3 CHFs developed 46 months after the completion of iFAC chemotherapy, clinicians need to follow patients long-term after the completion of iFAC chemotherapy, and more efforts are needed to identify factors predisposing CHF. A cardioprotectant may be another option for patients with a high-risk of CHF. The author did not evaluate the cardiac function of all patients before iFAC chemotherapy at the beginning of the study, but did perform cardiac function evaluation at baseline from the mid-1990's, and advocate that patients with impaired or borderline cardiac function at baseline

need to be monitored closely.

Inflammatory breast cancer is known to have an extremely poor prognosis. In this study, the IBC group showed no differences with respect to downstaging, clinical response, and survival rates versus the non-inflammatory cases (data not-shown). The comparable outcome of the IBC group in the current study may have been due to difficulties distinguishing between a primary IBC and large tumors with secondary inflammatory changes. In many patients with large tumors characterized by slow, progressive growth, inflammatory changes may appear months or years after the breast mass is first detected. LABC with secondary inflammatory changes usually does not have poor prognosis[5].

As is known that pCR correlates with patients' best outcome[21, 22], good pathologic response group in our study (5 pCRs and 2 microRDs) had a trend for higher DFS and OS, which did not reach statistical significance due to small number of the group (data not-shown). Moreover many significant prognostic factors were reported as follow: the number of axillary nodes involved[28-30], a good pathologic response[31], a clinical response[11], the duration of neoadjuvant chemotherapy, and the size of the primary tumor[32-34]. In our study, early responsiveness was a common prognostic factor of LRFS, DRFS, and DFS, whereas clinical response per se was not a prognostic factor because late responders showed poor prognosis among responders. To

improve overall outcome, alternate chemotherapy strategies are needed for late responders as well as no-responders to iFAC neoadjuvant chemotherapy. Moreover, differences were not observed between survivals of resected and unresected patients after an alternate salvage regimen and/or radiation (5Y-OS: 40.0% versus 40.0%, p=0.58), which suggests that delayed change to the salvage regimen does not improve clinical outcome.

As early responders had a favorable clinical outcome in the present study, the author subsequently analyzed for factors predicting an early response. Early responsiveness was found to inversely correlate with initial tumor size, in other words tumor size of <10 cm was the predictor of an early response. Because initial tumor size correlates with treatment response and prognosis in the neoadjuvant chemotherapy of LABC, tumor size needs to be considered as an important clinical parameter for the selection of patients for neoadjuvant chemotherapay.

Why are early responders associated with a favorable outcome? A high pCR rate by neoadjuvant chemotherapy in LABC is a well-known favorable prognosticator[21], and the pCR rate was higher in early responders than in late/no responders (9.3% versus 4.7%), but the numbers of pCRs in these groups (4/43 versus 1/21) were too small to explain the different prognoses. The most valuable pathologic prognostic factors in breast cancer are the number of positive axillary nodes and

tumor size in general. The proportion of positive axillary nodes ≤3 was higher and the mean tumor size was smaller in early responders than in late/no responders. The favorable prognosis of early responders probably resulted from favorable pathologic prognostic factors in part. Since an early response itself was identified as an independent favorable prognostic factor, based on the multivariate analysis of prognostic factors, another mechanism may cause the correlation between an early response and a favorable prognosis. The main question is ' does an early response to chemotherapy simply identify patients with a biologically predetermined excellent prognosis, or can the early definitive local therapy alter the disease course?' In the NSABP protocol B-18, which compared preoperative and postoperative chemotherapy in operable cancer, it was speculated that the treatment modality could not alter the disease course. Thus an early response to iFAC may represent a biologically predetermined good prognosis. Further efforts need to be focussed on elucidating the molecular mechanisms associated with an early response. Attempts are under way to identify molecular predictors of response to neoadjuvant chemotherapy[35-36]. In addition, it is worth searching for the molecular mechanisms associated with an early response, since these may serve as predictive markers for treatment response and as a basis for individualized therapy.

Several molecular markers have been evaluated as prognostic

or predictive markers. Determination of c-erbB-2 status is now an integral part of the clinico-pathological workup of breast cancer as a predictor of benefit from adriamycin-based chemotherapy, as a predictor of benefit from the anti-c-erbB-2 antibody, trastuzumab therapy, and as a prognostic factor of survival. c-erbB-2 gene amplification or protein overexpression is observed in 20~40% of breast cancer patients[37-39], which was similar to our result. A recommended testing algorithm for c-erbB-2 determination is most efficient by using IHC, and FISH is performed for cancers with indeterminate results[40]. 3+ intensity of c-erbB-2 by IHC is known to correlate well with FISH-positive, and thus actually the anti-c-erbB-2 antibody therapy is indicated for tumors with 3+ of c-erbB-2 by IHC. c-erbB-2 protein overexpression is usually a direct consequence of gene amplification[37], and thus gene-protein correlation studies in general showed good concordance[41]. FISH/IHC concordance rate was also high in our study, and yet FISH-negative/IHC-positive discordance was observed in 2 patients. Suggested reasons are i) the possible exaggeration of staining intensity by non-specific binding of polyclonal c-erbB-2 antibody used in IHC, ii) amplification in the level of transcription or translation through unknown mechanism, or iii) technical variations.

Positive c-erbB-2 status was reported to have association with good clinical response to adriamycin-based chemotherapy,

but controversy still remains[42]. However, since in the present study, c-erbB-2 was determined not in biopsy specimen but in surgical specimen, c-erbB-2 determination would not be valuable for predicting clinical response to adriamycin-based chemotherapy. In addition, early responsiveness did not correlate with c-erbB-2 status, either. Poor clinical response in axillary node in c-erbB-2-positive patients might be one possible reason why c-erbB-2-positive patients have poor prognosis.

Since c-erbB-2 protein is an oncoprotein, c-erbB-2-positive patients are generally considered to have poor prognosis. However, in the current study, DFS and OS did not correlate with the c-erbB-2 status by FISH/IHC probably due to the small number of patients. However, in c-erbB-2 determination by only IHC, c-erbB-2-positive patients had a trend for an adverse prognosis of DFS and OS. It was speculated that c-erbB-2 protein rather than c-erbB-2 gene might act as an effector on the true biological basis. Disadvantages of FISH method include high reagent cost, and longer procedure and interpretation time[43] unlikely IHC method. Considering possibility of c-erbB-2 as a prognostic factor, FISH-positivity in all tumors expressing 3+ by IHC, and relatively easy accessability of IHC method, 3+ intensity of c-erbB-2 by IHC might be taken into account as a selection criteria of patients for adjuvant iFAC chemotherapay.

V. CONCLUSION

In conclusion, neoadjuvant chemotherapy with iFAC was found to have a comparable response rate with that of bolus FAC and acceptable toxicity in LABC. Moreover, an early response to neoadjuvant iFAC chemotherapy was a favorable prognostic factor, and initial tumor size was the only significant predictor of the early response.

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Abstract (in Korean)

국소 진행성 유방암에서

연속정주 5-fluorouracil, adriamycin, cyclophosphamide

병합약제를 이용한 술전 항암화학요법

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문용화

목적: 국소 진행성 유방암에서 술전 항암화학요법 (neoadjuvant chemotherapy)의 목적은 병기감소이다. 저자는 국소 진행성 유방암 환자 82례에서 연속 정주 5-fluorouracil, adriamycin, cyclophosphamide 병합약제 (iFAC)를 이용한 술전 항암화학요법의 효능과 안전성를 평가하고자 하였다.

방법: 국소 진행성 유방암 환자 82명에서 iFAC으로 술전 항암화학요법을 시행하였다. iFAC regimen 은 5-fluorouracil 은 체표면적당 1000mg을 제 1일부터 제 3일까지 연속 정주, adriamycin은 체표면적당 40mg을 제 1일에 정주, clophosphamide는 체표면적당 600mg을 제 1일에 정주하는 방법이며 3주마다 반복하였다. 종양 반응이 최대에 이르렀을 때 수술을 시행하였고 수술 후 iFAC로 보조 항암요법 (adjuvant chemotherapy)과 방사선 치료를 시행하였다. 호르몬 수용체가 양성이거나 폐경 상태인 환자들은 호르몬 치료도 시행하였다.

29명의 수술 조직에서 c-erbB-2 상태를 immunohistochemistry (IHC)와 fluorescence *in situ* hybridization (FISH) 방법으로 조사하였다.

결과: 82명중 병기감소는 71명 (86.6%)에서 있었으나 그 중 4명은 액와 림프절 크기 증가, 유방에 새로운 병변 발생, 액와 림프절 고정의 불변 등으로 인해 절제불가능하였다. 반면에 병기감소가 없는 11명중에서 5명은 유방 종양의 크기 감소로 인해 절제가능하여, 결과적으로 총 72명이 절제가능한 상태였다 (절제가능율, 87.8%). 임상적 반응율은 84.2% (완전반응, 17.1%; 부분반응, 67.1%), 그리고 병리학적 완전 반응율은 7.8%였다. 임상적 반응을 보인 69 명중 조기 반응율 (iFAC 3주기내에 최대반응을 나타낸 경우)은 73.9%였고 지연 반응율 (iFAC 3주기 이후에 최대 반응을 나타낸 경우)은 26.1%였다. 총 891 주기의 iFAC 항암치료 동안 WHO 등급 3/4의 혈액학적 독성은 백혈구 감소증 36.0%, 빈혈 36.0%, 혈소판 감소증 0.5%였다. 1명은 폐렴으로 인한 패혈성 쇼크가 발생하였으며 3명은 울혈성 심부전증을 나타내었으나, 항암치료와 연관되어 사망한 경우는 없었다. 중앙 추적관찰 기간 51개월 동안, 82명의 중앙 총 생존기간은 66개월, 수술을 시행한 64명의 중앙 무병 생존기간은 45개월, 중앙 총 생존기간은 89개월이었다. IHC와 FISH에 의한 c-erbB-2의 양성률은 각각 31.0%, 37.9% 였고, 두 방법의 일치도는 93.1% (27/29)였으며, IHC 음성인 경우에 무병 생존기간 및 총 생존기간이 연장되는 경향을 나타내었다. iFAC 항암치료에 대한 조기 반응이 국소 재발 억제, 원격 재발 억제, 무병 생존 및 총 생존률에 대해서 공통적으로 좋은 예후 인자였으며, 유방 종괴의

크기가 10cm 이하인 경우가 조기 반응에 대한 좋은 예측 인자였다 (hazard ratio=0.1, p=0.003).

결론: 국소 진행성 유방암에서 술전 iFAC 항암화학요법은 FAC 정주법과 비슷한 반응율을 보이며 독성도 수용가능하였다. iFAC 술전 항암화학요법에 대한 조기 반응은 좋은 예후 인자였으며, 치료 초기 종양의 크기가 조기 반응 여부에 대해 유일한 예측 인자였다.