The degree of P-gp expression also strongly correlated with the degree of drug resistance observed.[8]

Chemotherapy remains the treatment of choice for women with hormone receptor–negative and hormone-refractory breast cancer disease.[9-11] However, many tumors that are initially responsive to chemotherapy frequently relapse and develop resistance to the broad spectrum of cytotoxic drugs currently employed.[8,12,13] Consequently, MDR remains a major reason for treatment failure in patients with metastatic breast cancer and highlights the urgent need for MDR modifiers in breast cancer chemotherapy.

Since the discovery of verapamil as an MDR-reversing agent,[14] many compounds have been investigated as MDR inhibitors.[14-16] Dofequidar fumarate (Fig 1), is a novel, orally active, quinoline-derived inhibitor of MDR.[17] In preclinical studies, dofequidar reversed MDR in P-gp– and MRP-1–expressing cancer cells in vitro (1 to 3 μmol/L), as well as enhancing the antitumor effects of doxorubicin in MDR tumor–bearing mice.[17-19] A phase I trial in healthy volunteers showed dofequidar to be well tolerated (10 to 1,200 mg) with no dose-limiting toxicities and an effective plasma concentration was maintained for 8 hours at 900 mg (data on file, Schering AG, Berlin, Germany). In a phase II combination trial in patients with recurrent breast cancer, dofequidar potentiated the antitumor effects of CAF (cyclophosphamide, doxorubicin, and fluorouracil) therapy; patients who had not responded to treatment with three cycles of CAF responded to subsequent treatment with dofequidar plus CAF. The numbers of patients with an objective response were two of seven at 600 mg and two of six at 900 mg dofequidar, though dose escalation was stopped at 1,200 mg due to increased hematologic toxicity (data on file, Schering AG). On the basis of this result, this phase III study was conducted to compare the efficacy and safety of dofequidar plus CAF with placebo plus CAF in patients with advanced or recurrent breast cancer.

Study Design

This was a randomized, multicenter, double-blind, placebo-controlled trial conducted at 46 centers across Japan, comparing the efficacy and safety of dofequidar plus CAF with placebo plus CAF. Female patients (age 20 to 70 years) with advanced (stage IV at diagnosis with an intact primary tumor) or recurrent breast cancer were enrolled onto the study. Other inclusion criteria included a histologically defined, measurable or assessable primary lesion; two or fewer regimens of prior chemotherapy in both neo/adjuvant and metastatic settings, (excluding prior endocrine or single-agent fluorouracil therapy); 180 mg/m^2 anthracyclines (doxorubicin equivalent) or less previously; a performance status of 0 to 2; and adequate bone marrow, renal, hepatic and cardiac functions. Patients who progressed or had a recurrence in less than 6 months with anthracycline-containing chemotherapy, and those who had a history of major cardiac disease, uncontrolled hypertension, symptomatic brain metastasis, or simultaneous malignancy were excluded. The trial was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki (1996). All patients provided written informed consent before study entry.

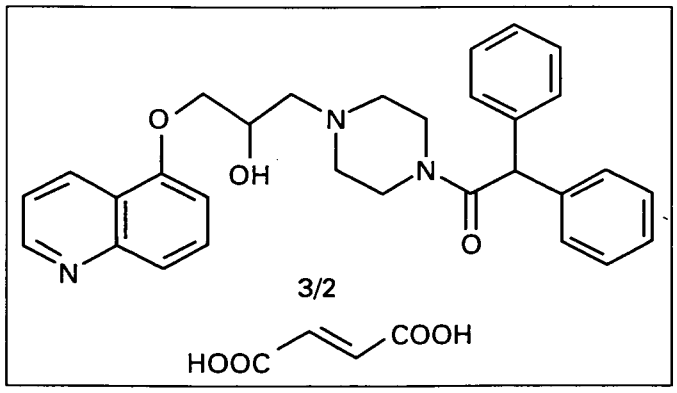

Fig 1. Structure of dofequidar (MS-209).

Dosing and Dose Modification for Toxicity

Patients were treated with six cycles of CAF therapy with dofequidar or placebo, and each treatment cycle lasted for 28 days; drugs were administered as follows: days 1 and 8, doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2), each infused over 15 minutes; days 1 through 14, cyclophosphamide (100 mg orally [PO]); dofequidar (900 mg/d; 3 × 300 mg tablets) or placebo administered 30 minutes before each doxorubicin dose to ensure adequate blood concentration of dofequidar. The doses of doxorubicin and fluorouracil were reduced to 20 mg/m^2 and 400 mg/m^2, respectively, if any of the following criteria were met: grade 3 nonhematologic toxicity (except nausea and vomiting); grade 3 or worse neutropenia (< 1,000/mm^3) maintained for at least 5 days with an episode of fever of 38.5°C or higher; grade 3 or worse thrombocytopenia (< 50,000/mm^3); and grade 4 neutropenia (< 500/mm^3). The next cycle was postponed for 3 weeks unless the patient had a WBC count of at least 4,000/mm^3, or a neutrophil count of at least 2,000/mm^3 and a platelet count of at least 100,000/mm^3. Patients were followed up for 3 months after completion or discontinuation of treatment.

Treatment Assignment

Patients were randomly assigned to their treatment by the Trial Register Center. Treatment assignment was securely stored and coded until completion of the study. Investigators were also blinded to the assigned treatment. Patients were stratified by the number of prior chemotherapy regimens, including adjuvant chemotherapy, by a history of prior use of anthracyclines, and by the presence of liver metastases.

Efficacy

The primary study end point was the overall response rate (ORR) in the full analysis set (FAS; all patients who received treatment at least once and met all inclusion/exclusion criteria). Efficacy assessment by lesion and ORR assessment were made at each treatment cycle (every 4 weeks) and at treatment completion. Objective responses were assessed through blinded reading of radiographs by an independent expert panel. The secondary study end points included complete response rate (CR), time to treatment failure (TTF), time to progression (TTP), and progression-free survival (PFS).

Subgroup analyses were conducted to assess PFS within specific patient subpopulations, including premenopausal women, patients who had no prior therapy, and patients who had advanced primary breast cancer.

Safety and Tolerability

Adverse events (AEs) were recorded at the end of each treatment cycle and at the end of the study period using data from the safety population (all patients who received treatment at least once in the study). AEs were categorized according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2. The incidence of significant decreases in left ventricular ejection fraction (LVEF) and serious AEs were recorded. The CBC was evaluated weekly. Serum chemistries and urinalysis were evaluated every 2 weeks. The minimum hematology values and LVEF in each treatment cycle were also recorded and analyzed in the per-protocol set (PPS; all patients who received treatment at least once and had no protocol deviations).

Pharmacokinetics

To assess the effect of concomitant dofequidar use on the pharmacokinetics of doxorubicin, the plasma doxorubicin concentration on day 1 of cycle 1 was compared between treatment groups. Blood samples were taken at baseline and at 15 minutes, 30 minutes, and 1, 2, 4, and 6 hours after the start of doxorubicin administration. Plasma doxorubicin concentrations were determined by reversed-phase high-performance liquid chromatography. Area

under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule.

Statistical Analyses

The primary end point was analyzed using the Fisher's exact test at a significance level of 2.5% in a one-sided test. A difference in response rates of 20% between the two treatment groups was used as the basis for a statistically significant difference. CR, TTF, TTP and PFS were analyzed by the log-rank test at a significance level of 5% in a two-sided test. The CR, TTF, TTP and PFS were analyzed in the FAS, and the pharmacokinetic data analyzed in the PPS.

RESULTS

Patient Characteristics

A total of 227 patients were recruited onto the study (Fig A1, online only), of which 225 patients were included in the safety analysis (n = 113 for the dofequidar group; n = 112 for the placebo group); two patients did not receive the study treatment and were thus excluded. Four patients did not meet the inclusion/exclusion criteria; therefore, the FAS consisted of 221 patients (n = 113 for the dofequidar group; n = 108 for the placebo group). The PPS consisted of 199 patients (n = 100 for the dofequidar group; n = 99 for the placebo group). There were 22 patients excluded from the PPS analysis due to protocol deviations. Baseline patient characteristics were well balanced between the two treatment arms (Table 1). Most patients had predominantly recurrent disease and had received prior chemotherapy plus endocrine therapy. Also, many patients who had advanced primary breast cancer had received no prior therapy.

Table 1. Patient Demographics (full analysis set)

Characteristic	Dofequidar + CAF (n = 113) No.	%	Placebo + CAF (n = 108) No.	%
Age, years				
Mean	54.4		52.4	
SD	7.69		8.97	
Medical history known	65	57.5	60	55.6
Weight, kg				
Mean	56.2		54.1	
SD	7.52		7.73	
Height, cm				
Mean	154.7		154.7	
SD	5.71		5.61	
Body surface area, m^2				
Mean	1.5		1.5	
SD	0.11		0.11	
Disease state				
Recurrent	81	71.7	80	74.1
Advanced	32	28.3	28	25.9
Prior therapy				
Radiotherapy + chemotherapy + endocrine therapy	32	22.1	32	29.6
Chemotherapy + endocrine therapy	55	48.7	54	50.0
Radiotherapy	1	0.9	1	0.9
No prior therapy	25	22.1	21	19.4
Menopausal status				
Premenopausal	24	21.2	26	24.1
Postmenopausal	88	77.9	79	73.1

Abbreviations: CAF, cyclophosphamide, doxorubicin, and fluorouracil; SD, standard deviation.

Efficacy

The ORR, rated as CR or partial response rate, was 42.6% for CAF plus placebo versus 53.1% for dofequidar plus CAF (Table 2). Although this represents a 24.6% relative improvement and a 10.5% absolute increase in response rate for patients receiving dofequidar plus CAF compared with those receiving CAF plus placebo, this response was not statistically significant ($P = .077$). A higher value was observed in the dofequidar treatment group for all secondary end points compared with placebo, though these results were not statistically significant. Among them, Figure 2 shows a trend for prolonged PFS (median, 241 days for CAF plus placebo *v* 366 days for dofequidar plus CAF; $P = .145$).

Dofequidar plus CAF significantly improved PFS in several patient subgroups, including patients who were premenopausal ($P = .046$; Fig 3A), patients who had not received prior therapy ($P = .0007$; Fig 3B), and patients who had advanced primary breast cancer ($P = .017$; Fig 3C). An extended follow-up showed that dofequidar plus CAF also significantly improved overall survival ($P = .0034$; Fig 3D) in patients who had no prior therapy.

Safety and Tolerability

A similar number of patients completed six treatment cycles in both groups (n = 53 for the dofequidar group; n = 51 for the placebo group). The mean number of treatment cycles was 4.5 in the dofequidar group and 4.3 in the placebo group. More than half of patients in both groups included in each cycle from cycle 2 onward had a delay in treatment, mostly due to prolonged hematologic toxicities.

Dofequidar plus CAF was well tolerated throughout the study. No statistically significant excess of grade 3/4 AEs, except for neutropenia ($P = .006$) and leukopenia ($P = .005$), was found in the dofequidar group compared with placebo (Table A1, online only). Importantly, there was no marked difference in the incidence of neutropenia-related morbidity, such as febrile neutropenia or infection, between the two treatment groups. No significant differences in the incidence of cardiac AEs were found between the two treatment groups. In addition, dose intensities of chemotherapeutic agents were similar in both treatment arms. No significant difference in the incidence of serious AEs (SAEs) was observed between either group. However, there was a trend for a higher incidence of SAEs from leukopenia in the dofequidar group than in the placebo group ($P = .060$; Fisher's exact test); five leukopenia cases were reported for dofequidar, whereas no such case was reported for placebo.

A total of 124 patients discontinued the study (n = 61 for the dofequidar group; n = 63 for the placebo group). The major reasons for discontinuation were progressive disease (n = 23 for the dofequidar group; n = 28 for the placebo group), grade 4 hematologic toxicity (n = 20 for the dofequidar group; n = 6 for the placebo group), failure to meet treatment continuation criteria (n = 6 for the dofequidar group; n = 8 for the placebo group), and consent withdrawal (n = 6 for the dofequidar group; n = 12 for the placebo group). Of the 225 patients who received treatment in the study, 14 patients died during the treatment period (n = 3), the follow-up period (n = 2), or the follow-up period after study termination (n = 9). There were 49 other serious AEs in 32 patients during the study and follow-up period.

Pharmacokinetics

The mean plasma concentrations of doxorubicin in the dofequidar- and placebo-treatment groups at 15 minutes postadministration reached 0.997 μg/mL and 1.259 μg/mL, respectively, followed by biphasic elimination in both treatment groups. Mean plasma concentrations in

Table 2. Response Rates for Patients Treated With Dofequidar Plus CAF (n = 113) or Placebo Plus CAF (n = 108)

Treatment Group	Parameter (No. of patients)					Overall Response Rate (%)	95% CI
	Complete Response	Partial Response	No Change (stable disease)	Progressive Disease	Not Assessable		
Dofequidar	5	55	40	10	3	53.1	43.5 to 62.5
Placebo	4	42	41	14	7	42.6	33.1 to 52.5

NOTE. Odds ratio = 1.53 (range, 0.87-2.69); $P = .077$ for dofequidar *v* placebo.
Abbreviation: CAF, cyclophosphamide, doxorubicin, and fluorouracil.

the dofequidar and placebo groups remained similar at 1, 2, 4, and 6 hours after the start of doxorubicin administration. Thus the elimination pattern for the first 6 hours after the start of administration was similar in both groups. The plasma concentrations of doxorubicin in the terminal phase (4 and 6 hours postadministration) were slightly higher in the dofequidar group compared with placebo (1.2- to 1.3-fold). However, AUC (0 to 6 hours) values showed no statistically significant difference between the dofequidar and placebo groups (mean, 0.480 $\mu g \cdot h/mL$; standard deviation [SD], 0.324; range, 0.237-1.692; and mean, 0.407 $\mu g \cdot h/mL$; SD, 0.062; and range, 0.289-0.500, respectively). Therefore, treatment with dofequidar did not affect the plasma concentrations of doxorubicin in patients (Fig 4).

DISCUSSION

Chemotherapy remains the preferred adjuvant treatment for patients with hormone receptor–negative disease and for patients with more aggressive, hormone receptor–positive tumors.[11,20] However, despite the use of conventional adjuvant chemotherapy regimens, a significant proportion of patients with breast cancer still experience disease recurrence because of inherent or acquired drug resistance.[12] In this randomized phase III trial, the efficacy and safety of the multidrug resistance inhibitor dofequidar plus CAF was compared with CAF plus placebo in patients with recurrent or advanced breast cancer. Although, there was an observed relative improvement and absolute increase in response rate for patients who received dofequidar plus CAF, these results did not reach statistical significance. This improvement in response rate may have been reflected in the observation that there was a trend for prolonged PFS, which favored patients in the dofequidar plus CAF group.

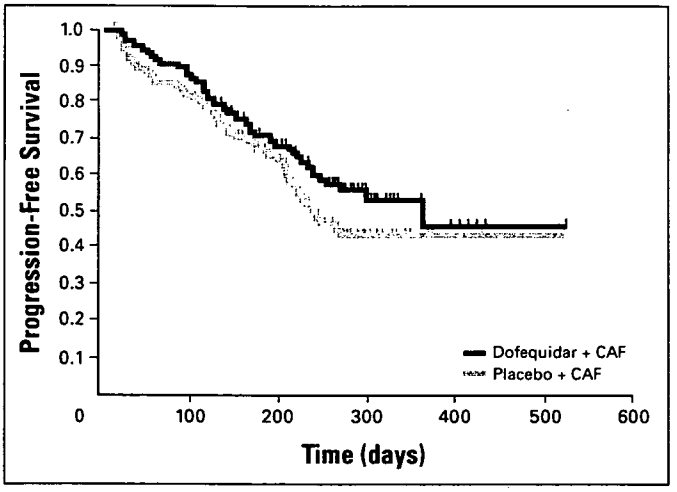

Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF ($P = .145$).

To date, only two randomized trials have examined the efficacy of a P-gp inhibitor in combination with chemotherapy in breast cancer patients. Wishart et al[21] examined quinidine combined with epirubicin in patients with advanced breast cancer, but failed to show any significant difference in overall survival or PFS compared with placebo. In a more recent prospective study of patients with anthracycline-resistant metastatic breast cancer (n = 99), verapamil combined with vindesine and fluorouracil resulted in a significantly longer overall survival and a higher response rate compared with patients who did not receive the P-gp inhibitor (median survival, 323 *v* 209 days; $P = .036$, respectively; ORR, 27% *v* 11%; $P = .04$, respectively).[22]

In the subgroup analyses, dofequidar in combination with CAF displayed a significantly increased PFS in patients who had not received prior therapy, who had advanced primary breast cancer or who were premenopausal. In addition, dofequidar also significantly improved overall survival in the patient group who had no prior therapy. Although the patient numbers in these analyses were small, the results remain important within these clinically significant patient populations. Both preclinical and clinical data have indicated that newer-generation MDR modulators can prevent the development of resistance.[23,24] A phase I/II trial in patients with acute myeloid leukemia showed that dosing with cyclosporine before and in combination with daunorubicin prevented chemotherapy resistance, while also resulting in a decrease in MDR-1 RNA expression.[24] Our results may highlight one potential treatment approach to MDR tumors that has not yet been fully exploited in the clinical environment, specifically the prevention of the emergence of resistance through the early use of P-gp inhibitors.[1-3] It seems reasonable that agents such as dofequidar may be useful in the adjuvant or even neoadjuvant setting with the goal of preventing or delaying the induction of MDR associated with chemotherapy.

The potential clinical significance of P-gp and MRP expression in breast cancer is supported by the results from a number of studies. For example in a study of primary breast cancer patients (n = 259), MRP expression was associated with an increased risk of treatment failure in patients with small tumors (T1) and node-positive patients who received adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy but not in node-negative patients.[25] Burger et al[12] reported that the expression of MDR1 mRNA in primary breast tumors was inversely correlated with the efficacy of first-line chemotherapy. Additionally, the high level of MDR1 expression was suggested to be a significant predictor of poor prognosis in patients

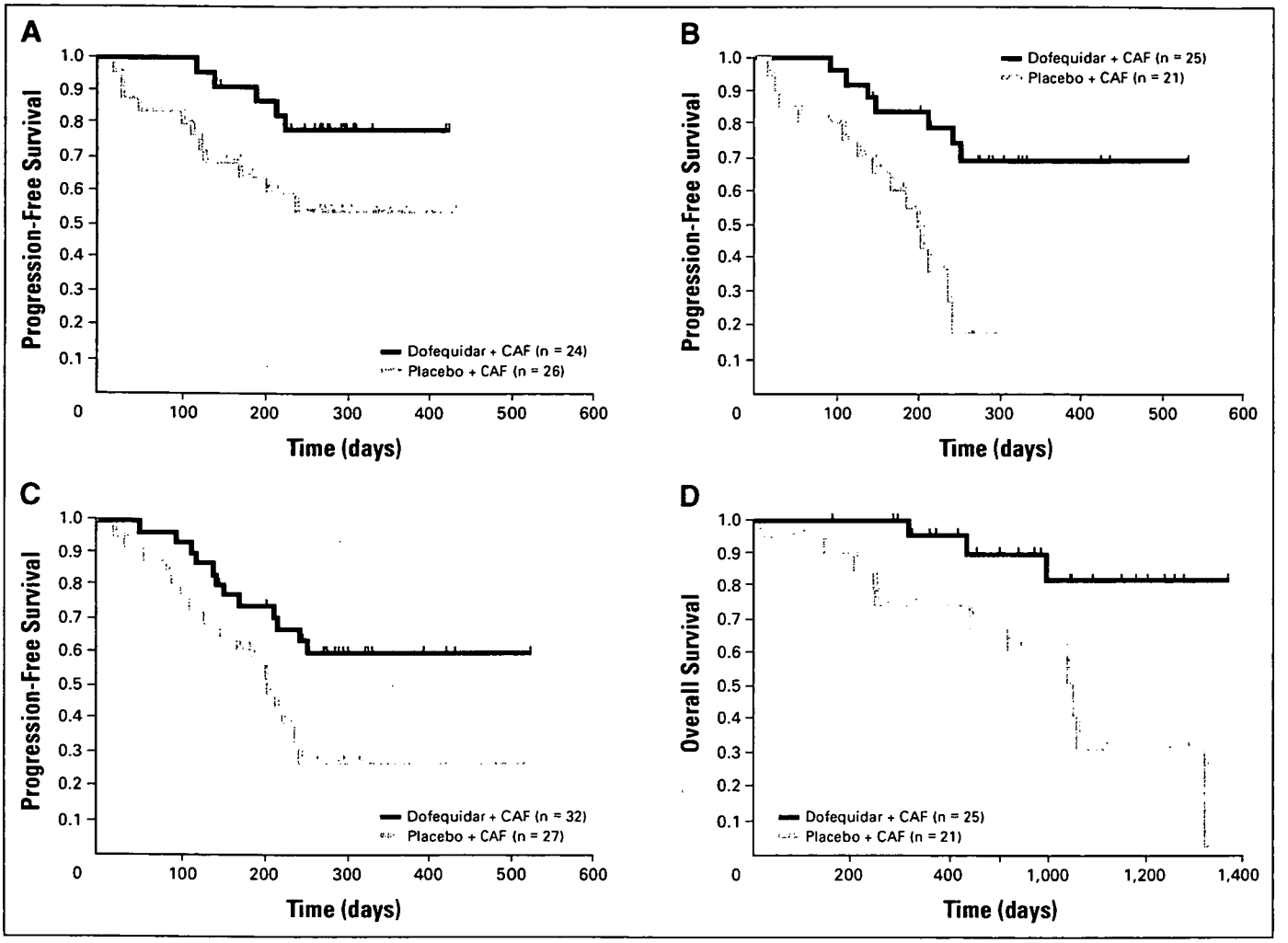

Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall survival in patients who had no prior therapy (P = .0034).

with advanced disease.[12] Significantly increased expression of P-gp and MRP-1 has also been reported in an immunohistochemical study of patients treated with preoperative chemotherapy, whereas pretreatment expression of MRP-1 was associated with significantly shorter PFS in patients.[26] In a more recent study, MRP-1 expression was shown to be an independent predictor for shorter relapse-free survival and overall survival, after adjuvant CMF treatment, in premenopausal, hormone receptor–positive patients.[27] However, MRP-1 expression did not affect patients' response to adjuvant tamoxifen plus goserelin treatment.[27]

These findings and our results support the view of Leonard et al,[3] who indicate that future patients will need to be carefully selected for the identification and development of effective drug-resistance modulators. Patient populations who may derive maximal benefit from MDR inhibition, for example, the no-prior-therapy, advanced-disease, or premenopausal patient group in the present study, could quite easily be overlooked or lost within a large, heterogeneous trial population.[3] Furthermore, by refining future clinical trials to incorporate specific disease and patient characteristics, a clearer picture of drug resistance in cancer will be obtained and the most effective MDR inhibitor/chemotherapeutic agent(s) selected.

Many MDR inhibitors have required high serum concentrations for MDR reversal, which resulted in unacceptable toxicity, thereby limiting their clinical impact.[7,28-32] Although more recent agents have shown improved tolerability profiles, this has been countered by unpredictable pharmacokinetic interactions with other transporter molecules (eg, cytochrome P450–mediated drug metabolism and excretion, necessitating dose reductions in chemotherapy agents and leading to inconsistent chemotherapy dosing among patients).[1,5] Similarly, the addition of the MDR-modulating agent valspodar (PSC 833) to chemotherapy agents did not improve treatment outcome.[33,34] Toxicity was increased in the valspodar-treated group compared with chemotherapy agents alone, despite the reduction of chemotherapy doses in the valspodar-containing regimen. In our study, dofequidar was well tolerated, with no indication of the unacceptable toxicity associated with early MDR inhibitors. Importantly, dofequidar did not affect the plasma concentrations of doxorubicin in patients during the study and displayed an acceptable pharmacokinetic profile.

In conclusion, this study suggests that treatment with dofequidar resulted in possible clinical benefit for patients who had not received prior therapy, who were premenopausal, or who were stage IV at diagnosis with an intact primary tumor. Dofequidar was also well

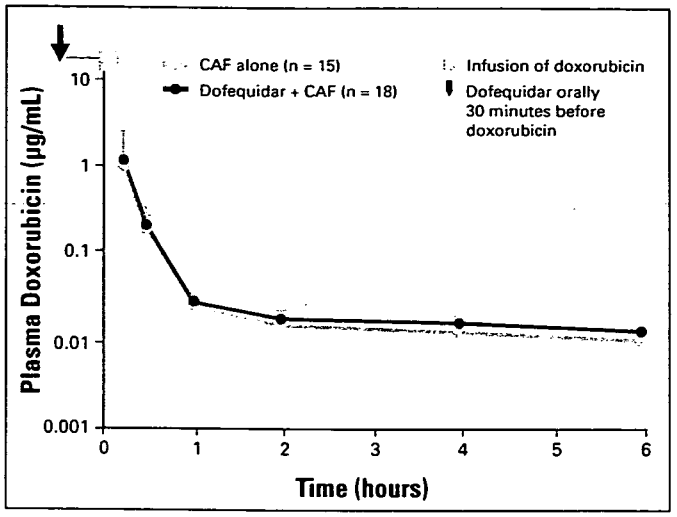

Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

tolerated in the clinical setting and had no impact on doxorubicin pharmacokinetics. Further studies are merited to assess the effect of dofequidar in specific patient populations with breast cancer.

Although all authors completed the disclosure declaration, the following author or immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment: N/A **Leadership:** N/A **Consultant:** Toshiaki Saeki, Nihon Schering; Takashi Tsuruo, Nihon Schering **Stock:** N/A **Honoraria:** N/A **Research Funds:** N/A **Testimony:** N/A **Other:** N/A

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Financial support: Wakao Sato

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Acknowledgment

We thank the investigators (physicians and staff) at the participating institutions; Shunzo Kobayashi, Tomoo Tajima, and Chikuma Hamada (Independent Monitoring Committee); Shigeto Miura, Morihiko Kimura, Hideo Inaji, Izo Kimijima, and Hirokazu Watanabe (Efficacy Evaluation Committeee); and Nihon Schering K.K. for their help.

Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

各論　化学療法

術前化学療法の適応と限界

Indications and limitations of primary systemic therapy for operable breast cancer

山本尚人　　鈴木正人　　田辺直人

Key words：術前化学療法，手術可能原発性乳癌，primary systemic therapy，operable breast cancer

はじめに

手術可能な原発性乳癌に対する治療戦略は，21世紀に入り大きな転換期を迎えた．早期原発性乳癌に対しては，従来から根治手術後に術後補助化学療法が行われてきたが，化学療法を術前に施行しても，術後に施行しても，無病生存率および全生存率に有意差は認めないという結果が，National Surgical Adjuvant Breast and Bowel Project(NSABP) B-18トライアル[1]およびEuropean Organization for Research and Treatment of Cancer(EORTC) 10902トライアル[2]から2001年に報告された．また，術前化学療法を行い病理学的完全奏効(pathological complete response: pCR)が得られた症例は，それ以外の症例と比較して有意に無再発生存期間の延長が認められ，pCRは生存に代わるsurrogate endpointとして用いられるようになり，術前化学療法の臨床的有用性が明らかとなった[3]．このような背景から近年，術前化学療法の重要性を加味してその名称を従来のneoadjuvant/preoperative therapyからprimary systemic therapy(PST)とするように提唱されている．

本稿では，手術可能な原発性乳癌に対するPSTをこれまでのエビデンスから考察し，その適応と限界について言及する．

1. PSTの目的

a. 腫瘍縮小による乳房温存療法の適応拡大

PSTによって原発腫瘍を縮小させ，乳房温存することを目的とする．NSABP B-18トライアル[3]では，PST施行群では乳房温存率が67.8%であったのに対し，手術先行群では59.8%で有意に前者の方の温存率が高かった．ただし，乳房内再発率は前者が14.5%，後者が6.9%で，PST後の乳房内再発は約2倍であると報告された[1]．しかし，最近ではPST後でも乳房内再発率は高くならないという報告もみられる．いずれにしても，PST後の乳房温存療法の適応はMDCTやMRIなどの画像診断を駆使して慎重に決定し，乳房内再発を防止するためには病理組織学的断端陰性を確保することが重要である．

b. 長期無再発生存可能な症例の選別

NASBP B-18[1]およびB-27[5]トライアルにおけるpCR症例は，それ以外の症例と比較して有意に無再発生存期間の延長が認められた．すなわち，原発巣がpCRであれば全身への微小転移も同時に根絶されたと考えられ良好な長期生存が期待できるため，pCRは生存に代わるsurrogate endpointとして用いられるようになった．

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0047-1852/07/¥40/頁/JCLS

表1 術前化学療法における代表的無作為化臨床試験

トライアル/著者, 年	n	対 象	レジメン	ORR (%)	pCR率 (%)	生存率 (%)
NSABP B-18/ Fisher ら[3], 1998 Wolmark ら[1], 2001	1,523	T1-3N0-1M0	AC×4→S vs S→AC×4	79	13.0	69 70 (9.5年)#
EORTC 10902/ von der Hage ら[2], 2001	698	T1c-4bN0-1M0	FEC×4→S vs S→FEC×4	NA	4.0	82 84 (4.7年)#
NSABP B-27/ Bear ら, 2003[4], 2006[5]	2,411	T1c-3N0-1M0	AC×4→S vs AC×4→TXT×4→S vs AC×4→S→TXT×4	85.5 91.1 85.5	13.7 26.1 13.7	81 82 (6.5年)#
Aberdeen/ Smith ら[6], 2002 Hutcheon ら[7], 2003	162	T2-4N0-2M0 (T>3cm)	CVAP×4→NR→TXT×4 vs CVAP×4→R→TXT×4 vs CVAP×4→R→CVAP×4	47.0 85.0 64.0	1.8 30.8 15.4	NA 97 (5.4年)# 78
MD Anderson/ Green ら[8], 2005	258	T1-3N0-1M0	TXLqw×12→FAC×4 vs TXLq3w×4→FAC×4	NA	28.8* 13.6*	NA NA
GEPARDUO/ von Minckwitz ら[9], 2005 Raab ら[10], 2004	913	T2-3N0-2M0 ≧2cm	AC×4→TXT×4 vs dose dense AT q2w	85 75	14.3* 7.0*	85 81 (5年)#

A: doxorubicin, C: cyclophosphamide, E: epirubicin, S: surgery, TXT: docetaxel, V: vincristine, P: prednisolone, NR: no response, R: response, TXL: paclitaxel, F: fluorouracil, NA: not available, *including nodal status, #(median follow up period)

NSABPトライアルおよびその他の代表的無作為化臨床試験を**表1**に示す.

2. PSTの適応

術後補助化学療法の適応となるすべての症例がPSTの適応になり得る. すなわち, 2005年のザンクトガレンのコンセンサスミーティングにおけるリスク分類でintermediate risk以上に入る症例である. 臨床的には35歳未満, 明らかなリンパ節転移あり, 病理学的には腫瘍径(浸潤径)2cm以上, 組織学的異型度II以上, 高度脈管侵襲およびHER2/neu(HER2)陽性であり, 以上のうち1つでも該当するものは適応になり得る.

しかし, 現状では臨床的な条件でその適応を決定するのが一般的であり, Stage IIAでも腫瘍径3cm以上の浸潤癌およびStage IIB以上は適応になる.

3. 至適レジメンと至適投与期間

大多数のトライアルで確認されたことは, アンスラサイクリン(An)系抗癌剤にタキサン(Tx)系抗癌剤を上乗せした方が, pCRを得る割合が高くなることで, おおよそ20%以上のpCRが得られている. 特にAn系抗癌剤の効果が認められている場合でも, 同じ治療法を継続するよりもTx系抗癌剤に治療法を変更した方がより高い抗腫瘍効果が期待できることがAberdeenトライアル[6]で確認され, 非交差耐性薬剤を早期に導入することが重要であると考えられている.

至適投与期間に関しては, 様々なトライアルで8-36週の間で計画され, トライアルによっては手術前後に化学療法を施行するように計画されているものもあるが, 少なくとも4サイクルは術前に施行すべきである.

4. pCRの定義

a. 原発巣に対する効果判定

欧米では, 癌細胞がすべて消失した場合か乳管内病巣のみが残存した場合, すなわち浸潤巣が消失していればpCRと定義していることが

多い．癌細胞が完全に消失した場合{pCR(all)}と浸潤巣が消失し乳管内病巣のみが残存した場合{pCR(inv)}のpCR率は，同一トライアルの中でもかなりの差がある．NSABP B-27トライアル[4]では，AC(doxorubicin, cyclophosphamide) 4サイクルのレジメンとAC 4サイクルにdocetaxel 4サイクルを加えたレジメンのpCR(all)率はそれぞれ9.6%，18.9%であるのに対し，pCR(inv)率はそれぞれ13.7%，26.1%となり，約1.5倍pCR率が上昇した．トライアル間でのpCR率の比較や，その予後に関する評価も十分注意する必要がある．

b. 腋窩リンパ節(Ax LN)に対する効果判定

NSABPのトライアルでは原発巣がpCRであればAx LN転移が残存していてもpCRと定義しているが，MD Anderson[8]やGEPARDUO[9,10]のトライアルでは原発巣とAx LN転移がともに消失した場合をpCRと判定している．NSABP B-18トライアル[3]においてAx LN転移を考慮しない場合のpCR率は13%で，考慮した場合は11%であった．同様にNSABP B-27トライアル[4]ではAC→docetaxel群におけるAx LNを考慮しない場合のpCR率は26%であるが，考慮した場合は22%であった．GEPARDUOトライアル[9]では，dose dense AT(doxorubicin, docetaxel)とAC→docetaxelのpCR率を比較し，それぞれ7%と14%であったが，Ax LNを考慮しない場合それぞれ12%と22%で，Ax LNをpCRの条件に組み入れるか否かでpCR率に大きな差が出ている．

また，今までのトライアルではPST前にAx LN転移の有無を確実に評価できていなかったため，Ax LNをpCRの判定に組み込むことによりPST前からAx LN転移がなかった症例もpCRに判定された可能性がある．Hennessyら[11]は，5つの前向きPST臨床試験において術前穿刺吸引細胞診にてAx LN転移が確認されたStage II/III原発性乳癌403症例についてAx LNに対する効果をみたところ，22%の症例にpCRが得られたと報告している．pCR群とnon-pCR群の5年無再発生存率はそれぞれ87%，60%で，生存率はそれぞれ93%，72%であり，pCR群で有意に予後良好であった．また，Ax LNのpCR症例の予後は，原発巣のpCR達成の有無に影響されなかった．すなわち，PST後にAx LNのpCRが達成できれば残存原発巣が認められても予後は良好であることから，原発巣と転移巣には生物学的な違いがあることが示唆された．

5. 腫瘍の生物学的特性による治療効果予測

a. ホルモンレセプター(HR)の有無

大部分のトライアルにおけるHR陰性乳癌に対するPSTによるpCR率は，それぞれ陽性乳癌の約2-4倍と高く，16-42%であった．また，Nakamuraら[12]は，202例の手術可能原発性乳癌に対してFEC100(fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2) 4サイクルとdocetaxel 75 mg/m^2 4サイクルの順次投与を行い，全体のpCR率は23%であったが，HR陽性かつHER2陰性乳癌のpCR率は13%と低率であったのに対し，HR陰性かつHER2陽性乳癌のpCR率は65%と高率であったと報告している．

b. HER2, topoisomerase IIα および Ki-67の発現

HER2とtopoisomerase IIα (Topo II)遺伝子は17番染色体の長腕(q12-q21)領域に近接して存在し，HER2遺伝子の過剰発現した乳癌の20-30%程度にTopo II遺伝子の過剰発現がある．An系抗癌剤はTopo IIを阻害することで抗腫瘍効果をもたらすので，Topo II遺伝子の過剰発現は，An系抗癌剤を含む化学療法に対する効果予測因子となると考えられている．PSTにおいても同時増幅例に対してはAn系抗癌剤によるpCR率の向上が期待される．

一方Petitら[13]は，免疫染色によるKi-67の高発現(20%以上)は，高い細胞増殖能を反映しAn系抗癌剤を含むPSTの効果予測因子として重要であることを報告している．また，Bozzettiら[14]は，An系抗癌剤の投与量の違い(低用量と高用量)，HER2遺伝子の過剰発現の有無，HRの有無および免疫染色によるKi-67の高発現の有無の因子間でPSTの臨床効果について

表2 HER2過剰発現を呈する原発性乳癌に対するtrastuzumab併用術前化学療法

著者，年	n	対 象	レジメン	cRR(%)	pCR(%)
Burstein ら[18]，2003	40	T1-3N0-1M0	12Hqw+4P(175)q3w	75	18
Coudert ら[19]，2006	33	T1-3N0-1M0	18Hqw+6D(100)q3w	96	41
Hurley ら[20]，2006	48	Stage II，III，炎症性	12Hqw +4{D(70)q3w+Cp(70)}q3w	100	23
佐野ら[21]，2006	21	T>3cm or N+	12Hqw+4D(75)q3w	90	21
Buzdar ら[22]，2005	42*	T1-3N0-1M0	4P(225)q3w→4FEC(75)q3w with or without Hqw	87 vs 47	65 vs 26

P: paclitaxel, D: docetaxel, H: trastuzumab, Cp: cisplatin, FEC: fluorouracil, epirubicin and cyclophosphamide, *randomized, (dose) mg/m^2

多変量解析した結果，免疫染色によるKi-67の高発現の有無が独立した臨床効果予測因子であったと報告している．

c. Triple negative(TN)腫瘍

エストロゲンおよびプロゲステロンレセプター(ERおよびPgR)陰性，かつHER2過剰発現のない乳癌をTN乳癌と呼称している．ER/PgRとHER2により定義された腫瘍タイプ間での遺伝子発現プロファイルが異なることは幾つかの報告で明らかとなった．Sorlie ら[15]は，乳癌のサブタイプを確認するように設計された固有の遺伝子リストを確認し，luminal(管腔)，basal-like(基底膜様)，HER2サブタイプなど幾つかの確認可能なクラスターに分類した．更に，固有の遺伝子リストによって確認された乳癌サブタイプは臨床上の特徴，転帰および治療に対する反応が異なることが示された．なかでもTN乳癌のおおよそ80%はbasal-like腫瘍であり，予後不良である．これらは内分泌療法やtrastuzumab療法などの乳癌標的治療の対象とならずに化学療法のみが治療手段として残る．PSTにおいて，MD Andersonの試験[16]では遺伝子プロファイリングが行われた原発性乳癌83例のpCR率は，luminal腫瘍よりbasal-like腫瘍が有意に高かった．また，UNC試験[17]では105例に対して免疫組織化学的にサブタイプ分類が行われ，luminal，basal-like，HER2タイプはそれぞれ52%，27%，21%であった．術前AC療法を行った結果，pCR率はそれぞれ13%，30%，27%であり，basal-like腫瘍で一番高かったと報告している．化学療法に対する感受性を考えるとbasal-like腫瘍の予後が不良なのは逆説的にみえるが，UNC試験[17]における観察期間2.5年においてbasal-like腫瘍はluminal腫瘍と比較して無遠隔転移生存率が低く，全生存率で有意に悪かった．これは，PSTに奏効しなかったbasal-like腫瘍は他の化学療法にも反応を示さずに不良な予後をたどることを示唆している．

basal-like腫瘍に代表されるTN腫瘍に対するPSTは，現在の標準的レジメンであるAn系抗癌剤とTx系抗癌剤を用いることを基本として今後更に有効なレジメンの開発が必要である．

6. HER2過剰発現を呈する原発性乳癌に対するtrastuzumab併用PST

Tx系抗癌剤とtrastuzumabの併用療法でpCR率が報告されている主なphase IIトライアルを**表2**に示す．対象症例はStage II以上で，なかにはStage IIIbや炎症性乳癌を対象とした試験もあるためそれぞれの効果の比較は困難であるが，pCR率は18-41%[18-21]と比較的高かった．Tx系抗癌剤は，4サイクル[18,20,21]または6サイクル[19]投与され，6サイクル投与でより高いpCR

率が得られる傾向があった．本レジメンは，期待される治療法であるが症例数も20-50例程度と少数であり，今後予後を含めた多数例での検討が必要である．

Buzdarら[22]は，術前にpaclitaxel 225 mg/m²を3週ごと4サイクルのあとFEC75(fluorouracil 500 mg/m², epirubicin 75 mg/m², cyclophosphamide 500 mg/m²) 3週ごと4サイクル投与するレジメンと，そのレジメンにtrastuzumabを毎週24回併用したレジメンの2群に無作為に分け，化学療法に対するtrastuzumabの併用効果を検討した．最終登録数は42例で化学療法単独群のpCR率は26.3%であるのに対し，trastuzumab併用群は65.2%と有意に高率であった．安全性の面で心機能に関しては両群間で差はなかったが，血液学的毒性に関してはGrade 4の好中球減少が有意にtrastuzumab併用群で多かった．An系抗癌剤とtrastuzumabの併用レジメンの効果は期待されるが，安全性に関してはいまだ確立されたとはいえない．

現在，paclitaxel毎週投与12回に続くFEC 4サイクル化学療法に最初からtrastuzumabを24回併用する群と，FEC 4サイクル後にpaclitaxelとtrastuzumabを毎週12回併用する群で，大規模なランダム化トライアル(NSABP B-41)が行われており，その結果が待たれる．

また，Nakamuraら[12]が報告したようなHR陰性かつHER2陽性乳癌である化学療法に感受性の高い症例に対しては，まずAn系抗癌剤を4サイクル行い，その治療効果によってTx系抗癌剤にtrastuzumabを併用するかどうかを考慮するという治療戦略も考えられる．

7. pCR例に対する予後予測

NSABP B-27トライアルにおけるBearら[5]の報告では，pCR症例についてそれぞれAx LN転移個数別(0個，1-3個，4-9個，10個以上)に予後を検討した結果，原発巣がpCRでもAx LN転移が多いほど予後不良であった．また，非pCR症例においても同様の結果であった．すなわち，Ax LN転移個数は原発巣のpCRとは独立した強力な予後因子であったと述べている．

また，MD Anderson Cancer CenterにおけるPST後にAx LNも含むpCRを得た原発性乳癌226例のレトロスペクティブな多変量解析の検討では，遠隔転移再発に影響を及ぼす独立した因子はStage IIIB，IIICおよび炎症性乳癌，閉経前，Ax LN郭清個数10個以下の3つであった[23]．3つの独立した予測因子に1つも当てはまらない群，1因子の群，2因子の群および3因子の群に分類すると，10年無遠隔再発率はそれぞれ97%，88%，77%，31%で，各群間で有意差を認めた．原発巣およびAx LNでpCRを得た比較的予後良好な症例でも，閉経前の局所進行乳癌症例は遠隔再発に対する注意が必要であろう．

おわりに

手術可能原発性乳癌に対するPSTについて最近のエビデンスを中心に述べ，考察した．今までと同様に高いpCR率を目指すレジメンの開発が進められる一方で，腫瘍の生物学的特性に合わせたテーラーメイド医療を実現するための探索が今後更に期待される．また，pCRの有無にかかわらずPST後の予後予測因子を更に検討し，それに基づいた術後補助療法の適応と治療戦略を探求することが今後の重要な課題である．

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特集　術前薬物療法のbreak through

JBCRG03:Docetaxel 75mg/m² followed by FEC100mg/m² による術前化学療法 —JBCRG01, 02からのreviewとbreakthrough—

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JBCRG03 trial：Primary Systemic Chemotherapy Docetaxel 75mg/m² followed by FEC100 mg/m² for Operable Breast Cancer —Review and Breakthrough Resulting from JBCRG01-02-：Ksahiwaba M*1,2, Wakabayashi G*1, Nakamura S*2, Kuroi K*2, Iwata H*2, Ohno S*2, Masuda N*2, Sato N*2, Asaga T*2, Yamamoto N*2, Aogi K*2, Sato Y*2, Kurosumi M*2, Tsuda H*2, Akiyama F*2 and Toi M*2 (*1Iwate Medical University, *2Japan Breast Cancer Research Group -JBCRG-)

Here we report on the concept and design of Japan Breast Cancer Research Group (JBCRG) 03 trail which resulted from the experience of JBCRG01；FEC100 mg/m² followed by docetaxel 75mg/m² and JBCRG02；FEC100 mg/m² followed by docetaxel 100mg/m². Our goal is to find the ultimate primary systemic therapy for operable breast cancer. JBCRG01 trial was started in 2002 to evaluate the efficacy and safety of FEC100 followed by docetaxel 75mg/m² for operable primary breast cancer. The subsequent JBCGR02 trial used increasing docetaxel from 75 mg/m² to 100mg/m² to try and improve results obtained in the interim analysis of JBCRG01. Our current study, JBCRG03 was designed as a reverse regimen to resolve some important issues arising from these previous studies. Here we discuss the issues encountered and the rational for our methodology in this new trial. Further studies will maximize the results obtained in JBCRG01-03.

Key words：Breast cancer, Primary systemic chemotherapy, Clinical trial
Jpn J Breast Cancer **22**(5)：372～375, 2007

はじめに

乳癌治療において，Fisherの報告を例に挙げるまでもなく一定の条件からは全身性疾患の性格を有し，根治的手術後であっても多くの患者では補助療法が有用である[1]．また，メタアナリシスの結果はホルモン療法・化学療法とも再発・死亡のリスクを低下させることを支持している[2]．

歴史的には，locally-advanceの患者にreduction chemotherapyとして実施してきた術前化学療法は，今や1）*in vivo*での化学療法の感受性試験，2）腫瘍縮小によるbreast conserving rate（BCR）の向上，3）病理学的検索による予後のsurrogate markerとして実臨床にも浸透している．われわれはJBCRGで実施したJBCRG01から03の術前化学療法のtrialを通して得られた知見を報告する．

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1. JBCRG01～03の術前化学療法の歴史的背景

1) 世界の術前化学療法の歴史

術前化学療法の歴史においてNational Surgical Adjuvant Breast and Bowel Project (NSABP) の2つのtrialの結果は今日の治療のmilestoneとして世界に大きなインパクトを与えた．1つはNSABP-B18であり，切除可能乳癌(T_{1-3}, N_{0-1}, M_0)1,523例に対し，AC療法(doxorubicin 60mg/m²+cyclophosphamide 600mg/m²) 4サイクルを手術前後に実施した試験である．この試験は1）手術前後での化学療法実施のタイミングの違いは無再発生存，全生存に差を生じない，2）術前AC群でpCR (病理学的寛解＝癌の完全消失＋DCISのみの遺残)が得られた患者では非pCRの患者に比較し無再発生存が有意に良好であるという重要な結果をもたらした[3]．もう1つはNSABP-B27であり，切除可能乳癌 ($T1_{c-3}$, N_{0-1}, M_0) 2,400例に対し，AC療法4サイクル後にdocetaxel 100mg/m² 4サイクルを術前に投与した場合，pCR (no tumor＋DCIS) rateが13.7%から26.1%まで飛躍的に改善することを示し，術前化学療法におけるtaxaneの重要性を位置付けた[4]．

2) 当時のわが国における術前化学療法の現状とJBCRG01の成り立ち

NSABP B-27の結果が発表された2001年当時，多くの乳腺治療医にとって，術前化学療法は手探り的な状態にあったことが同年の癌治療学会のアンケートから伺える．これによるとanthracyclineに関してはAC，EC (epirubicin＋cyclophosphamide)，FEC (fluorouracil＋epirubicin＋cyclophosphamide)と種々のレジメンが用いられていたが，心毒性への懸念から効果よりも安全性が重要視され，ACよりもFECが選択される傾向がみられた．しかしepirubicinの汎用doseは20～90mg/m²と様々でglobal trialの結果へ照合し使用する姿勢には乏しかった様である．Taxaneに関してはsequentialではFEC→Taxane (T)，AC→T，EC→Tが，concurrentではTAC (docetaxel＋doxorubicin＋cyclophosphamide) に関心が向けられていた．sequentialとconcurrentの投与法による毒性の違いやNSABP-B27とTACの結果からsequentialレジメンの浸透がみられようになってきた．これらのtrialの結果や社会事情を受けて，JBCRGではJBCRG01 studyとして切除可能乳癌 (T_{1c-3}, N_{0-1}, M_0) に対してFEC100mg/m²×4 followed by docetaxel 75mg/m²× 4の安全性と有効性を検討する臨床試験をデザインした (**図1**)．

3) JBCRG02への発展

世界的に鑑みてdocetaxelのstandard doseは100mg/m²であり，JBCRG 01での75mg/m²の設定は国外のtrialへの整合性が問われていた．また，JBCRG 01での中間解析の病理学的検討において浸潤癌の瘢痕組織の中に高度の変化を有する癌細胞がごく少量残存した所見，つまり僅かにpCRに至らない症例が確認されたことから1）anthracyclineとdocetaxelによるsequential chemotherapyでのglobal dose100 mg/m²の検証，2）docetaxelの高用量投与によるpCR rateの向上の検討の為にJBCRG02試験としてFEC100mg/m²× 4 followed by docetaxel 100mg/m²× 4が実施された．この試験にはJBCRG01参加施設の内コンセプトに合意した数施設によって実施された (**図1**)．

3) JBCRG03のrationaleとstudy design

さらに，JBCRG01の中間解析による結果は，種々の疑問と考察を生み出した．1)FEC先行の治療ではその毒性により引き続くdocetaxelの減量が認められ，忍容性および効果を低下させている可能性があること，2）FEC responderがdocetaxel変更後に増悪した症例が認められたこと，また3）docetaxel先行regimenが海外の試験で発表されており[5]，わが国でのdocetaxel先行の術前化学療法を検討する余地があること，等からJBCRG01のreverse regimenであるdocetaxel 75mg/m²× 4 followed by FEC 100mg/m²× 4がJBCRG03試験として実施されるに至った (**図1**)．Primary endpointは病理組織学的効果 (pCR rate)，secondary endpointは安全性，臨床効果，乳房温存率，3年無再発生存率であり，予定症例数は130名，予定登録期間は2005年8月から2006年7月までの12カ月であった．

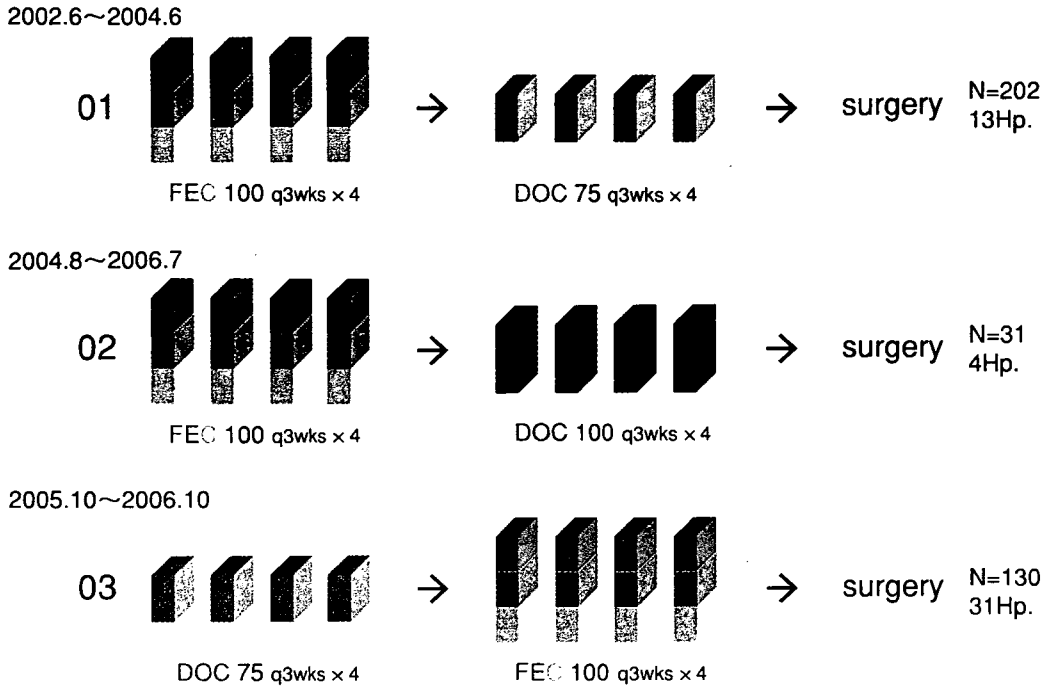

図1　JBCRG neoadjuvant 01-03 study design

2. 結　果

JBCRG01およびJBCRG02の結果については，既に報告されているが[6,7]，JBCRG03については予定症例数130例に対し，実際の登録期間は2005年10月から2006年10月までの13カ月で137例の登録を得た．今回の検討は137例中111例での解析のため，最終的な効果・認容性に関しては言及しないが，患者背景では閉経前，T2，ERかつ/あるいはPgR陽性が多く登録されていた．血液毒性では白血球・好中球減少はgrade3-4もみられたが，発熱性好中球減少はdocetaxel投与中，FEC投与中いずれも10%前後であった．一方，非血液毒性ではFECにて疲労，悪心・嘔吐および食欲不振においてgrade3が認められたが，他はgrade1-2であり忍容性が高かった．全体的に評価するとFECでは血液毒性と消化器症状の発生頻度とgradeが高く，docetaxelでは皮膚症状や浮腫，末梢神経障害などの症状が特徴的であったが，いずれもgradeは低く忍容性は高いと考えられた．

3. 考　察

1) 01-03から得られた知見

3つの臨床試験から得られた知見をまとめてみる．JBCRG01では当時はわが国においてmanageableか解らなかった世界的標準治療であるFEC100を組み入れ，anthracycline→taxaneでの臨床効果の有用性を検証，JBCRG02ではglobal doseであったdocetaxel 100mg/m²へのdose upによる病理学的効果の向上を検証，JBCRG03ではdocetaxel先行による忍容性の向上が臨床的，病理学的効果の向上に寄与するか，reverse regimenとした場合に増悪症例がみられるかが検証されたと言える．

JBCRG01が開始された2002年当時では画期的であったFEC100の実施，pCRを目標とした術前化学療法による臨床試験，中間解析での予想を上回る臨床・病理学的効果等が魅力的であったためか，JBCRG01開始時13施設であったJBCRG参加施設もJBCRG03では31施設まで拡がりをみせた．また，JBCRG01，JBCRG02，JBCRG03の登録期間と症例数を見直すと，JBCRG01では2002年6月から2004年6月までの25カ月で202症例がenrollされたが，JBCRG02試験では24カ月で50症例，JBCRG03では13カ月で130例と症例登録がスムースとなり，global trialの趨勢を意識した臨床試験参加へのinvestigatorの意欲と術前

化学療法自体の浸透が伺えた．

2) 今後の課題と展望

JBCRG01からJBCRG03までの試験の結果は非常に示唆に富むものであった．1）強力であるFEC100 mg/m²やDocetaxel 75mg/m²を用いても，明らかなchemo resistanceは存在し，この症例における予後の改善が求められる．術前化学療法後に残存病変が有った症例に対する術後治療を検証する試験が存在しないため，われわれはJBCRG04として残存病変がある症例に対するcapecitabine追加投与の有無という試験を計画，始動している．2）JBCRG01試験において治療前に得られた腫瘍の免疫染色の結果から，ER陽性群はpCR rateが低い点が明らかとなった．この結果を受け，特に化学療法の恩恵が乏しいと考えられる閉経患者を対象にaromatase inhibitorの逐次投与の臨床試験が準備されている．3）同様にJBCRG01試験において諸家が指摘している様にHER2陽性群に高いpCR rateが得られたが，最近特に着目されるtrastuzumabやlapatinib等のHER2 inhibitor，分子標的療法の追加による更なるpCR rateの向上が期待されている．4）JBCRG01，JBCRG03いずれにおいてdocetaxelのnon-responderが観察されたことから01-03の臨床データとTranslational Research（TR）での種々の情報を組み合わせて，術前化学療法のbest selectionが必須と理解された．

結　語

今回われわれはJBCRGを基盤とした3つの臨床試験から得られた知見を総合的に検証した．種々のコンセプトのもと，3種類のregimenが術前化学療法の試験として遂行され，予定症例数も完了可能であった．慎重な観察のもと，多くが高い忍容性を維持し諸家が報告したpCR rateと同等以上の結果が得られた．これらの試験を通じて，JBCRGは日本での医師主導型臨床試験の普及に寄与したとともに，付随した科学的検証の為にTRを併行するglobal typeの試験の骨子を確立したと言える．

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Breast Cancer
Vol. 13 No. 3 July 2006

Original Article

Analysis of Non-Genetic Risk Factors for Adverse Skin Reactions to Radiotherapy among 284 Breast Cancer Patients

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***Objectves*:** We analyzed non-genetic risk factors for adverse skin reactions to irradiation at 4 collaborating Japanese institutions, to design future investigation into genetic risk factors for adverse skin reactions to irradiation in a multicenter setting.

***Methods*:** From April 2001, 284 breast cancer patients, who underwent radiotherapy with breast-conserving surgery, were enrolled from 4 collaborating institutions in Japan. We graded skin reactions according to international scoring systems. Clinical factors were tested against adverse effects.

***Results*:** Grade 1+ skin reactions were observed in 261 (92%) of the patients in less than 3 months, 118 (42%) at 3 months, and 29 (10%) at 6 months in the late phase. Univariate analysis of treatment risk factors (such as the use of a multi-leaf colimeter, wedge-filter, or immobilization device) for skin reactions revealed a significant association ($p<0.0001$). After a variable selection procedure with logistic regression, the institution, operative procedure, and magnitude of photon energy remained significantly associated with acute skin reactions. Only the institution was an explanatory variable for skin reactions at 3 and 6 months in the final logistic model.

***Conclusion*:** After stratification, substantial remaining variations in the occurrence of skin reactions of a given level suggested that individual genetic factors contribute markedly to individual radiosensitivity. Analysis of genetic factors associated with adverse effects would be possible by stratifying patients according to institution. Selection of eligible institutions, where appropriate treatment modalities could be performed, would also be possible when planning such a study.

Breast Cancer 13:300-307, 2006.

Key words: Radiation therapy, Adverse effects, Breast cancer

The number of breast cancer patients has been increasing in Japan[1]. Breast-conserving surgery followed by radiotherapy is the most common form of primary breast cancer treatment for patients with early-stage breast cancer[2,3]. Radiotherapy for breast cancer patients occasionally induces adverse effects, such as poor cosmetic outcome[4], fibrosis or thickening of the dermis[5] and radiation pneumonitis[6], but the risk factors are not yet understood[7-9]. Extrinsic factors such as age and smoking habit are associated with radiation damage of the skin[10-13], and intrinsic factors such as the cellular radiosensitivity of normal fibroblasts[14], the level of TGF-beta polymorphisms[15,16], and the radiosensitivity of lymphocytes[17], are associated with variability in the occurrence of subcutaneous fibrosis. Safwat analyzed the occurrence of telangiectasia of the skin in patients treated with bilater-

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Abbreviations:
NIRS, National Institute of Radiological Sciences; NCI-CTC, The National Cancer Institute Common Toxicity Criteria; RTOG/EORTC, The Radiation Therapy Oncology Group and European Organization for Research and Treatment of Cancer; MLC, Multileaf collimater; Dmax, Depth of maximum dose; KW, Kruskal-Wallis test; FE, Fisher's exact test; CS, Chi-square test; CA, Cochran-Armitage test; Bq, Quadrantectomy; Bp, Partial exision

Received August 22, 2005; accepted February 3, 2006

Table 1. Clinical Factors Used in Analysis of Skin Reaction

clinical factors		
Institution	Tumor occupation	Irradiation fractions (fr)
Birth date	Size of tumor (volume)	Irradiation duaration (days)
Number of pregnancy	TMN classification (T)	Number of directions
Number of delivery	TMN classification (N)	Direction code
Age of menarche	TMN classification (M)	Irradiation field (area)
Age of menopause	Stage	Multileaf collimater
Number of children	Pathological diagnosis	Wedge filter
Diabetes Mellitus	Tumor margin yes or no	Bolus
Collagen disease	Receptor (breast cancer) ER	Immobilization devise
Past history of cancer	Receptor (breast cancer) PgR	Boost therapy
Past history of radiotherapy	Receptor (breast cancer) HER2	Boost irradiated dose (Gy)
Smoking index	Chemotherapy	Boost duration (days)
Alcohol habbit	Involved field	Direction code of boost therapy
Family history	Irradiation method	Irradiation field (area)
Operation procedure	Irradiation dose (Gy)	Boost bolus

al internal mammary fields, and found that approximately 90% of the variability in radio-responsiveness in the right-sided field was explained by radio-responsiveness in the left-sided field[18]. These facts suggest that genetic factors play a major role in individual radiosensitivity and have stimulated investigations into genetic risk factors for adverse reactions to radiotherapy. We have collected information on patient characteristics and treatment details potentially related to adverse reactions to radiotherapy, and have been analyzing blood samples obtained from all patients to investigate genetic polymorphisms such as single nucleotide polymorphisms (SNPs). Although the final goal of the research is to identify genetic factors predictive of adverse reactions to radiotherapy, identification of non-genetic factors associated with adverse events remains important, as such factors need to be taken into consideration when designing, analyzing and interpreting data from research into genetic factors. The present study undertook an exploratory analysis of non-genetic risk factors in 284 breast cancer patients from 4 collaborating institutions in Japan.

Patients and Methods

Patients

Beginning in April 2001, we enrolled 284 breast cancer patients from 4 collaborating institutions in Japan: the Research Center for Charged Particle Therapy of the National Institute of Radiological Sciences (NIRS), Chiba Cancer Center, Chiba University Hospital, and Toyonaka Municipal Hospital. All patients underwent radiotherapy after breast-conserving surgery between 1989 and 2002 and were followed for more than 8 months. All patients provided written informed consent.

Ethical Review

The study was approved by the Ethical Committee at the NIRS and by each collaborating institution. Patient privacy was safeguarded to the highest level possible. A double anonymity system was utilized for patient records, whereby each patient was assigned a clinical number, a random number, and a sample number at each step requiring information, so that all names and identifying information were deleted from the dataset. Identifying information was managed at the Medical Information Processing Office of the Research Center for Charged Particle Therapy, NIRS[20].

Radiotherapy

Radiotherapy was delivered to the breast using the tangential irradiation technique. The total irradiation dose ranged from 42-60 Gy for all patients, and 46-50 Gy for 253 (89%) patients. Fractionation was 2.0 Gy, 5 times/week in most cases. The total duration of irradiation was 30-40 days for 233 patients (82%). More than 60% of the patients were treated with a 4-MV linear accelerator, and 30% were treated with a 6-MV linear accelerator. The primary site was given a boost dose in 100 patients (35%). In 85 of them, this was accomplished with an electron beam to give an additional 10 Gy.

Table 2. Patient Characteristics by Institution

Characteristics	Institution				Total (n = 284)	Institutional difference
	A (n = 66)	B (n = 90)	C (n = 64)	D (n = 64)		*p*-value*
Age at radiotherapy: Mean (Range)	52.5 (36-74)	51.0 (33-73)	54.6 (27-77)	53.5 (26-76)	52.7 (26-77)	0.1070 (KW)
Complications						
Collagen disease	1 (2%)	2 (2%)	2 (3%)	–	5 (2%)	0.6243 (FE)
Familiy history of breast cancer	35 (53%)	36 (40%)	25 (39%)	36 (56%)	132 (47%)	0.0894 (FE)
TNM stage classification						0.0284 (CS)
I	–	6 (7%)	1 (2%)	3 (5%)	10 (4%)	
IIa	51 (77%)	55 (61%)	32 (50%)	38 (59%)	176 (62%)	
IIb	13 (20%)	13 (14%)	22 (34%)	17 (27%)	65 (23%)	
IIIa	1 (2%)	7 (8%)	5 (8%)	5 (8%)	18 (6%)	
IIIb	–	3 (3%)	1 (2%)	–	4 (1%)	
IV	–	2 (2%)	1 (2%)	–	3 (1%)	
Unknown	1 (2%)	4 (4%)	2 (3%)	1 (2%)	8 (3%)	

*KW: Kruskal-Wallis test, FE: Fisher's Exact test, CS: Chi-Square test

Table 3. Treatment Details by Institution

Treatment	Institution				Total (n = 284)	Institutional difference
	A (n = 66)	B (n = 90)	C (n = 64)	D (n = 64)		*p*-value (Test*)
Drug therapy						<0.0001 (CS)
Chemotherapy	–	6 (7%)	17 (27%)	6 (9%)	29 (10%)	
Hormone therapy	49 (74%)	19 (21%)	30 (47%)	7 (11%)	105 (37%)	
Both	17 (26%)	10 (11%)	10 (16%)	1 (2%)	38 (13%)	
No drug	–	55 (61%)	7 (11%)	50 (78%)	112 (39%)	
Radiotherapy						
Multi-leaf collimator	1 (2%)	89 (99%)	53 (83%)	2 (3%)	145 (51%)	<0.0001 (FE)
Wedge filter	45 (68%)	87 (97%)	62 (97%)	13 (20%)	207 (73%)	<0.0001 (FE)
Bolus	13 (20%)	–	–	–	13 (5%)	<0.0001 (FE)
Immobilization device	–	87 (97%)	64 (100%)	64 (100%)	215 (76%)	<0.0001 (FE)
Boost	23 (35%)	37 (41%)	20 (31%)	20 (31%)	100 (35%)	0.5275 (FE)

*KW: Kruskal-Wallis test, FE: Fisher's Exact test, CS: Chi-Square test

Patient Characteristics and Treatment Details

Table 1 lists the 45 clinical factors that we tested with regard to adverse effects. Table 2 lists the patient's characteristics by institution, and Table 3 lists the treatment details. The mean age ± SD was similar for all institutions, but smoking habits (Table 5), TNM stage, and invasiveness differed significantly, as did the type of breast conserving surgery (Table 5) and drug therapy.

Grade of Skin Reaction; Clinical Radiosensitivity

We graded adverse skin effects according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) scoring system, version 2 (http://ctep.info.nih.gov), within 3 months of starting radiotherapy, and according to the Radiation Therapy Oncology Group and European Organization for Research and Treatment of Cancer (RTOG/EORTC) grading system at 3 or 6 months after starting radiotherapy. These scoring systems offer quantitative descriptive grades from 0 to 4.