Table 3. Association of SNPs between high- and low-grade groups

				Allele (M/m)				Genotype (MM/Mm/mm)		Dominant model		Recessive model	
rsSNP ID	Gene	Chr	Mm	HG (n = 218)	LG (n = 580)	p	OR (95% CI)	HG (n = 109)	LG (n = 290)	p	OR (95% CI)	p	OR (95% CI)
rs2294638	MAD2L2	1	GC	141/77	318/262	0.013	1.51 (1.10–2.09)	48/45/16	86/146/58	0.25	1.45 (0.83–2.90)	0.0087	1.87 (1.18–2.97)
rs913060	TGFBR3	1	GA	166/52	480/100	0.043	0.67 (0.45–0.98)	65/36/8	203/74/13	0.31	0.59 (0.23–1.74)	0.056	0.63 (0.40–1.01)
rs1926261	TGFBR3	1	CT	116/102	361/219	0.023	0.69 (0.51–0.95)	35/46/28	120/121/49	0.063	0.59 (0.35–1.03)	0.11	0.67 (0.41–1.06)
rs1806649	NFE2L2	2	CT	199/19	552/28	0.043	0.53 (0.29–1.01)	90/19/0	262/28/0	NC	NC	0.037	0.51 (0.27–0.99)
rs2075747	OGG1	3	GA	155/63	388/190	0.31	1.20 (0.86–1.71)	51/53/5	137/114/38	0.017	3.15 (1.38–13.92)	1.0	0.98 (0.62–1.52)
rs3805169	NEIL3	4	TC	170/48	473/107	0.27	0.80 (0.55–1.19)	70/30/9	188/97/5	0.0036	0.19 (0.04–0.59)	0.91	0.97 (0.62–1.56)
rs3756402	RAD17	5	TC	199/19	500/80	0.054	1.68 (1.01–3.05)	91/17/1	213/74/3	1.0	1.13 (0.18–2.28)	0.036	1.83 (1.07–3.51)
rs3811999	PTTG1	5	CT	195/23	476/104	0.012	1.85 (1.20–3.12)	88/19/2	199/78/13	0.37	2.51 (0.74–7.15)	0.018	1.92 (1.15–3.46)
rs2961950	PTTG1	5	AG	159/59	380/200	0.051	1.42 (1.02–2.00)	59/41/9	122/136/32	0.47	1.38 (0.67–3.60)	0.033	1.62 (1.04–2.54)
rs2961952	PTTG1	5	GA	126/92	383/197	0.032	0.70 (0.51–0.98)	35/56/18	127/129/34	0.24	0.67 (0.36–1.31)	0.040	0.61 (0.37–0.95)
rs3757244	MAP3K7	6	CT	211/7	539/41	0.045	2.29 (1.13–7.36)	102/7/0	249/41/0	NC	NC	0.038	2.40 (1.15–7.54)
rs190246	REV3L	6	GT	113/105	353/227	0.024	0.69 (0.50–0.94)	31/51/27	104/145/41	0.016	0.50 (0.29–0.88)	0.19	0.71 (0.43–1.14)
rs240962	REV3L	6	GT	104/114	335/245	0.013	0.67 (0.49–0.91)	24/56/29	97/141/52	0.069	0.60 (0.36–1.03)	0.028	0.56 (0.32–0.92)
rs818707	ALAD	9	CT	208/10	528/52	0.039	2.05 (1.11–4.94)	100/8/1	242/44/4	1.0	1.51 (0.19–3.06)	0.037	2.20 (1.14–5.74)
rs2282367	MAT1A	10	GA	205/13	525/55	0.12	1.65 (0.95–3.55)	98/9/2	236/53/1	0.21	0.208 (0.08–1.26)	0.047	2.04 (1.09–4.67)
rs8193	CD44	11	CT	118/100	369/211	0.018	0.67 (0.49–0.92)	27/64/18	118/133/39	0.43	0.79 (0.44–1.54)	0.0034	0.48 (0.28–0.76)
rs2286620	RAD9A	11	TC	183/35	432/148	0.0045	1.79 (1.22–2.77)	76/31/2	161/110/19	0.077	3.75 (1.13–10.19)	0.012	1.85 (1.17–3.04)
rs917570	RAD9A	11	CG	196/22	488/92	0.041	1.68 (1.06–2.93)	87/22/0	203/82/5	0.33	NC	0.058	1.69 (1.03–3.03)
rs2268622	TGFB3	14	TC	113/105	347/233	0.045	0.72 (0.52–0.99)	29/55/25	108/131/51	0.25	0.72 (0.42–1.29)	0.058	0.61 (0.36–0.98)
rs3744355	LIG3	17	GC	144/74	435/145	0.013	0.65 (0.46–0.92)	49/46/14	158/119/13	0.0060	0.32 (0.13–0.73)	0.093	0.68 (0.43–1.06)
rs73234	SH3GL1	19	CG	131/79	390/178	0.10	0.76 (0.54–1.07)	39/53/13	141/108/35	1.0	0.99 (0.52–2.12)	0.030	0.60 (0.37–0.95)
rs243336	SH3GL1	19	GC	126/92	377/203	0.070	0.74 (0.54–1.02)	34/58/17	130/117/43	0.88	0.94 (0.52–1.86)	0.016	0.56 (0.34–0.89)
rs25487	XRCC1	19	CT	154/64	443/137	0.10	0.74 (0.53–1.06)	59/36/14	168/107/5	0.015	0.37 (0.17–0.84)	0.50	0.86 (0.54–1.34)
rs918546	BAX	19	GT	126/92	362/216	0.22	0.82 (0.59–1.13)	30/66/13	117/128/44	0.52	1.33 (0.70–2.84)	0.020	0.56 (0.33–0.89)
rs3087869	COMT	22	AG	154/64	390/190	0.39	1.17 (0.85–1.66)	52/50/7	142/106/42	0.039	2.47 (1.18–7.82)	0.91	0.95 (0.61–1.49)

Abbreviations: rsSNP = reference single nucleotide polymorphism; ID = identifier; LG = low grade; HG = high grade; Chr = chromosome; M = major allele; m = minor allele; OR = odds ratio; CI = confidence interval; NC = insufficient sample size to perform calculation.

SNPAlyze software used for Fisher exact test and calculation of OR and its 95% CI (bootstrap method).

Table 4. Haplotype association and FDR

Gene	SNPs for haplotype	p*	FDR
RAD9A	rs2255990, rs2286620, rs917570	0.015	0.033
PTTG1	rs2910190, rs3811999, rs1862391, rs2961951	0.016	0.033
LIG3	rs3744355, rs2074518, rs3744357	0.017	0.033
REV3L	rs190246, rs240962	0.023	0.033
CD44	rs187116, rs3794116, rs3794107, rs8193	0.026	0.033
MAD2L2	rs2294638, rs746218	0.040	0.041
NFE2L2	rs1806649, rs2364724	0.067	0.053
TGFBR3	rs1926261, rs2296620	0.068	0.053
ALAD	rs818707, rs1805312	0.091	0.055
TGFB3	rs2268622, rs3917145	0.10	0.055
SH3GL1	rs2705, rs243387	0.11	0.055
RAD17	rs3756402, rs299081	0.11	0.055
OGG1	rs1801129, rs2075747	0.11	0.055
XRCC1	rs25487, rs2682585	0.17	0.075
NEIL3	rs3805169, rs13112358	0.22	0.092
MAT1A	rs2993763, rs2282367	0.28	0.11
MAP3K7	rs1144158, rs157692, rs205343, rs3757244, rs282065	0.38	0.14
BAX	rs918546, rs3745693	0.43	0.15
COMT	rs2020917, rs3087869	0.63	0.21

Abbreviations: SNP = single nucleotide polymorphism; FDR = false-discovery rate.

Estimates of FDR based on q values (http://faculty.washington.edu/~jstorey/qvalue/); tuning parameter, $\lambda = 0.2$.

* p value for global statistic corresponds to test for overall association between haplotypes and risk of adverse skin reactions.

in each locus. In the *CD44* gene, the haplotype GGTT significantly increased the risk of EASRs compared with the most common haplotype GGTC (OR = 2.17; 95% CI, 1.07–4.43). The overall difference in the haplotype distribution was assessed in *REV3L* (simulation-based $p = 0.023$), but no stratum with a significant risk haplotype was observed. The haplotypes CG in *MAD2L2* (OR = 0.55; 95% CI, 0.35–0.87), GTTG in *PTTG1* (OR = 0.48; 95% CI, 0.24–0.96), TCC (OR = 0.48; 95% CI, 0.26–0.89), and CCG (OR = 0.50; 95% CI, 0.27–0.92) in *RAD9A*, and GCT in *LIG3* (OR = 0.46; 95% CI, 0.22–0.93) were associated with a reduced risk of EASRs compared with the most common haplotype in each locus. These results have suggested that the group of breast cancer patients in this study could be stratified by specific haplotypes and that the individuals with the haplotype GGTT in *CD44* were at a significantly greater risk of EASRs compared with those with haplotypes CG in *MAD2L2*, GTTG in *PTTG1*, TCC or CCG in RAD9A, and GCT in *LIG3*.

DISCUSSION

The ultimate goals of our ongoing research are to find genetic variations that are associated with radiosensitivity and to use this information to identify genetic markers. In this report we analyzed the haplotypes of genes that were candidates for affecting the risk of EASRs after RT. Variations in the candidate genes were considered as haplotypes because the statistical power of the association tests using phased data is likely to increase (39–42). A total of 123 genes covering 510 SNPs were subjected to the first screening. From the LD maps for the 19 genes selected by the screening, the haplo-tag SNPs were selected for each locus. Global haplotype analysis ($p < 0.05$) and consideration of FDR ($q < 0.05$) showed that *CD44, MAD2L2, PTTG1, RAD9A, LIG3*, and *REV3L* loci were associated with EASR risk. We found haplotypes associated with an increased risk of EASRs in *CD44* and other haplotypes associated with a reduced risk of EASRs in *MAD2L2, PTTG1, RAD9A*, and *LIG3*. No significant risk was observed for any haplotype in *REV3L*.

Combinations of these haplotypes in multiple loci might determine the complexity of an individual's radiosensitivity. Andreassen *et al.* (9) suggested a model consisting of multiple genes with different effects on clinical radiosensitivity to explain patient-to-patient variability in normal tissue reactions after RT. The identification in the present study of five genes with different contributions to clinical radiosensitivity seems to support their model. Because a haplotype of one gene increased the risk of EASRs but other haplotypes of other genes reduced the risk of EASRs, the combined effect of haplotype contribution should be considered patient by patient. The present haplotypes, however, could only be estimated statistically. Therefore, the real haplotypes of these genes must be determined experimentally. This would provide an understanding of the mechanisms underlying the genetic variation in radiation sensitivity or resistance among the population and would enable the prediction of the risk of EASRs before RT. It might be possible to perform the required experiments using a recently reported new method for haplotype determination (52).

Five of the six genes shown in Table 5 (*REV3L, MAD2L2, PTTG1, RAD9A*, and *LIG3*) encode for proteins that act in the nucleus. The functions of three of them (*MAD2L2, REV3L*, and *PTTG1*) are related to chromosome maintenance, involving sister chromatid separation and the mitotic spindle checkpoint (see Nasmyth [37] for review). This suggests that functional variation might cause the malfunction of cell cycle regulation and lead to genome instability, including aneuploidy. The functions attributed to the identified genes appear consistent with the early damage that results from the death of a large number of cells in the epidermal layer of the skin (17). CD44 is a transmembrane adhesion receptor that is the major cell surface receptor for the nonsulfated glycosaminoglycan hyaluronan and is reported to be involved in lymphocyte extravasation (53). Appropriate repair after radiation injury and inflammation requires a resolution of the inflammatory response and removal of extracellular matrix breakdown products. We have previously analyzed interstrain variations in irradiated murine lung and found an increase in the number of CD44-positive cells in radioresistant mice (29). Therefore, the finding that this haplotype is associated with patients who developed EASRs was of great interest.

Our previous *in vitro* study showed that genes in the base-excision repair system, such as *LIG1* (data not shown) and *PCNA*, were likely candidates for genotyping (31). This base-excision repair system has been suggested to play a role in repairing DNA damaged by ultraviolet light and

Table 5. Estimated frequency of haplotypes and association with risk of EASRs

		Estimated frequency†			Effect	
Gene	Haplotype*	Pool (n = 798)	LG (n = 580)	HG (n = 218)	OR (95% CI)‡	p§
CD44	GGTC	0.40	0.42	0.33	1.0 (Reference)	
	GGTT	0.25	0.22	0.30	2.17 (1.07–4.43)	0.010
	AGTC	0.13	0.12	0.14	1.79 (0.82–3.90)	0.18
	AGTT	0.068	0.058	0.093	2.09 (0.93–4.67)	0.040
	AGAC	0.049	0.054	0.034	1.07 (0.36–3.18)	0.68
	AGAT	0.048	0.052	0.037	0.80 (0.27–2.40)	1.0
	AATC	0.038	0.041	0.030	0.88 (0.26–3.01)	0.82
	AATT	0.023	0.026	0.014	0.84 (0.12–5.83)	0.77
REV3L	GC	0.54	0.56	0.48	1.0 (Reference)	
	TT	0.41	0.38	0.48	1.47 (0.90–2.39)	0.014
	GT	0.044	0.045	0.041	0.96 (0.43–2.14)	0.84
	TC	0.010	0.014	NA	NA	NA
MAD2L2	GG	0.57	0.54	0.64	1.0 (Reference)	
	CG	0.31	0.34	0.24	0.55 (0.35–0.87)	0.0044
	CA	0.11	0.12	0.11	0.81 (0.46–1.42)	0.45
PTTG1	GCTG	0.24	0.23	0.27	1.0 (Reference)	
	ACTG	0.21	0.22	0.19	0.94 (0.51–1.71)	0.29
	ACTT	0.20	0.19	0.23	1.29 (0.73–2.27)	0.82
	GTTG	0.13	0.15	0.079	0.48 (0.24–0.96)	0.0081
	GCGG	0.090	0.094	0.076	0.86 (0.41–1.82)	0.29
	ACGG	0.064	0.064	0.061	0.86 (0.39–1.90)	0.60
	GCTT	0.036	0.025	0.059	2.17 (0.78–6.03)	0.13
	GTTT	0.016	0.022	NA	NA	NA
RAD9A	CTC	0.74	0.71	0.82	1.0 (Reference)	
	TCC	0.11	0.13	0.073	0.48 (0.26–0.89)	0.017
	CCG	0.11	0.12	0.073	0.50 (0.27–0.92)	0.022
	CTG	0.033	0.036	0.023	0.51 (0.18–1.39)	0.28
LIG3	GCC	0.42	0.41	0.45	1.0 (Reference)	
	CCC	0.25	0.24	0.29	1.14 (0.68–1.90)	0.63
	GTC	0.20	0.21	0.15	0.76 (0.45–1.29)	0.10
	GCT	0.11	0.13	0.041	0.46 (0.22–0.93)	0.0004
	CCT	0.015	0.007	0.033	3.73 (0.66–21.05)	0.021

Abbreviations: EASRs = early adverse skin reactions; NA = not applicable; other abbreviations as in Table 3.

* Haplotypes observed with >1% frequency in pool.

† Haplotype frequency was estimated using haplo.cc function of the haplo.stats.

‡ Odds ratio obtained using recursively the estimated posterior probabilities of pairs of haplotypes per subjects as weights in the logistic model (haplo.cc function of the haplo.stats).

§ The p-value based on the score statistics corresponds to the test for association between the specific haplotype and the risk of ASRs.

ionizing radiation (54, 55). The SNPs on the members of this pathway, *NEIL3, APEX1, POLB, POLD1, POLE, XRCC1, LIG1, LIG3, PARP1, PNKP, PCNA*, and *FEN1*, were subjected to genotyping analysis. Allelic and/or genotypic associations were observed between the SNPs on *NEIL3, LIG3*, and *XRCC1* and EASR risk, but an association between the haplotypes of these genes and EASR risk was suggested only for the *LIG3* locus (Tables 3–5).

RAD9A was selected because it was reported to act as a damage sensor in the DNA damage checkpoint response (56). This gene product is a subunit of the heterotrimeric RAD9-RAD1-HUS1 complex. RAD9A also interacts with the anti-apoptotic bcl-2 family of proteins (BCL-2/BCL-x_L) suggesting a role for RAD9A in regulating apoptosis after DNA damage (57). We propose that if cells contained unusual activity of these gene products, the cell cycle would not be completed after RT. The increased number of defective cells could activate the immune system, but not the apoptotic system, and cause inflammation.

A total of 27 cSNPs for *CD44, LIG3*, and *REV3L* and none for *MAD2L2, PTTG1*, and *RAD9A* genes were recorded in the jSNP or dbSNP database when this research began. However, because of a lower allele frequency (not polymorphic or <5%) in our patient group, these cSNPs in *CD44, LIG3*, and *REV3L* were not used for the haplotype analysis. At present, we cannot explain how the specific haplotypes with a greater risk affected the function of the gene products. It is possible that SNPs in regulatory regions (rSNPs) or in untranslated regions of mRNA contribute to functional differences in genes. With respect to *PTTG1*, the SNPs in the estimated haplotype (rs3811999 and rs1862391) were located within 2 kb from the transcriptional start site, suggesting that they might affect transcription of the *PTTG1* gene. Regarding *CD44*, rs8193 was located in the 3′ untranslated region of its mRNA,

indicating that this SNP might cause stability of *CD44* mRNA. Searching functional SNPs in the genes identified in the present study would facilitate the understanding of mechanisms contributing to variations in radiation susceptibility.

The rs25487 (Arg399Gln) polymorphism on *XRCC1* reportedly associates with the risk of early skin reactions after RT in breast cancer patients (19). Our findings suggest an association between the Arg399Gln genotype in *XRCC1* and the risk of EASRs (dominant model in Table 3). However, no overall significant difference in the haplotype distribution in the *XRCC1* gene was detected between the HG and LG groups (Table 4). Associations between the markers C-509T (rs1800469) in *TGFB1* (20, 22, 23) and Val16Ala (rs4880) in *SOD2* (20) and late adverse effects in breast cancer patients have been reported. To test whether these polymorphisms were also associated with early skin reactions, we examined these polymorphisms in our subjects. However, no association between these SNPs and EASRs was detected.

We also analyzed in detail the SNPs within and surrounding the *ATM* gene. The marker Asp1853Asn (rs1801516), which is associated with late reactions (24, 26), was not polymorphic in our 399 breast cancer patients and 115 healthy Japanese women. The marker IVS22-77 T>C (rs664677), which has also been reported to associate with late reactions (21), was not associated with EASRs in our study. Furthermore, we analyzed this region spanning approximately 200 kb with 53 SNPs selected from the jSNP and dbSNP databases; 34 SNPs were not polymorphic and minor allele frequency of 6 SNPs were <0.05. The remaining 13 SNPs were not associated with the risk of EASRs. Because the *ATM* gene has been reported to lie within a large single LD block according to the HapMap data (D' >0.8) with HAN Chinese and Japanese populations (58), these markers seemed to cover most of the *ATM* gene. The distinctive features of our study, which could have influenced the results, were the following: *(1)* that we analyzed EASRs that occurred within 3 months of starting RT; *(2)* that we tested the reported SNPs that were able to be typed using our matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based technique; and *(3)* the Japanese ethnicity of our subjects, which might have had some bearing on their radiosensitivity.

The use of selected genes imposes some limitations on our findings. The candidate genes for genotyping were selected using a limited number of gene expression analyses, in addition to the published genes we were interested in. Because the HapMap data has only been available recently, it might be able to perform a genome-wide association study for radiosensitivity in cancer patients. Furthermore, we emphasize the importance of a subsequent association study with a large number of patients and/or a meta-analysis of multiple populations.

CONCLUSION

We identified six novel haplotypes associated with the risk for EASRs after RT using a large-scale candidate-genes approach. Focused investigations of functions related to the haplotypes in these genes will improve our understanding further of how genetic factors contribute to individual radiosensitivity.

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ORIGINAL REPORT

Dofequidar Fumarate (MS-209) in Combination With Cyclophosphamide, Doxorubicin, and Fluorouracil for Patients With Advanced or Recurrent Breast Cancer

Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, and Takashi Tsuruo

From the National Shikoku Cancer Center, Matsuyama; Hoshi General Hospital, Koriyama; Japanese Foundation for Cancer Research, Tokyo; School of Medicine, Osaka University, Osaka; Nihon Schering K.K., Osaka; Kanagawa Cancer Center, Yokohama; National Cancer Center Hospital East, Kashiwa; Chiba Cancer Center, Chiba; Tokyo Metropolitan Komagome Hospital; The Jikei University School of Medicine; School of Medicine, Keio University; Biostatistics/Epidemiology and Preventive Health Sciences, University of Tokyo; Institute of Molecular and Cellular Biosciences, University of Tokyo; and Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan.

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Address reprint requests to Toshiaki Saeki, MD, PhD, Saitama Medical School Hospital, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan; e-mail: tsaeki@saitama-med.ac.jp.

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ABSTRACT

Purpose
To evaluate the efficacy and tolerability of dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) therapy in comparison with CAF alone, in patients with advanced or recurrent breast cancer. Dofequidar is a novel, orally active quinoline derivative that reverses multidrug resistance.

Patients and Methods
In this randomized, double-blind, placebo-controlled trial, patients were treated with six cycles of CAF therapy: 28 days/cycle, with doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2) administered on days 1 and 8 and cyclophosphamide (100 mg orally [PO]) administered on day 1 through 14. Patients received dofequidar (900 mg PO) 30 minutes before each dose of doxorubicin. Primary end point was overall response rate (ORR; partial or complete response). In total, 221 patients were assessable.

Results
ORR was 42.6% for CAF compared with 53.1% for dofequidar + CAF, a 24.6% relative improvement and 10.5% absolute increase (P = .077). There was a trend for prolonged progression-free survival (PFS; median 241 days for CAF *v* 366 days for dofequidar + CAF; P = .145). In retrospectively defined subgroups, significant improvement in PFS in favor of dofequidar was observed in patients who were premenopausal, had no prior therapy, and were stage IV at diagnosis with an intact primary tumor. Except for neutropenia and leukopenia, there was no statistically significant excess of grade 3/4 adverse events compared with CAF. Treatment with dofequidar did not affect the plasma concentration of doxorubicin.

Conclusion
Dofequidar + CAF was well tolerated and is suggested to have efficacy in patients who had not received prior therapy.

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INTRODUCTION

Despite the advances in chemotherapeutic intervention, many cancers are either inherently resistant or develop resistance to chemotherapy.[1,2] Consequently, multidrug resistance (MDR) remains a major obstacle to the successful treatment of cancer.[1,3,4] One mechanism by which MDR operates is via the increased cellular efflux of cytotoxic compounds due to increased expression of membrane transport proteins such as P-glycoprotein (P-gp) and MDR-associated protein (MRP).[1,4,5] MDR affects many structurally and functionally unrelated agents including cytotoxic drugs that are hydrophobic, natural products, such as taxanes, vinca alkaloids, anthracyclines, epipodophyllotoxins, topotecan, dactinomycin, and mitomycin.[1,6,7] These represent some of the most commonly used chemotherapeutic agents.

In tumors with low levels of P-gp expression at baseline or diagnosis, P-gp expression increases after exposure to chemotherapy agents, thus leading to the development of MDR. In breast cancer patients who had received prior chemotherapy, P-gp expression has been shown to increase from 11% in untreated patients to 30% after chemotherapy.[8] Furthermore, compared with P-gp–negative tumors, a significant increase in resistance to paclitaxel and doxorubicin was reported in P-gp positive breast cancer tissue, irrespective of prior therapy.

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.

PATIENTS AND METHODS

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.

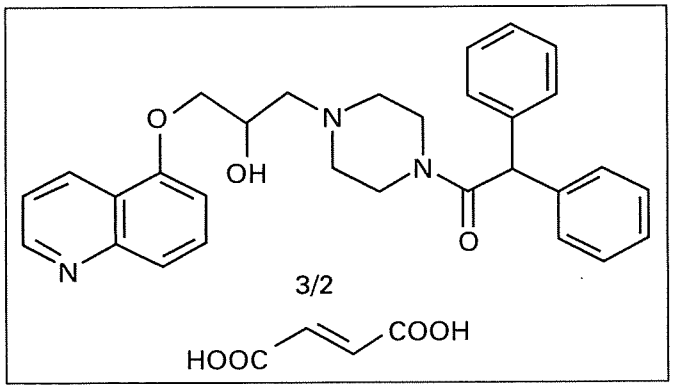

Fig 1. Structure of dofequidar (MS-209).

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)		Placebo + CAF (n = 108)	
	No.	%	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.

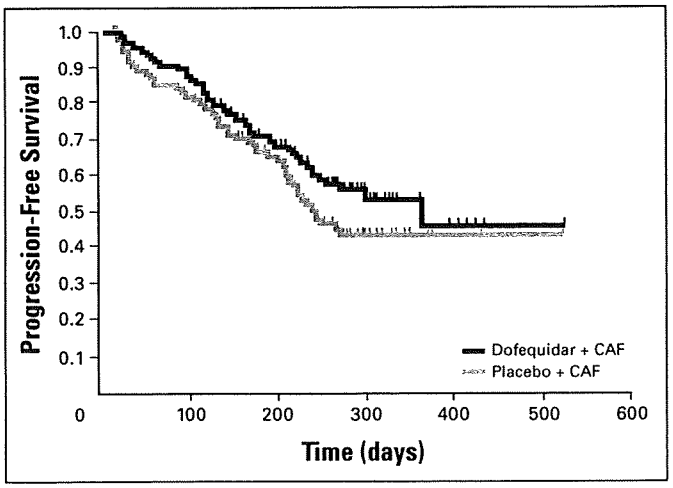

Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF ($P = .145$).

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

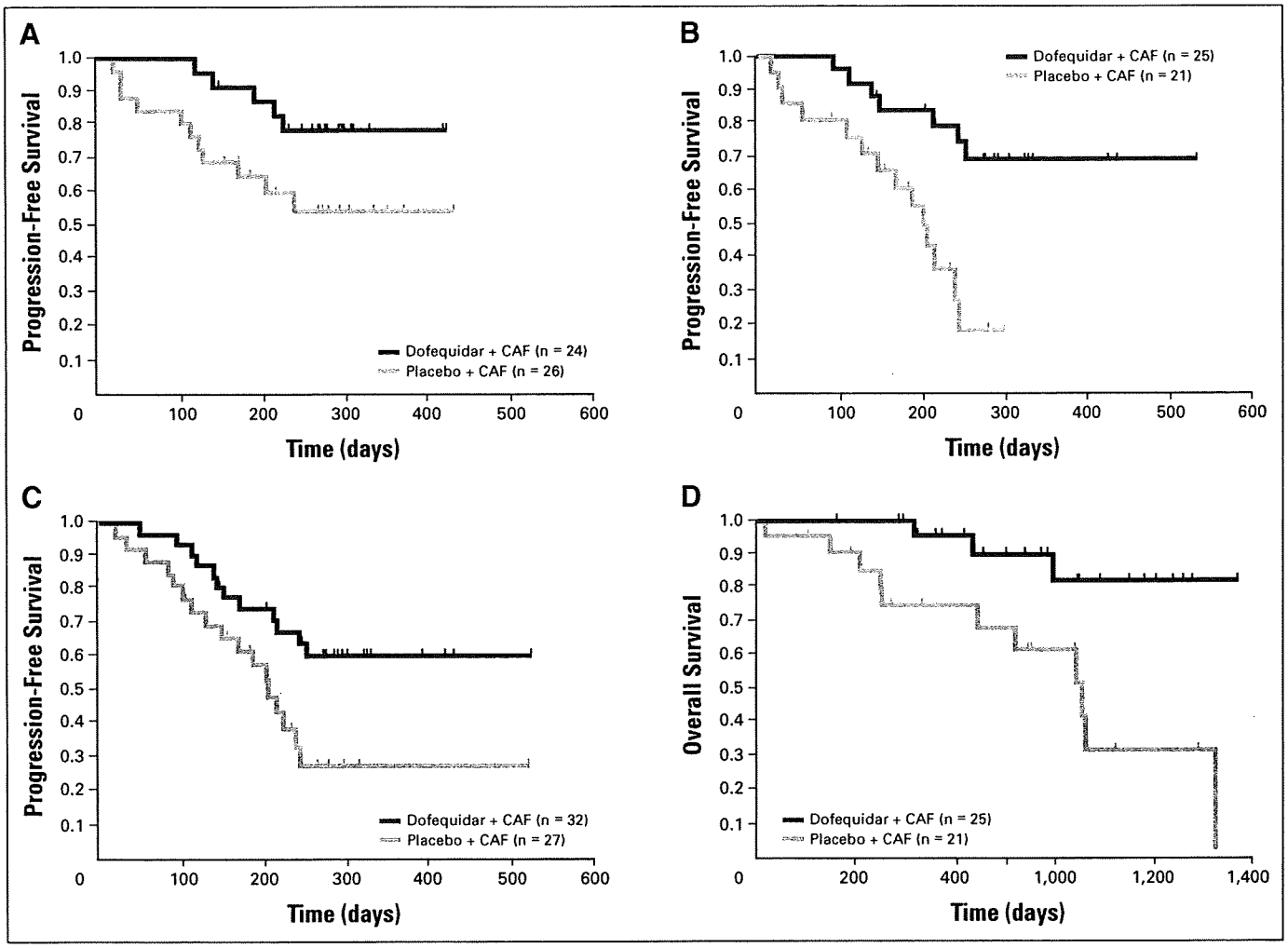

Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall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

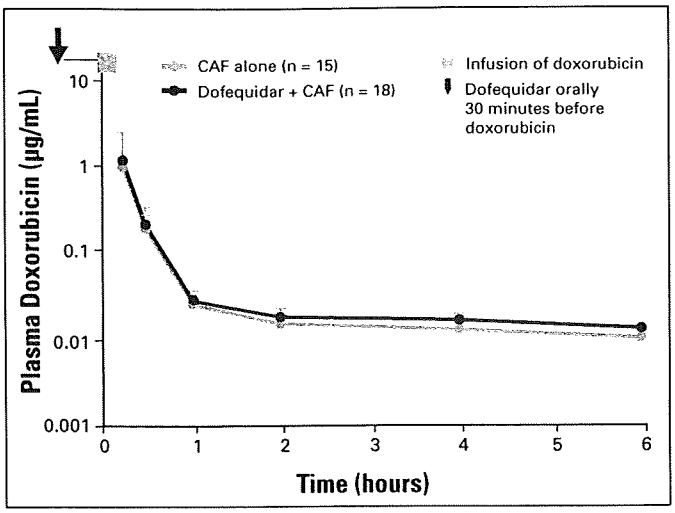

Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

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.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Toshiaki Saeki, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Hironobu Minami, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, Takashi Tsuruo

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Provision of study materials or patients: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda

Collection and assembly of data: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi

Data analysis and interpretation: Toshiaki Saeki, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Hironobu Minami, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato

Manuscript writing: Toshiaki Saeki, Wakao Sato

Final approval of manuscript: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, Takashi Tsuruo

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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．

Naohito Yamamoto, Masato Suzuki, Naoto Tanabe: Division of Breast Surgery, Chiba Cancer Center　千葉県がんセンター　乳腺外科

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>3 cm)	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 ≧2 cm	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)2 cm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3 cm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>3 cm 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—

柏葉匡寛[*1,2]　若林　剛[*1]　中村清吾[*2]　黒井克昌[*2]
岩田広治[*2]　大野真司[*2]　増田慎三[*2]　佐藤信昭[*2]
麻賀太郎[*2]　山本尚人[*2]　青儀健二郎[*2]　佐藤康幸[*2]
黒住昌史[*2]　津田　均[*2]　秋山　太[*2]　戸井雅和[*2]

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]([*1]Iwate Medical University, [*2]Japan 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
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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．

*1 岩手医科大学
*2 Japan Breast Cancer Research Group（JBCRG）