because patients without *UGT1A1*28* or **6* do experience severe irinotecan toxicity. Several studies have examined associations between irinotecan toxicity and *UGT1A* haplotypes in addition to each genotype of *UGT1A* (17-19). However, determining the haplotype or diplotype for each patient is difficult; moreover, most haplotypes and diplotypes are too rare to constitute a group large enough for meaningful statistical analysis. Moreover, gender and age of patients each reportedly have an impact on irinotecan toxicity (20-22). Hence, these factors should be also taken into consideration when developing a system designed to predict irinotecan toxicity.

The aim of this study was to evaluate whether the combinations of *UGT1A* genotypes, but not haplotypes, together with patient characteristics might be useful in predicting the risk to patients with mCRC treated of irinotecan-containing regimens. Here, we investigated the genotypes of 123 patients at six loci: *UGT1A1*6* (211G>A, rs4148323), *UGT1A1*28* (TA_6>TA_7, rs8175347), *UGT1A1*60* (-3279T>G, rs4124874), *UGT1A7* (387T>G, rs17868323), *UGT1A7* (622T>C, rs11692021), and *UGT1A9*1b* (-118T_9>T_{10}, rs35426722, also called *UGT1A9*22*) (23). Next, we evaluated the contribution of each *UGT1A* genotype, haplotype, and diplotype to the risk of irinotecan toxicity. Furthermore, we developed a new system for predicting the risk that a patient will experience irinotecan toxicity; this system uses sequential forward floating selection (SFFS) algorithm based on statistical pattern recognition to select the combinations of *UGT1A* genotypes, gender and age. SFFS is a sequential search method characterized by a dynamically changing number of features included or eliminated at each step of an individual analysis (24). This is the first study conducted to assess the role of the combination of genotypes at six polymorphic sites in *UGT1A* and clinical features constructed by SFFS on the risk of irinotecan toxicity.

Materials and methods

Patients. In this study, 123 mCRC patients were examined for association between *UGT1A* genotypes and irinotecan toxicity (Table I). This study was performed as an ancillary investigation; data collected from three prospective studies [FLIGHT1 (5), FLIGHT2 (5) and FRUTIRI (6)] and from consecutive patients who received FOLFIRI at the Department of Digestive Surgery and Surgical Oncology, Yamaguchi University Graduate School of Medicine, Japan. Each participant received irinotecan at the dose of 150 mg/m^2, which has been approved in Japan.

FLIGHT1 (UMIN000002388) and FLIGHT2 (UMIN000002476) were phase II studies of first line and second line chemotherapy, respectively, for mCRC. Study designs and key eligibility and exclusion criteria have been described in detail (5,25,26). Briefly, each regimen consisted of irinotecan on day 1 +400 mg/m^2 fluorouracil bolus followed by 2,400 mg/m^2 fluorouracil continuous infusion during 46 h + 200 mg/m^2 leucovorin on day 1 every 2 weeks. Of all patients from the FLIGHT1 and FLIGHT2 studies, 38 and 35, respectively, participated in this ancillary investigation and use; these 73 patients constituted the training population. FLIGHT1 or FLIGHT2 patients homozygous for *UGT1A1*28* were excluded from the training population because these patients received a lower starting dose of irinotecan (100 mg/m^2) (5).

The validation population comprised 50 patients from two different study groups: 22 patients who participated in FRUTIRI (UMIN000005011), a phase II study of a combination therapy comprised irinotecan and 5'-deoxy-5-fluorouridine (5'-DFUR) (6) and 28 consecutive patients who underwent second-line FOLFILI treatment between October, 2008 and July, 2012 in the Department of Digestive Surgery and Surgical Oncology, Yamaguchi University Graduate School of Medicine, Japan. Detail treatment regimen tested in FRUTIRI was described previously (6). Briefly, irinotecan was administered every two weeks, and 400 mg 5'-DFUR was administered every week orally twice a day on five consecutive days that were followed by a weekly 2-day washout. The 28 consecutive patients undergoing FOLFIRI treatment were following the protocol used in FLIGHT2 (26). In a validation population, patients with *UGT1A1*28* homozygous were not found in the FRUTIRI study (n=28). Additionally, patients heterozygous for *UGT1A1*28* (n=6) were excluded from the FRUTIRI study because these patients received lower starting dose of irinotecan 70 mg/m^2. Among the 28 consecutive patients who received second-line FOLFILI therapy, homozygous for *UGT1A1*6* or **28* and those compound heterozygous for *UGT1A1*6* and *UGT1A1*28* been excluded from this ancillary study. The training (n=73) and validation (n=50) populations did not differ significantly with regard to the distribution of any clinical feature or genotype that is listed in Table I except for the distributions of the *UGT1A7 (387T>G)* and *UGT1A9*1b* alleles (data not shown).

In this study, we defined patients who exhibited hematologic toxicity greater than grade 3 during the entire course of therapy as experiencing irinotecan toxicity. The study protocols were approved by the Institutional Review Board at Yamaguchi University Graduate School of Medicine, and were carried out in accordance with the Helsinki declaration on experimentation on human subjects. Each patient gave written, informed consent for their participation in this study.

Genotyping of UGT1A and haplotype construction. A conventional sodium iodide (NaI) method was used to extract genomic DNA from peripheral blood samples (27). The number of TA repeats in the *UGT1A1* promoter region was determined by the fragment size analysis followed by direct sequencing as described previously (4). The TaqMan technique with a hydrolysis probe was used to determine the *UGT1A1*6* genotype as described previously (28); similarly, hydrolysis probes were used to determine the genotypes at *UGT1A1*60*; a direct sequencing method was also used to determine the genotypes at *UGT1A7* (387T>G and 622T>C) and *UGT1A9*1b*.

Each nucleotide variant was evaluated to determine whether it was in Hardy-Weinberg equilibrium; Haploview 4.2 software was used to perform the linkage disequilibrium (LD) and case-control haplotype analyses (29). Lewontin's coefficient D' and correlation coefficient r^2 were calculated as measures of LD.

Construction of toxicity prediction system by genotype combinations. To predict severe toxicities of irinotecan, the age, the gender and a comprehensive 6-site *UGT1A* genotype were determined for each of the 73 patients in the training population. SFFS, a method of statistical pattern recognition, was

Table I. Characteristics of the patients.

Clinical features and genotypes	Total (n=123)	Sub-population (treatment regimen)				
		FLIGHT1[a] (n=38)	FLIGHT2[a] (n=35)	FRUTIRI[b] (n=22)	2nd-line FOLFILI[c] (n=28)	
Toxicity of irinotecan						
No	72	20	19	16	17	NS[d]
Yes	51	18	16	6	11	
Gender						
Male	78	24	24	17	13	NS[d]
Female	45	14	11	5	15	
Age						
≤60	50	14	14	9	13	NS[d]
>60	73	24	21	13	15	
*UGT1A1*6*						
-/-	84	25	23	15	21	NS[d]
-/**6*	36	12	11	6	7	
**6/*6*	3	1	1	1	0[c]	
*UGT1A1*28*						
-/-	103	32	27	22	22	NS[d]
-/**28*	20	6	8	0[b]	6	
**28/*28*	0	0[a]	0[a]	0[b]	0[c]	
*UGT1A1*60*						
-/-	71	19	21	15	16	NS[d]
-/**60*	46	17	12	6	11	
**60/*60*	6	2	2	1	1	
UGT1A7						
387T/T	41	13	12	8	8	NS[d]
387T/G	69	18	18	13	20	
387G/G	13	7	5	1	0	
UGT1A7						
387T/T	70	21	19	14	16	NS[d]
387T/G	48	15	13	8	12	
387G/G	5	2	3	0	0	
*UGT1A9*1b*						
**1b/*1b*	43	14	12	9	8	NS[d]
-/**1b*	67	17	18	12	20	
-/-	13	7	5	1	0	

The following patients were not enrolled in this study as described in Materials and methods. [a]Patients bearing *UGT1A1*28* homozygous were excluded from the FLIGHT1 and FLIGHT2 studies. [b]Homozygous and heterozygous of *UGT1A1*28* were not enrolled in the FRUTIRI study. [c]Homozygous of *UGT1A1*6* and **28* and compound heterozygous of *UGT1A1*6* and **28* were not included in the consecutive patients received second-line FOLFILI therapy. [d]NS, not significant among 4 groups by Fisher's exact test.

then used to determine the optimal genotype combinations for predicting the risk of irinotecan toxicity. The statistical pattern recognition, SFFS, identified the genotype combinations with the 'maximum number of cases' and 'maximum prediction rate' to maximize overall diagnostic accuracy (24). Briefly, the algorithm of the SFFS used in this study was as follows: i) Suppose that at stage k we have a set of $X_1, \ldots, X_k$ of sizes 1 to k, respectively. ii) Let the corresponding values of the feature selection criteria be J_1 to J_k, where $J_i = J(X_i)$, for the feature selection criterion $J(.)$. iii) Let the total set of features be X. Then at the kth stage of the SFFS procedure follow these steps: Step 1, select the feature x_j from X-X_k that increases the value of J to the greatest degree and add it to the current set: $X_{(k+1)} = X_k + x_j$. Step 2, find the feature x_r in the current set $X_{(k+1)}$ that reduces the value of J the least; if this feature is the same as x_j then set $J_{(k+1)} = J(X_{(k+1)})$; increment k; go to step 1; otherwise

Table II. Minor allele frequency and Hardy-Weinberg equilibrium in 123 patients.

	103 patients[a]		123 patients[b]	
	MAF	HWp	MAF	HWp
*UGT1A1*6* [211 (G>A)]	0.18	1.00	0.17	1.00
*UGT1A1*28* [$(TA)_6$>$(TA)_7$]	0.12	0.80	0.08	0.86
*UGT1A1*60* [-3279 (T>G)]	0.27	0.99	0.24	0.92
UGT1A7 [387 (T>G)]	0.42	1.00	0.39	0.07
UGT1A7 [622 (T>C)]	0.27	0.61	0.24	0.54
*UGT1A9*1b* [-118 (T_9>T_{10})]	0.41	0.84	0.38	0.13

[a]Patients enrolled in the FLIGHT1, FLIGHT2 and FRUTIRI studies (patients received lower starting dose of irinotecan were not excluded). [b]Patients subjected to case-control study (patients received lower starting dose of irinotecan were excluded). MAF, minor allele frequency. HWp, p-value of Hardy-Weinberg equilibrium.

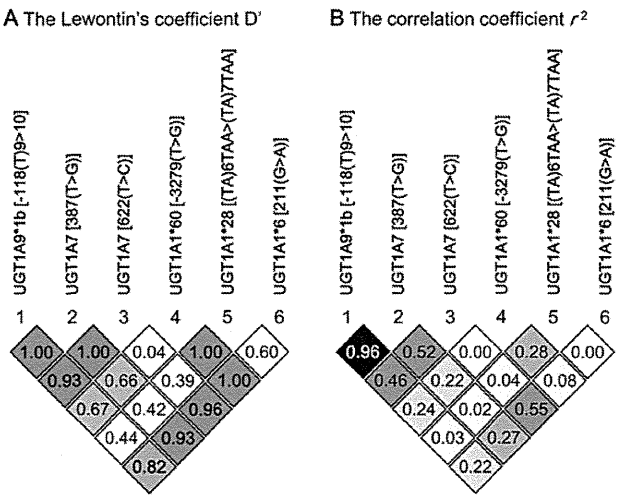

Figure 1. Pairwise linkage disequilibrium relationships between the *UGT1A* variants. (A) The Lewontin's coefficient D' and (B) the correlation coefficient r^2 are represented as values and colors [in panel A, log of the odds (LOD) ≥2 shades of pink/red, LOD <2 and D'=1 is blue, and LOD <2 and D' <1 is white. In panel B, r^2<0.01 is white, 0.01≤r^2 <0.95 is shades of grey, and r^2≥0.95 is black] in each box.

remove it from the set to from $X'_k = X_{(k+1)} - x_r$. Step 3, continue removing features from the set X'_k to form reduced sets $X'_{(k-1)}$ while $J(X'_{(k-1)}) > J_{(k-1)}$; $k = k - 1$; until $k = 2$; then continue with step 1. The algorithm is initialized by setting $k = 0$ and $X_0 = \emptyset$.

Statistical analysis. Fisher's exact test was used to assess the relationship between toxicity and each *UGT1A* variant. The Cochran-Armitage trend test was used to examine the linearity of the relationship between *UGT1A* genotypes and irinotecan toxicity. SPSS Statics 17.0 software (IBM, Tokyo, Japan) and R version 2.13.0 software were used to perform the calculations (30). p<0.05 was considered statistically significant.

Results

UGT1A allele and haplotype frequencies. The minor allele frequencies (MAF) of each *UGT1A* allele among the 103 patients without genetic bias; all patients regardless of the starting dose of irinotecan enrolled in FLIGHT1, FLIGHT2, and FRUTIRI studies, and 123 patients received a starting dose of 150 mg/m^2 for case-control study participating in this study are listed in Table II. In this study, the MAFs of *UGT1A1*28* and *UGT1A1*6* were approximately 0.117 and 0.184, respectively. The MAF for each other *UGT1A* SNP examined in this study was greater than 0.20. Among all patients, the Hardy-Weinberg equilibrium p-value for each locus examined in this study was higher than 0.05. LD analysis with 103 patients showed that high LD (r^2>0.9) was evident between *UGT1A7* (*387T>G*) and *UGT1A9*1b* (Fig. 1). We found 12 *UGT1A* haplotypes (*Hp-I* to *Hp-XII*) using 6 loci in 103 patients: *UGT1A1*6*, **28*, **60*, *UGT1A7* (*387T>G*), *UGT1A7* (*622T>C*), and *UGT1A9*1b* (Table III). Three common haplotypes (*Hp-I*, *Hp-II* and *Hp-III*) accounted for 82.5% of all haplotypes identified in this study.

Associations between UGT1A genotypes/haplotypes and irinotecan toxicity. We examined associations between individual *UGT1A* genotypes or haplotypes and severe irinotecan toxicity among 123 patients with mCRC who receive chemotherapy that included irinotecan (Table IV). Each of four *UGT1A* genotypes [*UGT1A1*6*, *UGT1A7* (*387T>G*), *UGT1A7* (*622T>C*) and *UGT1A9*1b*] showed a significant association to irinotecan toxicity and linear trend (p<0.05). Similarly, two haplotypes (*Hp-I* and *Hp-II*) each showed a significant association to and linear trend with irinotecan toxicity (p<0.05). Among two patients received a starting dose of 100 mg/m^2 irinotecan, diplotype of *Hp-IV/V* did not show toxicity and diplotype of *Hp-V/V* showed toxicity. Six patients excluded from FRUTIRI study did not show toxicity of irinotecan (a starting dose of 70 mg/m^2; *UGT1A* diplotypes of *Hp-I/V*, *II/IV* and *III/IV* were found in 2, 3 and 1 patients). Regarding non-hematological toxicities, only 5 patients developed grade 3 diarrhea (*UGT1A* diplotype of these 5 patients consists of 4 *Hp-I/II* and 1 *Hp-II/XII*).

Performances of the toxicity prediction system by genotype combination. To construct a system for predicting the risk of severe irinotecan toxicity, genetic data from 73 patients that constituted the training population were analyzed exhaustively; specifically, SFFS was used to assess gender, age and the individual genotypes at six polymorphic *UGT1A* sites (Fig. 2). In addition to the three possible genotypes (wild-type homozygous, heterozygous, variant homozygous), a fourth option for each site (designated 'unspecified genotype') was included into the algorithm. Similarly, patient gender (male, female, regardless of gender) and age (≤60, >60 years old, regardless of age) were assessed. The cutoff value for age (60 years) was determined by Youden index obtained by the receiver operating characteristic (ROC) curve analysis with the training population. Among possible combinations (4^6 x 3^2 - 1 = 36,863), the following cases were excluded: cases not found, single cases, and cases that represented positive or negative predictive values <80%. In order to optimize the combinations,

Table III. Haplotype frequency.

Haplotypes	*UGT1A* alleles						Allele frequencies	
	UGT1A9	*UGT1A7*			*UGT1A1*			
	**1b*	387T>G	622T>C	**60*	**28*	**6*	(n=103)[b]	(n=123)[c]
Hp-I	T_{10}	T	T	T	TA_6	G	0.524	0.573
Hp-II	T_9[a]	G[a]	C[a]	T	TA_6	A[a]	0.170	0.159
Hp-III	T_9[a]	G[a]	T	G[a]	TA_6	G	0.131	0.134
Hp-IV	T_9[a]	G[a]	C[a]	G[a]	TA_7[a]	G	0.063	0.041
Hp-V	T_{10}	T	T	G[a]	TA_7[a]	G	0.044	0.028
Hp-VI	T_9[a]	G[a]	C[a]	T	TA_6	G	0.015	0.016
Hp-VII	T_9[a]	G[a]	C[a]	G[a]	TA_6	G	0.015	0.012
Hp-VIII	T_9[a]	G[a]	T	G[a]	TA_7[a]	G	0.010	0.012
Hp-IX	T_{10}	T	T	G[a]	TA_6	G	0.010	0.008
Hp-X	T_{10}	G[a]	C[a]	T	TA_6	A[a]	0.010	0.008
Hp-XI	T_9[a]	G[a]	T	T	TA_6	G	0.005	0.004
Hp-XII	T_{10}	T	T	T	TA_6	A[a]	0.005	0.004

[a]Association of the alleles with toxicity of irinotecan. [b]Patients enrolled in the FLIGHT1, FLIGHT2 and FRUTIRI studies (patients received lower starting dose of irinotecan were not excluded). [c]Patients subjected to the case-control study (patients received lower starting dose of irinotecan were excluded).

A Combinations for cases with toxicity (Positive)

Name	*UGT* genotypes: *1A1*6* 211(G>A)	*1A1*28* $(TA)_6>(TA)_7$	*1A1*60* -3279(T>G)	*1A7* 387(T>G)	*1A7* 622(T>C)	*1A9*1b* $-118(T)_{9>10}$	Clinical features: Sex	Age	G3, G4/Total *n*	(%)
P-I	**	TA_6/TA_7	**	**	**	**	Female	>60	4/4	(100.0)
P-II	G/A	**	**	**	**	T_9/T_{10}	Male	>60	4/4	(100.0)
P-III	A/A	**	**	**	**	**	**	**	2/2	(100.0)
P-IV	**	TA_6/TA_7	**	G/G	T/C	**	**	**	2/2	(100.0)
P-V	**	TA_6/TA_6	T/G	**	T/C	**	**	>60	2/2	(100.0)
P-VI	G/A	**	**	**	**	T_9/T_{10}	**	>60	9/10	(90.0)
P-VII	**	**	**	**	T/C	**	**	>60	14/17	(82.4)
P-VIII	**	**	**	G/G	T/C	T_9/T_9	**	**	4/5	(80.0)
									19/22	**(86.4)**

B Combinations for cases without toxicity (Negative)

Name	*UGT* genotypes: *1A1*6* 211(G>A)	*1A1*28* $(TA)_6>(TA)_7$	*1A1*60* -3279(T>G)	*1A7* 387(T>G)	*1A7* 622(T>C)	*1A9*1b* $-118(T)_{9>10}$	Clinical features: Sex	Age	G0-G2/Total *n*	(%)
N-I	**	**	**	T/T	**	**	Male	≦60	6/6	(100.0)
N-II	G/G	TA_6/TA_7	**	**	**	**	**	≦60	5/5	(100.0)
N-III	G/G	TA_6/TA_6	T/G	**	**	**	Female	**	3/3	(100.0)
N-IV	G/G	TA_6/TA_7	T/G	**	**	**	Male	**	3/3	(100.0)
N-V	G/G	**	T/T	T/G	**	**	**	**	2/2	(100.0)
N-VI	G/A	**	**	**	C/C	**	**	**	2/2	(100.0)
N-VII	G/G	**	T/T	**	**	**	Male	**	12/15	(80.0)
N-VIII	**	**	T/T	**	**	T_{10}/T_{10}	Male	**	12/15	(80.0)
N-IX	**	**	T/T	**	**	**	**	≦60	12/15	(80.0)
N-X	G/G	TA_6/TA_6	**	**	**	**	Female	**	8/10	(80.0)
									33/40	**(82.5)**

Figure 2. The *UGT1A* genotype combinations that predict the presence or absence of severe irinotecan toxicity based on statistical pattern recognition. (A) A total of 8 combinations (P-I to P-VIII) for positive prediction of the toxicity and (B) 10 combinations (N-I to N-X) for negative prediction are presented. (A) The 8 combinations (P-I to P-VIII) that predict the presence of irinotecan toxicity are shown. (B) The 10 combinations (N-I to N-X) that predict the absence of irinotecan toxicity are shown. Eight factors-patient age, patient gender and genotypes at six *UGT1A* sites [*UGT1A1*6*, **28*, **60*, *1A7* (*387T>G*), *1A7* (*622T>C*) and *1A9*1b*] were used with sequential floating forward selection (SFFS) for statistical pattern recognition as described in Materials and methods. Homozygosity for alleles associated with irinotecan toxicity, heterozygosity and homozygosity for alleles not associated with irinotecan toxicity are indicated by red, blue and green cells, respectively. **The un-specified categories (regardless of genotypes, gender or age).

Table IV. Associations between *UGT1A* genotypes/haplotypes and irinotecan toxicity.

		Toxicity			p-value	
		Yes	No	(% of yes)	Fisher's exact	CA trend
Genotypes						
*UGT1A1*6*	-/-	27	57	(32.1)	0.002	0.001
	-/**6*	21	15	(58.3)		
	**6/*6*	3	0	(100.0)		
*UGT1A1*28*	-/-	40	63	(38.8)	0.218	-
	-/*1*28*	11	9	(55.0)		
	*1*28/1*28*	-	-	-		
*UGT1A1*60*	-/-	27	44	(38.0)	0.349	0.219
	-/*1*60*	20	26	(43.5)		
	*1*60/1*60*	4	2	(66.7)		
UGT1A7 (387T>G)	387T/T	9	32	(22.0)	0.005	0.002
	387T/G	34	35	(49.3)		
	387G/G	8	5	(61.5)		
UGT1A7 (622T>C)	622T/T	18	52	(25.7)	<0.001	<0.001
	622T/C	31	17	(64.6)		
	622C/C	2	3	(40.0)		
*UGT1A9*1b*	*9*1b/9*1b*	9	34	(20.9)	0.003	0.001
	-/*9*1b*	34	33	(50.7)		
	-/-	8	5	(61.5)		
Haplotypes						
Hp-I	0[a]	12	6	(66.7)	0.002	<0.001
	1[a]	32	37	(46.4)		
	2[a]	7	29	(19.4)		
Hp-II	0[a]	27	59	(31.4)	0.001	<0.001
	1[a]	22	13	(62.9)		
	2[a]	2	0	(100.0)		
Hp-III	0[a]	38	53	(41.8)	0.517	0.900
	1[a]	12	19	(38.7)		
	2[a]	1	0	(100.0)		
Clinical features						
Gender	Male	31	47	(39.7)	0.705	-
	Female	20	25	(44.4)		
Age	≤60	15	35	(30.0)	0.027	-
	>60	36	37	(49.3)		

[a]Number of alleles carried by the patient. CA, Cochran-Armitage trend test.

categorization according to predictive value and exclusion of redundant combinations in each category were performed. As a result, 8 combinations (P-I to P-VIII, Fig. 1A) appeared to predict an increased risk of toxicity, and 10 combinations (N-I to N-X, Fig. 1B) appeared to predict a lack of toxicity.

The system for predicting irinotecan toxicity based on combinations of 8 factors (6 genotypes, gender and age) was generated using data from of all 73 patients in the training population. The system was then applied to data from 84.9 and 86.0% of the patients in the training and validation populations, respectively (Table V). This prediction system showed 83.9% accuracy (positive predictive value, 86.4%; negative predictive value, 82.5%) for the training population (n=62) and 72.1% accuracy (positive predictive value, 70.0%; negative predictive value, 72.7%) for the validation population (n=43). When patients who were not applied to the combinations were included, the performance of the system was 71.2% accuracy (sensitivity, 55.9%; specificity, 84.6%) in training population (n=73) and 62.0% accuracy (sensitivity, 41.2%; specificity, 72.7%) in validation population (n=50). Odds ratios of positive prediction for irinotecan toxicity for this prediction system were 8.0 (95% CI, 1.5-42.5) and 16.3 (95% CI, 2.2-121.4) in training and validation populations, respectively ($p<0.05$, Table VI).

Patients with either of three *UGT1A* alleles [*UGT1A1*6*, *UGT1A7* (*622T>C*) or *UGT1A9*1b*], *UGT1A haplotype-I* or *haplotype-II* showed significant association to severe irinotecan toxicity ($p<0.05$) in both the training and validation populations (data not shown).

Table V. Predicitive performance for irinotecan toxicity by the genotype combinations.

	Training (n=73)		Validation (n=50)	
	n	(%)	n	(%)
Matched with the combination[a]	62/73	(84.9)	43/50	(86.0)
Accuracy in applied patients	52/62	(83.9)	31/43	(72.1)
Positive predictive value[b]	19/22	(86.4)	7/10	(70.0)
Negative predictive value[b]	33/40	(82.5)	24/33	(72.7)
Accuracy	52/73	(71.2)	31/50	(62.0)
Sensitivity	19/34	(55.9)	7/17	(41.2)
Specificity	33/39	(84.6)	24/33	(72.7)

[a]The combination consists of 8 factors; 6 genotypes [*UGT1A1*6*, *UGT1A1*28*, *UGT1A1*60*, *UGT1A7* (387T>G), *UGT1A7* (622T>C) and *UGT1A9*1b*], gender and age. [b]Prediction of severe toxicity is positive and prediction of no severe toxicity is negative.

Discussion

The novel system for predicting severe irinotecan toxicity described here was based on genotypes at 6 polymorphic sites in *UGT1A* and 2 basic clinical features; notably, it showed high predictive performance even though the treatment regimens differed among the training and validation patients (Tables V and VI). The odds ratio of positive prediction for severe irinotecan toxicity was higher for this prediction system than for that of any other haplotype or for that of any genotype (Table VI). The performance of this prediction system was reduced from the 83.9% accuracy seen with applied patients to this system in the training population to 72.1% accuracy in the validation. With regard to positive prediction, the inconsistency in accuracy between training and validation populations was seen when the combinations included the *UGT1A9*1b* site and patient age (P-II, VI and VII in Fig. 2). The frequencies of *UGT1A9*1b* genotype differed between the training and validation populations; moreover, the *UGT1A9*1b* alleles were not in Hardy-Weinberg equilibrium in the validation population (data not shown). The cutoff value for patient age (60 years old) was determined by a ROC curve generated with data from the training population; however, previous studies used a cutoff age of 65 years (20,21). Indeed, one patient without toxicity, but predicted as presence of toxicity in this system, was aged 63 years.

Some genotypic combinations decreased the performance of negative prediction for sever irinotecan toxicity in the validation population relative to the training population (N-II, IV, and V in Fig. 2). Specifically, 36.4% (n=4/11) of patients in training population with a combined genotype that included heterozygous for *UGT1A1*28* alleles and *UGT1A1*6* (-/-) experienced severe irinotecan toxicity, but 66.7% (n=4/6) of the patients in validation population with the same genotype combinations (*UGT1A1*6*, -/- and *UGT1A1*28*, -/+) showed severe toxicity. Of the 73 patients in the training population and the 50 in the validation population, 11 (15.1%) and 7 (14.0%), respectively, were matched with neither of the combination in our prediction system. Interestingly, the incidence of severe toxicity among patients who were not matched with either combination identified by this prediction system was 72.7% (training population) and 14.3% (validation population) (Table VI). Therefore, the frequency of the irinotecan toxicity among patients who do not have any combination of *UGT1A* variants identified by this novel prediction system might be due to factors other than *UGT1A* polymorphisms.

Many published studies have focused on associations between irinotecan toxicity, irinotecan efficacy, or both and any one or more of each *UGT1A* variants examined here (10-19,31,32). Patients, especially Asian patients, homozygous for *UGT1A1*6* or **28* or compound heterozygous for these variants are at high risk for hematologic toxicity (13,33,34). In this study, each patient homozygous for *UGT1A1*6* (n=3) and those compound heterozygous for *UGT1A1*6* and **28* (n=3) showed severe hematologic toxicity; however, 45 patients of the remaining 117 patients still exhibited severe irinotecan toxicity. *UGT1A1*6* and **28* each have strong effects on UGT1A1 activity and expression, but frequency of each allele is low; moreover, the frequencies of each allele differ between races (11,14,35-37). Among the patients that lacked these rare, highly effective variants, this novel prediction system could accurately predict whether there is severe irinotecan toxicity.

Here, as in previous studies, each identified *UGT1A* haplotypes was useful for precisely predicting the presence or absence of severe irinotecan toxicity (14,18,38-40). Consistent with our study, Cecchin *et al* reported that a haplotype comprising *UGT1A1*28* (-), *UGT1A1*60* (-), *UGT1A7* (*387T* and *622T*), and *UGT1A9*1b* (+) was a predictor of severe hematologic toxicity during the entire course of therapy (18). However, determining the haplotypes for any one patient is a difficult clinical measurement. Therefore, the genotypes at each of the 6 sites (rather than the haplotype or diplotype) could be used for clinical assessments.

Our prediction system depend not only on *UGT1A* genotypes but also on patient gender and age. Previous studies showed that patient gender and age were related to the risk of irinotecan toxicity (20-22). In the training population, patient age was associated with severe irinotecan toxicity, but patient gender was not (Table IV). Interestingly, when patient age, patient gender or both the patient age and gender were excluded from the factors used by the prediction system, the

Table VI. Associations between UGT1A genotypes/haplotypes and irinotecan toxicity in training and validation sub-populations.

	Training (n=73)						Validation (n=50)					
	Toxicity			Fisher's exact test			Toxicity			Fisher's exact test		
	Yes	No	(% of yes)	OR	(95% CI)	p-value	Yes	No	(% of yes)	OR	(95% CI)	p-value
Haplotypes												
Hp-I (+/+)	5	15	(25.0)	1[c]			2	14	(12.5)	1[c]		
Hp-I (-/-, -/+)	29	24	(54.7)	3.63	(1.15-11.42)	0.035	15	19	(44.1)	5.53	(1.08-28.18)	0.053
Hp-II (+/+, -/+)	16	8	(66.7)	0.64	(1.60-22.48)	0.008	8	5	(61.5)	11.20	(1.75-71.64)	0.016
The predicition system[a]												
Negative for toxicity	7	33	(17.5)	0.64	(0.17-2.34)	0.511	9	24	(27.3)	2.63	(0.50-13.92)	0.300
Positive for toxicity	19	3	(86.4)	8.00	(1.51-42.45)	0.021	7	3	(70.0)	16.33	(2.20-121.43)	0.009
Not matched[b]	8	3	(72.7)				1	6	(14.3)			
Genotypes												
*UGT1A1*6* (+/+, -/+)	16	9	(64.0)	5.33	(1.45-19.58)	0.016	8	6	(57.1)	9.33	(1.51-57.65)	0.019
*UGT1A1*28* (-/+)	7	7	(50.0)	3.00	(0.70-12.88)	0.163	4	2	(66.7)	14.00	(1.47-133.23)	0.025
*UGT1A1*60* (+/+, -/+)	18	15	(54.5)	3.60	(1.06-12.22)	0.048	6	13	(31.6)	3.23	(0.55-18.96)	0.244
UGT1A7 (387G/G, T/G)	27	21	(56.3)	3.86	(1.21-12.33)	0.032	15	19	(44.1)	5.53	(1.08-28.18)	0.053
UGT1A7 (622C/C, T/C)	20	13	(60.6)	4.62	(1.35-15.78)	0.022	13	7	(65.0)	13.00	(2.27-74.32)	0.002
*UGT1A9*1b* (-/-, -/+)	27	20	(57.4)	4.05	(1.26-12.99)	0.018	15	18	(45.5)	5.83	(1.14-29.84)	0.028

[a]The prediction system consisted of the combinations of 8 factors (6 genotypes, gender and age). [b]In training and validation populations, 9/73 (12.3%) and 7/50 (14.0%) patients were not matched with the combinations of the prediction system. [c]Reference category.

number of patients that matched with the prediction system decreased, although the system maintained the high positive and negative predictive values (data not shown).

The SFFS algorithm could be modified to include other factors (e.g., mutations in the tumor, patients' clinical characteristics, additional genetic variants, etc.) to improve the prediction performance. Such modifications may result in a system that could meaningfully predict clinical outcomes, including tumor response. Recent advances in technology for sequencing whole genomes of individuals may lead to substantial increases in information that might be useful for personalized therapy. However, such complicated information could not be efficiently or fully utilized in the currently available formats. SFFS could easily construct a system that can utilize huge data sets such as whole-genome sequences. Our strategy for developing SFFS-based systems for clinical use could serve as a powerful tool for advancing personalized therapy, although additional prospective study of this prediction system is needed.

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Clinical Trial Note

Observational Study of Axilla Treatment for Breast Cancer Patients with 1–3 Positive Micrometastases or Macrometastases in Sentinel Lymph Nodes

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Sentinel node biopsy is a standard procedure in clinically node-negative breast cancer patients. It has eliminated unnecessary axillary lymph node dissection in more than half of the early breast cancers. However, one of the unresolved issues in sentinel node biopsy is how to manage axilla surgery for sentinel node-positive patients and clinically node-negative patients. To evaluate the outcome of no axillary lymph node dissection in sentinel node-positive breast cancer, a prospective cohort study registering early breast cancer patients with positive sentinel nodes has been conducted (UMIN 000011782). Patients with 1–3 positive micrometastases or macrometastases in sentinel lymph nodes are eligible for the study. The primary endpoint is the recurrence rate of regional lymph nodes in patients treated with sentinel node biopsy. Patients treated with sentinel node biopsy followed by axillary lymph node dissection are also registered simultaneously to compare the prognosis. The propensity score matching is used to make the distributions of baseline risk factors comparable.

Key words: sentinel node biopsy – breast cancer – propensity score – lymph node dissection

INTRODUCTION

Until the 21st century, axillary lymph node dissection (ALND) was a standard procedure for operable breast cancer patients. However, it can cause lymphedema, peripheral nerve injury, shoulder dysfunction and other complications that compromise functional activity and quality of life. Sentinel node biopsy (SNB) is the most accurate method for detecting axillary lymph node metastases in early breast cancer. Large clinical trials that compared SNB with ALND were launched in the 1990s. The National Surgical Adjuvant Breast & Bowel Project (NASBP) B32 showed that SNB provided an outcome equivalent to that of SNB + ALND for sentinel node-negative patients (1). In this study, occult metastases that were found in negative sentinel lymph nodes with a detailed histological examination had a very small impact on the prognosis, since adjuvant therapy could have reduced systemic dissemination of cancer cells (2).

Other randomized trials showed that ALND provided no clinical benefit in some breast cancer patients with positive sentinel lymph nodes. The American College of Surgeons Oncology Group (ACOSOG) Z0011 demonstrated that in patients with micrometastases or 1–2 macrometastases in sentinel lymph nodes who received SNB, breast-conserving surgery, breast irradiation and adjuvant therapy, the 5-year recurrence rate of axillary lymph nodes was 0.9% and not different from patients treated with ALND (3). Based on the results of the International Breast Cancer Study Group (IBCSG) 23-01, patients with micrometastases should be treated with SNB alone, since the regional recurrence rate at 5 years was 1% (4). The AMAROS trial suggested that regional node irradiation was effective for loco-regional control in sentinel node-positive breast cancer patients treated with SNB alone instead of SNB + ALND (5). In some institutes, patients who are eligible for ACOSOG Z0011 undergo no ALND in clinical practice (6). However, each one of these trials had some limitations. Patients registered in ACOSOG Z0011 and AMAROS tended to have small breast tumors and a small tumor burden in sentinel lymph nodes. Such patients might have no additional metastases in non-sentinel lymph nodes. How to incorporate these results into clinical practice is still unclear (7). In IBCSG 23-01, the histological diagnosis of micrometastases was strictly performed using lymph node specimens cut at 50–200 μm. This method is far from actual clinical practice. Indeed, a SNB registry trial in the Netherlands found that the 5-year regional recurrence rate after SNB alone in patients with micrometastases in sentinel lymph nodes was 5.6% (8).

On the other hand, personalized medicine based on the intrinsic subtype of breast cancer can eliminate breast tumor and nodal metastases. In ~30% of cases of triple-negative breast cancer and HER2-enriched breast cancer, neoadjuvant chemotherapy could achieve a complete pathological response of breast tumor and nodal metastases (9). These results again raised the question of whether ALND is always needed for node-positive breast cancer patients after neoadjuvant chemotherapy. The Japanese Society for Sentinel Node Navigation Surgery was founded in 1996 and a prospective study on SNB in breast cancer was reported (10). To evaluate the clinical benefit of SNB without ALND in sentinel node-positive breast cancer, we planned a cohort study to register patients with positive sentinel nodes prospectively. This study was approved by the institutional review board at Kyorin University in September 2013 and registered at the UMIN Clinical Trials Registry as UMIN000011782 (http://www.umin.ac.jp/ctr/index.htm).

PROTOCOL DIGEST OF THE STUDY

Objectives

The purpose of this study is to evaluate the prognosis of sentinel node-positive breast cancer patients treated with SNB alone. The secondary purpose is to compare the prognosis of the patients treated with SNB alone to those treated with SNB followed by ALND. To reduce the bias associated with the lack of randomization, we use the propensity score matching method to adjust unbalanced clinicopathological factors in the both groups.

Study Setting

A multi-institutional prospective cohort study.

Endpoints

The primary endpoint is the recurrence rate of regional lymph nodes for patients treated with SNB after primary treatment of breast cancer. The secondary endpoint is the 5-year overall survival. Primary treatment is defined as breast surgery including SNB with or without ALND, neoadjuvant therapy or SNB to diagnose lymph node metastases prior to neoadjuvant therapy. The time to regional lymph node recurrence is counted from the date of primary treatment. It is censored at the earliest day of either local recurrence, contralateral breast cancer, distant metastases, other malignant disease or death from any cause. Overall survival is defined as the duration from primary treatment to death from any cause. It is censored at the last day when the patient is alive.

Eligibility Criteria

Inclusion Criteria

(i) Female patients aged 20–70 years.
(ii) T1-3N0-1M0 in the eighth edition of the UICC TNM classification.
(iii) Histological confirmation of invasive disease in the breast.
(iv) SNB was performed or scheduled after 1 January 2012.
(v) SNB or SNB followed by ALND should be performed. SNB and the sampling of Level I lymph nodes is acceptable and considered SNB.
(vi) One to three positive sentinel lymph nodes with micrometastases and/or macrometastases confirmed by histological or molecular diagnosis.

Exclusion Criteria

(i) Ductal carcinoma *in situ* or lobular carcinoma *in situ* in the breast.
(ii) Synchronous or metachronous bilateral breast cancer.
(iii) Four or more sentinel lymph nodes with micrometastases and/or macrometastases except for isolated tumor cells
(iv) Past history of invasive disease within 5 years before registration.
(v) Physician's discretion due to the patient's condition (e.g. severe co-morbidity, psychiatric disorder, pregnancy, refusal to undergo appropriate surgery for breast cancer).

(vi) Failure of SNB, or histologically false-negative sentinel lymph nodes.

Treatment Methods

Breast cancer treatment consists of breast surgery, adjuvant therapy and radiation therapy. Breast surgery includes SNB, ALND or both, and partial or total mastectomy with or without breast reconstruction. Adjuvant therapy includes chemotherapy, endocrine therapy and anti-HER2 therapy before and after breast surgery. Radiation therapy covers fields that include the breast, chest wall or regional lymph nodes. In this study, physicians will follow clinical practice for breast cancer patients according to imaging diagnosis and the intrinsic subtype of breast cancer as confirmed by core-needle biopsy or resected specimens. There is no surgical protocol with regard to SNB, ALND, type of mastectomy or breast reconstruction. Adjuvant therapy and radiation therapy also depend on the physician's discretion.

A histological diagnosis of sentinel lymph nodes is performed following the institution protocol, but it is recommended that physicians use lymph node specimens sliced at 2 mm intervals and stained with hematoxylin–eosin. Molecular diagnosis by the one-step nuclear amplification (OSNA) method is used worldwide instead of the histological examination of sentinel lymph nodes, and is allowed in this study (11).

Observation

The participants will be followed-up every 6 months until 5 years after primary treatment. Routine examination is recommended following the American Society of Clinical Oncology Clinical Practice Guidelines. If recurrence is suspected, an appropriate imaging diagnosis and histological confirmation should be performed.

Study Design

Our objectives are to estimate regional lymph node recurrence of the patients treated with SNB and to compare them to patients treated with SNB followed by ALND. Although an observational study cannot provide the same definitive evidence as a randomized trial, some statistical methods should be able to reduce the bias associated with the lack of randomization. In this study, we use the propensity score matching method to compare SNB to SNB followed by ALND in sentinel node-positive patients.

Utility of the Propensity Score Matching Method

In an observational study, treatment selection could be influenced by the patient's characteristics. Therefore, the distributions of risk factors such as age, stage, and severity differ between the treatment groups. To compare the outcome between groups without the effect of bias due to treatment selection, we use a propensity score matching method in this study. The propensity score is defined as a patient's probability of receiving a specific treatment conditional on the observed risk factors (12). In this study, an individual probability of being treated with SNB alone is the propensity score. It is estimated for each patient using the logistic model based on the observed risk factors. Risk factors included in the logistic model are selected from the observed baseline data after the close of enrollment. We plan to implement 1:1 or 1:2 greedy matching (13) with the propensity score.

Statistical Analysis

Based on an estimated regional lymph node recurrence rate of 5% at 5 years among patients treated with SNB, 240 patients are needed to give 80% power to reject the null hypothesis that the recurrence rate is 10% with a one-sided type I error rate of 2.5%. If we consider that some patients will be lost to follow-up or become ineligible, a total of 250 patients treated with SNB only will be needed to comprise the sample. At the same time, as many eligible patients as possible who are treated with SNB followed by ALND are also enrolled to constitute a control pool for comparison of regional lymph node recurrence.

Regional lymph node recurrence is estimated by considering all eligible patients with SNB only by the Kaplan–Meier method and the 95% confidence interval (CI) is computed using Greenwood's formula (14). The hazard ratio and its 95% CI of regional lymph node recurrence is estimated by the Cox regression model and a robust sandwich estimate of variance with matched samples. Basically, multivariate adjustment with other prognostic variables is not planned for comparison the patients treated with SNB only and SNB followed by ALND.

Participating Institutions

Asahikawa Medical University, Keio University, Kyorin University, Kyoto Prefectural University of Medicine, National Cancer Center Hospital East, Osaka National Hospital, Tonan Hospital, University of Kurume Faculty of Medicine.

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Conflict of interest statement

Toh has received consulting fees from Chugai Pharma and AstraZeneca plc. Other authors have no conflict of interest to declare.

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APPENDIX

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Phase II Study of S-1 in Combination with Trastuzumab for HER2-positive Metastatic Breast Cancer

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Abstract. *Aim: We undertook a prospective phase II study to evaluate the efficacy of S-1 plus trastuzumab combination regimen for human epidermal-growth factor receptor-2 (HER2)-positive metastatic breast cancer (MBC). Patients and Methods: HER2-positive MBC patients received oral administration of S-1 (80 mg/m^2/day, days 1 to 28, every 6 weeks) and intravenous weekly trastuzumab (2 mg/kg), according to the results of a prior Phase I trial of our group. Results: A total of 28 patients were enrolled and received a median of 3.5 (range 1-10) cycles of treatment. Overall response rate and clinical benefit rate were 53.6% and 75.0%, respectively. Progression-free survival was 30 weeks. With regard to grade 3 and 4 adverse effects, leucopenia, neutropenia, increase in serum alanine aminotransferase, and diarrhea were observed. Conclusion: Combination of S-1 and trastuzumab was tolerable and had excellent efficacy with good response and disease control in this trial.*

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Key Words: HER2-positivity, metastatic breast cancer, S-1, trastuzumab, first-line therapy.

Trastuzumab, a humanized monoclonal antibody against the extracellular domain of human epidermal growth factor receptor type-2, has shown high clinical efficacy in combination with cytotoxic agents for HER2-overexpressing breast cancer. After this agent was approved, prognosis of the patients with HER2-positive advanced or metastatic disease has become superior to that of estrogen receptor-negative disease (1).

According to the Japanese Breast Cancer Society guidelines (2) and National Comprehensive Cancer network guidelines (3) chemotherapy is recommended for advanced or metastatic breast cancer that is refractory to hormonal therapy. The first and second choices of cytotoxic agents are either anthracyclines or taxanes. The options for the third-line or later treatment comprised of capecitabine, S-1, vinorelbine, irinotecan, gemcitabine, and eribulin. For HER2-positive

disease, paclitaxel was the first established cytotoxic agent to be combined with trastuzumab (4). *In vitro* studies demonstrated additive antitumor effect of the combination with docetaxel, vinorelbine, platinum derivatives as synergistic effects, however fluorinated pyrimidine showed antagonistic effect (5). A similar pre-clinical study using a xenografted HER2-positive tumor demonstrated less effect when trastuzumab was combined with fluorinated pyrimidine agents, therefore, this combination was not commonly used in upfront lines, and clinical data has not been sufficient (5).

Capecitabine combined with trastuzumab was investigated in a prospective analysis of 40 consecutive heavily-treated patients as salvage therapy. Contrary to the *in vitro* tests (5), clinical outcome of patients treated by the capecitabine-plus-trastuzumab regimen in this study demonstrated a favorable effect of 20% in overall response rate (ORR) and 70% in clinical benefit rate (CBR) with acceptable toxicity (6).

Encouraged by this result of capecitabine, another fluorinated pyrimidine agent S-1 was highlighted as a promising cytotoxic companion for trastuzumab. S-1 was originally developed for the treatment of gastrointestinal tract cancers and has been widely used in Asian countries including Japan, especially as the key drug for the treatment of gastric cancer both in advanced (7, 8) and adjuvant setting (9).

In vitro studies of S-1 in combination with trastuzumab have already shown its favorable efficacy against several cancer cell types (10, 11). In a clinical setting, the result of our phase I trial demonstrated that the trastuzumab-plus-S-1 regimen had fewer serious or unpleasant adverse events, such as alopecia, nausea, vomiting, and febrile neutropenia, compared to other cytotoxic regimens commonly combined with trastuzumab (12). Based on the findings of this phase I study, we underwent the present phase II trial to test the clinical efficacy of S-1 in combination with trastuzumab for metastatic breast cancer (MBC).

Patients and Methods

Patients. Patients with inoperable or recurrent breast cancer from 12 participating Institutions were enrolled in the study, to be treated with S-1 in combination with trastuzumab from February 2007 to February 2012.

A total of 37 assessable patients were required to test the null hypothesis that the true confirmed response rate is at most 30% *versus* the alternative that it is at least 50%.

Unfortunately, analysis at the point in time was urged by independent data monitoring committee because the accumulation of the cases did not reach to the projected numbers at 5 years after the initiation of the trial.

Women aged ≥20 years with a histological or cytological diagnosis of HER-2-positive (IHC 3+ or IHC 2+/FISH+) MBC were considered to be eligible for the trial. Eligibility required measurable cancer by RECIST criteria version 1.1 (13): baseline left ventricular ejection fraction (LVEF) >55%; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2; expected survival >6 months; adequate organ function defined as hemoglobin >9 g/dl, leukocyte count 3,000-12,000/mm^3, neutrophil count >1,500/mm^3, platelet count >100,000/mm^3, serum total bilirubin level less than the upper level of normal in each institution x1.5, and serum creatinine level less than upper limit of normal. Treatment within one prior chemotherapy regimen was permitted for those with metastatic disease.

Patients with any of the following were excluded: lung metastasis with dyspnea; brain metastasis with symptoms; a second primary cancer; serious concomitant illness; cardiac abnormalities; or cases with possible infection. The protocol was reviewed and approved by the Institutional Review Boards at all participating centers. Written informed consent was obtained from all the patients.

Treatment. Patients received S-1 in combination with trastuzumab according to the recommended dose defined by the phase I trial (12). Trastuzumab was administered intravenously every week. The first dose was 4 mg per kilogram (kg) of body weight and the subsequent doses 2 mg per kg, administered over periods of 90 and 60 min, respectively. S-1 was administered in a daily dose of 80 mg/m^2 orally, divided two after meals for 4 weeks, followed by a 2 weeks rest as one course. Study treatments continued until disease progression, appearance of unmanageable toxicity, or patients' request to withdraw from the study.

Safety. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (14). Hematology and biochemistry assessments were performed before the start of each treatment cycle. LVEF was monitored by echocardiography at least every 12 weeks. Chemotherapy dose adjustments were allowed. Trastuzumab toxicity was managed by treatment interruptions.

Efficacy. The primary end-points were ORR and CBR, that were evaluated according to the RECIST criteria version 1.1 (13) until disease progression. ORR was defined as the proportion of all patients with complete response (CR) and partial response (PR). CBR was defined as the proportion of the patients with CR+PR +stable disease (SD) continued longer than 24 weeks. CR and PR required confirmation at least 4 weeks after first being reported.

The secondary end-points are overall survival (OS); progression-free survival (PFS); safety profile; CBR; and health related quality of life (HRQOL). HRQOL was accessed before starting the chemotherapy and every each course of the chemotherapy by EORTC QLQ-C30 questionnaire (15). The data of each questionnaire were processed along the EORTC QLQ-C30 scoring manual (16).

Results

Patients' characteristics. A total of 28 patients were enrolled. A completed survey form was collected from all patients. All patients were treated with a fixed dose (initial dose: 4 mg/kg, maintenance treatment dose: 2 mg/kg) of trastuzumab in combination with 80 mg/m^2/day of S-1. The baseline clinical characteristics are summarized in Table I. The median age was 62 (range 45-78) years. Eight patients had distant metastasis when breast cancer was first diagnosed, and 20 patients had recurrent breast cancer. Fifteen patients had hormonal receptor (HR)-positive (ER+ and/or PR+); 13

Table I. *Baseline patient characteristics (N=28).*

Patients' characteristics	
Age median (Range)	62 (45-78)
ECOG Performance Status	
PS 0	14
PS 1	12
PS 2	2
Hormonal Receptor	
Positive	13
Negative	15
Progesteron Receptor	
Positive	8
Negative	19
Unknown	1
Metastatic site	
Lung	9
Bone	5
Liver	7
Lymph node	18
Others	2
Prior Surgery	
Yes	20
No	8
Adjuvant Therapy	
Chemotherapy	17
Endocrine Therapy	12
Prior Therapy for Metastatic Disease	
Cytotoxic Chemotherapy	2
Trastuzumab Monotherapy	3
Endocrine Therapy	9

Table II. *Tumor response (N=28).*

Response rates	
CR	3 (10.7%)
PR	12 (42.9%)
SD≥24wks	6 (21.4%)
SD<24wks	4 (14.3%)
PD	1 (3.5%)
NE	2 (7.1%)

patients had HR-negative tumors. The sites of metastatic disease were the bone and/or soft tissues in 24 patients and visceral sites in 17 patients, including duplication. The majority of patients (n=23) received S-1+ trastuzumab treatment as first-line therapy for MBC. Only 2 patients received cytotoxic chemotherapy and 3 patients received trastuzumab monotherapy before registration. Nine patients received endocrine therapy for MBC before registration.

Efficacy measured by response rate and clinical benefit rate. All the patients had measurable lesions and received the recommended dose according to the result of the phase I trial (12). Three patients achieved CR, 12 patients showed PR, and 10 patients showed SD (6 patients longer than 24 weeks and 4 patients shorter than 24 weeks). A patient resulted in progression without response and 2 patients could not evaluate the efficacy. Therefore the ORR was 53.6% (15/28), and the CBR was 75.0% (21/28) (Table II). In the sub-group of patients without previous cytotoxic chemotherapy for MBC, ORR was 74.3% (15/21). These results of the primary end-points indicate that the combination therapy regimen was highly effective to HER-2-positive breast cancers, especially for patients who had never received cytotoxic chemotherapy for metastatic disease. No difference was demonstrated between the HR positive sub-group and negative sub-group (HR +; CR 1, PR 6, SD 5, PD1. HR–; CR 2, PR 6, SD 5, PD 0, NE 2).

Prognosis estimated by overall and progression-free survival. By the time of data cut-off, PFS events had been observed in 22 patients (79%). Median PFS was 30 weeks (95% CI= 26-59 weeks) (Figure 1). Nine out of 10 patients died from breast cancer. Median OS was 227 weeks (95% CI= 141- ∞ weeks) (Figure 2).

Toxicity. All the patients were assessed for toxicities during the treatment cycles. No grade 4, and a few grade 3 toxicities were observed in all enrolled patients. Although Grade 3 hematological toxicities were observed in 7 patients including leukopenia and neutropenia, they were not critically severe. With regard to non-hematological toxicities, the most common events were nausea, diarrhea, and elevation of serum alkaline phosphatase. The hematological and non–hematological events in all the 28 patients are listed in Table III.

Clinical benefit response and health-related quality of life. Sixteen patients cooperated with the first and the later questionnaire survey. In this QLQ-C30 questionnaire, Osoba *et al.* (17) reported that the patients who reported 'very much' change for better or worse on a particular scale corresponded to a change greater than 20. Our study demonstrated the patients who showed improvement of QOL with reduction of score more than 20 points were 6.3% in physical function, 12.5% in role function and emotional function, 18.8% in cognitive function, 25% in social function and 0% in general health status.

Discussion

Although the most important objective of metastatic breast cancer treatment is to prolong survival with good patients' QOL (18), chemotherapeutic agents have been chosen in the

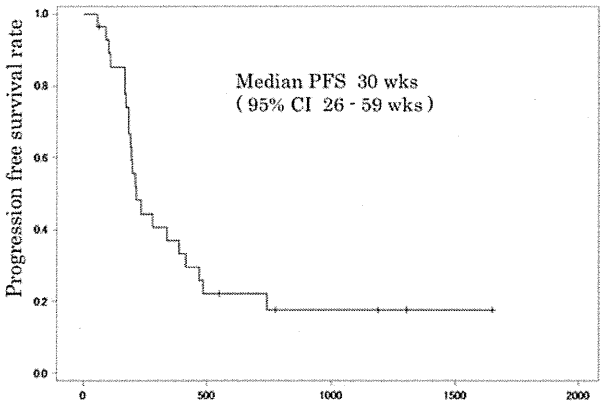

Figure 1. *Kaplan–Meier Estimates of Progression-free Survival Rates. Bars represent patients whose data were censored.*

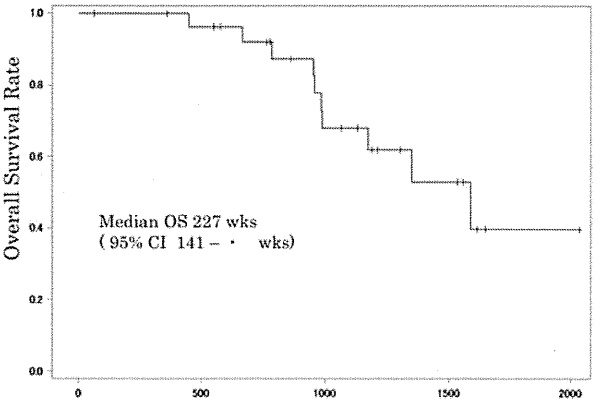

Figure 2. *Kaplan–Meier Estimates of Overall Survival Rates. Bars represent patients whose data were censored.*

order of their effectiveness for shrinking tumors, rather than their intensity of side-effects. In line with this objective, less toxic treatment should be chosen in up-front line as long as the treatment could control disease progression. Generally, response and duration of efficacy of the chemotherapeutic agent are better when it administered in up-front line than late line. Therefore, the patients can have more comfortable lifetime if they responded to less toxic therapy. By such a viewpoint, SELECT BC trial that compares OS of the HER2-negative metastatic breast cancer patients administered S-1 or taxanes as the first chemotherapy is ongoing in Japan (19). This study may give a solution for this issue.

The present study strongly suggests that combination therapy with S-1 and trastuzumab appears to be an effective treatment option with a manageable toxicity profile for MBC. Efficacy rates of S-1 alone for MBC patients were 21.8% and 41.7% in two Japanese phase II trials (20, 21) and 30% in a retrospective study (22), S-1 is expected to be one of the promising fluorinated pyrimidines for the treatment of breast cancers. It should be emphasized that the rate may improve when the agent is applied at an up-front line of the treatment sequence. Sub-group analysis of the patients who were treated with S-1 relatively early, *i.e.* in the first and second lines, showed response rates as high as 45.5% (22). This result is compatible to the efficacy rate of taxanes that were used as the standard therapy in first line. Additionally, compared with anthracyclines and taxanes, adverse events such as hair loss are significantly rare, and its oral dosage form is supposed to be helpful in improving QOL of the patients. Actually, the patients whom HRQOL score decreased were few in this series. This result suggested that the combination of S-1 and trastuzumab was useful for maintenance of HRQOL.

Table III. *Common adverse events in the enrolled patients (N=28).*

Adverse Event	All Grades	≥Grade 3
Leukocytopenia	17	3
Neutropenia	9	4
Thrombocytopenia	14	0
Anemia	18	0
Increasing Total Bilirubin	12	0
Increasing AST	12	0
Increasing ALT	11	2
Nausea	12	1
Vomiting	8	1
Diarrhea	15	4
Anorexia	8	0
Stomatitis	8	0
Fatigue	14	0
Skin pigmentation	8	0
Skin rash	3	0
Pyrexia	6	0

Although capecitabine is another orally-available fluorinated pyrimidine anticancer agent and its efficacy has also been proved by several phase II studies (23, 24), its toxicity profiles are different from those of S-1. Results of a randomized phase II trial comparing these two agents for gastric cancer demonstrated almost identical efficacy but high incidence of hand-foot syndrome and stomatitis decrease the dose intensity of capecitabine in comparison with S-1 (25).

A combination of capecitabine and trastuzumab in HER2-positive breast cancer after failure of previous trastuzumab-containing regimen was investigated in randomized phase III

study. One hundred and fifty-six patients were enrolled in the trial, and the combination of trastuzumab and capecitabine showed survival benefit against capecitabine monotherapy although no significant difference was demonstrated (26). Another clinical trial investigating the efficacy and feasibility of the combination regimen after failure of anthracyclines and docetaxel or vinorelbine demonstrated good response rate and favorable impact for prognosis for heavily pre-treated MBC (6).

With regard to S-1 and trastuzumab combination regimen, several case studies reported efficacy of the regimen in heavily pre-treated HER2-positive MBC including trastuzumab treatment in their prior therapy (27-30). According to those anecdotal reports, S-1 in combination with trastuzumab indicated outstanding effects on heavily pre-treated patients without serious toxicity. In a large retrospective study which enrolled 1,468 breast cancer patients, whole adverse events and grade 3 or 4 adverse events of the combination therapy were occurred in 55.9% and 16.1%, which is comparable to that of S-1 alone, 63.5% and 14.6%, respectively (31). Like our result of the phase I trial, toxicity profile of the S-1 plus trastuzumab combination seems to be acceptable.

The present study was in line with previous reports in terms of the safety of the regimen. Our report is the result of the first prospective clinical trial evaluating efficacy of the S-1 plus trastuzumab combination regimen for MBC. With regard to the efficacy of the combination regimen, an ORR of 53.6% and an CBR of 75.0%, obtained from this phase II study should give a strong support to apply this regimen for a common clinical practice.

In conclusion, combination of trastuzumab and S-1 is an effective regimen with feasible toxicity and valuable to maintain patient's HRQOL. This combination therapy can be considered as a new standard option of the first- or second-line chemotherapy in patients with HER2-positive MBC.

Conflicts of Interest

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Sequential paclitaxel followed by tegafur and uracil (UFT) or S-1 versus UFT or S-1 monotherapy as adjuvant chemotherapy for T4a/b gastric cancer (SAMIT): a phase 3 factorial randomised controlled trial

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Summary

Background The prognosis for locally advanced gastric cancer is poor despite advances in adjuvant chemotherapy. We did the Stomach cancer Adjuvant Multi-Institutional group Trial (SAMIT) to assess the superiority of sequential treatment (paclitaxel then tegafur and uracil [UFT] or paclitaxel then S-1) compared with monotherapy (UFT or S-1) and also the non-inferiority of UFT compared with S-1.

Methods We did this randomised phase 3 trial with a two-by-two factorial design at 230 hospitals in Japan. We enrolled patients aged 20–80 years with T4a or T4b gastric cancer, who had had D2 dissection and a ECOG performance score of 0–1. Patients were randomly assigned to one of four treatment groups with minimisation for tumour size, lymph node metastasis, and study site. Patients received UFT only (267 mg/m² per day), S-1 only (80 mg/m² per day) for 14 days, with a 7-day rest period or three courses of intermittent weekly paclitaxel (80 mg/m²) followed by either UFT, or S-1. Treatment lasted 48 weeks in monotherapy groups and 49 weeks in the sequential treatment groups. The primary endpoint was disease-free survival assessed by intention to treat. We assessed whether UFT was non-inferior to S-1 with a non-inferiority margin of 1·33. This trial was registered at UMIN Clinical Trials Registry, number C000000082.

Findings We randomly assigned 1495 patients between Aug 3, 2004, and Sept 29, 2009. 374 patients were assigned to receive UFT alone, 374 to receive S-1 alone, 374 to received paclitaxel then UFT, and 373 to receive paclitaxel then S-1. We included 1433 patients in the primary analysis after at least 3 years of follow-up (359, 364, 355, and 355 in each group respectively). Protocol treatment was completed by 215 (60%) patients in the UFT group, 224 (62%) in the S-1 group, 242 (68%) in the paclitaxel then UFT group, and 250 (70%) in the paclitaxel then S-1 group. 3-year disease-free survival for monotherapy was 54·0% (95% CI 50·2–57·6) and that of sequential treatment was 57·2% (53·4–60·8; hazard ratio [HR] 0·92, 95% CI 0·80–1·07, p=0·273). 3-year disease-free survival for the UFT group was 53·0% (95% CI 49·2–56·6) and that of the S-1 group was 58·2% (54·4–61·8; HR 0·81, 95% CI 0·70–0·93, p=0·0048; $p_{non\text{-}inferiority}$=0·151). The most common grade 3–4 haematological adverse event was neutropenia (41 [11%] of 359 patients in the UFT group, 48 [13%] of 363 in the S-1 group, 46 [13%] of 355 in the paclitaxel then UFT group, and 83 [23%] of 356 in the paclitaxel then S-1 group). The most common grade 3–4 non-haematological adverse event was anorexia (21 [6%], 24 [7%], seven [2%], and 18 [5%], respectively).

Interpretation Sequential treatment did not improve disease-free survival, and UFT was not non-inferior to S-1 (and S-1 was superior to UFT), therefore S-1 monotherapy should remain the standard treatment for locally advanced gastric cancer in Japan.

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Introduction

Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer mortality.[1] The prognosis of locally advanced tumours remains poor despite advances in adjuvant chemotherapy.[2–4] More effective adjuvant chemotherapy treatments are needed for curatively resected but locally advanced gastric cancer.

Oral fluoropyrimidines and taxanes are often used to treat both gastric and breast cancer,[5–7] and taxanes are important drugs for breast and ovarian cancer given singly or in combination.[8,9] Docetaxel provided modest but better survival than did paclitaxel every 3 weeks in a trial of advanced breast cancer; but its toxic effects were worse,[10] and it has not been compared with dose-dense paclitaxel once per week, which is superior to paclitaxel every 3 weeks for ovarian cancer.[9,11] Sequential treatment with single taxanes is often preferred owing to fewer toxic effects and quality of life seems to be better.[12,13]

Peritoneal metastasis are the most common site of relapse in patients with gastric cancer,[3] for whom serosal