moderate ($P = .0372$, JT test) but was remarkable in combination with **60* (**6/*60*) or **28* (**6/*28*) as compared with the **1* group (**1/*1*) ($P = .049$ and $P = .0071$, respectively; nonparametric Dunnett test). *Conclusion:* This study identified several *UGT1A1* haplotypes significantly associated with the reduced AUC ratio (**28* and **6*) and with the increased total bilirubin level (**28*, **60*, and **1B*) and suggested that the novel haplotype **1B* might be functionally important. These findings will be useful for further pharmacogenetic studies on adverse reactions to irinotecan. (Clin Pharmacol Ther 2004;75:501-15.)

Irinotecan, a camptothecin derivative, is a prodrug with strong antitumor activity. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor,[1,2] is formed by hydrolysis of the parent compound with carboxylesterases.[3] SN-38 is subsequently conjugated by uridine diphosphate–glucuronosyltransferases (UGTs) to form an inactive metabolite, SN-38 glucuronide (SN-38G), in the liver,[4] which is excreted into bile and urine (Fig 1). The principal dose-limiting toxicities of irinotecan therapy are severe diarrhea and leukopenia.[5] Because the biliary SN-38G excreted into the small intestine could be cleaved by bacterial glucuronidases in the colon, the regenerated SN-38 is assumed to be one of the mechanisms of late-onset diarrhea.[6]

Pharmacokinetic studies of irinotecan have shown that there are large variations between individuals in the area under the concentration-time curves (AUCs) of SN-38 and SN-38G.[7,8] Among the UGT isoforms, UGT1A1 is thought to contribute predominantly to SN-38G formation,[4,9] and the wide interindividual variability in SN-38G formation in hepatic tissues was shown to correlate with a *UGT1A1* genetic factor.[4,10] Therefore *UGT1A1* polymorphisms seem to be one of the most important factors for irinotecan efficacy and toxicity.

The most extensively studied polymorphism seen was a variation of the TATA box [$A(TA)_6TAA > A(TA)_{5/7/8}TAA$], which is associated with enhanced [$(TA)_5$] or reduced [$(TA)_{7/8}$] *UGT1A1* transcription. The pathogenesis of Gilbert syndrome is associated with the variant $(TA)_7$ (**28*),[11,12] and reduced glucuronidation of SN-38 and bilirubin in hepatic tissues from the **28* patients has also been shown.[13] A substitution of T with G at nucleotide −3279 (the adenine of the translational start codon in GenBank Accession No. AF297093.1 is numbered +1) in the phenobarbital-responsive enhancer module of *UGT1A1* (**60*) was shown to reduce its transcriptional activity and to be associated with an increase in plasma bilirubin levels in the Japanese population.[14] However, this nucleotide change was associated with only a marginal reduction in SN-38G formation in hepatic samples from white subjects.[15] Reduced SN-38 glucuronidation in vitro was also shown by the exonic nonsynonymous single nucleotide polymorphisms (SNPs) 211G>A (G71R, **6*), 686C>A (P229Q, **27*), and 1456T>G (Y486D, **7*).[16,17] Several clinical studies have shown an association of **28* with reduced SN-38G formation[18] and with severe adverse reactions.[19]

Recent pharmacogenomic studies have suggested an advantage to the use of haplotypes, linked combinations of SNPs rather than individual SNPs, to investigate the associations between genotypes and phenotypes.[20] Innocenti et al[15] conducted haplotyping of the *UGT1A1* enhancer/promoter region on hepatic samples from white and black subjects, and this study showed a significant reduction in the SN-38 glucuronidation activity in the **28* variants. We have previously determined the allelic frequencies of the common marker SNPs, namely, *UGT1A1*6*, **7*, **27*, **28*, **60*, and **62* alleles, using 48 samples from Japanese individuals.[21] However, no haplotype analysis has been done on the entire *UGT1A1* gene, including SNPs located in the exons, such as 211G>A (G71R, **6*), which is more common in Asian populations. In this study with 195 Japanese subjects, we sequenced the *UGT1A1* gene, including the enhancer/promoter regions, all exons and their flanking regions, and assigned haplotypes using the detected SNPs. Next the association of these haplotypes with *UGT1A1*-related phenotypes, AUC ratios (SN-38G to SN-38), and pretreatment levels of serum total bilirubin was investigated in 85 patients with cancer who received irinotecan.

METHODS

Materials. SN-38 and SN-38G were kindly supplied by Yakult Honsha Co Ltd (Tokyo, Japan).

Patients. The 195 Japanese subjects in this study consisted of 88 patients with various cancers who were administered irinotecan and 107 patients with cardiovascular disease who were administered β-blockers (the 48 patients described in the previous report[21] were included). The sample size of 195 subjects was estimated to be sufficient to detect SNPs with rare frequencies (>0.01) and to determine allelic frequencies of SNPs accurately with confidence intervals of less than ±5%. Deoxyribonucleic acid was extracted from blood

leukocytes and used for sequencing. The ethics committees of the National Cancer Center, Tokyo, Japan, the National Cardiovascular Center, Suita, Japan, and the National Institute of Health Sciences, Tokyo, Japan, approved this study. Written informed consent was obtained from all participants.

Deoxyribonucleic acid sequencing, linkage disequilibrium, and haplotype analysis. Sequencing was performed as previously described,[22] by use of the primers listed in Table I, except that annealing in the second amplification was done at 58°C for the enhancer region. Linkage disequilibrium (LD) analysis was carried out with the program SNPAlyze 2.2 (Dynacom Co Ltd, Yokohama, Japan), and a pairwise 2-dimensional map between SNPs was obtained for the χ^2 and ρ^2 values. On the basis of the data, the *UGT1A1* gene was divided into 2 blocks (block 1 and block 2). First, haplotyping was done individually within each block (in-block haplotyping). Separately, the diplotype configurations (combinations of haplotypes) were inferred by an expectation-maximization–based program, LDSUPPORT, which determines the posterior probability distribution of the diplotype configuration for each subject, on the basis of the estimated haplotype frequencies.[23] The diplotype configurations of all subjects had a probability (certainty) of greater than 0.99 for all 195 subjects in block 1 and for 193 subjects in block 2. Next, diplotype configurations were inferred by use of all SNPs in both blocks; this whole-gene haplotyping confirmed the in-block haplotyping in blocks 1 and 2 and further assigned the 2 unidentified haplotypes in block 2.

Patient demographic profiles and eligibility for irinotecan treatment. Pharmacokinetic data obtained from 85 cancer patients who received irinotecan in the National Cancer Center Hospitals (Tokyo and Chiba, Japan) were used for the evaluation of the phenotype-genotype association in this study. None of the patients had previously received irinotecan chemotherapy, and some subjects were also given other anticancer drugs, such as cisplatin, mitomycin, 5-fluorouracil, or amrubicin. The eligibility criteria for irinotecan therapy were as follows: total bilirubin, ≤2 mg/dL; AST, ≤105 IU/L; ALT, ≤120 IU/L; creatinine, ≤1.5 mg/dL; and performance status (PS), 0 to 2. Detailed patient demographics (age, sex, PS, tumor type, previous treatment, and combination therapy) are summarized in Table II.

Pharmacokinetics. Each patient received a 90-minute intravenous infusion at doses of 60 to 150 mg/m^2. Heparinized blood was collected before administration of irinotecan and at 0 minutes, 20 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after infusion. The pretreatment levels of serum total bilirubin were determined by a kit (VL T-BIL; Azwell Inc, Osaka, Japan) according to an enzymatic method with the use of bilirubin oxidase.[24] Concentrations of SN-38 and SN-38G in the plasma were determined by the HPLC method previously described.[25] The AUCs from time 0 to infinity of the 2 metabolites were calculated on the basis of the linear-up/log-down rule (linear trapezoidal rule up to maximum concentration and log trapezoidal rule after maximum concentration) by use of the 202 noncompartmental model for a constant infusion in WinNonlin version 4.01 (Pharsight Corporation, Mountain View, Calif).

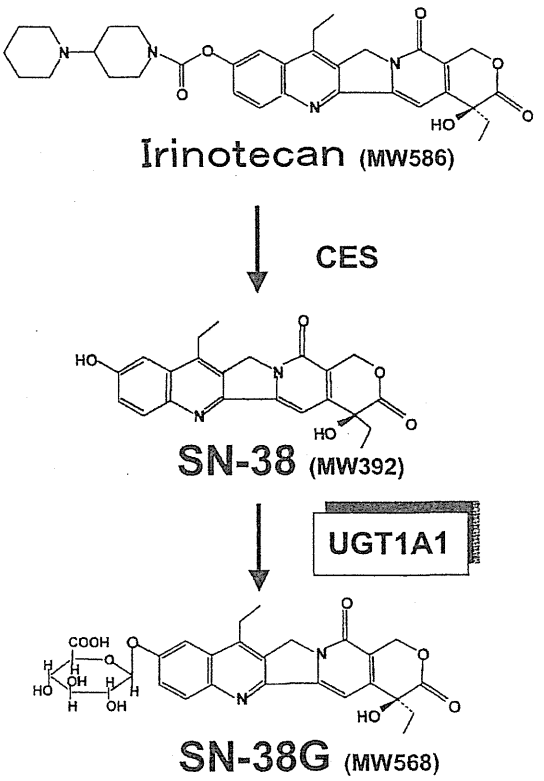

Fig 1. A role of uridine diphosphate–glucuronosyltransferase 1A1 (UGT1A1) in inactivating an irinotecan metabolite. The active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) is produced by carboxylesterase (CES) and is subsequently conjugated by UGT1A1 to form an inactive metabolite, SN-38 glucuronide (SN-38G).

Statistical analysis. Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) and total bilirubin levels among *UGT1A1* genotypes was performed with the Kruskal-Wallis test followed by the nonparametric Dunnett multiple comparison test or

Table I. Primers for *UGT1A1* amplification and sequencing

	Site	*Primer name*	*Sequence (5′ to 3′)*
First amplification			
Forward		UGT1A1CAR-Ex5ZF	GGTGGTGGGAGTGAGTTTAGT
Reverse		UGT1A1CAR-Ex5ZR	AGAGGGAAATAGTGGACAGAA
Second amplification			
Forward	CAR binding site	UGTCAR1stF2	AAGAACATTCTAACGGTTCATAA
Reverse	CAR binding site	UGTCAR1stR2	TGAATCATTGCATCGGCTGCCCA
Forward	HNF binding site	UGTHNF1stF	CACGATTTCTAAGTTCCTGCTC
Reverse	HNF binding site	UGTHNF1stR	ATCAACAGTATCTTCCCAGCAT
Forward	Exon 1	UGT1A11stF	TATCTCTGAAAGTGAACTCCCTG
Reverse	Exon 1	UGT1A11stR	GCACACAGAGTAAAATGTCCAA
Forward	Exon 2	UGT1AEx2-1stF-2	TTGCTCTGTGTTCTCTAAGTGGA
Reverse	Exon 2	UGT1AEx2R1st	TGATACTTCTGAGTGTGGTGGAT
Forward	Exons 3 and 4	UGT1AEx3-4-1stF	AGTTGCCAGTCCTCAGAAGC
Reverse	Exons 3 and 4	UGT1AEx3-4-1stR	TTGAAACAACGCTATTAAATGC
Forward	Exon 5	UGT1AEx5-F5-2	CTGGGCAACACAATAAGACCT
Reverse	Exon 5	UGT1AEx5-1stR	CTCAAATACACCACCCACCAA
Sequencing			
Forward	CAR binding site	UGTCAR1stF2	AAGAACATTCTAACGGTTCATAA
Reverse	CAR binding site	CAR-Rseq2	TGCCCACCTGAATAAACCCCACC
Forward	HNF binding site	UGTHNFseqF	CACTACATAGTCGTCCTTCTTCC
Reverse	HNF binding site	UGTHNFseqR	ATATGGCAAAAACCAATCGATA
Forward	Exon 1	UT1A11F1	GAACCTCTGGCAGGAGCAAAG
Reverse	Exon 1	UGT1A1R3-2	ATGCCCGAGACTAACAAAAGAC
Forward	Exon 1	UT1A11F4	ATCAGAGACGGAGCATTTTACACC
Reverse	Exon 1	UT1A11R4	CACGTAGGAGAATGGGTTGGG
Forward	Exon 1	UT1A11F5	AGTACCTGTCTCTGCCCAC
Reverse	Exon 1	UT1A11R5	AGTGGATTTTGGTGAAGGCAG
Forward	Exon 1	UT1A11F6	TGCTCATTGCCTTTTCACAG
Reverse	Exon 1	UGT1A1seqR1-2	TCAGATACCAAGAAATCATCCA
Forward	Exon 2	UGT1AEx2F	CTCTATCTCAAACACGCATGCC
Reverse	Exon 2	UGT1AEx2R	GGAAGTCTGGGATTAGCGCTC
Reverse	Exons 3 and 4	UT1AEx3-4seq3R	CAGAAGAAAATGTGGGTTGAGA
Forward	Exons 3 and 4	UT1AEx3-4seq4F	TCTCAACCCACATTTTCTTCTG
Forward	Exons 3 and 4	UGT1AEx3-4-F1-2	GTTCTGCTCTTTTTGCCCC
Reverse	Exons 3 and 4	UT1AEx3-4R2	CATGAATGCCATGACCAAAG
Reverse	Exon 5	UGT1AEx5-R5-2	ACCTTTGAATCCCGCACTC
Forward	Exon 5	UGT1AEx5seqF1	GTTTGGAAAATCTGGTAGTCTTC
Forward	Exon 5	UGT1AEx5seqF2	AAATGTTGTGCTTATGGCTACC
Reverse	Exon 5	UGT1AEx5seqR1	TCTTGGATTTGTGGGCTTTCT

UGT1A1, Uridine diphosphate–glucuronosyl transferase 1A1; CAR, constitutive androstane receptor; HNF, hepatocyte nuclear factor.

with the Mann-Whitney *U* test (1-tailed), and the analysis of a gene-dose effect of each haplotype was performed with the Jonckheere-Terpstra (JT) test (1-tailed) in the SAS system, version 5.0 (SAS Institute, Inc, Cary, NC). A P value of .05 was set as a significant level. Regarding significance levels for the JT test, the corrected significance levels of $P = .017$ and $P = .025$ in block 1 and block 2, respectively, were used on the basis of the number of comparisons.

Before a multiple regression analysis considering the *UGT1A1* genetic polymorphisms was carried out, other possible contributing factors from the patient backgrounds (age, sex, PS, tumor type, previous treatment, combination therapy, and irinotecan dosage) for the AUC ratios and for the bilirubin levels were assessed by the Kruskal-Wallis test, the Mann-Whitney test, or the Spearman correlation coefficient. Because no significant contribution from these factors was found, except for a correlation of age with AUC ratio ($r_s = 0.3690$, $P = .0006$), the genetic factors (the marker polymorphisms of the haplotypes) and age were incorporated as variables in a multiple regression analysis. The variables in the final model were chosen by the forward and backward stepwise procedure at the sig-

nificance levels of .25 and .05, respectively, by use of JMP version 5.0 software (SAS Institute, Inc).

On the basis of the results obtained in this study, we confirmed the adequacy of the sample size with power higher than 80% as explained later. We assumed that the 50% decrease in the mean AUC ratio was a minimum detectable difference that might be responsible for a reduction in neutrophil count.[18] We also assumed that the difference between the upper limit of the normal value for total bilirubin level (1.0 mg/dL) and the mean value for total bilirubin level in a control group (0.52 mg/dL in block 1 or 0.53 mg/dL in block 2) was a minimum detectable difference. Then we estimated the required total number of subjects necessary for detecting the specified minimum detectable differences at $\alpha = .05$ and $\beta = .20$ by use of JMP version 5.0 software (SAS Institute, Inc). We obtained the required total numbers of 20 and 18 for AUC ratios in blocks 1 and 2, respectively, and the numbers of 15 and 9 for total bilirubin levels in blocks 1 and 2, respectively. Regarding the total number required, this study (85 and 58 subjects in blocks 1 and 2, respectively) meets the estimated total sample size.

RESULTS

UGT1A1 polymorphisms detected in a Japanese population. The *UGT1A1* enhancer region, promoter region, and all exons and their flanking introns were sequenced for 195 Japanese subjects. All of the allele frequencies were in Hardy-Weinberg equilibrium ($P = .527$ or higher). A total of 25 polymorphisms were detected in this study. Among them, 10 SNPs were novel, including a nonsynonymous SNP (1598A>C [H533P] in exon 5) and 2 SNPs in the 3'-UTR (untranslated region) (1813C>T and 2021T>C). In addition, we detected the known SNPs causing reduced enzymatic activity, −3279T>G (**60*), −40_−39insTA (**28*), 211G>A (**6*, G71R), and 686C>A (**27*, P229Q) with frequencies of 0.262, 0.131, 0.151, and 0.005, respectively. These frequencies were similar to those previously reported in Asian populations.[14,26-28] A previously reported nonsynonymous SNP, 1091C>T (P364L),[28] was also found. Because each SNP frequency between the cancer and arrhythmic patients was similar, the combined data were used for the following LD and haplotype analyses.

LD analysis. Using the detected SNPs, we next performed LD analysis and obtained the pairwise values of ρ^2 and χ^2. As the data for χ^2 and ρ^2 were almost equivalent, the data for ρ^2 are depicted in Fig 2. A strong LD was seen among −3279T>G (**60*), −3156G>A, −364C>T, and −40_−39insTA (**28*).

Table II. Demographic profiles of patients with cancer receiving irinotecan therapy

	No.
Age (y)	
62 (56-69)*	85
Sex	
Male	65
Female	20
Performance status	
0	29
1	55
2	1
Tumor type	
Small cell lung	34
Non–small cell lung	12
Colon	19
Stomach	16
Others	4
Previous treatment	
1. None	27
2. Other chemotherapy	12
3. Surgery	9
4. Radiotherapy	1
5. 2 and 3	26
6. 2 and 4	8
7. 2, 3, and 4	2
Combination therapy and dose of irinotecan (mg/m^2)*	
Irinotecan alone, 140 (100-150)	24
With platinum antineoplastics, 60 (60)	43
With 5-fluorouracil, 100 (82-100)	7
With mitomycin C, 150 (150)	9
With amrubicin, 60 (60)	2

*The values are expressed as the median with the interquartile range in parentheses.

Another close linkage in the enhancer/promoter regions was found among −3177C>G, −3175A>G, and −64G>C. A close association among 1813C>T (211 bases downstream of the stop codon), 1941C>G (339 bases from the stop codon), and 2042C>G (440 bases from the stop codon) was also prominent, and they showed a perfect linkage. IVS2+15T>C was also weakly associated with the 3 SNPs. Thus the *UGT1A1* gene can be divided into at least 2 blocks, each having closely associated SNP groups. Considering that exons 2 to 5 are common among *UGT1A* families and that exons 1 and 2 are fairly distant from each other, we divided the gene into the following 2 blocks: block 1, corresponding to the enhancer and promoter regions and exon 1, and block 2, corresponding to exons 2 to 5.

Haplotype analysis. First, haplotyping was done within each block (in-block haplotyping). In block 1 the haplotypes **1b*, **6a*, **60a*, **60b*, and **28b* were unambiguously assigned. We separately estimated the

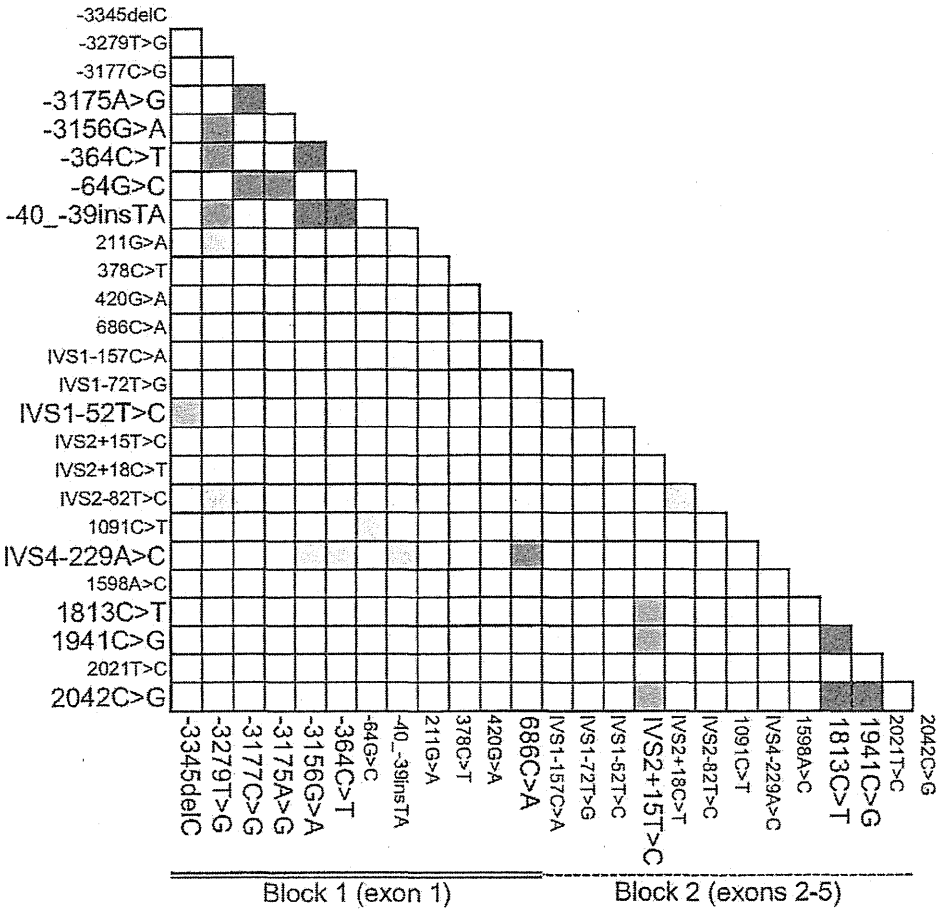

Fig 2. Linkage disequilibrium (LD) analysis for *UGT1A1* single nucleotide polymorphisms (SNPs). Pairwise LD as ρ^2 (from 0 to 1) is expressed as a 10-graded blue color. The denser color represents a higher linkage.

diplotype configuration (a combination of haplotypes) for each subject by using LDSUPPORT software. On the basis of the known key SNPs, the haplotypes inferred in block 1 were classified into 4 groups (**1*, **6*, **28*, and **60*). The additionally assigned haplotypes were 2 **6* subtypes (**6b* and **6c*), 2 **28* subtypes (**28c* and **28d*), and 1 **60* subtype (**60c*) (Fig 3). We classified the haplotypes having both **28* and **60* into the **28* group (**28b* and **28c* in Fig 3). The **28c* haplotype was inferred to have −3279T>G (**60*), −40_−39insTA (**28*), and 686C>A (**27*) on the same chromosome. The most frequent haplotype was **1a* (wild type, frequency = 0.564), followed by **6a* (0.146) and **28b* (0.121). It must be noted that 96% of the subjects with **28* also had **60* (−3279T>G), namely, in **28b* and **28c*.

In block 2 the haplotypes **1b* to **1i* and **364L* (the haplotype bearing P364L was tentatively named **364L*) were unambiguously identified. The additionally assigned haplotypes by the program were 3 **1* subtypes (**1j*, **1k*, and **1L*) and **533P* (H533P-bearing haplotype) (Fig 4). The most frequent haplotype was **1a* (frequency = 0.810), followed by **1b* (0.079) and **1c* (0.036). As shown in Fig 2, 1813C>T, 1941C>G, and 2042C>G (consisting of **1b*) showed a strong linkage. As described later, the **1* subtypes having the 3 SNPs were named **IB* as a group, and the others were named **IA*.

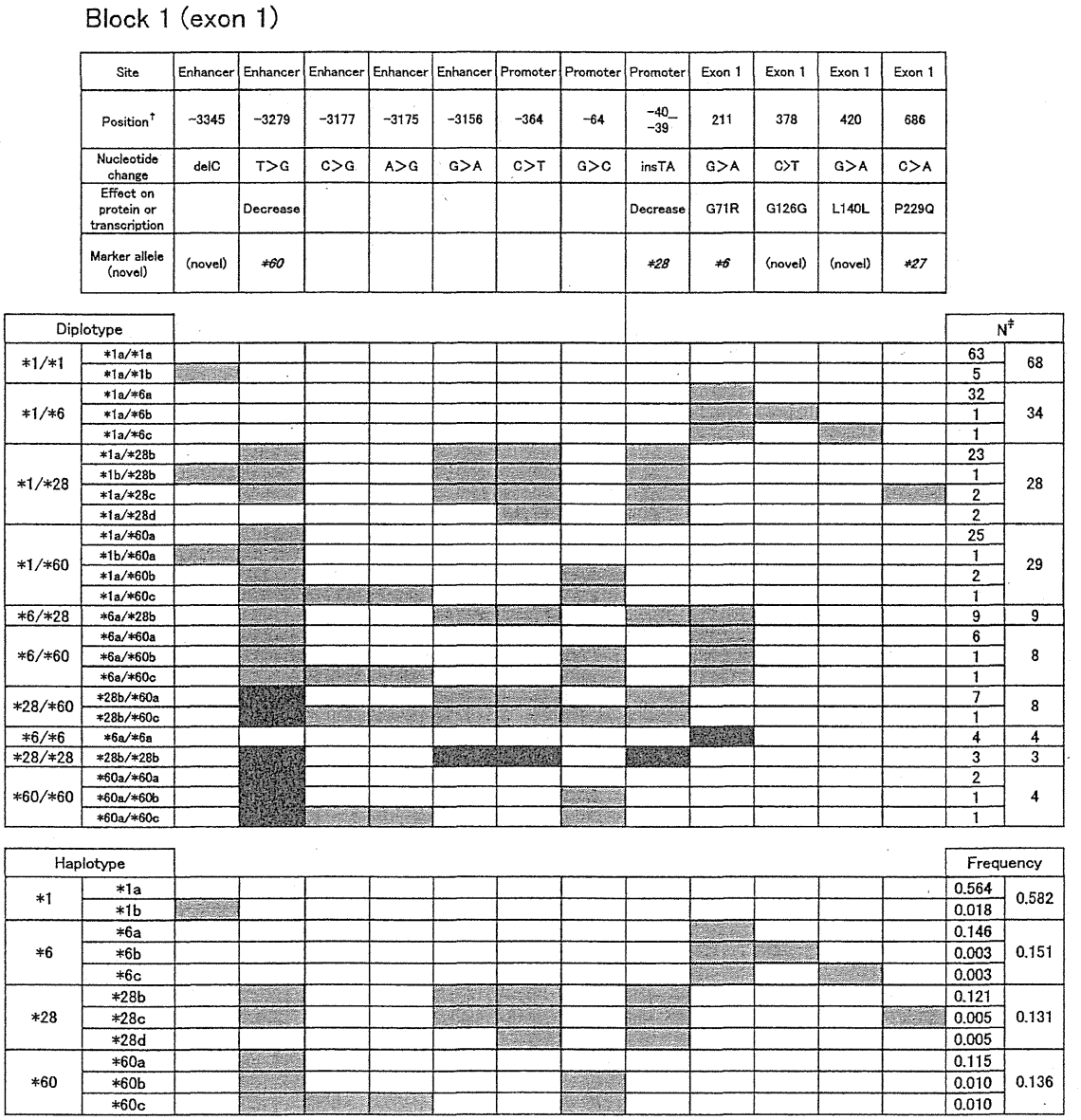

Fig 3. Diplotypes and haplotypes in block 1 (the enhancer/promoter regions and exon 1) of *UGT1A1* for 195 Japanese subjects. The haplotype groups are named according to previous reports, and individual haplotypes are described with small alphabetic letters. *Dagger*, Position in complementary deoxyribonucleic acid (cDNA) or from adenine of the translational initiation codon ATG; *double dagger*, number of subjects.

In addition, the combination of haplotypes was determined with both blocks 1 and 2 covered on the same chromosome (whole-gene haplotyping) (data not shown). Interestingly, **IB* in block 2 existed only on the chromosome having either **1a* or **60a* (or probably **6a*) in block 1 but not **28*. The haplotype combinations (block 1–block 2) were limited to **1a-*1b(*1c)* and **60a-*1b(*1j)* (and rarely **6a-*1c*). For 10 subjects with heterozygous block 1 **60a* and block 2 **1b* (or **533P*) and 5 subjects with heterozygous block 1

Block 2 (exons 2–5)

Site	Intron 1	Intron 1	Intron 1	Intron 2	Intron 2	Intron 2	Exon 4	Intron 4	Exon 5	3'-UTR	3'-UTR	3'-UTR	3'-UTR
Position†	IVS1 −157	IVS1 −72	IVS1 −52	IVS2 +15	IVS2 +18	IVS2 −82	1091	IVS4 −229	1598	1813 (211)	1941 (339)	2021 (419)	2042 (440)
Nucleotide change	C>A	T>G	T>C	T>C	C>T	T>C	C>T	A>C	A>C	C>T	C>G	T>C	C>G
Effect on protein							P364L		H533P				
Marker allele (novel)		(novel)	(novel)		(novel)		*364L	(novel)	*533P (novel)	(novel)		(novel)	

Diplotype		N‡	
*IA/*IA	*1a/*1a	126	149
	*1a/*1d	11	
	*1a/*1e	3	
	*1a/*1f	3	
	*1a/*1g	1	
	*1a/*1h	1	
	*1a/*1i	1	
	*1a/*1L	1	
	*1d/*1e	1	
	*1a/*364L	1	
*IA/*IB	*1a/*1b	27	44
	*1a/*1c	12	
	*1a/*1j	1	
	*1a/*1k	1	
	*1c/*1e	1	
	*1b/*1d	1	
	*1a/*533P	1	
*IB/*IB	*1b/*1b	1	2
	*1b/*1c	1	

Haplotype		Frequency	
*IA	*1a	0.810	0.877
	*1d	0.033	
	*1e	0.013	
	*1f	0.008	
	*1g	0.003	
	*1h	0.003	
	*1i	0.003	
	*1L	0.003	
	*364L	0.003	
*IB	*1b	0.079	0.123
	*1c	0.036	
	*1j	0.003	
	*1k	0.003	
	*533P	0.003	

Fig 4. Diplotypes and haplotypes in block 2 (exons 2 to 5) of *UGT1A1* for 195 Japanese subjects. The individual haplotypes were divided into either **IA* or **IB*. *Dagger*, Position in cDNA or from the nearest exon (with the *number in parentheses* indicating the position from the termination codon); *double dagger*, number of subjects.

**6a* and block 2 **1c*, it could not be determined whether both haplotypes were or were not on the same chromosome. However, most of the block 1 **6* haplotypes were linked to block 2 **1a* (data not shown).

UGT1A1 genotype–dependent SN-38 glucuronidation and serum bilirubin levels in irinotecan-administered patients. Next, we investigated the relationship between *UGT1A1* genotypes and AUC ratios (SN-38G/SN-38) or pretreatment levels of serum total bilirubin in cancer patients who were administered irinotecan. The median values of the AUC ratios and the total bilirubin levels in irinotecan-administered patients were 4.35 (interquartile range, 3.16-6.30) and 0.6 mg/dL (interquartile range, 0.5-0.8 mg/dL), respectively. We first assessed a possible influence of patient background factors listed in Table II and irinotecan

Table III. Influence of sex and coadministered drugs on AUC ratios and serum total bilirubin levels of patients with cancer who were administered irinotecan

	*AUC ratio (SN-38G/SN-38)**	*No.*	*Total bilirubin level (mg/dL)**	*No.*
Sex				
Male	4.45 (3.29-6.40)	64†	0.60 (0.50-0.80)	65
Female	4.06 (2.78-6.11)	20	0.65 (0.50-0.85)	20
Coadministered drug				
Irinotecan alone	5.22 (4.35-8.27)	23†	0.60 (0.50-0.90)	24
Platinum antineoplastics	4.00 (3.35-6.13)	43	0.60 (0.50-0.80)	43
5-Fluorouracil	2.88 (2.73-3.43)	7	0.70 (0.50-0.80)	7
Mitomycin C	3.41 (2.44-8.53)	9	0.50 (0.45-0.80)	9
Amrubicin	4.72	2	0.45	2

There were no significant differences in the AUC ratios and total bilirubin levels between sexes or among coadministered drugs.
AUC, Area under concentration-time curve.
*The values are expressed as the median with the interquartile range in parentheses.
†The analysis of AUC was not achieved for 1 patient.

dosage on these parameters and found no significant differences among them (Mann-Whitney and Kruskal-Wallis tests or Spearman correlation coefficient), except for a correlation of age with AUC ratio (r_s = 0.3690, P = .0006). However, we found no significant difference in the median values of age among the diplotypes in both blocks 1 and 2 (P = .5798 and P = .8593, respectively; Kruskal-Wallis test). Then we assessed the relationship between the genotype and the AUC ratio without normalization by age. We also confirmed that the diplotype configurations of both block 1 and block 2 were not significantly different with respect to the patient background factors (χ^2 test). On the basis of these findings, we assessed the correlation between the genotypes and phenotypes with all of the irinotecan-administered patients considered as a single group. The data for the effects of sex and coadministered drugs on the phenotypes are summarized in Table III.

As for block 1, the AUC ratios and total bilirubin levels were compared among all haplotype combinations (diplotypes) by use of the 4 haplotype groups, **1*, **60*, **6*, and **28*, and analyzed by the Kruskal-Wallis test (Fig 5). The observed P values were .0070 for the AUC ratio and .0503 for the total bilirubin level, respectively. A significant reduction in the AUC ratio was observed in the **6/*60* and **28/*6* haplotype groups (P = .049 and P = .0071, respectively; nonparametric Dunnett multiple comparison test) compared with the **1/*1* group. We separately evaluated the gene-dose effects of each haplotype by use of a nonparametric trend test (JT test) and summarized the results in Table IV. In the patients carrying the **60* haplotypes, although a **60*-dependent decreasing tendency in the AUC ratio was observed and the median AUC ratio for **60/*60* was reduced to 48% of that for the **1/*1* diplotypes, this trend was not statistically significant (P = .0724, JT test). As for the subjects with the **6* haplotypes, a moderate decrease was detected (P = .0372, JT test), and the **6/*6* diplotypes showed 67% of the median AUC ratio of **1/*1*. For the patients with the **28* haplotypes, a significant **28*-dependent decrease was observed among the **1/*1*, **28/*1*, and **28/*28* diplotypes (P = .0014, JT test). The median AUC ratio in **28/*28* was 56% of that of **1/*1*. In the **28/*1* patients, there was 1 heterozygote, with the **28c* haplotype having an additional nonsynonymous SNP, 686C>A (P229Q, **27*), and there were 2 heterozygotes of the **28d* haplotype lacking −3279T>G (**60*). The AUC ratios of these subjects fell within an interquartile range of the **28/*1* group. Thus the effects of these additional SNPs seemed not to be significant as compared with the effect of **28* alone. Regarding the effects of block 1 haplotype combinations, a further decreasing effect of the **6* haplotypes on the AUC ratios in the **60* or **28* group was evident when the **6/*60* diplotype was compared with **60/*1* or when the **6/*28* diplotype was compared with **28/*1* (P = .0149 and P = .0486, respectively; Mann-Whitney test). However, no apparent effect of the **60* haplotypes on the **28* heterozygotes was seen when **28/*1* and **28/*60* were compared (P = .2768, Mann-Whitney test) (Fig 5, *A*).

The effect of the *UGT1A1* block 1 haplotypes on the total bilirubin level was also evaluated. A significant increase in the bilirubin levels was observed in the patients with the **60* haplotypes (P = .0048, JT test), and a 1.2-fold increase in the median value of the **60/*60* diplotypes was observed compared with that of

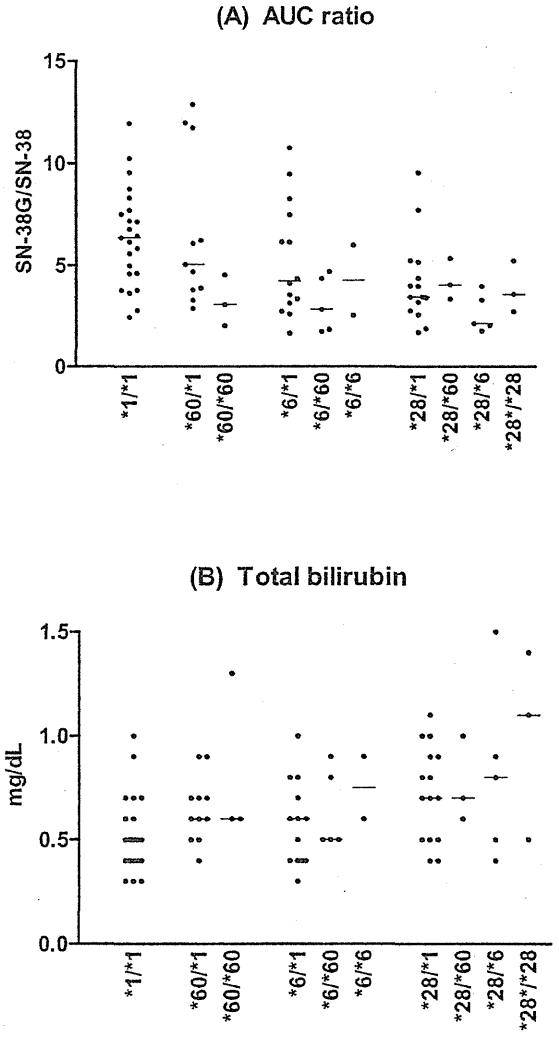

Fig 5. Association of the *UGT1A1* diplotypes in block 1 with the reduced area under the concentration-time curve (AUC) ratio (SN-38G/SN-38) (**A**) and with the increased serum total bilirubin levels (pretreatment) (**B**) in Japanese cancer patients who received irinotecan (84 patients for **A** and 85 for **B**). Each *point* represents an individual, and the median is indicated by a *bar*. The Kruskal-Wallis test for the full data sets (10 diplotypes) yielded *P* values of .0070 for **A** and .0503 for **B**. Significant reduction in the AUC ratio was detected in the **6/*60* and **28/*6* groups ($P = .049$ and $P = .0071$, respectively; nonparametric Dunnett multiple comparison test) compared with the **1/*1* group. Statistical analysis of gene-dose effect of each haplotype was separately conducted and is summarized in Table IV.

1/*1*. The bilirubin levels in the subjects with the **6* haplotypes were also elevated to 1.2- and 1.5-fold for the heterozygotes (6/*1*) and homozygotes (**6/*6*), respectively, although this trend was not statistically significant ($P = .0988$, JT test). For the **28* haplotypes, a significant genotype-dependent increase was evident when the **1/*1*, **28/*1*, and **28/*28* diplotypes were compared ($P = .0007$, JT test). The median values of the heterozygotes and homozygotes were elevated 1.4- and 2.2-fold, respectively, compared with the value for **1/*1* (Table IV). The additional effect (1.1-fold elevation) of the **6* haplotype on the increased levels of the **28* heterozygotes was also observed when **28/*1* and **28/*6* were compared, although it was not significant ($P = .4476$, Mann-Whitney test) (Fig 5, *B*). No apparent effect of **60* haplotypes on **28* was seen between **28/*1* and **28/*60* ($P = .4294$, Mann-Whitney test). Thus the effects of the haplotypes in block 1 on the bilirubin levels correlated well with those on the metabolic ratios.

As for block 2, we first overviewed the effect of each haplotype on the AUC ratios and the total bilirubin levels and found that these parameters for the subjects with **1b*, **1c*, **1j*, and **533P* (**1k* was not found in irinotecan-administered patients), which share the 3 SNPs 1813C>T, 1941C>G, and 2042C>G, were lower for the AUC ratios and higher for the bilirubin levels than those of **1a/*1a*. Moreover, significant differences among **1b*, **1c*, **1j*, and **533P* could not be detected. Therefore we joined these haplotypes into the **IB* haplotypes. Similarly, the non-**IB* haplotypes were combined as the **IA* haplotypes. Among the 21 patients who carried the **IB* haplotypes in block 2, only 2 were heterozygous for **28b* (their whole-gene diplotypes were **1a-*1c/*28b-*1a* and **1a-*1c/*28b-*1e*). Thus the majority of the subjects with **IB* had the non-**28* haplotypes in block 1; the effect of the **IB* haplotypes in block 2 was then analyzed among the non-**28* population. We further surveyed an additional effect of block 2 **IB* on the block 1 haplotypes and found that the **IB*-dependent reduction in the AUC ratios was remarkable in the **60*-bearing patients. Accordingly, we separately evaluated the **60*-positive subjects with **IB* in block 2. The AUC ratios and total bilirubin levels were compared among the diplotypes in block 2 and analyzed by the Kruskal-Wallis test, with *P* values of .3355 obtained for the AUC ratio and .0650 for the total bilirubin, respectively (Fig 6). We also evaluated the gene-dose effect of **IB* haplotypes, as summarized in Table IV. Although a **IB*-dependent decreasing tendency in the AUC ratios was observed and the median AUC ratio in **IB* homozygotes was

Table IV. Gene-dose effects of *UGT1A1* haplotypes on AUC ratio and serum total bilirubin levels

	Haplotype group	*No.*	*AUC ratio (SN-38G/SN-38)*	*Total bilirubin*
Block 1	**1/*1*	23	6.36 (4.59-8.00)	0.50 (0.40-0.65)
	**60/*1*	11	5.05 (3.84-11.87)	0.60 (0.55-0.80)
	**60/*60*	3	3.06	0.6
	P value		.0724 (NS)	.0048 ($P < .017$‡)
	**1/*1*	23	6.36 (4.59-8.00)	0.50 (0.40-0.65)
	**6/*1*	14 (15)†	4.23 (2.94-7.88)	0.60 (0.40-0.75)
	**6/*6*	2	4.27	0.75
	P value		.0372 (NS)	.0988 (NS)
	**1/*1*	23	6.36 (4.59-8.00)	0.50 (0.40-0.65)
	**28/*1*	15	3.45 (2.97-5.20)	0.7 (0.50-0.95)
	**28/*28*	3	3.57	1.1
	P value		.0014 ($P < .017$‡)	.0007 ($P < .017$‡)
Block 2	Non-**28*			
	**IA/*IA*	34 (35)†	6.04 (3.25-7.61)	0.50 (0.40-0.70)
	**IB/*IA*	22	4.65 (3.42-6.19)	0.60 (0.50-0.85)
	**IB/*IB*	2	3.37	0.90
	P value		.1551 (NS)	.0224 ($P < .025$§)
	60* in non-28*			
	**IA/*IA*	4	9.04	0.65
	**IB/*IA*	8	3.84 (3.18-5.64)	0.60 (0.55-0.80)
	**IB/*IB*	2	3.37	0.90
	P value		.0173 ($P < .025$§)	.4744 (NS)

Gene-dose effects of each haplotype were evaluated by the Jonckheere-Terpstra test. Values are expressed as the median with the interquartile range in parentheses. NS, Not significant.
†The number in parentheses represents total bilirubin.
‡Significant level corrected by the number of comparisons in block 1 that corresponds to .05 of the type I risk.
§Significant level corrected by number of comparisons in block 2 that corresponds to .05 of the type I risk.

reduced to 56% of the **IA/*IA* group, it was not statistically significant ($P = .1551$, JT test). For the serum bilirubin level, a significant **IB*-dependent increase was observed ($P = .0224$, JT test); a 1.8-fold increase in **IB/*IB* was observed compared with **IA/*IA* (Fig 6, *B*, and Table IV).

Regarding the effect of the **IB* in the **60*-bearing subjects, a significant **IB*-dependent decrease in the AUC ratio was observed ($P = .0173$, JT test) (Table IV). The AUC ratios (median) of the **60*-bearing subjects in **IB/*IA* and **IB/*IB* were 42% and 37% of those of the non-**IB* subjects, respectively (Fig 6, *A*, and Table IV). We then attempted to evaluate the combined effects of **60* and **IB* on the same chromosome (**60-*IB*). Among the 10 **IB*-bearing subjects with **60/*1* or **60/*60*, however, only 4 were unambiguously assigned to have **60-*IB* as their whole-gene haplotypes. Therefore the effect of **60-*IB* could not be fully analyzed. Nonetheless, it must be noted that the values of the homozygote of **60-*IB* were the lowest with regard to the AUC ratio and the highest with regard to the bilirubin level among the **60/*60* group in block 1 (Fig 6).

To further assess the responsible factors for alteration of these phenotypes, we attempted a multiple regression analysis considering genetic polymorphisms. For the AUC ratio, there is a significant correlation with age ($P = .0094$) and with the polymorphisms of −40_−39insTA ($P = .0012$) and 211G>A ($P = .0065$), which are the markers for the **28* and **6* haplotypes, respectively (Table V). The positive correlation of age with AUC ratio may be a result of the decrease in renal function with age, which may cause a reduction in renal excretion of SN-38G and thereby its increase in the plasma. Regarding total bilirubin level, a significant relationship was detected in the polymorphisms of −40_−39insTA ($P < .0001$) and 1813C>T ($P = .0270$), which are the markers for the **28* and **IB* haplotypes, respectively (Table V). These findings confirmed the effects of **28*, **6*, and **IB*.

DISCUSSION

Our sequencing data revealed 25 polymorphisms, including 10 novel ones. On the basis of LD analysis of these polymorphisms, we divided the gene into 2

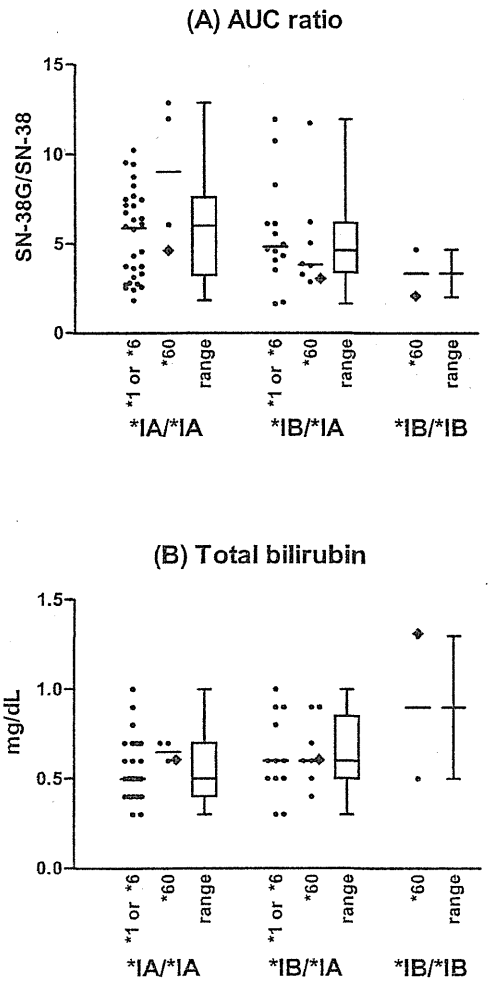

Fig 6. Association of the *UGT1A1* diplotypes in block 2 with the reduced AUC ratio (SN-38G/SN-38) (**A**) and with the increased serum total bilirubin levels (pretreatment) (**B**) in the non-**28* group of Japanese cancer patients administered irinotecan (58 patients for **A** and 59 for **B**). The distribution of the **1B* genotypes in block 2 was shown by a *box* representing the 25th to 75th percentiles with a *line* at the median and by *bars* representing the highest and lowest values. The patients having the **60* haplotype in block 1 are distinguished in each group, and the homozygotes of **60* are marked as *diamonds*. The Kruskal-Wallis test for the full data sets (5 diplotypes) yielded *P* values of .3355 for **A** and .0650 for **B**. Statistical analysis of gene-dose effect of **1B* was separately conducted and is summarized in Table IV.

blocks and designated haplotype groups (**6*, **27*, **28*, and **60*) in block 1 using the previously reported definitions. In block 2 (exons 2-5), we detected 3 perfectly linked SNPs in the 3'-UTR region (1813C>T, 1941C>G, and 2042C>G) and subsequently named the group having the 3 linked SNPs **1B*. In addition, we attempted to assign the haplotype combinations throughout both blocks (whole-gene haplotyping), but the assigned combinations were not complete; 17 subjects among 195 were not assigned to the block 1–block 2 combination. Interestingly, however, we found that block 2 **1B* exists with either block 1 **1a* or **60a* (or probably **6*) but not **28*.

Regarding the haplotypes in block 1, the impact of the **28* haplotype was the most significant in terms of the decreased AUC ratio (SN-38G/SN-38) and the increased total bilirubin level (Fig 5 and Table IV). Significant effects of −40_−39insTA (the marker of the **28* haplotype) on the AUC ratio and bilirubin level were also confirmed by multiple regression analysis (Table V). Our results were consistent with previous reports that showed correlations of **28* with hyperbilirubinemia in Gilbert syndrome or in cancer patients[11,12,19] and with decreased SN-38G formation in both in vitro and in vivo studies.[13,18,19]

The previous reports on **6* (G71R) showed a close linkage between hyperbilirubinemia and the genotype in Japanese infants[26] or decreased SN-38G formation in the *UGT1A1*6*-expressing cell line.[16,17] However, there were only limited pharmacokinetic data on irinotecan-administered subjects with the **6* haplotype. In this study it was shown that block 1 **6* was always linked to block 2 **1a* (or **1L*) and probably to block 2 **1c*. The patients bearing **6* alone showed trends for a decrease in the AUC ratios (SN-38G/SN-38) and an increase in total bilirubin levels, although the trends were not statistically significant (Table IV). It is noteworthy, however, that the AUC ratios in the patients bearing the **6* haplotype together with the **28* or **60* haplotype were significantly low compared with the **1/*1* group (Fig 5, *A*). The multiple regression analysis also indicated that 211G>A (**6* marker) had a significant effect on the AUC ratio (Table V). Ando et al[19] have also reported that the **6* allele alone is not a good predictor of severe toxicity of irinotecan but considered that it might enhance the **28*-associated irinotecan toxicity. Given that our haplotype analysis revealed that **28* and **6* were present on a mutually exclusive chromosome, **28* and **6* should exert an additive effect on irinotecan toxicity.

Regarding the **60* allele (−3279T>G), a decreasing tendency in the AUC ratio (SN-38G/SN-38) and a significant increase in total bilirubin levels were observed in the subjects bearing the **60* haplotypes (Fig 5 and Table IV), although multiple regression analysis

Table V. Multiple regression analysis of AUC ratio and total bilirubin level concerning *UGT1A1* polymorphisms

	Coefficient	*F value*	*P value*	R^2	*Intercept*
AUC ratio (SN-38G/SN-38)				0.2174	1.726
Variable					
Age	0.0722	7.08	.0094		
−40_−39insTA (**28*)†	−1.666	11.27	.0012		
211G>A (**6*)†	−1.461	7.80	.0065		
Total bilirubin				0.2029	0.539
Variable					
−40_−39insTA (**28*)†	0.213	20.10	<.0001		
1813C>T (**IB*)†	0.112	5.07	.0270		

The numbers of the cancer patients used for the analyses were 84 for AUC ratio and 85 for total bilirubin, respectively.
†The corresponding haplotype is described in parentheses.

revealed no significant relevance of the marker SNP of **60* (−3279T>G) to those phenotypic parameters. There is a possibility that the multiple regression analysis might miss the **60* marker, because this site is frequently associated with other marker sites of **28* or **IB*.

In this study we showed for the first time the effect of novel **IB* haplotypes on *UGT1A1*-related phenotypes. We found a **IB*-dependent decreasing trend in the AUC ratio (SN-38G/SN-38), although not significant, and a significant increase in serum total bilirubin levels (Fig 6 and Table IV). The multiple regression analysis also confirmed the significant relationship of the marker site of **IB* (1813C>T) to total bilirubin level (Table V). When the AUC ratios of **1a-*IA/*1a-*IA* and **1a-*IB/*1a-*IA* were compared, the difference (the effect of **1a-*IB*) was not clear (data not shown). However, the presence of **IB* together with **60* haplotypes in block 1 enhanced a trend for decreased AUC ratios (Fig 6, *A* and Table IV). Thus it is likely that the effects of **IB* could be more evidently manifested in the concurrent presence of **60*. One possible mechanism of the reduced UGT activity in the **IB* haplotypes might be an instability of messenger ribonucleic acid as a result of the nucleotide substitutions in the 3'-UTR. Because the 3'-UTR in *UGT1A1* is shared by other UGT1A isoforms, the **IB* haplotypes might also affect their activities.

We found that there were 2 types in the **60* haplotypes, **60-*IA* and **60-*IB* (data not shown). Because **60a* is known to partially reduce *UGT1A1* transcriptional levels,[14] the presence of **IB* on the same chromosome might cause a synergistic reduction in *UGT1A1* transcription, possibly through a **IB*-induced messenger ribonucleic acid instability. Thus we attempted to evaluate the combined effects of **60* and **IB* on the same chromosome (**60-*IB*). Among the 10 **IB*-bearing subjects with the **60* groups, the block 1−block 2 haplotype combinations for 6 patients could not be determined either to be on the same chromosome or not. Although this ambiguity hampered the precise evaluation of the effect of **60-*IB*, it is noteworthy that the diplotype of more than half of the **60*-bearing patients was estimated to have **60-*IB* and that values of the **60-*IB* homozygotes were the lowest with regard to the AUC ratio and the highest with regard to the bilirubin level among the **60/*60* group in block 1 (Fig 6).

On further studies of the association of the *UGT1A1* haplotypes with irinotecan-induced toxicity, such as neutropenia, we realized that the association should be evaluated among the patients who received irinotecan alone, because the coadministered drugs significantly potentiated the neutropenia. Because the number of patients administered irinotecan alone was still small, a precise evaluation was not available in this study. However, regarding the **IB* in block 2 (in which **IB/*IB* was not available), our preliminary observation revealed a significant decrease in the neutrophil count nadir in the **1B* heterozygotes ($P = 0.0044$, Mann-Whitney test); their median value was 1100 counts/μL (n = 7) versus 2500 counts/μL (n = 10) in the **IA* group. The elucidation of the clinical outcome of **IB*, as well as the other *UGT1A1* haplotypes, awaits further studies.

In this study we attempted to evaluate the functional significance of *UGT1A1* haplotypes by analyzing *UGT1A1*-related phenotypic parameters in irinotecan-administered patients and identified several *UGT1A1* haplotypes significantly associated with the reduced AUC ratio (**28* and **6*) and with the increased total bilirubin level (**28*, **60*, and **IB*). We also suggested that the novel haplotype **IB* might be functionally important, although this is still hypothetical. The clin-

ical significance of those haplotypes should be evaluated in a future study.

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Phase II Study of Oral S-1 for Treatment of Metastatic Colorectal Carcinoma

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BACKGROUND. The goal of the current study was to evaluate the objective response rate and toxicity associated with the oral fluoropyrimidine S-1 (a combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate) in patients with previously untreated metastatic colorectal carcinoma.

METHODS. Thirty-eight patients were enrolled in the study. S-1 was administered orally at a dose of 40 mg/m^2 twice daily for 28 days, followed by a 14-day rest period. Treatment was repeated every 6 weeks unless disease progression was observed.

RESULTS. A combined total of 173 courses of S-1 were administered to the 38 enrolled patients. The median number of courses administered to a given patient was 3.5 (range, 1–18). Although no patient exhibited a complete response to treatment, 15 had partial responses (response rate, 39.5%; 95% confidence interval, 24.0–56.6%). In addition, 5 patients had minor responses, and 14 had stable disease. Four patients were found to have progressive disease after two courses of treatment. The median survival time was 358 days (95% confidence interval, 305–490 days), and the 1-year survival rate was 47.4%. The most common adverse reactions included myelosuppression and gastrointestinal toxicity; most cases involved Grade 1 or 2 toxicity, but Grade 3 toxicities (anemia [7.9% of patients], neutropenia [5.3% of patients], diarrhea [2.6% of patients], and abnormal bilirubin levels [7.9% of patients]) also were noted. Neither Grade 4 toxicity nor treatment-related death was observed during the study.

CONCLUSIONS. Orally administered S-1 is active against metastatic colorectal carcinoma and has an acceptable toxicity profile. This promising agent has the potential to become a valuable chemotherapeutic option. ***Cancer* 2004;100: 2355–61.** © *2004 American Cancer Society.*

KEYWORDS: colorectal carcinoma, S-1, 5-fluorouracil derivative, oral fluoropyrimidine, Phase II study.

Colorectal carcinoma is one of the most common causes of malignancy-related death in the United States, Japan, and most European countries. The median survival duration for patients with metastatic colorectal carcinoma treated with supportive care alone is approximately 4–6 months.[1] Systemic chemotherapy with 5-fluorouracil (5-FU) recently was shown to prolong survival, with a median

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survival time of 17–21 months associated with such treatment.[2,3] The administration of irinotecan together with 5-FU and leucovorin (LV) as first-line treatment for metastatic disease also has been shown to produce a survival benefit,[2,4] but recently, concern has been raised regarding the toxicity of the weekly bolus combination of these agents.[5]

A randomized cooperative group study has yielded preliminary data supporting the role of 5-FU and LV administered via continuous intravenous infusion (CVI) as the backbone of treatment strategies for metastatic colorectal carcinoma.[6] Nonetheless, CVI performed using a portable pump and an indwelling catheter is challenging and may induce phlebitis or infection originating at the injection site and requiring long-term hospitalization; thus, oral anticancer agents have been developed to address this problem.[7] The results of large Phase III studies of oral capecitabine and the combination of tegafur + uracil (UFT) with LV were reported recently and demonstrated survival benefits that were equivalent to those achieved using intravenous 5-FU + LV.[8–11] Oral chemotherapy has major advantages over intravenously administered treatment in terms of pharmacoeconomic considerations and patient preferences, because oral treatment can be administered on an outpatient basis, thereby reducing the length of patients' hospital stays.[12] Over time, the role of oral chemotherapy in the treatment of malignant disease is expected to become increasingly significant.

Gastrointestinal side effects represent the dose-limiting toxicity associated with 5-FU in a long-term administration schedule (i.e., a CVI schedule).[7] Therefore, to maximize the therapeutic effects of 5-FU, prevention of gastrointestinal toxicity is of primary importance. A new oral fluoropyrimidine, S-1, has been developed by Taiho Pharmaceutical Co. (Tokyo, Japan) and adapted for use in the treatment of advanced gastric[13–15] and head and neck malignancies[16]; at present, this agent is used widely throughout Japan. S-1 consists of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1.[17] Tegafur is a precursor of 5-FU and functions as an effector. As an enhancer of the antitumor activity of tegafur, CDHP is prescribed to potently and reversibly inhibit the 5-FU degradation enzyme dihydropyrimidine dehydrogenase (DPD); by inhibiting DPD, CDHP induced the long-term retention of an increased concentration of 5-FU in the blood.[18] Orally administered potassium oxonate is selectively distributed to the gastrointestinal tract with high concentration and inhibits orotate phosphoribosyltransferase, which phosphorylates 5-FU to yield the active metabolite form of 5-FU in humans.[19]

In rats bearing subcutaneous Yoshida sarcoma compared with UFT administered at an equally harmful dose to the rats, S-1 tended to maintain the concentration of 5-FU in plasma and tumor tissue for a longer duration and with less gastrointestinal toxicity.[20] Furthermore, compared with tegafur, UFT, and other fluoropyrimidines, S-1 exhibited greater therapeutic efficacy against various rat tumors and human xenografts.[21]

In a Phase I study involving Japanese patients, S-1 was administered orally for 28 days. The maximum allowed dose of S-1 was 150 mg once daily or 75 mg twice daily, and leukopenia was the resulting dose-limiting toxicity. The pharmacokinetic profile of S-1 revealed that twice-daily administration preserved therapeutic 5-FU levels without increasing the maximum 5-FU concentration in the blood.[22,23] Therefore, oral administration of S-1 at a dose of 75 mg twice daily for 28 consecutive days, with a subsequent 14-day rest period, was recommended. Two Phase II studies of twice-daily S-1 administered as a single agent for the treatment of metastatic gastric malignancy yielded response rates of approximately 50%, with minimal toxicity.[13–15]

Based on these results, two Phase II studies of S-1 in the treatment of metastatic colorectal carcinoma were initiated. Response rates of 17% and 35% were observed in these two trials.[13,24] To verify the reproducibility of these findings, we performed our own Phase II study of S-1 in the treatment of Japanese patients with metastatic colorectal carcinoma.

MATERIALS AND METHODS

Eligibility

Patients were entered into the study only if they fulfilled the following eligibility requirements: 1) histologically confirmed colorectal carcinoma; 2) inoperable metastatic disease or recurrent metastatic disease after surgery; 3) the presence of measurable or evaluable lesions; 4) age $\geq$ 20 years but $<$ 75 years; 5) Eastern Cooperative Oncology Group performance status (PS) $\leq$ 2; 6) no previous chemotherapy or radiotherapy for advanced disease (with any adjuvant chemotherapy for colorectal carcinoma required to have been completed $\geq$ 6 months before enrollment); 7) adequate bone marrow function (hemoglobin concentration $\geq$ 9.0 mg/dL, white blood cell count $\geq$ 4000/μL but $\leq$ 12,000/μL, and platelet count $\geq$ 100,000/μL); 8) adequate liver function (serum bilirubin levels $\leq$ 1.5 mg/dL, serum transaminase levels $\leq$ 100 international units per liter, and serum alkaline phosphatase levels $<$ 2 times the upper limit of normal); 9) adequate renal function (serum creatinine levels within normal limits); 10) no other severe med-

ical conditions; and 11) no other active malignancies. In addition, patients were required to provide written informed consent, and pregnant women were excluded from the study.

Treatment Schedule

S-1 was administered at a dose of 40 mg/m^2 twice daily for 28 consecutive days, with a subsequent 14-day rest period. Patients were assigned on the basis of body surface area (BSA) to receive one of the following doses twice daily: 40 mg (BSA < 1.25 m^2), 50 mg (BSA ≤ 125 to < 1.50 m^2), or 60 mg (BSA > 1.50 m^2). S-1 was supplied by Taiho Pharmaceutical Co. in the form of 20 and 25 mg capsules (i.e., 20 and 25 mg tegafur). A course of therapy was defined as 28 consecutive days of treatment followed by a 14-day rest period, and courses were repeated every 6 weeks until either disease progression or unacceptable toxicity was observed. Patients whose toxicities necessitated a rest period of more than 4 weeks were withdrawn from treatment. Prophylactic use of antiemetic agents was not allowed. For all patients, treatment compliance and receipt of treatment without hospitalization were verified by patient interviews conducted on a regular schedule.

Evaluation

Before entry into the study, patients were evaluated using appropriate investigational methods to determine the extent of disease. A complete blood cell count, liver function testing, renal function testing, and urinalysis were performed at least once every 2 weeks during treatment. Appropriate investigation was repeated as necessary to evaluate target lesion sites before every treatment course. Antitumor activity was evaluated in accordance with the general rules, based on the corresponding World Health Organization criteria, set forth by the Japanese Research Society for Colorectal Carcinoma.[25] Complete response (CR) was defined as the disappearance of all evidence of malignant disease for more than 4 weeks. Partial response (PR) was defined as a reduction (lasting longer than 4 weeks) of greater than 50% in the sum over all lesions of the product of the longest perpendicular tumor dimensions, with no evidence of new lesions or of the progression of any preexisting lesion. Stable disease (SD) was defined as a reduction of less than 50% or an increase of less than 25% in the sum over all lesions of the product of the longest perpendicular tumor dimensions, with no evidence of new lesions. Progressive disease (PD) was defined by increases of greater than 25% in sum overall lesions of the product of the longest perpendicular tumor dimensions or the appearance of new lesions. The toxicity criteria of the Japan Society for Cancer Therapy, which were based (with some modification) on the World Health Organization criteria, were used to evaluate treatment-related toxicity.[26] The eligibility and suitability of patients for assessment and the responses of patients to treatment were reviewed extramurally.

TABLE 1
Patient Characteristics

Characteristic	No. of patients
No. of eligible patients	38
Median age in yrs (range)	58.5 (28–74)
Gender (%)	
Male	18 (47)
Female	20 (53)
ECOG PS (%)	
0	18 (47)
1	20 (53)
Primary lesion site (%)	
Colon	23 (61)
Rectum	15 (39)
Histology (%)	
Well/moderately differentiated	33 (87)
Poorly differentiated	5 (13)
Previous therapy (%)	
Surgery	23 (61)
Surgery + adjuvant chemotherapy	4 (11)
Surgery + radiotherapy	2 (5)
None	9 (24)
Mean body surface area in m^2 (range)	1.53 (1.26–1.85)

ECOG PS: Eastern Cooperative Oncology Group Performance Status.

Statistical Methods

Previous Phase II studies have reported a 35.5% response rate for metastatic colorectal carcinoma treated with S-1. The current study was designed to have a target activity level of 35% and a minimum activity level of 15%, with an α error of 0.05 and a β error of 0.2; thus, a minimum of 38 patients were required. Survival was calculated from the date of treatment initiation using the Kaplan–Meier method.

Ethical Considerations

The current trial was approved by the institutional review boards of the clinical oncology programs at all participating hospitals. Approval was based on the 1975 revision of the Helsinki Declaration. Oral and written statements of informed consent were acquired from all patients.

RESULTS

Thirty-eight patients (18 men and 20 women) with advanced metastatic colorectal carcinoma were en-

TABLE 2
Body Surface Area and Corresponding S-1 Dose

BSA (m^2)	S-1 dose[a] (mg)	No. of patients (%)
< 1.25	40	0
≤1.25 to < 1.50	50	15 (39)
≥1.50	60	23 (61)

BSA: body surface area.
[a] Dose administered twice daily.

TABLE 3
Objective Response Data

Response type	No. of patients
Complete response	0
Partial response	15
Minor response	5
Stable disease	14
Progressive disease	4
Overall response rate[a]	39.5% (15/38)
95% confidence interval	24.0–56.6%

[a] Includes complete responses and partial responses.

tered into the trial between June 1999 and December 2000. Patient characteristics are summarized in Table 1. The median patient age was 58.5 years (range, 28–74 years). Eighteen patients had PS 0, and the remaining 20 had PS 1. The primary tumor was located in the colon in 23 patients (61%) and in the rectum in 15 patients (39%). Thirty-three patients (87%) had well or moderately differentiated adenocarcinoma, whereas 5 (13%) had poorly differentiated adenocarcinoma. Of the 38 patients in the current study, 29 (76%) had undergone surgery before entry, 4 (11%) had received 5-FU-based adjuvant chemotherapy, and 2 had received pelvic radiotherapy.

The mean BSA in the current study population was 1.53 m^2 (range, 1.26–1.85 m^2). Daily S-1 doses according to BSA are shown in Table 2. The median S-1 dose was 60 mg administered twice daily. A combined total of 173 treatment courses were administered to the 38 patients enrolled in the study. The median number of courses per patient was 3.5 (range, 1–18), and the median cumulative S-1 dose per patient was 10,080 mg (range, 2660–44,660 mg).

Response

All 38 patients had measurable metastatic lesions. Although no patient experienced a CR, 15 patients had PRs (response rate, 39.5%; 95% confidence interval, 24.0–56.6%) (Table 3). Among these 15 patients, the median time required for a 50% reduction in tumor

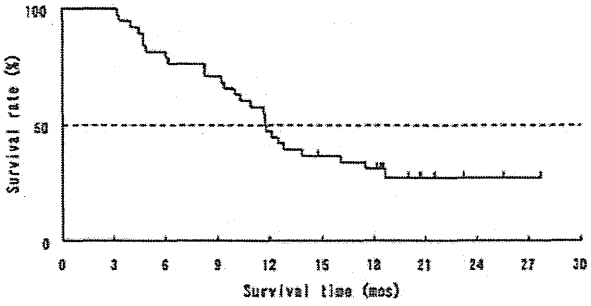

FIGURE 1. Overall survival of 38 patients treated with S-1 for previously untreated metastatic colorectal carcinoma. Median survival time, 358 days (95% confidence interval, 305–490 days).

size was 68 days (range, 29–130 days), and the median duration of response was 232 days (range, 96–679 days). Five patients had minor responses, and 14 had SD. The remaining four patients were found to have PD after two courses of treatment. Response rates according to metastatic site were as follows: liver, 38% (9 of 24 patients); lung, 27% (4 of 15 patients); and lymph nodes, 30% (3 of 10 patients). The response rate among patients with colon carcinoma was 44% (10 of 23 patients), and the response rate among patients with rectal carcinoma was 33% (5 of 15 patients). The response rate at the primary site as evaluated using the roentgenographic evaluation criteria proposed by the Japanese Society for Cancer of the Colon and Rectum was 43% (3 of 7 patients). One of the four patients who had a history of adjuvant chemotherapy achieved a PR.

At the close of the trial, the median time to evidence of disease progression was 162 days (range, 118–254 days). The median survival time from the beginning of treatment was 358 days (median follow-up, 666 days; 95% confidence interval, 305–490 days) for the overall study cohort, and the 1-year survival rate was 47.4% (Fig. 1).

Toxicity

For each toxicity, the patient distribution with respect to highest observed grade is summarized in Table 4. The most common adverse reactions included myelosuppression and gastrointestinal toxicity, although these events generally were mild, and no cumulative toxicity was noted. Neither Grade 4 toxicity nor treatment-related death was observed during the study. Toxicity incidence rates were as follows: anemia, 45% (17 of 38 patients); leukopenia, 45% (17 of 38 patients); neutropenia, 42% (16 of 38 patients); and thrombocytopenia, 13% (5 of 38 patients). Nonetheless, Grade ≥ 3 toxicities were noted in less than 8% of patients.

TABLE 4
Toxicity Data

Toxicity	Grade 1	2	3	4	Grade ≥3 (%)
Anemia	7	7	3	0	7.9
Leukopenia	7	10	0	0	0
Neutropenia	4	10	2	0	5.3
Thrombocytopenia	4	1	0	0	0
Diarrhea	5	8	1	0	2.6
Nausea/vomiting	8	7	0	0	0
Anorexia	15	4	0	0	0
Stomatitis	11	3	0	0	0
Hand-foot syndrome	2	0	0	0	0
Pigmentation	15	0	0	0	0
Malaise	17	2	0	0	0
Bilirubinemia	—[a]	14	3	0	7.9

[a] Grade 1 bilirubinemia is not defined in the toxicity criteria of the Japan Society for Cancer Therapy. (See: Japan Society for Cancer Therapy. Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. *J Jpn Soc Cancer Ther.* 1993;28:101–130.[26])

The overall incidence rate for diarrhea was 37% (14 of 38 patients), with Grade 3 diarrhea noted in 3% of the study cohort (1 of 38 patients). The overall stomatitis incidence rate was 37% (14 of 38 patients); however, Grade ≥ 3 stomatitis was not observed. The incidence rate for hand-foot syndrome (palmar-plantar erythrodysesthesia) was 5% (2 of 38 patients); Grade 1 erythrodysesthesia was noted in both cases. Overall, abnormal bilirubin levels were noted in 45% of the study cohort (17 of 38 patients), with an incidence rate of 8% (3 of 38 patients) for Grade 3 bilirubin abnormalities. Nonetheless, no Grade ≥ 3 elevation of aspartate aminotransferase or alanine aminotransferase levels was observed in the current study.

Toxicity caused two patients to discontinue S-1 treatment. One of these two was hospitalized for abdominal pain (Grade 2), nausea with vomiting (Grade 2), and anorexia (Grade 2) during the third treatment course, and S-1 treatment subsequently was discontinued. The other patient withdrew from the study during the second treatment course due to diarrhea (Grade 3) and neutropenia (Grade 2). Discontinuation of treatment was not considered necessary for any of the other patients who experienced Grade 2 or Grade 3 toxicities; instead, these patients were able to continue receiving treatment after a brief interruption or after dose reduction. Thirty-five of 38 patients (92%) were treated as outpatients, a finding that indicates extremely good compliance. Of the 173 courses that were administered overall, 163 (94%) were administered at ≥ 75% of the protocol-defined dose.

DISCUSSION

The current study was conducted to evaluate the objective response rate and toxicity associated with an oral regimen of S-1 for patients with previously untreated metastatic colorectal carcinoma. We observed a response rate of 39.5%, which was equal to or greater than the corresponding response rates associated with 5-FU alone and with 5-FU + LV. In an earlier Phase II study of S-1, an overall response rate of 35% was reported for patients who had not previously received chemotherapy.[24] That earlier study and the current one were similar in terms of dosing and scheduling of S-1, eligibility criteria, and response criteria, and both studies also reported similar response rates and survival times; these similarities suggest that the activity of oral S-1 against metastatic colorectal carcinoma represents a reproducible finding.

In a previous Phase I study involving Japanese patients, S-1 was administered orally for 28 consecutive days.[22] The maximum allowable S-1 dose was 150 mg once daily or 75 mg twice daily, and myelosuppression (primarily leukopenia) was found to be the dose-limiting toxicity. This daily dose of 150 mg per day is equivalent to 100 mg/m^2 per day for the average Japanese patient, who has a BSA of 1.5 m^2. For the current study, we selected an S-1 dose of 80 mg/m^2 per day (40 mg/m^2 twice daily), which was slightly less than the maximum allowable dose identified by Phase I trials.[22] The most commonly observed adverse reactions in the current study were myelosuppression and gastrointestinal toxicity; these events generally were mild, with no Grade 4 toxicity noted. Although a small number of cases of Grade 4 myelosuppression have been reported in other Phase II studies in which a total daily dose of 80 mg/m^2 S-1 was used to treat malignant disease (gastric,[14,15] colorectal,[24] head and neck,[16] lung,[27] or breast[28]), the incidence and degree of toxicity observed in those studies did not differ substantially from what was documented in the current study.

The toxicity profile of 5-FU is schedule dependent. Myelosuppression is the primary toxic effect observed in patients receiving bolus 5-FU schedules, whereas hand-foot syndrome, stomatitis, neurotoxicity, and cardiotoxicity are associated with continuous infusion of 5-FU.[7] Hand-foot syndrome, in addition to being a typical side effect of prolonged 5-FU administration via CVI,[29] is commonly associated with the oral administration of other fluoropyrimidines, such as capecitabine.[10,11] The mechanism involved in the development of hand-foot syndrome has not been completely elucidated; however, some 5-FU catabolites are believed to be inducers of this condition.[30]

Thus, the low incidence of hand-foot syndrome associated with UFT use is consistent with the observation of low plasma levels of 5-FU catabolites in patients receiving UFT.[31] In the current trial, hand-foot syndrome was observed in only 5% of the study cohort (2 of 38 patients); furthermore, both of these cases involved reversible, Grade 1 hand-foot syndrome. In other trials, only mild S-1-induced hand-foot syndrome, which was not suggestive of dose-limiting toxicity, has been reported. These findings may reflect the inhibitory effect of CDHP on DPD.

The pharmacokinetic characteristics of prolonged S-1 administration were believed to be consistent with the use of CVI; however, the dose-limiting toxicity induced by S-1 was myelosuppression, which is associated with the bolus dose protocol. In a previous Phase I study, the maximum plasma 5-FU concentration was estimated to be approximately 230 ng/mL for Japanese patients who received S-1 at a dose of 75 mg per day.[22] This relatively high peak plasma 5-FU concentration may result in myelotoxicity, rather than gastrointestinal toxicity, in spite of the prolonged S-1 administration protocol. The low severity of gastrointestinal toxicity, even in the face of a relatively high peak plasma 5-FU concentration[22,23] and area under the plasma concentration–time curve, suggests the usefulness (previously noted in rats[19]) of potassium oxonate in humans. The toxicity observed in the current trial, in which S-1 was administered at a dose of 80 mg/m^2 per day (40 mg/m^2 twice daily), was mild and reversible, and yet the observed activity was remarkable, being equal to or greater than the activity of 5-FU alone.

Oral chemotherapy, for which only limited hospitalization is necessary, has major advantages over intravenously administered treatment in terms of pharmacoeconomic considerations and patient preference, as well as compliance.[12] In one study, it was reported that more than 90% of patients with advanced solid malignancies preferred oral agents over infusional agents when both types of treatment provided comparable efficacy.[32] Furthermore, a randomized crossover trial involving patients with advanced colorectal carcinoma found that oral UFT + LV compared favorably with intravenous 5-FU + LV in terms of toxicity and patient preference.[31]

In the current study, the S-1 regimen was administered successfully, with good treatment compliance, on an outpatient basis. Due to the absence of severe toxicity, especially with regard to symptoms such as nausea, vomiting, and diarrhea, almost all patients received $\geq$ 75% of the full protocol-defined S-1 dose; it is clear that good compliance increases the likelihood of favorable therapeutic responses. Thus, the findings of the current study indicate that S-1 is a promising agent that has the potential to become a valuable oral treatment option, along with capecitabine and UFT + LV, for patients with colorectal carcinoma. Clinical studies of S-1 in the treatment of metastatic colorectal and gastric malignancies[33,34] also suggest that S-1 possesses superior therapeutic activity compared with other regimens.

The combination of irinotecan or oxaliplatin with 5-FU + LV recently has been identified as a candidate regimen for the standard treatment of metastatic colorectal carcinoma. To determine which of these chemotherapeutic agents are most suitable for use in combination with S-1, clinical trials are essential. Three Phase I/II trials of S-1 with LV irinotecan or oxaliplatin for the treatment of metastatic colorectal carcinoma have been scheduled. In addition, a Phase III study of adjuvant chemotherapy (surgery alone vs. surgery followed by S-1) in the treatment of gastric tumors and a Phase III study comparing the use of S-1 alone with the use of S-1 + cisplatin in the treatment of metastatic gastric malignancies are ongoing. In another ongoing Phase III trial involving patients with gastric malignancies, the Japan Clinical Oncology Group is comparing 5-FU, which currently is the standard treatment agent, with single-agent S-1 and with cisplatin + irinotecan.

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