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Association of *carboxylesterase 1A* genotypes with irinotecan pharmacokinetics in Japanese cancer patients

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Association of *UDP-glucuronosyltransferase 1A1* (*UGT1A1*) genetic polymorphisms **6* and **28* with reduced clearance of SN-38 and severe neutropenia in irinotecan therapy was demonstrated in Japanese cancer patients.
- The detailed gene structure of *CES1* has been characterized.
- Possible functional SNPs in the promoter region have been reported.

WHAT THIS STUDY ADDS

- Association of functional *CES1* gene number with AUC ratio [(SN-38 + SN-38G)/irinotecan], an *in vivo* index of CES activity, was observed in patients with irinotecan monotherapy.
- No significant effects of major *CES1* SNPs on irinotecan PK were detected.

AIMS

Human carboxylesterase 1 (CES1) hydrolyzes irinotecan to produce an active metabolite SN-38 in the liver. The human *CES1* gene family consists of two functional genes, *CES1A1* (*1A1*) and *CES1A2* (*1A2*), which are located tail-to-tail on chromosome 16q13-q22.1 (*CES1A2-1A1*). The pseudogene *CES1A3* (*1A3*) and a chimeric *CES1A1* variant (*var1A1*) are also found as polymorphic isoforms of *1A2* and *1A1*, respectively. In this study, roles of *CES1* genotypes and major SNPs in irinotecan pharmacokinetics were investigated in Japanese cancer patients.

METHODS

CES1A diplotypes [combinations of haplotypes A (*1A3-1A1*), B (*1A2-1A1*), C (*1A3-var1A1*) and D (*1A2-var1A1*)] and the major SNPs (−75T>G and −30G>A in *1A1*, and −816A>C in *1A2* and *1A3*) were determined in 177 Japanese cancer patients. Associations of *CES1* genotypes, number of functional *CES1* genes (*1A1*, *1A2* and *var1A1*) and major SNPs, with the AUC ratio of (SN-38 + SN-38G)/irinotecan, a parameter of *in vivo* CES activity, were analyzed for 58 patients treated with irinotecan monotherapy.

RESULTS

The median AUC ratio of patients having three or four functional *CES1* genes (diplotypes A/B, A/D or B/C, C/D, B/B and B/D; $n = 35$) was 1.24-fold of that in patients with two functional *CES1* genes (diplotypes A/A, A/C and C/C; $n = 23$) [median (25th–75th percentiles): 0.31 (0.25–0.38) *vs.* 0.25 (0.20–0.32), $P = 0.0134$]. No significant effects of *var1A1* and the major SNPs examined were observed.

CONCLUSION

This study suggests a gene-dose effect of functional *CES1A* genes on SN-38 formation in irinotecan-treated Japanese cancer patients.

Introduction

Human carboxylesterases (CESs) are members of the α/β-hydrolase-fold family and are localized in the endoplasmic reticulum of many different cell types. These enzymes efficiently catalyze the hydrolysis of a variety of ester- and amide-containing chemicals as well as drugs (including prodrugs) to the respective free acids. They are involved in detoxification or metabolic activation of various drugs, environmental toxicants and carcinogens. CESs also catalyze the hydrolysis of endogenous compounds such as short- and long-chain acyl-glycerols, long-chain acyl-carnitine, and long-chain acyl-CoA esters. The two major CES families CES1 and CES2 have been identified in human tissues. CES1 is abundant in the liver and lung but not in the intestine, while CES2 is highly expressed in the intestine and kidney but has low expression in the liver and lung [1].

Human CES1 and CES2 are involved in producing a topoisomerase I inhibitor SN-38, an active metabolite of irinotecan which is clinically used for colorectal, lung and other cancers [2]. SN-38 is further inactivated by UDP-glucuronosyltransferase 1As (UGT1As) to produce SN-38 glucuronide (SN-38G). Irinotecan is also converted by cytochrome P450 3A4 (CYP3A4) to an inactive compound 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) (Figure 1).

Recent pharmacogenetic studies on irinotecan have revealed significant associations of *UGT1A1* polymorphisms **28* [–54_39A(TA)$_6$TAA>A(TA)$_7$TAA or –40_39insTA] and **6* [211G>A (G71R)], the latter being specifically detected in East Asians, with reduced clearance of SN-38 resulting in severe neutropenia [3–8]. These findings have led to the clinical application of genetic testing for *UGT1A1*28* in the United States (since August 2005) and for *UGT1A1*6* and **28* in Japan (since March 2009). In addition, possible additive effects of genotypes of the transporters for irinotecan and its metabolites, such as *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1*, have been suggested [9–12]. We previously analyzed *CES2* polymorphisms in a Japanese

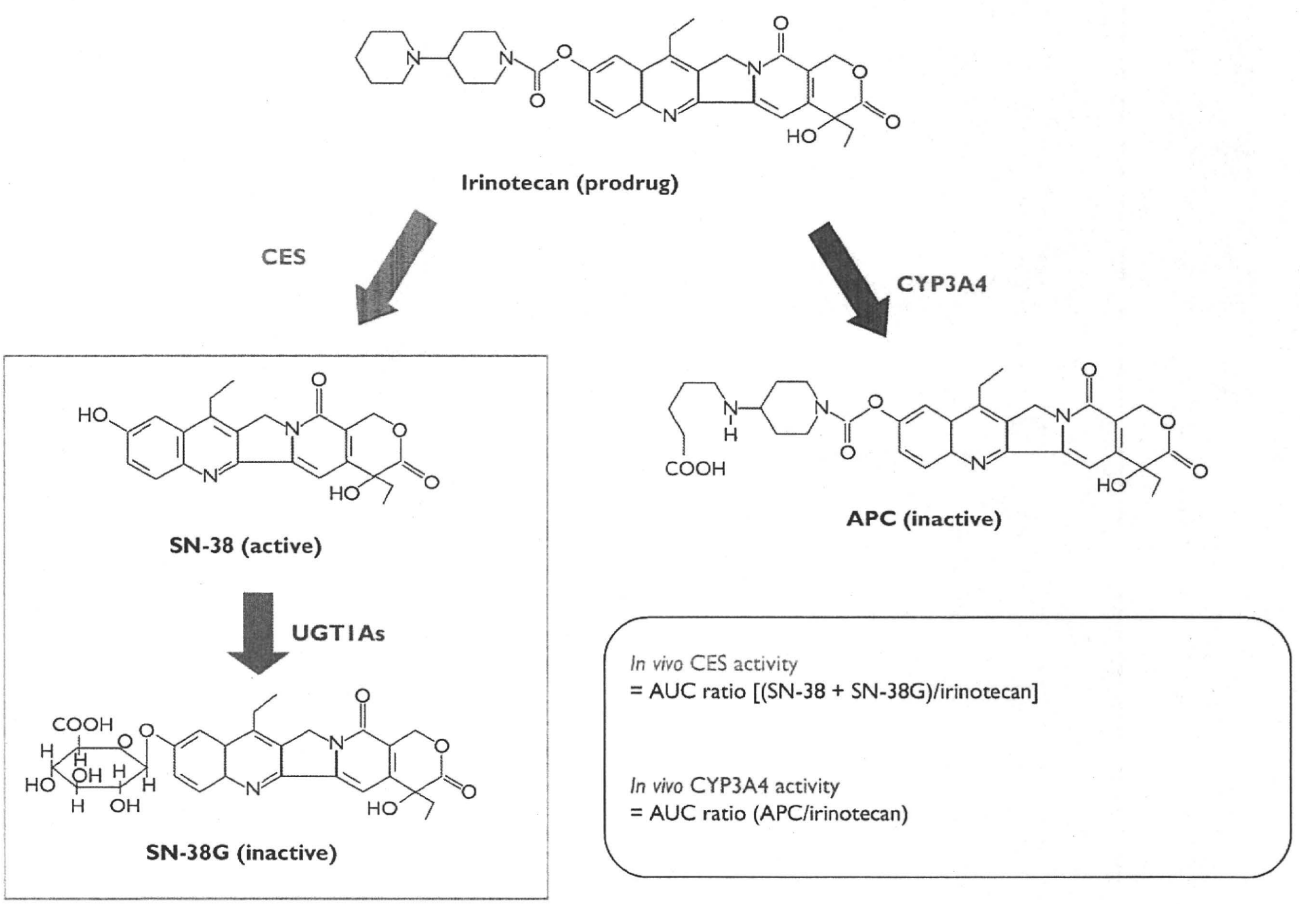

Figure 1

Metabolic pathway of irinotecan. The prodrug irinotecan is hydrolyzed by carboxylesterase (CES) to produce an active metabolite SN-38, and subsequently detoxified by UDP-glucuronosyltransferase 1As (UGT1As) to produce an inactive metabolite SN-38 glucuronide (SN-38G). Irinotecan is also metabolized by cytochrome P450 3A4 (CYP3A4) to produce another inactive metabolite APC

population and identified minor genetic variations which were associated with lower expression/function *in vitro* and *in vivo* [13, 14]. However, major *CES2* haplotypes (**1b* and **1c*) did not affect irinotecan pharmacokinetics (PK) [14]. Since CES1 is expressed at higher levels in the liver, a major organ for activating irinotecan, it is possible that *CES1* genotypes affect the plasma concentrations of irinotecan metabolites. However, their clinical relevance to irinotecan pharmacokinetics/pharmacodynamics has not yet been fully investigated.

Functional human *CES1* genes include *CES1A1* (*1A1*) and *CES1A2* (*1A2*), which are inversely located (tail-to-tail) on chromosome 16q13-q22.1 (*1A2-1A1*). Both *1A1* and *1A2* consist of 14 exons encoding 567 amino acids, and they have 98% homology with 5 nucleotide (4 amino acid) differences in exon 1, which encodes a signal peptide [1]. Recent studies also identified *CES1A1* variants (*var1A1*), in which exon 1 was replaced with exon 1 of *CES1A2*, and a pseudogene *CES1A3* (*1A3*; formerly referred to as *CES4*) replacing *CES1A2* [15, 16]. The *1A3* sequence from the promoter region to exon 1 is the same as that of *CES1A2*, but contains a stop codon in exon 3. The sequence downstream from exon 11 is highly homologous with that of *1A1* (NT_010498) [16]. Ethnic differences in these *CES1* genes (*1A1*, *var1A1*, *1A2* and *1A3*) have been reported [16].

Expression levels of CES1A2 mRNA were lower than those of CES1A1 mRNA in several tissues. This CES1A1 up-regulation could be mediated by additional Sp1 and C/EBP binding sites in the promoter region [17]. Transcript levels of CES1A2 derived from *var1A1* were reported to be higher than those from the original *1A2* [15, 16]. These findings suggest that polymorphisms in the upstream region of *CES1A1* or *var1A1* could affect their expression.

In addition to structural variations of the *CES1* gene family, several single nucleotide polymorphisms (SNPs) and small deletion/insertion variants were found. –816C in the *CES1A2* promoter region was reported to be associated with enhanced CES1A2 expression and imidapril efficacy [18]. Furthermore, –816A>C was found to be linked with several SNPs (–62T>C, –47G>C, –46G>T, –41C>G, –40A>G, –37G>C, –34del/G and –32G>T) in the proximal promoter region, leading to two additional Sp1 binding sites, and these additional sites were suggested to increase transcription of *1A2* [19].

In this context, this study investigated the clinical significance of *CES1* genotypes in irinotecan therapy. For this purpose, we analyzed the *CES1* genotypes (combinations of four *CES1A* isoforms) and major SNPs in the *CES1A1* exon 1 with its adjacent region and in the *CES1A2* and *1A3* promoter regions, which could be important for CES1 expression or function, in Japanese cancer patients treated with irinotecan, and then examined the associations of these *CES1* genotypes or SNPs with irinotecan PK.

Methods

Patients

Genetic analysis of 177 Japanese cancer patients who received irinotecan therapy at the National Cancer Center in Japan was performed. The patients were the same as those described in our previous study [7], where details on eligibility criteria for irinotecan therapy, patient profiles and irinotecan regimens were described. Since the AUC ratio [(SN-38 + SN-38G) : irinotecan], a parameter of *in vivo* CES activity, was influenced by irinotecan regimens [14], 58 patients receiving irinotecan monotherapy (100 mg m^{-2} weekly or 150 mg m^{-2} biweekly) from the 177 patients were primarily used for analysis of the association between *CES1* genotypes and irinotecan PK parameters. The patient set was the same as used in our previous study on *CES2* [14]. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Determination of CES1 genotypes and SNPs

For describing the *CES1* gene family, haplotypes A to D designated by Fukami *et al.* [16] were used (Figure 2): haplotype A, *CES1A3-CES1A1* (*1A3-1A1*); haplotype B, *CES1A2-CES1A1* (*1A2-1A1*); haplotype C, *CES1A3-CES1A1* variant (*1A3-var1A1*); and haplotype D, *CES1A2-CES1A1* variant (*1A2-var1A1*). To determine the diplotypes, combinations of haplotypes A to D, we sequenced *1A1/var1A1* exon 1 and its flanking region and the *1A2/1A3* promoter region of 177 patients. These regions are indicated in Figure 2, and a list of primers/probes is shown in Table 1.

For discrimination between *1A1* and *var1A1*, their exon 1s and flanking regions were sequenced (Figure 2a). Briefly, the first PCR was performed using 25 ng of genomic DNA with 0.625 units of Ex-Taq (Takara Bio. Inc., Shiga, Japan) and 0.2 μM of primers, Ces1-FP and Ces1-RP (Table 1a, first PCR). The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Then, the second PCR was performed with the primers, Ces1_seqF and Ces1_seqR (Table 1a, second PCR) under the same reaction conditions described above. The PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in Table 1a (sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). The conditions of the PCR and sequencing procedures described in the following section were the same as described above unless otherwise noted.

1A2 and *1A3* were discriminated by the restriction fragment length polymorphism (RFLP) method for exon 5

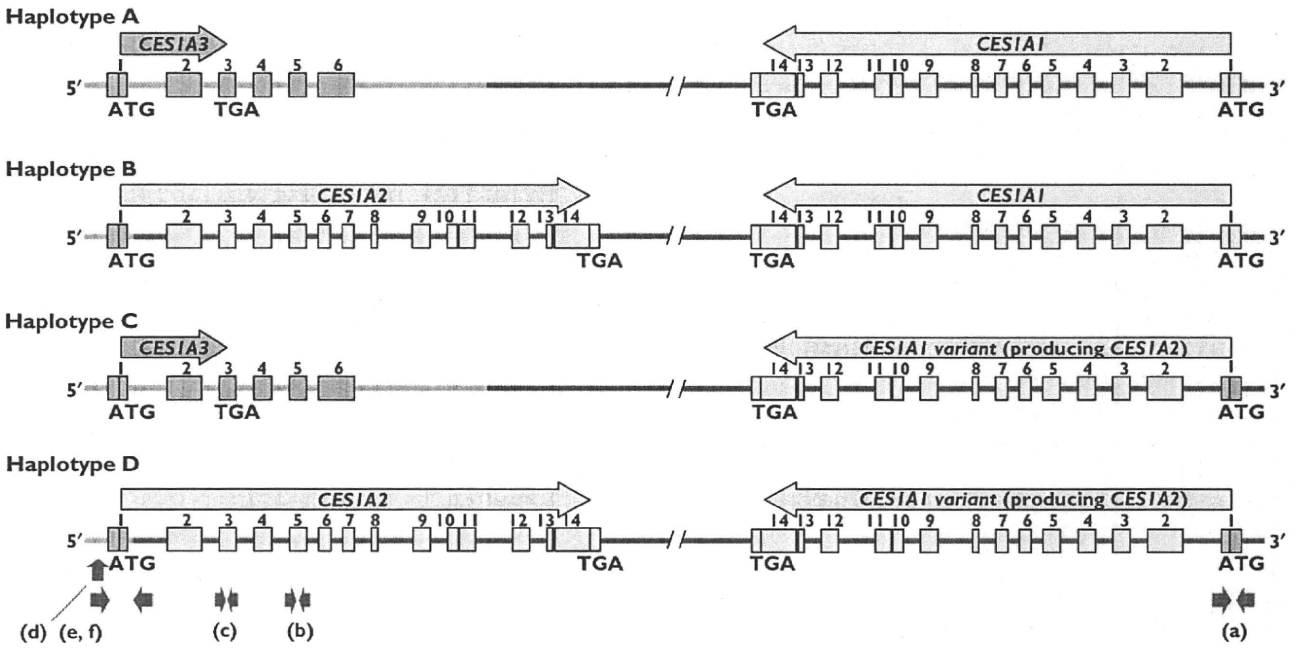

Figure 2

CES1 gene structure and haplotypes. The regions used for haplotype determination in this study are indicated with arrows (a–f)

reported by Fukami *et al.* [16] (Figure 2b). Briefly, the PCR was performed using a primer set (1A-int4F and 1A-int5AS) (Table 1b), and then the PCR products were digested with *Pvu*II to produce *CES1A3*-derived fragments (409 bp and 248 bp). UV intensity of the fragments stained with ethidium bromide was measured after electrophoresis (2% agarose gel). The number of *1A3* (0, 1 or 2) was also confirmed by direct sequencing of exon 5 using the same primer set. To verify that the *1A3* sequence is derived from the pseudogene, we confirmed the existence of a stop codon at codon 105 of *1A3* exon 3 (Figure 2c) in 11 randomly selected patients (heterozygous or homozygous) by amplification and sequencing using primers listed in Table 1c.

Genotyping for −816A>C in the *1A2* and *1A3* promoter region (Figure 2d) was conducted by the TaqMan method of Geshi *et al.* [18] (Table 1d) in all patients. We also examined attribution of −816C to *1A2* or *1A3* by specific amplifications from 5′-regions to intron 1 of the *1A2* and *1A3* (Figure 2e,f) in 23 randomly selected heterozygous patients. For specific amplifications, primers CES1A3-1A2_F1 and CES1A2 R1 for *CES1A2* (Table 1e) and primers CES1A3-1A2_F1 and CES1A3 R1 for *1A3* (Table 1f, first PCR) were used with 0.05 U μl^{-1} LA-Taq with GC buffer I (Takara Bio. Inc.); and for *1A3*, the second PCR using primers CES1A3-1A2_F2 and CES1A3 R2 (Table 1f, second PCR) was also conducted with 0.05 U μl^{-1} Ex-taq. Then, direct sequencing of the *1A2* and *1A3* PCR products was performed. Complete linkage among −816A>C and several SNPs in the proximal promoter region (between −62 to −32) [19] was confirmed for 11 randomly selected subjects.

All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. GenBank NT_010498.15 was used as the reference sequence for *CES1A1*, *CES1A3* and the promoter region of *CES1A2*, and AB119998.1 was used for exon 1 and its downstream region of *CES1A2*. The translational initiation site was designated as +1 to describe the polymorphism positions. Diplotype configuration was estimated with the LDSUPPORT software [20]. The diplotypes A/D and B/C could not be distinguished.

Pharmacokinetic data and association analysis

The area under the concentration–time curve (AUC) values for irinotecan and its metabolites, SN-38, SN-38G and APC, were previously obtained [4, 21]. The AUC ratio of SN-38 plus SN-38G to irinotecan [$AUC_{(SN\text{-}38\ +\ SN\text{-}38G)}/AUC_{irinotecan}$] was used as a parameter reflecting *in vivo* CES activity [14]. The AUC ratio of APC to irinotecan [$AUC_{APC}/AUC_{irinotecan}$] was used as a parameter for *in vivo* CYP3A4 activity [21].

Statistical significance (two-sided, $P < 0.05$) for associations between AUC ratios (or AUC/dose) and *CES1* genotypes or SNPs was determined by the Mann-Whitney test or the Jonckheere-Terpstra (JT) test using Prism version 4.0 (GraphPad Prism Software Inc. San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Correla-

Table 1
Primers and probes used in this study

Region (indicated in Figure 2)	Primer	Primer sequence	Reference
(a) *CES1A1* exon 1 and promoter region			This study
First PCR	Ces1-FP	5′-CCAGGCAAAACCTAGGAGTG-3′	
	Ces1-RP	5′-AGTACAGGGCGATCTCAGGA-3′	
Second PCR	Ces1_seqF	5′-GTATTTCCTTAGCCAGCGGTA-3′	
	Ces1_seqR	5′-CAGAGCCGGACCTGTTGT-3′	
Sequencing	Ces1_SF2	5′-AGAGCCTGGAAAGCTATGAAAA-3′	
	Ces1_SR	5′-TTTCTACGCATCTGCGCCCACC-3′	
(b) *CES1A1, 1A2* and *1A3* exon 5			[16]
PCR and sequencing	1A-int4F	5′-GCTCAGTAAATAGTTGCCAGTT-3′	
	1A-int5AS	5′-TCTCATCAGCATCACATCAAG-3′	
(c) *CES1A3* exon 3			This study
PCR and sequencing	CES1A3-15183F	5′-CAGGGAAGATCGTTGTATTGGTTT-3′	
	CES1A3-15974R	5′-TTCCTTCCACCACTAACATTATTG-3′	
Sequencing (additional primer)	CES1A3-15823R	5′-AAGATGTTCATTAAAGATGCACAG-3′	
(d) *CES1A2* and *1A3* -816A>C genotyping			[18]
PCR	F	5′-CCTTAATTTGGTGATTTCACATTGC-3′	
	R	5′-CAAGACATGGTTCAGCTTCTCAAG-3′	
TaqMan probe	FAM	5′-CATCACCCCTACTGC-3′	
	VIC	5′-CATCACACCTACTGCT-3′	
(e) *CES1A2* promoter region			This study
PCR	CES1A3-CES1A2_F1	5′-ATGATTTCCAGCTTCATCTACA-3′	
	CES1A2_R1	5′-GAGAGAACGTTCCCATGTCTTT-3′	
(f) *CES1A3* promoter region			This study
First PCR	CES1A3-CES1A2_F1	5′-ATGATTTCCAGCTTCATCTACA-3′	
	CES1A3_R1	5′-GCTTGAGTTTTCTTTACAGACA-3′	
Second PCR	CES1A3-CES1A2_F2	5′-AACAGTTTATAACCTGTTATTTTT-3′	
	CES1A3_R2	5′-TGCTTTGGATAAAGACAAGATGTT-3′	
Seqeuncing of *CES1A2/1A3* promoter region			
	CES1A3-CES1A2_F2	5′-AACAGTTTATAACCTGTTATTTTT-3′	
	CES1A3-CES1A2_R1	5′-CACACTTCCAATCTCAGGTAAA-3′	
	CES1A3-CES1A2_F3	5′-TTATGCCACAAGCAGTTGGGCG-3′	
	CES1A3-CES1A2_R2	5′-TCCAAGTCCAATTCCAAGTACGGA-3′	

NT_010498.15 was used as the reference sequence for *CES1A1*, *CES1A3* and the promoter region of *CES1A2*, and AB119998.1 was used for exon 1 and its downstream region of *CES1A2*.

tions between the AUC ratios [$AUC_{(SN\text{-}38\ +\ SN\text{-}38G)}/AUC_{irinotecan}$] and [$AUC_{APC}/AUC_{irinotecan}$] were analyzed by Spearman's rank correlation test. Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to the AUC ratios [$AUC_{(SN\text{-}38\ +\ SN\text{-}38G)}/AUC_{irinotecan}$] were further determined by multiple regression analysis after logarithmic transformation of the AUC ratio. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, *CES1* genotypes and SNPs, *CES2*2* [100C>T(R34W)] or **5* [1A>T (M1L)] [13, 14], *UGT1A1*6* or **28* [7, 8], and the transporter haplotypes, *ABCB1*2* [2677G>T(A893A)], *ABCC2*1A* (−1774delG), *ABCG2#IIB* [421C>A (Q141K) and IVS12+49G>T] and *SLCO1A1*15·17* [521T>C (V174A)] [10]. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.10 using JMP version 7.0.0 (SAS Institute, Inc., Cary, NC, USA). *UGT1A1*6 or *28* was grouped as '+' for stratifying patients: for example, homozygous *UGT1A1 *6 or *28* was depicted as *UGT+/+*.

Results

Genotypes and SNPs of CES1 gene family in Japanese

Frequencies of individual *CES1* genes and *CES1* diplotypes stratified according to the number of functional *CES1* genes are summarized in Table 2. The frequencies of the patients with two, three and four functional *CES1* genes were 44%, 47% and 9%, respectively, in all 177 patients.

By sequencing *1A1* and *var1A1* exon 1s and their flanking region, we detected four novel variations; three in the 5′-flanking region and one in the 5′-untranslated region (5′-UTR) (Table 3): −258C>T (allele frequency: 0.014), −233C>A (0.003), −161A>G (0.006) and −30G>A (0.042). Eleven nucleotide substitutions from the 5′-UTR to intron 1 at allele frequencies of 0.294–0.299 were closely linked with *var1A1* (Table 3). The SNP −816A>C found in the *1A2* and *1A3* promoter regions was genotyped by a TaqMan method [18], and the allele frequency of −816A>C in 177 subjects was 0.249 (Table 4). It was noted that −816C was detected only in patients with *1A3* (*1A3/1A2* and *1A3/1A3*),

Table 2

Frequency of *CES1* genes and diplotypes in Japanese cancer patients

CES1 diplotype	Number of *CES1* gene *1A1*	*var1A1*	*1A2*	*1A3*	Total*	Frequency (n = 177)†		Frequency (monotherapy: n = 58)†	
A/A	2	0	0	2	2	0.203	0.441	0.138	0.397
A/C	1	1	0	2		0.220		0.241	
C/C	0	2	0	2		0.017		0.017	
A/B	2	0	1	1	3	0.237	0.469	0.293	0.534
A/D or B/C	1	1	1	1		0.192		0.190	
C/D	0	2	1	1		0.040		0.052	
B/B	2	0	2	0	4	0.040	0.090	0.017	0.069
B/D	1	1	2	0		0.034		0.052	
D/D	0	2	2	0		0.017		0.000	
Frequency (n = 354)‡	0.703	0.297	0.325	0.675					
(monotherapy: n = 116)‡	0.690	0.310	0.336	0.664					

*Number of functional genes. †Number of subjects. ‡Number of chromosomes.

but not in the *1A2* homozygotes (*1A2/1A2*). In the *1A2/1A3* patients, 38 of the 39 patients having −816C were heterozygous for −816C (Table 4). These findings suggested a close association between −816C with *1A3*. Following specific amplifications of the regions from 5′-regions to intron 1 in *1A2* and *1A3* (Figure 2e,f) of 23 patients randomly selected from the 38 patients with −816A/C and *1A2/1A3*, we confirmed that −816C resided in the *1A3* gene (data not shown). Thus, −816A>C is the major SNP of *1A3* but very rare in *1A2*. In addition, the SNPs, −62T>C, −47G>C, −46G>T, −41C>G, −40A>G, −37G>C, −34del/G and −32G>T, in the proximal promoter region reported to be linked with −816A>C [19] were found to be completely linked with *1A3* (data not shown).

Association of CES1 genotypes with in vivo CES activity

CES1 diplotypes In patients treated with irinotecan monotherapy, we found the AUC ratios of patients with haplotypes A or C (having the *1A3* pseudogene) were lower than those without A or C, indicating functional *CES1* gene number dependency. The median AUC ratio of patients having three or four functional *CES1* genes was 1.24-fold of that in patients with two functional *CES1* genes [median (25th–75th percentiles): 0.31 (0.25–0.38) *vs.* 0.25 (0.20–0.32), P = 0.0134, Mann-Whitney test)] (Figure 3a). No significant differences were observed between *1A1* and *var1A1* (among *1A1/1A1*, *var1A1/1A1* and *var1A1/var1A1*). As we previously reported, the *CES2* variations, *CES2*5* [1A>T(M1L)] and *CES2*2* [100C>T(R34W)] [13, 14] showed low CES activity as indicated in Figure 3a.

Platinum-containing regimens themselves enhance renal excretion of irinotecan and its metabolites, especially SN-38G. No significant effect of *CES1* gene number on the AUC ratio was observed. However, it was noted that the median renal excretion ratio [(SN-38 + SN-38G)/irinotecan] in patients with four functional *CES1* genes was 1.37-fold higher than that in patients with two or three functional genes (P = 0.0217, Mann-Whitney test) (data not shown).

To exclude the possibility that the higher AUC ratio observed above (Figure 3a) was biased by CYP3A4, another metabolic enzyme for irinotecan, we analyzed the association between the (SN-38 + SN-38G)/irinotecan AUC ratio and the APC/irinotecan AUC ratio, an *in vivo* parameter of CYP3A4 activity [21], in patients treated with irinotecan monotherapy. The result showed no correlation between the two parameters (Spearman r = 0.126, P = 0.345).

CES1 SNPs Next, associations of the two *1A1* SNPs, −75G>T and −30G>A (Table 3) and *1A3*-816A>C with the AUC ratio [(SN-38 + SN-38G)/irinotecan] were analyzed. The effects of the SNPs were analyzed in patients stratified by the functional *CES1* gene number and also in all the patients receiving monotherapy. A −75G>T-dependent increase in the AUC ratio was observed in the whole group of patients (P = 0.027, JT test) (Figure 3b), and this trend was remarkable in patients with three or four functional *CES1* genes. No significant effect of −30G>A was observed (Figure 3c). As for −816C in *1A3*, no association between this SNP and the AUC ratio was evident in patients with two or three functional *CES1* genes (Figure 3d). In the platinum-containing regimens, no significant effects of these SNPs on the AUC ratio or the renal recovery ratio were observed (data not shown).

Multivariate analysis The contribution of *CES1* genotypes to the AUC ratio was further analyzed by multivariate analysis, using the patient background factors and polymorphisms including the haplotypes of *CES2*, *UGT1A1* and transporters as variables [7, 8, 10, 13, 14]. The final model revealed a significant association of the functional *CES1* gene number (n = 3 or 4) with the AUC ratio. Contributions of smoking history, irinotecan dose, hepatic and renal function were also detected while that of *ABCB1*2* (+/+) was

Table 3

Summary of genetic variations of *CES1A1* and *var 1A1* exon 1s and their flanking regions detected in this study

SNP identification				Position					
This study	NCBI (dbSNP)	JSNP	Location	NT_010498.15	From the translational initiation site or the nearest exon	Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele frequency (n = 354)*	*CES1A1* variant (*CES1A2* type)
MPJ6_CS1001†			5′-flank	9481424	−258	ttgggcaagtttacagctct**C/T**tgtaatctgacagtagagtc		0.014	
MPJ6_CS1002†			5′-flank	9481399	−233	atctgacagtagagtccaga**C/A**tggtttgatgaaagagggta		0.003	
MPJ6_CS1003†			5′-flank	9481327	−161	tagaagcccagggagatctg**A/G**ggaaagggagggctttttctg		0.006	
MPJ6_CS1004	rs3815583	IMS-JST175949	Exon1(5′-UTR)	9481241	−75	aactctgggcggggctgggc**G/T**ccagggctggacagcacagt		0.41	
MPJ6_CS1005	rs28429139		Exon1(5′-UTR)	9481212	−46	ggacagcacagtccctctga**A/G**ctgcacagagacctcgcagg		0.299	*var1A1*
MPJ6_CS1006	rs28494177		Exon1(5′-UTR)	9481205	−39	acagtccctctgaactgcac**A/G**gagacctcgcaggccccgag		0.299	*var1A1*
MPJ6_CS1007†			Exon1(5′-UTR)	9481196	−30	ctgaactgcacagagacctc**G/A**caggccccgagaactgtcgc		0.042	
MPJ6_CS1008	rs28520463		Exon1(5′-UTR)	9481187	−21	acagagacctcgcaggcccc**G/C**agaactgtcgcccttccacg		0.297	*var1A1*
MPJ6_CS1009	rs28499065		Exon1(5′-UTR)	9481186	−20	cagagacctcgcaggccccg**A/G**gaactgtcgcccttccacga		0.297	*var1A1*
MPJ6_CS1010	rs28515828		Exon1(5′-UTR)	9481168	−2	cgagaactgtcgcccttcca**C/G**gatgtggctccgtgccttta		0.299	*var1A1*
MPJ6_CS1011			Exon 1	9481156	11	cccttccacgatgtggctcc**G/C**tgcctttatcctggccactc	Arg4Pro	0.297	*var1A1*
MPJ6_CS1012			Exon 1	9481152	15	tccacgatgtggctccgtgc**C/T**tttatcctggccactctctc	Ala5Ala	0.297	*var1A1*
MPJ6_CS1013			Exon 1	9481151	16	ccacgatgtggctccgtgcc**T/C**ttatcctggccactctctct	Phe6Leu	0.297	*var1A1*
MPJ6_CS1014			Exon 1	9481148	19	cgatgtggctccgtgcctt**A/G**tcctggccactctctctgct	Ile7Val	0.297	*var1A1*
MPJ6_CS1015	rs28563878		Exon 1	9481133	34	tgcctttatcctggccactct**C/G**ctgcttccgcggcttggggt	Ser12Ala	0.297	*var1A1*
MPJ6_CS1016	rs12149359		Intron 1	9481099	IVS1+16	ttggggtgagtccttctgaa**A/G**tcaaaatgcggggcacttttt		0.294	*var1A1*

*Number of chromosomes. †Novel variation detected in this study.

Table 4

Frequency of *CES1A2(/1A3)* promoter SNP −816A>C in Japanese cancer patients

CES1A2 and *1A3* Genotype	-816A>C Genotype	Number of subjects	Allele frequency
1A2/1A2	A/A	16	0/32 (0%)
	A/C	0	
	C/C	0	
1A2/1A3	A/A	44	40/166 (24.1%)
	A/C	38	
	C/C	1	
1A3/1A3	A/A	41	48/156 (30.8%)
	A/C	26	
	C/C	11	
Total		177	88/354 (24.9%)

not significant (Table 5). The *CES1* genotypes explained 22.6% of variability in the final model among all the variables and 11.3% of total variability in the AUC ratio.

Effects of CES1 genotypes on SN-38 AUC and toxicity

To clarify the clinical importance of *CES1* genotyping for irinotecan therapy, the effects of *CES1* genotypes or SNPs on AUC levels of the active metabolite SN-38 and neutropenia were examined in the non-*UGT*+/+ patients. In this non-*UGT*+/+ population, significantly higher AUC ratios of (SN-38 + SN-38G)/irinotecan were also observed in the patients with three or four functional *CES1* genes (P = 0.0234, Mann-Whitney test) as observed in all the patients treated with irinotecan monotherapy (Figure 3a). With increased number of functional *CES1* genes, an increasing trend of SN-38 AUC/dose was observed in patients receiving irinotecan monotherapy (1.4-fold for four genes *vs.* two genes; P = 0.080, JT test) (Figure 4). However, multiple regression analysis revealed no statistically significant contribution of *CES1* genotypes to SN-38 AUC/dose although *UGT1A1"*6 or *28"* and *ABCB1*2/*2* showed significant contributions [10]. Regarding neutropenia, a higher incidence (though statistically insignificant) for grade 3/4 neutropenia in patients with four functional *CES1* genes was observed (50% for four genes and 16% for two or three genes, P = 0.09, Fisher's exact test). The effects of the SNPs (−75G>T, −30G>A and −816A>C) on SN-38 AUC or incidence grade 3/4 neutropenia were not significant (data not shown). In platinum-containing regimens, no significant effects of the *CES1* genotypes on SN-38 AUC/dose or incidence of grade 3/4 neutropenia were detected in the non-*UGT*+/+ patients (data not shown).

Discussion

Recent pharmacogenetic studies on irinotecan have shown the clinical significance of *UGT1A1 *6* and **28* in Japanese patients [7,8] and *UGT1A1*28* in Caucasians [5,6] for severe neutropenia. Subsequent studies have revealed additional genetic factors including transporters [10–12]. However, the clinical importance of genotypes of the irinotecan-activating enzymes *CES1* and *CES2* is still uncertain.

Since the hydrolytic activity of CES2 for irinotecan was reported to be much higher than that of CES1 [2], most studies have focused on the clinical significance of *CES2* polymorphisms in irinotecan therapy [13, 14, 22]. We previously identified minor *CES2* genetic variations in Japanese, including *CES2*2* [100C>T (R34W)] and *CES2*5* [1A>T (M1L)] which caused low *in vitro* expression/function of CES2 [13, 14] and also exhibited reduced *in vivo* CES activity in irinotecan-treated patients [14] (also see Figure 3a). However, the major *CES2* haplotypes in Japanese, **1b* (IVS10-108G>A and 1749A>G, frequency = 0.233) and **1c* (−363C>G, IVS10-108G>A and IVS10-87G>A, frequency = 0.027), did not show any significant effects on irinotecan PK [14]. No clinical significance of *CES2* polymorphisms has been reported in Caucasians [22]. Neither *CES1* nor *CES2* SNPs affecting their mRNA expression in normal colonic mucosa were found in European and African populations [23]. Since precise structures of the *CES1* genes and their promoter regions had not been elucidated, evaluation of the roles of the *CES1* genotypes in irinotecan therapy has been rather difficult.

In the present study, the frequencies of individual *CES1* genes (*1A1*, *var1A1*, *1A2* and *1A3*) (Table 2) were almost comparable with the previous report in the Japanese population (0.748, 0.252, 0.313 and 0.687, respectively) [16]. To our knowledge, the present study is the first report suggesting a possible effect of *CES1* genotypes on irinotecan PK. This study showed that the AUC ratio [(SN-38 + SN-38G)/irinotecan], and probably *in vivo* CES activity, was elevated depending on the number of functional *CES1* genes (*1A1*, *var1A1* and *1A2*) in patients treated by irinotecan monotherapy (100 or 150 mg m^{-2} irinotecan) (Figure 3a). This gene-dose effect was not clearly shown in the platinum-containing combination therapy (60–70 mg m^{-2} irinotecan), where renal excretion of irinotecan and its metabolites (especially SN-38G) is highly enhanced by a large volume of infusion fluid. However, the median renal excretion ratio [(SN-38 + SN-38G)/irinotecan] in patients with four functional genes was 1.37-fold higher than that in patients with two or three functional genes in the platinum-containing therapy (data not shown), supporting a partial but significant contribution of the CES1s to activate irinotecan. The present study showed no significant differences in the AUC ratios between *1A1* and *var1A1* (Figure 3a), indicating a common upstream region may be involved in regulation of gene expression of *1A1* and *var1A1*. The previous reports showed the expression levels of CES1A2 were lower than those of CES1A1 [17] and suggested that CES1A2 mRNA was derived mainly from transcription of *var1A1* rather than the original *1A2* [15, 16]. The present study, on the other hand, has suggested that the

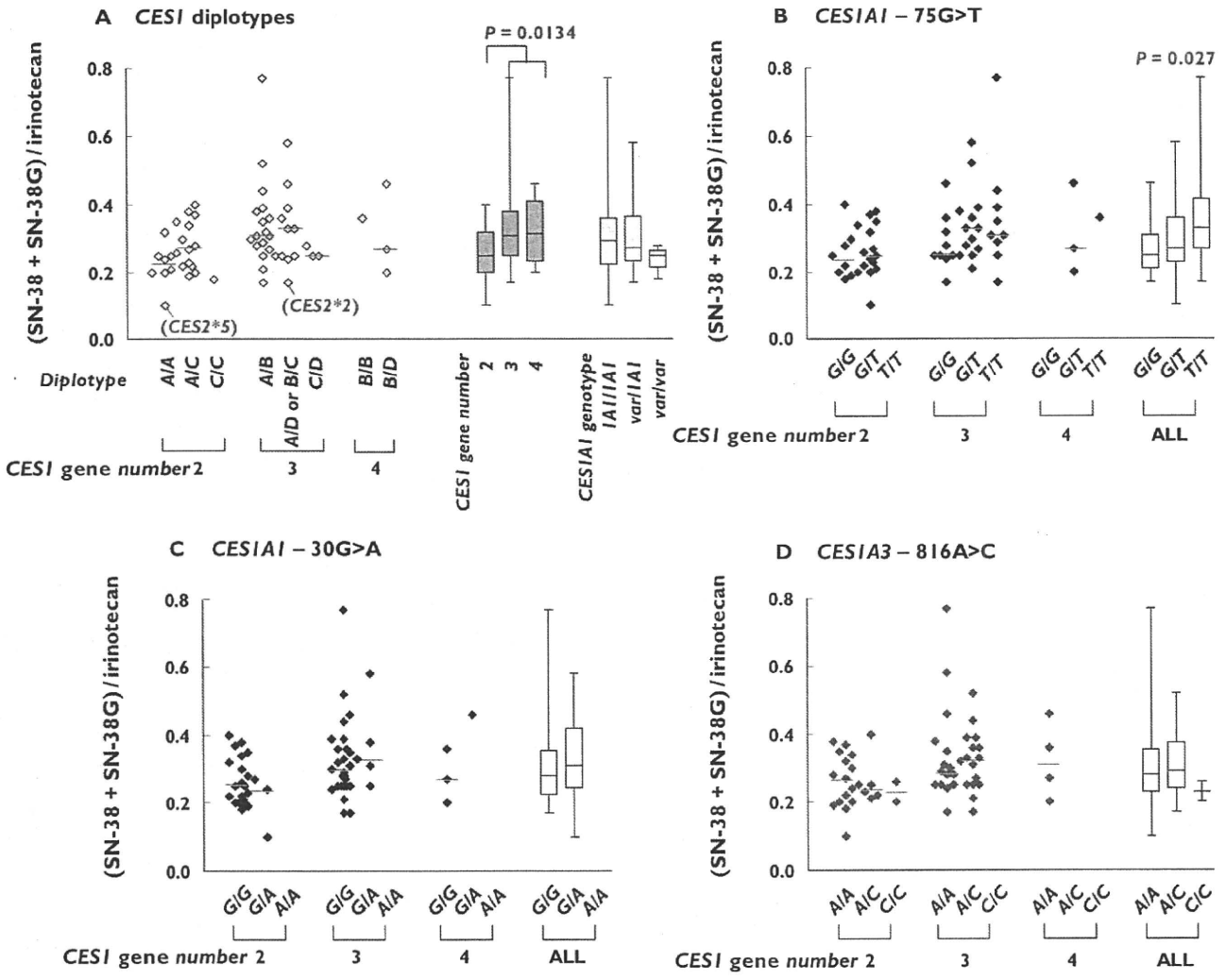

Figure 3

Association of *CES1* diplotypes (A) or SNPs (B–D) with AUC ratio [(SN-38 + SN-38G)/irinotecan], an *in vivo* index of CES activity, in Japanese cancer patients treated with irinotecan monotherapy (n = 58).'*CES1* gene number' means the number of functional genes (*1A1*, *var1A1* and *1A2*). Higher AUC ratios were observed in patients with three or four functional *CES1* genes than with two functional genes (P = 0.0134, Mann-Whitney test) in (A). Patients with *CES2*5* [*CES2* 1A>T (M1L)] (*CES2*5*) and *CES2*2* [*CES2* 100C>T (R34W)] (*CES2*2*) were found to have reduced CES activity in our previous study [13, 14]

Table 5

Multiple regression analysis of AUC ratio [(SN-38 + SN-38G)/irinotecan]* in Japanese cancer patients treated with irinotecan monotherapy

Variable	Coefficient	SE	P value
Smoking	0.073	0.034	0.0375
Initial dose of irinotecan (mg m^{-2})	−0.002	0.001	0.0005
Serum GOT and ALP†	0.082	0.027	0.0038
Serum creatinine (mg dl^{-1})	0.130	0.062	0.0399
***ABCB1*2*‡ (+/+)**	0.042	0.024	0.0831
***CES1* functional gene (n = 3 or 4)**	0.038	0.016	0.0215

r^2 = 0.500, Intercept = −0.248, n = 58. * Values after logarithmic conversion were used. † Grade 1 or greater for both GOT and ALP ‡ *ABCB1*2* [2677G>T (A893S)].

1A2 transcript could contribute to the total CES activity because the [(SN-38 + SN-38G)/irinotecan] AUC ratios of patients without *1A2* (with two functional *CES1* genes) were lower than those with *1A2* (with three or four functional genes) (Figure 3a). However, it must be noted that the increase in the AUC ratio by three or four functional *CES1* genes was only 20% compared with two functional genes (Figure 3a), and that such alterations might be masked by other non-genetic factors. In fact, hepatic and renal function, irinotecan dosage and smoking history were found to be potent contributors to this parameter (Table 5).

–816A>C SNP in *1A2* was reported to be associated with imidapril efficacy and a higher promoter activity for

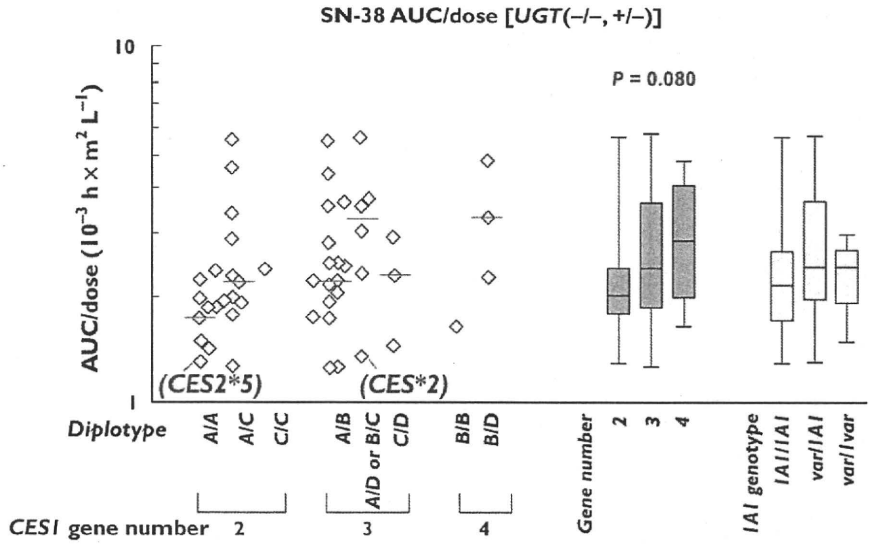

Figure 4

Association of *CES1* genotypes with SN-38 AUC/dose in *UGT*(−/− and +/−) patients treated with irinotecan monotherapy ($n = 51$). '*CES1* gene number' means the number of functional genes (*1A1*, *var1A1* and *1A2*). One patient with an outlying value who had *ABCB1*2* [2677G>T (A893S)] and **14* [2677G>T (A893S) and 1345G>A 230 (E448K)] was excluded from this analysis [10]. A slightly increasing trend in SN-38 AUC(/dose) was observed depending on functional *CES1* gene number. ($P = 0.080$, Jonckheere-Terpstra test). The patients with *CES2*5* [*CES2* 1A>T (M1L)] (*CES2*5*) and *CES2*2* [*CES2* 100C>T (R34W)] (*CES2*2*) [13, 14] are marked

CES1A2 [18] and had strong linkage with SNPs in the proximal promoter region (between −62 to −32) which resulted in additional Sp1 binding sites in the *1A2* promoter region [19]. However, our current study showed no significant effect of −816A>C on the AUC ratio. This can be explained by our finding that −816C and several linked SNPs were mostly located on the *CES1A3* psuedogene but not the functional *1A2* gene.

We newly detected three SNPs (−258C>T, −233C>A and −161A>G) in the 5'-flanking region and one SNP (−30 G>A) in the 5'-UTR of *CES1A1* (Table 3). The effect of −30 G>A on the AUC ratio was not significant (Figure 3c). The frequencies of three other SNPs in the 5'-flanking region were very low (0.003–0.014) which made statistical analysis difficult. These SNPs are not located in the putative transcriptional regulatory regions of *CES1A1*, the binding sites of transcription factors Sp1 and C/EBP [17]. The AUC ratios of the patients with these SNPs were within the 25th–75th percentiles except that slightly higher values were shown in the two -258T patients who received platinum-combination therapy (data not shown). Thus, clinical impact of these SNPs would be small.

With respect to the clinical importance of *CES1* genotyping for irinotecan therapy, the effects of *CES1* genotypes on the AUC level of the active metabolite SN-38 and incidence of grade 3/4 neutropenia should be considered. Since the patients homozygous for *UGT1A1*6 or *28* (*UGT+/+*: **6/*6*, **6/*28* and **28/*28*) showed higher SN-38 AUC/dose levels and severe neutropenia [7], we examined the effects of *CES1* genotypes and SNPs in the non-*UGT+/+* patients. Increasing trends of SN-38 AUC/dose (Figure 4) and incidence of grade 3/4 neutropenia were observed depending on the functional *CES1* gene number in patients with irinotecan monotherapy although statistical significance was not obtained. For the platinum-containing regimens, no significant effects of *CES1* genotypes were shown. Thus, although possible effects of the *CES1* genotypes on neutropenia could not be excluded in irinotecan monotherapy, this study was still insufficient to establish the clinical importance of *CES1* genotyping in irinotecan therapy. Since the sample size will be twice that of the present study to detect a statistically significant decrease of absolute neutrophil counts in the patients with four functional *CES1* genes, future clinical data obtained in a larger number of patients could clarify this point.

In conclusion, this study suggests that the total number of functional *CES1A* genes could influence the formation of the active metabolite of irinotecan in Japanese cancer patients.

Competing interests

HK has received lecture honorarium from Yakult Honsha, the manufacturer of irinotecan. HM has been paid by Yakult Honsha, the manufacturer of irinotecan, for speaking and research.

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Severe Interstitial Lung Disease Associated with Amrubicin Treatment

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Background: Amrubicin is a novel anthracycline agent that is well known to exert significant activity against small cell lung cancer (SCLC), but the adverse pulmonary effects of amrubicin are less well known. We investigated the incidence of acute interstitial lung disease (ILD) in SCLC patients who had been treated with amrubicin.

Methods: Medical records were used to retrospectively investigate a total of 100 cases of SCLC patients treated with single-agent amrubicin therapy at the National Cancer Center Hospital East between June 2003 and March 2008. The patients' radiographic records and clinical data were reviewed to identify patients who had developed acute ILD after being treated with amrubicin.

Results: After receiving amrubicin, seven of the 100 SCLC patients subsequently developed pulmonary infiltrates, and they were identified as cases of acute ILD associated with amrubicin. Of the seven patients who developed ILD, six were treated with corticosteroids, and the ILD improved in three of them, but the other three patients died of respiratory failure. The incidence of ILD was 33% (4/12) among the patients with pre-existing pulmonary fibrosis (PF) and 3% (3/88) among the patients without PF, and the difference between the two groups was statistically significant ($P = 0.0036$).

Conclusions: The results of this study indicated that amrubicin may cause severe ILD and that pre-existing PF was associated with a higher rate of ILD among SCLC patients treated with amrubicin. We recommend not administering amrubicin in the treatment of SCLC patients with pre-existing PF.

Key Words: Amrubicin, Interstitial lung disease, Toxicity, Small cell lung cancer, Chemotherapy.

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Amrubicin is a novel, totally synthetic 9-aminoanthracycline that is converted to an active metabolite, amrubicinol, as a result of reduction of its C-13 ketone group to a hydroxy group. Despite the similarity between the chemical structure of amrubicin and doxorubicin, amrubicin has a different mode of action. Amrubicin and amrubicinol are DNA topoisomerase II inhibitors, which exert their cytotoxic effects by stabilizing a topoisomerase II-mediated cleavable complex, and they are approximately 1/10 weaker than doxorubicin as a DNA intercalator. The in vitro cytotoxic activity of amrubicinol is 18 to 220 times more potent than that of its parent compound, amrubicin.[1,2] An in vivo comparison with doxorubicin showed that amrubicin has a more potent antitumor effect and lower toxic effects on the heart, which is a site of delayed toxicity with doxorubicin, and on the liver and kidneys.[3–5]

Amrubicin is a promising agent for the treatment of small cell lung cancer (SCLC).[6] Most patients with SCLC treated with standard chemotherapy, such as cisplatin plus etposide or cisplatin plus irinotecan, tend to experience a relapse within a year of the completion of treatment, and patients with relapsed SCLC historically have a poor outcome.[4,7] Some multicenter phase II trials in Japan or North America have shown that amrubicin has significant activity in patients with refractory or relapsed SCLC.[8,9] Randomized controlled trials with amrubicin for the treatment of SCLC patients are ongoing in the United States. The major toxicity of amrubicin is hematologic, and more than half of the patients treated with amrubicin develop grade 3 or 4 neutropenia. Nonhematologic toxicities, such as gastrointestinal toxicity or alopecia, are relatively mild. Surprisingly, several patients in Japanese phase II trials developed interstitial lung disease (ILD).[10,11] However, because the adverse pulmonary effects of amrubicin are less well known, in this study, we investigated the incidence of acute ILD in SCLC patients who had been treated with amrubicin.

PATIENTS AND METHODS

Medical records were used to retrospectively investigate a total of 100 consecutive cases of SCLC treated with single-agent amrubicin therapy at the National Cancer Center Hospital East between June 2003 and March 2008. The patients' radiologic reports and clinical data were reviewed to identify patients who had developed acute ILD after being treated with amrubicin. The study was approved by the institutional review board of our institution.

Three independent pulmonologists (K.Y., H.K., and Y.Y.) who had no knowledge of the patients' outcome diagnosed pre-existing lung conditions, i.e., pulmonary fibrosis (PF) and emphysematous change, based on the chest radiographic and

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computed tomographic (CT) findings before the start of amrubicin therapy. The diagnostic criteria for PF were a linear, ground-glass attenuation, or reticular shadows on chest radiographs and CT scans that were predominant in the lower zone of the lung. ILD was diagnosed on the basis of chest radiograph and CT findings (diffuse ground-glass opacity, reticular shadow, or consolidation without segmental distribution and honey-comb pattern), a serum lactate dehydrogenase (LDH) and/or KL-6, which is a mucin-like high-molecular-weight glycoprotein and shown to correlate well with the activities of several different kinds of interstitial pneumonia, elevation, and no evidence of underlying heart disease, infection, or lymphangitic carcinomatosis. Objective tumor response was assessed as complete response, partial response, stable disease ≥8 weeks, or progressive disease according to the Response Evaluation Criteria in Solid Tumors. Toxicity was graded by using the Common Terminology Criteria for Adverse Events version 3.0.

Univariate and multivariate analyses were performed to identify risk factors for ILD associated with amrubicin therapy. All comparisons between proportions were performed by the χ^2 test or Fisher's exact test, as appropriate. Multivariate analyses were performed using the logistic regression procedure to assess the relationship between several factors and the onset of ILD. *P* values less than 0.05 were considered statistically significant. Two-sided statistical tests were used in all analyses.

TABLE 1. Patient Characteristics

	Patients (n = 100)	
	N	%
Age (yr)		
Median	66	
Range	48–81	
Sex		
Female	17	17
Male	83	83
Performance status		
0/1	3/76	77
2/3	20/1	21
Smoking history		
Current/former smoker	98	98
Never smoker	2	2
No. of prior chemotherapy regimens		
1	43	43
2/3	51/6	57
Prior thoracic radiotherapy		
Yes	42	42
No	58	58
Pre-existing pulmonary fibrosis		
Yes	12	12
No	88	88
Pulmonary emphysematous change		
Yes	41	41
No	59	59
Amrubicin dose per square meter body surface area		
45 mg/m^2	37	37
40/35/30 mg/m^2	48/12/3	63

RESULTS

Patient Characteristics

The patients' characteristics are listed in Table 1. Their median age was 66 (range, 48–81) years, 17% of them were women, and 77% had an Eastern Cooperative Oncology Group performance status 0 and 1. Current smokers or exsmokers accounted for 98% of the patients, and emphysematous change was detected in 41% of the patients. Pre-existing PF was detected in 12% of the patients, but none of them had dyspnea. Amrubicin was used as a second-line treatment in 43% of the patients, and 57% had received two or more prior chemotherapy regimens. Amrubicin was diluted in 50 ml of normal saline and administered as a 5-minute daily intravenous injection at a dose of 30 to 45 mg/m^2 on 3 consecutive days, every 3 to 4 weeks.

Incidence and Outcome of ILD

After receiving amrubicin, 7 (7%) of the 100 SCLC patients developed pulmonary infiltrates in the absence of un-

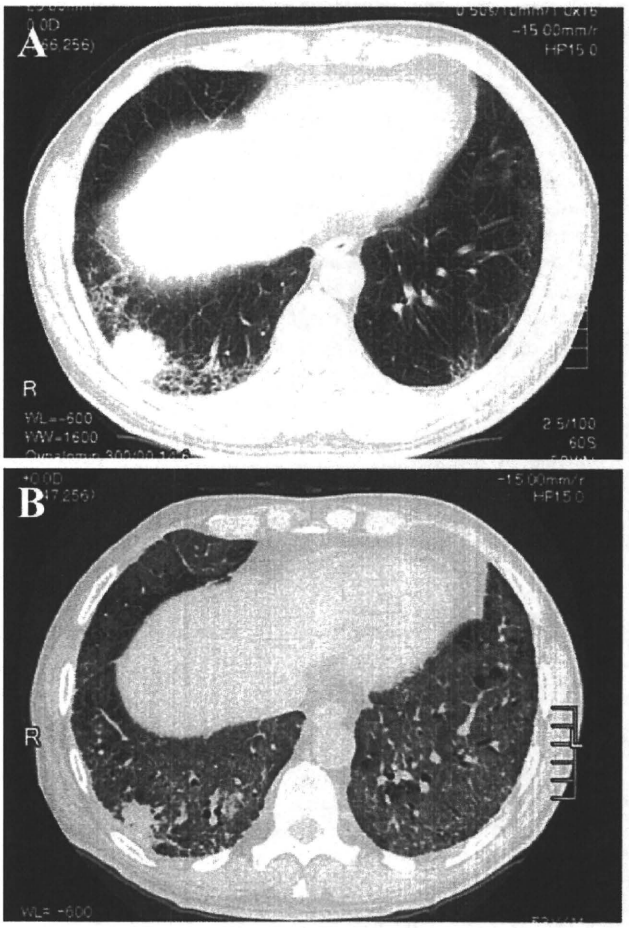

FIGURE 1. Computed tomography (CT) scans of the chest before and after treatment with amrubicin (patient 2 in Table 2). *A*, This CT scan of the chest before treatment with amrubicin shows a bilateral reticular shadow just beneath the pleura and a primary tumor in the right lower lobe. *B*, CT scan of the chest on day 17 of the first course of amrubicin therapy showing bilateral diffuse ground-glass opacities.

TABLE 2. Summary of Patients Who Developed Interstitial Lung Disease Associated with Amrubicin Therapy

No.	Age (yr)	Sex	PS	Smoking History	Prior Chemotherapy	Prior TRT	PF	Time to ILD From First AMR (d)	Initial Manifestations	ILD Status	Time to Death From Last AMR (d)
1	78	M	1	Yes	Carboplatin Etoposide	No	No	15 (day 15 in cycle 1)	Dyspnea, cough, hypoxemia	Died	23
2	53	M	2	Yes	Cisplatin Etoposide Irinotecan	No	Yes	17 (day 17 in cycle 1)	Dyspnea, fever, hypoxemia	Died	30
3	55	M	1	Yes	Cisplatin Etoposide Irinotecan	No	Yes	21 (day 21 in cycle 1)	Dyspnea, hypoxemia	Improved	—
4	70	M	1	Yes	Carboplatin Etoposide	No	Yes	22 (day 22 in cycle 1)	Dyspnea, fever, hypoxemia	Improved	—
5	64	M	1	No	Cisplatin Etoposide Irinotecan	Yes	No	43 (day 15 in cycle 2)	Cough, fever	Improved	—
6	62	M	1	Yes	Cisplatin Etoposide Irinotecan	No	Yes	72 (day 18 in cycle 3)	Dyspnea, hypoxemia	Improved	—
7	63	M	1	Yes	Carboplatin Etoposide	No	No	94 (day 21 in cycle 4)	Dyspnea, fever, hypoxemia	Died	36

PS, performance status; TRT, thoracic radiotherapy; PF, pre-existing pulmonary fibrosis; ILD, interstitial lung disease; AMR, amrubicin.

derlying heart disease, and they were identified as cases of acute ILD associated with amrubicin (Fig. 1). The characteristics of the seven patients with ILD are listed in Table 2. The median time between the start of amrubicin therapy and the diagnosis of ILD was 22 days (range, 15–94 days). All seven patients experienced acute onset or exacerbation of respiratory symptoms, and chest CT scans revealed the new diffuse interstitial changes in both lungs with ground-glass opacity and/or consolidation in all seven patients. Six of the seven patients who developed ILD, the exception being patient 6, received corticosteroid therapy consisting of 500 to 1000 mg methylprednisolone for 3 days, and the ILD improved in four of them. Three patients died of respiratory failure as a result of the ILD, but no autopsy was permitted in any of these three patients.

The results of the univariate analysis of risk factors for ILD associated with amrubicin therapy are shown in Table 3. The incidence of ILD associated with amrubicin was 33% (4/12) in patients with pre-existing PF and 3% (3/88) in patients without PF, and the difference in incidence between the two groups was statistically significant ($P = 0.004$). Based on the results of the univariate analysis, a multivariate analysis was performed using two variables (Pre-existing PF and LDH) and the results showed that pre-existing PF (odds ratio: 10.9, 95% confidence interval: 2.0–66.8) was a significant independent variable correlated with increased risk of ILD associated with amrubicin therapy ($P = 0.006$). LDH was not a significant independent variable (odds ratio: 3.3, 95% confidence interval: 0.44–66.3, $P = 0.30$).

Efficacy of Amrubicin Therapy

The median number of cycles per patient was 2 (range, 1–6). The responses of all 100 patients were assessed, and the results showed a partial response in 32 patients, stable disease in 17 patients, and progressive disease in 51 patients. Thus, the overall response rate was 32% (32/100). The response rate of the chemotherapy-sensitive relapse (defined as relapse at an interval of ≥90 days after the completion of prior chemotherapy) group was 44% (18/41), which is higher than that in the refractory relapse (defined as relapse within 90 days after completion of prior chemotherapy) group (24% [14/59], $P = 0.034$). By contrast, the response rate of the group with pre-existing PF was 25% (3/12), as opposed to 33% (29/88) in the group without PF ($P = 0.74$).

TABLE 3. Relationship Between Clinical Variables and Interstitial Lung Disease Associated with Amrubicin Therapy

Variables	No. of Patients	Incidence of ILD (%)	p
Total	100	7	
Age			
<70 yr	65	7.7	>0.99
≥70 yr	35	5.7	
Sex			
Female	17	0	0.59
Male	83	8.4	
Performance status			
0/1	79	7.6	>0.99
2/3	21	4.8	
Smoking history			
Current/former smoker	98	6.1	0.13
Never smoker	2	50	
No. of prior chemotherapy regimens			
1	43	7	>0.99
2/3	57	7	
Prior thoracic radiotherapy			
Yes	42	2.4	0.23
No	58	10.3	
Pre-existing pulmonary fibrosis			
Yes	12	33.3	0.004
No	88	3.4	
Pulmonary emphysematous change			
Yes	41	9.8	0.69
No	59	5.1	
LDH			
High (more than upper limit of normal)	55	10.9	0.09
Normal	45	2.2	

ILD, interstitial lung disease; LDH, lactate dehydrogenase.

DISCUSSION

Anticancer-agent-associated ILD is an important cause of respiratory failure during cancer chemotherapy.[12] Although the incidence of anticancer-agent-associated ILD seems low, more cases can be expected as increasing numbers of patients receive the new generations of anticancer agents, such as gemcitabine,[13] irinotecan,[14] docetaxel,[15] and gefitinib.[16] To our knowledge, this is the first review on the incidence of ILD in SCLC patients treated with amrubicin.

Amrubicin has already been tested as a treatment for advanced or relapsed SCLC in phase II trials and shown promising activity in Japan and North America. Yana et al.[11] reported finding that 1 (3%) of 33 previously untreated SCLC patients developed interstitial pneumonia after treatment with amrubicin. Inoue et al.[17] reported the results of a randomized phase II trial comparing amrubicin with topotecan in previously treated SCLC patients, and 1 (3.3%) of the 30 patients who received amrubicin had pneumonitis. No amrubicin-associated ILD was reported in two phase II trials of relapsed SCLC patients recently performed in the United States.[9,18] Based on the results of previous clinical trials, the risk of ILD seems to be around 0 to 3% in SCLC patients treated with amrubicin.

In this study, we found a relatively high incidence of ILD (7% of the patients) in SCLC patients treated with amrubicin, and it was higher than in previous clinical trials. The reason for the high incidence is thought to be the possibility of different background between the patients in the present and previous studies. Pre-existing PF has been reported to be the most significant risk factor for the development of anticancer-agent-associated ILD.[19] The patients in our study were treated with amrubicin as clinical practice and the incidence of pre-existing PF was 12%. In previous clinical trials, patients with pre-existing PF were ineligible and the incidence of pre-existing PF was unknown. We attempted to identify the risk factors for the development of amrubicin-associated ILD, and the results showed that pre-existing PF was associated with a significantly higher risk of amrubicin-associated ILD. In our study, six of the seven patients who developed amrubicin-associated ILD received corticosteroid therapy and the ILD improved in four of them. We speculate that patients who developed ILD may benefit partly from corticosteroids.

A major limitation of this study was that none of the patients diagnosed with amrubicin-associated ILD had undergone a lung biopsies during bronchoscopy and no autopsies were performed that would have enabled histologic confirmation of ILD. Therefore, we cannot completely exclude the possibility that the patients had developed lymphangitic carcinomatosis or other diseases and not ILD. However, because the clinical course and radiographic findings of these patients were consistent with drug-induced ILD, we made the diagnosis of amrubicin-associated ILD. In our study, only two patients underwent bronchoalveolar lavage culture. The bronchoalveolar lavage culture obtained from two patients showed no evidence of infection. The exact pathogenetic mechanism of amrubicin-associated ILD is unclear, and further investigation is needed to confirm this finding and evaluate associations between amrubicin-associated ILD and genetic or ethnic factors.

In conclusion, our findings indicated that amrubicin may cause severe ILD and that pre-existing PF was associated with a higher rate of amrubicin-associated ILD. We recommend not administering amrubicin in the treatment of SCLC patients with pre-existing PF. Physicians should have a caution and appropriate management to prevent the development of ILD when using amrubicin to treat patients with pre-existing PF.

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Original Articles

Lung Cancer Working Group Report

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Asia needs a guideline for non-small-cell lung cancer because of differences in medical care, medical care insurance, ethnic variation and drug approval lag within Asian countries and compared with Western countries. Due to ethnic differences, drug dosages are often higher in the USA than in Japan. EGFR mutation in non-small-cell lung cancer was detected in 32% of Asians but only 6% of non-Asians, while differences in irinotecan metabolism cause higher frequencies of toxicity (leukopenia, diarrhea) in Asians. Pharmacodynamic ethnic differences in relation to paclitaxel/carboplatin resulted in longer median survival and a higher 1-year survival rate for Japanese-advanced non-small-cell lung cancer patients compared with Americans. To solve the problem of drug lag, pharmaceutical companies must perform multinational Asian clinical trials with quick accrual of patients, while regulatory authorities must establish high-quality, efficient approval processes, and achieve regulatory harmonization. The National Comprehensive Cancer Network promotes creation of national clinical practice guidelines, and Korea, China and Thailand adapted the National Comprehensive Cancer Network guidelines. Many Asian countries still lack such guidelines, and there are no pan-Asian guidelines for non-small-cell lung cancer. Japan developed its own non-small-cell lung cancer guidelines and also a gefitinib guidance. The study group members concluded that immediate establishment of an Asian non-small-cell lung cancer guideline will be difficult because of the differences among the countries. Asian collaborative trials on treatment of non-small-cell lung cancer need to be started at an early date to generate Asian data.

Key words: non-small-cell lung cancer – EGFR mutation – ethnic differences

GUIDELINES

Asia needs a guideline for non-small-cell lung cancer (NSCLC) (1,2). One reason is the differences in medical care for lung cancer within Asian countries (3–9), such as performance of systematic lymph node dissection versus sampling only. There are also differences in medical care insurance and the economic situations among Asian countries. Ethnic variation in pharmacogenomics is yet another reason for needing an Asian guideline (10–14). Differences exist in the selection of validated data, such as for histology, that is, non-squamous versus squamous, biomarkers such as ERCC1, RRM1 and MSH2 (15–23). The concept of consolidation/maintenance therapy also differs between Western and Asian countries. Drug lag in some Asian countries is another important factor affecting treatment of NSCLC (Table 1).

With regard to ethnic differences, the ICH-E5 guideline states that, 'Although ethnic differences among populations may cause differences in a medicine's safety, efficacy, dosage or dose regimen, many medicines have comparable characteristics and effects across regions.' However, comparison

Table 1. Why do we need Asian guideline for lung cancer?

Difference in medical care for lung cancer
Systematic LN dissection versus sampling
Difference in Medical Care Insurance and economical situation
Ethnic difference of PGX
Evidence obtained specifically from Asian (Japanese) patients (trials)
UFT adjuvant (Stage 1B)
Gefitinib and erlotinib (advanced)
Irinotecan (small and non-small)
Difference in the selection of validated data
Histology: non-squamous versus squamous
Biomarker: ERCC1, RRM1, MSH2
Consolidation/maintenance therapy
Drug lag

between the US and Japan revealed that the US daily doses were higher than those in Japan for 33% of several cardiovascular and other drugs. In addition, ethnic differences are seen in regard to the molecular target, with the EGFR mutation rate being different, as well as drug metabolism and receptor sites.

Concerning molecular targeting, gefitinib monotherapy data can be compared between geographic regions on the basis of the IDEAL I and II Phase II studies (24,25), which were carried out in Japanese and non-Japanese populations, and in Americans, respectively. The patient characteristics were exactly the same in the three populations, but the response rate was significantly higher in the Japanese population, the median survival duration was also higher and the 1-year survival rate was double that of Americans. EGFR mutation in NSCLC was detected at a higher incidence in Asians than in non-Asians, by 32 to 6%. Moreover, the frequency of EGFR mutations was higher in every clinical subgroup, i.e. smokers, non-smokers, adenocarcinoma, males, females, etc., of East-Asian patients compared with non-East-Asian patients (1,26). Gefitinib is known to induce pulmonary toxicity. In Japanese studies, the frequency of gefitinib-induced interstitial lung disease (ILD) ranged from 3.5 to 5.8%, and the ILD mortality ranged from 1.6 to 3.6% (1). In contrast, the frequency of ILD was very low in the USA and other Asian countries, i.e. 0.36 and 0.34% (Table 2).

Irinotecan is another example of ethnic differences is in drug metabolism. Irinotecan is activated to SN-38 by carboxyesterase and then converted to SN-38G by beta-glucuronidase. UGT1A1 is an enzyme that converts SN-38 to SN-38G by glucuronidation. The UGT1A1 promoter shows polymorphism (4,5). When the UGT1A1 promoter has a genotype of 7/7, SN-38 glucuronidation is greatly decreased, and bilirubin glucuronidation is also somewhat decreased. Thus, patients with the 7/7 genotype show higher frequencies of toxicity, such as grade 4 leukopenia and/or grade 3 or higher diarrhea, compared with other UGT1A1 genotypes. In patients with the 7/7 genotype, the AUC of SN-38 is higher compared with other genotypes, while the SN-38G/SN-38 ratio is significantly lower. The distributions of the UGT1A1*28 promoter genotypes differ among racial groups. The 7/7 genotype was observed in only 3% of Japanese and Asian populations, whereas it was present at significantly higher rates of 17% in Canadians, 12% in Caucasians and 23% in Africans (3).

A common-arm analysis was performed to detect pharmacodynamic ethnic differences in paclitaxel plus carboplatin in the treatment of advanced NSCLC in Japan and the USA (27,28). Three trials were included in the analysis: the FACS, JMTO (LC00-03) and SWOG (S0003). The common arm was paclitaxel/carboplatin. The patient characteristics (age, gender and percentages of Stage IV and non-squamous cell carcinoma) were compared and were almost the same in the three studies. The toxicity of the treatment was analyzed with regard to the frequencies of neutropenia and febrile neutropenia, both of which were significantly higher in the Japanese population compared with the American population. When the same dose and same schedule were employed and the efficacy was analyzed, the response rate was almost the same in each of the studies. However, the median survival was 12 and 14 months in the two Japanese studies compared with 9 months in the American study (Tables 3 and 4). The 1-year survival rate was also higher in the Japanese populations compared with the American

Table 2. ILD by EGFR-TKI

	Number of patients	ILD (%)	ILD mortality (%)	Risk factors
WJTOG	1976	70 (3.5)	31 (1.6)	Male, smoker, pulmonary fibrosis
Prospective study of AZ	3322	193 (5.8)	75 (2.5%)	Poor PS, smoker, pulmonary fibrosis, prior CT
Okayama study group	330	15 (4.5)	8 (2.4)	
NCCH	112	6 (5.4)	4 (3.6)	
USA	~24 000	0.36	0.06	
AZ (Asian patient excluding Japanese)	53 150	0.34	0.11	
Korea	111	0		
China	31	0		